

OVERWINTERING AND LOW TEMPERATURE BIOLOGY
OF CEREAL APHIDS

by

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ABSTRACT

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The aphids Sitobion avenae F., Metopolophium dirhodum Wlk. and Rhopalosiphum padi L. are important pests of cereal crops. More knowledge of their biology during autumn, winter and spring is needed to improve the control of summer outbreaks. The work described in this thesis was undertaken to investigate the species' low temperature biology and overwintering ecology. All three species can survive the winter as eggs (holocyclic overwintering) or as viviparae (anholocyclic overwintering). Most of the work in this study investigated anholocyclic overwintering. It concentrated particularly on the central role played by low temperatures in winter development, reproduction and survival.

The host plant in most of the experiments on viviparae was winter wheat, the crop on which cereal aphids cause most damage. Environmental and intrinsic factors affecting low temperature mortality were investigated in the laboratory. As extensive winter sampling of natural populations in Hampshire was being undertaken by S.C. Hand, fieldwork on anholocyclic overwintering in the present study involved experiments on artificially established populations. In these the processes of mortality, development and reproduction were analysed separately, which enabled relatively precise mathematical relationships to be determined. Mortality due to weather factors was quantified in a field experiment which also allowed comparisons between low temperature mortality under laboratory and field conditions to be made. Detailed monitoring of clip-caged S. avenae viviparae on winter wheat from September to March enabled relationships between development rate, reproduction rate and temperature and between survival, reproduction rate and age to be determined accurately. A less detailed investigation of differences in winter performance between S. avenae and R. padi on winter wheat and perennial rye grass was also carried out.

Nearly all the work on holocyclic overwintering concerned M. dirhodum on its primary hosts, Rosa spp. Population surveys in spring and autumn in a west Hampshire study area were undertaken to estimate population densities and characterize favourable overwintering sites. The surveys were done in collaboration with S.C. Hand, who undertook the great bulk of the analysis involved. Colonies of M. dirhodum on selected rose bushes were monitored to determine the timing of the holocyclic cycle and obtain information on mortality factors. The monitoring and surveys allowed the importance of holocyclic overwintering in M. dirhodum to be assessed. A small amount of laboratory work on holocyclic life-stages was also undertaken.

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ABBREVIATIONS USED

- $^{\circ}\text{C} > 3^{\circ}$: Degrees centigrade measured above 3°C
- $\text{day}^{\circ}\text{C} > 3^{\circ}$: Day-degrees centigrade measured using a threshold of 3°C
- d.d.r : Day-degree requirement ($\text{day}^{\circ}\text{C} > 3^{\circ}$ needed to complete a particular stage or process)
- LT50 : Temperature which kills 50% of a tested batch of animals under defined conditions
- n : Sample size
- $\bar{x}$: Mean
- S.E. : Standard error (standard deviation/ $\sqrt{n}$)
- 95% C.I. or C.L.: 95% confidence interval or confidence limits
- P : Probability
- D.F. : Degrees of freedom
- b : Regression coefficient or partial regression coefficient
- r : Correlation coefficient or Multiple correlation coefficient
- $S_{y.x}^2/S_y^2$: $\frac{\text{Variance of deviations of } y \text{ from regression line}}{\text{Total variance of } y}$
for a regression line fitted through the origin
- τ : Kendall's rank correlation coefficient
- r_m : Intrinsic rate of increase
- R_o : Net reproductive rate

min, h : minutes, hours

mm, cm, m, km : millimetres, centimetres, metres, kilometres

ha : hectares

CHAPTER ONE

CHAPTER ONE

GENERAL INTRODUCTION

1.1 THE SPECIES STUDIED AND THEIR PEST STATUS

Seven species of Aphididae are regularly found on cereal and grass crops in Britain. Of these the three commonest species on cereals, and the ones with which this study was concerned, are the English grain aphid Sitobion avenae (F.), the rose-grain aphid Metopolophium dirhodum (Walk.) and the bird cherry-oat aphid Rhopalosiphum padi (L.). All three are important pests of cereal crops, capable of causing significant yield losses directly, by feeding on the plants' sap in large numbers, and indirectly, by infecting plants with virus (Vickerman & Wratten, 1979; Carter, McLean, Watt & Dixon, 1980).

In Britain S. avenae is usually the most abundant species on cereals. The first major outbreak of this species in Britain was recorded in the summer of 1968 and since then summer populations have reached pest status in 1975, 1976 and 1977 (Vickerman & Wratten, 1979; Carter *et al.*, 1980). Though M. dirhodum is generally less common it reached pest status in summer 1970 (George, 1974; Carter *et al.*, 1980) and reached plague proportions in 1979, during the present study. In this country winter cereals, particularly winter wheat, are the crops which most frequently suffer attack (McLean, Carter & Watt, 1977) though M. dirhodum may be important on spring barley (George, 1974; Bardner, French & Dupuch, 1980). Yield losses on wheat and barley caused by moderate to heavy infestations of S. avenae or M. dirhodum have usually been estimated at between 7 and 15% (Kolbe, 1970; Wratten, 1975; George & Gair, 1979; Dean, Dewar, Powell & Wilding, 1980) though with exceptionally high populations losses of up to 42% have been reported (Kolbe, 1969). In addition to reducing yields, aphid infestations have detrimental effects on grain quality (Wratten, 1975; Lee, Stevens, Stokes & Wratten, 1981).

Though more abundant than the other species in Finland and Sweden, R. padi is rarely numerous enough on

cereals in Britain to cause direct damage (Vickerman & Wratten, 1979; Carter et al., 1980). However it is particularly important as a vector of barley yellow dwarf virus (BYDV) because it transmits severe strains of the virus more readily than do the other species (A'brook, 1974; Plumb, 1977). Moreover, its rapid reproduction on grasses means that winged forms, capable of carrying the virus over long distances, are often very abundant in summer and autumn. It is extremely difficult to assess yield loss due to virus but it may be at least as important as direct aphid damage, especially if plants are infected not *long* after germination (A'brook, 1974; Plumb, 1977).

Though some investigations of cereal aphids (chiefly in their capacity as vectors of BYDV) were started in mainland Europe and North America in the 1950s (Vickerman & Wratten, 1979; Carter et al., 1980) very little data on population levels on crops were recorded before the late 1960s. Consequently, the apparent trend towards more severe and widespread cereal aphid outbreaks in recent years cannot be proved (Taylor, 1977; Vickerman, 1977). Nevertheless, several authors have pointed out recent changes in agricultural practice which would appear to favour the pests, often by reducing their natural controlling agents and generally acting to simplify and consequently de-stabilize the cereal ecosystem (Baranyovits, 1973; Kolbe, 1973; Potts & Vickerman, 1974; Vickerman, 1977; Powell, Dean, Dewar & Wilding, 1981). The changes implicated include the increased use of insecticides, herbicides, fungicides and fertilizers, the trend towards monoculture, less rigid adherence to crop rotations, increased use of minimal cultivation techniques, increased straw-burning and earlier sowing of winter cereals.

1.2 WHY STUDY OVERWINTERING?

Though firm evidence to support the view that outbreaks are increasing is lacking, there is no doubt that since 1968 the use of insecticides to combat cereal aphids has greatly increased in Britain (Plumb, 1977; Vickerman & Wratten, 1979; Bardner et al., 1980; Powell

et al., 1981). In 1976 and 1977, the last two years in which severe outbreaks of S. avenae occurred, it was estimated that nearly half the winter wheat crop was treated (Plumb, 1977; Bardner et al., 1980). Moreover, there has been an increased tendency, encouraged by a rise in cereal prices, to apply insecticides as a prophylactic measure.

The heavy and indiscriminate use of insecticides is not only economically wasteful in non-outbreak years but is likely to have less obvious but more serious long-term consequences for two reasons. Firstly, most insecticides are not very specific and so kill non-target animals, including the aphids' predators and parasites and alternative prey which sustains natural enemy populations when the pests themselves are rare. With their natural enemies reduced, cereal aphid outbreaks are likely to occur with increased frequency (Baranyovits, 1973; Potts & Vickerman, 1974; Powell et al., 1981). Secondly, extensive use of insecticides produces strong selection pressure for insecticide-resistance (Baranyovits, 1973; Plumb, 1977; Taylor, 1977; Powell et al., 1981). This has already developed in populations of the peach-potato aphid Myzus persicae in Britain and over much of Europe, and in several other aphid species (Baranyovits, 1973; Taylor, 1977). Cases of pest resurgence and the appearance of new pests due to removal of natural enemies and insecticide resistance are well documented for many crops in which intensive insecticide use was implemented during the 1950s and 1960s (Metcalf, 1980).

There are thus clear incentives for moving away from prophylactic chemical control and towards an integrated pest management approach, in which the use of cultural and biological control methods is maximized and pesticides are applied only when absolutely necessary. However, this strategy requires a detailed knowledge of cereal aphid ecology, both to enhance non-chemical controls and to develop prediction systems needed to give adequate warning of outbreaks which require insecticide treatment. After 1968 several studies were initiated in Britain and Europe to gather the required information. Naturally enough the

initial studies were predominantly concerned with cereal aphids on the crop in the summer. They investigated economic damage thresholds (eg. Kolbe, 1969; George, 1974; Wratten, 1975), the effectiveness of natural enemies (eg. Dean, 1974c; Vickerman & Sunderland, 1975) and the development of short-term prediction systems (eg. Carter, 1978; Rabbinge, Ankersmit & Pak, 1979).

For some aphid species, such as Myzus persicae and other sugar beet virus vectors (Watson, 1966) and Myzus ascolonicus (Hurst, 1969), relationships have been found between summer numbers and weather conditions during the previous winter, while for others, such as Aphis fabae (Way & Cammell, 1974) and Rhopalosiphum insertum (Taylor, 1977) autumn or winter population samples can be used to predict spring or summer infestations. This knowledge helped to stimulate a little research on the overwintering biology of cereal aphids. Results of this early work, particularly the discovery that cereal aphids could overwinter on winter cereal crops under suitable conditions (Dean, 1974a; George, 1974) and the determination of relationships between the timing and size of summer infestations and winter and spring temperatures (Sparrow, 1974; Vickerman, 1977) suggested that events during autumn, winter and spring could have important effects on summer numbers and pest status. Consequently the need for more information on the overwintering of cereal aphids was realized.

The present study was one of two research projects started at the University of Southampton to help fill this important gap in our knowledge. The other was funded by the Natural Environment Research Council and was undertaken by S.C. Hand. Though neither project was aimed specifically at developing a long-term prediction system (enabling summers of high or low outbreak-risk to be identified on the basis of abiotic and biotic conditions in the previous autumn, winter and spring) it was hoped that the data obtained would aid the ultimate development of such a system.

1.3 APHID LIFE-CYCLES AND OVERWINTERING STRATEGIES

Aphids have complex life-cycles involving several different morphs. The terminology introduced here and summarized in the glossary of aphidological terms (Appendix 1.1) follows that of Hille Ris Lambers (1966) and Blackman (1974a).

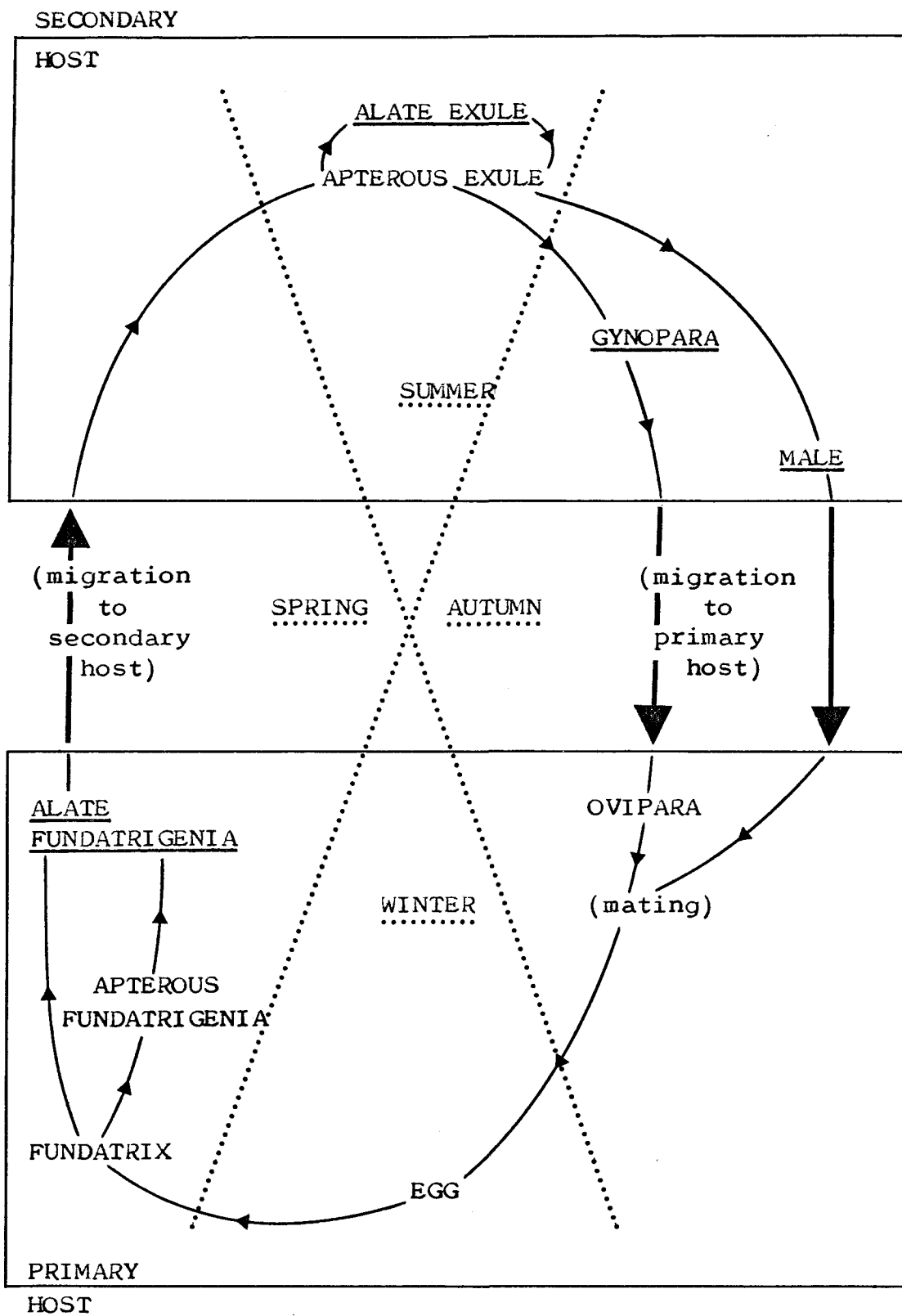
The three species studied all occur on cereals and grasses in the summer as viviparae - asexually reproducing females which give birth to live young by parthenogenesis. However, all three are capable of a 'holocyclic' life-cycle, involving the production of males and sexually reproducing females which lay overwintering eggs.

S. avenae is said to be 'monoecious' as all phases of its holocyclic life-cycle take place on Gramineae but both M. dirhodum and R. padi are 'heteroecious'; that is, their holocyclic life-cycle involves alternation between 'primary' and 'secondary' hosts. The secondary hosts are Gramineae, while the primary hosts are woody plants on which sexual reproduction takes place and eggs are laid. The only known primary host of R. padi in Britain is the bird cherry tree (Prunus padus L.) (Rogerson, 1947). The usual primary hosts of M. dirhodum are species of rose (Rosa spp.) though holocyclic stages have occasionally been found on other Rosaceae (Hille Ris Lambers, 1947; Elkhider, 1979).

The holocyclic life-cycle of a heteroecious aphid species is summarized in Fig. 1.1. The viviparae which occur on the secondary hosts during the summer are known as 'exules' or 'alienicolae'. As adults they may be wingless (apterae*) or winged (alatae*). Alate exules are usually produced when colonies become crowded and the host plant becomes less suitable as a food source (often as a result of heavy aphid infestation) (Blackman, 1974a). They are usually less fecund than apterae but enable the species to escape from an unfavourable situation and colonize fresh host plants. In the autumn the colonies of exules produce alate 'gynoparae' and alate males in response to short day-length (Blackman, 1974a), the males

*The terms 'apterae' and 'apterous' can be applied to any wingless morph and the terms 'alatae' and 'alate' to any winged morph.

Fig. 1.1 Generalized holocyclic life-cycle of a heteroecious aphid species.
(Winged morphs are underlined)



later than the gynoparae.

The gynoparae fly from the secondary to the primary hosts where they give birth by parthenogenesis to sexual, egg-laying females called 'oviparae'. The males also migrate to the primary host and mate with the oviparae, which then lay eggs on the stems. The eggs remain in diapause until early spring when apterous parthenogenetic 'fundatrices' hatch from them. These feed on buds and new leaves and initiate clonal colonies of parthenogenetic 'fundatrigeniae'* on the primary host. The fundatrigeniae may be wingless or winged. Winged fundatrigeniae, sometimes known as spring migrants or emigrants, fly from the primary host and start the first summer colonies of exules on secondary hosts.

In R. padi the first generation of fundatrigeniae is predominantly apterous but the second generation is predominantly alate (Rogerson, 1947). In M. dirhodum the first generation can vary from nearly completely apterous to nearly completely alate but the second is completely alate (Hille Ris Lambers, 1947). Thus more than two generations of fundatrigeniae are exceptional for both species. Though S. avenae does not migrate between primary and secondary hosts its holocyclic life-cycle is otherwise similar to that described. The males are always winged but gynoparae need not be (Hille Ris Lambers, 1939). There is no clear distinction between fundatrigeniae and subsequent generations of viviparae and the term exule is not used.

All three species can survive the winter as eggs when following the holocyclic life-cycle. However they are also potentially capable of 'anholocyclic' overwintering, that is persisting through the winter as parthenogenetic viviparae on Gramineae. Though aphid eggs are markedly more cold-hardy than viviparae (see Chapter 2 for data in the literature on which this conclusion is based) an anholocyclic overwintering strategy has the advantage that reproduction may be possible during mild winters and, for the heteroecious species, no migration between primary

*Some authors use the term fundatrigeniae for the progeny of fundatrices but not for subsequent generations on the primary host.

and secondary hosts is needed.

1.4 THE WORK UNDERTAKEN

As mentioned earlier, the present research project ran concurrently with another on the overwintering biology of cereal aphids, undertaken by S.C. Hand (Hand, 1982). The two studies were designed to be complementary, concentrating on different but related aspects of overwintering. To ensure that unnecessary overlap did not occur subject areas were allocated to one or other project at the outset.

There is a natural division between work on holocyclic overwintering and that on anholocyclic overwintering. Subjects studied within these two broad divisions are described below.

1.4.1 Anholocyclic overwintering

Bruehl (1961) has suggested that overwintering by viviparae is a more efficient way of producing many alatae capable of invading crops early in the year than holocyclic overwintering, and several other workers have suggested links between direct or indirect aphid damage to crops and mild winter weather which allows viviparae to survive (Andre & Tate, 1933; George, 1974; Breniaux, Lescar & Moreau, 1976; Plumb, 1977; Turl, 1980). Especially on spring-sown crops, the potential importance of anholocyclic overwintering to crop damage is accentuated because seedling cereals are particularly susceptible to BYDV (Plumb, 1977). These strong suggestions of a link between pest status and the winter survival of viviparae were the main reason why both Hand and myself concentrated particularly on this mode of overwintering.

Hand undertook sampling of natural populations on a wide range of Gramineae during autumn, winter and spring in a study area in west Hampshire, mainly using a Dietrich vacuum insect net. The work aimed to determine the relative importance of different overwintering sites to the different species and to investigate mortality factors acting during the winter. It constituted the largest single component of Hand's work. The larger part of the

present study was also on anholocyclic overwintering but it did not involve natural populations. In all of it the emphasis was placed on an experimental rather than a descriptive approach, in which the population processes of mortality, development and reproduction were separated and analysed independently. Temperature plays a central role in aphid population dynamics (Gilbert, Gutierrez, Frazer & Jones, 1976) and it is especially important to populations of viviparae during the winter, when it severely limits development and reproduction rates and may also be an important mortality factor (Powell, 1974; Powell & Parry, 1976). A very large proportion of the work on anholocyclic overwintering in the present study was concerned with temperature effects. The work comprised:

1. Laboratory experiments on the nature of low temperature mortality in all three species and the roles of intrinsic and environmental factors which might affect low temperature survival in the field (Chapter 2).
2. A field experiment on winter weather mortality using artificially established populations on winter wheat, aimed particularly at testing conclusions on low temperature mortality derived from the laboratory experiments (Chapter 3).
3. A winter field experiment on clip-caged S. avenae on winter wheat to determine relationships between development, reproduction and temperature (Chapter 4).
4. A winter field experiment on species differences in reproduction, development and survival using R. padi and S. avenae clip-caged on winter wheat and perennial rye grass (Chapter 5).

Effort was concentrated on S. avenae because it appears to be a more important pest in southern England

than the other two species and because the literature and Hand's early sampling (see section 1.5) suggested that anholocycly was particularly important for this species. Also, a single species study on R. padi, including work on overwintering, was being done at the University of East Anglia (Leather, 1980b).

Winter wheat was used as the host plant in the large majority of experiments because it is the main cereal crop and the one most seriously affected by aphid attack. Successful overwintering by viviparae on the crop itself, rather than on grasses, has special implications, firstly because it allows aphids to spread virus and possibly cause damage directly right through the winter. Secondly, and probably more importantly, it facilitates early population build-up on the crop because the aphids do not have to fly in in the spring. The trend towards early sowing of winter cereals (Plumb, 1977) is likely to increase the importance of overwintering on cereal crops as those which emerge early are usually more heavily colonized by autumn alatae before low temperatures prevent further migration (Kendall, Smith, Hazell, Mathiae, Hammock, Chin, March & Young, 1979; Smith, 1981; Hand, 1982).

Practical considerations were also involved in the choice of winter wheat rather than a grass species as the main host, as it is easier to culture and clip-cage aphids on cereals. Also, when plants are at the densities normal in agriculture it is very difficult to count aphids accurately (as had to be done for the weather mortality experiment) if they are on grasses. Though for these reasons grasses were not used much in the present study, it should be stressed that they form the bulk of the Gramineae available to overwintering viviparae in most areas and they are important as winter hosts, particularly for R. padi (Hand, 1982).

It is clear that in aphid ecology, as in ecology generally, a major obstacle to progress has been the tendency of researchers not to define experimental conditions accurately enough and to describe ecological relationships in rather inexact, unmathematical terms. The result is that later workers find it difficult or impossible to

use the relevant data already available and a large amount of potentially useful information is simply discarded. In the study of aphid population dynamics the need to define ecological relationships in mathematical terms has been underlined recently by the increased use of computer simulation modelling as a research tool (eg. Gilbert et al., 1976; Barlow & Dixon, 1980; Carter, Rabbinge & Dixon, 1981). Though the present study was not aimed at developing a simulation model, a special effort was made to present relationships determined in the field experiments in exact, mathematical terms wherever possible.

1.4.2 Holocyclic overwintering

Though aphids collected during Hand's winter sampling of Gramineae could have included holocyclic S. avenae, nearly all the fieldwork in Hand's study and the present one designed specifically to investigate holocyclic life-cycles concerned the overwintering of M. dirhodum on rose. Current controversy as to the relative importance of holocycly and anholocycly in M. dirhodum (see section 1.5.2), the extreme scarcity of the primary host of R. padi in southern England and the intractable problem of sampling for S. avenae eggs on Gramineae were the main reasons for this choice.

The fieldwork undertaken consisted of:

1. Population surveys of M. dirhodum on wild rose in spring and autumn in Hand's west Hampshire study area to obtain population estimates and characterize favourable overwintering sites (Chapter 6, section 6.2).
2. The monitoring of colonies on selected bushes to determine the timing of the holocyclic cycle and obtain information on mortality factors (Chapter 6, sections 6.4 and 6.5).

In addition a small amount of laboratory work on the fecundity of oviparae was undertaken (Chapter 6, section 6.3). The population surveys were undertaken jointly

with S.C. Hand. This collaboration was needed to maximize the amount of rose sampled during the brief periods when spring and autumn holocyclic populations were near their peaks. Though Hand and myself had approximately equal roles in the planning and execution of the field-work, Hand undertook the great bulk of data collation, analysis and presentation, as well as detailed identification work. The only work undertaken in the present study on holocyclic stages of other species was cold-hardiness tests on eggs and fundatrices (Chapter 2, sections 2.5.3 and 2.5.4) and some observations on the production of eggs by artificially established populations of S. avenae in the field (Appendix 1.2). Hand (1982) undertook laboratory work on sexual morph determination in S. avenae and biometric analysis of holocyclic and anholocyclic M. dirhodum morphs.

No work on natural enemies was undertaken in the present study or in Hand's, except for a small amount during the monitoring of M. dirhodum on rose. However, this was not due to any assumption that their role in aphid population dynamics during the winter was unimportant. It was simply felt that, given the very small amount of data on any aspect of overwintering, the more basic aspects should be tackled first. A study of the role of natural enemies at a time of year when both they and the aphids are at low density is likely to present severe methodological difficulties and demands at least one research project in its own right.

1.5 EVIDENCE FOR HOLOCYCLIC AND ANHOLOCYCLIC OVERWINTERING BY THE SPECIES STUDIED

A holocyclic overwintering strategy involves sexual morphs, eggs and specialized spring generations, life-stages with biological characteristics very different from viviparae overwintering anholocyclically. Also, in the case of the two heteroecious species, the two types of overwintering take place on completely different host plants. Holocyclic and anholocyclic populations are

therefore affected very differently by biotic and abiotic factors during autumn, winter and spring. Consequently, even though the present study was not concerned with sampling natural populations, except for the work on holocyclic M. dirhodum, consideration of the relative extent of holocyclic and anholocyclic overwintering in natural populations was of central importance. The relatively small amount of information available at the start of the present study is reviewed below, together with extra data which appeared in the literature between 1977 and 1981. In addition some results of the winter sampling of Gramineae undertaken in Hampshire by Hand (1982) are briefly summarized. Though Hand's study ran concurrently with the present one, conclusions based on the 1977/78 winter sampling were available early on and were not greatly modified by results from the two subsequent field seasons.

1.5.1 Overwintering by S. avenae

Unlike the other two species, holocyclic populations of S. avenae are not concentrated on relatively uncommon primary hosts and could, as far as is known, occur on a wide range of Gramineae. Moreover, the eggs are cemented to the leaves so that they are very unlikely to be found by sampling with a vacuum insect net. This was the main sampling technique used by Hand and is the only method available for sampling large areas of Gramineae which is not extremely labour intensive. The eggs are also small and inconspicuous and so are unlikely to be found by plant searching. Nevertheless, three were found in this way during Hand's study. Perhaps not surprisingly this find apparently constitutes the only direct evidence for holocyclic overwintering by S. avenae in England, though Turl (1980) stated that limited observations in south-east Scotland suggested that the species was holocyclic there.

Some indirect evidence for the occurrence of holocyclic and anholocyclic overwintering by S. avenae in Britain has been obtained from laboratory and field experiments, (Dean, 1974a, 1978; Singer, Smith, Kendall, March, Mathias & Halfacree, 1977; Watt, 1979b). Singer

et al. (1977) kept clones of S. avenae collected in the summer under short daylength conditions in the laboratory and found that most did not produce sexual forms and some members of all clones continued to produce viviparae. Similarly, Watt (1979b) found some clones were completely anholocyclic and that a holocyclic clone kept under short daylength conditions did not switch completely to the production of sexual morphs. These results were confirmed by Hand (1982). Dean (1978) monitored colonies of S. avenae and M. dirhodum established on ten pots of wheat seedlings in early September until late October 1970. The S. avenae continued to produce viviparae throughout the period and though a small number of oviparae were produced in mid-October no males were found and the few eggs laid failed to hatch.

Dean (1974a) also monitored colonies of all three species from November 1970 to March 1971 on two pots of wheat, one sheltered from wind, rain and snow and the other exposed near a hedge. Each week ten adult apterae of each species were added to each pot. S. avenae survived better than the other species but died out before March on the exposed pot. From his experiments Dean concluded that all three species could overwinter successfully as viviparae in grass tussocks or hedgerows where they were sheltered (Dean, 1974a) and that S. avenae was largely or completely anholocyclic in southern England (Dean, 1978). Also in southern England artificially established colonies of S. avenae on potted wheat were monitored by Smith (1981) through the winters of 1977/78 and 1978/79, some in shelter cages and some exposed. Unlike Dean's, these colonies were not supplemented each week and all declined steadily from the time they were established in November. In both winters all unsheltered colonies were extinct by late March and the sheltered ones had declined to under 10% of initial numbers. Winter monitoring by Dean (1974a) and Smith (1981) is discussed in more detail in Chapter 3.

George (1974) and co-workers undertook the only extensive winter sampling of Gramineae before Hand's work. This involved nationwide surveys of winter cereal crops

during March-April 1972 and 1973 and November 1972, with 20 0.3 m lengths of row examined in each sampled field. S. avenae viviparae were the commonest aphids and, along with the other species found, were concentrated in Wales, south and south-west England and, especially, in the west Midlands, where every field examined was infested with aphids. Overall, 40 of the 213 fields sampled in early spring 1972 were infested. No aphids were found in Scotland or in northern and eastern England in the spring surveys. George suggested that overwintering viviparae were concentrated in the south and west because the weather was milder and winter crops, being at a more advanced growth stage, provided more shelter. The importance of anholocyclic overwintering in the south was confirmed by Hand (1982) who regularly found S. avenae viviparae on both cereals and grasses in Hampshire through all three winters in which he sampled. It was the commonest species on cereals in the winter. Though the finding of three eggs showed that some holocyclic overwintering occurred, no oviparae and only one male were found and Hand concluded that in Hampshire holocycly was probably not widespread or important compared to anholocycly.

Elsewhere in western Europe holocyclic overwintering by S. avenae has been recorded rarely (Hille Ris Lambers, 1939) and Remaudière, Latge & Michel (1980) and Dedryver (1978) considered the species was completely anholocyclic in western France. But Grigorov (1976) and Müller (1977), working in Bulgaria and East Germany respectively, recorded holocycly on cereals and grasses and found no evidence of anholocyclic overwintering. This apparent regional difference may well be linked to the lower winter temperatures in central Europe.

In the U.S.A. Phillips (1916) was able to find large numbers of S. avenae eggs in Indiana. However few of these hatched and he also observed anholocyclic overwintering. He considered that both modes were usual in the northern states but only anholocycly occurred south of latitude 35°. Greene (1966) and Wallis & Turner (1972) recorded winter finds of S. avenae viviparae in Oregon and Washington State respectively, though in the latter

case the aphids were found in an unusually warm micro-environment produced by warm spring water. There are also reports of successful anholocyclic overwintering in Canada from the provinces of New Brunswick (Adams & Drew, 1964) and British Columbia (Forbes, 1962), both coastal areas with relatively mild winters.

1.5.2 Overwintering by *M. dirhodum*

There are records of *M. dirhodum* overwintering holocyclically on *Rosa* from Britain, Europe and North America (Patch, 1938; Hille Ris Lambers, 1947; Stroyan, 1952; Forbes, 1962; Robinson & Hsu, 1963; Kolbe, 1969). Hille Ris Lambers (pers. comm.) who described the species' holocyclic life-cycle in detail, considered that holocycly was normally obligatory and that anholocyclic overwintering only occurred where colonies on Gramineae were exposed to artificial light so that natural short photoperiods in autumn or winter were not experienced.

However, considerable confusion has arisen recently as to the extent of anholocyclic overwintering in *M. dirhodum*. Prior (1976) reported "a number of finds" of viviparae during winters since 1972 and quoted one by Turl in Scotland well away from sources of artificial light. Later Turl (1980) stated that "a considerable amount of evidence" collected between 1975 and 1977 showed that anholocyclic populations survived the winter in south-east Scotland away from light sources. Vickerman (pers. comm.) also recorded *M. dirhodum* overwintering on winter oats and barley in the 1971/72 and 1972/73 winters in west Sussex away from artificial light. Singer *et al.* (1977) suggested that in the Bristol area "most *M. dirhodum* did not spend the winter as eggs on roses but remained on grasses throughout the year", though the evidence on which this conclusion was based was not given.

As well as the discoveries of viviparae during the winter mentioned above there are records of males during spring and summer (Markkula & Myllymaki, 1963; Dean, 1978) and of oviparae on Gramineae (Orlob & Medler, 1960; Elkhider, 1979). Prior (1976) stated that the occurrence of males during spring and summer is associated with

anholocyclic lineages in some other species such as Metopolophium festucae, and cited it as additional evidence that anholocycly is a recent and increasing phenomenon in M. dirhodum. He suggested that this may have been encouraged by increased exposure to artificial light.

Dean's monitoring of artificially established colonies from November 1970 to March 1971 suggested that M. dirhodum viviparae were capable of surviving the winter in sheltered conditions, though a little less easily than S. avenae (Dean, 1974a). However Smith (1981) monitored M. dirhodum alongside S. avenae between mid-November 1977 and late March 1978 and found the former survived better, actually increasing through the winter if sheltered. Neither Dean nor Smith mentioned finding sexual morphs in these experiments. But the colonies of M. dirhodum established by Dean (1978) in early September 1970 (which, unlike those established in November, were not added to each week) became extinct by mid-November due mainly to the production of large numbers of males. Alatae, which may have been gynoparae, were also produced in mid-October. Nevertheless Dean (1978) failed to find natural populations of M. dirhodum on rose in the autumn and concluded that the species was "largely or completely anholocyclic, with probably few or no overwintering eggs", despite the fact that this appeared to contradict his own observations of autumn colonies on wheat.

In France Remaudière et al. (1980) found M. dirhodum capable of anholocyclic overwintering in Normandy and Dedryver (1978) considered it completely anholocyclic in the west of the country. In the U.S.A., Greene (1966) found the species surviving through the winter on grasses and cereals in Oregon.

Despite the above evidence for anholocyclic overwintering, George (1974) did not mention finding any M. dirhodum viviparae during his nationwide winter surveys of cereals. It should be noted that this paper has been misquoted in two recent reviews (Vickerman & Wratten, 1979; Carter et al., 1980) which stated that M. dirhodum was found. Hand (1982) also failed to find any

M. dirhodum during his extensive winter sampling of cereals and grasses in his west Hampshire study area between 1977 and 1980 though a colony was found nearby at the Game Conservancy, Fordingbridge. However it was close to laboratories and so was subject to an artificially long photoperiod. Vorley (pers. comm.) did find seven M. dirhodum viviparae in the west Hampshire study area between mid-December 1980 and late March 1981 (all in a single field of winter barley) but it was quite clear that in the winter M. dirhodum was much less common on Gramineae in this area than either S. avenae or R. padi.

1.5.3 Overwintering by R. padi

The primary host of R. padi, the bird-cherry tree (Prunus padus) is widespread and rather common in Scotland, northern England and Wales, reaching Gloucester, Derby and east Yorkshire (Clapham, Tutin & Warburg, 1968). It is rare elsewhere in Britain and does not occur naturally in southern England, though it is occasionally planted. There are many records of the overwintering of R. padi on bird cherry (Rogerson, 1947; Gair, 1953; Dixon, 1971; Leather, 1980a, 1980b; Turl, 1980) and holocyclic populations are often found at high density.

It has been suggested that the usually low summer numbers of R. padi in southern England are due to the absence of the primary host (Carter et al., 1980), which implies that anholocyclic overwintering is unimportant. However, it has been recorded from Avon (Singer et al., 1977), Pembroke (A'brook, 1974) Derbyshire (Gair, 1953) and south-east Scotland (Turl, 1980). Also, Stroyan (unpublished data, quoted in Carter et al., 1980), using biometric analysis to distinguish alatae derived from primary and secondary hosts, found that in spring 1972 up to 71% of R. padi caught in Rothamsted Insect Survey suction traps were exules even in areas where Prunus padus was abundant. Moreover, George (1974) found R. padi quite commonly in his winter surveys of cereals, particularly in central Wales where densities of more than 50 per 0.3 m of row were recorded in some fields.

In Hampshire, Hand (1982) also found high densities

of R. padi, particularly on mature grasses in early winter when hundreds per square metre were regularly recorded. However, populations declined more sharply through the winter than those of S. avenae suggesting, as Dean's winter monitoring had, that viviparae of R. padi were in some way less suited to surviving the winter. Leather (1980b) also monitored artificially established colonies on potted cereals and grasses through the winter at Norwich. He found that all the colonies eventually died out (though the date of extinction varied from late October to early April) and concluded that in Norfolk R. padi could overwinter successfully as viviparae in very mild winters only.

In Finland, where Prunus padus is common and R. padi a pest, overwintering is probably entirely holocyclic as the winters are much colder than those in Britain (Leather & Lehti, 1981). In France, however, anholocycly has been reported by several authors (Breniaux et al., 1976; Dedryver, 1978; Remaudière et al., 1980). In North America there are records from Oregon, U.S.A. (Greene, 1966) and British Columbia (Forbes, 1962), New Brunswick (Adams & Drew, 1964) and Alberta (Harper & Blakely, 1968), Canada. The last record was from a warm microenvironment inside piles of sprouting grain.

CHAPTER TWO

CHAPTER TWO

LABORATORY EXPERIMENTS ON LOW-TEMPERATURE MORTALITY

2.1 INTRODUCTION

Many studies of low temperature mortality in insects have been undertaken solely to answer physiological questions, of theoretical rather than applied significance, on the nature and mechanisms of cold-hardiness. However, the present laboratory work, on three important agricultural pests, had the more applied aim of providing insight into how low temperatures cause mortality in natural overwintering populations. Consequently, rather than an in-depth study of physiological mechanisms, a broad survey was undertaken of the environmental and intrinsic factors likely to affect cereal aphid low temperature mortality in the field. The information this provided was used to interpret results from a field experiment on abiotic winter mortality factors (see Chapter 3) and to assess the usefulness of quick laboratory-based tests in predicting low temperature mortality in the field. Although the original aim was to compare all three species in all laboratory experiments, lack of time led to several experiments being conducted on S. avenae only. The emphasis in the laboratory experiments is on anholocyclic overwintering and the cold-hardiness of viviparae. Only two small experiments were conducted on life-stages from the holocyclic part of the life cycle (see sections 2.5.3 and 2.5.4).

The introduction below provides a summary of the principles of insect cold-hardiness needed to understand why particular factors were chosen for investigation and how they are thought to affect cold-tolerance in other insect species. Relatively little work has been undertaken on aphid cold-hardiness. Table 2.1 lists the main laboratory studies so far undertaken. Some evidence from these is cited in the introduction below and more detail is given in the descriptions of individual experiments. Terms relating to cold-hardiness are usually defined as they are introduced but the reader should also refer to the glossary in Appendix 2.1.

Table 2.1 Laboratory studies on aphid low temperature mortality.

Author and date	Aphid species
(a) <u>Papers on viviparae:</u>	
Adams (1962)	<u>Aulacorthum solani</u> (Kltb.), <u>Myzus ornatus</u> (Laing), <u>Macrosiphum euphorbiae</u> (Thos.), <u>Myzus persicae</u> (Sulz.), <u>Aphis gossypii</u> Glover, <u>Rhopalosiphum padi</u> (L.), <u>Sitobion avenae</u> (F.), <u>Myzus lythri</u> (Schrank) <u>Brevicoryne brassicae</u> (L.)
Carter (1972)	<u>Elatobium abietinum</u> (Walker)
Griffiths & Wratten (1979)	<u>Sitobion avenae</u> (F.) <u>Metopolophium dirhodum</u> (Wlk.) <u>Rhopalosiphum padi</u> (L.)
Harrison & Barlow (1973)	<u>Acyrtosiphon pisum</u> (Harris)
Parry (1978)	<u>Myzus persicae</u> (Sulz.)
Parry (1979a)	<u>Elatobium abietinum</u> (Walker)
Parry (1980)	<u>Adelges cooleyi</u> (Gillette)
Powell (1973) (Ph.D.thesis)	<u>Elatobium abietinum</u> (Walker)
Powell (1974)	<u>Elatobium abietinum</u> (Walker)
Powell (1976)	<u>Elatobium abietinum</u> (Walker) <u>Drepanosiphum platanoides</u> (Schr.) <u>Eucallipterus tiliae</u> (L.)
(b) <u>Papers on eggs:</u>	
James & Luff (1982)	<u>Rhopalosiphum insertum</u> (Walker)
Parry (1979b)	<u>Cinara pilicornis</u> (Hartig)
Somme (1964)	<u>Pterocomma smithia</u> (Monell)
Somme (1969)	<u>Hyalopterus pruni</u> (Geoff.) <u>Aphis pomi</u> (De Geer) <u>Rhopalosiphum padi</u> (L.)
Way & Banks (1964)	<u>Aphis fabae</u> (Scop.)

2.1.1 Types of insect cold-hardiness

Insects can be divided into three categories according to their ability to survive low temperatures (Mellanby, 1959; Salt, 1961; Bursell, 1964; Wigglesworth, 1965). These are:

1. "Chill-susceptible" insects: which are killed by low temperature exposure even when this does not result in ice forming inside the insect's body.
2. "Freezing-susceptible" insects: which survive low temperature exposure as long as ice does not form in the insect's body but are killed when it does.
3. "Freezing-tolerant" or "freezing-resistant" insects: which can survive at least some internal ice formation.

It should be noted that in freezing-susceptible insects, freezing and death due to low temperatures always go together but this is not true of the other two categories of insect.

(a) Chill-susceptible insects

Most of the insects shown to be chill-susceptible are species which are rarely if ever subjected to sub-zero temperatures in their natural habitats. Many are tropical or sub-tropical species, such as the fruit fly Ceratitis capitata (Andrewartha & Birch, 1954) and the mosquito Aedes aegyptii (Mellanby, 1960). Others include stored-product insects, such as the beetle Calandra oryzae (Robinson, 1928), or household pests like the bed-bug Cimex (Andrewartha & Birch, 1954). Many chill-susceptible insects suffer cold-injury at temperatures several degrees above 0°C. For example, the tsetse fly Glossina can be killed by chilling to +5°C for a few hours (Burnett, 1957), while some Cimex eggs fail to hatch after cooling to +10°C (Andrewartha & Birch, 1954).

Although few of the temperate species investigated so far have been found to be chill-susceptible this is

partly a consequence of researchers tending to concentrate on life-stages which have to survive particularly rigorous low-temperature conditions. These often show interesting and well-developed mechanisms for avoiding cold-injury but may well give a biased idea of the extent of cold-hardiness in temperate insects as a whole. Also, in several studies on apparently freezing-susceptible insects, too little attention has been paid to the possibility of chill-injury.

(b) Freezing-susceptible insects

Most insects from temperate regions are freezing-susceptible, even in the life-stages with which they overwinter. Nevertheless, many are capable of surviving temperatures many degrees below zero, avoiding ice-formation by 'supercooling' (see section 2.1.2 below). The most extreme example of freezing avoidance by supercooling in an insect is probably the gall midge Rhabdophaga sp. from arctic Canada, which is reported to supercool to -66°C (Ring & Tesar, 1981). However, lethal low temperatures for freezing-susceptible insects are usually in the range -10° to -30°C . All the aphids studied have been found to be killed by freezing and thus have been categorised as freezing-susceptible. But in several of these studies scant attention was paid to the possibility that aphids could be at least partially chill-susceptible.

(c) Freezing-tolerant species

Freezing-tolerance is generally confined to insects which experience very low winter temperatures and is, even then, usually restricted to the overwintering stages. The great bulk of the species shown to be freezing-tolerant come from the orders Coleoptera, Lepidoptera and Hymenoptera and in the large majority of cases, the freezing-tolerant stage is an overwintering pupa or larva that does not feed (Asahina, 1969). Many of these undergo an obligatory diapause. Recently, however, several examples of freezing tolerance in adult insects have been discovered. These include the Alaskan beetles Pterostichus brevicornis (Miller, 1969), Eleodes blanchardi

(Zachariassen, Hammel & Schmidek, 1979) and Upis ceramboides (Miller, 1978a) and overwintering queens of the hornet Vespula maculata (Duman & Patterson, 1978). Freezing tolerance does not imply complete immunity from the damaging effects of internal ice-formation. Indeed, a number of adult Coleoptera and Hymenoptera are known which, although they survive internal freezing, die when the temperature falls much below -10°C (Ohshima & Asahina, 1972). At the other extreme are very freezing-tolerant species such as Monema flavescens (Lepidoptera) prepupae of which, with suitable low temperature pre-treatment, can survive storage in liquid nitrogen at -190°C (Asahina, 1969).

(d) Divisions between the cold-hardiness categories

The divisions between chill-susceptible, freezing-susceptible and freezing-tolerant insects may not be as clear cut as is often implied in the literature. The fact that different life-stages of a single species often belong to different cold-hardiness categories has already been mentioned. A few species are also known in which seasonal changes or differences in low-temperature pre-treatment can change a particular life-stage from freezing-susceptible to freezing-tolerant or vice versa (Hanec & Beck, 1960; Somme, 1965; Baust & Miller, 1972). Even within an apparently homogeneous batch of a species exposed to low temperatures together, the possibility exists that some individuals may die as a result of chill-injury while others are unharmed until they freeze (Andrewartha & Birch, 1954).

(e) Regional differences in cold-hardiness

In several studies, populations of the same species from different regions have been found to differ with respect to cold-hardiness (Pantuyukov, 1964; Somme, 1965; Sullivan, 1965). In two North American populations of the gall fly Eurosta solidaginis the larvae not only showed differences in cold-hardiness but also in the type of protective strategy used to avoid cold-death (Baust, 1981). Generally speaking, where regional differences

are found, the more cold-hardy populations come from the areas with colder winters (Casagrande & Haynes, 1976). This has been found in clones of S. avenae from Spain, Switzerland and Norway and R. padi from Norway and Finland (Griffiths & Wratten, 1979).

2.1.2 Freezing in insects: the basic principles

The majority of temperate zone insects, including all the aphid species so far studied, are killed by freezing. Consequently, an understanding of the principles of ice-formation in biological systems is essential to the study of insect cold-hardiness.

(a) Freezing and supercooling points

The freezing point of an aqueous solution is defined as the temperature at which water in the solution is in thermodynamic equilibrium with ice. Thus an ice crystal placed in a solution at its freezing point will neither increase nor decrease in size. If the temperature is raised fractionally the crystal will melt. If it is lowered the crystal will grow as water freezes out of the solution. A solution's freezing point is depressed below 0°C (the freezing point of pure water) by the presence of solute molecules and the greater the concentration of solutes, the more the freezing point is lowered.

In insect body fluids the various solutes present usually depress the freezing point by only a few degrees Celsius (Salt, 1958b). However, if an insect's body fluid, or any other aqueous solution, is cooled below the freezing point it will not freeze immediately. But it will do so as soon as an ice crystal 'nucleus' of sufficient size has formed, or if a ready-formed ice crystal is introduced. A solution in this metastable condition below its freezing point is said to be 'supercooled' and the temperature at which it eventually freezes is known as its 'supercooling point' (Salt, 1961). Unlike the freezing point this is not a fixed temperature for a particular solution.

(b) Nucleative freezing

The formation of an ice crystal nucleus of sufficient size to initiate freezing can take place in two distinct ways, known as 'homogeneous' and 'heterogeneous' nucleation (Salt, 1961; Meryman, 1966; Asahina, 1969). In homogeneous nucleation only water molecules are involved. The ice crystal nucleus consists of an aggregation of water molecules which by chance, due to their random movement, take on an ice-like orientation and can thus form a nucleus for ice crystal growth. The nucleus will be under continual bombardment by surrounding water molecules. A water molecule hitting the nucleus may make it larger - by becoming part of its ice-like structure - or may help to break it down - by displacing molecules already in it.

The growth or breakdown of a nucleus is a stochastic or 'chance' process dependent on the random movement of water molecules. The probability of a nucleus being added to rather than diminished at any stage depends partly on its size (Meryman, 1966). As its size increases the further growth of a nucleus becomes more and more likely. Thus most nuclei will be destroyed soon after they form. Only a few will grow to 'critical' size - the size at which further growth is as likely as destruction - and of these the first to start to form an ice crystal will freeze the solution. The probability of a nucleus growing to form an ice crystal is also dependent on the kinetic energy of the surrounding water molecules. At temperatures just below the freezing point the water molecules hitting a newly formed nucleus have large amounts of energy and are much more likely to destroy it than become part of it. As a solution is cooled its water molecules lose energy and become progressively more likely to join a nucleus. Hence, the probability of a nucleus growing to form an ice crystal increases as the temperature of a supercooled solution is lowered. As nucleus formation and growth are stochastic processes, the probability of successful nucleation in a supercooled solution is equal in each unit time interval, as long as the temperature and other conditions are held constant. Thus the probability of a solution freezing is dependent on time as well as

temperature, increasing with the length of time it is kept supercooled.

Heterogeneous nucleation, like homogeneous nucleation, depends on the random movement of water molecules and is subject to the laws of probability. Consequently the probability of a supercooled solution freezing due to heterogeneous nucleation is also dependent on its temperature and the length of time it is kept supercooled. However, while homogeneous nucleation involves water molecules only, in heterogeneous nucleation an ice crystal nucleus is formed by the addition of supercooled water molecules onto a foreign (non-aqueous) surface or particle known as a 'nucleator' or 'nucleating agent' (Salt, 1961; Meryman, 1966). The molecular configuration of the nucleator's surface is such that it tends to attract water molecules and bring them together in an ice-like configuration. A sample of pure water can be cooled to as low as -45°C before it freezes provided special precautions are taken to eliminate possible nucleating agents (Meryman, 1966). In this case freezing may well be due to homogeneous nucleation.

However, in insects and other biological systems water is intimately associated with a wide variety of potential nucleating agents. Consequently, homogeneous nucleation probably occurs infrequently in nature (Salt, 1958a; Asahina, 1969) and the supercooling points of insects are generally well above -40°C . The exceptions are usually the overwintering stages of species which have to survive very low winter temperatures. In these forms natural selection has presumably led to the virtual elimination of all but the least efficient nucleating agents.

When a variety of nucleating agents are present, as in insect tissues, those most efficient at ice-nucleation will limit the supercooling ability of the solution in question. The probability of nucleation and subsequent freezing in a supercooled insect is thus dependent on the quantity and quality of the nucleating agents present in its body, as well as the temperature to which it is supercooled and the time it is kept supercooled.

(c) Inoculative freezing

As well as the spontaneous nucleative freezing described above, freezing in a supercooled insect may result from its inoculation by ice crystals formed outside the body. This usually involves penetration through the cuticle by ice formed on the body surface (Salt, 1961; Asahina, 1969). In the relatively few instances in which inoculative freezing has been demonstrated, considerable delay has usually been observed between the freezing of surface moisture and the inoculation of the insect (Salt, 1963; Asahina, 1969). This was probably due to the effectiveness of the cuticle as a barrier to ice crystal growth. It is likely that only minute pathways are available through the cuticle. These are probably air-filled and may be lined with hydrophobic molecules (Salt, 1963).

The importance of inoculative freezing by surface ice is likely to vary in different groups of insects depending on the structure of the cuticle and the frequency with which ice forms on the body surface, which will in turn depend on the habitats and microhabitats used. Asahina (1969) stated that, except in aquatic species, freezing in insects is generally presumed to be by spontaneous nucleative freezing rather than by inoculation as the latter has rarely been demonstrated even when the overwintering stages have been removed from coverings such as cocoons, hibernacula and puparia which often protect them from surface ice.

However, aphids overwintering anholocyclically are likely to be more susceptible to inoculative freezing than most other insects because of their relatively thin cuticle and their often unprotected overwintering sites. In addition, feeding aphids may be inoculated with ice from the host plant because a bridge of plant sap extends from the host's phloem to the aphid's gut via its stylets. Inoculative freezing from the host has been demonstrated in the green spruce aphid Elatobium abietinum (Powell, 1974). Further evidence on the occurrence of inoculative freezing in aphids and other insects is given below (sections 2.4.5 and 2.4.7).

(d) Intracellular and extracellular freezing

Ice may form either inside cells or in the extracellular body fluids. However, several authors consider that when organisms are subject to slow cooling, as they are in the natural environment, intracellular freezing is rare even in freezing-susceptible organisms (Meryman, 1956, 1966; Mazur, 1963; Asahina, 1969). This view is based on direct microscopic observation of freezing and on theoretical calculations. Salt (1959, 1962) has demonstrated ice crystals inside the fat body and labial gland cells of Eurosta and Cephus larvae after rapid freezing but no intracellular ice was found if the insects were cooled slowly.

The reason why intracellular ice has been observed so rarely after slow cooling is evident if one considers the freezing process. If freezing starts somewhere in the extracellular space, the removal of water and its incorporation into ice crystals will concentrate the solutes in the extracellular fluid. This will cause water to move out of the cells by osmosis, so that both intracellular and extracellular fluid will become progressively concentrated as ice forms. Consequently, the freezing point of the intracellular fluid is very rapidly depressed once extracellular freezing has started and the fluid will remain at its freezing point if cooling proceeds further.

Thus spontaneous nucleative freezing inside the cells will be rendered impossible almost as soon as extracellular freezing starts. Only if the cooling rate is very rapid will water be unable to leave the cells fast enough to keep the intracellular fluid at its freezing point (Mazur, 1963). If the first ice crystal forms inside a cell, not in the extracellular fluid, intracellular freezing should still not be extensive, as water will then move into that cell and be added to the ice crystal until it grows too large for the cell, ruptures it and inoculates the extracellular fluid (Meryman, 1966).

From theoretical considerations it therefore appears that extensive intracellular freezing is very unlikely unless extracellular ice crystals are capable of penetrating cell membranes. Direct observation of frozen tissues

suggests that this is rare (Smith, 1961; Asahina, 1969). But Salt (1961) considers it likely when an insect which is supercooled many degrees eventually freezes and an ice-front sweeps very rapidly through the body. This certainly remains a possibility, as the observations which led to the conclusion that extracellular ice cannot penetrate membranes were made on dissected insects or on cells in vitro, neither of which would supercool as much as an intact insect.

2.1.3 Testing insect cold-hardiness in the laboratory

The most obvious methods for assessing insect cold-hardiness in the laboratory involve the direct assessment of low temperature mortality. The usual technique is to run a series of tests each of which involves exposing a batch of the species in question to a constant low temperature for a fixed time period. The number of animals killed by the low temperature can then be counted and expressed as a proportion of the total number in the batch. After a series of such tests using different temperatures is complete the relationship between per cent mortality and temperature can be plotted. The relative cold-hardiness of different species, different life-stages within a species or different treatments of a particular life-stage can then be assessed, usually by comparing the temperatures needed to kill a certain proportion of the individuals. The temperature needed to kill 50% of the animals (the 'LT50') is usually used.

An alternative method is to run a series of tests keeping the test temperature constant while varying the duration of low temperature exposure in each test, to determine the relationship between per cent mortality and time of exposure at the chosen temperature. Species or treatments can then be compared on the basis of the time needed to kill a certain proportion of the animals, again usually 50%. But tests in which exposure time is held constant while temperature is varied are the more widely used. This is primarily because the relationship between mortality and temperature which is revealed is usually a

sigmoid curve (due to the fact that variation in lower lethal temperatures in a population usually approximates to the normal distribution). Probit analysis (originally developed for toxicology tests) can be employed to transform such a sigmoid relationship into a linear one from which it is relatively easy to determine the necessary statistical parameters, such as LT50s, and their confidence limits.

In freezing-susceptible insects, low temperature death is inextricably linked to freezing. Consequently, the measurement of supercooling points provides a potential alternative way of assessing cold-hardiness which does not involve the direct assessment of mortality. Supercooling points are easy to determine because when an insect freezes the body water being converted to ice gives out its latent heat of fusion. Thus, if the temperature of an insect's body surface is monitored using a small thermo-couple while the insect is cooled, its freezing will be registered as an abrupt rise in temperature, often of several degrees (see Fig. 2.5). The supercooling point is the temperature reached immediately before the latent heat of fusion is given out.

The only important variable which must be standardized when determining supercooling points is the rate at which the insect is cooled and even this has only a small modifying effect. Salt (1966b) proposed a standard cooling rate of $1^{\circ}\text{C}/\text{min}$ for supercooling point determinations and workers since then have usually followed this. Thus supercooling points from many different studies are directly comparable. This is in sharp contrast to studies involving the direct assessment of low temperature mortality. It is rare to find two such studies in which the same cooling rates, exposure times and criteria for death are used. Consequently direct comparisons are usually impossible.

Another advantage of supercooling point determinations over methods involving direct assessment of mortality is that the resultant data are easier to analyse statistically. The mean supercooling point of the members of a batch of insects is usually used as the measure of the

batch's cold-hardiness. If the supercooling points of batch members are approximately normally distributed - which they often are - Student's t-tests or analysis of variance can be used to test for differences between batches. If not, simple non-parametric tests are available. A third advantage is that supercooling points are quick to determine, provided they are not too low. Lastly, mean supercooling points with acceptably narrow confidence intervals can often be determined from a relatively small sample of insects. By contrast, the accurate determination of an LT50 using direct mortality assessment methods usually requires hundreds of individuals. This last is probably the crucial factor which has led so many workers to use supercooling point determinations when studying insect cold-hardiness.

However, supercooling points have two important disadvantages. Firstly, they provide a good measure of low temperature tolerance only if the insects studied are freezing-susceptible in the strictest sense. That is, if they can never survive freezing and never suffer fatal chill-injury. In several studies in which supercooling points have been used to assess cold-hardiness very little or no evidence is presented to show that the species in question cannot be killed by chill-injury. A second disadvantage is that it is often difficult to relate the mean supercooling point of a species to the temperature at which it will freeze under field conditions because insects in their natural habitats are cooled at much slower rates than $1^{\circ}\text{C}/\text{min}$ and often experience sub-zero temperatures for several hours at a time. It must be added that the same fault can also be true of laboratory tests involving the direct assessment of mortality but is less of a problem usually, as test conditions can be adjusted to approximate to those in the field. In such tests the exposure times are frequently chosen so that they approximately correspond to those in the field but cooling and re-warming rates of only a few degrees an hour are very seldom used. Most researchers lack the equipment needed to recreate field low temperature regimes in an accurately reproducible way in the laboratory.

2.1.4 The nature of cold-injury

(a) Chill-injury

Chill-injury is injury due to low temperatures but not involving the freezing of water inside the organism. Very little work has been done on the nature of chill-injury in insects. Although several authors have speculated as to the causes of chill-death very little good evidence is available for any of the proposed theories.

A variety of potentially lethal metabolic imbalances in chill-susceptible insects may well be created by the differential response of different enzyme reactions to temperature. The effective lower limit of some reactions may lie above 0°C due to reduced enzyme activity and molecular movement (Precht, 1973). Several authors have attributed chill-death in insects to the accumulation of toxic metabolites which would be eliminated at normal temperatures (Wigglesworth, 1965; Chapman, 1971). In the honey bee, Apis mellifica, it has been suggested that chill-death is due to starvation because absorption of sugars from the gut is arrested (Wigglesworth, 1965). Colhoun (1954) noted that irreversible disturbances seemed to have occurred in the nervous systems of cold-immobilised cockroaches (Blatella germanica). Brandts (1967) suggested protein denaturation due to the weakening of hydrophobic interactions as an important cause of disruption in biological systems at low temperatures and has observed denaturation in several proteins at temperatures above 0°C.

From the available information on chill-injury in plants, Lyons (1973) proposed a unified theory of chill-injury which seems compatible with the few observations on insects. He suggested that the primary response to cold which produces chill-death is a reversible physical phase transition in cell membranes from a normal, flexible liquid-crystal structure to a solid gel. This change probably results in contraction of the membrane resulting in the creation of 'holes' and increased permeability which leads to solute leakage and disrupted ion balance. Lyons also suggested that such a change in membrane structure would increase activation energies of membrane-bound

enzymes. This would result in various metabolic imbalances, in particular the disruption of respiratory metabolism which involves both membrane-bound and cytoplasmic enzyme systems. This provides an explanation for the observed build up of pyruvate, acetaldehyde and ethanol which are at the 'interface' between glycolysis and mitochondria-based enzyme systems. It would also lead to a reduced ATP supply which could account for the cessation of protoplasmic streaming observed in chill-sensitive plant cells. With time the various metabolic disorders created would become lethal.

Rapid cooling to temperatures near 0°C has a harmful effect on a variety of cells to which a gradual temperature change over the same range would be harmless (Chapman, 1967). This phenomenon, known as 'thermal shock' may lead to chill-injury in animals which are not normally chill-susceptible. Lovelock (1955) proposed that differential thermal expansion of the lipid and protein components of the cell membrane may be responsible for thermal shock-induced haemolysis in erythrocytes. At the other extreme, prolonged exposure to temperatures near 0°C may also cause chill-death in species normally regarded as chill-tolerant (Hanec & Beck, 1960; Salt, 1961). This mortality may be caused by dehydration or the exhaustion of metabolic reserves which continue to be utilised slowly even in a supercooled insect (Salt, 1950).

(b) Freezing-injury

A large amount of research has been undertaken on the freezing of biological systems. As a result many different theories of freezing-injury have been proposed and in the past there has been controversy over which of the proposed mechanisms are responsible. However, as freezing inevitably results in a large number of interdependent changes at the cellular level, many of which might cause damage, it seems most likely that several different mechanisms, acting more or less simultaneously, are involved in freezing-injury (Salt, 1962; Meryman, 1966; Baust, 1973). Few of the mechanisms proposed so far are incompatible.

The freezing process basically consists of two

linked events:

1. The removal of water from the system (to form ice crystals).
2. The appearance and growth of inert foreign bodies (the ice crystals) within or between cells.

All of the diverse and complex changes which occur when an organism freezes are due, directly or indirectly, to one or other of these events. The six main ways in which freezing may cause damage have been summarized by Meryman (1966). Of these, only one is a result of ice crystal growth. All the others are due to the removal of water. The mechanisms of freezing-injury listed by Meryman are:

1. Mechanical rupture of structural elements through ice crystal growth.
2. Increases in electrolyte concentration causing denaturation of macromolecules.
3. Changes in pH causing denaturation of macromolecules.
4. Precipitation of proteins.
5. Contact between protein molecules allowing abnormal, irreversible cross-linkage.
6. Removal of structurally important water.

The five 'dehydration effects' listed above (2 to 6) all cause chemical changes in essential macromolecules in one way or another. Proteins, particularly lipoproteins, which are important constituents of cell membranes, are thought to be especially susceptible to these effects (Meryman, 1966; Smith, 1961). Lipoproteins are particularly susceptible because unlike most simple proteins they are not held together by strong covalent bonds, but by much

weaker associations. They may thus dissociate following small changes in the environment which would be harmless to other proteins (Lovelock, 1957).

Some enzymes such as dehydrogenases are sensitive to freezing although others are insensitive and are actually more active in frozen systems than in supercooled ones because they are concentrated in the latter (Meryman, 1966). One particularly lethal effect of freezing and thawing is that on the lipoproteins in the membranes of lysosomes. Increased permeability or disruption of lysosomal membranes will release the broad spectrum, hydrolytic enzymes they contain causing intracellular digestion (Tappel, 1966).

Of the dehydration effects, damage due to increased electrolyte concentrations as water is incorporated into ice crystals has received the most attention. This 'salt concentration theory' was first proposed by Lovelock (1953). He showed that in erythrocytes increased salt concentration was associated with increased membrane permeability and loss of phospholipid. Similar relationships between freezing-injury and salt concentration have been shown in many other systems (Meryman, 1966). Asahina (1969) considered salt concentration an unimportant effect in insects and cites as evidence the survival of isolated insect hearts in concentrated sodium chloride solutions. Smith (1961) also considered this mechanism of freezing injury unlikely but other authors have suggested it is important (Meryman, 1956, 1966; Parkes, 1958; Mazur, 1969). There is little doubt, however, that other dehydration effects are also involved (Meryman, 1966). Apart from those listed above, others which have received less attention include increases in metabolic biproducts or intermediates to toxic levels, and ion imbalance due to the differential precipitation of ions of different solubilities. Nervous tissue is liable to be particularly susceptible to the latter effect (Pascoe, 1957).

Although mechanical damage by ice crystals is the most obvious cause of freezing-injury several authors have suggested that it is probably not the major cause (Smith, 1961; Meryman, 1966; Mazur, 1969). The extent of

mechanical damage probably depends on whether or not ice forms inside cells. No good evidence that extracellular ice causes mechanical damage is available but cells frozen internally after rapid cooling do show signs of mechanical disruption (Smith, 1961). However, it is not known whether this is the cause of cell death. Nevertheless, there is a wide concensus that intracellular freezing is usually, if not invariably, lethal, even in freezing-tolerant organisms (Levitt, 1956; Smith, 1961; Meryman, 1966; Asahina, 1969; Mazur, 1969; Zachariassen & Hammel, 1976; Duman & Patterson, 1978).

The fact that cells in freezing-tolerant species are killed by intracellular freezing but can survive extracellular ice leads to the conclusion that, in these species at least, intracellular ice probably exerts its lethal effect by causing mechanical damage rather than by the various consequences of dehydration listed above. This follows from the fact that cell membranes are permeable to water so that dehydration effects should be equally damaging whether ice is formed inside cells or in the extracellular fluid. However, as explained above (section 2.1.2) it is generally believed that intracellular freezing is rare unless cooling is very rapid (Meryman, 1956, 1966; Mazur, 1963; Asahina, 1969).

(c) Delayed mortality and sub-lethal effects

Although insects exposed to low temperatures usually appear completely unaffected if they are not killed immediately, in some cases low temperatures produce damaging sub-lethal effects which often cause mortality after a delay. In holometabolous insects the most common sub-lethal freezing-injury is failure to complete metamorphosis (Asahina, 1969). Overwintering larvae or pupae are often apparently unaffected by freezing but are incapable of developing further and, in the laboratory, may remain in the larval or pupal stage for many years (Asahina & Takehara, 1964). Sometimes they can transform to adult under the pupal cuticle but are unable to shed it (Asahina, 1966). Morphological abnormalities may also occur after ecdysis (Asahina, 1969; Salt, 1961). In the pink bollworm Pectinophora gossypiella death at pupation can

account for nearly 90% of the mortality due to low temperature exposure (Wright & Graham, 1978).

Insects affected more severely by cold show loss of co-ordinated movement (Salt, 1961). For example, in the beetle Rhyzopertha dominica exposure to low temperature can result in 'stress-induced lethargy' characterized by only feeble movements of legs, antennae and mouthparts and the inability to walk or feed (Swain, 1975). One of the most extreme examples of a delayed low temperature effect has been shown in the pea aphid Acyrtosiphon pisum. After sub-lethal exposure to -12°C not only did aphids show decreased fecundity and longevity and increased development time compared to controls but these effects were also shown by their offspring (Harrison & Barlow, 1972).

2.1.5 Feeding and cold-hardiness

It is generally agreed that in feeding stages of insects the usual site of nucleative freezing is the digestive tract, due to the presence of efficient nucleating agents in the food (Salt, 1953, 1961; Asahina, 1969; Krunic & Radovic, 1974). This view is supported by several different types of evidence:

1. Within a species, non-feeding stages generally have a greater ability to supercool than do feeding stages (Salt, 1953).
2. In several species, enforced starvation results in an increased ability to supercool (Cloudsley-Thompson, 1973; Somme, 1976; Block, 1981; Block & Somme, 1982).
3. In some species changes in food quality are associated with changes in the insect's supercooling ability. Species in which this has been demonstrated include the aphids Elatobium abietinum (Powell, 1974), Myzus persicae (Parry, 1978), Drepanosiphum platanoides and Eucallipterus tiliae (Powell, 1976) as well as

the solitary bee Megachile rotunda (Kronic, 1971) and the collembolan Cryptopygus antarcticus (Somme & Block, 1982).

4. In several collembolans and mites which do not feed during the winter, the resumption of feeding in the spring coincides with a marked decline in supercooling ability (Somme & Conradi-Larsen, 1977; Block, Young, Conradi-Larsen & Somme, 1978; Block, 1981).
5. Newly hatched larvae of some species show a large decrease in supercooling ability once they have started to feed. They include first-instar nymphs of the aphids Elatobium abietinum (Powell, 1974) and Myzus persicae (Parry, 1978) as well as larvae of the flour moth Anagasta kuhniella (Salt, 1966a).
6. When the carpenter ant Camponotus obscuripes is cooled it shows two well-separated supercooling points. Differential thermal analysis and freeze sectioning strongly suggest that the first is due to nucleative freezing in the foregut, while the rest of the insect remains unfrozen until the second is reached (Ohyama & Asahina, 1972).

From the fact that the gut is likely to be the normal site of inoculative freezing it follows that the quality and quantity of the food in the digestive tract of a freezing-susceptible insect plays a vital role in limiting cold-hardiness. Many species avoid such limitations by ceasing to feed at the onset of winter and eliminating efficient nucleators by emptying the gut as completely as possible. Possibly nucleators in the gut can also be changed chemically or 'masked' in some way (Salt, 1966a). Only after gut nucleators are eliminated will other physiological changes which tend to lower supercooling points be effective. Other insects overcome the problem by overwintering as eggs, as aphids do when they follow a holocyclic life-cycle.

However, anholocyclic overwintering by aphids requires the low temperature survival of viviparae which appear to feed more or less continuously through the winter. Furthermore, even if they cease feeding, aphids tend to retain food in their digestive tract (Auclair, 1963) and are thus unable to eliminate gut nucleators. The only two species tested to date, Myzus persicae and Elatobium abietinum, show no increase in their ability to supercool when starved artificially (Powell, 1974; Parry, 1978). The inability of aphids to eliminate gut nucleators is probably partially compensated for by their 'piercing and sucking' mode of feeding, which is likely to minimize contamination of the food with dust particles. These are known to be very efficient nucleators (Salt, 1961) and are probably the usual cause of nucleation in the active stages of insects. But despite their mode of feeding it is clear that the quality and quantity of nucleators in the host plant's phloem sap plays a vital role in limiting the supercooling ability of aphids (Powell, 1974, 1976; Parry, 1978, 1979a, 1980).

2.1.6 Cold-hardening

Cold-hardening, a physiological response in an organism resulting in an increase in its ability to survive low temperatures, has been demonstrated in many insects. Cold-hardening which occurs in the field - where a complex variety of environmental factors are changing simultaneously - is known as 'cold-acclimatization'. The term 'cold-acclimation' is restricted to cold-hardening under laboratory conditions in which all factors except temperature are held more or less constant. This usage follows that of Folk (1974).

Chill-susceptible, freezing-susceptible and freezing-tolerant species have been shown to cold-harden and sometimes cold-hardening changes an insect from chill-susceptible to chill-tolerant, or from freezing-susceptible to freezing-tolerant (see section 2.1.1). Often cold-hardening is evident after only a few hours or days at a low temperature. For example the locust Schistocerca

gregaria kept at 22°C has a mean supercooling point of -1.7°C but those pre-treated at 5°C for only 18 hours have a mean supercooling point of -4.9°C (Cloudsley-Thompson, 1973). Anderson and Mutchmor (1971) suggested that the rapidity of cold-hardening in an insect is related to the type of thermal environment the species usually experiences. Species which live in places where temperatures are relatively stable usually cold-harden slowly but those from areas where temperatures vary rapidly cold-harden much faster. Not only can cold-hardening be a rapid process, but it can often occur at temperatures well above 0°C (Somme, 1967). For example, females of the freezing-susceptible mosquito Culiseta inornata reared at 20°C show a significant drop in supercooling points after only 24 hours at 10°C (Hudson, 1978).

Some diapausing insects do not lose their cold-hardiness until diapause has ended whatever temperature conditions they are subjected to (Somme, 1967) but in other species, acquired cold-hardiness can be lost very quickly by exposure to higher temperatures. For instance the beetle Pterostichus brevicornis collected in mid-winter and stored at -22°C loses all its freezing-tolerance after only one day at 21°C and the lower lethal temperature rises from below -30°C to about -8°C (Baust & Miller, 1972).

Generally speaking, the longer an insect is held at a low temperature or the lower this temperature is, the more it will cold-harden. But several examples are known in which this general rule does not hold. For example, although Culiseta inornata reared at 20°C has significantly lower supercooling points after 24 hours at 10°C, it does not after 24 hours at 2°C (Hudson, 1978). This type of effect is probably caused by chill-injury during low-temperature pre-treatment off-setting or preventing cold-acclimation (Salt, 1961). Nothing is known of cold-hardening mechanisms in chill-susceptible species but in freezing-susceptible and freezing-tolerant insects cold-hardening is often associated with the cessation of feeding (see section 2.1.5) and with increases in the concentration of 'cryoprotectant' substances, whose role in cold-hardening is discussed below (section 2.1.7).

The Aphididae are one of the few groups of insects yet studied in which there is some controversy over the occurrence and survival-value of cold-hardening mechanisms. This is because in freezing-susceptible insects the elimination of efficient ice nucleators in the gut - which usually limit supercooling ability - appears to be required before cold-hardening mechanisms can become effective. Aphids appear unable to do this and consequently it has been suggested by Powell (1974) and Parry (1978, 1979a) that cold-hardening mechanisms are unlikely to have been developed in this group of insects. Both authors have also argued that even if aphids do show cold-hardening mechanisms these would have a negligible effect on winter survival as their potentially beneficial influence on supercooling ability would be overridden by the continued presence of efficient nucleators in the gut. Furthermore, Elatobium abietinum, the species studied most intensively, has supercooling points lower than those of the Picea sitchensis needles on which it feeds and, when the host's needles freeze, feeding aphids are inoculated with ice (Powell, 1974).

Increased cold-tolerance after low temperature conditioning has been demonstrated in Elatobium abietinum (Carter, 1972), Myzus persicae (Adams, 1962), Acyrtosiphon pisum (Harrison & Barlow, 1973) and Sitobion avenae (Griffiths & Wratten, 1979). However, Powell (1974) and Parry (1978, 1979a) suggest^{ed} that such changes in the cold-tolerance of feeding aphids are not a consequence of a true cold-hardening mechanism but are due to the aphids' acquiring cold-hardiness from the host in a passive way, due to changes in the quality of the phloem sap they imbibe. If aphids are freezing-susceptible in the strict sense of the word, as the evidence accumulated so far has suggested, this argument is persuasive.

2.1.7 Cryoprotectants

Cryoprotectants are substances whose presence in an organism increases its cold-hardiness. In insects the polyhydric alcohol glycerol was the first to be discovered

and is by far the most widespread. Early reports of glycerol in insects came in the late 1950s (Chino, 1957; Salt, 1958b; Wyatt & Meyer, 1959). As the substance was already widely used to protect a variety of mammalian cells stored at low temperatures from freezing injury, its role as a cryoprotectant was immediately suspected. Since then it has been demonstrated in a wide variety of freezing-susceptible and freezing-tolerant insects, mainly eggs, larvae and pupae of Lepidoptera, Coleoptera, Hymenoptera and Diptera.

In many species a link has been demonstrated between the production of glycerol and low temperature exposure in the laboratory or the field (Somme, 1964, 1965; Takehara, 1966; Wyatt, 1967; Baust & Miller, 1970; Kronic & Salt, 1971; Ohyama & Asahina, 1972; Frankos & Platt, 1976; Duman & Patterson, 1978; Werner, 1978). A few other polyhydric alcohols have also been found in insects, often in association with glycerol. They probably serve a similar cryoprotectant function. Of these, the commonest is sorbitol (Somme, 1964; Miller & Smith, 1975; Morrissey & Baust, 1976; Somme & Block, 1982). Sugars, particularly trehalose, are also implicated as cryoprotectants in a few species (Tanno & Asahina, 1964; Wyatt, 1967; Morrissey & Baust, 1976; Somme & Block, 1982).

Glycerol is an effective cryoprotectant due to its ability to permeate cells rapidly and completely, as well as its high solubility, low molecular weight and non-toxicity, even in high concentrations (Smith, 1961; Precht, 1973). It is believed to act in two distinct ways to increase cold-hardiness:

1. By lowering supercooling points (in freezing-susceptible and freezing-tolerant insects).
2. By protecting against freezing-injury (in freezing-tolerant insects).

A third mode of action - as an enzyme stabilizer at low temperatures - has been suggested (Frankos & Platt, 1976; Ring & Tesar, 1981) as glycerol is used as such for

storage of enzymes in vitro. Unlike the two types of action mentioned above, this could operate to increase cold-hardiness in chill-susceptible insects.

Lowered supercooling points have been shown to be associated with increased glycerol concentration in several insects (eg. Salt, 1958b; Somme, 1964, 1965; Baust & Miller, 1970; Kronic & Salt, 1971; Werner, 1978). The addition of any solute lowers a solution's freezing point and, in general, this results in a lowering of the supercooling point by approximately the same amount (Meryman, 1956). However, in several insects and in the mite Alaskozetes antarcticus glycerol has been found to lower supercooling points by approximately double the calculated freezing point depression (Salt, 1958b; Somme, 1964, 1965; Block & Young, 1979). This is probably due to glycerol's ability to form hydrogen bonds which may allow it to 'bind' water molecules (Salt, 1958b) or to compete with water at ice nucleation sites and thus 'mask' nucleators (Baust, 1973; Ring & Tesar, 1981).

Glycerol's importance in increasing freezing-tolerance is less clear than its role in lowering supercooling points. Although it is often found in freezing-tolerant insects there is a poor correlation between glycerol content and degree of freezing-tolerance in different species. Some insects can survive freezing at moderately low temperatures without glycerol while others cannot survive freezing even when they contain large amounts (Asahina, 1969). However, theoretically glycerol could protect against freezing-injury in several ways. Firstly its presence inside and outside cells increases the solute concentration of the body fluids. Consequently, at a particular temperature below the supercooling point, a smaller proportion of the body's water will be frozen out of solution than if glycerol were absent (Lovelock, 1953; Salt, 1961; Smith, 1961; Baust, 1973; Zachariassen, 1979). Thus the various dehydration effects likely to produce freezing-injury (listed in section 2.1.4) will be mitigated.

Glycerol has also been shown to slow the rate of growth of ice crystals and to alter their shape, producing

blunt crystals less likely to damage cell membranes mechanically (Baust, 1973). This is probably due to glycerol increasing the viscosity of the body fluids (Baust, 1973) and perhaps also to its ability to form hydrogen bonds, allowing it to interfere with the incorporation of water molecules into ice crystals (Precht, 1973; Ring & Tesar, 1981). Lastly, glycerol may act by lowering eutectic points, minimizing the risk of the precipitation of salts (Baust, 1973).

The presence of cryoprotectant polyhydric alcohols has been investigated in only one species of aphid, Elatobium abietinum (Parry, 1979a). Viviparae overwintering in the field were found to contain the polyhydric alcohol mannitol but lost it all after three days at 15°C. Unfed first-instar nymphs born in the field during the winter did have significantly lower supercooling points than those produced at 15°C, the difference in mean supercooling points being about 4°C. However, there was no change in supercooling points associated with the loss of mannitol in aphids which had begun to feed, presumably because the potentially beneficial effect of mannitol on supercooling points was nullified by the presence of efficient ice-nucleators in the gut. The supercooling points of fed aphids were considerably higher than those of unfed nymphs. Glycerol has been found in the eggs of the aphids Aphis pomi, Rhopalosiphum padi, and Hyalopterus pruni (Somme, 1969), and in Pterocomma smithia (Somme, 1964), and Cinara pilicornis (Parry, 1979b). Mannitol was found in the eggs of C. pilicornis (Parry, 1979b) and H. pruni (Somme, 1969) and was probably also present in eggs of P. smithia (Somme, 1964). In the eggs of the four species studied by Somme, increased polyhydric alcohol levels were associated with lowered supercooling points. It is likely that glycerol and possibly mannitol contributed to the extremely low supercooling points found, which were below -35°C in all species in mid-winter. In C. pilicornis eggs, however, which were also cold-hardy, seasonal changes in supercooling ability were not related to changes in the levels of glycerol or mannitol (Parry, 1979b).

Although there is a general concensus that glycerol and other polyhydric alcohols are important in insect cold-hardiness it is also apparent, from the very imperfect correlation between the levels of polyhydric alcohols and cold-hardiness in different species, that other factors are also involved (Asahina, 1969). Other organic compounds may well play a role by increasing the amount of 'bound' water and thus mitigating dehydration effects (Baust, 1981; Storey, Baust & Buescher, 1981). Recently, proteins produced during the winter and associated with lowered supercooling points have been found in the haemolymph of several species of Coleoptera (Duman, 1979).

2.1.8 The cold-hardiness of plants

It is evident from the summary of insect cold-hardiness given above that the host plant influences the low temperature mortality of aphids in a number of ways. Firstly, feeding aphids may be killed by ice entering them from the host via the stylets. Secondly, characteristics of the host's sap govern the quality and quantity of ice nucleators in the aphid's gut. Thirdly, an aphid feeding on a cold-susceptible plant is likely to starve when the host dies, even if the aphid itself is much more cold-hardy. It is clear, therefore, that the host plant's cold-hardiness is an important factor in the low temperature survival of aphids and must be taken into consideration. The summary below concentrates on cold-hardiness in temperate zone herbaceous plants, like the Gramineae on which cereal aphid viviparae overwinter.

The cold-hardiness strategy of most temperate zone plants differs from that of most temperate zone insects, including aphids, in two important respects. Firstly, while the majority of insects supercool extensively, herbaceous plants and most of the tissues of woody plants do not supercool more than a few degrees below their freezing points which, like the freezing points of insects, generally lie only one or two degrees below 0°C (Burke, Gusta, Quamme, Weiser & Li, 1976). Secondly, while most insects are killed by freezing, most herbaceous and woody

perennials possess considerable freezing tolerance when acclimatized (Levitt, 1966; Burke et al., 1976; Sutcliffe, 1977). The generally poor supercooling ability of plant tissue is due to the presence of large bodies of water in the xylem and between cells, and to the ease with which the intercellular water can be inoculated by external ice crystals via stomates, lenticels or wounds (Burke et al., 1976).

As in animals, intracellular freezing in plants is almost invariably lethal (Levitt, 1966; Meryman, 1966; Burke et al., 1976; Sutcliffe, 1977) but it is very rarely observed in cold-hardened perennials if they are cooled slowly (Levitt, 1966; Mazur, 1969; Burke et al., 1976; Chen & Gusta, 1978). Once extracellular freezing has started cold-hardened tissues are extremely unlikely to freeze intracellularly because water will move out of the cells, quickly depressing the freezing point of the cell sap (see section 2.1.2). As in animals, freezing-injury in plant tissues is thought to be due primarily to dehydration effects rather than mechanical disruption (Burke et al., 1976; Mazur, 1969). Cell membranes are thought to be particularly susceptible to freezing-injury (Mazur, 1969; Burke et al., 1976).

Plants often show greatly increased cold-hardiness when grown at relatively low temperatures. Winter wheat grown at about 20°C in a glasshouse can be killed by freezing well above -10°C but cold-hardened crowns of hardy cultivars held at -3°C for 16 h and then frozen slowly can readily tolerate -18°C to -20°C (Chen & Gusta, 1978). However, as with many insects, the cold-hardiness of winter wheat and other plants can be lost rapidly (Langridge & McWilliam, 1967).

As plants cold-harden, moisture content usually decreases, while sap concentration and sugar content increase (Levitt, 1948; Burke et al., 1976; Chen & Gusta, 1978). Sugars, particularly sucrose, may well protect plant tissues against damaging dehydration effects after freezing, in the same way that glycerol does in insects (see section 2.1.7). Cold-hardening in plants is also often associated with an increase in soluble proteins,

which may protect sensitive sites from damaging concentrations of salts (Burke et al., 1976). Cold-hardened plant cells also show increased permeability (Levitt, 1948; Mazur, 1969) - minimizing the risk of intracellular freezing - and may show increased elasticity - making mechanical damage less likely (Burke et al., 1976).

2.1.9 Summary of factors likely to affect cold-hardiness in aphids

From the summary of insect cold-hardiness given above it is clear that a wide variety of factors are likely to affect the low temperature mortality of aphids. These can be divided into environmental factors - pertaining to the environmental conditions before or during low temperature exposure - and intrinsic factors - pertaining to the aphid itself. A few factors could arguably be put in either category. The main environmental and intrinsic factors likely to alter cold-hardiness are listed below with a brief explanatory note where necessary.

(a) Environmental factors

1. Temperature - the probability of injury increases with decreased temperature.
2. Duration of exposure to low temperature - the probability of injury increases with increased exposure time at a particular temperature.
3. Previous low temperature conditioning - allows cold-hardening to occur.
4. Rates of cooling and re-warming - fast rates increase the chance of 'thermal shock' effects and minimize the chance of rapid cold-hardening.
5. Freezing in the host plant - increases the chance of inoculative freezing.

6. Characteristics of the host's phloem sap - govern the quality and quantity of ice nucleators in the gut.
 - *7. Cold-susceptibility of the host - cold-damage/death of a cold-susceptible host will probably lead to the aphid's death from starvation.
 8. Ice on the body surface - increases the chance of inoculative freezing.
- (b) Intrinsic factors
1. Species.
 2. Clone/local population.
 3. Life-stage.
- *4. Presence of food in the gut - increases the chance of nucleative freezing in the gut.

All but two of these factors were investigated to some extent in the present study. Further details of evidence in the literature for their effects on aphids are given below in the descriptions of each experiment.

2.1.10 Organization of the laboratory work

The laboratory work on low temperature mortality is described below in four main sections. The first of these (2.2) gives details of materials and methods common to most experiments. Other details of methodology are given in the descriptions of the individual experiments which are divided into three sections (2.3, 2.4 and 2.5). The first of these describes three preliminary experiments in which the relative cold-hardiness of the three aphid species was

*factors not investigated in the present study.

investigated together with the relationship between lower lethal temperatures and supercooling points. Results of these first three experiments profoundly affected the methodology used in the later tests, all of which investigated factors likely to affect cold-hardiness. They are divided between section 2.4 - dealing with environmental factors - and section 2.5 - dealing with intrinsic ones.

2.2 GENERAL MATERIALS AND METHODS

The cold-hardiness of freezing-susceptible insects can be investigated either by the direct assessment of mortality after low temperature exposure or by supercooling point determinations (see section 2.1.3). All but one of the experiments conducted in the present study involved the direct assessment of mortality. In most of these experiments batches of aphids were exposed to different low temperatures for a standard time and the results analysed using probit analysis. In the following sections, experiments are referred to by their section numbers.

2.2.1 Aphids used

Two sets of clones were used in these experiments. Each set consisted of three clones, one of each species. In the early experiments, clones obtained in 1977 were used (referred to as the 'old' clones) but these were replaced in the later experiments by three collected in July 1980 (referred to as the 'new' clones). The old clones of S. avenae and M. dirhodum were obtained from the Plant Breeding Institute, Cambridge and the old clone of R. padi from winter wheat in Southampton University Biology Department glasshouses. All three old clones were restarted from single viviparae in late 1977.

The new clones of all three species were built up from single viviparae collected from winter wheat in West Hampshire. In nearly all the experiments either old clones or new clones were used but in one (2.5.3) old and

new clones were tested together. The old clone of S. avenae was of a brown colour morph but the new one was green. Additional S. avenae clones used in experiment 2.5.2 were kindly provided by Dr. H. Loxdale of Rothamsted Experimental Station, Harpenden, Hertfordshire. Although it was originally intended that all three species should be used in all experiments, lack of time led to several experiments being run on S. avenae alone.

In the first three experiments adult apterae were tested but in later experiments second- to fourth-instar nymphs (excluding alatiform fourth-instars) were used as they showed lower and less variable 'control' mortality than adults. First instars were excluded because their small size increased the chance of loss or handling damage and because some might not have started feeding when tested. Evidence in the literature suggested these would be atypically cold-hardy (Powell, 1974). Alatiform fourth-instar nymphs were excluded because they moulted to alatae between testing and mortality assessment. Alatae were difficult to handle in large numbers and showed higher control mortality than did apterous adults or nymphs because of their restless behaviour. Differences in cold-hardiness between adult apterae and different types of nymph were determined in experiment 2.5.1.

The aphid species, clones and instars used in each experiment are stated at the beginning of each 'Methods' section. All the aphids were taken from stock cultures kept on winter wheat (cv. Hobbit) at 20°C (with maximum fluctuations of 1.5°C) and 16 h daylength. The culturing procedure was rigidly standardised to minimize variability and is described in Appendix 2.2.

2.2.2 Cooling apparatus

Two types of cooling apparatus, a cold chamber and a cold bath, were used. The cold chamber was cooled by a refrigeration unit kindly loaned by Mr. D. Bevan and Mr. C.I. Carter of the Forestry Commission's Alice Holt Research Station. The unit consisted of a set of refrig-

erator-cooled plates and an integral fan which passed air over the plates and into the test chamber via an insulated tube. After circulating through the chamber air was returned to the refrigerator for recooling via a second tube. Cooled air flowed round the chamber between an outer wall thickly insulated with expanded polystyrene and an inner metal wall and it passed into the chamber itself through holes in the inner wall. The internal dimensions of the chamber were 60 x 60 x 40 cm high. The temperature in the chamber was controlled by a thermostat which switched the refrigerator on or off but left the cooling fan running.

This apparatus was used in experiments 2.3.1, 2.4.5, 2.4.7 and 2.5.4. In all four experiments the aphids were tested in plastic containers placed in the chamber. Exact details of the containers are given in the descriptions of each experiment. The test temperature was monitored using a thermistor connected through a YSI Telethermometer to a Servoscribe potentiometric chart recorder. The thermistor was fixed inside the test container or in an identical container placed alongside. In experiment 2.3.2 the aphids were held in a closed container without vents and in this experiment the test temperature they experienced fluctuated $\pm 0.75^{\circ}\text{C}$ or less. But in the other three experiments aphid containers with gauze vents were required. These allowed air to circulate through as well as round the test container and consequently fluctuations of up to $\pm 1.5^{\circ}\text{C}$ were recorded. In all four experiments the chamber was cooled to the test temperature before aphids were placed inside. The interior of the aphids' container took approximately 20 min to cool to the test temperature, which was taken as the mean temperature during the test ignoring the initial 20 min cooling period and was calculated from the Servoscribe chart recorder trace. After the test aphids were taken out of the chamber and re-warmed to room temperature rapidly.

To allow finer temperature control and enable lower temperatures to be achieved a cold bath was used in most of the later experiments. The bath had internal dimensions 32 x 30 x 22.3 cm deep. It was filled with 60% industrial alcohol cooled by a Grant Instruments cooling

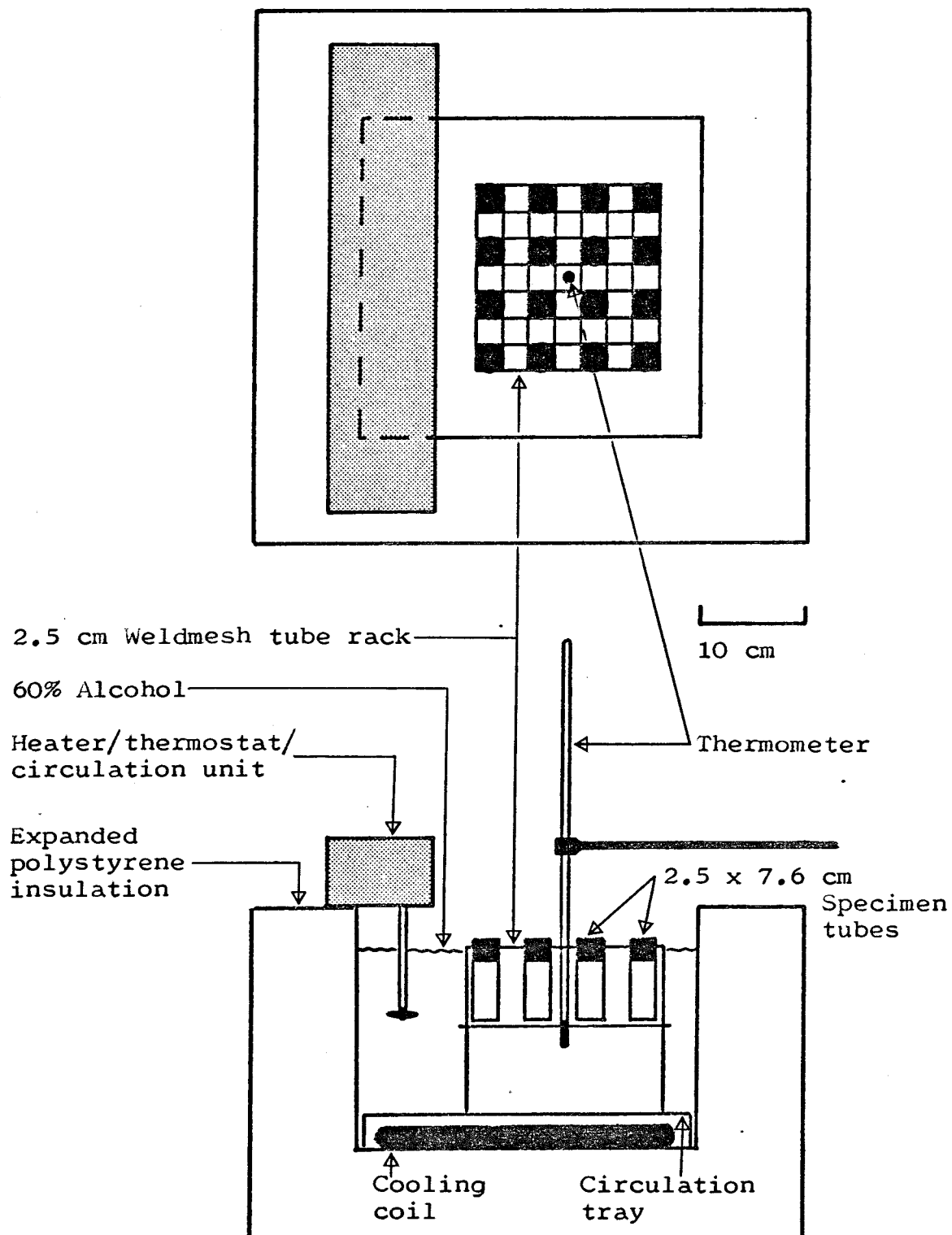
coil (model CC20) which had been modified to allow cooling to below -30°C . The temperature was controlled by a Grant Instruments heater/thermostat/circulation unit (type UL3). Details of the degree of temperature control achieved are given below (section 2.2.3). The cold bath was used for supercooling point determinations (experiment 2.3.2) and for all experiments involving the direct assessment of mortality, apart from experiments 2.3.1, 2.4.5, 2.4.7 and 2.5.4. The standard cold-hardiness test procedure which was used in most experiments is described below.

2.2.3 Standard test procedure

In the standard cold-hardiness test batches of aphids were exposed to low temperatures in the cold bath in 7.6×2.5 cm glass specimen tubes held in a weldmesh tube rack (see Fig. 2.1). The rack was placed centrally in the bath and held the tubes immersed to within a few millimetres of their rims. Sixteen tube positions were available in the rack, each tube separated from its neighbours by a 2.5 cm gap to allow thorough circulation of the coolant. The temperature was monitored using a 0° to -30°C mercury thermometer graduated to 0.1°C placed vertically through the centre of the rack with its bulb just below the tubes' bases.

Spatial and temporal variations in temperature were very small. Testing temperature differences between the four corners of the tube rack showed mean differences of 0.01°C or less at 7.6 cm depth. Temperature checks at 10 min intervals through all experiments showed fluctuations with time of 0.05°C or less. In the standard cold-hardiness tests tubes containing aphids were placed in the cold bath after it had been cooled to the test temperature and were taken out and left at room temperature immediately after the test. To assess the cooling and re-warming rates the aphids experienced and the difference between the test temperature inside the tubes and that of the bath, a thermocouple was used embedded in a 1.5 mm ball of plasticine resting on the floor of a tube. These tests used bath temperatures over the range -1° to -13°C . They showed

Fig. 2.1 The cold bath, set up for standard cold-hardiness tests.



that the test temperature experienced by the aphids varied from 0.3°C warmer than the bath at -13°C to 0.15°C warmer at -1°C . The interior of a tube had cooled to 0.6° to 0.8°C above the test temperature 10 min after it was placed in the bath, irrespective of the bath temperature, and to 0.1° to 0.2°C above test temperature 10 min later. When the bath temperature was held at -7°C a tube taken from it re-warmed to 0°C in 1.5 min.

In standard cold-hardiness tests aphids were shaken off cut culture plants onto a tray and sorted into specimen tubes with a fine brush. One replicate of a treatment consisted of a batch of 50 aphids in a single tube. Different treatments were not put in the same tube. Tubes were numbered and allocated their positions in the cold bath's tube rack using tables giving random permutations of 20 numbers. If only a few tubes were used in a test, only positions near the centre of the rack were allocated. Immediately before each tube was placed in the cold bath it was tapped against the bench to knock all the aphids to the bottom (the rapid cooling ensured that none climbed the tube walls before they were immobilized by cold). The tube's plastic cap was then quickly replaced with a rubber bung smeared with silicone grease and the tube was placed in the bath, which had already been cooled to the test temperature. The rubber bungs weighed tubes down in the bath and gave an air-tight seal - ensuring that aphids were not affected by alcohol fumes from the bath coolant. The placing and removal of the tubes was separated at 1 min intervals to ensure accurate timing. In most experiments the tubes were left in the bath for 90 min. The test temperature was taken as the mean of thermometer readings taken at 10 min intervals.

After a test the tubes were taken out of the bath, their rubber bungs removed, any silicone grease wiped off and their plastic caps replaced. The aphids were left at room temperature (19° to 24°C) for 30 min to 1 h and then transferred to small Perspex boxes 5 cm high and 2.5 cm in diameter. These contained damp filter paper and one or two freshly cut wheat leaves (cv. Hobbit). Mortality was not assessed until 72 h after each test, as a preliminary

experiment showed that considerable delayed mortality occurred (see experiment 2.3.1 and Fig. 2.3). In the interval between testing and assessing mortality aphids were kept in a growth room at 18° to 22°C and 16 h day-length. Each day condensation was wiped off the container walls, a fresh wheat leaf was added and the filter paper was moistened if necessary.

To assess mortality, aphids were tipped onto a sheet of paper and examined under a bench lamp. They were kept under observation for 10 min or more and touched gently with a fine brush to stimulate movement. They were then sorted into three categories: those showing no signs of movement, those showing some movement but incapable of walking, and those capable of walking. As long as aphids were not sorted within a few hours of low temperature exposure very few moved but were incapable of walking and most of those not moving were obviously discoloured, so that categorization was easy. In all experiments described below aphids were classified as 'dead' if they were incapable of walking. In some experiments mortality was assessed 24 h as well as 72 h after low temperature exposure. 'Control' mortality was assessed in each experiment by treating batches of aphids exactly as described above but keeping them in tubes at room temperature while 'treatment' batches were in the cold bath. Control mortalities were nearly always well below 10%. In the descriptions of individual experiments the method followed the standard test procedure given above except where stated otherwise.

2.2.4 Analysis

To correct for occasional miscounting or loss of aphids, mortalities have been expressed as percentages. In some analyses percentage mortalities were compared using statistical tests based on the normal distribution. In all these cases the distribution of the percentages was rendered normal using the arcsin transformation ($\arcsin\sqrt{\text{percentage}}$) but values given in Tables and Figures are untransformed.

In most experiments cold-hardiness was assessed by determining the LT50 of each treatment (the temperature needed to kill 50% of the aphids tested). LT50s were calculated from test temperature, number exposed and number killed in each test and mean percentage mortality in controls, using a probit analysis computer program written by Dr. R.J. White of the Biology Department, University of Southampton. This program calculated the intercept and slope of the best-fit probit line, the expected response (mortality) at each dose level (temperature) and the median effective dose (LT50) with its confidence limits. It also gave results of a chi-squared heterogeneity test used to assess the significance of the deviation of the observed data from the expected sigmoid response curve.

If the chi-squared heterogeneity test shows a significant difference ($P < 0.05$) between the data and the best-fit sigmoid curve a plot of the data (transformed to probits) about the probit line should be drawn to determine whether or not the heterogeneity is random. If it is, the program can be re-run incorporating the appropriate 'heterogeneity factor' (chi-squared value/degrees of freedom associated with the chi-squared) which widens the confidence limits of the LT50. But if random heterogeneity is too great the computer program is unable to calculate confidence limits. If a plot of the probits and the probit line shows that the heterogeneity is systematic, the analysis can still be used provided an appropriate transformation which eliminates it can be found. An option allowing log transformation - useful if the sigmoid dose-response curve is skewed to the left - is given in the program. The computer program used is written in Algol and was run on an ICL 2970 computer. It is listed in White & White (1981) together with details of the form of the input and output and a brief summary of probit analysis.

In the analyses below, test temperatures (nearly always below 0°C) were input as positive values because the program cannot accept negative 'dose' values and because mortality increases as temperature decreases. Occasionally log transformations were used where scatter plots showed

that mortality-temperature curves were skewed. In many analyses the χ^2 heterogeneity test revealed significant ($P < 0.05$) heterogeneity in the data which, from scatter plots, appeared random. The degree of heterogeneity was surprising considering the rigid standardization of culture conditions and suggests that small differences in rearing temperature or plant age affect cold-hardiness. The effects of heterogeneity were accentuated by the steep temperature-mortality curves. Expected and observed mortalities corrected for the mean mortality in controls (which were given in the program output) were used in drawing temperature-mortality plots with best-fit probit curves.

2.3 LOW TEMPERATURE MORTALITY AND SUPERCOOLING POINTS OF UNACCLIMATED VIVIPARAE

The three experiments described below were undertaken to investigate the relative cold-hardiness of the three aphid species under study and the relationship between their lower lethal temperatures (determined by the direct assessment of mortality and expressed as LT50s) and mean supercooling points. A comparison of LT50s and supercooling points was needed to determine whether or not the aphids were chill-susceptible and hence whether supercooling point determination was a valid method for assessing cold-hardiness (see section 2.1.3). In all three experiments adult apterae from the stock cultures at 20°C were tested without any low temperature pre-treatment.

2.3.1 Relative cold-hardiness of the three species Introduction

The only previous laboratory experiments on the cold-hardiness of S. avenae, M. dirhodum and R. padi were undertaken by Griffiths & Wratten (1979). In that investigation clones of S. avenae from Spain, Switzerland and Norway, M. dirhodum from Switzerland and R. padi from Norway and Finland were tested, but no British clones were used. Moreover, the results showed that a clone's place

of origin was an important variable governing cold-hardiness, particularly in the case of R. padi. Although a little additional information is available from field studies on differences in winter colony survival between the species (Dean, 1974a; Smith, 1981) it is impossible to tell from these whether the observed species differences were a result of differences in low temperature mortality or were due to the differential action of other mortality factors or differences in development and reproduction rates.

The present experiment was undertaken to determine LT50s for English clones of the three species to allow preliminary assessment of their relative cold-hardiness and to provide a measure of their lower lethal temperatures with which the species' mean supercooling points could be compared.

Methods

Species used: S. avenae, M. dirhodum, R. padi.

Clones used: old. Instars used: adults (apterae).

Cooling apparatus used: cold chamber.

The experiment consisted of a series of tests at different low temperatures from which probit lines and LT50s for each species were determined. Adult apterae were removed from culture plants immediately before use and were tested in batches of 50 in a 9 cm diameter petri dish which was raised on 1 cm legs to ensure even cooling when placed in the cold chamber. The temperature inside the petri dish was monitored using a thermistor positioned on the centre of the dish floor. Aphids were put into the petri dish immediately before it was placed in the cold chamber, which had already been cooled to the required test temperature. They were left in the chamber for 3 h and then removed to room temperature. Subsequent treatment and assessment of mortality after 3 days followed the standard test procedure (see section 2.2.3) as did the determination of control mortality. Batches of R. padi were usually tested on their own but those of S. avenae and M. dirhodum (which were similar in cold-hardiness) were usually tested together, although some additional tests using S. avenae only were also run.

The time aphids were left between low temperature exposure and the assessment of mortality in this and later experiments (3 days) was arrived at after a preliminary test in which batches of all three species were exposed to -7.7°C for 3 h and then assessed for mortality 0.5, 3, 24, 48, 73 and 98 h later. This showed that additional delayed mortality was negligible 73 h after low temperature exposure.

Results and discussion

Table 2.2 and Fig. 2.2 give the results of probit analyses for each species. The data for R. padi clearly followed a skewed sigmoid relationship and so the 'dose' (test temperature) was subtracted from $+7^{\circ}\text{C}$ (to make all values positive, as the highest test temperature was $+6^{\circ}\text{C}$) and logged before probit analysis. When cultured at 20°C the English clones of S. avenae and M. dirhodum tested here showed no significant difference in cold-hardiness but R. padi was much less cold-hardy and its response curve was of a different shape. The response curves of S. avenae and M. dirhodum in particular were extremely steep, so that a lowering of the test temperature by less than 3°C increased mortality from below 10% to around 90%, and differences of fractions of one degree near the LT50s had large effects.

Assessment of the differences between the English clones studied here and the European clones studied by Griffiths and Wratten (1979) is difficult because the test procedures differed. Except for the Spanish clone of S. avenae, LT50s for the European clones were lower than those for the English ones. This may be because they came from Switzerland, Norway and Finland, all of which have a colder winter climate than England. But the difference may simply be due to the fact that in the tests by Griffiths and Wratten aphids were cultured at 17°C and the test temperature was taken as the minimum temperature achieved after slow cooling from room temperature for 3 h. In the present study aphids were cooled to the test temperature in 20 min and then held at that temperature for 2 h 40 min. In comparing the two sets of LT50s it is noticeable that while those for European and English clones of S. avenae

Table 2.2 Probit analysis summaries for adult apterae cultured at 20°C and exposed to low temperature for 3 h.

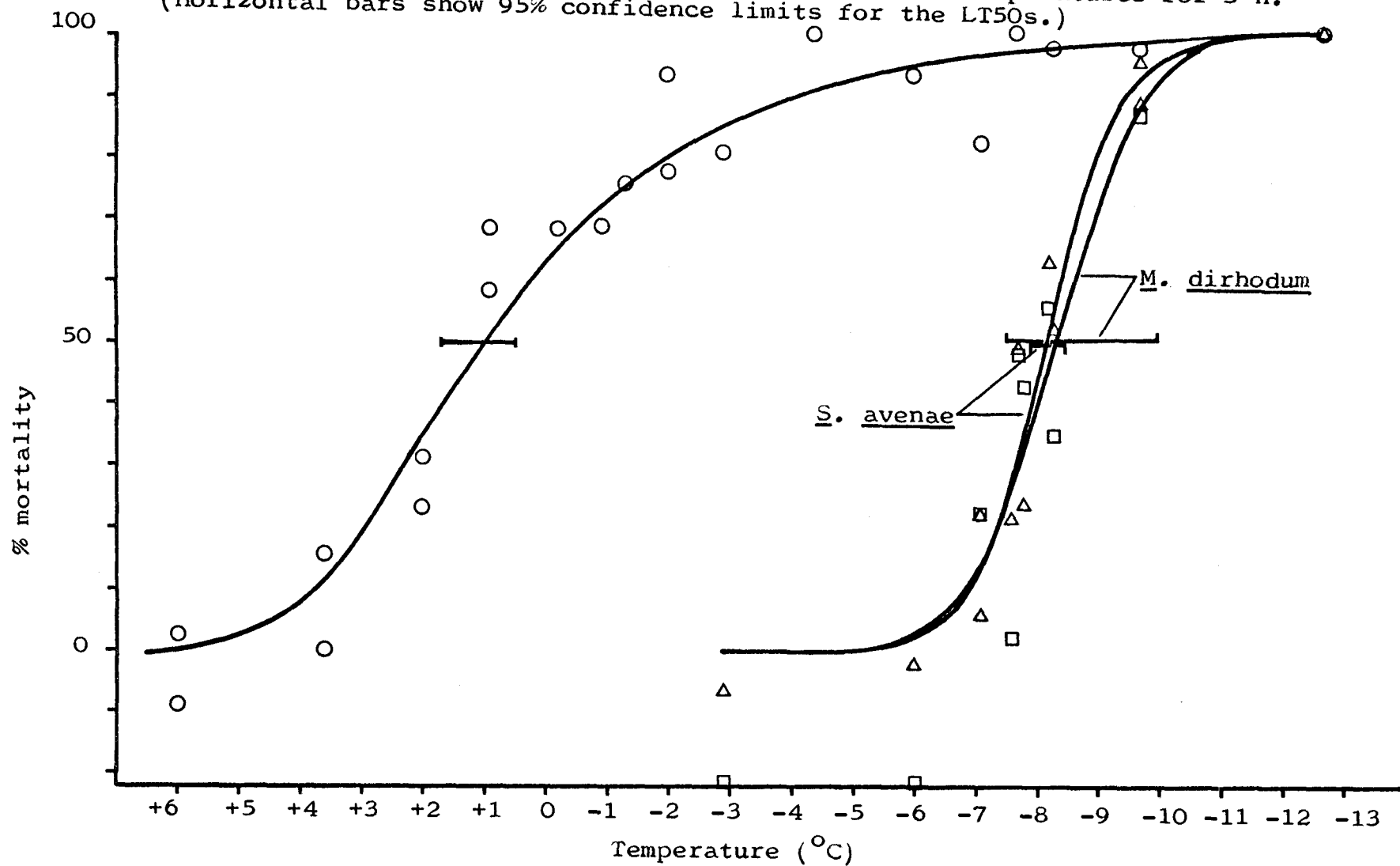
	<u>S. avenae</u>	<u>M. dirhodum</u>	<u>R. padi</u>
LT50	-8.17	-8.33	+1.03
95% confidence limits	-7.91 to -8.46	-7.56 to -9.98	+1.69 to +0.46
Probit line: intercept	-7.9876	-7.1305	-3.7506
slope	0.9778 0.0985	0.8565 0.1382	4.8348 0.3436
Heterogeneity χ^2	19.02*	33.56***	35.63**
Degrees of freedom	9	7	16
Number of: batches	12	9	21
test temperatures	10	9	16
Transformation	-temp.	-temp.	Log (7-temp.)

*: $P < 0.05$

** : $P < 0.01$

***: $P < 0.001$

Fig. 2.2 Mortality - temperature curves for adult apterae of S. avenae (Δ), M. dirhodum ($\square$) and R. padi (O) cultured at 20°C and exposed to low temperatures for 3 h. (Horizontal bars show 95% confidence limits for the LT50s.)



and M. dirhodum differed by 3.5°C or less, the English clone of R. padi tested here had an LT50 5.8°C higher than the more cold-susceptible of the two clones tested by Griffiths and Wratten. This suggests that increasing cooling rate and/or exposure time has a greater injurious effect on R. padi than on the other two species.

The greater cold-susceptibility of R. padi compared with the other species is in accord with the results of Dean (1974a) who found artificial colonies of S. avenae and M. dirhodum persisted through the winter better than those of R. padi. It also agrees with the results from the extensive winter sampling of Gramineae undertaken by Hand (1982) concurrently with the present study. Hand found, from multiple regression analysis of changes in aphid numbers on a variety of environmental variables, that R. padi appeared to be more susceptible to low temperatures than S. avenae. In the laboratory study by Griffiths and Wratten (1979) Norwegian S. avenae showed a lower LT50 than Norwegian R. padi (-8.37°C and -4.80°C respectively). However, R. padi from Finland were the most cold-hardy clone tested, although other species from Finland were not available for direct comparison. Only one clone of M. dirhodum, from Switzerland, was tested but this showed a significantly lower LT50 than S. avenae from the same place of origin (-11.67°C and -10.68°C respectively) whereas the English clones tested here had nearly identical LT50s.

The fact that in this experiment R. padi exhibited considerable mortality above 0°C shows that under these conditions the species could be killed by chill-injury. However, comparison with the winter field data of Dean (1974a), George (1974), Hand (1982) and Smith (1981) clearly demonstrates that R. padi must show considerably greater cold-hardiness under winter field conditions. An English field population with an LT50 as high as 1°C would be exterminated early in the autumn. Further experiments to investigate increased cold-hardiness after low temperature pre-treatment and using slower cooling rates are described below (experiments 2.4.1, 2.4.2 and 2.4.3). Although only one English clone of each species was tested in the present

experiment the pattern of cold-hardiness shown was confirmed by later tests on the new clones collected in 1980 (see experiment 2.4.2).

The preliminary test to determine how long aphids should be left between low-temperature exposure and the assessment of mortality revealed considerable delayed mortality (Fig. 2.3). In this and later experiments aphids often showed uncoordinated movement and/or partial paralysis a few hours after a test and died hours or days later. Others were unable to shed partially moulted skins and died during ecdysis. Some survived ecdysis but showed morphological abnormalities. The most usual deformity was elongated, weak tarsi with many segments. These types of cold-injury symptoms have also been found in other insects (Salt, 1961; Asahina, 1969; Swain, 1975).

2.3.2 Supercooling points

Introduction

Mean supercooling points for each species were determined to discover whether or not they resembled the species' LT50s. From this comparison the usefulness of mean supercooling points as an easily-obtained index of cold-hardiness could be assessed and evidence gained on whether or not S. avenae and M. dirhodum were, like R. padi, chill-susceptible when reared at 20°C.

Methods

Species used: S. avenae, M. dirhodum, R. padi.

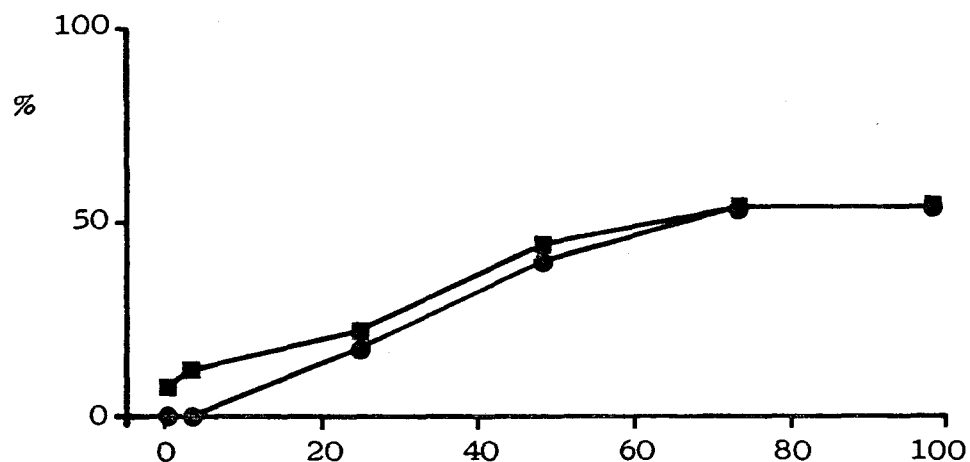
Clones used: old. Instar used: adults (apterae).

Cooling apparatus used: cold bath.

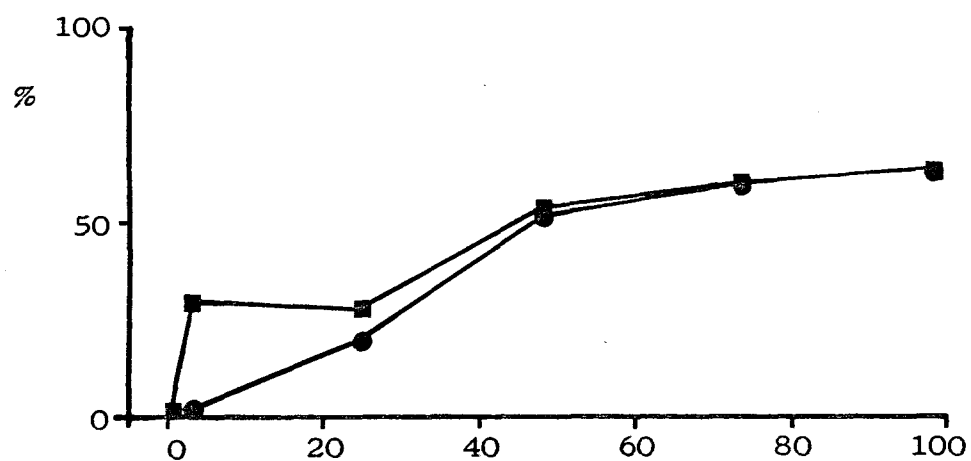
The method and apparatus were very similar to those used by Powell (1974) to determine supercooling points of Elatobium abietinum. Aphid temperatures were monitored using a 30 gauge copper-constantan thermocouple connected to a Servoscribe potentiometric chart recorder. The thermocouple's reference junction was taped to a thermometer immersed in a vacuum flask of melting ice. Individual aphids were attached to the test junction with a small amount of petroleum jelly and enclosed in a gelatin

Fig. 2.3 Relationship between the proportion of aphids capable of movement (■) and walking (●) and the time since low temperature exposure (-7.7°C for 3 h).

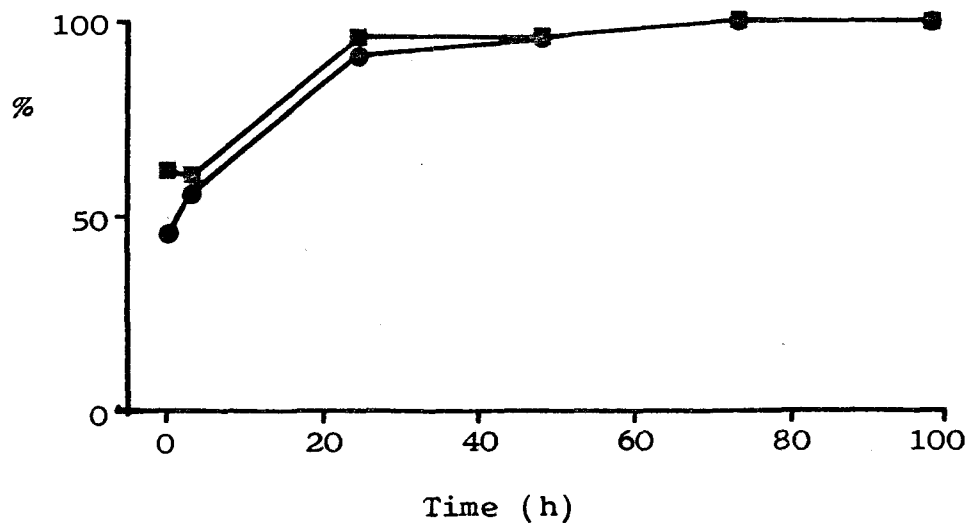
(a) S. avenae



(b) M. dirhodum



(c) R. padi



capsule held partly immersed in 90% ethyl alcohol in a boiling tube, as shown in Fig. 2.4. A steady cooling rate of $1^{\circ}\text{C}/\text{min}$ was achieved by slowly lowering the boiling tube into the cold bath kept at -30°C . The tube was lowered in a standardized way (initially determined by trial and error) using the graduated scale on the tube's holder for reference. The steady cooling rate was monitored on the Servoscribe chart and when the aphid froze it gave out its latent heat of fusion causing a sharp rise in temperature. Immediately after freezing aphids were taken out of the cold bath and examined for signs of life.

The supercooling points of 20 adult apterae of each species taken from the 20°C stock cultures were determined. The tests were run over a 15 day period and single individuals of each species were tested in turn to ensure that subtle changes in culture conditions from day to day did not produce spurious species differences. Before and after each day's supercooling point determinations the thermocouple's calibration was checked by placing its test junction alongside a thermometer in the cold bath.

Results and discussion

A typical chart record from a supercooling point determination is shown in Fig. 2.5. The frequency distributions, means and standard errors of each species' supercooling points are shown in Fig. 2.6. The distributions for S. avenae and M. dirhodum both seem to deviate from normality. Consequently, a non-parametric test - the Mann-Whitney U test (Siegel, 1956) - was used to test the significance of species differences. All species differences were highly significant ($n_1 = n_2 = 20$, $U \leq 97$, $P < 0.01$ in all comparisons). No aphids showed any movement after freezing.

All three species had mean supercooling points at least 14°C below their 3 h LT50s. The LT50s might be expected to be a few degrees above the supercooling points because of the longer duration of low temperature exposure during the LT50 tests but differences of this magnitude suggest very strongly that all three species died as a result of chill-injury long before they froze, when they

Fig. 2.4 Apparatus for the determination of supercooling points.

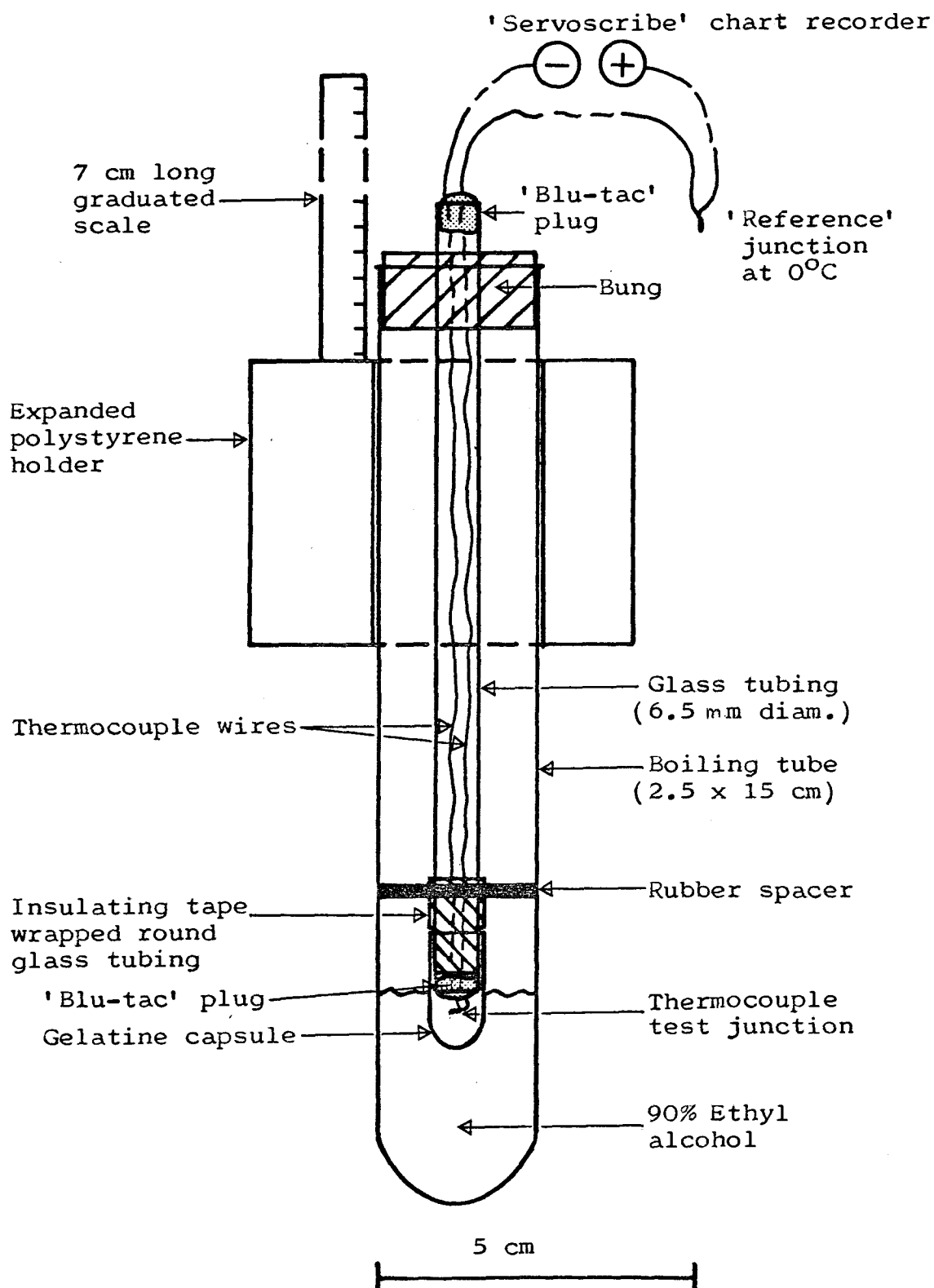


Fig. 2.5 Chart recorder trace of the body surface temperature of an apterous adult S. avenae cooled at $1^{\circ}\text{C}/\text{min}$, with supercooling point at -22.5°C .

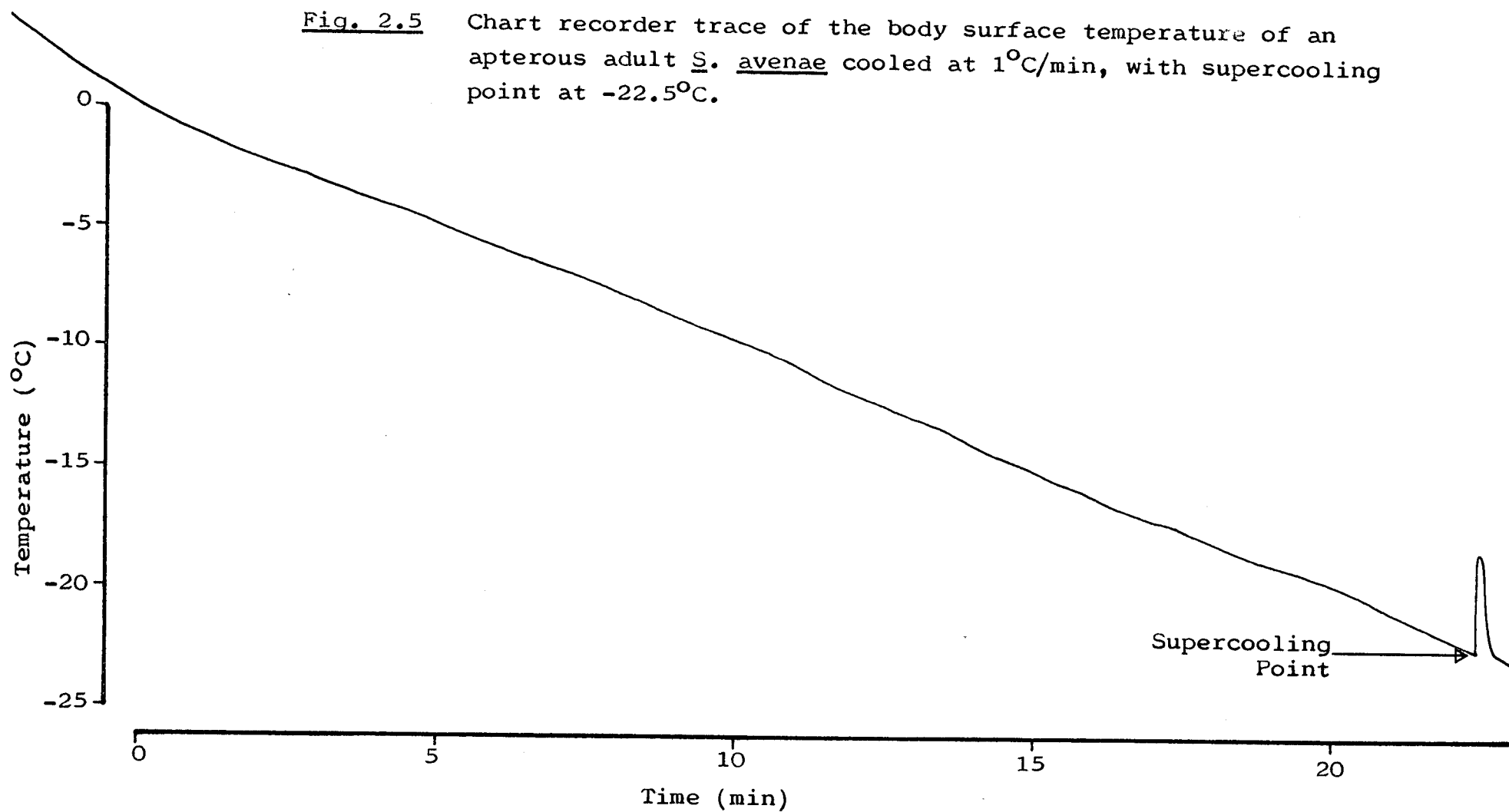
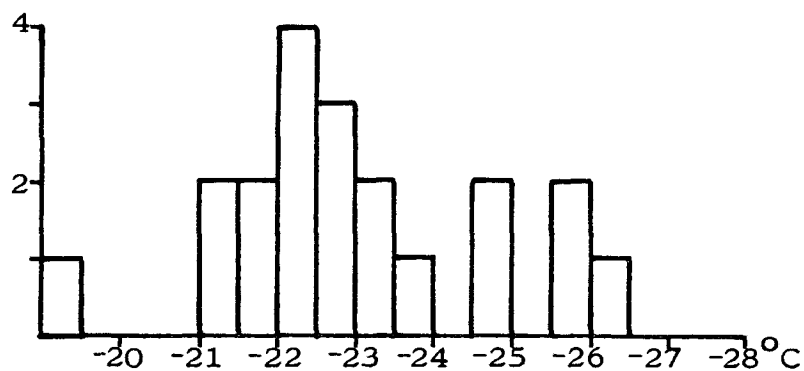


Fig. 2.6 Supercooling point frequency distributions.

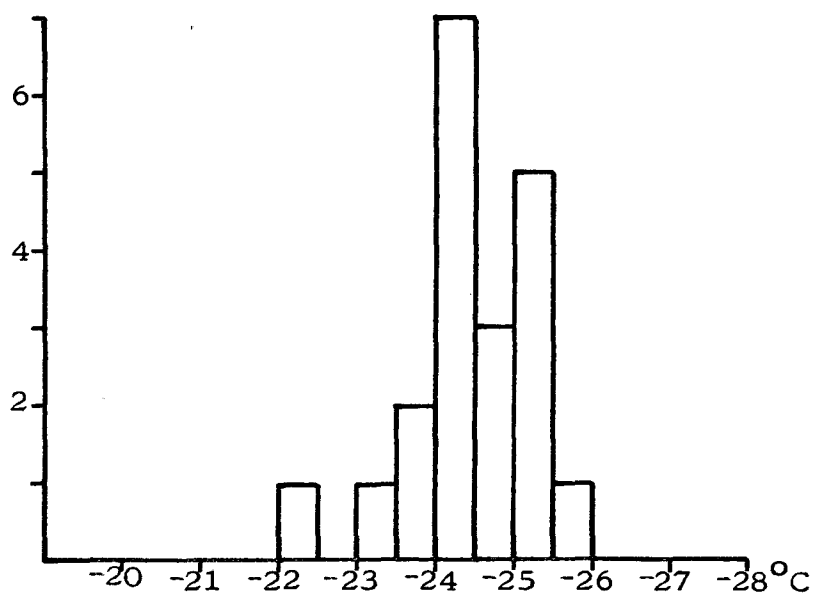
(a) S. avenae

$\bar{x} = -23.0^{\circ}\text{C}$ S.E. = 0.389



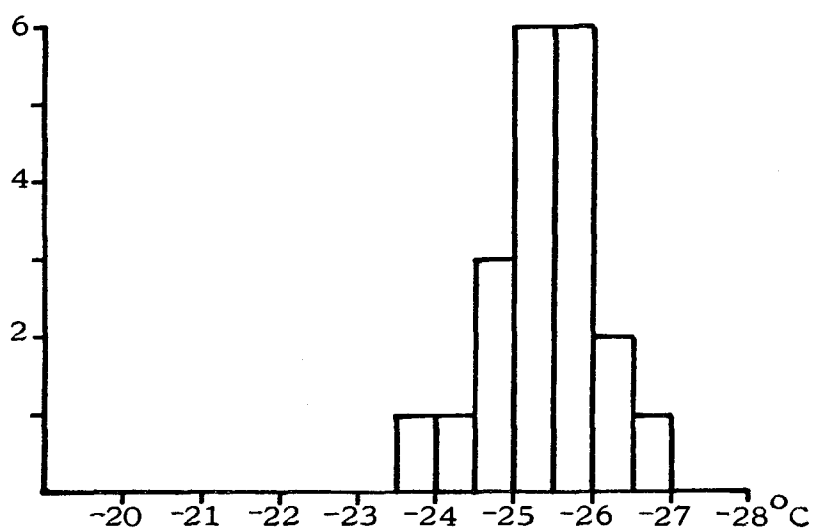
(b) M. dirhodum

$\bar{x} = -24.4^{\circ}\text{C}$ S.E. = 0.177



(c) R. padi

$\bar{x} = -25.4^{\circ}\text{C}$ S.E. = 0.170



were reared at 20°C. Not only were mean supercooling points much lower than LT50s but the difference between the two was far from constant. Thus R. padi, which was much less cold-hardy than the other two species, had the lowest mean supercooling point.

The supercooling points determined here are lower than those found for adult aphids of other species. For example adults of Elatobium abietinum and Myzus persicae (both reared at 15°C) had mean supercooling points of -15.7°C and -12.0°C respectively (Powell, 1974; Parry, 1978). Alatae of Drepanosiphum platanoides collected in July showed a bimodal supercooling point distribution with peaks around -17°C and -22°C while alatae of Eucallipterus tiliiae collected at the same time showed a less clearly bimodal supercooling point distribution with the major peak at about -19°C and a minor one at around -25°C (Powell, 1976). It seems, therefore, that the phloem sap of the winter wheat on which the cereal aphids were reared contained less efficient nucleating agents than the food of the other species. Cereal aphids feeding on a different host might show higher supercooling points.

The supercooling points of S. avenae, M. dirhodum and R. padi reared at 20°C were only slightly higher than those of freezing-susceptible Antarctic collembolans and mites which had eliminated ice nucleators from their digestive tracts (Block et al., 1978; Somme, 1978a, 1978b; Block, 1982; Block & Somme, 1982). Thus, if chill-injury and inoculative freezing could be overcome, all three cereal aphids seem pre-adapted to survival at very low temperatures.

2.3.3 Chill-susceptibility

Introduction

Several previous studies on aphid cold-hardiness have used supercooling points to assess low temperature tolerance - a method which is only applicable if the aphids are freezing-susceptible and not chill-susceptible (Powell, 1974, 1976; Parry, 1978, 1979a). But the results of the previous two experiments showed that R. padi was definitely

chill-susceptible when reared at 20°C (as its LT50 was above 0°C). These experiments also revealed that there were discrepancies of 14°C or more between the LT50s and supercooling points of all three species, strongly suggesting that all were chill-susceptible. However, despite these results the possibility remained that the supercooling abilities of S. avenae and M. dirhodum were drastically reduced by increased cooling rates and exposure times. If this were so the aphids might have frozen during LT50 tests, in which they were exposed to temperatures around -8°C or below for over two hours after rapid cooling, despite the fact that when cooled steadily at 1°C/min during supercooling point tests they did not freeze above -20°C. To provide conclusive evidence on whether or not these aphids could die well before they freeze, they were cooled at the same rate as in supercooling point determinations (1°C/min) but returned to room temperature well before the supercooling point range was reached and then examined for signs of life.

Methods

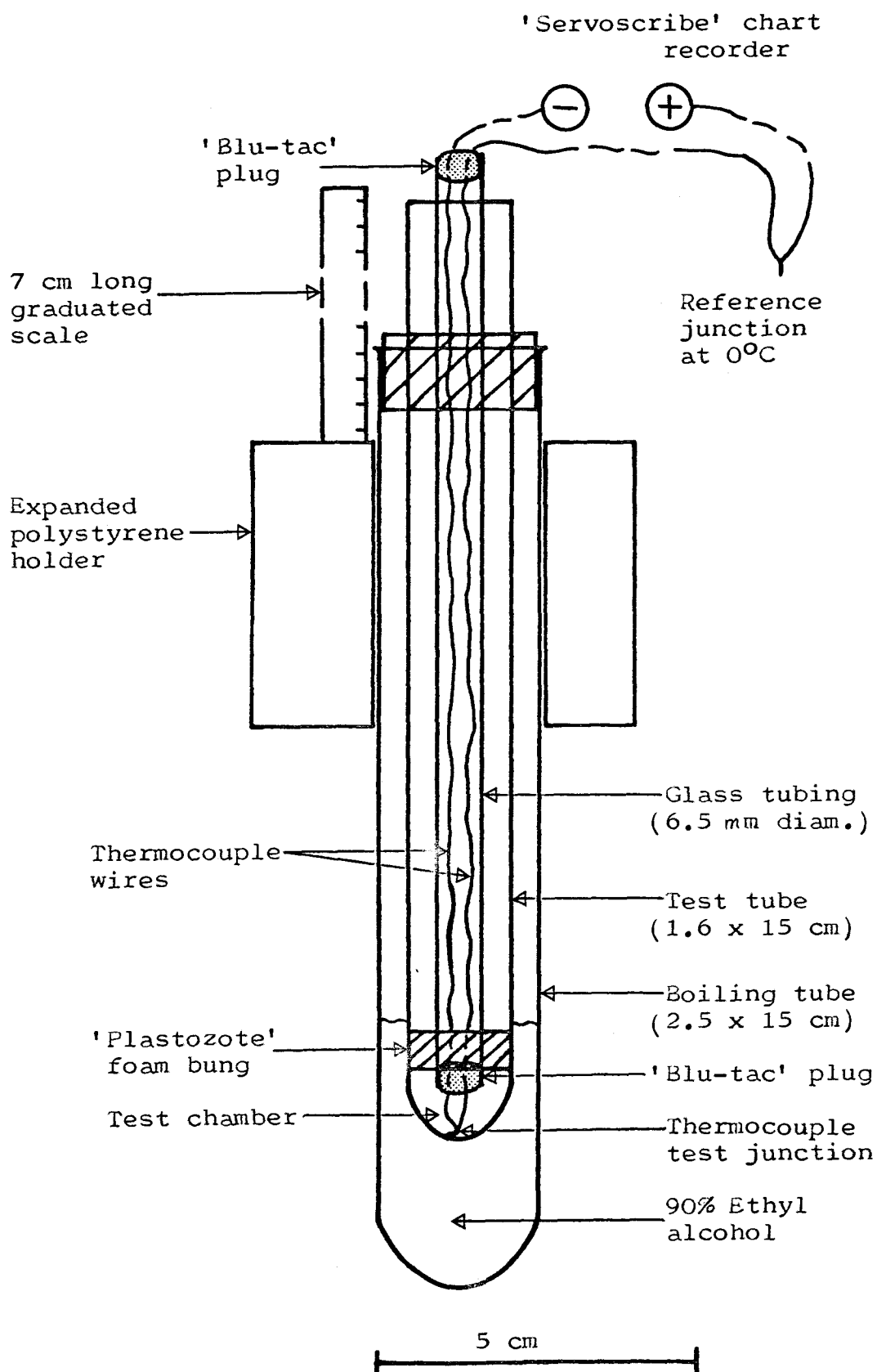
Species used: S. avenae, M. dirhodum, R. padi.

Clones used: old. Instars used: adult apterae.

Cooling apparatus used: cold bath.

The apparatus was very similar to that used for supercooling point determinations but in this experiment aphids were not attached to the thermocouple and six (two of each species) were tested together, enclosed in a small test chamber formed by the end of a test tube (see Fig. 2.7). The thermocouple's test junction was pressed against the end of the test tube and so recorded temperatures that were, if anything, slightly colder than those experienced by the aphids themselves. Cooling at a steady 1°C/min was achieved by lowering the boiling tube slowly into the cold bath held at -30°C as before. When -15.5°C was reached the aphids were taken out of the cold bath and left at room temperature. Mortality was assessed according to the standard test procedure (see section 2.2.3) 30 min after testing and again 3 days later. Between testing and the final assessment of mortality aphids

Fig. 2.7 Apparatus used for cooling small batches of aphids at $1^{\circ}\text{C}/\text{min}$.



were kept in plastic containers in the standard way. A total of 20 adults of each species were tested.

Results and discussion

Unlike aphids cooled below their supercooling points, over half the aphids showed some movement 30 min after testing, although only three could walk (Table 2.3). But after 3 days all the aphids were dead. This proved that when cultured at 20°C and cooled at 1°C/min, all three species were killed by chill-injury long before they froze. The results do not exclude the possibility that the aphids could lose their chill-susceptibility when cooled at slower rates or given suitable low temperature pre-treatment, or when some other aspect of conditions before or during low temperature exposure was changed. But the fact that all three species were chill-susceptible under one set of conditions makes it likely that they will be so, though perhaps to a lesser extent, under other conditions. Because of this it is clear that for these three species mean supercooling points are an unsuitable measure of cold-hardiness. Consequently, although it was originally intended to use supercooling points when investigating factors likely to modify cold-hardiness (sections 2.4 and 2.5 below) LT50s were used instead. This change of plan proved sound as in none of the later experiments in which cooling rates, low temperature pre-treatment and other variables were altered, did the species' LT50s approach their supercooling points.

2.4 ENVIRONMENTAL FACTORS AFFECTING LOW TEMPERATURE MORTALITY

Experiments on the following environmental influences are described below:

1. Cooling and re-warming rates (experiment 2.4.1).
2. Low temperature pre-treatment (experiments 2.4.2 and 2.4.3).

Table 2.3 Total numbers of aphids
 (a) walking, (b) moving but not walking,
 (c) not moving, 30 min after cooling to
 -15.5°C at $1^{\circ}\text{C}/\text{min}$.

	<u>S. avenae</u>	<u>M. dirhodum</u>	<u>R. padi</u>
Walking	2	0	1
Moving but not walking	9	10	11
Not moving	9	10	8
	20	20	20

3. Exposure time (experiment 2.4.4).
4. Host plant effects (experiments 2.4.5 and 2.4.6).
5. Surface ice (experiment 2.4.7).

Although these constitute most of the important environmental factors likely to influence cold-hardiness, none were investigated exhaustively. The experiments were primarily designed to test whether or not each factor had any effect on cereal aphids and, if so, to determine roughly the nature and magnitude of the effect. Some preliminary investigation of these factors had already been undertaken by Griffiths & Wratten (1979).

As cereal aphids can be killed by chill-injury, supercooling point determination cannot be considered a reliable laboratory technique for assessing low temperature mortality in natural populations but LT50 determination provides a possible alternative method which is reasonably quick and easy to perform. The results of the laboratory experiments described below (sections 2.4 and 2.5), taken together with those from the fieldwork described in Chapters 3 and 4, allowed the usefulness of this method to be assessed. In addition, laboratory experiments 2.4.2 and 2.4.3 were directly linked to the assessment of results from the field experiment on winter mortality (Chapter 3).

2.4.1 Cooling and re-warming rates

Introduction

Cooling and re-warming rates experienced in the field involve changes of only a few degrees Celsius per hour but those usually used in supercooling point and LT50 determinations - including the standard LT50 test procedure used in the present study - are much more rapid, often involving temperature changes of several degrees per minute. Salt (1966b) found that mean supercooling points were affected very little by cooling rate but in chill-susceptible insects rapid cooling may produce thermal shock or allow

insufficient time for rapid cold-hardening mechanisms to operate, so that laboratory tested animals may show artificially high mortality. The present experiment was undertaken to determine whether or not slow cooling and re-warming increased survival in laboratory tests on the three cereal aphid species under study.

Methods

Species used: S. avenae, M. dirhodum, R. padi.

Clones used: old. Instars used: 2nd-4th.

Cooling apparatus used: cold bath.

Each species was tested separately, S. avenae and M. dirhodum at their 3 h LT50s (determined in experiment 2.3.1) and R. padi, which has a shallower, skewed response curve, at -2.0°C - a temperature estimated to give about 80% mortality when aphids were cooled rapidly. In each species' test three treatments were exposed to low temperatures simultaneously, each consisting of four replicates of 50 aphids. The treatments were:

1. Slow cooling and re-warming: aphids were placed in the cold bath at a temperature 10°C above the test temperature and cooled to the test temperature at 2°C/h by slowly altering the bath's thermostat setting. They were left at the test temperature for 3 h and then re-warmed at 2°C/h to the starting temperature before being taken out of the bath.
2. Intermediate cooling and re-warming: aphids were placed in the bath when it had cooled to the threshold for low temperature mortality (estimated from experiment 2.3.1) and cooled down at 2°C/h with the previous treatment to the test temperature. They were also held at the test temperature for 3 h and then re-warmed at the same rate to the estimated threshold for mortality before being taken out of the bath.
3. Fast cooling and re-warming: aphids were placed in the bath when it had reached the test temperature, left for 3 h and then taken out. The treatments

and temperatures used for each species are summarised in Fig. 2.8 and Table 2.4.

The aphids used in all three treatments were taken off culture plants at the same time before the test and put in plastic containers with wheat leaves at the same time after the test. Two types of control were run, each replicated four times. In both, aphids were kept in specimen tubes for 13 h (the time all treatment aphids were off plants), in the first at $20^{\circ}\pm 2^{\circ}\text{C}$ and in the second at 10°C above the test temperature. All other experimental details followed the standard test procedure (see section 2.2.3).

Results and discussion

One-way analyses of variance on the arcsin transformed percentage mortalities revealed highly significant differences between the treatments for all three species ($F = 158.7$ (S. avenae), $F = 27.9$ (M. dirhodum), $F = 502.8$ (R. padi), D.F. = 2 & 9 for all species, $P < 0.001$ for all species). However, Bartlett's test of homogeneity of variances revealed significant differences between treatment variances in the case of S. avenae ($M/C = 7.46$, D.F. = 2, $P < 0.05$). For all three species mortality was lowest in the slow cooled/re-warmed treatment and highest in the fast cooled/re-warmed treatment and mortality in both controls was always below 7% (Fig. 2.9). Planned comparisons between treatments (Table 2.5) revealed that all the differences between treatments were significant ($P < 0.05$) except for that between intermediate and fast cooled M. dirhodum. In all species slow-cooled treatments showed significantly higher mortality ($P < 0.05$) than low temperature controls. In R. padi mortality in the intermediate-cooled treatment was close to that in the slow-cooled treatment, while in S. avenae and M. dirhodum it was closer to the fast-cooled (Fig. 2.9) but this was not a real species difference. It was due to the estimated threshold for low temperature mortality lying 6°C above the test temperature for R. padi but only 2°C above the test temperature for the other two species.

For all three species a slow cooling and re-warming

Fig. 2.8 Treatments used in the experiment on cooling and re-warming rates.

TREATMENTS: S = Slow cooling/re-warming rate

I = Intermediate cooling/re-warming rate

F = Fast cooling/re-warming rate

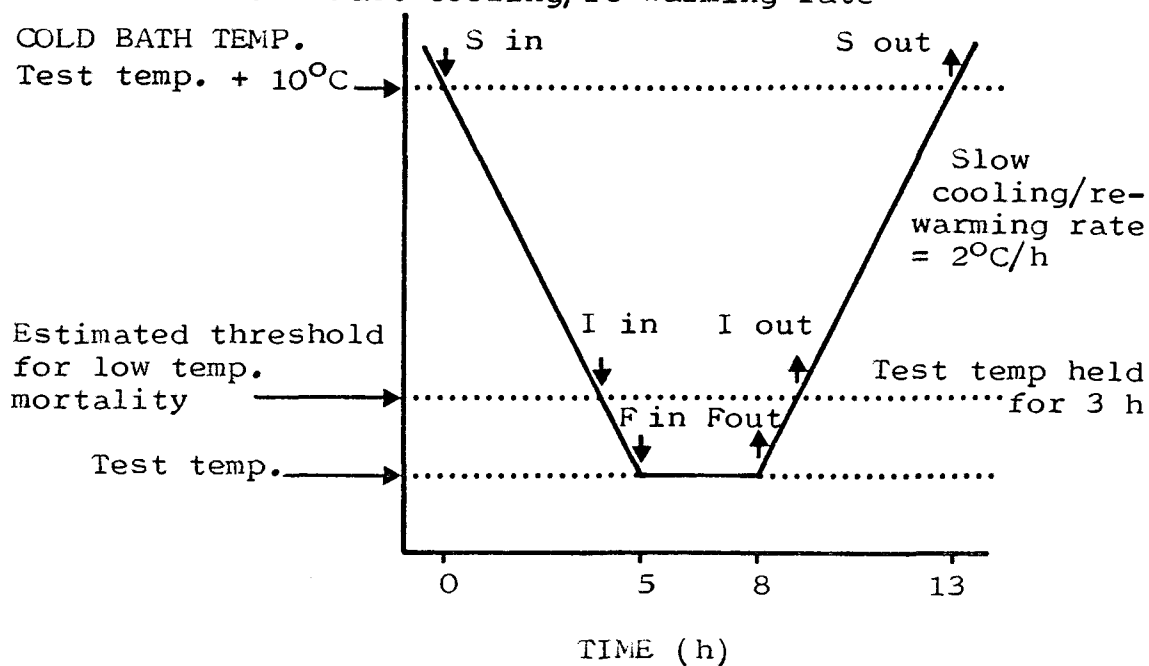


Table 2.4 Temperatures ($^{\circ}\text{C}$) used in the experiment on cooling and re-warming rates.

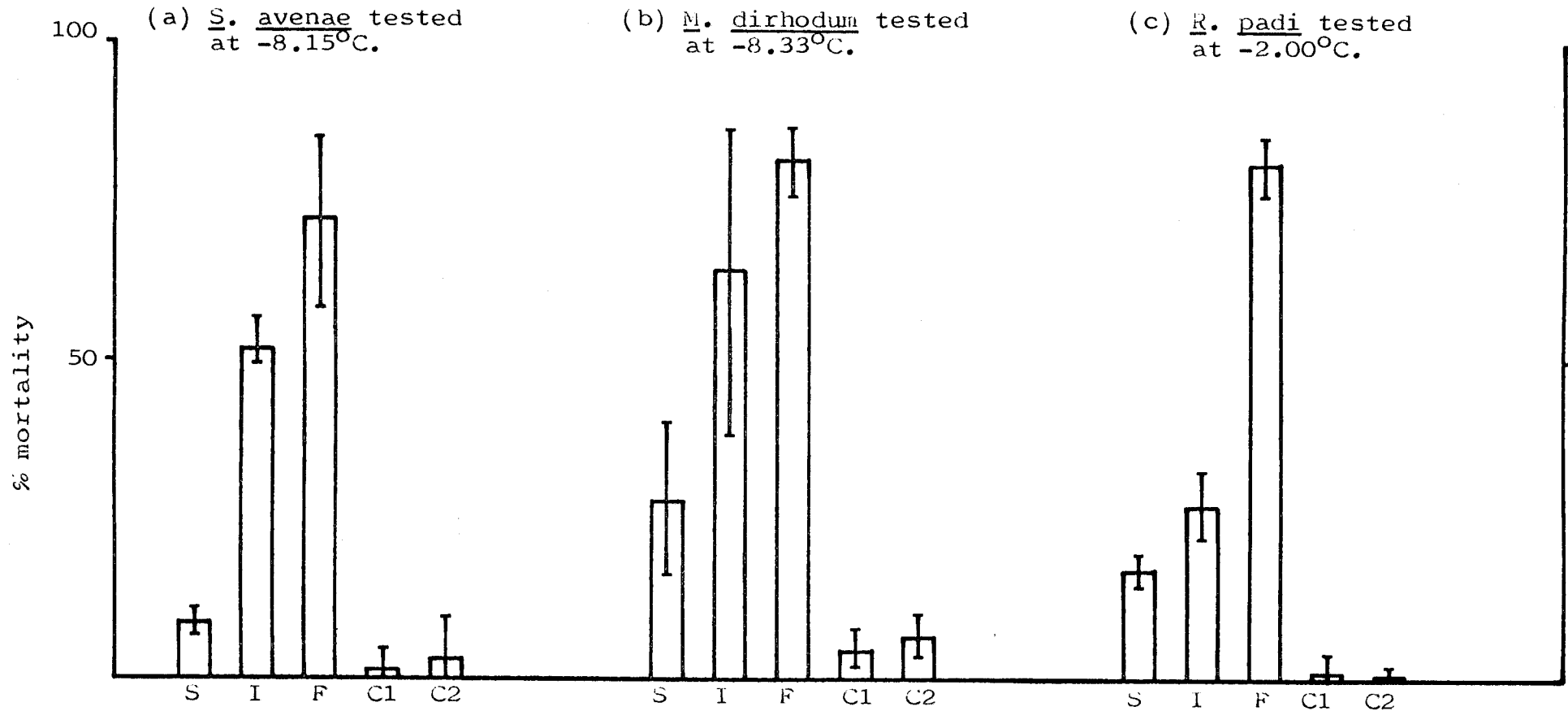
	<u>S. avenae</u>	<u>M. dirhodum</u>	<u>R. padi</u>
Starting temperature	+2	+2	+8
Estimated threshold for low temperature mortality	-6	-6	+4
Test temperature	-8.15	-8.33	-2.00
Low temperature control	+2	+2	+8

Table 2.5 Results of t-tests on pairs of treatments used in the experiment on cooling and re-warming rates.

Comparison	<u>S. avenae</u>	<u>M. dirhodum</u>	<u>R. padi</u>
S v. I	***	**	**
S v. F	***	***	***
I v. F	*	NS	***
S v. low temp. control	*	**	*

(NS: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$)

Fig. 2.9 Mortality in low temperature tests using slow (S), intermediate (I) and fast (F) cooling rates and in controls kept at 20°C (C1) and at 10°C above the test temperature (C2). (Vertical bars are 95% confidence intervals.)



rate (similar to that experienced in the field) increased survival very significantly. But it is not known whether this was due to the operation of an active cold-hardening mechanism or whether the high mortality in fast-cooled treatments was due to thermal shock caused by the artificially rapid cooling and re-warming rates. If a cold-hardening mechanism was responsible it must have had a very rapid effect as intermediate cooled treatments of S. avenae and M. dirhodum were subject to cooling at 2°C/min for only 1 h before the test temperature was reached but still showed significantly improved survival.

Although cooling and/or re-warming rates had highly significant effects, it appears - from the response curves determined in experiment 2.3.1 - that using fast instead of slow rates is equivalent to a shift of test temperature of only about 2°C for S. avenae and M. dirhodum. However, in the case of R. padi the difference is equivalent to a shift of about 5°C, suggesting that this species is more sensitive to fast cooling/re-warming. Consequently its cold-hardiness relative to the other species will be underestimated in laboratory tests using fast cooling/re-warming rates. This provides one explanation for the observation that laboratory tested R. padi showed considerably less cold-hardiness than one would predict from the species' occurrence in the field in winter (see experiment 2.3.1). However, although the use of fast cooling and re-warming rates over emphasizes the cold-susceptibility of R. padi, there is no doubt that R. padi remains much less cold-hardy than the other two species even when slow cooled, because mortality in the slow cooled treatment was significantly greater than in controls ($P < 0.05$) despite the high test temperature.

The use of artificially fast cooling and re-warming rates clearly increases cold-susceptibility and distorts species differences. However, because no means of automatically regulating cooling rates were available (making tests using slow cooling rates extremely time consuming) and because the discrepancies between fast and slow cooled treatments were equivalent to shifts in test temperature of a few degrees only, it was decided to continue using the

standard fast cooling test procedure in later experiments. However, it was realised that the results of such tests provide an index of cold-hardiness rather than an accurate prediction of low temperature mortality in the field. Despite the artificiality of fast cooling, the standard LT50 determination procedure still appeared a much better method for assessing cold-hardiness for the three species under study than supercooling points.

Lastly, it should be noted that the mortalities in fast cooled treatments of S. avenae and M. dirhodum were higher than predicted from the results of experiment 2.3.1. This is probably partly due to the fact that temperature control in the cold chamber used in that experiment was not very accurate ($\pm 0.75^{\circ}\text{C}$) so that the response curves were unlikely to be accurate to fractions of a degree. In addition, adults were used to determine the response curves, whereas nymphs - shown in experiment 2.5.1 to be a little less cold-hardy - were used in the present experiment.

2.4.2 Short-term low temperature pre-treatment in the laboratory

Introduction

The experiments described above all involved the testing of aphids reared at $20^{\circ} \pm 2^{\circ}\text{C}$. However, natural populations subjected to sub-zero temperatures in the field will have previously experienced temperatures considerably lower than 20°C , which may serve to increase cold-hardiness. Low temperature pre-treatment has been shown in laboratory tests to increase cold-hardiness of S. avenae clones from Spain and Norway (Griffiths & Wratten, 1979) and the same effect has also been demonstrated for three other aphid species (Adams, 1962; Carter, 1972; Harrison & Barlow, 1973). The present experiment was undertaken to test whether an increase in cold-hardiness after low temperature pre-treatment is shown by the three cereal aphid species under study and to determine whether the different species increased their cold-hardiness by different amounts - as

measured by LT50 values - when cultured together and given the same low temperature pre-treatment. The low temperature pre-treatment given - a 6 day exposure to 6°C - did not mimic field conditions but longer-term acclimatization in the field was investigated in a later experiment (2.4.3). The particular temperature and exposure time used for pre-treatment was chosen because it was the same as that given to aphids used in the field experiment on winter mortality described in Chapter 3.

Based mainly on their findings with Elatobium abietinum, Powell (1974) and Parry (1978, 1979a) considered that the increases in aphid cold-hardiness after low temperature pre-treatment demonstrated by earlier researchers could not be due to a true, active cold-hardening mechanism in the aphids but were due to additional cold-hardiness being acquired passively from the host plant (see section 2.1.6 for further explanation). To test whether or not S. avenae could cold-harden independently of the host an additional experiment was run in which individuals of S. avenae were given a short low temperature pre-treatment after they had been removed from culture plants.

Methods

Species used: S. avenae, M. dirhodum, R. padi (main experiment); S. avenae only (subsidiary experiment involving off-plant low temperature pre-treatment).

Clones used: new. Instars used: 2nd-4th.

Cooling apparatus used: cold bath.

The main experiment involved determination of LT50s using the standard test procedure (see section 2.2.3) with a 90 min exposure time. The two treatments were:

1. 'Unacclimated': aphids were taken from cultures at $20^{\circ} \pm 1.5^{\circ}\text{C}$ and tested immediately.
2. 'Acclimated': aphid cultures were placed in an LEEC controlled-temperature cabinet at $6^{\circ} \pm 2.2^{\circ}\text{C}$ and 16 h photoperiod for 6 days before testing. Aphids were then shaken off culture plants and sorted into replicates of 50 nymphs in a cold room at 6°C.

They were exposed to normal laboratory temperature for only 30 min before testing, thus minimizing the chances of rapid loss of acquired cold-hardiness.

Acclimated and unacclimated aphids were not tested together and tests of each treatment were divided between two cultures because of the numbers of aphids required, so that altogether four cultures set up one after the other were used. The first and third of these were used for acclimated tests and the second and fourth for unacclimated tests. The control mortality of acclimated and unacclimated aphids was determined using four replicates for each treatment of each species, which were held at room temperature (19 to 24°C) while treatment aphids were in the cold bath.

In the subsidiary experiment in which S. avenae nymphs were given low temperature pre-treatment after removal from culture plants, the acclimated aphids were kept in an LEEC cabinet in specimen tubes for 23 h at $0^{\circ} \pm 1^{\circ}\text{C}$. The pre-treatment period was short as aphids kept off plants for longer might have suffered stress due to starvation or desiccation. Again acclimated aphids were put in the cold bath after 30 min at room temperature. Unacclimated aphids taken straight from the stock cultures were tested with them. Four tests were run at different temperatures, each using two replicates of each treatment. Four control replicates of each treatment were also run.

Results and discussion

The LT50s and response curves determined from probit analyses showed that the cold-hardiness of all three species was increased significantly by low temperature pre-treatment at 6°C for 6 days (Table 2.6, Figs 2.10 to 2.12). The shifts in LT50 values were as follows: S. avenae: 3.0°C; M. dirhodum: 1.9°C; R. padi: 4.8°C. Changes of this magnitude suggest that increases in cold-hardiness after low temperature experience are likely to be important in the winter survival of anholocyclic field populations, particularly those of R. padi. The results show that only a few days at a temperature well above 0°C can produce a useful increase in cold-hardiness.

Table 2.6 Summary of probit analyses for nymphs
(a) cold-acclimated at 6°C for 6 days.
(b) not cold-acclimated.

	<u>S. avenae</u>		<u>M. dirhodum</u>		<u>R. padi</u>	
	not cold-acc.	cold-acc.	not cold-acc.	cold-acc.	not cold-acc.	cold-acc.
LT50	-7.13	-10.12	-6.73	-8.62	-1.06	-5.87
LT50 95% confidence limits	-6.74 to -7.53	- 9.97 to -10.25	-6.37 to -7.05	-8.43 to -8.81	-0.09 to -1.67	-5.55 to -6.18
Probit line: intercept	-3.7651	-15.1713	-4.0966	-7.0646	-0.4688	-3.7792
slope	0.5278	1.4994	0.6089	0.8193	0.4411	0.6438
Heterogeneity χ^2 D.F.	46.97*** 14	10.79 8	32.57** 13	1.68 6	41.73*** 11	18.69* 10
Number of: batches tested	16	12	16	11	16	13
test temps.	8	6	8	6	10	7
Transformation .	-temp.	-temp.	-temp.	-temp.	-temp.	-temp.

(*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$)

Fig. 2.10 Mortality - temperature curves for *S. avenae* nymphs (a) cold-acclimated at 6°C for 6 days (○) (b) not cold-acclimated (●). (Horizontal bars show 95% confidence limits for the LT50s.)

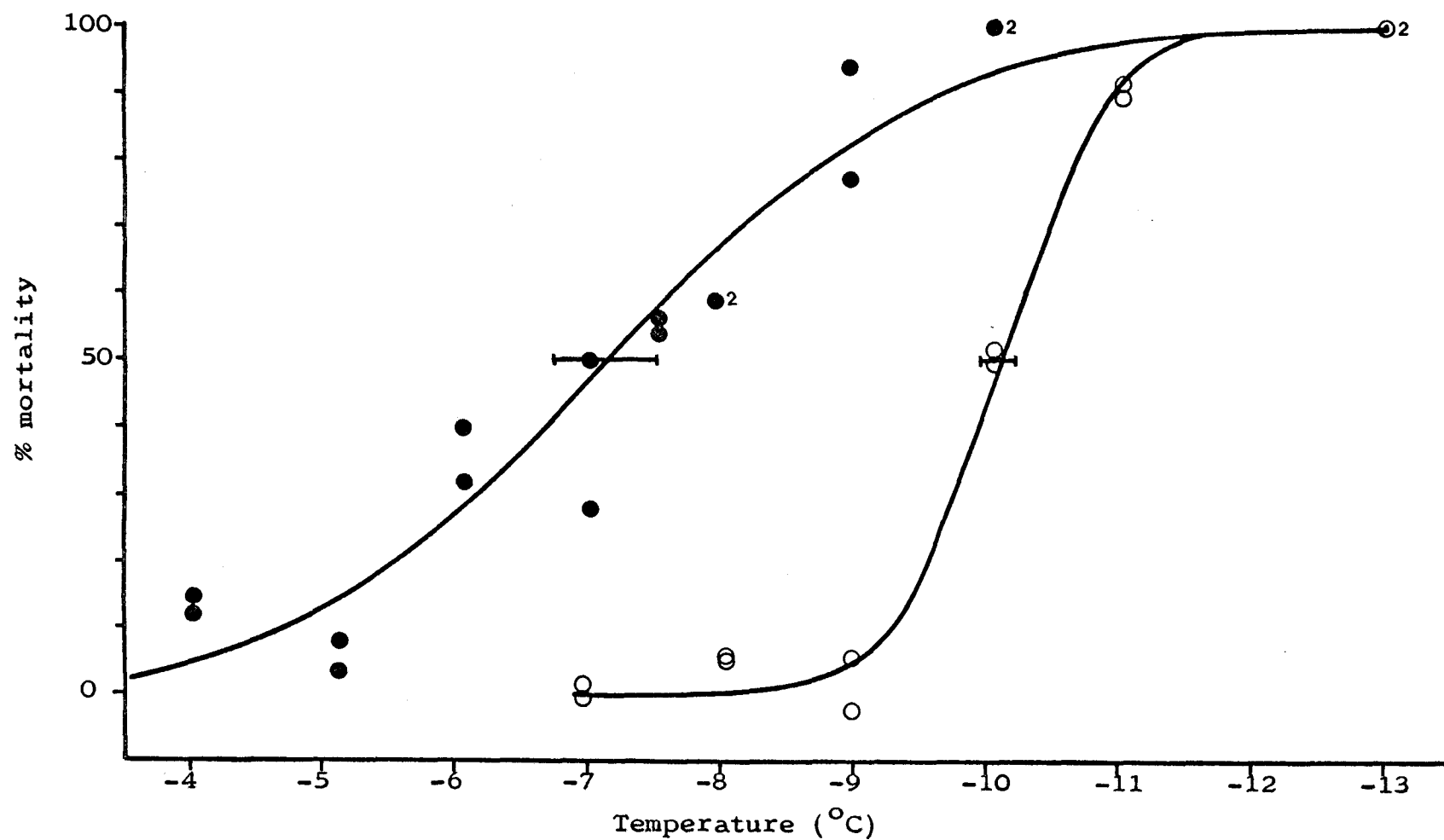


Fig. 2.11 Mortality - temperature curves for *M. dirhodum* nymphs (a) cold-acclimated at 6°C for 6 days (○) (b) not cold-acclimated (●). (Horizontal bars show 95% confidence limits for the LT50s.)

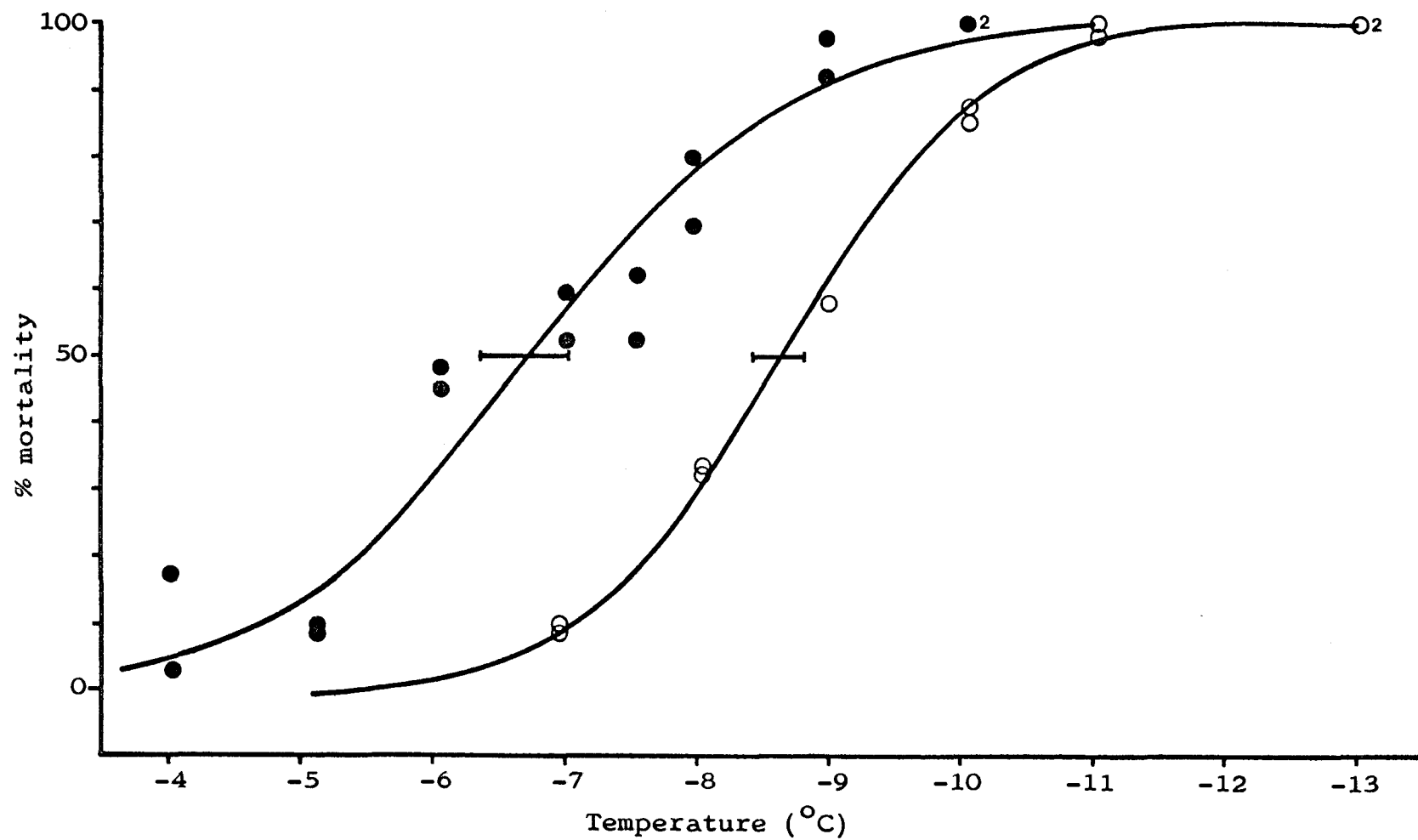
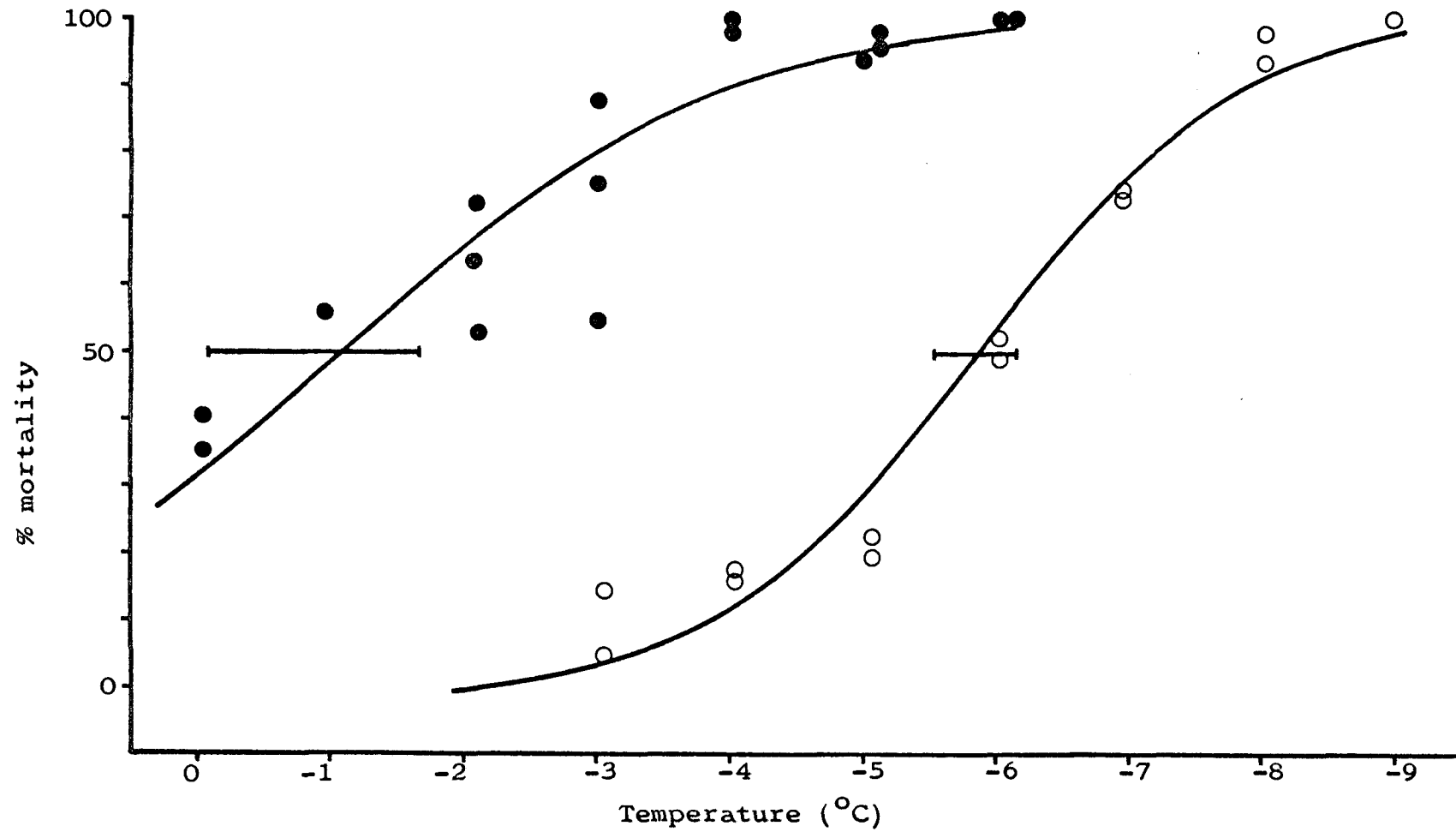


Fig 2.12 Mortality - temperature curves for *R. padi* nymphs (a) cold-acclimated at 6°C for 6 days (○) (b) not cold-acclimated (●). (Horizontal bars show 95% confidence limits for the LT50s.)



Given the standard low temperature pre-treatment the three species acclimate to different extents. It is perhaps to be expected that M. dirhodum viviparae showed the smallest improvement in cold-hardiness as anholocyclic overwintering appears rare in this species and viviparae possess good inherent cold-hardiness even when cultured at high temperatures. In southern England - where the clones under study were collected - the primary host of R. padi is rare and high densities of viviparae may be found on Gramineae during late autumn and winter (Hand, 1982). Viviparae of the species have a relatively high lower lethal temperature range so that low temperature mortality is likely to occur regularly in most winters. Consequently, selection pressure for a cold-hardening mechanism is particularly high. This may explain why R. padi shows the greatest improvement in performance but it also appears that some characteristic of the species' physiology prevents it from matching the cold-hardiness of S. avenae and M. dirhodum even after low temperature pre-treatment.

The study by Griffiths & Wratten (1979) provides comparative data on cold-acclimation in two European clones of S. avenae, from Spain and Norway. The data in this study was presented as regression equations of LT50 on exposure time for aphids kept at 5°, 10° and 28°C for one week before testing, after culturing at 17°C. From the equations it can be calculated that Spanish S. avenae kept at 5°C have a 90 min LT50 2.2°C lower than those kept at 28°C, while for the Norwegian clone the difference is 2.1°C. The English clone used in the present experiment showed a larger shift in LT50s (3.0°C), despite a slightly shorter low temperature pre-treatment time and a smaller difference between pre-treatment temperatures. This suggests that the earlier study underestimated the importance of cold-hardening somewhat, although the results of the two studies agree reasonably well, given the different clones and test procedures used.

By varying the time of exposure to sub-zero temperatures, Griffiths & Wratten (1979) showed that differences in survival between S. avenae acclimated at 5°, 10° and 28°C diminished as exposure time increased over the range

30 min to 3 h, so that cold-acclimation apparently delays lethal cold-injury rather than reducing it absolutely. From their regression equations the LT50s of Norwegian S. avenae acclimated at 5°C and 28°C still differed by 1.6°C after tests of 10 h duration but those of the Spanish clone differed by only 0.24°C. The latter's poor ability to 'hold-off' cold-injury for several hours was probably due to the mild winter climate of its place of origin. British S. avenae have a lower lethal temperature zone below -5°C even when reared at 20°C and so are very unlikely to experience temperatures in the lethal zone for more than a few hours at a time. Consequently cold-acclimatization should be effective in reducing low temperature mortality in the field even if it only acts to delay cold-injury to the extent shown by the Norwegian clone studied by Griffiths & Wratten (1979).

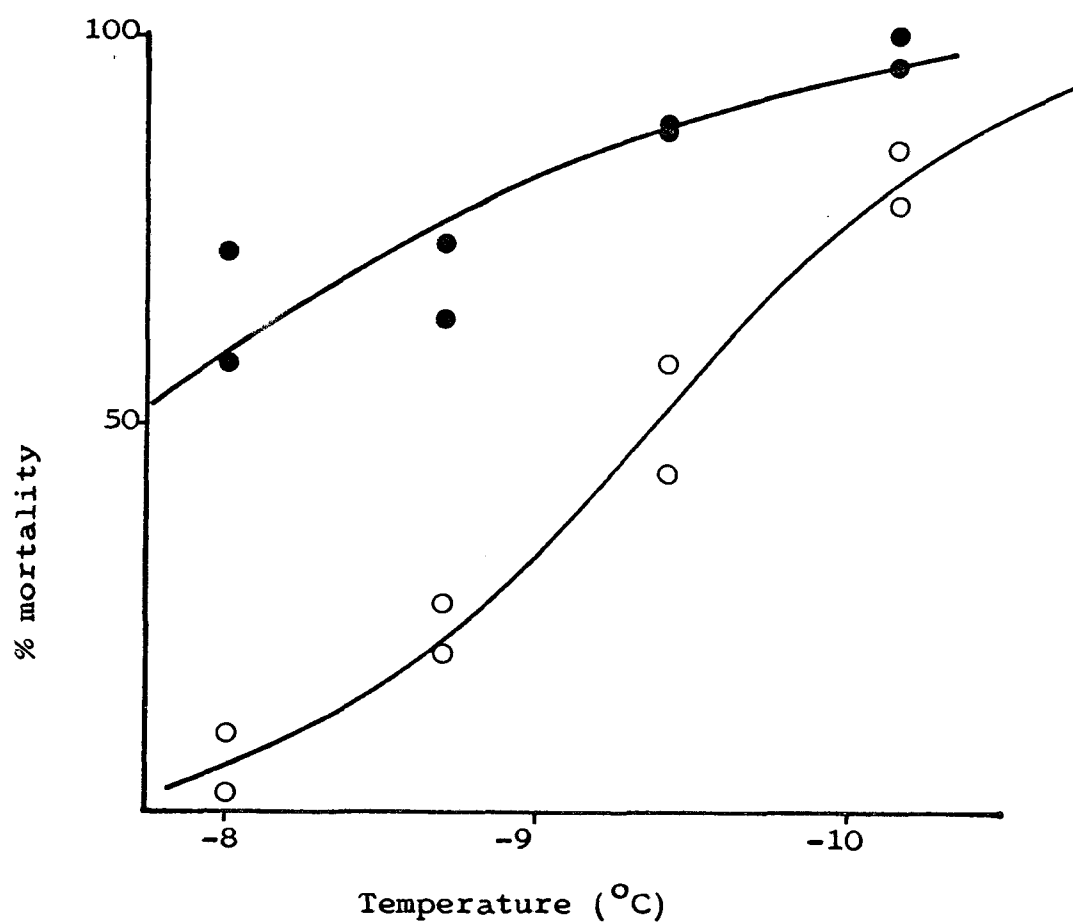
Although the term acclimation has been used in this discussion, the lowering of LT50s after low temperature pre-treatment described above does not prove that the cereal aphids have an active, physiological acclimation or cold-hardening mechanism, because they were given low temperature pre-treatment while on the host plants, and could have passively acquired additional cold-hardiness from them as Powell (1974) and Parry (1978, 1979a) suggested. However, the results of the subsidiary experiment in which S. avenae were removed from the host plants before a low temperature pre-treatment of 0°C for 23 h proved that this species, at least, does not simply acquire cold-hardiness passively from its host, as the aphids given low temperature pre-treatment had a significantly lower LT50 (Table 2.7, Fig. 2.13).

In fact it is likely that all three species under study possess true cold-hardening mechanisms because the argument against a cold-hardening mechanism in aphids is based on the premise that they do not die until their supercooling points are reached and that supercooling points are determined by efficient ice-nucleators in the gut which cannot be eliminated. However, experiments 2.3.1, 2.3.2 and 2.3.3 above proved that all three species studied here can die of chill-injury well above their supercooling

Table 2.7 Summary of probit analyses for S. avenae nymphs (a) cold-acclimated off the host plant at 0°C for 23 h. (b) not cold-acclimated.

	Cold-Acclimated	Not cold-Acclimated
LT50	-9.37	-7.68
95% confidence limits	-9.23 to -9.52	-7.17 to -8.00
Probit line: intercept slope	-10.5412 1.1250	-5.3819 0.7005
Heterogeneity χ^2 Degrees of freedom	4.52 6	10.43 6
Number of: batches tested test temperatures	8 4	8 4
Transformation	-temp.	-temp.

Fig. 2.13 Mortality-temperature curves for S. avenae nymphs (a) cold-acclimated off the host plant at 0°C for 23 h (○) (b) not cold-acclimated (●).



points, which are thus irrelevant to their cold-hardiness. Thus a cold-hardening mechanism in these species must involve changes to reduce chill-injury in order to be effective, but need not alter the aphids' supercooling points at all.

If the 90 min LT50s for unacclimated aphids determined in this experiment are compared with the 3 h LT50s determined in experiment 2.3.1 it can be seen that all are slightly but significantly higher, the differences varying from just over 1°C - for S. avenae - to just over 2°C - for R. padi. The pattern of species differences remained much the same, although in the earlier experiment the LT50 of M. dirhodum was marginally lower than that of S. avenae, while in the present experiment it was a little higher. These small differences may be genuine differences between clones of the same species but may also be due to the different instars tested - instar differences were studied in experiment 2.5.1, which showed adult apterae were a little more cold-hardy than nymphs. Other subtle differences in experimental procedure or cooling rates in the two types of apparatus may also contribute. However, the two sets of data are reassuringly close and suggest that LT50s are a reasonably robust index of aphid cold-hardiness. The fact that the 3 h LT50s are a little lower, rather than higher than the 90 min. LT50s suggests that changes in exposure time over this range have very little effect on mortality. This was investigated further in experiment 2.4.4.

2.4.3 Long-term low temperature pre-treatment in the field

Introduction

Although the experiment above demonstrated that low temperature pre-treatment could cause important changes in cold-hardiness, the pre-treatment used did not approximate to field conditions. To assess the extent of winter cold-hardening in the field and investigate possible changes in cold-hardiness through the winter aphids were caged out-of-doors and tested at three times during the 1980-81 winter.

Although it was originally intended to test all three species, field cultures of M. dirhodum were severely reduced by Entomophthora during the autumn and could not be used.

Methods

Species used: S. avenae and R. padi.

Clones used: new. Instars used: large 1st-4th.

Cooling apparatus used: cold bath.

Cultures were established in field plots of late August-sown winter wheat (cv. Hobbit) on 23 and 24 September 1980. The aphids were kept in cylindrical shelter cages 22 cm high and 26 cm in diameter with clear polythene roofs and gauze walls on a plastic framework. These were pegged down over the infested plants with their bases buried to prevent natural enemies entering. Five cages of each species were established, each with 100 to 200 aphids initially. After the first series of tests more cultures were started on wheat sown in mid-September. A thermistor connected to a Grant Instruments model D temperature recorder was used to record the temperature inside a shelter cage at hourly intervals through the winter. The thermistor was fixed in the centre of the cage 4 cm above ground level under a metal shade. Comparison with records from a shaded thermistor at the same height outside showed shelter cages raised maximum and mean temperatures by several degrees on clear days but had little effect on minima.

The LT50s of both species were assessed using the standard test procedure (see section 2.2.3) at three times:

1. 31 October - 5 November 1980.
2. 1-5 January 1981.
3. 7-16 March 1981.

On the day aphids were tested infested plants were cut and kept in polythene bags in the shade out-of-doors. The aphids were brought inside and sorted 30 min to 3 h before testing. Large first instar nymphs were used as well as second to fourth instars because of a shortage of aphids.

Fig. 2.14 Shade temperatures inside a shelter cage during the 1980/81 winter.
(5-day period means and extremes shown).

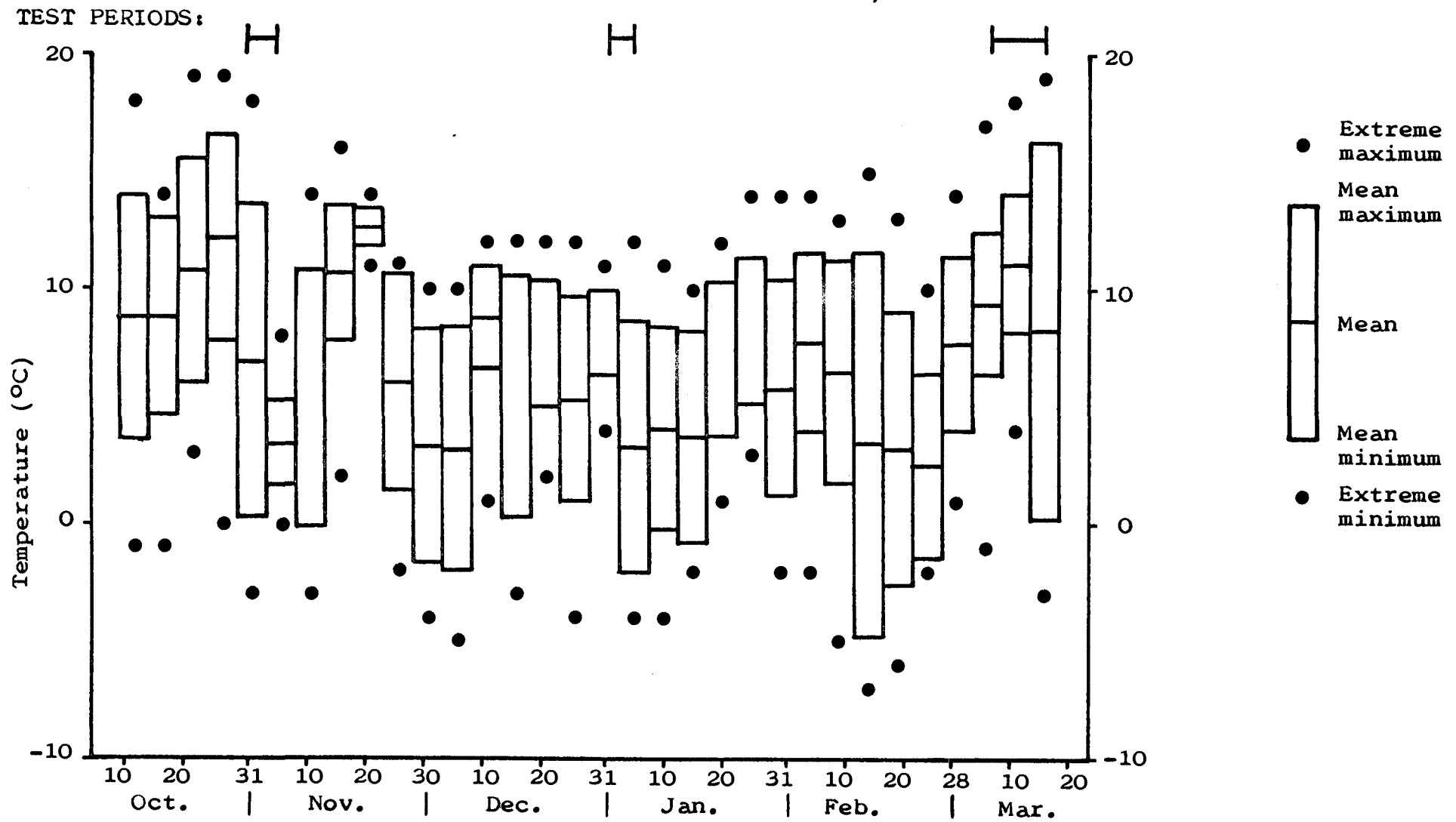


Table 2.8 Summary of probit analyses for field acclimatized S. avenae nymphs with mortality assessed one day after testing.

Test period	Oct./Nov.	Jan.	Mar.	All Combined
LT50	-12.81	-13.75	-13.40	-13.36
95% confidence limits	-11.60 to -14.40	-13.30 to -14.22	-12.29 to -16.09	-12.83 to -14.01
Probit line: intercept	-5.7704	-5.9421	-5.5792	-5.3096
slope	0.4505	0.4322	0.4164	0.3973
Heterogeneity χ^2	20.84***	5.17	25.86***	63.75***
Degrees of freedom	5	2	5	16
Number of: batches tested	7	4	7	18
test temperatures	6	4	7	17
Transformation	-temp.	-temp.	-temp.	-temp.

(***: $P < 0.001$)

Table 2.9 Summary of probit analyses for field acclimatized S. avenae nymphs with mortality assessed three days after testing. (October/November tests omitted due to high control mortality).

Test period	Jan.	Mar.	Both combined
LT50	-12.21	-11.62	-11.77
95% confidence limits	-11.63 to -12.59	-	-10.96 to -12.28
Probit line: intercept	-9.1068	-10.2120	-22.8597
slope	0.7456	0.8787	21.3458
Heterogeneity χ^2	1.56	24.10***	32.60***
Degrees of freedom	1	3	7
Number of: batches tested	4	7	11
test temperatures	4	7	11
Transformation	-temp.	-temp.	log (-temp.)

(***: $P < 0.001$)

Table 2.10 Summary of probit analyses for field acclimatized R. padi nymphs with mortality assessed one day after testing.

Test period	Oct/Nov.	Jan.	Mar.	All combined
LT50	-7.38	-7.57	-8.99	-8.12
95% confidence limits	-6.61 to -7.96	-7.21 to -7.90	-	-7.65 to -8.57
Probit line: intercept	-4.3335	-4.9336	-5.0797	-0.7457
slope	0.5875	0.6516	0.5653	2.7213
Heterogeneity χ^2	40.99***	3.32	26.11***	89.34***
Degrees of freedom	10	2	2	
Number of: batches tested	12	4	4	20
test temperatures	8	4	3	15
Transformation	-temp.	-temp.	-temp.	log (10+temp.) and number 'responding' = number surviving

(***: $P < 0.001$)

Table 2.11 Summary of probit analyses for field acclimatized R. padi nymphs with mortality assessed three days after testing.

Test period	Oct/Nov.	Jan.	Mar.	All combined
LT50	-6.62	-7.17	-7.41	-7.25
95% confidence limits	-4.87 to -7.53	-	-	-6.55 to -7.79
Probit line: intercept	-3.1249	-4.7822	-3.4554	2.5446
slope	0.4720	0.6666	0.4665	0.2026
Heterogeneity χ^2	67.21***	6.46*	10.77**	70.17***
Degrees of freedom	10	2	2	16
Number of: batches tested	12	4	4	20
test temperatures	8	4	3	15
Transformation	-temp.	-temp.	-temp.	log (10+temp.) and number 'responding' = number surviving

(*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$)

Fig. 2.15 Mortality - temperature curves for field acclimatized S. avenae and R. padi with mortality assessed 1 day after testing and data for 3 test periods combined. **KEY:** R. padi: solid symbols; S. avenae: hollow symbols; ● ○ Oct/Nov. tests; ■ □ Jan. tests; ▲ △ Mar. tests. Horizontal bars show 95% confidence limits for LT50s.

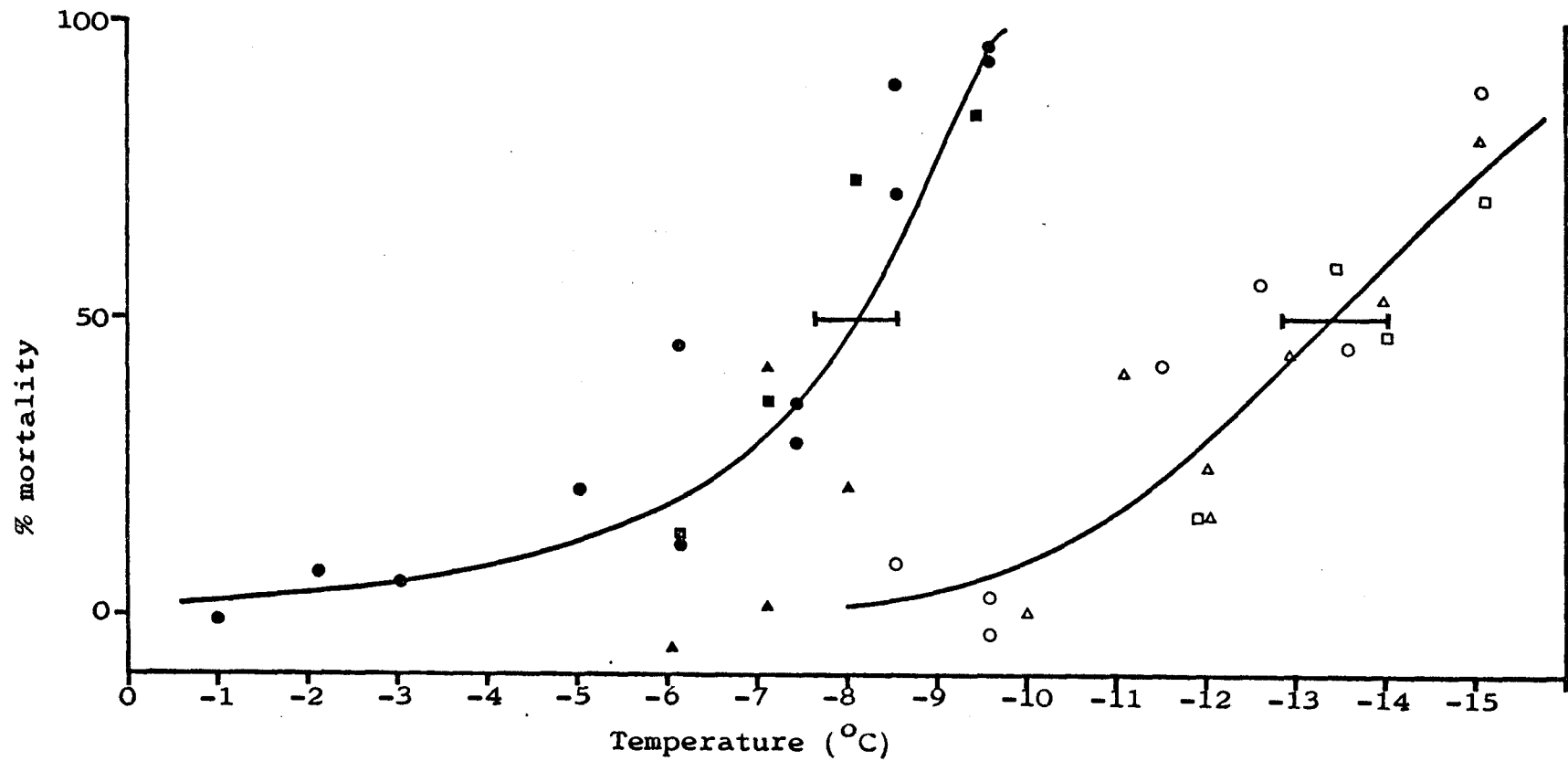
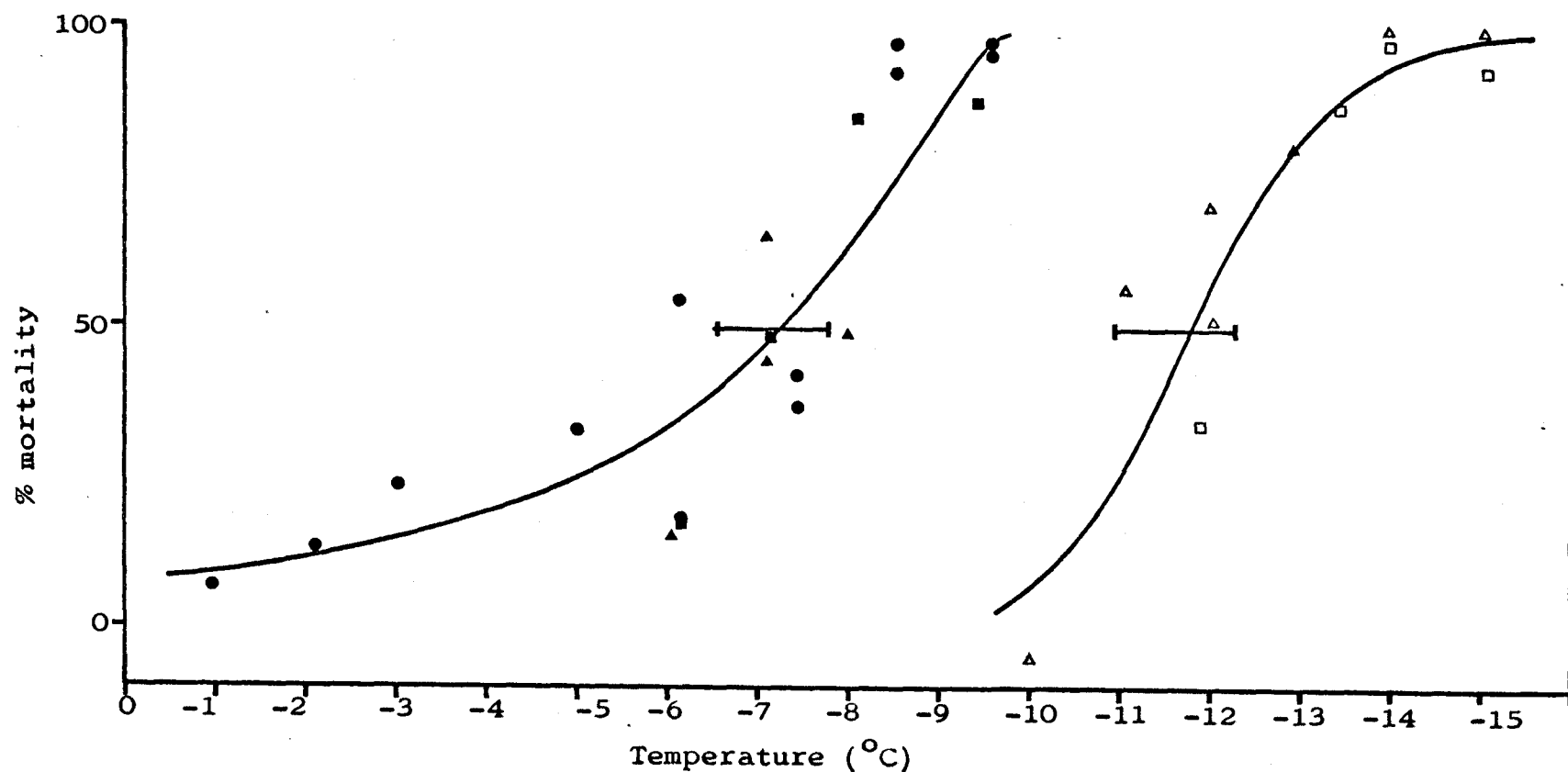


Fig. 2.16 Mortality - temperature curves for field acclimatized S. avenae and R. padi with mortality assessed 3 days after testing and data for 3 test periods combined. **KEY:** R. padi: solid symbols; S. avenae: hollow symbols; ● ○ Oct./Nov. tests; ■ □ Jan. tests; ▲ △ Mar. tests. (Data from S. avenae Oct/Nov. tests excluded.) Horizontal bars show 95% confidence limits for LT50s.



This was caused by Entomophthora infection in some S. avenae field cultures in the autumn and later by poor winter survival of R. padi colonies. Because of the shortage of field acclimatized aphids LT50s could not be determined as accurately as in the previous experiments.

Entomophthora infection caused unacceptably high mortality in preliminary tests on S. avenae in late October because keeping the aphids at 20°C and high humidity for 3 days between testing and assessment of mortality provided ideal conditions for the fungus. However, controls kept for only 1 day at 20°C showed little control mortality. Because of this, aphid mortality was assessed 1 day and 3 days after all tests on both species, so that some comparative data from the late October/early November tests with S. avenae were available. In all tests on R. padi, and in tests on S. avenae in January and March, Entomophthora was not a problem and control mortalities after 3 days were all below 13%.

Results and discussion

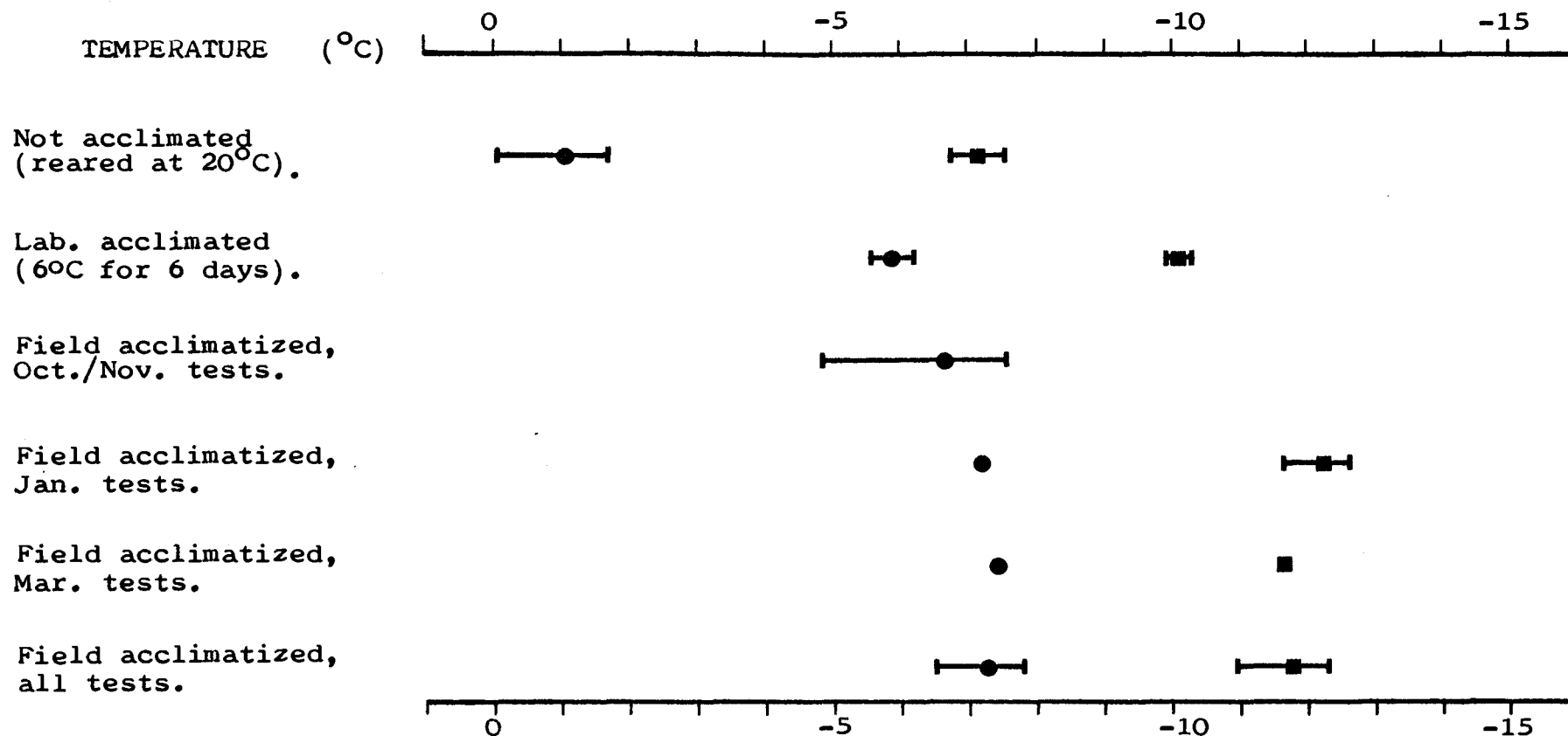
For each species, probit analyses were run on the results of each series of tests separately and then on all the data combined, using mortalities recorded 1 day after testing (Tables 2.8 & 2.10 and Fig. 2.15), and 3 days after testing (Tables 2.9 & 2.11 and Fig. 2.16). The two sets of results showed the same trends but, as expected, LT50s determined when mortality was assessed 3 days after testing were higher, due to some delayed mortality. In three of the probit analyses in which results from all tests on a species were combined, scatter plots showed that the temperature-mortality curves were skewed sigmoids and so appropriate log transformations were used before fitting probit lines. Chi-squared tests showed a high degree of heterogeneity in the data (Tables 2.8 to 2.11). This was probably largely because the tests in a series could not be run at the same time, so that aphids in each test had slightly different pre-test temperature experience. The degree of heterogeneity and shortage of aphids meant that in some tests the 95% confidence limits of LT50 values could not be calculated. The 1980/81 winter, particularly the latter half, was mild and the lowest temperature experienced inside shelter cages was -7°C (Fig. 2.14).

Combining the results of all three series of tests, the LT50 values of field acclimatized S. avenae and R. padi were less than 2°C below those of aphids given a short laboratory pre-treatment of 6°C for only 6 days (Fig. 2.17). Both species underwent extensive acclimatization between late September and early November, despite the fact that in this interval temperatures never dropped below -3°C and 5-day mean temperatures were at or above 7°C (Fig. 2.14). There was little sign of changes in LT50 values between November and March. The small shifts shown (Fig. 2.17) were not statistically significant as 95% confidence limits, where these could be calculated, overlapped widely. The results showed that field populations should quickly be able to acquire a useful amount of cold-hardiness early in the winter without previous exposure to lethal minimum temperatures, an acclimatization strategy which minimizes mortality due to sudden, early-autumn frosts.

Results for R. padi suggested a slight downward trend in LT50s from autumn to spring, while those for S. avenae showed that the January LT50 was slightly lower than the March LT50. This difference may have been due to differences in cold-hardiness between the species. R. padi was not very cold-hardy and was liable to have suffered some low temperature mortality at temperatures of -4°C or below - which were experienced regularly from December to late February. Consequently the less cold-hardy individuals in the field colonies of this species should have been gradually eliminated. This would have resulted in a gradual lowering of the LT50 of the surviving population, provided individuals with above average cold-hardiness retained their cold-hardiness for life. S. avenae, however, was considerably more cold-hardy and probably suffered little if any low temperature mortality during the 1980/81 winter. Consequently, no selection for cold-hardy individuals should have occurred and changes in LT50 probably reflected differences in temperature experience in the few days or weeks before testing.

Although changes in cold-hardiness through the 1980/81 winter were negligible, this might be expected given the unusually even temperatures experienced through this relat-

Fig. 2.17 Summary of LT50 values for S. avenae (■) and R. padi (●) with different temperature pre-treatments: (Horizontal bars show 95% confidence limits; where none are shown they cannot be calculated due to heterogeneity).



ively mild winter. Larger seasonal changes might occur in single-clone populations, such as those studied in the present experiment, in a more severe winter. However, in a single-clone population (all members of which should be genetically identical) differences in cold-hardiness between individuals are presumably due to subtle differences in the environmental conditions they experience before testing. Unless aphids have some kind of cytoplasmic inheritance mechanism for cold-hardiness, there is no reason to expect the offspring of a particularly cold-hardy individual to inherit their parent's cold-hardiness. Thus, although seasonal cold-hardiness changes may occur in a clonal population due to changes in environmental conditions, as a result of selective mortality of the more cold-susceptible individuals, these changes cannot be long-lived. They may not occur at all if the cold-hardiness of an aphid changes with time relative to that of other members of the clone. In contrast, in a natural field population of overwintering viviparae containing individuals from many genetically different clones, selective mortality of more cold-susceptible individuals during the winter may produce large, genetically-based shifts in the population's cold-hardiness, provided the clones involved show genetic differences which affect cold-hardiness.

The large differences between LT50 values for aphids kept at 20°C and those acclimatized in the field demonstrate that tests on the former cannot be used as any more than a very rough measure of field cold-hardiness. Results from such tests could lead to serious errors if used on their own. For example, the LT50 of R. padi reared at 20°C was so high that one might conclude from it that anholocyclic overwintering by this species in England is never successful. But field acclimatized R. padi had an LT50 more than 6°C lower, from which one would conclude that some viviparae could survive on Gramineae in most English winters. The relatively close agreement between LT50s for laboratory acclimated and field acclimatized aphids shows that a brief period of low temperature pre-treatment will produce sufficient cold-hardening to mimic field acclimatization quite well, but to what extent this is true of other aphid species is unknown.

From experiment 2.4.1 it was discovered that the use of slow instead of fast cooling and re-warming rates appeared to lower the mortality-temperature relationship for S. avenae by about 2°C but that for R. padi by about 5°C. If this cooling rate effect and the acclimation/acclimatization effect are independent and additive then the field LT50 for natural populations of R. padi will lie only 1.5°C above that for S. avenae rather than 4.5°C above, as found in this experiment. However, it is likely that the two effects are not independent and additive as they probably both act to stimulate the same cold-hardening processes in the aphid. Nevertheless, the differences between the field LT50s of S. avenae and R. padi may be less than the 4.5°C determined in this experiment. Some field data on the two species' low temperature mortality are presented in Chapter 3.

Although the LT50s of laboratory-reared S. avenae and R. padi kept at 20°C differ considerably from those determined after acclimatization, they are still a much better index of field cold-hardiness than the species' supercooling points. Even for S. avenae - with the highest supercooling points and lowest LT50 values - the mean supercooling point lies 11°C below the 90 min LT50 for acclimatized aphids. Thus it appears that even after winter acclimatization S. avenae and R. padi die well above their supercooling points. No data are available for field-acclimatized M. dirhodum but their similarity to S. avenae in previous LT50 tests suggests that they also remain chill-susceptible under field conditions. Although these results suggest that chill-injury is an important cause of cold-death in the field, freezing may also be important as inoculative freezing (see section 2.1.2) may well occur at temperatures well above the supercooling point when aphids experience sub-zero temperatures while feeding and/or while covered with frozen dew or hoar frost. Inoculative freezing was investigated in experiments 2.4.5 and 2.4.7.

2.4.4 Exposure time

Introduction

This experiment was undertaken to assess the extent to which low temperature mortality is influenced by changes in exposure time at a fixed sub-zero temperature near the 90 min LT50. If cereal aphid mortality is sensitive to exposure time changes, conclusions based on LT50 determinations at one exposure time only are of limited use, especially if different species are affected to differing extents by the changes. In addition, equations developed to predict low temperature mortality in field populations would need to include parameters for exposure time as well as temperature.

Methods

Species used: S. avenae, M. dirhodum, R. padi.

Clones used: new. Instars used: 2nd-4th.

Cooling apparatus used: cold bath.

As the species differed in their cold-hardiness they were tested separately and at different temperatures. S. avenae and M. dirhodum were tested at their 90 min LT50s as determined for unacclimated aphids in experiment 2.4.2 (-7.1°C and -6.7°C respectively). R. padi was tested at -2.0°C , about 1°C below its 90 min LT50. The same four exposure times were used in each species' test: 37.5 min, 1 h 30 min, 5 h and 19 h. Subtracting the 20 min cooling period these give times at the test temperature of 17.5 min, 1h 10 min, 4 h 40 min and 18 h 40 min. Two-hundred aphids (four tubes of 50 each) were used for each of the four exposure time treatments and all four treatments were placed in the cold bath together. Other details follow the standard test procedure (see section 2.2.3).

Results and discussion

Of the three species, M. dirhodum was the most sensitive to changes in exposure time (Table 2.12, Fig. 2.18). S. avenae appeared a little more sensitive than R. padi but results from these two species were not directly comparable because the mortality of R. padi at -2°C was lower than that of S. avenae at -7.1°C for all the exposure

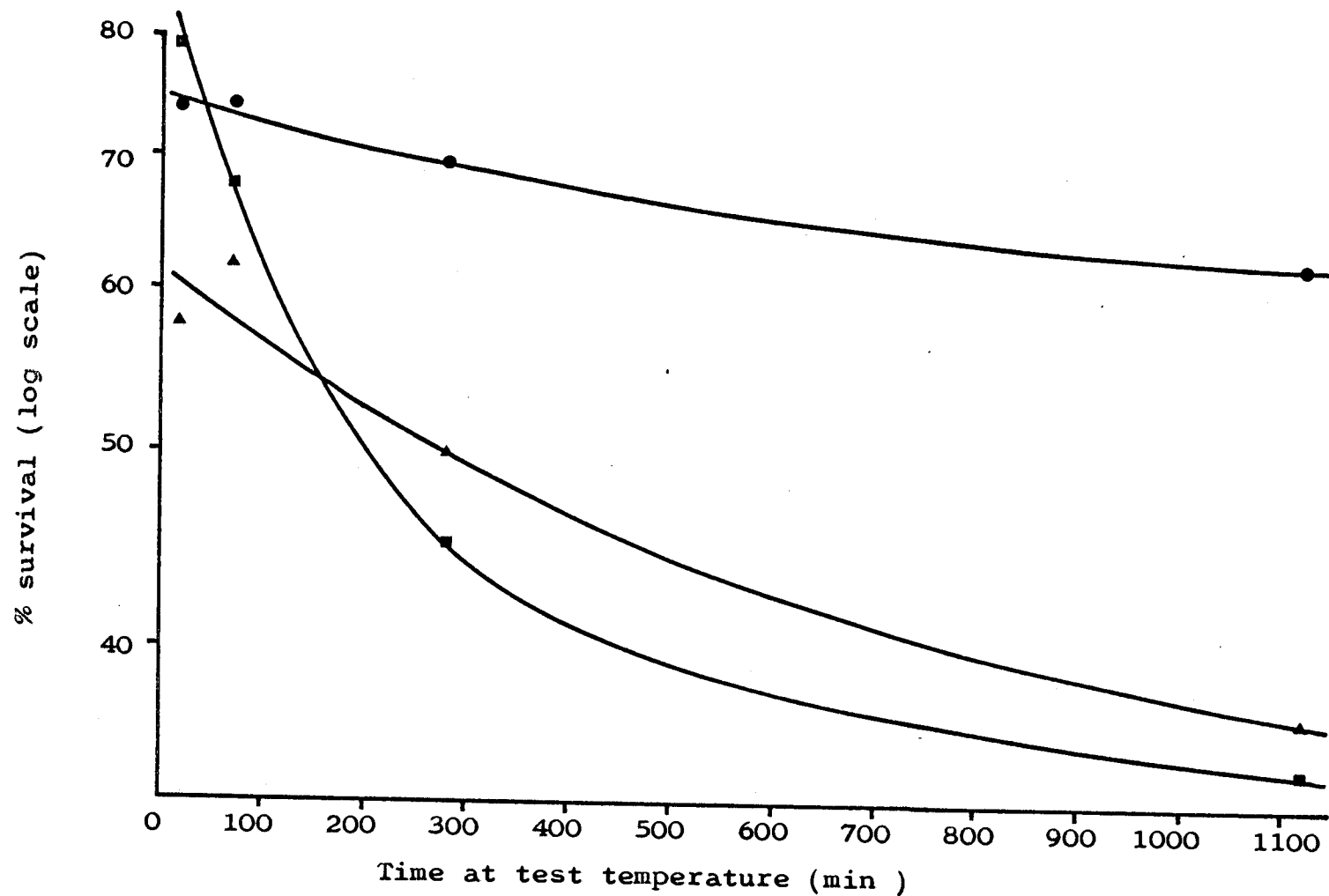
Table 2.12 Mean percentage mortality for each exposure time. (95% confidence limits in brackets)

Exposure time	<u>S. avenae</u> at -7.1°C	<u>M. dirhodum</u> at -6.7°C	<u>R. padi</u> at -2.0°C
17½ min	42.3 (22.9-62.8)	20.6 (12.9-28.9)	26.1 (10.9-44.1)
1h 10min	38.3 (31.2-45.3)	32.4 (23.5-41.2)	25.9 (16.4-36.3)
4h 40min	50.0 (37.6-62.5)	54.9 (43.2-66.9)	30.5 (15.7-46.3)
18h 40min	62.9 (45.0-80.5)	65.0 (55.7-74.3)	37.5 (31.1-43.9)

Table 2.13 Regression coefficients for linear regressions of percentage mortality (after arcsin transformation) on exposure time (min).

Species	Test temperature	b	S.E.b	t (D.F.=14)	Significance level
<u>S.avenae</u>	-7.1°C	1.23×10^{-2}	4.17×10^{-3}	2.94	P < 0.05
<u>M.dirhodum</u>	-6.7°C	2.04×10^{-2}	4.36×10^{-3}	4.68	P < 0.001
<u>R.padi</u>	-2.0°C	6.58×10^{-3}	3.71×10^{-3}	1.77	P > 0.05

Fig. 2.18 Relationships between percentage survival and exposure time for S. avenae at -7.1°C ($\blacktriangle$), M. dirhodum at -6.7°C ($\blacksquare$) and R. padi at -2.0°C ($\bullet$).



times used. Significance tests on linear regression coefficients for percentage survival on exposure time (Table 2.13) showed that in S. avenae and M. dirhodum increases in mortality with increased exposure time were statistically significant ($P < 0.05$) but in R. padi they were not.

Although the three species differed in their sensitivity, the effects of changing exposure times were remarkably small in all three. Using the mortality-temperature curves for unacclimated aphids determined in experiment 2.4.2 (Figs 2.10 to 2.12) it can be seen that the changes in mortality observed in the present experiment when exposure time was increased from 17½ min to 18 h 40 min could have been produced by keeping exposure time constant and lowering the test temperature by approximately 1.0°C (S. avenae), 1.9°C (M. dirhodum) and 1.6°C (R. padi). Thus changes in exposure time of many hours are equivalent to changes in temperature of only 1° or 2°C. If the same is true of acclimatized aphids exposed to low temperatures in the field, natural low temperature mortality will be dominated by the minimum temperatures experienced and will be affected very little by differences in exposure time. Consequently, predictive equations for natural low temperature mortality using minimum temperature data alone may prove reasonably accurate. However, the possibility remains that exposure time plays a more important role under field conditions.

From theoretical considerations Salt (1961) has shown that in an 'ideal' population of a freezing-susceptible species in which all individuals have identical supercooling ability, exposure at a fixed sub-zero temperature should produce a relationship between mortality and exposure time which follows the compound interest law. This is because the probability of an individual freezing in each unit time interval of low temperature exposure is equal. Thus survival can be expressed as:

$$Y_t = Y_0 e^{-rt}$$

where Y_t is the number of individuals unfrozen at time t

out of an original population of Y_0 , e is the base of natural logarithms and r is a constant probability, expressed as a rate of freezing. From this it follows that a plot of $\log$ (percentage survival) against exposure time should be a straight line with negative slope and survival of 100% when $t = 0$. In real populations of freezing-susceptible insects however, the probability of individuals freezing in each unit time interval gradually decreases because individuals with high supercooling ability survive longer than more cold-susceptible ones, so that the relationship becomes a curve with gradually decreasing negative slope (Salt, 1961, 1966c).

None of the three species studied here, which are chill-susceptible rather than freezing-susceptible, showed survival-exposure time relationships remotely like that predicted from the compound interest equation given above (Fig. 2.18). In *S. avenae* and *R. padi* in particular the probability of dying was much higher in the first 20 min or so of low temperature exposure than subsequently. This was probably at least partly due to individual differences in chill-susceptibility, resulting in the more chill-susceptible aphids dying first so that aphids which survived a certain time were inevitably more cold-hardy than those which had already succumbed. It may also be due to the nature of chill-injury itself - in the 'fast-cool' test conditions used here this may be because chill-injury is a result of thermal shock. Alternatively, very fast-acting cold-hardening mechanisms may come into effect within a few minutes of exposure and so increase the chances of subsequent survival. It should be noted that the shapes of mortality-exposure time relationships may well change considerably when different test temperatures are used or other conditions are altered. Such changes are shown by adults of the Australian wheat weevil *Rhyzopertha dominica* (Swain, 1975) which shows sigmoid relationships that change with test temperature changes and pre-test starvation.

The mortalities of aphids left in the cold bath for 1 h 30 min in this experiment were noticeably lower than expected from the mortality-temperature curves determined



for unacclimated aphids in experiment 2.4.2 using the same exposure time. For S. avenae and M. dirhodum the discrepancies are only equivalent to LT50 changes of 0.5° and 0.7°C but in the case of R. padi the mortality shown at -2°C in the present experiment is expected at $+0.4^{\circ}\text{C}$ according to the results of experiment 2.4.2. No reason for this large difference can be found as culture conditions and test procedure were the same in the two experiments. It suggests that very subtle differences in pre-test temperatures and/or host plant condition can have relatively large influences on the cold-hardiness of R. padi, so that LT50 determinations for this species are less reliable than those for S. avenae and M. dirhodum.

It should be noted that the maximum exposure time used in this experiment was less than 1 day. Exposure for several days or weeks may well produce cold-death at considerably higher temperatures, possibly as a result of more indirect effects such as starvation or desiccation. Adams (1962) has shown gradual mortality in five species of aphids kept on excised leaves at -2°C for several months with 50% mortality reached only after exposure for 400 h or more. In Britain, aphids are very unlikely to experience such a constant sub-zero temperature for so long but could be subject to similar conditions for days on end if covered with snow.

2.4.5 Ice formation in the leaves of the host plant

Introduction

Powell (1974) showed that in the green spruce aphid Elatobium abietinum, 90% of aphids exposed to -12°C for 4 h while feeding on Sitka spruce (Picea sitchensis) were killed but all those detached from the host before testing survived. The test temperature used lay above the super-cooling points of detached aphids but below those of Sitka spruce needles, which, unlike aphids, can freeze and thaw without damage. From this Powell concluded that E. abietinum are killed by inoculative freezing when the needles they are feeding on freeze, due to the spread of an ice-front from the host's phloem cells up the aphid's stylets

and into its body. Furthermore, the aphids did not withdraw their stylets from the host when cooled slowly (Powell, 1974), so that inoculation from the host is probably common under field conditions also.

Powell's results show that in E. abietinum the low temperature survival of the aphid is severely limited by the supercooling ability of its host. If the overwintering viviparae of cereal aphids are as easily inoculated by the host, it can be seen that host plant supercooling ability might be the dominant factor limiting the success of anholocyclic overwintering and the cold-hardiness of the aphids themselves could be more or less irrelevant to their winter survival. Moreover the limiting effect of the host would probably be even more severe than in E. abietinum as the leaves of Sitka spruce supercool to about -10° to -12°C (Powell, 1974) whereas those of most other plants do not supercool to more than a few degrees below 0°C (Burke et al., 1976) and they rely on freezing-tolerance to survive.

However, in experiments on European clones of S. avenae, M. dirhodum and R. padi, Griffiths & Wratten (1979) showed that testing these species while feeding generally altered their LT50s towards the freezing point of the host plant used ($c.-8^{\circ}\text{C}$) so that, for three of the four clones tested, cold-hardiness was actually improved if aphids were tested while feeding. The exception was a Swiss clone of M. dirhodum, the LT50 of which was raised by a little over 3°C . The test time in these experiments was 3 h.

To investigate further the possibility of inoculation from the host, feeding and detached aphids were tested together for longer exposure times and at a variety of sub-zero temperatures in the present experiment. Apart from a preliminary test using S. avenae and M. dirhodum, only the former species was used.

Methods

Species used: S. avenae (all tests), M. dirhodum (preliminary test only).

Clones used: old (preliminary test), new (all other tests).

Instars used: 2nd-small 4th.

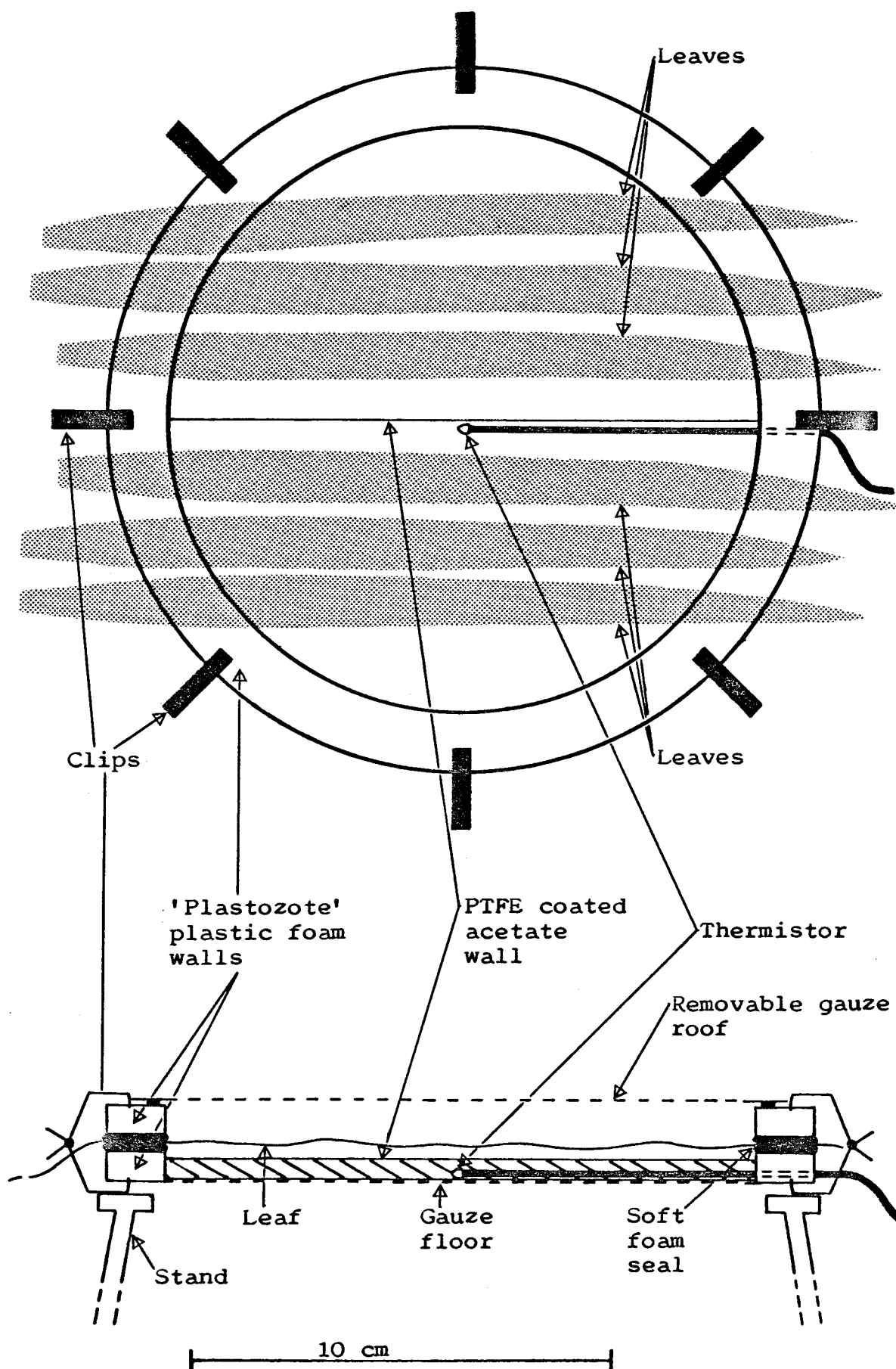
Cooling apparatus used: cold chamber.

Inoculation by the host plant is probably dependent on the extent and position of ice crystals in its leaves. To maximise the chances of demonstrating the effect, glass-house-grown winter wheat seedlings were used. These froze extensively at sub-zero temperatures above the LT50s of detached S. avenae and M. dirhodum and were invariably killed during the tests. The tests involved exposing feeding aphids to sub-zero temperatures alongside those detached from the host plant; these two treatments are referred to as 'on-plant' and 'off-plant'. The aphids were tested in the cold chamber inside a large clip-cage with an internal diameter of 14 cm and a depth of 2 cm (Fig. 2.19). The floor of the cage was divided in half by a 1 cm high acetate strip coated with a suspension of polytetrafluoroethylene (P.T.F.E.) so that aphids could not climb it. The cage walls were similarly coated. The cage was supported on a tray next to pots of winter wheat seedlings (cv. Hobbit) which had been grown at approximately 20°C and 16 h daylength. Leaves still attached to the plants were arranged across the cage parallel to the acetate barrier as shown in Fig. 2.19.

In the main series of tests on S. avenae approximately 100 nymphs were placed in each side of the cage and small pieces of gauze draped over the leaves to allow the aphids to climb onto them. The cage was left in a growth room at 20° ± 2°C and 16 h daylength for 24 h, by which time nearly all the aphids had settled on the leaves. Any which were still wandering were removed. The three leaves across one half of the cage were then cut and the aphids removed from them and placed on the cage floor on that side. The cage was immediately placed in the pre-cooled cold chamber and left for 12 h. It was then taken out and the 'on-plant' and 'off-plant' aphids were sorted in a cold room to prevent them becoming too active. As in other experiments they were kept in plastic containers with wheat leaves at 20°C and 16 h daylength for 3 days before assessment of mortality.

Five tests were run with S. avenae only, using temperatures between -6.8°C and -8.5°C. In the preliminary

Fig. 2.19 Clip-cage used to study the effect of host plant freezing.



test using S. avenae and M. dirhodum the same method was used with about 100 nymphs of each species but the exposure time was 21 h 30 min and the test temperature -6.4°C . In all tests the wheat leaves were all killed by freezing. Immediately after a test they were dark green and brittle but became completely flaccid when the ice in them had melted; they then wilted rapidly.

Results and discussion

In the preliminary test at -6.4°C nearly all the aphids survived and the mortality of those on plants was slightly lower than those off plants (Table 2.14), so that no inoculative freezing from the host appeared to have taken place despite the long exposure time. In the later series of tests on S. avenae only temperatures nearer the 'off-plant' LT50 were used. In these tests mortality of 'on-plant' aphids still on the leaves at the end of the test was always greater than that of 'off-plant' aphids tested alongside (Table 2.15, Fig. 2.20). Two-way analysis of variance on percentage mortalities after arcsin transformation showed that the increased mortality in the 'on-plant' treatment was significant ($F = 14.70$, D.F. = 1 & 4, $P < 0.05$). It appears, however, that inoculation from the host plant only kills about 10 to 20% of feeding aphids and only raises the LT50 by a fraction of 1°C even after 12 h exposure on an unnaturally cold-susceptible host, the leaves of which freeze completely. And from the preliminary test it appears that no inoculation from the host occurs in either S. avenae or M. dirhodum at or above -6.4°C . These results suggest that inoculation from the host is of little importance in the field, although the possibility remains that it occurs more commonly when the aphids are feeding on other hosts.

In all the tests, some 'on-plant' aphids fell off the plants during low temperature exposure. These were kept separately after the test. In four of the five tests they showed mortality lower than that of 'on-plant' aphids which had not fallen off the leaves, but the numbers involved were too small to test for statistically significant differences. In analysing differences between 'on-plant' and 'off-plant' treatments aphids which fell off the leaves were ignored.

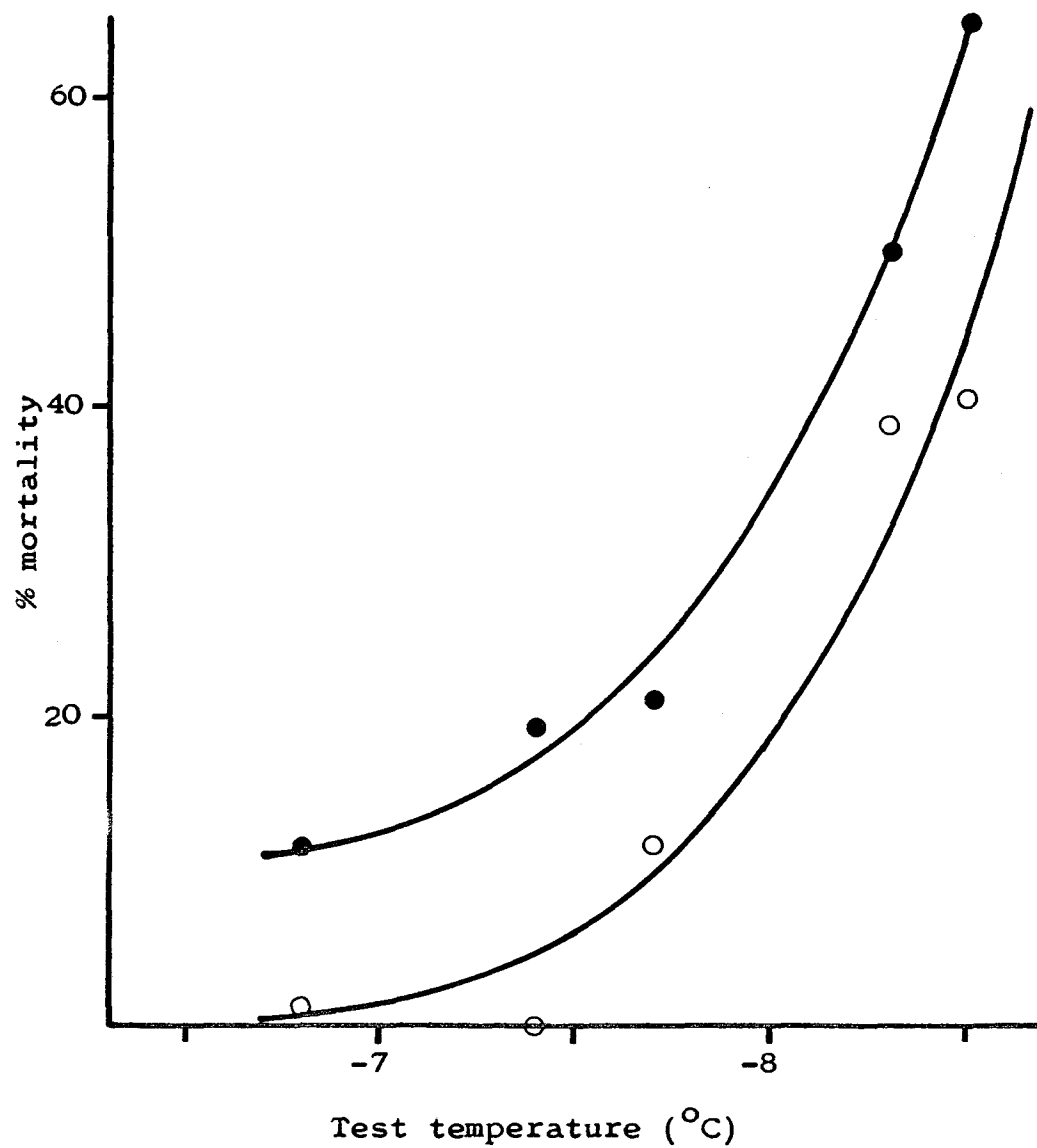
Table 2.14 Numbers of S. avenae and M. dirhodum nymphs alive and dead after exposure to -6.4°C for 21.5h (a) while on glass-house grown wheat, and (b) off the host plant.

SPECIES	(a) ON PLANT		(b) OFF PLANT	
	Alive	Dead	Alive	Dead
<u>S. avenae</u>	52	1	47	2
<u>M. dirhodum</u>	53	0	45	3

Table 2.15 Mortalities of S. avenae nymphs exposed to low temperatures (a) while on glass-house grown wheat and (b) off the host plant.

TEMP. ($^{\circ}\text{C}$)	(a) ON PLANT				(b) OFF PLANT			
	Alive	Dead	Total	% dead	Alive	Dead	Total	% dead
-6.8	38	5	43	11.6	78	1	79	1.3
-7.4	50	12	62	19.4	88	0	88	0
-7.7	49	13	62	21.0	99	13	112	11.6
-8.3	18	28	46	50.0	60	38	98	38.8
-8.5	24	44	68	64.7	53	36	89	40.5
Totals	179	102	281		378	88	466	

Fig. 2.20 Mortality-temperature curves for S. avenae tested on glasshouse grown winter wheat (●) and for those tested off the host plant (○). (Smoothed curves fitted by eye.)



Adams (1962) found aphids which survived low temperature exposure while feeding were sometimes unable to extricate their stylets from the frost-damaged leaves but aphids in the present experiment were not trapped in this way.

Superficially, it may appear surprising that little inoculation from the host occurs even when it freezes extensively and is killed as a result. However, freezing in plant tissues is nearly always confined to extracellular spaces (Levitt, 1966; Meryman, 1966; Burke *et al.*, 1976; Sutcliffe, 1977) so that phloem cells - from which aphids feed - should not normally freeze internally. Consequently the additional mortality observed in aphids tested while feeding is probably a direct result of the damage an aphid causes to a phloem cell as it inserts its stylets (Parry, 1979a). This will allow ice to penetrate the cell and from there it can inoculate the aphid via its stylets.

Comparing the results of the study by Powell (1974) with those from the present experiment and those of Griffiths & Wratten (1979) it appears that *E. abietinum* usually causes enough damage when probing cells to allow ice to penetrate, so that inoculation from the host is common. By contrast, *S. avenae* - and probably the other two cereal aphids also - must cause less damage, or are capable of effectively sealing the wounds they make with saliva, so that ice rarely penetrates the cells they are feeding from and inoculation from the host is uncommon. It may be that *E. abietinum* is atypical in its inability to prevent inoculative freezing from the host. This may be because the species has been completely or predominantly holocyclic until quite recently in its evolutionary history, as it still is in central Europe (Beier-Petersen, 1962). Also, the leaves of spruce have greater supercooling ability than those of other plants so that inoculation from the host presents less of a limitation to low temperature survival. Consequently there may have been less selection pressure for the evolution of a feeding technique which minimizes the incidence of inoculative freezing.

In three of the four clones tested by Griffiths & Wratten (1979) feeding aphids had lower LT50s than those detached from the host. The authors suggested that this

was because the host's sap confers better resistance to freezing in the more cold-susceptible clones. But this explanation appears inadequate for two reasons:

1. From experiments described above (section 2.3) it is clear that chill-injury rather than freezing is the normal cause of death in cereal aphids tested off the host plant.
2. It is difficult to conceive how feeding could confer an advantage when the test time is only 3 h and when the 'off-plant' and 'on-plant' aphids are kept under identical conditions until just before testing and are tested under identical conditions, as they should be for the results to be valid. A mechanism conferring advantage on feeding aphids under these conditions must be capable of producing a difference between the treatments' LT50s of as much as 3.5°C (Griffiths & Wratten, 1979) within a few hours at most.

The apparent beneficial effect of feeding demonstrated by Griffiths & Wratten (1979) certainly deserves further study, as the possibility exists that rapid physiological changes which reduce cold-hardiness occur as a result of the cessation of feeding. However, it may be that the differences they observed are due to small differences in the temperatures aphids experienced during tests. The cooling chambers used by Griffiths & Wratten were tested in some preliminary work in the present study. It was found that cooling rates and the final temperatures reached could not be controlled accurately, so that exact replication of test conditions was not possible. Also, significant vertical temperature gradients existed within the cold chambers so that, unless 'off-plant' and 'on-plant' aphids were tested at the same height in a chamber, spurious treatment differences might result.

Both the S. avenae clones tested by Griffiths & Wratten (1979) had their cold-hardiness increased when feeding but the clone studied here had its cold-hardiness reduced. However, this discrepancy may well be due to the

different clones, host plants and test conditions used.

2.4.6 Host plant effects other than leaf freezing Introduction

It is generally recognized that the gut contents are the normal site of spontaneous nucleative freezing in many insects due to the existence of efficient ice nucleators in the food (see section 2.1.5). Evidence for a relationship between supercooling points and food quality has been found for several aphid species. For example, Powell (1976) has shown that in Drepanosiphum platanoides and Eucallipterus tiliae, adults and nymphs have different supercooling points which appear to be linked to their different feeding positions on the host. He has also shown that Elatobium abietinum has different mean supercooling points when feeding on Sitka spruce (Picea sitchensis) and Norway spruce (Picea abies) (Powell, 1976) and that seasonal changes in the species' supercooling points on Sitka spruce closely follow supercooling point changes in the host's needles (Powell, 1974). Parry (1978) has found an association between feeding sites and supercooling points in Myzus persicae on radish (Raphanus sativus) and shown that the species' supercooling points can be changed by the manipulation of sucrose concentrations in artificial diets.

Although it has been shown that supercooling points are irrelevant to cold-hardiness in the cereal aphids under study here, differences in food quality could produce physiological changes which affect the extent of chill-injury or the aphid's ability to repair it after low temperature exposure. If this is so cereal aphids reared on different hosts should show differences in cold-hardiness even when exposed to low temperatures while off the host plants. To test for this effect, off-plant cold-hardiness tests were performed on S. avenae reared in the field on two important overwintering hosts - winter wheat (Triticum aestivum L.) and perennial rye grass (Lolium perenne L.).

Methods

Species used: S. avenae.

Clone used: new. Instars used: 2nd-4th.

Cooling apparatus used: cold bath.

Tests were run between 18 and 20 March 1981. The aphids used were from a culture kept in the field in clip-cages from early August 1980. The culture was maintained through the winter on field plots of winter wheat (cv. Hobbit) sown in August and September. In late November some alatiform fourth instars were taken from it to start a culture on a field plot of perennial rye grass (cv. S.23) sown in late August. Nymphs from rye grass tested in this experiment were the third generation on that host.

Aphids from the two hosts were removed from the plants and brought into the laboratory immediately before the tests. Only a limited number were available from perennial rye grass because of S. avenae's relatively poor performance on this host. Consequently only three tests were done, in each of which 25 nymphs from each host were compared at a different test temperature. The remaining aphids were used to assess control mortality. Testing followed the standard procedure (see section 2.2.3) but between testing and the assessment of mortality the aphids were kept in containers with leaves of both winter wheat and perennial rye grass.

Results and discussion

Mortality in controls from wheat and rye grass was not significantly different (Fisher exact probability test: $p = 0.145$, $P > 0.5$). The mean control mortality was 5.0%. In all three tests the mortality of aphids reared on wheat was lower than that of aphids from rye grass (Fig. 2.21). A χ^2 test on the combined results showed that the difference was significant ($P < 0.001$) (Table 2.16). Probit analysis on the results gave LT50 values for field acclimatized S. avenae on winter wheat and perennial rye grass of -11.7° and -10.6°C respectively. However, too few tests were done for a heterogeneity χ^2 -test and confidence limits for the LT50s to be calculated.

The results show that the host plant can produce changes in an aphid which affect its cold-hardiness even

Fig. 2.21 Low temperature mortality in acclimatized *S. avenae* nymphs reared on winter wheat (○) and perennial rye grass (●) and tested off plants for 90 min.

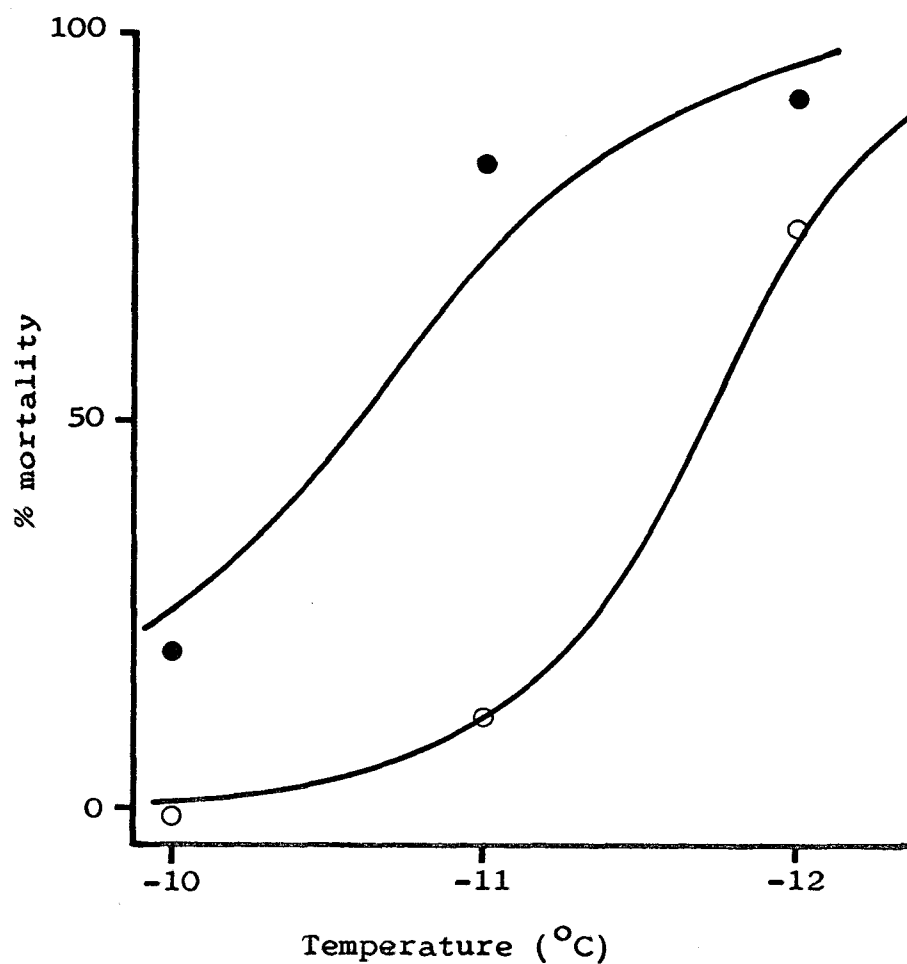


Table 2.16 Chi-squared test on the difference in mortality between *S. avenae* reared on winter wheat and perennial rye grass (results for 3 tests combined).

Host	Alive	Dead	Total
Wheat	51	24	75
Rye grass	25	50	75

$$\chi^2 = 16.67, \text{ D.F.} = 1, P < 0.001.$$

when it is not feeding during low temperature exposure. But for S. avenae from winter wheat and perennial rye grass the difference in cold-hardiness is not important outside the range -9° to -12°C . This experiment suggests that winter wheat is the better overwintering host but other differences in performance between aphids on rye grass and wheat are much more important (see section 5.3).

2.4.7 Ice formation on the aphid's cuticle

Introduction

Although Asahina (1966, 1969) considered that the inoculative freezing of an insect by ice on the body surface is a rare occurrence, except in aquatic species, the effect has been demonstrated in several terrestrial insects. These include the weevil Rhynchaenus fagi (Bale, 1980), larvae of the sawfly Cephus cinctus (Salt, 1958a) and the collembolan Tetracanthella wahlgreni (Somme, 1977), all of which have higher supercooling points if exposed to sub-zero temperatures when wet. Evidence for inoculation by surface ice also exists for a few aphid species. For example, nymphs of Rhopalosiphum insertum from apple buds showed increased mortality in laboratory tests if exposed to sub-zero temperatures after moistening (Evenhuis, 1968). For Elatobium abietinum unpublished work by Bevan (cited in Carter, 1972) suggested the same effect but in laboratory tests on the species by Carter (1972) the mortality of wet and dry aphids was not significantly different. Bevan & Carter (1980) stated that in Megoura viciae (Buckt.) relative humidity affected low temperature mortality, which suggests that surface ice plays a role. They showed that, due to its waxy filaments, the body surface of E. abietinum was more water-repellent than that of M. viciae and suggested that this was why the latter was prone to inoculative freezing while the former was not.

Smith (1981) concluded from winter field observations on S. avenae viviparae that the combination of dew and sub-zero temperatures accounted for significantly heavier mortality than low temperature alone, which suggests that inoculation by surface ice may be important. But in LT50

determinations in the laboratory Griffiths & Wratten (1979) found that when S. avenae and R. padi were moistened before testing LT50s were raised by only about 1°C, though the LT50 of a Swiss clone of M. dirhodum was raised by about 4°C. To investigate inoculative freezing by contact moisture further, the low temperature mortality of moistened and dry aphids was compared at several temperatures in the present experiment. In the main series of tests only S. avenae was used. But in two later tests S. avenae and M. dirhodum were tested together to determine whether M. dirhodum was more prone to inoculative freezing, as the results of Griffiths & Wratten (1979) suggest.

Methods

Species used: S. avenae (all tests), M. dirhodum (2 final tests only).

Clones used: new. Instars used: 2nd-4th.

Cooling apparatus used: cold chamber.

Six tests were run on S. avenae nymphs only, using temperatures between -1.7°C and -8.2°C. In each of these, two batches of 50 nymphs were tested together, each in a plastic container 5 cm in diameter and 2.5 cm high with a gauze roof and floor. Nymphs were placed in these containers immediately before a test. One of the containers (the 'wet' treatment) was then completely immersed in water for two seconds and then quickly drained and dried in a standardized way. In this way wet treatment aphids were evenly covered with small water droplets which froze during the test.

Then both wet and dry containers were placed in the pre-cooled cold chamber either side of an identical container holding a thermistor used to monitor the test temperature (Fig. 2.22). All three containers were raised 1 cm above the floor of the cold chamber to ensure even cooling. Aphids were left in the chamber for 14 h and were then kept for 3 days at 20°C before assessment of mortality, as in the standard test procedure (see section 2.2.3). Four wet and four dry control replicates were also run to determine whether wetting aphids without subjecting them to sub-zero temperatures increased mortality. In these, aphids were treated as described above but were placed in a cold

Fig. 2.22 Arrangement of aphid containers used in experiments on the effect of surface ice on cold-hardiness.

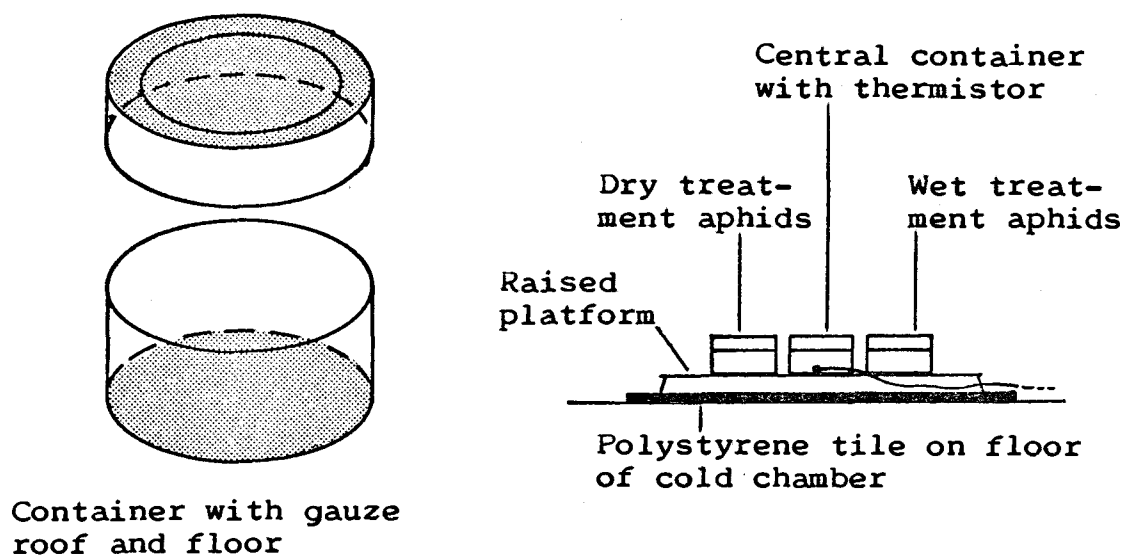
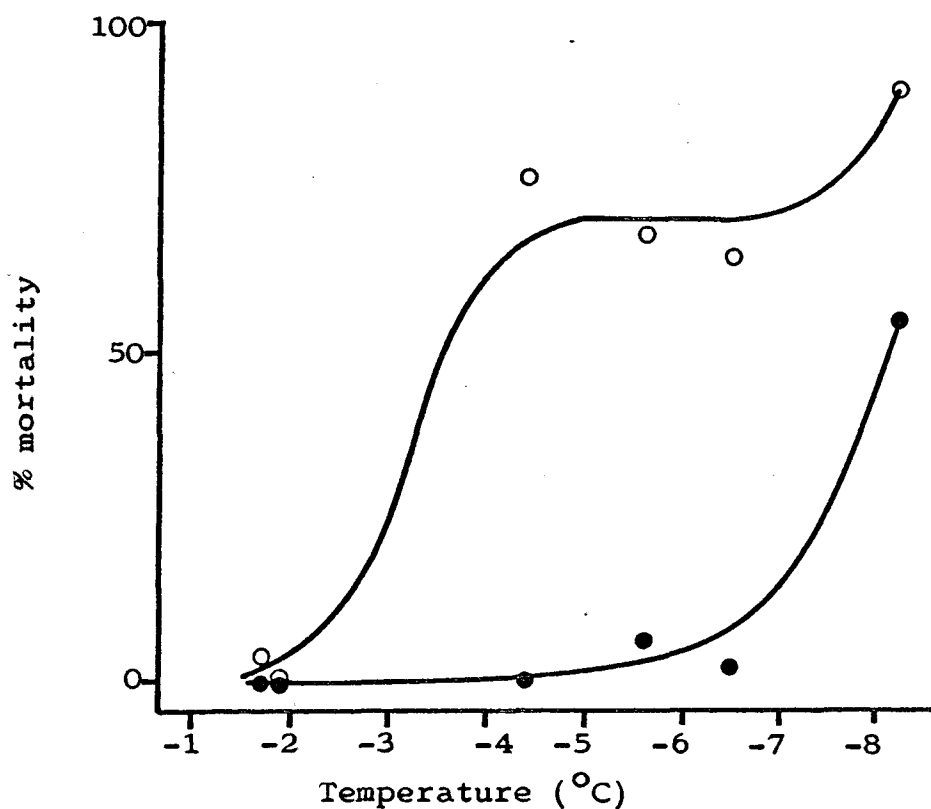


Fig. 2.23 Mortality-temperature curves for *S. avenae* nymphs exposed to low temperature for 14 h with (○) and without (●) ice on the body surface. (Smoothed curves fitted by eye).



room at $+6^{\circ}\text{C}$ for 14 h while treatment aphids were in the cold chamber.

The same test procedure was followed in the two tests using S. avenae and M. dirhodum nymphs together. In both these the test temperature was -4.6°C and, in each, 50 nymphs of each species were tested in each container. The two species were put in separate containers after the test. Two wet and two dry control replicates were also run, in which aphids were kept at $+6^{\circ}\text{C}$ for 14 h.

Results and discussion

The six tests on S. avenae showed that in all tests in which the temperature was lower than -4°C , nymphs with ice on their cuticle suffered substantially greater mortality than dry aphids. Mean mortalities in wet and dry controls were 2.0% and 3.6% respectively. A 2×2 χ^2 test on the combined results of the six tests showed the difference between wet and dry treatments was highly significant (χ^2 with Yates' correction = 111, D.F. = 1, $P < 0.001$).

Aphids with ice on the body surface did not show the normal sigmoid relationship between mortality and temperature (Fig. 2.23). A possible explanation for the form of the relationship shown is as follows: the threshold for mortality in the wet treatment, at about -2° or -3°C , probably represents the freezing point of aphid body fluids. Above this, aphids will not freeze even if ice does penetrate the cuticle. Between about -4° and -7°C no aphids are killed by chill-injury (as shown by the lack of mortality in the dry treatment) but a constant proportion of wet aphids (c.70%) are killed by inoculative freezing from surface ice. Successful ice penetration through the cuticle inevitably produces freezing in an aphid's body at any temperature below its freezing point. It appears to be independent of temperature but is probably dependent on the position of ice crystals on the body surface. The threshold temperature for chill-injury lies at about -7°C - as shown by the mortality in the dry treatment. Below -7°C the same proportion of aphids die of chill-injury in wet and dry treatments but in the wet treatment about 70% of the survivors of chill-injury die from inoculative freezing. From this hypothesis the proportion of wet

aphids surviving the test at -8.2°C is predicted as:

$$\begin{array}{lcl} \text{probability of surviving} & & \text{Constant probability of} \\ \text{chill-injury (0.45)} & \times & \text{avoiding inoculation (0.3)} \\ & & \\ = & 0.135. & \end{array}$$

The actual proportion of wet aphids surviving -8.2°C is 0.1 - very close to that expected.

Although the probability of inoculative freezing appears to be independent of temperature below -4°C it is probably dependent on exposure time as delay is usually observed between the freezing of surface moisture and inoculation (Salt, 1963; Asahina, 1969). Different types of ice deposition have different effectiveness in inoculating insects, and the position of ice crystals near cuticular pores or other openings must be important. Asahina (1966) notes that freezing of water moistening the surface of an insect's body is most effective, while contact between the dry surface of a cooled insect and an ice crystal has little effect. This suggests that in the field water droplets from rain or heavy dew which later freeze on an aphid (similar to the wet treatment in the present experiment) are most likely to produce inoculation, while hoar frost deposited after the body surface temperature has dropped below 0°C is less likely to do so.

The magnitude of the difference between wet and dry treatments in this experiment suggests that inoculation by surface ice may be important in the field but several factors may operate to mitigate its effect:

1. Temperatures are likely to remain below -3°C for less than 14 h (the exposure time used here) on many nights with frosts.
2. In the field, ice on the aphids' surface will usually form as hoar frost, not from frozen dew or rain. Even if water freezes on an aphid, contact between the ice and the insect's cuticle may not be as intimate as in the present experiment, in which aphids were actually immersed in water before testing.

3. Aphids may occupy sheltered feeding positions surrounded by foliage so that they are sheltered from hoar frost, dew and rain.
4. Aphids may be able to protect themselves against inoculation by developing a thicker, waxier and more water-repellent cuticle in the field. S. avenae reared in the field in winter do sometimes have a whitish, waxy bloom on their ventral surface.

The possibility of these mitigating factors makes extrapolation from laboratory to field conditions difficult. But the results of this experiment certainly suggest that S. avenae is much more likely to freeze as a result of surface ice than as a result of inoculation from the host plant (see experiment 2.4.5) and that surface ice may well be an important mortality factor because it can kill a significant proportion of aphids well above the threshold for chill-injury.

Analysis of results of the comparative tests using S. avenae and M. dirhodum together (Table 2.17) was complicated by the fact that mortalities in control replicates of the two species differ. Because of this, survival in wet and dry treatments was corrected assuming treatment and control mortality effects do not interact, so that:

$$\frac{\text{Corrected treatment}}{\% \text{ survival}} = \frac{\% \text{ survival in treatment}}{\% \text{ survival in control}} \times 100$$

The corrected values were used in the analysis below. Differences in the corrected dry treatment survivals showed that, as expected from experiment 2.4.2, M. dirhodum was a little more susceptible to chill-injury than S. avenae (Table 2.17). This complicated the comparison between species as any differences between the two may have been due to differences in their susceptibility to chill-injury, not to a difference in susceptibility to inoculative freezing.

The probability of a species surviving the wet treatment was dependent on two independent probabilities, being

Table 2.17 Survival (%) of wet and dry S. avenae and M. dirhodum nymphs in two low temperature tests at -4.6°C for 14 h.

Treatment	% survival at -4.6°C	% survival in controls	Corrected % survival at -4.6°C
<u>S.avenae</u> 'wet'	52.0 , 50.0	94.95	54.8 , 52.7
<u>S.avenae</u> 'dry'	97.9 , 93.9	100.00	97.9 , 93.9
<u>M.dirhodum</u> 'wet'	20.8 , 35.4	87.76	23.7 , 40.4
<u>M.dirhodum</u> 'dry'	51.9 , 69.4	92.78	56.0 , 74.8

Table 2.18 Analysis of variance table for logged percentage survivals in wet and dry treatments.

Source	Sum of squares	D.F.	Variance	F-ratio	Probability
Treatment effect	.1635	1	.1635	18.79	0.012
Species effect	.0841	1	.0841	9.67	0.036
Interaction	.0023	1	.0023	.27	0.632
Residual	.0348	4	.0087		
Total	.2847	7	.0407		

equal to:

$$\begin{array}{l} \text{probability of} \\ \text{surviving chill-injury} \end{array} \times \begin{array}{l} \text{probability of avoiding} \\ \text{inoculative freezing} \end{array}$$

Thus, for each species the probability of avoiding inoculative freezing was estimated as:

$$\frac{\text{mean \% survival in wet treatment}}{\text{mean \% survival in dry treatment}}$$

From this experiment the estimated probabilities of S. avenae and M. dirhodum avoiding inoculative freezing were very similar - 0.56 and 0.49 respectively. Both were a little lower than predicted from the earlier tests on S. avenae only. As shown above, even if the probability of inoculative freezing was equal for the two species, the two factors affecting survival in wet treatments (the species effect on chill-injury and the wet/dry treatment effect) act together in a multiplicative rather than an additive way. To allow species differences in the incidence of inoculative freezing to be tested statistically, these two factors were first rendered additive by taking logs of the corrected treatment survivals and then the significance of an interaction between them was tested using two-way analysis of variance (Table 2.18). This showed that there was no significant interaction ($F = 0.27$, D.F. = 1, $P > 0.5$). Thus the species did not show a significant difference in their ability to avoid inoculative freezing from ice on the body surface.

This result does not agree with those of Griffiths & Wratten (1979) as they found M. dirhodum more susceptible. The difference may be due to the different clones used but may also be due to the fact Griffiths & Wratten compared wet and dry treatments using LT50s determined by probit analysis. From the results of the present experiment the shapes of the mortality-temperature curves for the two treatments are different, so that LT50s are not a good means of comparison. Moreover, probit analysis on wet treatment data is probably not valid because the mortality-

temperature curve in the present experiment was not of the normal sigmoid type.

2.5 WITHIN-SPECIES INTRINSIC FACTORS AFFECTING LOW TEMPERATURE MORTALITY

Three of the four experiments described below investigated differences in cold-hardiness between life-stages within a clone. These are:

1. Instar/size differences in viviparae of all three species (experiment 2.5.1).
2. Fundatrix v. viviparae differences in S. avenae (experiment 2.5.3).
3. Egg v. viviparae differences in S. avenae and Metopolophium spp. (experiment 2.5.4).

The fourth experiment (2.5.2) investigated differences between British clones of S. avenae.

As in the experiments on environmental factors affecting cold-hardiness described above (section 2.4), the experiments described here were designed to determine the approximate magnitude of cold-hardiness differences but were not concerned with investigating the underlying physiological mechanisms involved. The results were primarily intended to shed light on which factors were likely to be important to the low temperature mortality of natural populations in the field. Also, in conjunction with results from winter fieldwork, these experiments helped in assessing the usefulness of laboratory LT50 tests for estimating cold-hardiness in field populations.

2.5.1 Instar/size differences Introduction

This experiment examined differences in cold-hardiness between viviparae all from the same clone but of different

sizes and instars. The main part of the experiment - three tests using aphids reared at 20°C - was conducted early in the study to test the validity of using mixed-instar batches of nymphs in later tests, instead of the adult apterae used in the first experiment (2.3.1). Nymphs were preferred for LT50 tests because they were much more numerous than adults, showed lower control mortality and could be guaranteed not to die of old age before the assessment of mortality. However, if different nymphal instars showed large differences in cold-hardiness, mixed instar batches could not be used, while selection of nymphs of a single instar would be very time consuming. As in experiment 2.3.1, the old clones of each species were used. In a small, subsidiary test the cold-hardiness of field-reared nymphs and adults from the new S. avenae clone were used to determine whether their relative cold-hardiness changed with acclimatization.

Methods

Species used: S. avenae, M. dirhodum, R. padi (tests on laboratory-reared aphids); S. avenae (test on field-reared aphids).

Clones used: old (tests on laboratory-reared aphids); new (test on field-reared S. avenae).

Instars used: 1st-4th and adult (apterae).

Cooling apparatus used: cold bath.

In the main series of tests on aphids reared at 20°C, each species was tested for 3 h near the 3 h LT50 for apterous adults determined in experiment 2.3.1. Three size/instar categories of nymphs were defined by body-length (Table 2.19). Four replicates of each category were tested with four replicates of adult apterae. Each replicate consisted of 25 aphids in a specimen tube. The three species were tested on consecutive days. All came from the same culture started 11 days before the first test. Nymphs were measured using the eye-piece graticule in a binocular microscope. Alatiform fourth instars were excluded. Other details of the method followed the standard test procedure (see section 2.2.3) using test temperatures of -7.95°C (S. avenae), -8.30°C (M. dirhodum)

and $+1.35^{\circ}\text{C}$ (*R. padi*). Control mortality was assessed in four replicates of each size/instar category of each species, which were treated as treatment replicates but exposed to $+20^{\circ}\text{C}$ for 3 h in the bath, instead of low temperatures.

In the subsidiary test on field acclimatized *S. avenae*, mixed batches of 'medium' and 'large' nymphs (see Table 2.19) were tested with apterous adults at -11.50°C for 90 min. The aphids were from a culture of the new *S. avenae* clone maintained in clip-cages on field plots of winter wheat (cv. Hobbit) from early August 1980 to 27 March 1981 (the date of the test). The adult apterae had all been born since 2 December 1980 and were all still producing nymphs. Three replicates of nymphs and three of adults were tested together, one replicate of each in a single tube. One replicate consisted of 30 nymphs or 10 adults as only a limited number were available. Control mortalities were assessed using 45 adults and 90 nymphs.

Results and discussion

In all three species, mean control mortalities of different size/instar categories were different (Table 2.20). In *S. avenae* and *M. dirhodum* medium-sized and large nymphs had control mortalities near zero but those of small nymphs and adults were higher. In *R. padi*, mortalities of adults and large nymphs were low and those of medium-sized and small nymphs higher. Treatment mortalities were corrected for differences in control mortality as follows:

$$\text{corrected treatment mortality (\%)} = 100 \left[1 - \frac{\text{treatment survival (\%)}}{\text{mean control survival (\%)}} \right]$$

Corrected mean treatment mortalities for each size/instar category are shown in Fig. 2.24. Confidence limits were calculated after first normalizing percentage mortalities using the arcsin transformation and then converting the calculated limits back to their untransformed values. The statistical significance of differences between pairs of means was tested using the Studentized Range, *Q*, (Sned-

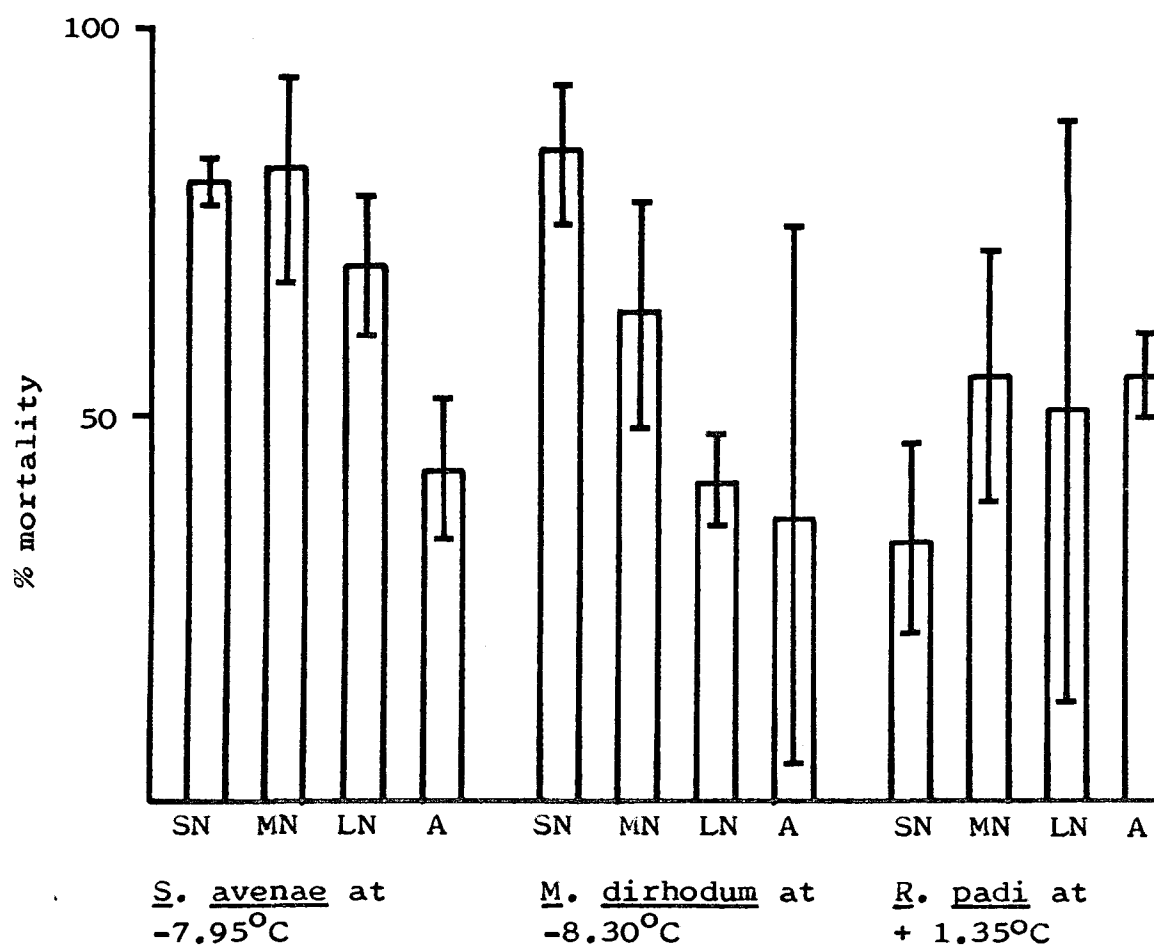
Table 2.19 Nymph size categories used in the experiment on instar/size differences.

Category	<u>S. avenae</u> & <u>M. dirhodum</u>	<u>R. padi</u>	Instar range
'small' (SN)	< 1.3	< .84	1st, small 2nd.
'medium-sized' (MN)	1.3-1.9	.84-1.22	2nd, 3rd, small 4th.
'large' (LN)	> 1.9	> 1.22	large 3rd, 4th.

Table 2.20 Mortalities (%) of control replicates in the experiment on instar/size differences.

	<u>S. avenae</u>	<u>M. dirhodum</u>	<u>R. padi</u>
Small nymphs (SN)	5.3	14.0	7.1
Medium-sized nymphs (MN)	0	0	5.1
Large nymphs (LN)	1.0	2.0	1.0
Adult apterae (A)	9.9	12.9	1.0

Fig. 2.24 Mean treatment mortalities for small nymphs (SN), medium-sized nymphs (MN), large nymphs (LN), and adult apterae (A) corrected for differences in control mortality.



(Vertical bars show 95% confidence limits)

ecor & Cochran, 1967) which, unlike tests based on the Least Significant Difference, takes the number of comparisons being made into account. These tests (Table 2.21) showed that in S. avenae, adults were significantly more cold-hardy than all categories of nymphs ($P < 0.01$) and large nymphs were significantly more cold-hardy than medium-sized and small nymphs ($P < 0.05$). In M. dirhodum, adults and large nymphs were significantly more cold-hardy than small nymphs ($P < 0.05$). In R. padi no categories were significantly different ($P > 0.05$). A disadvantage of Studentized Range and Least Significant Difference tests is that both use a pooled estimate of variance. In this experiment, M. dirhodum and R. padi showed significant heterogeneity in the variances of the different size/instar categories (Bartlett's test: $M/C > 9$, D.F. = 3, $P < 0.05$ in both species). Consequently, significance levels based on Studentized Range may not be very accurate for these two species. Confidence limits (Fig. 2.24) suggested that medium-sized and large nymphs of M. dirhodum may have had different cold-hardiness, as may small nymphs and adults of R. padi.

In S. avenae and M. dirhodum there was a tendency for the later instars to be more cold-hardy than early ones. Harrison & Barlow (1973) found a similar trend in Acyrtosiphon pisum at several acclimation temperatures. However, in Elatobium abietinum the only difference found between instars was a tendency for a few new-born nymphs to be significantly more cold-hardy than older aphids (Carter, 1972). Powell (1974) found in 'off-plant' tests that new-born first instars which had yet to start feeding had much lower supercooling points than aphids which had fed. If as suspected, the species is usually killed by nucleative freezing, this provides an explanation for the greater cold-hardiness of some young nymphs observed by Carter (1972). The difference in supercooling points was probably due to aphids taking in efficient ice nucleators with their first meal, which limited their supercooling ability subsequently (Powell, 1974) (see section 2.1.5).

The cereal aphids studied here, however, die of chill-injury well above their supercooling points (see

Table 2.21 Significance levels associated with differences in cold-hardiness between instar/size categories, based on 'Studentized Range' Q. (See Table 2.20 for abbreviations).

(a) <u>S. avenae</u>	MN	LN	A
SN	n.s.	*	**
MN		*	**
LN			**

(b) <u>M. dirhodum</u>	MN	LN	A
SN	n.s.	*	**
MN		n.s.	n.s.
LN			n.s.

n.s.: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$.

Table 2.22 Mortality of acclimatized nymphs (>1.3 mm) and adult apterae of S. avenae tested at -11.50°C for 90 min.

	Nymphs	Adult
Number alive	71	14
Number dead	18	16
% mortality	20.2	53.3
% mortality in controls	3.3	11.1
% mortality corrected for control mortality	17.5	47.5

section 2.3) and so new-born first instars should not show higher than normal cold-hardiness, even if their super-cooling points are lower than those of feeding aphids. But it should be noted that the results of the present experiment do not prove that nymphs which have yet to start feeding are no more cold-hardy than older ones. The great majority of small nymphs tested would already have started feeding and the test temperatures used were too high for exceptional cold-hardiness in a small number of individuals to be revealed.

Although younger instars of S. avenae and M. dirhodum showed less cold-hardiness than older ones, the differences were relatively small. The greatest difference - between adults and small nymphs of M. dirhodum - was equivalent to a temperature shift of only about 2°C. But, if the differences operate under field conditions, they are large enough for low temperature mortality to modify the age structure of a winter population towards older instars. The comparison between field acclimatized medium and large nymphs and adult apterae of S. avenae suggests, however, that the same differences do not hold in the field. The results (Table 2.22) showed that acclimatized nymphs were significantly more cold-hardy than adults ($\chi^2 = 10.48$, D.F. = 1, $P < 0.01$). Differences between the two were reduced slightly if treatment mortalities were corrected for the difference in control mortalities, but adult mortality was still 30% more than that for the nymphs. The results are not conclusive as the decreased cold-hardiness of adults may be due to the use of older adults in this test than in the tests on unacclimatized aphids. Adult cold-hardiness is known to decrease with age in several other species of insect (Davison, 1969). However, the difference may be genuine, due to differences in the nature of chill-injury in acclimatized and unacclimatized aphids. It was noticeable in earlier experiments that laboratory-reared nymphs quite often died after testing because they were unable to complete ecdysis. This type of delayed mortality, not shown by field acclimatized nymphs, tends to decrease the cold-hardiness of nymphs relative to adults.

From the results of the tests on laboratory-reared

aphids, it was decided to use mixed batches of medium-sized and large nymphs in later experiments as, in all three species, they had similar cold-hardiness (usually intermediate between that of small nymphs and adults) and low control mortalities. They were also more numerous and easier to handle than small nymphs and adults. Although no laboratory tests were run on alatae, some data on the cold-hardiness of this morph were gathered in the course of the winter field experiment described in Chapter 4. These suggested that field acclimatized alatae were a little less cold-hardy than apterae.

2.5.2 Differences between British clones

Introduction

Griffiths & Wratten (1979) demonstrated within-species, inter-clonal differences in cold-hardiness between three clones of S. avenae and two clones of R. padi from continental Europe. They also found that clones obtained from areas with colder winter climates showed greater cold-hardiness. From these results the authors suggested that similar differences may exist between different British clones. Such differences would have important implications for the development of an outbreak prediction system based on autumn or winter numbers, as they might necessitate modifications of such a system according to region, to take inter-clonal variation into account.

To investigate the possibility of inter-clonal differences, 12 British clones of S. avenae were tested together in the present experiment, along with a clone from S.W. Norway. All but one of these clones were kindly supplied by Dr. H. Loxdale of Rothamsted Experimental Station, Harpenden, Hertfordshire. They had been collected in the course of a study on inter-clonal genetic variation.

Methods

Species used: S. avenae.

Clones used: see Table 2.23. Instars used: 2nd-4th.

Cooling apparatus used: cold bath.

The places of origin, reference numbers and colour morphs of the 13 clones used are shown in Table 2.23.

Clones 1 to 10 were obtained from Dr. Loxdale. They had been collected from cereals or grasses during July, August and September 1979 and were kept at Rothamsted on wheat seedlings in a growth room at 18°C and 16 h daylength. A few individuals from each were transferred to Southampton in the summer of 1980. Clone 13 was the Norwegian clone used by Griffiths & Wratten (1979), which had been collected from the field in summer 1977 and kept at Rothamsted Experimental Station from summer 1978. Clone 11 was collected in July 1979 and kept at Southampton throughout. Clone 12 was the 'old' clone used in earlier experiments described above. It had been collected near Rothamsted in 1971 and cultured at Southampton since 1977. It was included here to determine whether it had lower cold-hardiness than the other clones because of the long period it had been maintained under high temperature, laboratory conditions. From summer 1980 until the following winter, when the tests were run, all 13 clones were maintained at Southampton in a culture room at $20 \pm 2^\circ\text{C}$ and 16 h daylength. Each was kept on a 5 cm-square pot of winter wheat seedlings (cv. Hobbit) covered with a cylindrical acetate and muslin cage. Before the tests aphid numbers were increased under the same conditions on 12.5 cm diameter pots of wheat, each covered with a clear plastic propagator with gauze vents.

Two series of tests were run, each consisting of three tests at different temperatures. In the first series, aphids were kept at 20°C until testing. All 13 clones were tested together at -7.5°C, -7.9°C and -8.25°C - temperatures giving mean mortalities between 25% and 75%. Control mortalities were estimated using 50 to 150 aphids from each clone. In the second series of three tests, four clones were tested after 7 days' cold-acclimation at $6^\circ\text{C} \pm 2^\circ\text{C}$ in an LEEC cabinet. These tests were run to determine whether clones differed in their acclimation ability despite having similar cold-hardiness when not cold-acclimated. Only four clones could be tested because of limited space in the LEEC cabinet, those used - numbers 3, 8, 9 and 13 - were chosen because their places of origin gave the widest range of winter temperatures.

In all tests, each clone was represented by one

replicate of approximately 50 aphids in a single tube. The clones' positions in the cold bath were re-allocated before each test using tables of random permutations of 20 numbers. Other test details followed the standard procedure given in section 2.2.3.

Results and discussion

Mortalities in controls varied from 1% to 16% in different clones, with a mean of 6.5%. However, for several clones the control mortalities were based on too few aphids to give accurate estimates and so test mortalities were not corrected for control mortality. In the tests on unacclimated aphids, different clones showed little difference in mortality (Table 2.23). The significance of between-clone differences was tested using two-way analysis of variance (Table 2.24) which revealed a significant clone effect ($P < 0.05$). However, the Least Significant Difference between clone means (45.4 with $P = 0.05$) was too large for any two clones to be significantly different. The significance of the clone effect was due to the low mortality shown by clone 6 - from Trumpington, Cambridge. If this clone was excluded, analysis of variance showed that the clone effect was no longer significant (F-ratio for clone effect = 1.36, D.F. = 11 & 22, $P > 0.25$).

To test for a relationship between mean test mortality and winter temperature in the place of origin, Kendall's rank correlation coefficients were calculated between mean mortality and nine different parameters for winter temperature. For the 12 British clones, parameters were calculated from data from the nearest meteorological station to the clones' places of origin. Three parameters were based on long-term mean temperatures (1941-1970), the rest were for the harsh 1978/79 winter only - the winter before clones 1 to 11 were collected - which may have played a dominant role in producing regional differences in cold-hardiness shown at the time of collection. January 1979 was the coldest January since 1963 for most parts of Britain and temperatures were below average from late November 1978 to late March 1979. Because clone 12 was collected before 1979 it was left out of correlations involving parameters

Table 2.23 Colour morph, place of origin and mean percentage mortalities for S. avenae clones used in comparative cold-hardiness tests using unacclimated aphids.

Number	Colour* morph	Place of origin	Mean % mortality
1	B	Aberdeen	64.6
2	B	Aberdeen	57.4
3	B	Dunfermline, Fife	71.1
4	B	Rotherham, S.Yorks.	52.1
5	B	Shardlow, nr.Derby	58.5
6	G	Trumpington, Cambridge	37.7
7	B	Norfolk Agricultural College, nr.Norwich	58.7
8	B	Bath, Somerset	51.0
9	B	Plas Gogerddan, nr. Aberystwyth, Dyfed	72.7
10	B	Patricxbourne, nr. Canterbury, Kent	52.3
11	G	Damerham, nr.Fordingbridge, Hants.	69.0
12	B	Rothamsted, Herts.	60.3
13	G	Vollebeck, S.W. Norway	64.4

*B = brown, G = green.

Table 2.24 Analysis of variance on percentage mortalities (after arcsin transformation) in 3 tests on 13 unacclimated clones of S. avenae.

Source	Sum of squares	D.F.	Variance	F-ratio	Probability
Clone effect	1175.7	12	98.0	2.42	0.031
Temperature effect	684.6	2	342.3	8.45	0.002
Residual	972.4	24	40.5		
Total	2832.7	38	74.6		

Least Significant Difference ($P = 0.05$) for comparing clone means = 45.4.

specific to the 1978/79 winter. The Norwegian clone was only included in the correlation in which the temperature parameter used was the long-term mean daily minimum for January. In this case the temperature was obtained from a climate map (Steinhauser, 1970). None of the nine rank correlations calculated was significant (Table 2.25) ($P > 0.1$ in all cases). Scatter plots for mean percentage mortality on two temperature parameters are shown in Figs 2.25 and 2.26. In the three tests on four clones after cold-acclimation, one clone (number 9) was not available for the final test. From mean mortalities for the two tests in which all four clones were used, there was no sign that clones from areas with low winter temperatures showed greater cold-hardiness than others after cold-acclimation (Table 2.26). Analysis of variance of the results (Table 2.27) showed no significant difference between clones ($P > 0.05$).

There was no evidence from this experiment to suggest that, for the clones tested, those from areas with colder winters had greater cold-hardiness, although this was shown for clones from Spain, Norway and Switzerland by Griffiths & Wratten (1979). It is unlikely that the clones tested here had lost differences in cold-hardiness during the 18 months most had been cultured in the laboratory, as clone 12, which had been collected nearly 10 years previously, showed similar cold-hardiness to the rest. Present evidence suggests that S. avenae is predominantly anholocyclic in Britain (Hand, 1982) while the winter temperatures experienced, particularly in the severe 1978/79 winter, are low enough to cause mortality in fully acclimatized S. avenae viviparae. From this it seems inevitable that if any genetic variation affecting low temperature tolerance exists - and the results of Griffiths & Wratten (1979) show that it does on a continent-wide scale - differential mortality of the less cold-tolerant clones will occur during at least some winters.

The lack of a relationship between cold-hardiness and winter temperatures among the 12 British clones tested here is probably a result of two factors. Firstly, regional variations in winter temperatures within the British Isles

Table 2.25 Kendall's Rank Correlation Coefficients
between mean percentage mortality for
different clones of S. avenae and various
winter temperature parameters.

TEMPERATURE PARAMETER	k	n	PROBABILITY (1-tailed test)
Jan.'79 mean daily minimum	.224	11	.173
Jan.'79 month's minimum	-.147	11	.266
Jan.'79 mean	.037	11	.437
Nov.'78 - March '79 mean daily minimum	.110	11	.320
Nov.'78 - March '79 mean of months' minima	-.073	11	.377
Nov.'78 - March '79 mean	.000	11	.500
**Long-term mean daily minimum for Jan.	-.092	13	.334
*Long-term mean of month's minimum for Jan.	.154	12	.245
*Long-term mean of means for Jan.	-.016	12	.472

** Clones 12 and 13 included.

* Clone 12 included.

Fig. 2.25 Scatter plot of mean mortality after low temperature exposure for 11 British clones of *S. avenae* on mean minimum temperature in January 1979 at their places of origin*.

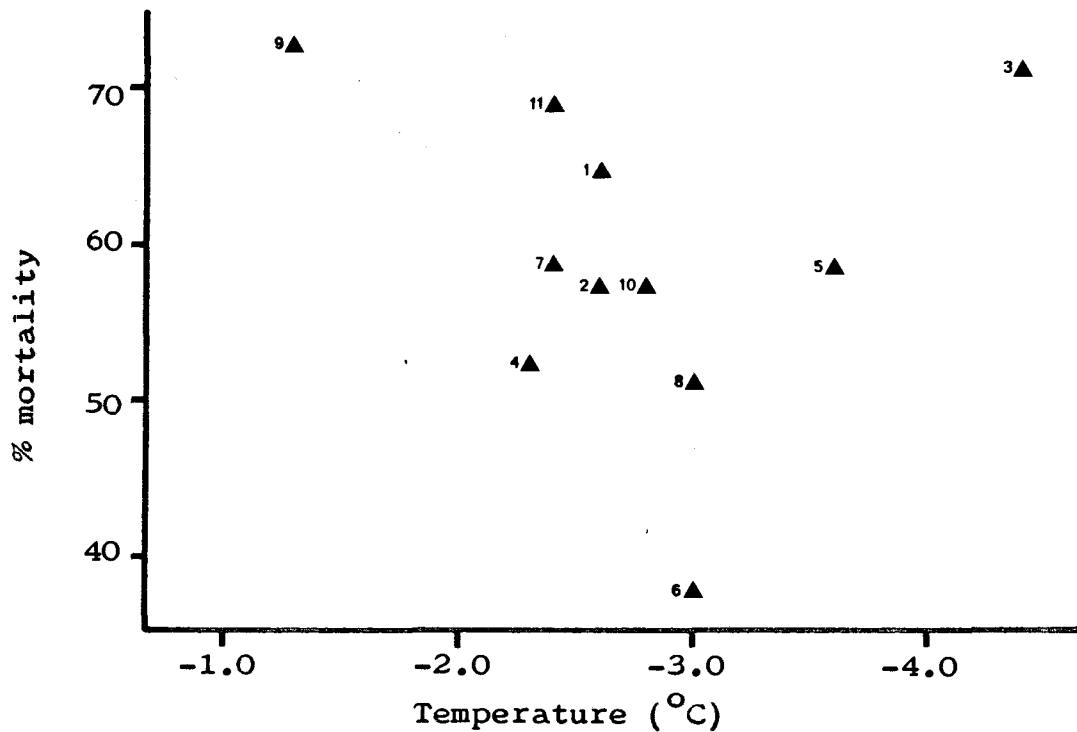
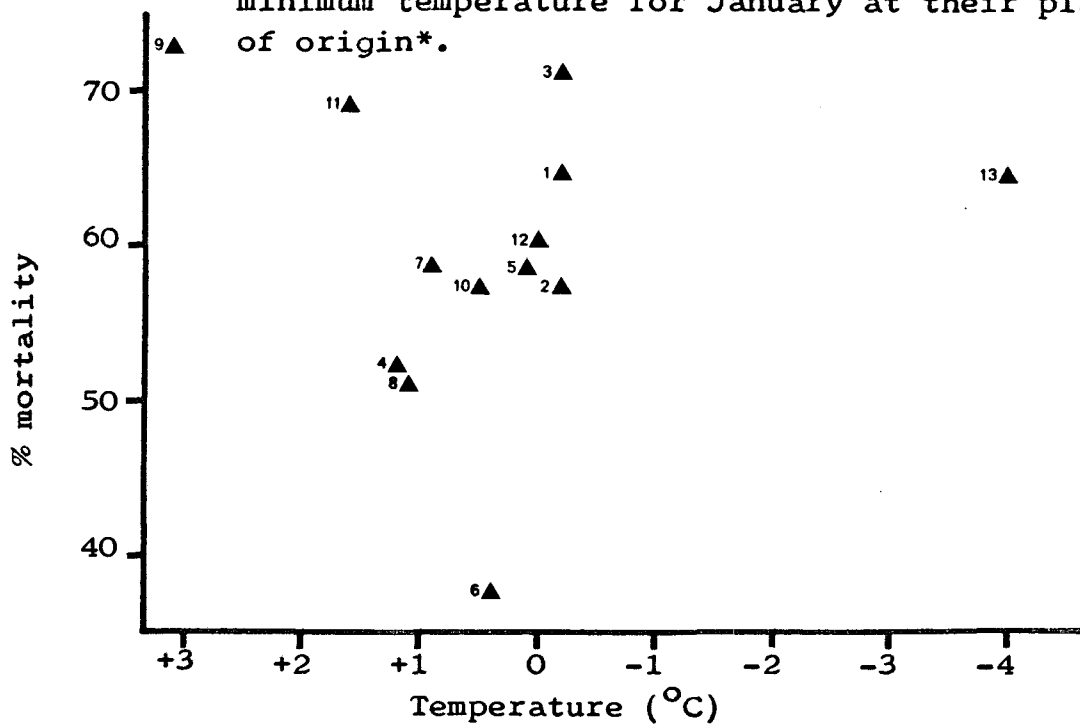


Fig. 2.26 Scatter plot of mean mortality after low temperature exposure for 12 British and one Norwegian clone of *S. avenae* on long-term mean minimum temperature for January at their places of origin*.



* see Table 2.23 for key to clone reference numbers.

Table 2.26 Mean mortalities (%) in two tests on four clones of S. avenae after cold-acclimation.

Clone number	Place of origin	Mean percentage mortality	Long-term mean January temperature in place of origin
3	Dunfermline, Fife	59.5	+2.7°C
8	Bath, Somerset	60.0	+4.1°C
13	Vollebeck, Norway	61.0	-4.0°C
9	nr. Aberystwyth, Dyfed	71.2	+4.7°C

Table 2.27 Analysis of variance of percentage mortalities (after arcsin transformation) in 3 tests on 4 clones of S. avenae after cold-acclimation.

Source	Sum of squares	D.F.	Variance	F-ratio	Probability
Clone effect	526.1	3	175.4	3.78	>0.05
Temperature effect	4559.3	2	2279.7	49.1	<0.01
Residual	232.0	5	46.4		
Total	5317.4	10*	531.7		

*1 missing value, therefore total degrees of freedom = 10, not 11.

are relatively limited compared with those existing over continental Europe. Secondly, the clones tested are probably too close geographically. Migration by alatae probably prevents populations in colder regions of Britain from remaining isolated from the rest from one winter to the next, so that they can never build up a significant amount of inheritable cold-hardiness. Even when a severe winter produces some genetically-based regional differences in cold-hardiness within a single season, migration by alatae during spring and summer probably produces mixing sufficient to eliminate these differences within a few months. In addition, there is the possibility that selection pressures acting on spring and summer populations select against cold-hardiness and so prevent the persistence of regional differences. For example, cold-adapted clones may show lower development and reproduction rates at high summer temperatures.

Caution should be exercised in comparing the Norwegian clone with the British ones, as it was collected two years earlier. However, its similar performance to British clones is probably genuine, as when tested by Griffiths & Wratten (1979) it had an LT50 similar to the two British clones used in earlier experiments in the present study. Its similarity to the British clones suggests that alate migration produces mixing of the populations in Britain and S.W. Norway, although the possibility exists that the species is predominantly holocyclic in Norway and so cold-hardiness of viviparae is not selected for.

2.5.3 Differences between fundatrices and viviparae Introduction

Most aphid eggs break diapause in late winter or early spring - those of S. avenae probably do so after mid-February (Hand, 1982). Consequently, the fundatrices which hatch from them must often experience temperatures well below those encountered by later generations of viviparae*. Because of this, fundatrices may show greater

*'Vivipara' and 'fundatrix' are used as mutually exclusive terms.

inherent cold-hardiness. To test this hypothesis the cold-hardiness of field-reared fundatrices and viviparae were compared in this experiment.

Methods

Species used: S. avenae.

Clones used: old (fundatrices), new (viviparae).

Instars used: 3rd-4th.

Cooling apparatus used: cold bath.

The fundatrices used were hatched from eggs of the old clone of S. avenae kindly donated by Dr. S.C. Hand. These had been produced in the laboratory at 10⁰- 15⁰C and short daylength but were kept in the field from early January 1981. After hatching in March the fundatrices were transferred to clip-cages on field-grown winter wheat seedlings alongside an outdoor culture of viviparae of the new clone of S. avenae, which had been kept in clip-cages in the field since early August 1980. The aphids were taken in and tested as third- and fourth-instar nymphs on 29 March 1981. Only 38 fundatrices were available. Twenty of these were tested with 30 third- and fourth-instar viviparae at -13.0⁰C for 90 min. Both types of aphid were tested together in the same specimen tube. The remaining fundatrices and 30 viviparae were used to assess control mortality. Other details of the test followed the standard procedure given in section 2.2.3.

Results and discussion

Although the numbers tested were too small to obtain more than a rough estimate of relative cold-hardiness, the fundatrices tested were not more cold-hardy than the viviparae, as they actually suffered heavier mortality (Table 2.28). Fisher's exact probability test showed no significant difference between the two morphs ($P = 0.067$) and control mortality was low for both (Table 2.28).

As the fundatrices and viviparae tested here came from different clones, it is possible that real differences between the morphs do exist but are masked by differences between the clones. However, from the results of experiment 2.5.2 this seems unlikely. It is more likely that, despite being an early spring morph, fundatrices of S. avenae have no inherent low temperature survival advant-

Table 2.28 Mortalities (%) of fundatrices and viviparae of S. avenae after exposure to -13°C for 90 min..

	Alive	Dead	% mortality
TREATMENT:			
Fundatrices	0	20	100.0
Viviparae	5	25	83.3
CONTROL:			
Fundatrices	17	1	5.6
Viviparae	30	0	0.0

ages over similarly acclimatized viviparae. This may be because S. avenae usually overwinters anholocyclically in England, so that there has been selection for cold-hardiness in viviparae as well as fundatrices. If so, fundatrices of other species which are predominantly or completely holocyclic may show inherently greater cold-hardiness than viviparae.

2.5.4 Cold-hardiness of post diapause eggs

Introduction

As eggs are the usual and for many species the only form in which aphids overwinter, it is generally agreed that they possess greater cold-hardiness than viviparae. Field observations and some experimental evidence exist to support this view. For example, Way & Banks (1964) found that hatching success of diapausing eggs of Aphis fabae was unaffected by 14 days at -10°C and 23% hatched after the same period at -20°C , thus showing a degree of cold-hardiness much greater than that of viviparae. The eggs of several other species have very low supercooling points (James & Luff, 1982; Parry, 1979b; Somme, 1964, 1969) - approaching -40°C in most cases. As inoculative freezing and chill-injury seem unlikely in eggs, these low supercooling points have been taken to indicate great cold-hardiness.

The present experiment was undertaken to determine whether, as expected, the eggs of S. avenae and M. dirhodum were more cold-hardy than viviparae. Only a limited number were available and so only a single test could be run on each species, using a temperature low enough to kill nearly all fully cold-hardened viviparae. The S. avenae eggs were kindly donated by Dr. S.C. Hand who also conducted some tests on their cold-hardiness himself (Hand, 1982). Eggs of M. dirhodum had to be obtained from oviparae collected from Rosa in autumn 1980. This was complicated by the fact that another species of Metopolophium (M. fasciatum sp. nov.), which is very similar to M. dirhodum and also alternates between Gramineae and Rosa, has recently been discovered (Stroyan, 1982). Oviparae of

the two species can only be separated by detailed morphometric analysis and, consequently, the Metopolophium oviparae used in this experiment could not be identified to species at the time of collection. Subsequently, morphometric analysis of a sample of these by Dr. Hand revealed that they were M. fasciatum, not M. dirhodum. Nevertheless, it was decided to go ahead with the test as eggs of the two closely related species are likely to have similar cold-hardiness and the abundance and pest status of the new species is little known at present due to its extreme similarity to M. dirhodum.

The eggs were tested in February and March, after the termination of diapause, because work on the supercooling points of eggs of other species suggests that eggs are less cold-hardy at this time than during diapause (Somme, 1964, 1969; Parry, 1979b; James & Luff, 1982).

Methods

Species used: S. avenae, M. fasciatum.

Clones used: old (S. avenae), field-collected (Metopolophium sp.).

Cooling apparatus used: cold chamber.

The S. avenae eggs were produced in the laboratory at 10 - 15°C and short daylength on winter wheat. They were transferred to a field cage in December 1980 and early January 1981 and tested on 4 March 1981. Eggs of M. fasciatum were produced by the field-collected oviparae which were kept with males on cut rose stems in a field cage. The eggs were laid in October and November 1980 and tested on 10 February 1981.

The eggs were tested for 90 min at -14.9°C (S. avenae) and -14.0°C (M. fasciatum). These temperatures are sufficiently low to kill over 90% of fully cold-hardened viviparae (see experiments 2.4.2 and 2.4.3). Only eggs which were apparently viable - black and not collapsed - were used. One hundred M. fasciatum eggs and 123 S. avenae eggs were tested and control batches of similar size were placed in a cold room at 6°C while test batches were in the cold chamber. The eggs were then kept in containers at 100% R.H. in a culture room at 12°C to 15°C and 16 h daylength. They were checked at 7 to 14 day intervals until

hatching had ceased.

Results and discussion

In both species the hatching success of test and control egg batches was very similar (Table 2.29 & 2.30). In neither species did the proportion hatched in test and control batches differ significantly (χ^2 tests: S. avenae: $\chi^2 = 0.70$, D.F. = 1, $P > 0.3$. M. fasciatum: $\chi^2 = 0.63$, D.F. = 1, $P > 0.3$). The results showed that a temperature nearly low enough to annihilate overwintering viviparae did not harm post-diapause eggs.

Hand (1982) conducted cold-hardiness tests on S. avenae eggs from the same clone at -10°C . He found that hatching success was not affected after 24 h at this temperature (again demonstrating the greater cold-hardiness of S. avenae eggs compared to viviparae) but was significantly reduced if eggs were left at -10°C for 2 wk. This suggests that S. avenae eggs are less cold-hardy than those of Aphis fabae (Way & Banks, 1964), but such extreme conditions would never be experienced by natural populations in Britain.

2.6 GENERAL DISCUSSION ON THE LABORATORY WORK ON COLD-HARDINESS

2.6.1 Conclusions on the characteristics of low temperature mortality in cereal aphids

The main conclusions on the action of low temperatures in producing mortality in the field are summarized below. They apply to all three species unless stated otherwise.

1. R. padi is the most cold-susceptible of the three species studied - the laboratory determined 90 min LT50 for acclimatized nymphs is 4.5°C higher than that for S. avenae.
2. S. avenae and M. dirhodum have similar cold-hardiness. Although S. avenae cold-hardens to a slightly greater extent, differences between viviparae of the two species are much too small to account for their very

Table 2.29 Hatching success (%) of S. avenae eggs after low temperature exposure.

	Hatched	Unhatched	% hatched
Treatment (-14.9°C)	68	55	55.3
Control (+6.0°C)	63	65	49.2

Table 2.30 Hatching success (%) of M. fasciatum eggs after low temperature exposure.

	Hatched	Unhatched	% hatched
Treatment (-14.0°C)	77	23	77.0
Control (+6.0°C)	74	30	71.2

different abundance on Gramineae during the winter (Hand, 1982).

3. When reared at 20°C all three species are killed by chill-injury rather than by nucleative freezing, and even when given low temperature pre-treatment their LT50s remain well above their supercooling points.
4. The use of unnaturally fast cooling and re-warming rates in laboratory tests produces artificially high low temperature mortality, particularly in R. padi.
5. Changes in exposure time have only small modifying effects on aphid mortality, which, around the LT50, is very sensitive to temperature.
6. Exposure to non-lethal low temperatures produces large improvements in cold-hardiness and affects different species to different extents. Consequently, tests on aphids kept at high temperatures will result in substantial errors if used to predict field mortality.
7. Substantial cold-hardening occurs after only a few days and at temperatures well above 0°C. Consequently, aphids will acclimatize appreciably quite early in the autumn, probably before the first hard frosts.
8. Inoculative freezing from host plant sap is rare in S. avenae and M. dirhodum feeding on winter wheat.
9. The chill-susceptibility of S. avenae is slightly increased when it is reared on perennial rye grass rather than winter wheat.
10. In the laboratory, inoculative freezing from ice on the body surface can kill about 70% of S.avenae but several factors may act to reduce this mortality in the field.

11. In laboratory-reared R. padi different sized nymphs and adult apterae have similar cold-hardiness. In laboratory-reared S. avenae and M. dirhodum there is a tendency for younger instars to be less cold-hardy but there is evidence that under field conditions adult apterae of S. avenae are less cold-hardy than nymphs.
12. There is little variation in cold-hardiness between different British clones of S. avenae and no relationship between cold-hardiness and the winter temperature in their places of origin.
13. Fundatrices of S. avenae are no more cold-hardy than viviparae.
14. Eggs of S. avenae and M. fasciatum are substantially more cold-hardy than viviparae and probably suffer little if any low temperature mortality in most British winters.

Of the environmental factors investigated, low temperature pre-treatment would appear to have the most important effect on field low temperature mortality, while exposure time and host plant effects probably have only minor, modifying influences. The importance of ice on the body surface is difficult to assess but it was important under laboratory conditions and may also be so in the field. The difference between cooling and re-warming rates used in the laboratory and those experienced in the field is an important factor to be taken into account when interpreting laboratory results.

2.6.2 The use of supercooling points

Although supercooling points have been used as indices of cold-hardiness in many studies, they can only be used as such for insects which are freezing-susceptible in the strictest sense. Thus they cannot be used for the three aphid species studied here, which can die of chill-injury

well above their supercooling points. Supercooling points have been overused, and possibly misused, in the past, as in several studies on aphid cold-hardiness in which they have been used extensively (Powell, 1974, 1976; Parry, 1978, 1979a) no evidence was presented to prove that the species under study were not chill-susceptible. This fact calls into doubt some of the conclusions from these studies. But for Elatobium abietinum at least, it is clear that even if some chill-death does occur, the relationship between supercooling points and death is much closer than in cereal aphids, because Powell (1974) has shown that when individuals of this species were tested off the host plant all survived -12°C for 4 h, and the species' mean supercooling point was only about 3° or 4°C below this.

However, the finding in the present study that the supercooling points of cereal aphids bear no relation to their LT50s demonstrated that if supercooling points are used in future studies on aphid cold-hardiness, lack of chill-susceptibility should be proved rather than assumed. Even for insects shown to be completely immune to chill-injury, supercooling points should not be taken as accurate predictions of field mortality because they do not take inoculative freezing into account and because, under field conditions, spontaneous nucleative freezing may occur in some individuals several degrees above the mean supercooling point.

2.6.3 The use of LT50s determined in the laboratory

Low temperature mortality is affected by so many factors that laboratory-determined LT50s cannot be taken as accurate predictions of field mortality and are best seen as indices of field cold-hardiness. They can be reasonably accurate if certain test conditions are met. The standard test procedure used in this study would appear to be a reasonable compromise between convenience and fidelity to field conditions, provided the aphids tested are given at least a few days to cold-harden at a temperature around 5°C or, preferably, are acclimatized in the

field for several weeks. Ideally, cooling and re-warming rates of only a few degrees an hour should be used and if this is impossible results must be corrected for the extra mortality produced by fast cooling before being applied to the field. Also, standard LT50 tests will not take death from inoculative freezing into account, nor the possibility of slow, 'chronic' mortality directly or indirectly related to long exposure (days or weeks) at low but not necessarily sub-zero temperatures. Some evidence for the latter type of mortality is given in Chapter 5.

2.6.4 Contrasts between the cold-hardiness of cereal aphids and that of *Elatobium abietinum*

More information on the cold-hardiness of the green spruce aphid, *Elatobium abietinum*, exists in the literature than for any other species of aphid. If the conclusions from studies on the species (Carter, 1972; Powell, 1973, 1974, 1976; Parry, 1979a) are compared with those from the present study, several important contrasts are evident, though to some extent the differences may be due to the extensive use of supercooling points in most of these studies. The main differences between the species as revealed by comparisons of the present study with those of Carter (1972), Powell (1973, 1974, 1976) and Parry (1979a) are as follows:

1. Laboratory-reared cereal aphids, and probably field acclimatized ones also, die of chill-injury well above their supercooling points. But in studies on *E. abietinum* (Powell, 1974, 1976; Parry, 1979a) the use of supercooling points implies that the authors believe the species is not chill-susceptible, so that freezing and death always occur together. Although no experiments proving the absence of lethal chill-injury in the species are reported, it is clear that, unlike cereal aphids, it can survive to within a few degrees of the mean supercooling point (Powell, 1974).

2. In cereal aphids low temperature pre-treatment produces substantial increases in cold-hardiness by reducing chill-susceptibility. In S. avenae at least, this additional cold-hardiness is not passively acquired from the host plant. But Powell (1974) and Parry (1979a) believe that low temperature pre-treatment does not produce additional cold-hardiness in E. abietinum although supercooling points may show seasonal changes related to similar changes in the host's leaves. They argue that a cold-hardening mechanism in the aphid could not be effective for two reasons. Firstly, when aphids are exposed to sub-zero temperatures while feeding, they are killed by inoculative freezing when the leaves of the host plant freeze (Powell, 1974), so that cold-hardening in the aphid is irrelevant to its survival. Secondly, it is believed that E. abietinum is freezing-susceptible, not chill-susceptible. If this is so, cold-hardiness can only be improved by lowering supercooling points. For E. abietinum, Parry (1979a) has shown that changes in acclimation temperature do not produce changes in supercooling point in aphids which have started feeding. It is argued that a cold-hardening mechanism could not produce a change in supercooling points because these are governed by efficient ice nucleators in the gut taken in with plant sap (Powell, 1974; Parry, 1979a). However, the possibility that low temperature pre-treatment affects chill-susceptibility in E. abietinum needs investigation. Carter (1972) found that lowering pre-treatment temperature from 19° to 15°C and from 5° to 0°C lowered LT50s of E. abietinum by several degrees, which suggests this type of acclimation does occur. But Powell (1974) and Parry (1979a) considered the observed changes to be the result of changes in the quality of host plant sap affecting supercooling points. Powell (1973) also criticizes several aspects of Carter's methodology.

3. S. avenae and M. dirhodum exposed to sub-zero temperatures while feeding rarely freeze due to inoculation with ice from the host plant but in E. abietinum inoculative freezing from frozen leaves occurs in the great majority of aphids within a few hours and is a major factor limiting low temperature survival (Powell, 1974).
4. In S. avenae and M. dirhodum ice on the body surface can produce inoculative freezing in a large proportion of aphids but in E. abietinum it appears to have no effect (Carter, 1972).

These contrasts highlight the usefulness of the laboratory work undertaken here. Without it several spurious assumptions on the nature of low temperature mortality in cereal aphids could easily have been made, based on evidence from E. abietinum. The discovery that cereal aphids are chill-susceptible, so that supercooling points are not a valid measure of their cold-hardiness, was particularly important and profoundly affected the direction and methodology of the project.

The laboratory results described above are compared with those from winter field experiments in Chapter 3. Thoughts on further laboratory studies on the cold-hardiness of cereal aphids are given in Chapter 7.

CHAPTER THREE

CHAPTER THREE

EFFECTS OF LOW TEMPERATURE AND OTHER WEATHER CONDITIONS
ON THE OVERWINTERING MORTALITY OF VIVIPARAE ON WINTER
WHEAT3.1 INTRODUCTION

The extensive winter sampling of Gramineae undertaken by Hand (1982) concurrently with this study provided the first good field data on the effects of weather on the mortality of natural overwintering populations of cereal aphid viviparae. However, the accuracy of such data obtained from natural populations is limited as several types of error cannot be avoided. For example:

1. Natural populations are at low density and patchily distributed and it is impossible to obtain density estimates as frequently and accurately as one can with artificial populations.
2. There is an unknown amount of mortality due to the action of natural enemies.
3. Variation in factors other than weather conditions is inevitable (for example crop type, growth stage and local physiography).
4. In natural populations mortality is continually being offset by reproduction, which is difficult to predict accurately.*

Consequently in the present work populations of viviparae were artificially established on winter wheat to provide more detailed, quantitative data on the winter mortality caused by rain, snow, high winds and low temperatures. Natural enemies were excluded from the artificial populations, site and crop variables were held constant and

*The data on low temperature development and reproduction presented in Chapter 4 were not available in time for Hand's analysis.

extra weather data were collected a few metres from the monitored aphids. Most observations were made on S. avenae and M. dirhodum viviparae, although some data on R. padi were also obtained. Particular attention was paid to differences between the species and the extent to which conclusions on the low temperature mortality of the three species, drawn from laboratory experiments (see Chapter 2) held good under natural, field conditions.

Artificially established populations of cereal aphid viviparae were used to study winter mortality in two previous studies (see Dean (1974a) and Smith (1981)). Dean's experiment involved weekly monitoring through the 1970/71 winter of mixed populations of S. avenae, M. dirhodum and R. padi on two pots of winter wheat seedlings (one protected from wind, rain and snow). In this experiment S. avenae survived best and R. padi worst in both pots but Dean failed to find relationships between mortality and weather conditions. This was, perhaps, not surprising given the small sample sizes and complication of the analysis due to the addition of 10 new adult apterae of each species each week.

Experiments undertaken by Smith (1981) in the winters of 1977/78 and 1978/79 were on a larger scale and better designed. They involved S. avenae and M. dirhodum in the first winter but only S. avenae in the second. Populations on pots of winter wheat sunk into the ground were counted at 1 or 2 week intervals, some exposed and some in various predator-exclusion and shelter cages, designed to separate the different mortality factors. In the first winter M. dirhodum survived better than S. avenae. Results from both winters showed that the aphids were unexpectedly low temperature tolerant and suggested that wind was the weather factor causing most mortality. However, in both these studies the population changes which were being recorded and related to weather conditions were not due to mortality alone, but were the result of mortality offset by a variable and unknown amount of reproduction. This failure to separate mortality and reproduction meant that the causes of species differences could not be pin-pointed, the results could

not be put to any predictive use and to some extent they had to be treated in a subjective, anecdotal way with only limited statistical analysis.

In order to examine weather mortality in isolation, without the confounding effect of reproduction, a new approach was adopted in the present study. Instead of monitoring the same mixed-age populations over several months, as Dean and Smith had, many batches of aphids were used one after the other and each was monitored for only 8 days before being replaced. The aphids were put out as nymphs and none had time to reach maturity and start to reproduce before they were replaced by a fresh batch. Thus no recruitment occurred in the monitored populations. Shelter cages were not used to try to separate weather effects as it was felt that it would be very difficult to design cages which eliminated one weather factor without affecting the others. Instead, multiple regression analyses of aphid mortality on a set of parameters for the various weather variables were used to quantify weather effects. The experimental method was labour-intensive and some artificiality was inevitable, though this was minimized by acclimating the aphids in the laboratory before exposure and by using field-grown plants. The method had the extra advantage of allowing standardization of the aphids' pre-exposure condition, thus minimizing another type of unwanted variation.

The experiment was run first from January to March 1979 using S. avenae and M. dirhodum only. It was continued at a different study site from December 1979 to March 1980 with more detailed weather monitoring on site and using R. padi as well in some replicates. The additional data obtained in the second winter allowed more detailed analysis, particularly of interactions between weather factors. The failure to use R. padi in the first winter was unfortunate. It was due to the assumption that the species' very sheltered feeding sites on wheat seedlings would make it very difficult to count but, in the event, it proved possible to do so.

3.2 MATERIALS AND METHODS

3.2.1 Field sites and plants used

The experiment was carried out from 26 January to 27 March 1979 at the Game Conservancy, near Fordingbridge, Hampshire (Grid Ref. SU153149). It was repeated with improved on-site weather monitoring between 5 December 1979 and 10 March 1980 at Chilworth Manor, near Southampton, Hampshire (Grid Ref. SU406183). In both field seasons aphids were exposed in a study plot about 20 m square in an open field, with no shelter from nearby buildings or hedges. Both plots were broadcast sown with winter wheat (cv. Hobbit) in early October at a sowing rate which conformed with that of commercially-grown wheat. The plants on which aphids were exposed were in 7.5 cm plastic pots so that aphids could be introduced onto them in the laboratory at a temperature high enough to allow them to find suitable feeding sites and start feeding before exposure to winter weather conditions. These plants were dug up from the edge of the experimental plot, or from an adjacent plot sown at the same time. They were potted-up up to 6 weeks before use.

The pots were sunk into the ground to within 1 cm of their rims so that aphids were not exposed above the level of the rest of the experimental plot. The holes, made by sinking empty 7.5 cm pots into the ground, were arranged in five rows of five each near the centre of the plot, with each hole 1 m from its neighbours. There were thus 25 possible positions for pots with aphids, though more than half of these were never used at once. The plants on which aphids were exposed varied in growth stage from 12, 20 to 17, 24 (Zadoks' scale). For batches of pots put out together, mean plant density varied from 4.0 to 7.5 per pot and the mean number of tillers from 1.1 to 2.7 per plant.

3.2.2 Aphids used and experimental routine

The experiment was mainly concerned with S. avenae and M. dirhodum. The description given here of the experimental routine, which was repeated with a fresh

batch of aphids in an 8-day cycle, applies to these two species. When used, R. padi was treated slightly differently (see below).

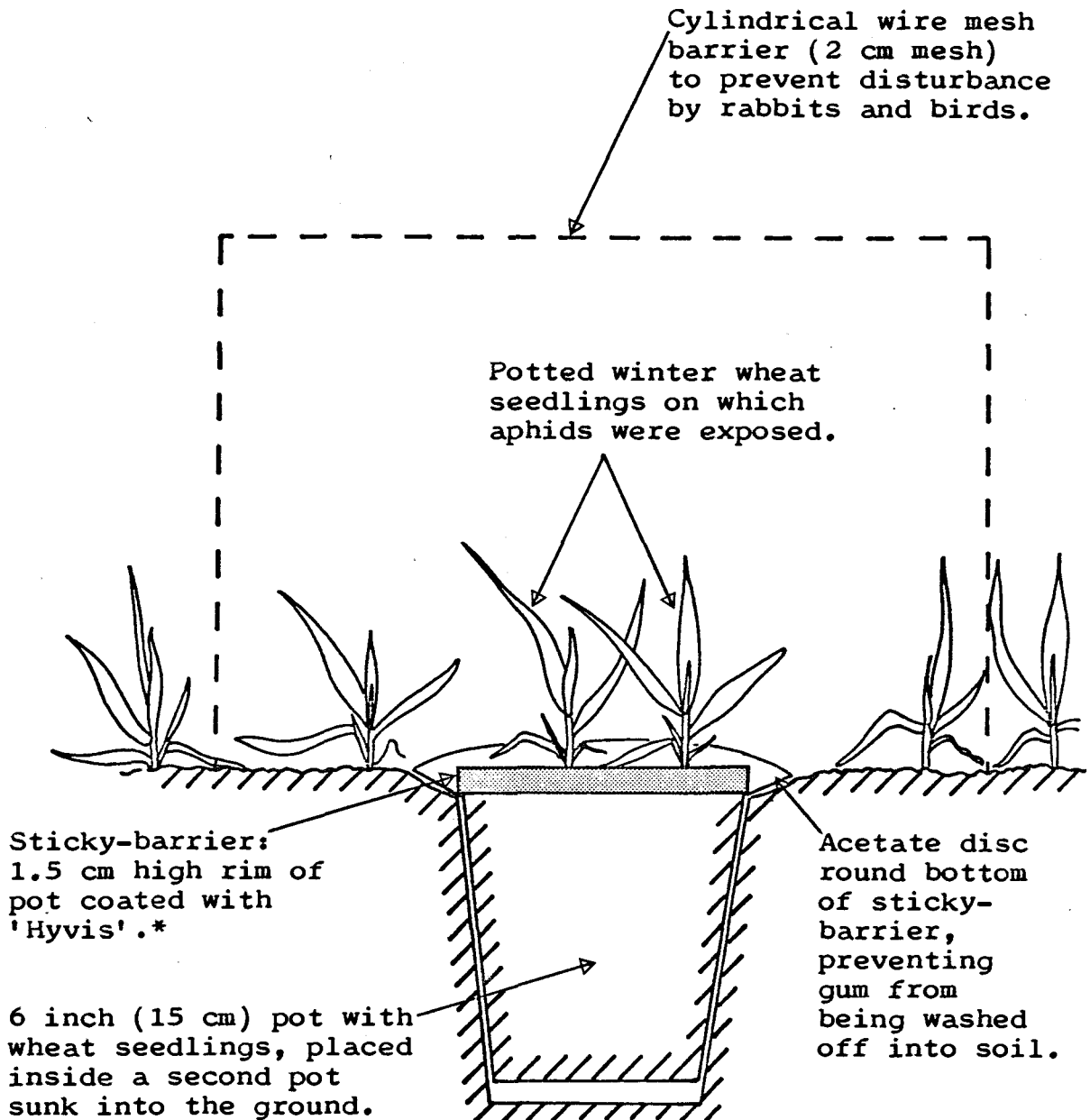
The aphids were cultured at $20^{\circ} \pm 1.5^{\circ}\text{C}$ and a 16 h photoperiod using the standard culturing method (Appendix 2.2). They were then acclimated at 6.5°C for 6 days, a treatment which, from laboratory experiments, was estimated to bring LT50s to within 1°C or 2°C of those of fully field-acclimatized animals (see Chapter 2, experiments 2.4.2 and 2.4.3). The aphids were then gently brushed off the culture plants and sorted. Only nymphs between 1.1 mm and 2.2 mm long were used, approximately corresponding to second, third and small fourth nymphal instars. A few aphids at either end of the size range were measured using a binocular microscope with an eyepiece graticule. These were used to gauge the size of the rest of the nymphs.

Eight pots of winter wheat seedlings were brought in from the field and infested, each with about 45 M. dirhodum and 35 S. avenae nymphs. The difference in the numbers used was necessary because M. dirhodum had a greater tendency to wander. The infested pots were left in the laboratory overnight, giving the aphids between 12 and 24 h at a temperature above 15°C in which to find suitable feeding positions. The next day the plants and soil surface in the eight pots were examined and all but 25 aphids of each species were removed from each pot.

The rim of each pot was then coated with 'Hyvis' sticky-barrier compound (see Fig. 3.1) to exclude arthropod predators and prevent aphids from wandering off while exposed in the field. Flying natural enemies could still have reached the aphids but in the cold winter conditions very few were active and none were ever seen on the pots. The positions of the pots in the experimental plot were assigned using random number tables. Cylindrical wire mesh barriers (mesh size 2 cm) 30 cm high by 40 cm in diameter were placed around the pots to prevent disturbance by birds or rabbits.

The pots were left out for 4 days and then brought in briefly and the aphids counted without removing any

Fig. 3.1 Arrangement used for exposing aphids to winter weather conditions on potted winter wheat seedlings in the experimental plot.



*supplier: The Wolfson Unit, The Biology Dept.,
Southampton University.

except those found dead. Provided care was taken and counts were done in an unheated laboratory the aphids could be counted accurately without disturbance. The pots were then returned to their previous positions in the experimental plot and left for a further 4 days before a final count, during which all the aphids remaining were removed. The period of the experiment was thus split into a series of 4-day intervals, for each of which mortality of each aphid species was known and could be related to the weather conditions during that period.

From January to March 1979 seven batches of aphids were exposed, giving a total of 14 4-day period records for each species. But results from one of the early periods (before wire mesh barriers were in use) were rejected due to disturbance of the plants by rabbits. From December 1979 to March 1980 nine batches of aphids were exposed, giving a total of 18 4-day period records for each species. During counting aphids were categorized as:

1. Alive on plants.
2. Dead on plants.
3. Alive off plants.
4. Dead off plants.
5. Dead on the sticky-barrier.

Very few aphids were found in the last three categories.

Aphids which had died on the plants were nearly always easy to tell from live ones as they soon became shrivelled and discoloured. They usually released their grip on the plants, but remained attached by their stylets. In some periods, particularly those in which aphids experienced lethal low temperatures the night before a count, low temperature mortality may have been underestimated due to delayed mortality, which was revealed in laboratory experiments (Chapter 2, section 2.3.1). However, observations during the experiment described here suggested that delayed mortality was less important in the field than in the laboratory as many aphids were obviously shrivelled and blackened the day after a particularly cold

night. This difference between field and laboratory observations may have been due to the lower relative humidity in the field (in laboratory experiments aphids were kept at 100% R.H. after testing). During nearly all counts it was found that a proportion of the aphids present initially were simply missing. Most of these had probably been blown, brushed or knocked off the plants onto the soil around the pots by wind or rain. However, some aphids knocked onto the soil inside the pots were probably missed during counts because S. avenae nymphs, being brown, were difficult to see against soil and also because both species could have been washed into cracks or covered with earth.

In the 1979/80 winter seven batches of R. padi nymphs were exposed with the S. avenae and M. dirhodum. The species was cultured with the other two and was treated identically except for the following details:

1. The nymphs used were 0.8 mm to 1.2 mm long. This size range corresponded, like that used for the other two species, to second, third and small fourth nymphal instars.
2. Each batch of R. padi comprised only 100 nymphs, not 200. These were exposed at 25 nymphs per pot on four separate pots.
3. For the first three batches no count was done on the fourth day of exposure, so that only the total mortality for the whole 8-day monitoring period could be calculated.
4. Extra care was taken with the counting as R. padi often occupied sheltered feeding positions around stem bases.

3.2.3 Weather monitoring

The results from both winters were analysed using a combination of weather data recorded next to the experi-

mental plot and data from local weather stations, provided by the Meteorological Office. In the 1978/79 winter the Met. Office data were obtained from Hurn Airport, near Bournemouth (Grid Ref. SU106985), 17 km S.S.W. of the Fordingbridge study site. In the 1979/80 winter most of the Met. Office data used were obtained from Southampton Weather Centre (Grid Ref. SU 420115), 7 km S.S.E. of the study site at Chilworth. The wind speed data used were recorded at Calshot, Hampshire (Grid Ref. SU 474015), 18 km S.S.E. of Chilworth.

In the 1978/79 winter the following weather data were recorded next to the experimental plot:

1. Temperature: maximum and minimum temperatures in each 4-day period were recorded by a max.-min. mercury thermometer in a Stevenson screen standing on the ground, with the thermometer about 5 cm above ground level. A model D Grant Temperature Recorder was also set up to record temperatures 2 cm above ground level in the study plot every hour but examination of the chart records after the experiment showed that the recorder was faulty and so the records were not used.
2. Rainfall: total rainfall in each 4-day period was measured using a 5 inch (12.7 cm) rain gauge.
3. Snow: days when snow was falling or lying were recorded.

In the 1979/80 winter more weather data were collected at the experimental plot:

1. Temperature: maximum and minimum temperatures for each 4-day period were recorded by two max.-min. mercury thermometers, one inside a Stevenson's screen at ground level and the other out in the experimental plot with its bulbs about 2 cm above ground level. This thermometer was at about the same height as the aphids and also at about the same

height as thermometers used to record standard 'grass minimum' temperatures. Hourly temperatures at this height were also recorded using a thermistor connected to a model D Grant Temperature Recorder.

2. Rainfall: a recording rain gauge was used to record rate of rainfall. Its calibration was checked using a 12.7 cm rain gauge.
3. Wind: a Woelfle type cup-anemometer recorded wind speed and direction 55 cm above ground level.

In the 1979/80 winter no snow fell during the experimental period.

3.2.4 Feeding positions

One criticism of the experiment's realism was that aphids introduced onto plants in the laboratory and then moved into the field might occupy more exposed feeding positions, and thus be more susceptible to weather mortality, than aphids in naturally occurring winter populations. To estimate the extent to which aphids moved into sheltered feeding sites or were selectively killed in more exposed sites, the feeding positions of S. avenae and M. dirhodum nymphs on a pot of winter wheat were recorded before exposure and after 8, 18 and 27 days in the experimental plot, starting on 1 March 1979. Feeding positions on a second pot were monitored in the same way before exposure and after 8 and 18 days, starting on 10 March 1979. Twelve variables were recorded, all related to the degree of shelter of the aphids' feeding sites.

3.2.5 Multiple regression analyses

Multiple regression analyses were a central part of the analysis. They were used to derive predictive equations from the results, of the general form:

$$y = a + b_1 x_1 + b_2 x_2 + b_3 x_3 \dots + b_i x_i$$

where:

y is the dependent variable, aphid mortality in each 4-day period;

$x_1, x_2, x_3 \dots x_i$ are the independent variables, representing the different weather factors (temperature, rain, wind, etc.);

$b_1, b_2, b_3 \dots b_i$ are their partial regression coefficients;

a is the intercept.

These analyses were done using a computer program which was part of the Statistical Package for the Social Sciences (S.P.S.S.) (Nie, Hull, Jenkins, Steinbrenner & Bent, 1975), run on the University of Southampton's ICL 2970 computer. Data for each species were always analysed separately. Before analysis the dependent variable (percentage aphid mortality after 4 days' exposure) was transformed according to the arcsin transformation ($\arcsin \sqrt{\text{percentage}}$) to normalize the distribution of percentages.

Using the S.P.S.S. program, independent variables could be incorporated into the multiple regression equation either all at once, or one at a time in a stepwise manner. When the stepwise inclusion option was chosen the independent variable incorporated at each step was the one that explained the greatest amount of the variance in the dependent variable not already explained by the regression equation. This was the variable (x_i) whose partial regression coefficient had the largest F-ratio, where:

$$F = \frac{\text{incremental sum of squares due to } x_i/1}{\text{residual sum of squares}/(N-k-1)}$$

with 1 and (N-k-1) degrees of freedom. (N = sample size (= number of 4-day periods) and k = number of independent variables in the regression equation).

A limit to the number of independent variables

included in the equation could be imposed by setting a maximum number of steps. Alternatively, a minimum value for the F-ratio could be set, so that only variables with partial regression coefficients significantly different from zero ($P < 0.05$) were included. The set of independent variables used was changed from one analysis to another. Often the set included several parameters representing the same weather factor. For example, rainfall might be represented by amount recorded in each period, duration, rate and amount x rate. Stepwise inclusion into the regression equation would then be used to determine which of these alternative parameters best modelled rainfall's influence on aphid mortality. Though it is assumed in multiple regression analysis that independent variables act independently and additively, non-additive interactions between weather factors could be modelled by combining two factors into a single variable before analysis. For example the product of mean wind speed and amount of rainfall could be used as a wind/rain interaction variable.

Care is needed when using multiple regression analysis. If large numbers of independent variables with little biological meaning are used, or if assumptions implicit in the method break down, then spurious but apparently significant relationships may be revealed. In this experiment, hypotheses and evidence on how weather factors act to produce mortality were used to select a relatively small set of 'meaningful' weather variables, and predictive equations were kept simple. After each analysis, residuals (differences between observed mortality and that predicted by the regression equation) were examined. A marked departure from a normal distribution of residuals shows that assumptions implicit in the use of regression analysis do not hold, but no obvious deviations from normality were found. Correlation coefficients between independent variables were also examined, as a high degree of inter-correlation leads to unstable equations whose variables and regression coefficients change markedly from sample to sample.

3.3 RESULTS AND ANALYSIS

Sections 3.3.1 to 3.3.5 below deal with the results for S. avenae and M. dirhodum only. The more limited data for R. padi are given in section 3.3.6.

3.3.1 'On-plant' and 'off-plant' mortality

Aphids dead or missing after a period of exposure could be divided into two categories:

1. Those found dead on the plants (Table 3.1 (c) and Table 3.2 (c)).
2. Those found dead on the soil or missing altogether (Table 3.1 (b) and Table 3.2 (b)).

These two categories are referred to below as 'on-plant' mortality and 'off-plant' mortality respectively, though the latter contained aphids which were missing rather than found dead, some of which must have been knocked off the pots but may have survived on adjacent plants in the study plot. The very few aphids that were found on the sticky-barriers were omitted from the analysis entirely (they were subtracted from the totals of aphids present before exposure).

It was thought that a few of the aphids found dead on the plants might have been drowned by rain but that the great majority killed by rain and wind were most probably washed, blown or brushed off the plants before death and so appeared as 'off-plant' rather than 'on-plant' mortality. Consequently, as snow only occurred in two 4-day periods, it was thought that low temperatures were the major cause of 'on-plant' mortality. To test this hypothesis the relationship between 'on-plant' mortality and low temperature was examined using scatter plots and simple regression analysis of 'percentage of aphids found dead on the plants' on grass minimum temperature. Grass minimum temperatures (rather than those in a Stevenson screen at ground-level or at 1.2 m) were used here because they were recorded by an unsheltered thermo-

Table 3.1 Mortalities in batches of S. avenae and M. dirhodum nymphs exposed for 4-day periods during the 1978/79 winter.

Period	Period code no.	(A) Percentage of aphids missing or found dead (= total mortality)		(B) Percentage of aphids missing or found dead off plants (= 'off-plant' mortality)		(C) Percentage of aphids found dead on plants (= uncorrected 'on-plant' mortality)		(D) Percentage of aphids found dead on plants, corrected for off-plant mortality (= corrected 'on-plant' mortality)	
		<u>S.avenae</u>	<u>M.dirhodum</u>	<u>S.avenae</u>	<u>M.dirhodum</u>	<u>S.avenae</u>	<u>M.dirhodum</u>	<u>S.avenae</u>	<u>M.dirhodum</u>
26/1-30/1	1	43.0	66.3	9.0	16.0	34.0	52.3	37.4	62.3
30/1- 3/2	2	30.8	45.9	13.0	1.6	17.8	44.3	20.5	45.0
3/2- 7/2	3	14.5	2.5	14.0	1.0	0.5	1.5	0.6	1.5
7/2-11/2	4	21.8	22.3	19.6	19.1	2.2	3.2	2.7	4.0
11/2-15/2	5	26.0	33.5	26.0	32.0	0	1.5	0	2.2
20/2-24/2	6	4.5	8.0	4.5	7.5	0	0.5	0	0.5
24/2-28/2	7	14.7	24.5	2.7	12.0	12.0	12.5	12.3	14.2
1/3- 5/3	8	5.0	4.5	5.0	4.0	0	0.5	0	0.5
5/3- 9/3	9	15.3	18.3	15.3	18.3	0	0	0	0
10/3-14/3	10	4.0	5.0	4.0	5.0	0	0	0	0
14/3-18/3	11	10.5	10.0	10.0	9.5	0.5	0.5	0.6	0.6
19/3-23/3	12	20.5	15.6	11.0	0	9.5	15.6	10.7	15.6
23/3-27/3	13	10.7	14.3	5.0	6.6	5.7	7.7	6.0	2.2
Means		17.0	20.8	10.7	10.2	6.3	10.8	7.0	11.4

Table 3.2 Mortalities in batches of S. avenae and M. dirhodum nymphs exposed for 4-day periods during the 1979/80 winter.

Period	Period code no.	(A) Percentage of aphids missing or found dead (= total mortality)		(B) Percentage of aphids missing or found dead off plants (= 'off-plant' mortality)		(C) Percentage of aphids found dead on plants (= uncorrected 'on-plant' mortality)		(D) Percentage of aphids found dead on plants, corrected for off-plant mortality (= corrected 'on-plant' mortality)	
		<u>S.avenae</u>	<u>M.dirhodum</u>	<u>S.avenae</u>	<u>M.dirhodum</u>	<u>S.avenae</u>	<u>M.dirhodum</u>	<u>S.avenae</u>	<u>M.dirhodum</u>
5/12- 9/12	14	15.6	14.0	15.6	14.0	0	0	0	0
9/12-13/12	15	42.5	33.3	41.9	33.3	0.6	0	1.0	0
15/12-19/12	16	27.6	8.6	27.6	8.6	0	0	0	0
19/12-23/12	17	10.6	7.9	10.6	7.9	0	0	0	0
31/12- 4/1	18	28.0	38.1	6.5	15.7	21.5	22.3	23.0	26.5
4/1 - 8/1	19	7.8	4.1	3.5	0	4.2	4.1	4.3	4.1
10/1 -14/1	20	9.7	10.0	2.8	1.0	6.8	9.0	7.0	9.1
14/1 -18/1	21	6.4	12.4	6.4	7.3	0	5.1	0	5.5
20/1 -24/1	22	53.7	67.5	53.1	64.5	0.6	3.0	1.3	8.5
24/1 -28/1	23	17.7	58.1	0	19.4	17.7	38.7	17.7	48.0
30/1 - 4/2	24	19.0	17.0	19.0	16.0	0	1.0	0	1.2
4/2 - 7/2	25	0	2.5	0	1.9	0	0.6	0	0.6
10/2 -14/2	26	0	2.0	0	2.0	0	0	0	0
14/2 -18/2	27	10.6	0	10.6	0	0	0	0	0
20/2 -24/2	28	1.5	4.5	0	2.0	1.5	2.5	1.5	2.6
24/2 -28/2	29	12.4	20.3	5.7	9.1	6.7	11.2	7.1	12.3
2/3 - 6/3	30	11.0	18.0	7.0	7.5	4.0	10.5	4.3	11.4
6/3 -10/3	31	4.5	1.2	4.5	0	0	1.2	0	1.2
Means		15.5	17.8	11.9	11.7	3.5	6.1	3.7	7.3

meter at approximately the same height as the aphids themselves.

The scatter plots (Figs. 3.2 and 3.3) and regression equations (Table 3.3 (a) and (b)) revealed clear, highly significant relationships between 'on-plant' mortality and grass minimum temperatures. The 'on-plant' mortality of M. dirhodum was higher than that of S. avenae in the colder periods but the threshold for 'on-plant' mortality was approximately -3°C for both species. Scatter plots and regression analyses also showed that no relationship existed between 'off-plant' mortality and grass minimum temperature (Figs. 3.4 and 3.5, Table 3.3 (c) and (d)).

3.3.2 Multiple regression analyses to derive predictive equations for aphid mortality using the full data set

To maximise the realism of the predictive equations the approach adopted was to derive two separate equations for each species, one to predict 'on-plant' mortality and the other 'off-plant' mortality. As the two types of mortality were to be predicted separately the mortality variables for the two equations had to be independent. However, as initially recorded, 'on-plant' mortality (the percentage of the aphids present initially which were found dead on the plants after exposure) was not independent of 'off-plant' mortality. For example, consider two periods in which 'on-plant' mortality factors acted with exactly equal strength, that is, they killed the same proportion of the aphids experiencing their effects in each period. Despite their identical strengths the amounts of 'on-plant' mortality actually recorded at the end of each period would not be identical if the extent of 'off-plant' mortality in the two periods differed. The recorded 'on-plant' mortality would be lower for the period in which 'off-plant' mortality factors acted more strongly because these would either remove more live aphids before conditions producing 'on-plant' mortality occurred, or they would remove more corpses initially killed by 'on-plant' mortality factors from the plants.

Fig. 3.2 Relationship between percentage of S. avenae nymphs found dead on wheat seedlings after exposure to winter weather conditions for a 4-day period and the grass minimum temperature for each period.

KEY □ : 4-day periods in the 1978/79 winter.
 ■ : 4-day periods in the 1979/80 winter.
 S□ : periods with snow.

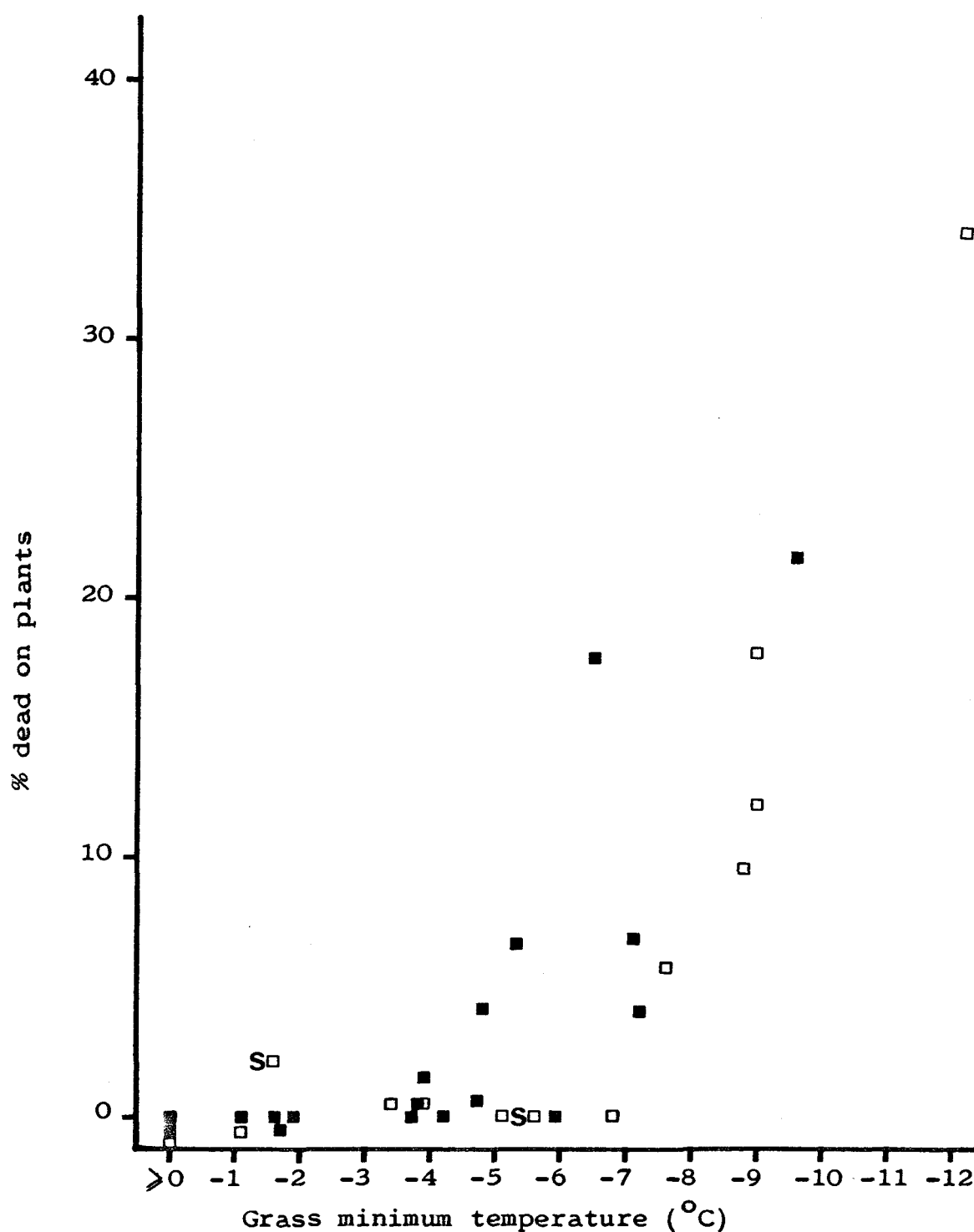


Fig. 3.3 Relationship between percentage of M. dirhodum nymphs found dead on wheat seedlings after exposure to winter weather conditions for a 4-day period and the grass minimum temperature for each period.

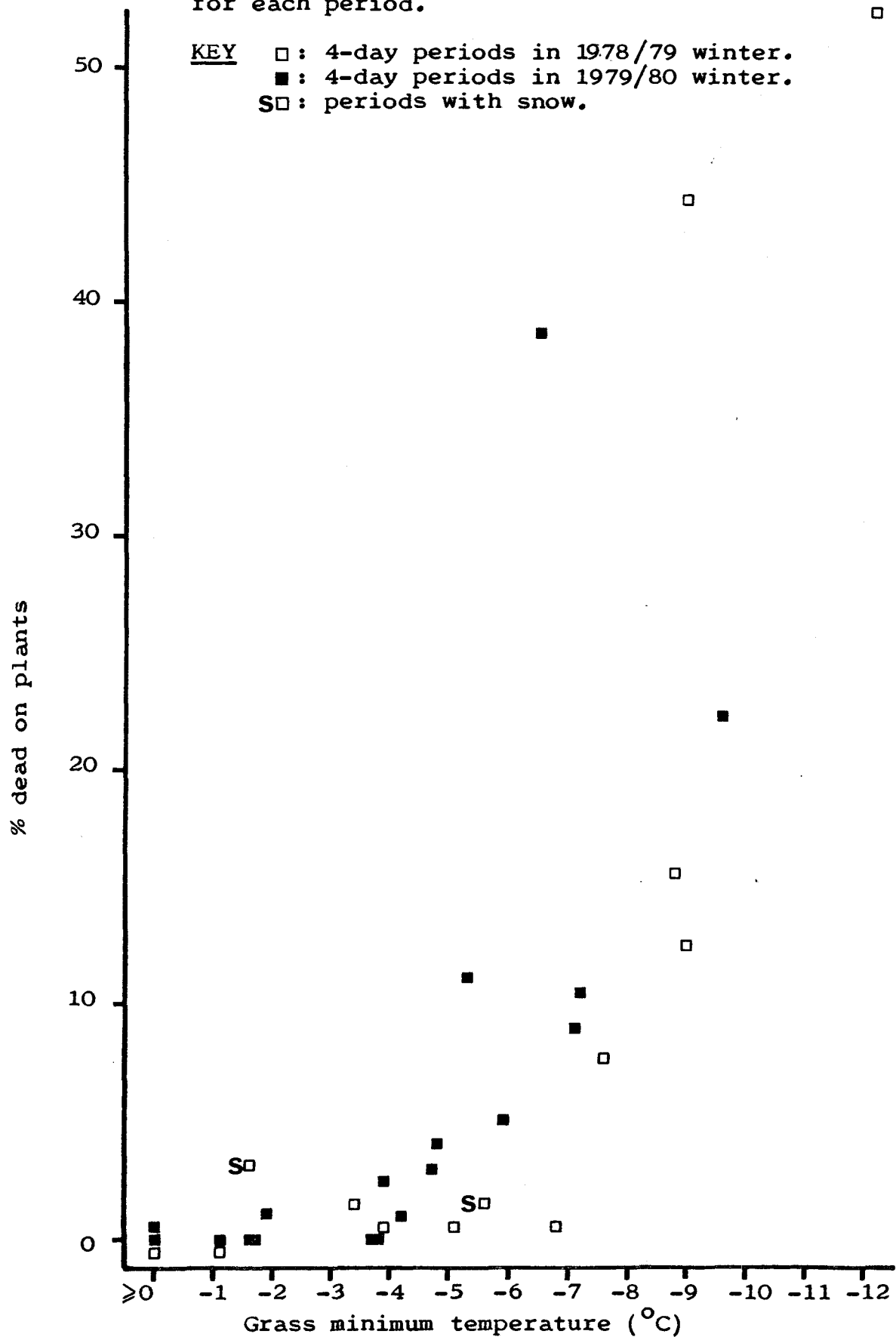


Table 3.3 Regression coefficients for mortalities of S. avenae and M. dirhodum nymphs (arcsin $\sqrt{\text{percentage mortality}}$) on grass minimum temperature ($^{\circ}\text{C}$).

Dependent variable	Regression coefficient for grass min. temp. (with S.E.)	F-ratio of regression coefficient (D.F. = 1 & 29)
(a) % <u>S. avenae</u> dead on plants	-1.99 (0.334)	35.17 (P<0.001)
(b) % <u>M. dirhodum</u> dead on plants	-2.55 (0.411)	38.61 (P<0.001)
(c) % <u>S. avenae</u> dead off plants or missing	0.13 (0.534)	0.056 (P>0.2)
(d) % <u>M. dirhodum</u> dead off plants or missing	-0.17 (0.576)	0.088 (P>0.2)

Fig. 3.4 Relationship between percentage of *S. avenae* nymphs found dead off wheat seedlings or missing after exposure to winter weather conditions for a 4-day period and the grass minimum temperature for each period.

KEY □ : 4-day periods in the 1978/79 winter.
 ■ : 4-day periods in the 1979/80 winter.
 S□ : periods with snow.

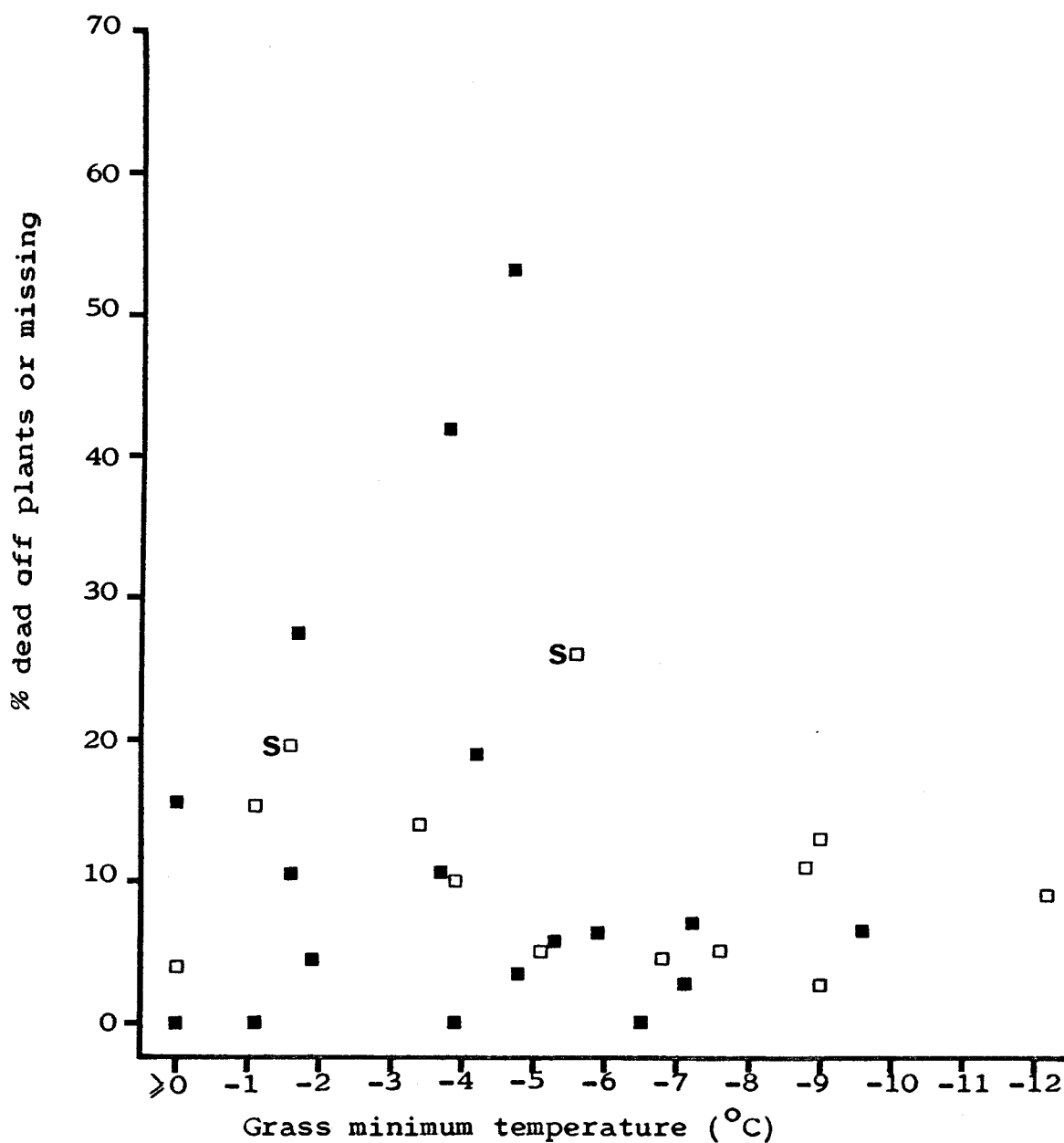
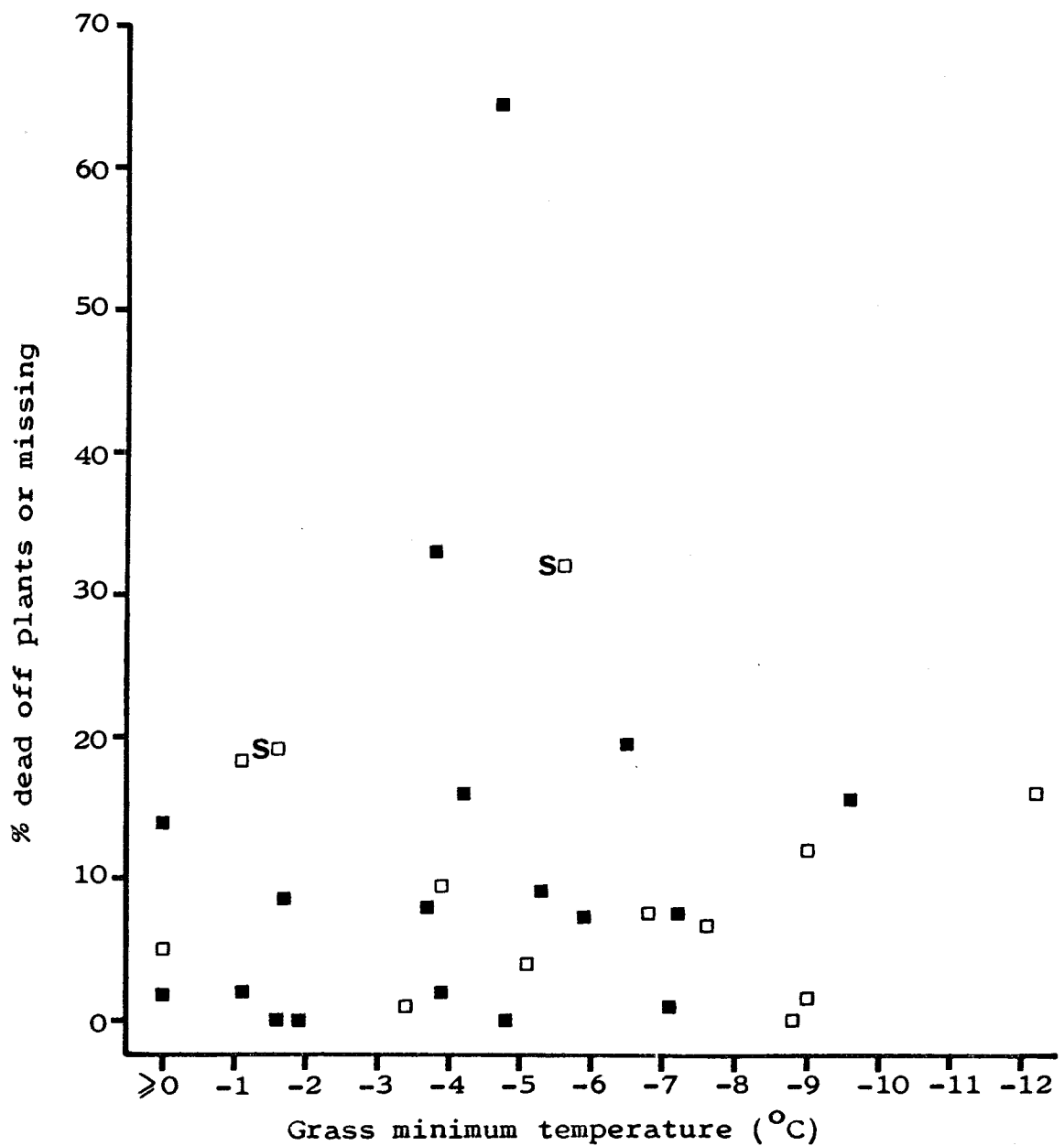


Fig. 3.5 Relationship between percentage of M. dirhodum nymphs found dead off wheat seedlings or missing after exposure to winter weather conditions for a 4-day period and the grass minimum temperature for each period.

KEY □ : 4-day periods in the 1978/79 winter.
 ■ : 4-day periods in the 1979/80 winter.
 S□ : periods with snow.



To eliminate this type of error an estimate had to be made of the proportion of the aphids present initially which would have been recorded dead on the plants after exposure if none had been removed, either before or after death, by 'off-plant' mortality factors. To do this it had to be assumed that living and dead aphids had approximately equal probabilities of being removed by 'off-plant' mortality factors. It is likely that dead aphids, attached by their stylets but usually not by their legs, were actually rather more likely to be removed than live ones, but given the short time interval between counts (4 days) the difference was unlikely to have produced important errors. Given this assumption it follows that:

Probability of an aphid present initially being found dead on the plants after exposure = (Probability of an aphid experiencing 'on-plant' mortality factors being killed by them) $\times$ (Probability of an aphid not being removed, either before or after death, by 'off-plant' mortality factors).

From this the corrected 'on-plant' mortality, expressed as a percentage = (percentage of aphids present initially actually found dead on the plants) $\div$ (100 - percentage of aphids found dead off the plants or missing).

In the event this correction produced only small changes in 'on-plant' mortalities (Tables 3.1 and 3.2; compare columns (C) and (D)) but in all analyses the corrected values were used. It should be noted that 'off-plant' mortality needed no equivalent correction, if it was assumed that living and dead aphids had approximately equal probabilities of being removed by 'off-plant' mortality factors.

The initial stage in deriving predictive equations for 'on-plant' and 'off-plant' mortality was to determine which of the main weather factors (temperature, rain, wind and snow) significantly influenced each type of mortality and to select suitable parameters to represent their effects. This was done by performing multiple regression analyses of 'on-plant' and 'off-plant' mortality (after arcsin transformation) on a set of weather variables

containing several alternative variables for each of the main weather factors (Table 3.4). This set only included variables which could be easily derived from standard weather station data. Whenever possible the variables used were recorded at the study site. To give an idea of the weather conditions during the experimental periods some are listed in Appendix 3.1.

Brief definitions of the variables are given in Table 3.4 but some require further explanation. The 'grass minimum temperature from -3°C ' (T2) was zero for all periods with minima of -3°C or above. For periods with minima below -3°C it was the difference between the minimum and -3°C , given as a positive value. The temperature variables involving means (T3, T4 and T5) were calculated from daily minima and/or maxima for each of the 4 days of a period. The mean and maximum temperature variables (T4 and T5) were included because it was thought that mean and maximum temperatures might modify the effects of other weather factors on the aphids. The wind speed data obtained from the Meteorological Office were mean wind speeds for each hour of the day and maximum gust speeds (instantaneous wind speeds) recorded in each hour, from which the wind variables W1 to W4 were easily obtained. Wind data recorded at the study site during the 1979/80 winter were not used in this part of the analysis because they were not directly comparable to Meteorological Office data, having been recorded at 55 cm, not at the standard height of 10 m.

Several of the variables used were designed specifically with the probable mode of action of a weather factor in mind. For example, R4 (rainfall amount $\times$ rate) was included because it was thought that heavy rainfall over a short period would knock more aphids off plants than a steady drizzle. Similarly, T2 (grass minimum temperature from -3°C) was included because scatter plots (Figs 3.2 and 3.3) had suggested an upper threshold for low temperature mortality of about -3°C .

As explained earlier, each batch of aphids was used for two consecutive 4-day periods, as it was counted 4 days after being put out and again 4 days later. Thus

Table 3.4 The set of weather variables used in regression analyses to determine predictive equations for 'on-plant' and 'off-plant' mortalities of S. avenae and M. dirhodum viviparae.

Code	Variable	Where recorded	
		1978/79	1979/80
	<u>TEMPERATURE</u> (°C)		
T1	Grass minimum	Hurn	Chilworth
T2	Grass minimum from -3.0°C	"	"
T3	Mean grass minimum	"	"
T4	Mean maximum*	"	Southampton
T5	Mean*	"	"
T6	Nights with grass minimum below -3.0°C	"	Chilworth
T7	Day-degrees below -3.0°C (grass minima)	"	"
	<u>RAIN</u>		
R1	Amount (mm)	Fording-bridge	Chilworth
R2	Duration (min.)	Hurn	"
R3	Mean rate (mm/100 min.)	"	"
R4	Amount x mean rate (mm ² /100 min.)	"	"
	<u>WIND</u> (knots)		
W1	Mean speed	Hurn	Calshot
W2	Maximum speed over 1 h	"	"
W3	Mean of maximum gusts recorded each h	"	"
W4	Maximum gust speed	"	"
	<u>SNOW</u>		**
S1	Presence/absence (0/1)	Fording-bridge	
S2	Days on which snow falling	"	
S3	Days on which snow falling	Hurn	
S4	Days on which snow lying at 9 a.m.	"	
S5	Sum of daily records of depth lying at 9 a.m. (cm)	"	
	<u>PERIOD</u>		
P1	1st/2nd period of a pair (1/2)		
P2	Winter (0 = 1978/79, 1 = 1979/80)		

* Recorded in a Stevenson screen at 1.2 m.

**No snow fell during the experimental period in the 1979/80 winter.

there was a possibility that aphids which had survived the first 4-day period would be significantly more hardy or sheltered than those initially put out (due to cold-hardening, selective mortality of less cold-hardy aphids or those in exposed feeding positions, or to movement to more sheltered positions). These effects might have been large enough to significantly change the relationships between mortality and weather conditions. To investigate this possibility a period variable (P1) was included with the set of weather variables. Periods representing an aphid batch's first 4 days of exposure were given a value of one and those representing the second were given a value of two. A second period variable (P2) was used in the same way to check for significant differences between results from the 1978/79 and the 1979/80 winters. Though these two period parameters were included with the weather parameters in all regression analyses they never showed partial regression coefficients significantly different from zero ($P > 0.05$).

In the analyses stepwise inclusion into the regression equations was used and variables whose partial regression coefficients were not significantly different from zero ($P > 0.05$) were not allowed in. In nearly all periods in which the grass minimum temperature was above -3°C there was no 'on-plant' mortality (Figs 3.2 and 3.3). Thus, to keep the distribution of residuals approximately normal (an assumption implicit in regression analysis) all regressions using 'on-plant' mortality as the dependent variable were performed omitting these warmer periods.

Regressions of 'on-plant' mortality on the set of variables shown in Table 3.4 revealed that the low temperature variable T2 (grass minimum from -3°C) had the most significant partial regression coefficient ($P < 0.001$) for both species (Tables 3.5 and 3.6). This variable alone explained approximately 70% of the total variation in 'on-plant' mortality ($r^2 \approx 0.7$ for both species). Once T2 was in the equations no other variables had significant partial regression coefficients ($P > 0.05$).

Regressions for each species using 'off-plant' mortality as the dependent variable revealed that both W2

Table 3.5 Results of multiple regression analysis of S. avenae 'on-plant' mortality on the set of variables shown in Table 3.2.

Variable code	Partial regression coefficient		
	b	S.E.	F-ratio
T2	3.878	0.5951	42.47***

D.F.= 1 & 20

***: $P < 0.001$

Intercept = -1.122

Multiple correlation coefficient (r) = 0.82. $r^2 = 0.68$.

Table 3.6 Results of multiple regression analysis of M. dirhodum 'on-plant' mortality on the set of variables shown in Table 3.2.

Variable code	Partial regression coefficient		
	b	S.E.	F-ratio
T2	4.760	0.6628	51.59***

D.F.= 1 & 20

***: $P < 0.001$

Intercept = -0.3465

Multiple correlation coefficient (r) = 0.85. $r^2 = 0.72$.

(maximum wind speed over 1 h) and SI (presence or absence of snow) had significant partial regression coefficients ($P < 0.05$) (Tables 3.7 and 3.8). Together they explained just under 60% of the total variation in 'off-plant' mortality for S. avenae, and just under 50% of that for M. dirhodum. All the rain variables had very insignificant partial regression coefficients when incorporated into the 'off-plant' mortality equations ($P > 0.2$ in all cases) (Table 3.9). For M. dirhodum the low temperature variable T6 (nights with grass minimum below -3°C) had a partial regression coefficient of 2.406 which was very nearly significant ($F = 4.21$, D.F. = 1 & 27, $P = 0.05$). This suggested a possible modifying effect of low temperature on 'off-plant' mortality.

Having determined the main weather factors with significant predictive value for each type of mortality, further analyses were undertaken with additional independent variables for two reasons:

1. To see if the relationships already determined were significantly curved, so that it was worth including quadratic (x^2) terms in the predictive equations. (This could not be investigated for snow, which only occurred in two periods.)
2. To see if interactions between weather factors produced significant modifying effects on mortality.

Six interaction variables were tested (Table 3.10), each the product of two other weather variables. The first three (TR1, TW1, RW1) represented possible low temperature/rain, low temperature/wind and rain/wind interactions. They were the products of the variables which best represented the effects of the three main weather factors, as determined from the previous regression analyses. The second three interaction variables (TT2, TR2, TW2) represented possible interactions between each of the three main weather factors and mean temperature. It was thought that low mean temperature might increase 'off-plant' mortality caused by rain or wind by reducing the

Table 3.7 Results of multiple regression analysis of S. avenae 'off-plant' mortality on the set of variables shown in Table 3.2

Variable code	Partial regression coefficient			
	b	S.E.	F-ratio	
W2	1.260	0.2135	34.84***	D.F. = 1 & 28 ***: $P < 0.001$ ** : $P < 0.01$
S1	14.983	5.3185	7.94**	

Intercept = -10.429

Multiple correlation coefficient (r) = 0.77. $r^2 = 0.59$.

Table 3.8 Results of multiple regression analysis of M. dirhodum 'off-plant' mortality on the set of variables shown in Table 3.2

Variable code	Partial regression coefficient			
	b	S.E.	F-ratio	
W2	1.179	0.2585	20.82***	D.F. = 1 & 28 ***: $P < 0.001$ * : $P < 0.05$
S1	17.400	6.4377	7.31*	

Intercept = -9.606

Multiple correlation coefficient (r) = 0.69. $r^2 = 0.48$

Table 3.9 F-ratios associated with the partial regression coefficients of rain variables when these variables were incorporated into regression equations of 'off-plant' mortality on W2 and S1.

Dependent variable	Rain variable			
	R1	R2	R3	R4
<u>S. avenae</u> off-plant mortality	0.10	0.92	0.75*	1.26*
<u>M. dirhodum</u> off-plant mortality	0.35	0.79	0.24	0.01

(D.F. = 1 & 27, $P > 0.2$ in all cases)

*partial regression coefficients positive.

Table 3.10 The set of interaction variables and first order polynomial terms used in regression analyses to determine predictive equations for 'on-plant' and 'off-plant' mortalities of S. avenae and M. dirhodum viviparae.

Code	Variable
	<u>INTERACTION VARIABLES</u>
TR1	Temperature (grass min. from -3°C) x Rainfall (amount x mean rate) (= T2 x R4)
TW1	Temperature (grass min. from -3°C) x Wind (max. speed over 1 h) (= T2 x W2)
RW1	Rainfall (amount x mean rate) x Wind (max. speed over 1 h) (= R4 x W2)
TT2	Temperature (mean) x Temperature (grass min. from -3°C) (= T5 x T2)
TR2	Temperature (mean) x Rainfall (amount x rate) (= T5 x R4)
TW2	Temperature (mean) x Wind (max. speed over 1 h) (= T5 x W2)
	<u>POLYNOMIAL TERMS</u>
$T2^2$	(Grass min. temp. from -3°C) ²
$W2^2$	(Max. wind speed over 1 h) ²

aphids' mobility, and might have altered the effects of low minimum temperatures either by producing additional cold-stress or by increasing cold-hardiness.

In the analyses of 'on-plant' mortality, T2 was entered into the regression equations, followed by the interaction variables and the quadratic term $T2^2$ if any of these had partial regression coefficients significantly different from zero ($P < 0.05$). However, no variable, apart from T2, had a significant partial regression coefficient for either species. In the analyses of 'off-plant' mortality, W2 and S1 were entered into the regression equations, followed by the interaction variables and the quadratic term $W2^2$ if any had partial regression coefficients significantly different from zero ($P < 0.05$). For both species no interaction variables had significant partial regression coefficients, but the quadratic term did (Tables 3.11 and 3.12), showing that the relationships between 'off-plant' mortality and maximum wind speed over 1 h were significantly curved for both species. Scatter plots of 'on-plant' mortality after arcsin transformation against grass minimum temperature and of 'off-plant' mortality after arcsin transformation against maximum wind speed over 1 h are shown for each species in Figs 3.6 to 3.9.

3.3.3 Additional regression analyses using data from the 1979/80 winter only

Extra analyses only using data from the 1979/80 winter were undertaken in order to try and identify more subtle weather mortality effects which might have failed to show up in the analyses of the full data set. Subtle effects could have remained hidden when the two winters' data were analysed together for two reasons. Firstly, many of the weather variables recorded during the 1978/79 winter were recorded away from the study site. Consequently, errors due to differences in weather conditions between the weather recording site (Hurn) and the study site (Fordingbridge) could have masked subtle mortality effects. Secondly, when the full data set was analysed,

Table 3.11 Results of multiple regression analysis of S. avenae 'off-plant' mortality on W2 (max. wind speed over 1 h), $W2^2$ and the set of interaction variables shown in Table 3.10.

Variable code	Partial regression coefficients		
	b	S.E.	F-ratio
W2	-1.412	1.1428	1.53
$W2^2$	0.05725	0.02411	5.64*
S1	16.158	4.9509	10.65**

D.F. = 1 & 27

* : $P < 0.05$

** : $P < 0.01$

Intercept = 18.282

Multiple correlation coefficient (r) = 0.81. $r^2 = 0.66$.

Table 3.12 Results of multiple regression analysis of M. dirhodum 'off-plant' mortality on W2 (max. wind speed over 1 h), $W2^2$ and the set of interaction variables shown in Table 3.10.

Variable code	Partial regression coefficients		
	b	S.E.	F-ratio
W2	-2.792	1.3080	4.56*
$W2^2$	0.08507	0.02760	9.50**
S1	19.146	5.6667	11.42**

D.F. = 1 & 27

* : $P < 0.05$

** : $P < 0.01$

Intercept = 33.054

Multiple correlation coefficient (r) = 0.79. $r^2 = 0.62$.

Fig. 3.6 Relationship between the arcsin transformed 'on-plant' mortality of *S. avenae* nymphs after exposure to winter weather conditions for a 4-day period and the grass minimum temperature for each period. (Periods with grass minima above -3°C omitted.)

KEY $\square$: 4-day periods in the 1978/79 winter.
 $\blacksquare$: 4-day periods in the 1979/80 winter.
 $\text{\$}$: period with snow.
 $\square$

Regression line: $y = -1.122 + 3.878x$

(where x = difference between grass min. temp. and -3°C)

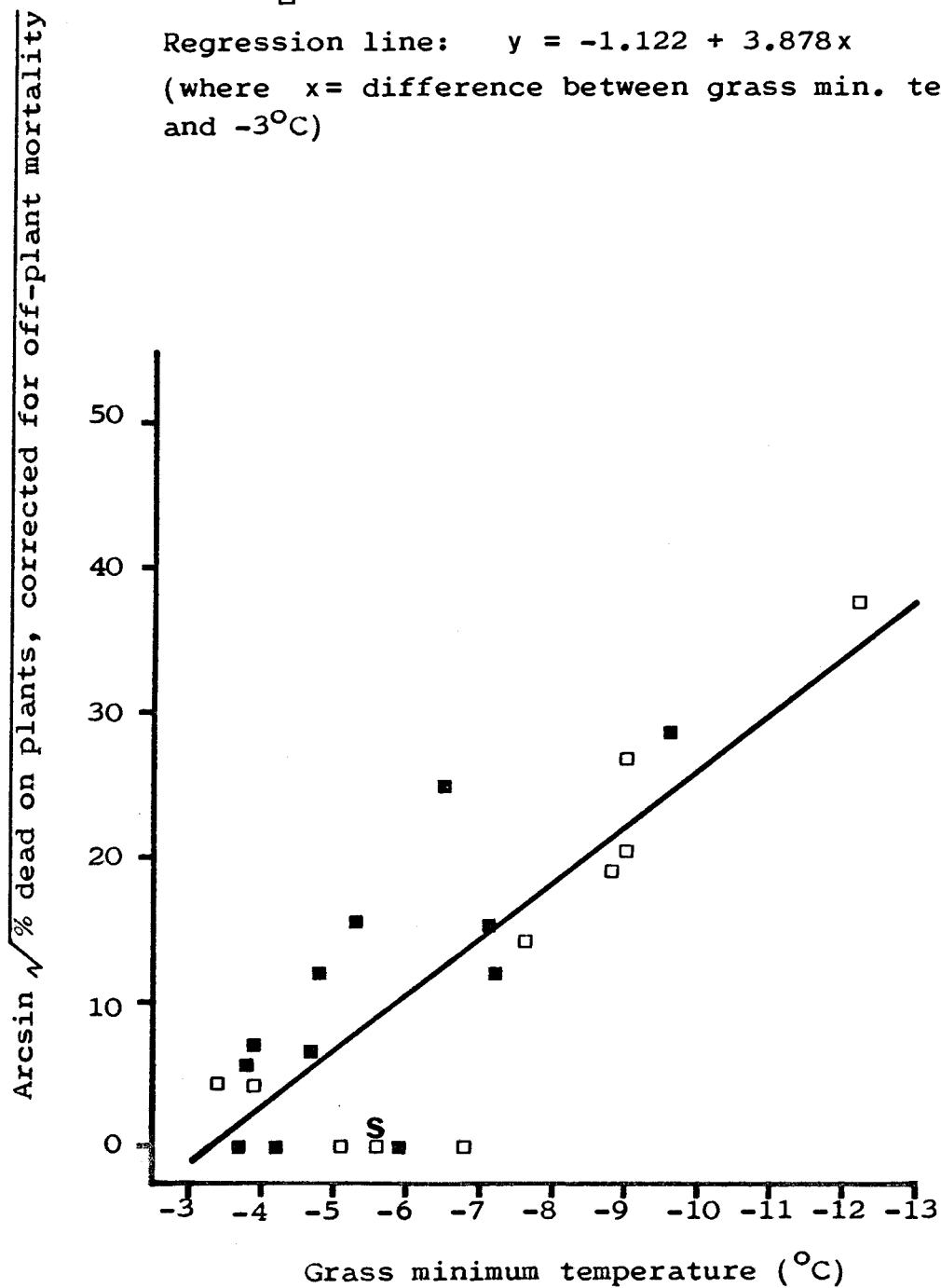


Fig. 3.7 Relationship between the arcsin transformed 'on-plant' mortality of *M. dirhodum* nymphs after exposure to winter weather conditions for a 4-day period and the grass minimum temperature for each period. (Periods with grass minima above -3°C omitted).

KEY $\square$: 4-day periods in the 1978/79 winter.
 $\blacksquare$: 4-day periods in the 1979/80 winter.
 $S\square$: period with snow.

Regression line: $y = -0.347 + 4.760x$

(where x = difference between grass min. temp. and -3°C)

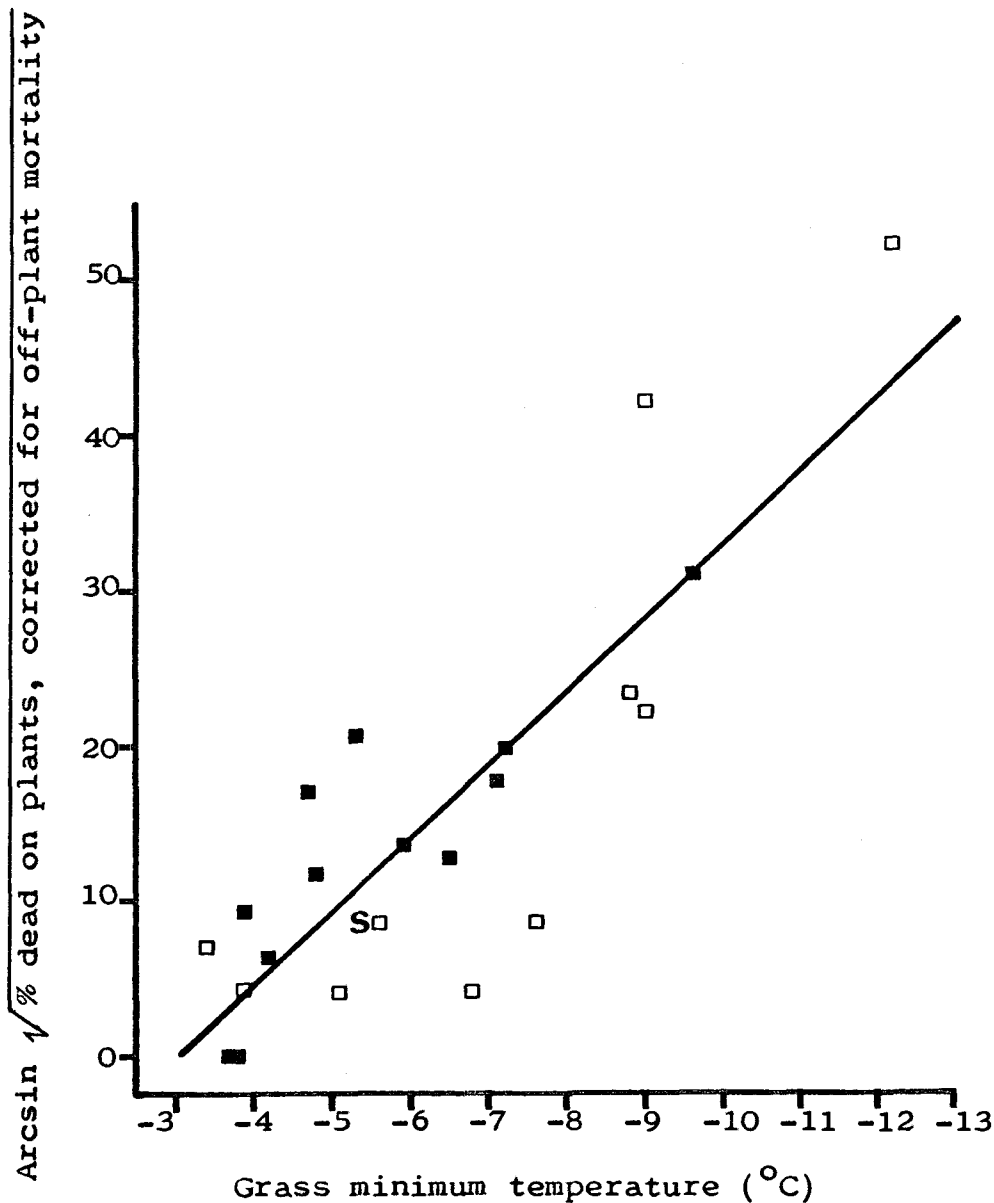


Fig. 3.8 Relationship between the arcsin transformed 'off-plant' mortality of *S. avenae* nymphs after exposure to winter weather conditions for a 4-day period and the maximum wind speed recorded over 1 h for each period.

KEY □ : 4-day periods in the 1978/79 winter.
 ■ : 4-day periods in the 1979/80 winter.
 S□ : periods with snow.

Regression line: $y = 18.282 - 1.412x + 0.05725x^2$

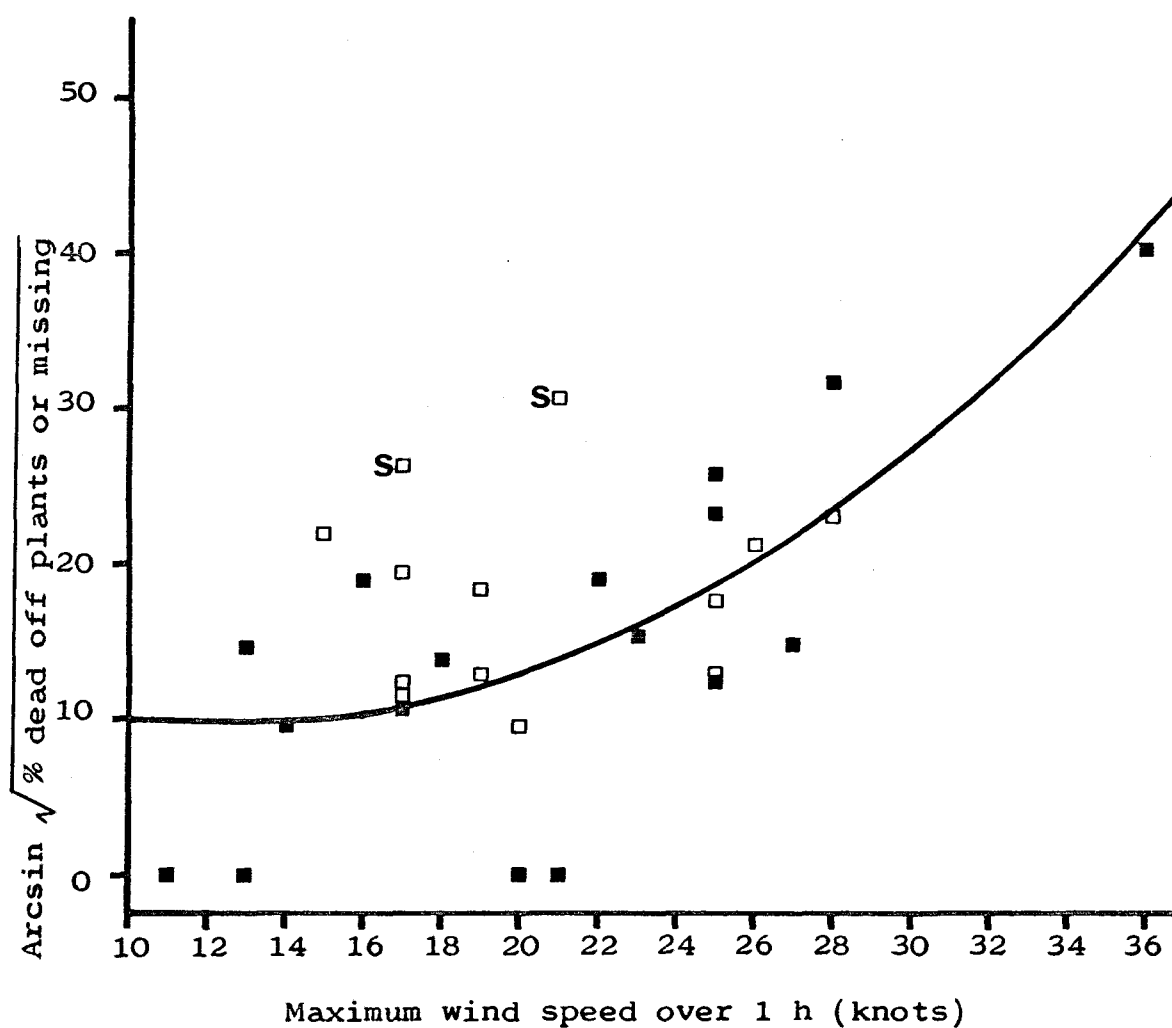
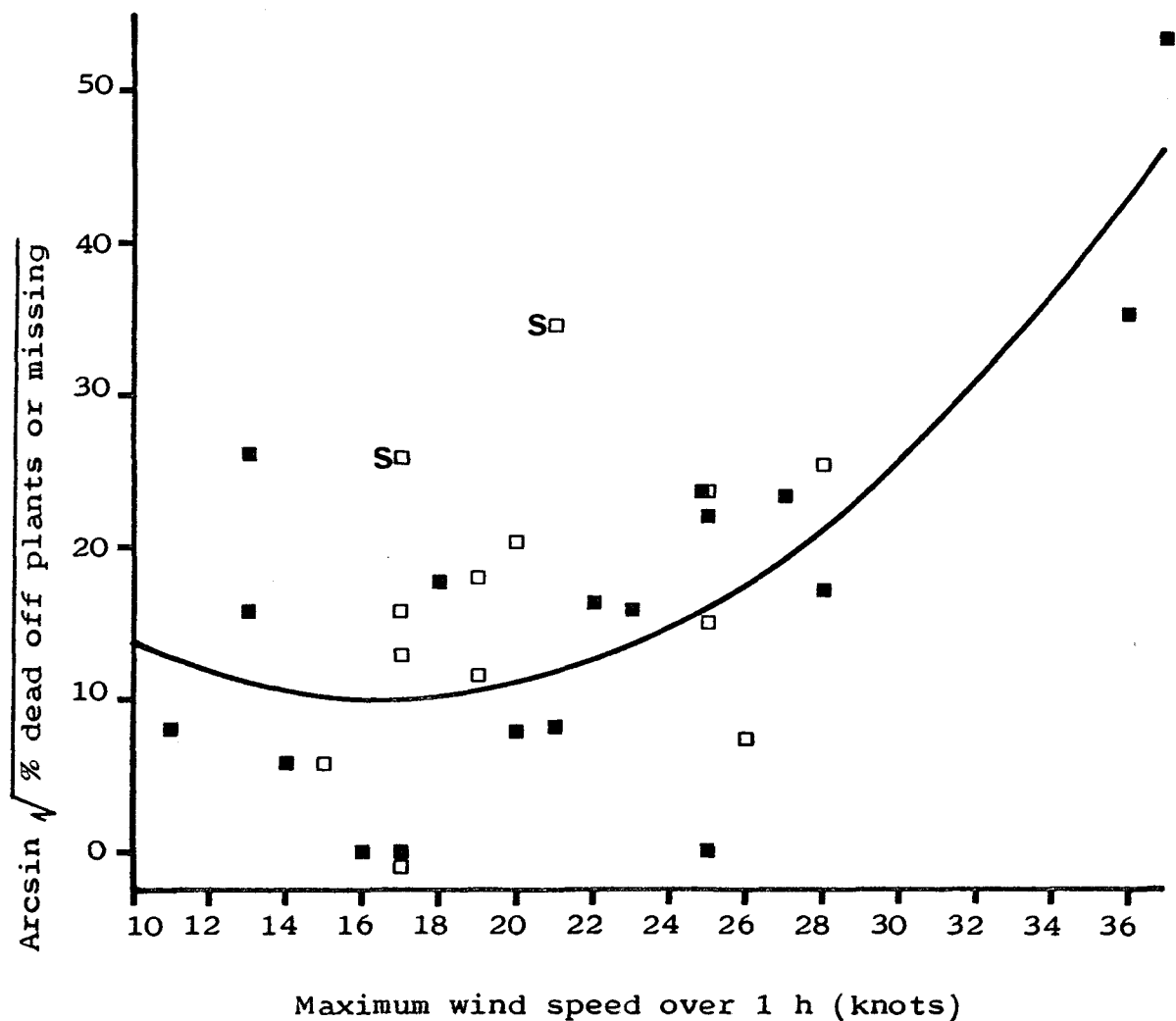


Fig. 3.9 Relationship between the arcsin transformed 'off-plant' mortality of *M. dirhodum* nymphs after exposure to winter weather conditions for a 4-day period and the maximum wind speed recorded over 1 h for each period.

KEY □ : 4-day periods in the 1978/79 winter.
 ■ : 4-day periods in the 1979/80 winter.
 S□ : periods with snow.

Regression line: $y = 33.054 - 2.792x + 0.08507x^2$



several extra weather variables recorded at the study site in the 1979/80 winter only could not be used because their values for the 1978/79 winter could not be derived from standard Meteorological Office data. The extra analyses of 'on-plant' and 'off-plant' mortality using the second winter's data only are described below.

(a) 'On-plant' mortality analyses

Firstly, regression analyses were undertaken of each species 'on-plant' mortality on the variables T2 to T7* (the set of temperature variables used in earlier analyses), P2* and an additional temperature variable, grass mean temperature (T8). This was calculated from daily maxima and minima recorded at the study site. As in the equivalent analyses of both winters' data, only periods with grass minimum temperatures below -3°C (the threshold for 'on-plant' mortality) were considered. The variables with significant ($P < 0.05$) partial regression coefficients were allowed into the equations in a stepwise manner. Results showed that for both species grass minimum temperature from -3°C (T2) was the best predictor of 'on-plant' mortality and once it was included in the equations no other variables had significant partial regression coefficients (Tables 3.13 and 3.14). Thus results using the 1979/80 winter's data agreed with those using the full data set. Intercepts, partial regression coefficients and multiple correlation coefficients were similar, but not identical, to those determined using both winters' data (compare Table 3.5 with Table 3.13 and Table 3.6 with Table 3.14).

Further analyses were undertaken on the 1979/80 data to test whether the relationships between 'on-plant' mortalities and low temperature were significantly curved, and whether any interactions between weather variables had significant effects. As with the equivalent regressions using both winters' data the best temperature variable (T2) was entered into the equations first, followed by various interaction variables (see Table 3.10)

*see Table 3.4 for definitions of all these variables.

Table 3.13 Results of multiple regression analysis of S. avenae 'on-plant' mortality on a set of temperature variables (1979/80 data only).

Variable code	Partial regression coefficient		
	b	S.E.	F-ratio
T2	4.075	1.0596	14.79**

D.F. = 1 & 10

** : $P < 0.01$

Intercept = 0.217

Multiple correlation coefficient (r) = 0.77. r^2 = 0.60.

Table 3.14 Results of multiple regression analysis of M. dirhodum 'on-plant' mortality on a set of temperature variables (1979/80 data only).

Variable code	Partial regression coefficient		
	b	S.E.	F-ratio
T2	4.255	0.807	27.79**

D.F. = 1 & 10

** : $P < 0.01$

Intercept = 2.397

Multiple correlation coefficient (r) = 0.86. r^2 = 0.74.

and the polynomial term $T2^2$ if any of these had significant partial regression coefficients. The results were the same as when both winters' data had been used: no variables apart from $T2$ had significant partial regression coefficients.

In laboratory tests dry, cold-hardened S. avenae and M. dirhodum had not suffered chill-injury above about -7°C (Chapter 2, experiments 2.4.2 and 2.4.3) but S. avenae had died at temperatures as high as -3°C when ice was present on the body surface, presumably due to inoculative freezing (Chapter 2, experiment 2.4.7). Comparing these two mortality thresholds derived from laboratory tests with the threshold of -3°C for 'on-plant' mortality determined in the present field experiment, strongly suggested that in the field aphids died from inoculative freezing as well as chill-injury. It was thus thought that the realism and predictive value of regression equations for 'on-plant' mortality might be improved if two separate variables were used, each modelling one of the two kinds of low temperature mortality. Analyses were undertaken to test this, using variables recorded at the study site during the 1979/80 winter.

In choosing a set of alternative variables to model inoculative freezing, the following assumptions were made (the first three are based on the results of laboratory experiments):

1. Inoculative freezing was predominantly due to ice on the body surface, not to inoculation from the host's phloem.
2. The threshold for inoculative freezing was about -3°C .
3. The chance of freezing was mainly dependent on the length of time aphids experienced surface ice and a temperature below -3°C but, if other conditions were constant, there was not a strong relationship between the chance of freezing and minimum temperature below -3°C .

4. Though surface ice may occasionally have been frozen rain drops or dew it was usually hoar frost.

No direct measure of hoar frost was available so indirect ones had to be calculated. Heavy hoar frost forms on nights when low temperatures and low wind speeds occur together. It is probably also increased by high relative humidity the preceding day but as no suitable humidity measures were available for the study site this factor had to be ignored. The five alternative variables chosen to represent inoculative freezing were thus all based on low wind speed and time below -3°C (Table 3.15). To model chill-injury, the laboratory determined upper threshold for acclimated aphids (-7°C) was assumed and so 'grass minimum temperature from -7°C ' (T9) was used.

For each species, regressions were undertaken in which the chill-injury variable T9 was included in predictive equations for 'on-plant' mortality, followed by the inoculative freezing variable with the highest partial regression coefficient (TLW1 to TLW5, see Table 3.15). For both species this proved to be TLW5 (hours with temperature $< -3^{\circ}\text{C}/(\text{mean wind speed})^2$) and its partial regression coefficient was significant ($P < 0.05$) (Tables 3.16 and 3.17). However, for M. dirhodum the new equation using T9 and TLW5 gave a worse fit to the data than did the old one using T2 only (multiple correlation coefficients were 0.82 and 0.86 respectively) and T9 had a very insignificant partial regression coefficient ($P > 0.2$). For S. avenae, T9 also had a very insignificant partial regression coefficient ($P > 0.2$) and though the multiple correlation coefficient for the new equation was greater than that for the old one using T2 (0.88 compared to 0.77) the way 'on-plant' mortality was predicted by the new equation was not realistic. The partial regression coefficient for T9 was negative (Table 3.16) suggesting that chill-injury increased with increasing temperature. Thus, though it might have a sound theoretical basis, there was little justification for using the new, more complex model for 'on-plant' mortality.

Table 3.15 The set of variables used to model the occurrence of hoar frost.

Variable code	Description
TLW1	Hours with temperature $< -3^{\circ}\text{C}$ and wind speed ≤ 2 knots.
TLW2	Hours with temperature $< -3^{\circ}\text{C}$ and wind speed ≤ 3 knots.
TLW3	Hours with temperature $< -3^{\circ}\text{C}$ and wind speed ≤ 4 knots.
TLW4	Hours with temperature $< -3^{\circ}\text{C}$ Mean wind speed during this time (knots)
TLW5	Hours with temperature $< -3^{\circ}\text{C}$ (Mean wind speed during this time) ²

Table 3.16 Results of multiple regression analysis using a new way of modelling S. avenae low temperature mortality with separate variables to represent the effects of chill-injury and inoculative freezing by hoar frost (1979/80 data only).

Variable code	Partial regression coefficient			D.F. = 1 & 10
	b	S.E.	F-ratio	
T9	-1.184	3.0660	0.15	
TLW5	1.220	0.3135	15.15**	** : $P < 0.01$

Intercept = 2.423

Multiple correlation coefficient (r) = 0.88. $r^2 = 0.77$.

Table 3.17 Results of multiple regression analysis using a new way of modelling M. dirhodum low temperature mortality with separate variables to represent the effects of chill-injury and inoculative freezing by hoar frost (1979/80 data only).

Variable code	Partial regression coefficient		
	b	S.E.	F-ratio
T9	1.215	3.4511	0.12
TLW5	0.8964	0.35282	6.45*

D.F. = 1 & 10

*: $P < 0.05$

Intercept = 6.741

Multiple correlation coefficient (r) = 0.82. $r^2 = 0.67$.

Table 3.18 The extra rain and wind variables used in the analysis of 'off-plant' mortality using the 1979/80 data only.

Variable code	Description
R5	$\sum (\text{mean rate for each bout}) (\text{mm}/100 \text{ min})$
R6	$\sum (\text{amount} \times \text{mean rate for each bout}) (\text{mm}^2/100 \text{ min})$
W5	Mean wind speed at 55 cm
W6	Max. wind speed over 1 h at 55 cm

(b) 'Off-plant' mortality analyses

Analyses of 'off-plant' mortality using data from the 1979/80 winter were similar to those in which data from both winters were used, but extra predictor variables were tested. Firstly, regressions of 'off-plant' mortality on a set of weather variables and the period variable P2 were undertaken. As before, stepwise inclusion was used and only variables with significant partial regression coefficients ($P < 0.05$) were allowed into the equations. The weather variables used were the four rain and four wind variables used earlier (R1 to R4 and W1 to W4, see Table 3.4) plus an additional two rain and two wind variables recorded at Chilworth (R5, R6, W5 and W6, see Table 3.18). No snow variables were included because no snow fell while aphids were exposed during the 1979/80 winter.

The two extra rain variables (R5 and R6) were calculated using the detailed information available from the recording rain gauge. For these, parameters were first calculated for each bout of rainfall separately, not for the 4-day period as a whole, and were then summed for all bouts in a particular 4-day period. A bout of rain was defined as a period during which at least 0.05 mm of rain fell and the rate never dropped below 0.035 mm/100 min (an extremely light drizzle). Despite the inclusion of the extra rain and wind variables recorded on-site, the results of these analyses were the same as those in which both winters' data were used: for both species the only variable to come into the predictive equations was W2 (maximum wind speed over 1 h, recorded at Calshot) (Tables 3.19 and 3.20). Intercepts, partial regression coefficients and multiple correlation coefficients were quite similar to those determined using the full data set.

More analyses were undertaken to test whether the relationship between 'off-plant' mortality and W2 was significantly curved and whether interactions between weather factors had significant effects. As in the equivalent analyses using the full data set, W2 was entered into the predictive equations first, followed by a set of interaction variables and the quadratic term $W2^2$, if any

Table 3.19 Results of multiple regression analysis of S. avenae 'off-plant' mortality on a set of rain and wind variables (1979/80 data only).

Variable code	Partial regression coefficient		
	b	S.E.	F-ratio
W2	1.472	0.2621	31.53***

D.F. = 1 & 16

***: $P < 0.001$

Intercept = -15.463

Multiple correlation coefficient (r) = 0.81. $r^2 = 0.66$.

Table 3.20 Results of multiple regression analysis of M. dirhodum 'off-plant' mortality on a set of rain and wind variables (1979/80 data only).

Variable code	Partial regression coefficient		
	b	S.E.	F-ratio
W2	1.219	0.335	13.19**

D.F. = 1 & 16

** : $P < 0.01$

Intercept = -10.027

Multiple correlation coefficient (r) = 0.67. $r^2 = 0.45$.

Table 3.21 Results of multiple regression analysis of M. dirhodum 'off-plant' mortality on W2 (max. wind speed over 1 h), $W2^2$ and a set of interaction variables (1979/80 data only).

Variable code	Partial regression coefficient		
	b	S.E.	F-ratio
W2	-3.350	1.5793	4.50
$W2^2$	0.09661	0.03288	8.63

D.F. = 1 & 15

*: $P < 0.05$

Intercept = 38.602.

Multiple correlation coefficient (r) = 0.81. $r^2 = 0.65$.

of these had significant partial regression coefficients. The interaction variables were the same six used earlier (see Table 3.10) plus an additional rain/wind interaction variable, RW2. This, unlike the previously used rain/wind variable (RW1) was based on parameters calculated for each bout of rainfall separately and excluded any measure of wind speed at times when no rain was falling. Bouts of rainfall were as defined above and the rain/wind parameter calculated for each bout was:

Amount of rain (mm) x mean rate of rainfall (mm/100 min) x mean wind speed during the bout (knots). This was summed for all bouts in a 4-day period to give the interaction variable RW2.

Despite the use of this more realistic rain/wind variable no interaction variables had significant partial regression coefficients for either species. For M. dirhodum the quadratic term $W2^2$ had a significant partial regression coefficient (Table 3.21). But for S. avenae, $W2^2$ was not significant (unlike the result of the equivalent analysis using both winters' data). For M. dirhodum the intercept, partial regression coefficients and multiple correlation coefficient for the equation including $W2$ and $W2^2$ were quite similar to those for the equivalent equation based on both winters' data.

3.3.4 Differences between total mortality of S. avenae and M. dirhodum in particular 4-day periods

Chi-squared tests on the proportions of each species dead or missing in each period revealed that the two species' total mortalities differed significantly ($\chi^2 > 3.84$, D.F. = 1, $P < 0.05$) in nine of the 31 4-day periods. Four of these were the four coldest periods, all with grass minimum temperatures below -8°C (Fig. 3.10) and in which the aphids suffered high 'on-plant' mortality (see Tables 3.1 and 3.2). In all of these the total mortality of M. dirhodum was significantly higher than that of S. avenae, strongly suggesting that in the field M. dirhodum was less cold-hardy.

Fig. 3.10 The relationship between total mortalities of S. avenae and M. dirhodum nymphs after exposure to winter weather conditions for 4-day periods (percentage dead or missing) and the grass minimum temperature in each period, showing those periods in which mortalities of the two species differed significantly ($P < 0.05$). (see Tables 3.1 & 3.2 for dates).

● or ○ = S. avenae mortality.

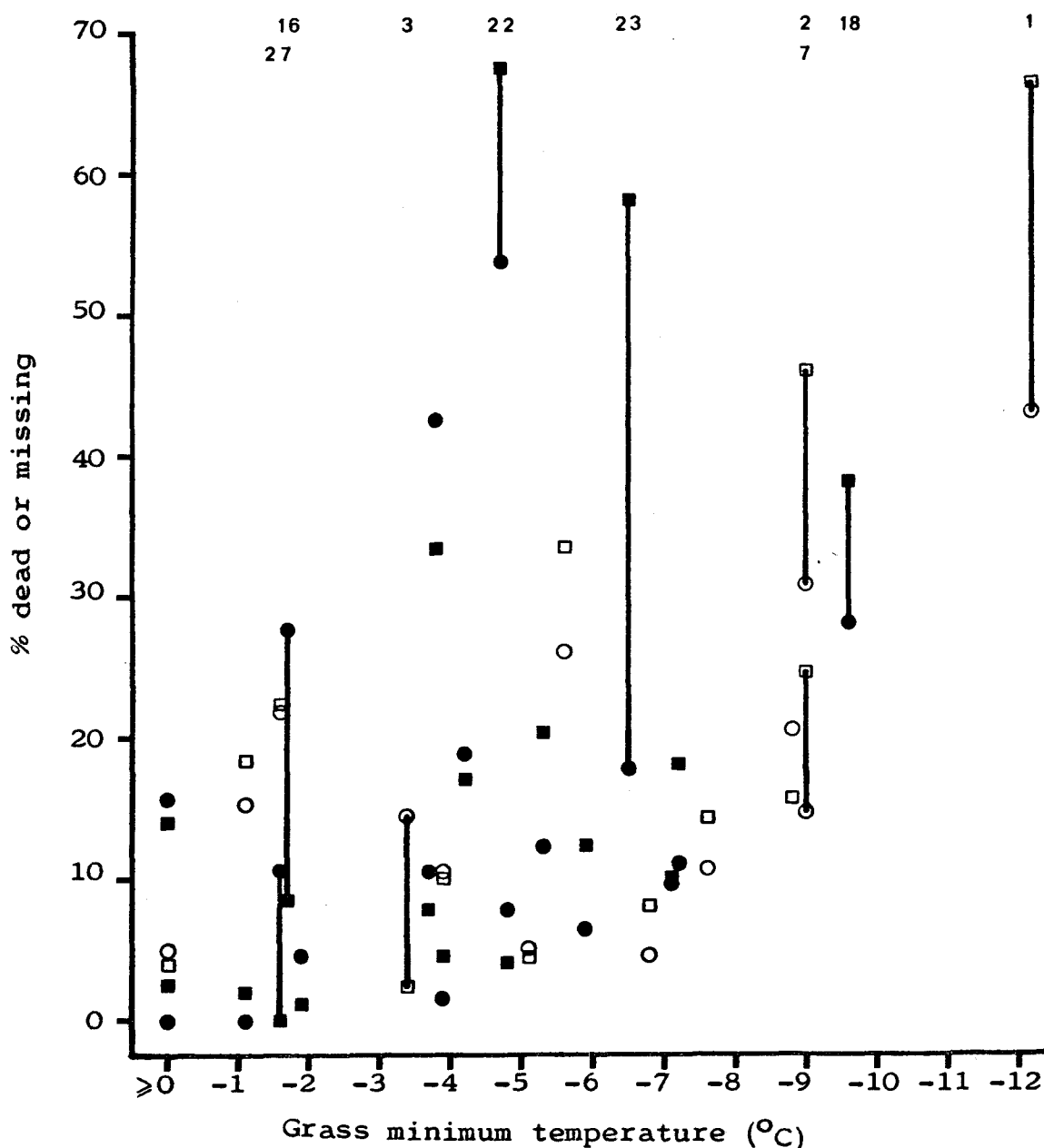
■ or □ = M. dirhodum mortality.

Hollow symbols = periods in the 1978/79 winter.

Solid symbols = periods in the 1979/80 winter.

Symbols joined by lines represent mortalities of S. avenae and M. dirhodum for periods in which the two species' mortalities differed significantly ($P < 0.05$).

CODE NUMBERS FOR PERIODS IN WHICH THE TWO SPECIES' MORTALITIES DIFFERED SIGNIFICANTLY:



For two of these four coldest periods (numbers 1 and 2) the differences between the species were due to differences in the amount of 'on-plant' mortality suffered (see Table 3.1), as was expected if low temperature was the factor responsible. But in the two other periods (numbers 7 and 18) the differences between the species were mainly due to differences in 'off-plant' mortalities, while 'on-plant' mortalities were similar (see Tables 3.1 and 3.2). This difference was not associated with higher wind speeds or rainfall in these two periods (see Appendix 3.1) but nevertheless suggests that M. dirhodum killed by low temperatures or in chill-coma were more likely to be knocked off the plants by wind or rain than were S. avenae. In one other period (number 23) differences in low temperature susceptibility were probably responsible for significantly greater mortality of M. dirhodum. This period had a grass minimum temperature of only -6.5°C , but all four nights were cold and the mean grass minimum, at -4.9°C , was the third lowest of any period. The differences in the species' total mortalities in this period were due to differences in both 'off-plant' and 'on-plant' mortality (see Table 3.2).

In the four remaining periods in which the species' total mortalities differed significantly grass minimum temperatures were above -5°C (Fig. 3.10), 'on-plant' mortalities were negligible and species differences were due to differences in 'off-plant' mortalities. In one of these periods (number 22) wind speeds were exceptionally high (maximum wind speed over 1 h = 37 knots) and mortalities of both species were high, with M. dirhodum significantly higher than S. avenae. But in the three remaining periods (numbers 3, 16 and 27), in which wind speeds were moderate to low, S. avenae suffered significantly more mortality than M. dirhodum. The possibility of a real difference between the two species' susceptibilities to weather factors other than low temperature was investigated further by examining differences between their total mortalities for the 23 periods in which 'on-plant' mortalities were less than 10%. Mean mortalities

for S. avenae and M. dirhodum in these periods were very similar (13.0% and 11.4% respectively) and a Wilcoxon's signed rank test revealed no evidence of a species difference ($T = 109.5$, $n = 23$, $P > 0.3$).

3.3.5 Changes in feeding position

Monitoring the feeding positions of S. avenae and M. dirhodum for several weeks during March 1979 (see section 3.2.4) showed that feeding positions did change in response to exposure to winter weather conditions, particularly in the first 8 days of exposure. The average distance of the aphids above the soil decreased (Table 3.22) and a greater proportion were found close to leaf axils (Fig. 3.11 (a)) and positioned on leaves so that they were protected from above (Fig. 3.11 (b)). All these changes should have resulted in a greater degree of shelter from wind and rain. Though a few aphids moved from leaves to leaf sheaths (which provided more sheltered conditions) the large majority remained on the leaves even after 18 days exposure (Fig. 3.11 (c)). The observed changes in feeding positions appeared to be due to movement to more sheltered positions and also selective mortality of aphids in more exposed positions.

3.3.6 The mortality of R. padi nymphs

The data available for R. padi were much more limited than those for the other two species, as only seven batches of R. padi were exposed (Table 3.23). Moreover, the first three batches of R. padi were not counted after 4 days of exposure, but only before exposure and after 8 days. Because of this, mortality and weather data for 8-day periods, rather than 4-day periods, were considered, so that data from all batches could be used. The amount of data was too small for regression analyses to be worthwhile but the relationship between total mortality of R. padi after 8 days of exposure and grass minimum temperatures was examined using a scatter plot (Fig. 3.12).

Table 3.22 Percentage of S. avenae and M. dirhodum at different heights above the ground on winter wheat seedlings, after exposure to winter weather conditions for different lengths of time.

(a) S. avenae

Height	Exposure time (days)			
	0	8	18	27
5-10 cm	16	0	0	0
2-5 cm	66	62	36	40
1-2 cm	16	24	33	40
0-1 cm	2	14	30	20
Sample size	50	42	33	5

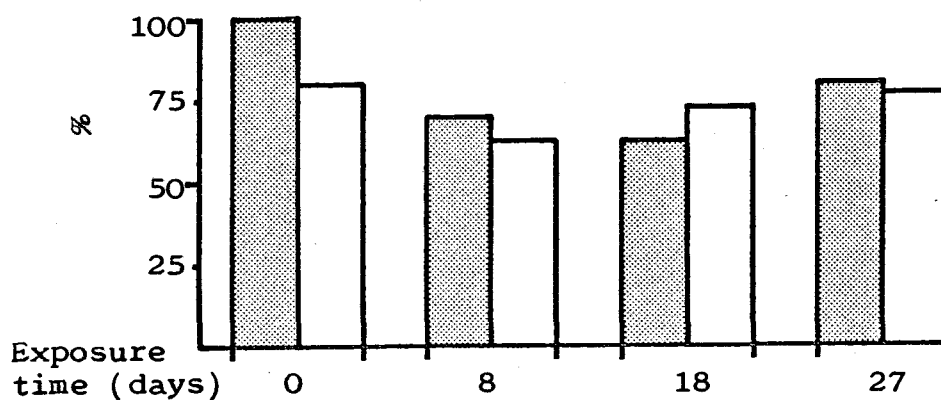
(b) M. dirhodum

Height	Exposure time (days)			
	0	8	18	27
5-10 cm	2	0	0	0
2-5 cm	56	51	24	44
1-2 cm	32	41	68	44
0-1 cm	10	8	8	11
Sample size	50	39	25	9

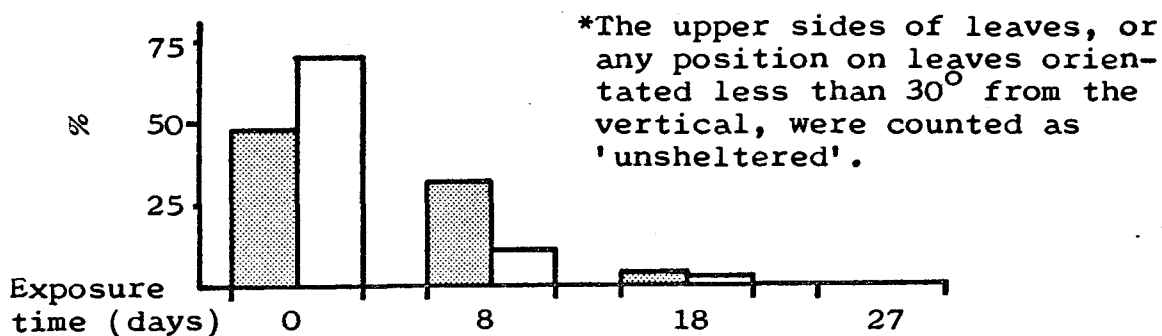
Fig. 3.11 Changes in the feeding position of S. avenae and M. dirhodum on winter wheat seedling after exposure to winter weather conditions for different lengths of time. (See Table 3.22 for sample sizes.)

■ = S. avenae □ = M. dirhodum

(a) Percentage of aphids more than 5 mm from a leaf axil.



(b) Percentage of aphids in unsheltered* feeding positions.



(c) Percentage of aphids feeding on leaves, rather than leaf sheaths.

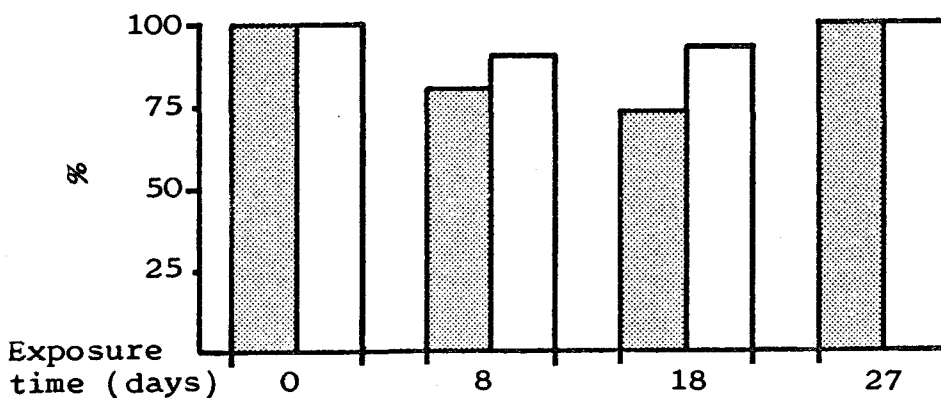


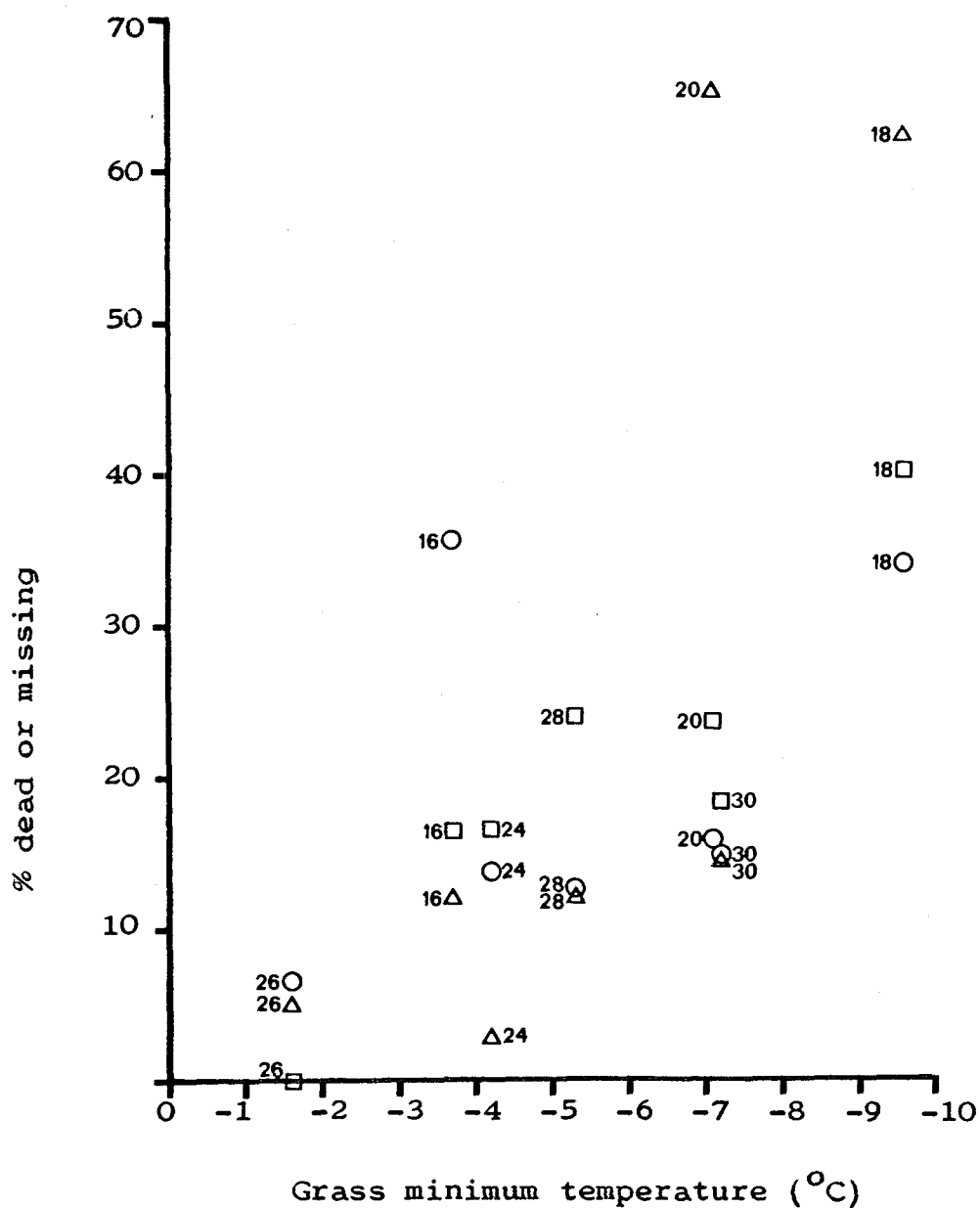
Table 3.23 Mortalities in batches of R. padi nymphs exposed in the field during the 1979/80 winter.

Batch number	Period	Period code no.	MORTALITIES		
			Total (% dead or missing)	'Off-plant' (% dead off plants or missing)	'On-plant'* (% dead on plants)
1	15/12-23/12	16 + 17	22.0	20.0	2.0
2	31/12- 8/1	18 + 19	62.0	38.0	24.0
3	10/1 -18/1	20 + 21	65.0	37.0	28.0
4	30/1 - 4/2	24	0	0	0
4	4/2 - 7/2	25	2.9	2.9	0
5	10/2 -14/2	26	6.0	6.0	0
5	14/2 -18/2	27	0	0	0
6	20/2 -24/2	28	0	0	0
6	24/2 -28/2	29	12.0	5.0	7.0
7	2/3 - 6/3	30	13.1	1.0	12.1
7	6/3 -10/3	31	0	0	0

* not corrected for 'off-plant' mortality.

Fig. 3.12 Relationships between the total mortalities of S. avenae (O), M. dirhodum (□) and R. padi (Δ) nymphs after exposure to winter weather conditions for 8 days (percentage dead or missing) and the grass minimum temperature recorded in each period.

Numbers are the code numbers for the first 4-day period of each 8-day period.



There was very little sign that total mortality increased as minimum temperature decreased, and little 'on-plant' mortality, unless temperatures dropped below -6°C (Fig. 3.12, Table 3.23). In two of the three periods in which minimum temperatures fell below -6° the mortality of R. padi was over 60%, much higher than that for S. avenae and M. dirhodum. These two periods had minimum temperatures of -9.6°C (period 18 + 19) and -7.1°C (period 20 + 21). However, in period 30 + 31, which had a minimum of -7.2°C , the mortality of R. padi was only 13.1%, slightly lower than that for the other two species.

Examination of other weather characteristics of these three periods showed that the discrepancy between the last cold period and the first two was attributable to less severe low temperature conditions in the last period, despite its low minimum temperature. In the first two cold periods, in which R. padi mortality was high, at least six of the eight nights had grass minima below -3°C and mean temperatures and mean grass minimum temperatures were low (Table 3.24). For the third cold period however, there were only two nights with grass minima below -3°C and mean temperatures and mean grass minimum temperatures were considerably higher.

In the four periods in which grass minimum temperatures stayed above -6°C there was very little 'on-plant' mortality for any species and the total mortality of R. padi was generally lower than that for S. avenae and M. dirhodum (Fig. 3.12), which suggested that R. padi suffered less mortality from wind or rain than the other two species did. The significance of this species difference was tested using 'off-plant' mortality data for the last four batches of aphids exposed. Unlike the first three batches R. padi in the last four were counted after 4 and 8 days of exposure, as were S. avenae and M. dirhodum, so that the data for all three species were exactly comparable. Chi-squared tests (Table 3.25) showed that in these batches the 'off-plant' mortality of R. padi was significantly lower than that for either S. avenae or M. dirhodum ($P < 0.01$ in both cases).

Table 3.24 Weather conditions in the three 8-day periods in which R. padi were exposed and grass min. temperatures fell below -6°C .

	PERIODS		
	18 + 19	20 + 21	30 + 31
Percentage of <u>R. padi</u> dead or missing	62.0	65.0	13.1
Grass minimum temperature ($^{\circ}\text{C}$)	-9.6	-7.1	-7.2
Mean grass minimum temperature ($^{\circ}\text{C}$)	-4.65	-5.65	-0.8
Mean temperature ($^{\circ}\text{C}$)	+3.7	+1.25	+6.3
Nights with min. temp. $< -3^{\circ}\text{C}$.	6	8	2
Mean wind speed (knots)	9.25	8.75	10.7
Maximum wind speed over 1 h (knots)	27	14	25
Rainfall (mm)	10.8	1.0	19.0

Table 3.25 Total numbers of S. avenae, M. dirhodum and R. padi (a) alive (b) dead off the plants or missing after exposure to winter weather conditions for 8 days. (Data for four separate batches of each species, covering the periods 24 to 31.)

Species	Number alive	Number dead off or missing	Total number
<u>S. avenae</u>	683	69	752
<u>M. dirhodum</u>	672	63	735
<u>R. padi</u>	372	16	388

Results of χ^2 tests on species differences:

R. padi v. S. avenae: $\chi^2 = 8.75$, D.F. = 1, $P < 0.01$.

R. padi v. M. dirhodum: $\chi^2 = 7.02$, D.F. = 1, $P < 0.01$.

3.4 DISCUSSION

3.4.1 The effect of low temperature

Analysis strongly suggested that low temperatures caused more or less all 'on-plant' mortality but little if any 'off-plant' mortality. For S. avenae and M. dirhodum enough field data on the relationship between 'on-plant' mortality and low temperature were available to allow comparisons with the results of earlier laboratory experiments described in Chapter 2.

When tested in the laboratory for 90 min after acclimation for 6 days at 6°C S. avenae and M. dirhodum nymphs had LT50s of -10.1°C and -8.6°C respectively (Chapter 2, experiment 2.4.2). From the 'on-plant' mortality equations determined in the present experiment (Tables 3.5 and 3.6), the LT50s for these species, given very similar laboratory acclimation but exposed to a natural winter temperature regime, were both several degrees lower: -14.9°C for S. avenae and -12.5°C for M. dirhodum. However, these shifts in LT50 values between laboratory and field were expected from the results of earlier investigations of the effects of varying the rates of cooling and re-warming in laboratory tests (Chapter 2, experiment 2.4.1). If it was assumed that the low temperature survival of the acclimated aphids used in the present field experiment would be improved to the same extent as that of unacclimated aphids when the artificially fast cooling regimes used in the laboratory tests were replaced by much slower, natural rates of temperature change, then the LT50s predicted for the field were close to those observed. From the results of the laboratory experiment on cooling and re-warming rates a downward shift in the LT50 of about 3.7°C* was expected for S. avenae, giving a predicted field LT50 of -13.8°C (= -10.1 - 3.7). For M. dirhodum the expected shift was 2.4°C*, giving a predicted field LT50 of -11.0°C (= -8.6 - 2.4). These predictions were both between 1°C and 2°C higher than the observed field LT50s. The small discrepancies were probably mainly due to additional cold-hardening in the field between the time of exposure and the time when lethal low temperatures were first experienced.

*estimated using mortality/temperature curves for unacclimated aphids determined in experiment 2.4.2.

On theoretical grounds, relationships between chill-death and low temperature should be sigmoidal in shape, if variation in cold-hardiness is normally distributed in aphid populations. In the present experiment 'on-plant' mortality rarely exceeded 50% and so the relationships between 'on-plant' mortality and grass minimum temperature should theoretically have been the lower halves of sigmoid curves. However, it was found that the relationships were not significantly curved. This was because using the arcsin transformation to normalize the distribution of percentage mortalities also had the effect of correcting curvature in the mortality/temperature relationships.

The close agreement between observed field LT50s and those predicted from earlier laboratory experiments tends to confirm several important conclusions from the laboratory work:

1. The laboratory test procedure can be used to approximately determine field low temperature susceptibility, provided the effect of using artificially rapid cooling and re-warming rates is allowed for.
2. Cold-hardened S. avenae viviparae are less susceptible to low temperatures than similarly cold-hardened M. dirhodum, but only slightly so.
3. The species' LT50s, however they are determined, lie well above their mean supercooling points. This strongly suggests that these species usually die of chill-injury or inoculative freezing long before spontaneous nucleative freezing can occur. (The mean supercooling points of S. avenae and M. dirhodum were determined as -23.0°C and -24.4°C respectively, see Chapter 2, experiment 2.3.2.)

Other features of 'on-plant' mortality also tend to confirm the findings of laboratory experiments. Firstly, the threshold for 'on-plant' mortality (about -3°C) agreed well with the threshold for inoculative freezing from surface ice observed for S. avenae in the laboratory

(Chapter 2, experiment 2.4.7) but was well above the upper threshold for chill-injury in acclimated aphids tested in the laboratory (c. -7°C or below, see Chapter 2, experiment 2.4.2). This supports the finding that both S. avenae and M. dirhodum can die from inoculative freezing as well as chill-injury. During periods when grass minimum temperatures were low enough for inoculative freezing but too high for chill-injury to be important (those with minimum temperatures lying between about -3°C and -7°C) percentage 'on-plant' mortalities were much lower than those found in the main series of laboratory tests in which S. avenae nymphs were tested while covered with ice: 'on-plant' mortality was nearly always under 10%, compared to c. 70% mortality in the laboratory tests. Several reasons why the laboratory tests probably exaggerated the importance of inoculative freezing have already been given (Chapter 2, experiment 2.4.7). The results of this field experiment suggest that these reasons apply and that in the field chill-injury is the most important cause of low temperature mortality.

Though the threshold for 'on-plant' mortality strongly suggested that inoculative freezing occurred in the field, attempts to improve predictive equations by modelling low temperature mortality using separate variables for inoculative freezing and chill-injury failed. However, this was not surprising given the very indirect measure of the occurrence of hoar frost which had to be used and the fact that low temperature mortality appears to be dominated by chill-injury, not inoculative freezing.

The fact that good predictive equations for 'on-plant' mortality could be based on grass minimum temperatures alone, supports another finding of the laboratory work: that the extent of low temperature mortality in S. avenae and M. dirhodum was largely governed by the minimum temperature experienced and that varying exposure time had little effect (Chapter 2, experiment 2.4.4). However, from theoretical considerations it was suggested earlier (Chapter 2, experiment 2.4.7) that when inoculative freezing rather than chill-injury was the cause of

low temperature mortality increased exposure time below -3°C should increase mortality. There was some supporting evidence for this from the present experiment. Examination of scatter plots (Figs 3.2 and 3.3) revealed that periods in the 1978/79 winter with grass minimum temperatures between -3° and -7°C generally had lower 'on-plant' mortality than those with similar minima in the 1979/80 winter. From the range of minimum temperatures, these were periods in which 'on-plant' mortality should have been caused mainly by inoculative freezing, not chill-injury. To investigate the apparent discrepancy between the two field seasons, the temperature conditions in the periods concerned were examined more closely. It was found that most periods in the 1979/80 winter (with relatively high 'on-plant' mortality) had three or four nights with minima below -3°C , while in the 1978/79 winter the periods concerned only had one or two nights below -3°C .

Evidence from this experiment suggested that exposure time had little influence on the low temperature mortality of S. avenae and M. dirhodum. However, as the aphids were only exposed for 8 days, the possibility remained that 'chronic' low temperature mortality due to cold-stress accumulated gradually over several weeks in the field might occur. Such mortality might well be more closely linked to mean temperatures and the duration of cold weather than to minimum temperatures. Consequently aphids in natural populations might suffer low temperature mortality even when temperatures do not drop below -3°C , the upper threshold observed here and in earlier laboratory work. Longer term field experiments on S. avenae and R. padi described in Chapter 5 produced evidence that such 'chronic' low temperature mortality was important for S. avenae on perennial rye grass and R. padi on perennial rye grass or winter wheat but was not shown by S. avenae on winter wheat. Unfortunately no comparable data were obtained for M. dirhodum but the results of Smith (1981) suggested that when on winter wheat 'chronic' low temperature mortality was not important for this species, as artificial winter colonies persisted as well as those of S. avenae.

Other features of the experimental design and analysis apart from the short exposure time in the field may have made the equations determined for 'on-plant' mortality only approximate predictors of low temperature mortality in natural populations:

1. Mortality due to chill-injury may have been overestimated because aphids had limited low temperature experience before exposure in the field (6.5°C for 6 days). However, in laboratory tests the LT50s of similarly laboratory acclimated aphids were found to differ from those given long-term field acclimatization by only 1.7°C (S. avenae) and 1.4°C (R. padi) (see Chapter 2, experiments 2.4.2 and 2.4.3).
2. Mortality due to inoculative freezing may have been overestimated because aphids occupied uncharacteristically exposed feeding positions due to their short exposure in the field (see section 3.3.5) and were thus atypically exposed to hoar frost.
3. It has been assumed that all low temperature mortality was 'on-plant' mortality, and corrected 'on-plant' mortalities were calculated assuming that living aphids and those killed on the plants were equally likely to be knocked off. However, it is likely that corpses were actually rather more easily dislodged than live aphids because they remained attached only by their stylets. For M. dirhodum this view is supported by the fact that the low temperature variable 'nights with grass minimum temperature below -3°C' was nearly significant as a predictor of 'off-plant' mortality ($P = 0.05$).

Though the amount of data for R. padi was too limited for the species' cold-hardiness in the field to be quantified at all accurately it was clear that, as in laboratory tests, the species was less cold-hardy than S. avenae or M. dirhodum. However, R. padi showed consider-

ably better low temperature tolerance in the field than in laboratory tests, with little 'on-plant' mortality unless temperatures fell below -6°C . As with S. avenae and M. dirhodum, this improvement was probably largely due to the much slower cooling and re-warming rates experienced in the field. From the laboratory experiment on cooling and re-warming rates (Chapter 2, experiment 2.4.1) the field LT50 of acclimated R. padi was expected to be about 4°C lower than that found in laboratory tests, that is about -10°C^* .

However, predictions of the low temperature mortality of R. padi in the field based on minimum temperatures may be quite unfeasible as the species showed very different mortalities in 8-day periods with similar, low minimum temperatures (see section 3.3.6 and Fig. 3.12). This was apparently due to heavy mortality linked to repeated low temperature exposure on successive nights, despite the fact that in laboratory tests (Chapter 2, experiment 2.4.4) R. padi mortality was insensitive to changes in exposure time. The discrepancy between laboratory and field results may have been due to R. padi being more susceptible to inoculative freezing than the other two species. Whatever the reason, it is clear that more field-work on R. padi is needed before low temperature mortality in natural populations can be predicted, particularly as longer term field experiments (Chapter 5) revealed that R. padi suffers 'chronic' low temperature mortality not closely correlated with minimum temperatures.

Evidence in the literature on the characteristics of low temperature mortality has already been discussed with reference to the laboratory experiments (Chapter 2) and need not be reiterated here. It is worth noting, however, that the two other intensive winter field studies on cereal aphid viviparae (Smith, 1981; Hand, 1982), though they did not aim to quantify low temperature mortality to the same extent, gave results in broad agreement with the present study. For example Hand (1982) found that declines in natural populations were associated with cold periods, particularly those with minima below -9° or -10°C and in the coldest periods R. padi appeared to suffer

*estimated using mortality/temperature curve for unacclimated R. padi determined in experiment 2.4.2.

to a greater extent than S. avenae.

Given the differences in methodology between the present experiment and that undertaken by Smith (1981), detailed comparisons of mortality/low temperature relationships in the two studies are very difficult. However, though Smith tended to play down the role of low temperature when discussing his results, percentage mortalities in his wind and rain protected colonies of S. avenae were not unexpectedly low compared to those in the present study, except in a period when the aphids were insulated from extreme temperatures by snow. Smith found aphids dead on the plants after severe cold but these never outnumbered those surviving and his conclusion that "temperatures as low as -10°C do not have what could be termed a catastrophic effect on S. avenae populations" is born out by the present study.

However, Smith did record exceptionally high mortality (60%) in exposed treatments when the aphids were subjected to -8°C while covered in frozen dew. This suggests much greater mortality due to inoculative freezing than was found in the present experiment, perhaps because frozen dew is a more efficient inoculator than hoar frost. This was also suggested by the high mortality of S. avenae tested in the laboratory while wet (Chapter 2, experiment 2.4.7). In the 1977/78 winter, when colonies of M. dirhodum as well as S. avenae were monitored, Smith found no evidence that S. avenae was more cold-hardy than M. dirhodum. M. dirhodum populations reproduced faster than S. avenae and actually increased when sheltered. However, conditions were relatively mild (temperatures dropped below -5°C on only six nights) so that the slightly greater low temperature mortality of M. dirhodum determined in the present study would have produced little effect.

3.4.2 The effects of wind and rain

Heavy rainfall has been implicated as an important mortality factor in several studies of natural aphid populations. For example in Britain high mortalities in

summer cereal aphid populations have been attributed to rainfall by Dean & Wilding (1971), Dransfield (1975) and Jones (1979). In the U.S.A. large reductions of Schizaphis graminum populations on cereals after heavy rain have been reported in both summer (Fenton & Fisher, 1940) and winter (Lowe, 1952). The observations of Fenton & Fisher suggested that it was the rate of rainfall, rather than the amount, that was decisive in causing mortality. In the Punjab, N. India, Grewal & Bains (1975) observed mortalities of over 50% in populations of Macrosiphum miscanthi and Rhopalosiphum maidis on wheat which they attributed to rainfall. Also in the Punjab, Dhaliwal & Singh (1975) reported "tremendous decreases" in natural populations of Macrosiphum miscanthi on wheat after rainfall, and in the laboratory simulated rain (10 or 20 mm) produced mortalities from 74% to over 90% if the aphids were exposed on plants which had not yet eared. Other authors who have suggested rain as an important mortality factor include Dunn & Wright (1955a) working on Acyrtosiphon pisum on lucerne in East Anglia, Jacob (1941) working on Myzus persicae on brassicas in North Wales, Lowe (1966) working on cereal aphids and the cabbage aphid Brevicoryne brassicae in New Zealand, Atwal, Chaudhary & Ramzan (1971) working on the cabbage aphid Lipaphis erysimi in India, Miskimen (1970) working on the sugar cane aphid Sipha flava in Puerto Rico and Leroux (1959) working on the apple aphid Aphis pomi in Canada.

Bakker & Robinson (1975) reported catastrophic losses in summer S. avenae populations on cereals in Canada due to high winds accompanied by heavy rain and hail. Also in Canada, Malyk & Robinson (1971) reported the same combined action of wind and rain for populations of Schizaphis graminum, S. avenae and M. dirhodum and this interaction between rain and wind has also been suggested by other authors (eg. Jacob, 1941; Leroux, 1959). It is also known that strong winds unaccompanied by rain can easily dislodge tree-living aphids such as the sycamore aphid Drepanosiphum platanoides (Dixon & McKay, 1970) which occupy high, exposed feeding positions. However, it has

rarely been suggested that wind acting alone can be an important cause of mortality for aphids on low-growing herbaceous hosts, though Jones (1979) has suggested that the build-up of summer populations of S. avenae and M. dirhodum on wheat and barley near Rothamsted, Hertfordshire was slowed appreciably by high winds in 1974 and 1975.

Thus from the literature one would conclude that heavy rainfall is generally a much more important mortality factor to aphids on cereals and other low plants than are strong winds. Nevertheless, the results of the present experiment very strongly suggest that the converse is true. Moreover, from his winter shelter cage experiment using S. avenae on seedling wheat, Smith (1981) also found that rainfall was much less important than high wind, which he concluded was the dominant winter mortality factor. There are several possible reasons for the discrepancy between the available information in the literature and the results of Smith (1981) and the present study:

1. Heavy rain is often accompanied by strong winds. Thus unless special efforts are made to separate their effects (as they were in the present study and that of Smith, but not in the others cited) the relative importance of the two factors can be easily confused.
2. Except for the overwintering populations of Schizaphis graminum on winter wheat observed by Lowe (1952) all the other aphids studied were not on low seedling crops but on relatively tall plants. Aphids in these less sheltered situations may have been more vulnerable to rain.
3. Several of the studies cited above were undertaken in parts of the world where rainfall is frequently very heavy by west European standards (eg. India and Puerto Rico).

4. In the other studies population declines after rain may have included indirect mortality due to Entomophthora infection, which may be important at summer temperatures.

Though it was clear from the present experiment and that of Smith (1981) that wind was much more important than rain as a mortality factor acting on the artificial populations studied, and though there was no sign of a significant wind/rain interaction, it is possible that the effect of rain on natural populations may be greater than suggested by the results. This is because the methodology of both experiments may have accentuated wind mortality somewhat and/or obscured a possible rain/wind interaction effect. In both experiments it was assumed that all aphids knocked off the test plants and onto adjacent parts of the study plot outside the pots were dead, whereas some may have found their way onto neighbouring plants and survived. Laboratory experiments by Smith suggested that large nymphs of S. avenae might be able to reinfest plants after they were dislodged, as they were capable of walking even at sub-zero temperatures and could survive off plants for at least 4 days at 5°C. Nevertheless, Smith rarely found aphids on seedlings beside infested test plants in the field, even though the test plants were not surrounded by sticky-barriers. He suggested that predation and mortality due to rainfall killed most dislodged aphids before they could regain plants. If rainfall was important in this respect this implies a real wind/rain interaction which would not have shown up in either his study or the present one.

A real wind/rain interaction was also suggested by the multiple regression analyses of data from the winter field samples taken by Hand (1982). Hand suggested from the results that high winds and heavy rainfall acted together to cause mortality, although periods when only one of these factors was extreme were rare, so that separating their effects was difficult. He pointed out that local topography may be important in determining the importance of rain, as rainwater may have a greater effect

on dislodged aphids (drowning them or hindering their resettling on plants) if it is running down a slope.

In laboratory experiments, Smith (1981) found that the probability of S. avenae being dislodged by wind increased with increases in mean wind speed and wind gustiness. In the present experiment it is likely that 'maximum wind speed over 1 h' proved to be the best wind variable for modelling 'off-plant' mortality because it represents a compromise between mean wind speed over the entire 4-day period and gustiness. The relationships between mortality and maximum wind speed over 1 h were markedly curved (Tables 3.11 and 3.12, Figs 3.8 and 3.9). Though the regression equations, particularly for M. dirhodum, predict increased 'off-plant' mortality as maximum hourly wind speed drops below 10 or 15 knots this is very unlikely to be a real effect. It is probably just a result of sampling error, as few 4-day periods with maximum hourly wind speeds below 15 knots were recorded. If the equations were being used for predictive purposes it would be more realistic to assume that 'off-plant' mortality is independent of maximum hourly wind speeds when these do not exceed 15 knots and that in these circumstances there is a constant wind mortality of 3% (= 10% after arcsin transformation, see Figs 3.8 and 3.9).

Several authors have suggested mortality due to an interaction between wind and low temperatures. Dean (1974a) suggested after monitoring artificially established colonies that a combination of wind and frost on most mornings between mid-March and mid-April 1971 caused S. avenae and M. dirhodum to fall off the plants. Way & Banks (1968) suggested that nymphal fundatrices of Aphis fabae on spindle trees (Euonymus europaeus) were killed in early spring because they were dessicated by dry winds in conditions too cold for them to feed and hence compensate for water loss. Smith (1981) also suggested this dessication effect as a possible reason why some S. avenae were found dead on winter wheat exposed to freezing winds, but inoculative freezing could have been responsible and a second experiment failed to confirm the suggested wind/low temperature interaction. From survival experiments

undertaken by Smith (1981) in the laboratory, in which S. avenae nymphs were kept at different temperatures and relative humidities, wind/low temperature mortality due to dessication appeared unlikely because even first instar nymphs could survive for about 70 h at 5°C and 0% R.H., a much lower humidity than would ever occur in the field.

In the present experiment there was little good evidence of a wind/low temperature interaction. For M. dirhodum there was a suggestion of an effect, in that this species sometimes showed higher 'off-plant' mortality than S. avenae in the coldest periods (see section 3.3.4) and also because 'nights with grass minimum temperatures below -3°C' was nearly significant ($P = 0.05$) as a predictor of M. dirhodum 'off-plant' mortality (see section 3.3.2). However these results would also have been produced if M. dirhodum corpses (initially killed by the action of low temperature alone) were more easily dislodged from plants than were live aphids.

Lastly, it was found that R. padi suffered less 'off-plant' mortality than did S. avenae or M. dirhodum. Hand (1982) also found that in natural overwintering populations R. padi suffered less from wind and rain than S. avenae. In both cases this species difference was probably due to the tendency of R. padi to feed lower down on the plants, in less exposed positions.

3.4.3 The effect of snow

Snow only occurred in two of the 31 experimental periods. Multiple regression analyses showed that it significantly increased 'off-plant' mortality of both S. avenae and M. dirhodum but the amount of data available was much too small to determine how it exerted its effect. A 'dummy' variable denoting its presence or absence was found to be the best of five alternative snow variables as a predictor of 'off-plant' mortality but it was probably selected only because three of the four others were not recorded at the study site. Dean (1974a) recorded 50 to 80% mortality in artificially established populations of S. avenae after a period of snow. Gair (1953) recorded

heavy mortality in natural populations of R. padi on grass plots after snow but found that on plots in which the aphids were afforded greater cover they suffered much less mortality.

From the results of Hand (1982) snow appeared to cause mortality in natural populations of R. padi and S. avenae, with the former species suffering more heavily. He suggested that this was because snow acted by drowning aphids during a thaw so that the basal feeding positions of R. padi made this species more vulnerable. There are several other ways in which snow might act; for example:

1. By knocking aphids off plants if there is drifting (as occurred on both occasions when snow fell in the present experiment).
2. By producing inoculative freezing due to contact between the aphid's cuticle and snow crystals.
3. By causing lethal cold-stress by maintaining covered aphids at more or less constant sub-zero temperatures until thawing.

Much more field data are needed to determine which of these possible effects is important.

Snow can also aid winter survival by protecting aphids from exceptionally low air temperatures as it is a very effective insulator. Even with air temperatures around 0°F (-18°C) the temperature under 15 cm of snow may be as high as 23°F (-5°C) (Green, 1962). Smith (1981) found surprisingly low mortality of S. avenae after a particularly harsh period and suggested this was because the aphids were protected from low temperatures and high winds by snow cover. Powell & Parry (1976) found winter survival of the spruce aphid Elatobium abietinum in Scotland was greater in the lower crowns of spruce trees than higher up and suggested this was because aphids lower down were insulated by snow during a critical cold period. The importance of snow cover to the survival of anholocyclic populations of a variety of aphid species in the

harsh winters of the northern U.S.A. and Canada has been noted by several authors (Andre & Tate, 1933; Lowe, 1952; Adams, 1962; Landis, Powell & Fox, 1972).

3.4.4 The relative importance of low temperature and the 'off-plant' mortality factors

From the evidence it seems justifiable to assume that low temperature produced nearly all 'on-plant' mortality but was responsible for little if any 'off-plant' mortality. Thus the relative importance of low temperatures, compared to the other weather factors (wind, snow and rain) during the experiment can be roughly assessed by comparing mean corrected 'on-plant' mortality with mean 'off-plant' mortality (Table 3.26). The comparison suggests that, except for M. dirhodum in the cold 1978/79 winter, low temperature was less important than the combined effect of the other factors (which appeared from regression analyses to be dominated by wind). However, the differences were relatively small except for S. avenae in the milder 1979/80 winter.

Smith (1981) concluded that low temperature was less important than wind as a mortality factor but implied that the difference between the two was greater than that found here. This disagreement may well be partly due to differences in methodology, with faults in both studies. The present experiment probably overestimated the importance of low temperature mortality somewhat as in natural populations aphids would have had longer to acclimatize. In addition, the average cold-hardiness of the population would be expected to steadily increase in harsh winters as low temperatures selectively removed the less hardy individuals.

In Smith's study it is likely that the importance of wind was exaggerated. This was firstly because using his shelter cage technique he was not able to quantify low temperature mortality properly. One can exclude wind and rain mortality using shelter cages but no cage can exclude low temperature mortality without altering development and reproduction rates. Secondly, from the photographs in

Table 3.26 Mean percentage 'on-plant' and 'off-plant' mortalities in batches of S. avenae and M. dirhodum nymphs exposed for 4-day periods during the 1978/79 and 1979/80 winters.

SPECIES	<u>S. avenae</u>		<u>M. dirhodum</u>	
MORTALITY	corrected 'on-plant'	'off-plant'	corrected 'on-plant'	'off-plant'
1978/79* winter	7.0	10.7	11.4	10.2
1979/80** winter	3.7	11.9	7.3	11.2
Both winters	5.1	11.4	9.0	11.1

*13 periods

**18 periods

his thesis, it appears that the potted test plants Smith used to expose aphids on were considerably taller than those in the surrounding experimental plot. Some aphids must thus have been exposed well above the boundary layer of slow-moving air created by the crop. An artificially high proportion were probably dislodged by wind as a result.

Even in the present experiment, in which special care was taken to use field-grown plants potted up not long before use, so that their height and growth form were as natural as possible, wind mortality was probably exaggerated because aphids occupied atypically exposed feeding positions due to their short time in the field (see section 3.3.5). Also, the assumption mentioned earlier, that all aphids knocked off the pots failed to regain plants may well have exaggerated wind mortality in both studies.

3.4.5 Improvements in the experimental design

If the same approach is used in future studies on winter weather mortality several improvements in the methodology are advisable:

1. On several occasions a thorough examination of the plants in the experimental plot close to pots on which aphids are being exposed should be undertaken to assess the extent to which aphids knocked off the pots can find their way onto neighbouring plants and survive. This is inevitably very labour intensive and was not attempted in the present experiment due to lack of time.
2. Weather monitoring should be expanded to include relative humidity and a direct assessment of the occurrence and severity of hoar frost and other types of surface ice.
3. If possible some aphid batches should be left out in the field for several weeks before culling

unwanted instars and monitoring, so that their feeding positions and degree of acclimatization are closer to those of natural populations. This was attempted in the 1979/80 winter but failed due to the unpredictability of the weather. In the 4 weeks between being put out and first counted the aphids were often found either to have been almost exterminated or to have increased so much as to severely damage the plants. Though a few batches were successfully monitored the data were too meagre to be worth presenting. To be successful it is probably necessary to put out many more pots of aphids than are needed, using a range of aphid densities. Some form of protection from extreme low temperatures may also be worthwhile.

3.4.6 Using the results

Though the artificialities mentioned above may have produced inaccuracies, the predictive equations derived here for S. avenae and M. dirhodum viviparae (Tables 3.5, 3.6, 3.11 and 3.12) should be of use to future researchers, particularly as the predictor variables used are easily derived from local Meteorological Office data.

However, the equations' limitations should not be forgotten and if possible they should be checked using independent data. Their use for other aphid species or for S. avenae and M. dirhodum viviparae on winter cereals at later growth stages or on other Gramineous hosts could well lead to large errors. This is particularly true for 'off-plant' mortality as the effect of wind is probably very dependent on plant height, growth form and density. It should also be stressed that the equations can only be applied if weather data are collected and mortality predicted for 4-day periods.

Predicting total mortality or survival in a 4-day period from the separate 'on-plant' and 'off-plant' mortality equations requires several stages:

1. Calculate predicted arcsin transformed 'on-plant'

and 'off-plant' percentage mortalities separately from the equations.

2. Change the predicted percentage mortalities from arcsin transformed values to untransformed values.
3. Convert them to probabilities of surviving ($= 1 - (\text{percentage mortality}/100)$).
4. Multiply the probabilities of surviving 'on-plant' and 'off-plant' mortalities to give the probability of surviving both types of mortality.

This process would be simpler if single equations predicting total mortality for each species had been calculated. In fact this approach was adopted initially but produced unrealistic results because, when dealing with the more complicated 'total mortality' equations (each involving three separate weather factors) the importance of including a quadratic term to model the effect of wind was missed.

Several other points concerning the use of the equations should be stressed. Firstly, though minimum temperatures are usually recorded in a Stevenson screen at 1.2 m, grass minima should be used to predict low temperature mortality as they reflect temperatures experienced by aphids on seedling winter cereals much more accurately. Standard Stevenson screen minima may be higher than grass minima recorded at the same time by as much as 3°C and the size of the difference may vary widely from one day to the next. When snow is lying the minimum temperature at plant height under the snow should be used. As already suggested (section 3.4.2) it is probably best to assume a constant wind mortality of 3% for 4-day periods in which the maximum wind speed over 1 h does not exceed 15 knots. If possible the significance of rain and rain/wind interaction effects should be re-tested. More discussion of the problems of predicting population change in anholo-cyclic winter populations is given in Chapters 4, 5 and 7.

CHAPTER FOUR

CHAPTER FOUR

DEVELOPMENT AND REPRODUCTION OF OVERWINTERING

S. AVENAE ON WINTER WHEAT4.1 INTRODUCTION

A knowledge of the relationships between development, reproduction and winter temperatures is essential to an understanding of the population dynamics of cereal aphid viviparae overwintering on secondary hosts. Without it, potential population growth in the absence of extrinsic mortality factors cannot be estimated and natality and mortality are inseparable. Consequently, the impact of abiotic and biotic mortality factors cannot be assessed in the quantitative way necessary if their effects on natural populations are to be predicted. The results of Smith (1981), discussed in Chapter 3, showed that data from population monitoring alone cannot be used to separate recruitment and mortality and analyse each process independently, even if the populations being studied are small, artificial ones, which can be monitored carefully and at frequent intervals.

In the winter mortality experiment described in Chapter 3 the problem of separating natality and mortality was overcome by determining changes in numbers in artificially established populations of nymphs of limited age-range over short time periods. Thus natality was eliminated completely and all changes in numbers were due to mortality. The experiment was successful in providing initial, approximate but quantitative assessments of the modes of action and effectiveness of winter weather conditions as mortality factors but the need to replace nymph batches every eight days meant that a degree of artificiality was inevitable with this experimental approach. It was realised that the next step in the investigation of the population dynamics of overwintering viviparae must be the separation of natality and mortality in natural populations. Hand (1982) adopted this approach in analysing the results of widespread winter 'D-vac' sampling of natural cereal aphid populations on

Gramineae. In assessing potential population increase between sampling dates he was forced to use algorithms for the temperature relations of development and reproduction derived from the work of Dean (1974b), which was done at relatively high, constant temperatures in the laboratory, because no data pertaining to winter temperature conditions were available. However, there is evidence in the literature (discussed below in sections 4.1.1 and 4.1.2) that for other species of insect, including several aphid species, the temperature relations of development and reproduction are different under low, fluctuating temperature conditions compared with intermediate, constant temperatures, so that extrapolation from laboratory data to winter field conditions may lead to large inaccuracies.

The experiment described here was undertaken primarily to determine the relationships between development rate, reproduction rate and temperature, as well as the relationships between reproduction rate, survival and age, for S. avenae viviparae on winter wheat under winter field conditions in southern England, which could be used with confidence in studies on the population dynamics of natural overwintering populations. To obtain the required data, the development, reproduction and survival of clip-caged viviparae and the ambient temperatures they experienced were monitored in the field from late September 1980 to late March 1981. The labour-intensive nature of the experiment and fairly arduous field conditions restricted it to a single aphid species on a single host plant. The results were too late for inclusion in Hand's analysis but should be of use to future researchers studying anholocyclic overwintering in S. avenae.

As well as fulfilling its central, applied aim, the experiment allowed several points of more theoretical interest to be investigated. For example:

1. Comparisons of the development, longevity and reproductive patterns of apterae and alatae at low temperatures with their performance under intermediate, 'summer' temperature conditions as determined

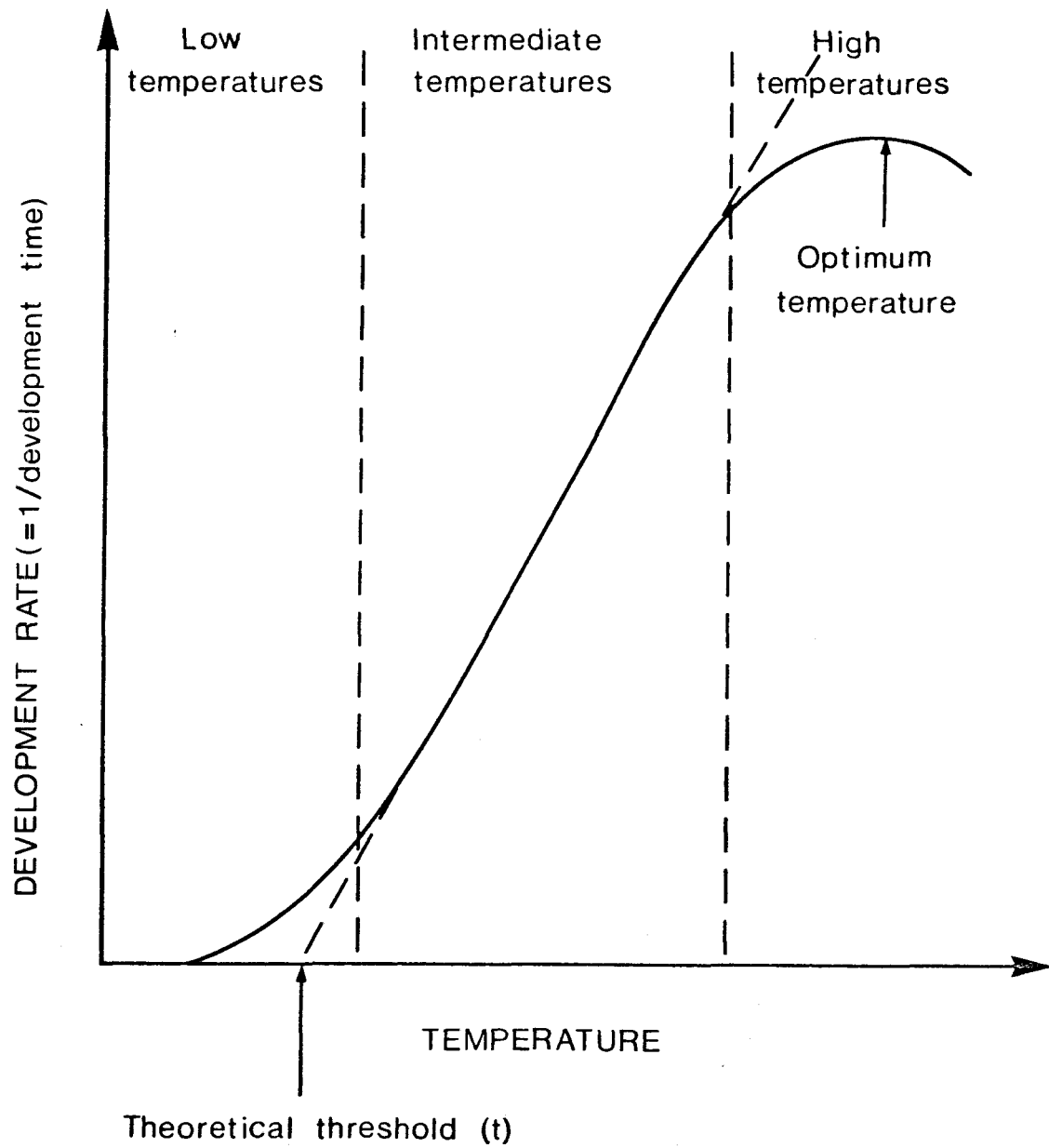
by earlier laboratory work (e.g. Dean, 1974b; Wratten, 1977; Watt, 1979b).

2. Seasonal changes in sexual morph production.
3. Possible modifications of reproduction and development temperature-relations from autumn to spring due to acclimatization or other phenomena.
4. The extent of curvilinearity in the relationships between development and reproduction rates and temperature at low temperatures.

4.1.1 Characteristics of the relationship between insect development and temperature

From the large amount of data on the development of eggs and larval stages of a wide variety of insects, including several aphid species, at various constant temperatures, it is now widely accepted that relationships between development rate (the reciprocal of the time taken to complete the development stage under study) and temperature are of the general form shown in Fig. 4.1; that is, a shallow sigmoid approximating to a straight line at intermediate temperatures and turning down above the optimum (Wigglesworth, 1965; Howe, 1967; Chapman, 1971; Varley, Gradwell & Hassell, 1973; Stinner, Gutierrez & Butler, 1974; Campbell, Frazer, Gilbert, Gutierrez & Mackauer, 1974; Gilbert, Gutierrez, Frazer & Jones, 1976). Many different types of equation have been used to describe the relationship between development rate and temperature (e.g. Krogh, 1914; Davidson, 1944; Fry, 1947; Eubank, Atmar & Ellington, 1973; Stinner et al., 1974). The variety is a reflection of differences in the exact form of the relationship in different insects, the range of temperatures over which data were obtained and also the aims and preconceptions of the researchers. The simplest type of equation used describes a straight line. Most of the early attempts to describe development rate/temperature

Fig. 4.1 Generalized relationship between the rate of insect development and temperature (after Campbell *et al.*, 1974).



relationships assumed linearity (e.g. Simpson, 1903; Sanderson, 1910; Blunck, 1914) and even the more sceptical of later authors agreed that a straight line gives an adequate fit over a range of intermediate temperatures (Andrewartha & Birch, 1954; Howe, 1967; Stinner et al., 1974).

If a linear relationship is assumed then there is a theoretical threshold temperature for development (t) which can be estimated by extrapolation from constant temperature data (Campbell et al., 1974). Below this threshold there is, theoretically, no development at all and above it the development rate is proportional to temperature measured from the threshold, that is:

$$\frac{1}{D} \propto (T-t)$$

where $1/D$ = reciprocal of development time
 = development rate.

T = temperature during development.

t = threshold for development.

From this, the product of development time and the temperature during development measured above the threshold ($T-t$, sometimes known as "effective temperature") is a constant:

$$D (T-t) = K$$

K is known as the "thermal constant" for development and represents the number of temperature-time units above the threshold (usually measured in 'day-degrees') which must be accumulated for development to be completed, irrespective of the actual temperature experienced. This concept of 'temperature summation', first developed by Simpson (1903), can be extended to variable temperature regimes. As long as the temperature fluctuates within a range over which the development rate/temperature relationship is linear, K should remain unchanged and thus the time for complete development can be calculated by summing the

temperatures measured above the threshold ($T-t$) in each time unit.

The concepts of thermal constants and temperature summation can also be extended, with varying degrees of justification, to other processes, such as reproduction and ageing, so that all the processes of population growth can be expressed on a 'physiological time' scale of temperature-time units rather than in calendar time.

Physiological time scales have been used in a number of well-known studies of insect population dynamics (e.g. Shelford, 1927; Hughes, 1963) and more recently in computer simulation models of aphid population dynamics (Frazer & Gilbert, 1976; Gilbert *et al.*, 1976).

The variety of more complex equations fitted to constant development rate/temperature data have generally been used to describe the relationship at high temperatures, near the optimum for development rate, where the assumption of linearity may lead to serious errors. Relatively little information is available on the exact form of the development rate/temperature relationship at constant temperatures close to the theoretical threshold because of the length of time needed for complete development at these low temperatures. Some authors have fitted curves which show decreasing slope as temperature drops towards the threshold, despite the fact that their experimental data showed little or no sign of this (e.g. Stinner *et al.*, 1974; Barlow & Dixon, 1980). However, there are theoretical reasons for assuming that the development rate/temperature relationship becomes increasingly curvilinear near the theoretical threshold and several studies with more data for lower temperatures show this to be so. These include studies on the aphids Acyrtosiphon pisum (Siddiqui, Barlow & Randolph, 1973), Therioaphis maculata (Messenger, 1964) and Myzus persicae (Barlow, 1962) as well as other insect groups (e.g. Birch, 1944; Davidson, 1944; Browning, 1952). Therefore, for low temperatures, the assumption that development rate is proportional to temperature above a threshold may lead to significant errors.

Although constant temperature regimes have been used in most studies on the temperature relations of insect development, this is an artificial simplification as, in their natural habitats, nearly all insects experience daily and seasonal temperature fluctuations. The literature on the effects of fluctuating temperatures consists, superficially at least, of a mass of confusing and seemingly conflicting results. However, the apparent contradictions are largely a result of fluctuating temperatures having different effects depending on the range of temperatures involved. Pradhan (1945) realized this and suggested the general principle that development times under fluctuating temperature conditions would be shorter than predicted from constant temperature data at low temperatures, similar at intermediate temperatures and greater at high temperatures. This general trend has been further vindicated by more recent work and is now widely accepted (Cloudsley-Thompson, 1953; Messenger, 1959; Howe, 1967).

There are several possible reasons why development times under fluctuating temperature regimes should deviate from those predicted from constant temperature data in this way, but researchers have seldom distinguished between these (Messenger, 1959). In the past apparent discrepancies between results from studies at constant temperatures and fluctuating temperatures have often simply been due to the incorrect calculation of 'expected' development times under fluctuating temperature conditions. The error made most commonly was the assumption that, if fluctuating temperatures have no stimulating or retarding influences, the development time of an insect experiencing fluctuating temperatures should equal that of one held at the mean temperature of the fluctuating temperature regime throughout its development. However, this would be so only if the development rate/constant temperature relationship was linear over the whole range of temperatures experienced under the fluctuating conditions, and this would be unlikely if the fluctuating conditions included high temperatures, near the optimum for development, or low temperatures, near the theoretical threshold (see Fig. 4.1).

This source of error can be removed if the development rate/constant temperature relationship is accurately known. Correct 'expected' development times in a fluctuating temperature regime can then be calculated by assuming that in each short time interval, over which temperature can be assumed constant, development will proceed at the same rate as when the insect is held at that temperature for its whole development period. The fractions of development predicted for each time unit are then summed to give a predicted development time which takes curvilinearity in the development rate/constant temperature relationship into account. Differences between predicted and observed development times may then be due to the following causes:

1. In fluctuating temperature conditions healthy development continues at temperatures which would be damaging or lethal if they were experienced continuously.
2. The extreme temperatures reached in fluctuating temperature conditions cause permanent damage which slows subsequent development.
3. Fluctuating temperatures have some additional stimulating or retarding effect within the temperature range for healthy development under constant temperature conditions.

Pradhan (1945), Cloudsley-Thompson (1953), Andre-wartha & Birch (1954) and Howe (1967) reviewed evidence for fluctuating temperature effects, but in most of the studies cited the exact cause of the effects was not determined. Of the four types of effect given above, the first, 'false' one (resulting from incorrect calculation of the development time expected if fluctuating temperatures produce no modifying effects) seems the commonest. But there is good evidence that healthy development below the lower limit for development as determined under constant temperature conditions was

significant in a number of non-aphid species (Ludwig & Cable, 1933; Harries, 1943; Lin, Hodgson & Richards, 1954; Messenger & Flitters, 1959). And in the spotted alfalfa aphid, Therioaphis maculata, continued development below the constant temperature limit for development appeared to be an important factor producing faster than expected development under fluctuating temperature conditions (Messenger, 1964).

Sometimes, when insects were subjected to hot, fluctuating temperature conditions, which included temperatures several degrees above the developmental optimum, the observed development times were longer than expected, even when the correct method for calculating the expected development times was used (taking the curvilinear nature of the development rate/temperature relationship above the optimum into account) (e.g. Ludwig & Cable, 1933; Messenger & Flitters, 1959). This phenomenon has been demonstrated in the pea aphid Acyrtosiphon pisum (Siddiqui *et al.*, 1973) but not in a similar study on Therioaphis maculata (Messenger, 1964). The most obvious explanation for this type of retardation is that the high maximum temperatures experienced produce permanent physiological damage. Theoretically, the same type of permanent retardation could also occur due to brief exposure to extreme low temperatures but this has rarely been demonstrated, probably because little work at sufficiently low, fluctuating temperatures has been done. It may also be that the temperatures required to produce such retardation lie well below those at which development proceeds at a measurable rate, and/or that the temperature needed to produce permanent retardation lies only just above that which kills the animals outright. This last hypothesis was suggested by work on fruit-fly egg development by Messenger and Flitters (1959). They found that observed development times were shorter than expected even under cold, fluctuating temperature conditions in which most eggs failed to hatch. However, Harrison and Barlow (1972) showed that exposing pre-reproductive adults of Acyrtosiphon pisum to -12°C for 65 min slightly increased their development time (defined as the time from birth to

the start of reproduction) and, interestingly, also that of their progeny. But these retarding effects were small compared to reductions of fecundity and longevity produced by the same low temperature treatment.

There is little evidence in the literature that fluctuating temperatures have real stimulating or retarding effects within the temperature range for healthy development under constant temperature conditions, although some stimulating effect is suggested by work on fruit-fly egg development (Messenger & Flitters, 1959) and for Therioaphis maculata (Messenger, 1964). The general lack of evidence for this type of effect must be at least partially due to the fact that it can only be demonstrated after all the other possible causes given above have been eliminated.

From the brief summary above there is good evidence to support the following statements:

1. Even in species in which the development rate/temperature relationship is nearly exactly linear at intermediate temperatures, it can be expected to become increasingly curvilinear at temperatures close to and below the theoretical threshold, so that using development rate data obtained at intermediate temperatures to predict development times at low temperatures may lead to large errors, even under constant temperature conditions.
2. Using a development rate/temperature relationship determined at constant temperatures to predict development times under variable-temperature, field conditions may lead to considerable errors when the fluctuations include temperatures outside the range over which development is approximately proportional to temperature, that is, around the optimum or close to and below the theoretical threshold.
3. All of the information available on the development of aphids at low and fluctuating temperatures shows that they are essentially similar to the other

insects studied (although the amount of data is rather small).

From these points it is obvious that, if the results are to be used to investigate changes in natural, field populations, the relationship between development and temperature for overwintering S. avenae viviparae should be determined under conditions which are as natural as possible.

4.1.2 Temperature relations of aphid reproduction and longevity

Like development, reproduction and longevity are strongly dependent on temperature. Little detailed work has been done on the temperature relations of longevity, although the general trend of increased longevity at lower temperatures, provided these remain above the lower lethal zone, has been stated often. Work on the temperature relations of reproduction in other insects is probably of little relevance to parthenogenesis by viviparous aphids, which are very much a special case. In nearly all other insects reproduction involves a much larger and more complex behavioural component (mate-finding, courtship, mating and often the location of suitable oviposition sites). Consequently it is likely to have much more complex temperature relations than parthenogenetic reproduction, especially by apterous aphids which are nearly always feeding on hosts suitable for their offspring.

Because of the simplicity of the reproductive process in viviparous aphids, the determination of the relationship between reproduction rate (nymphs produced per unit time) and temperature can be approached in the same way as the development rate/temperature relationship and most of the principles of that relationship (discussed above) can be applied. But there is the additional complication that adults of different ages will have different reproduction rates under the same temperature conditions, so that the relationship between reproduction

rate and age must be described, as well as that between reproduction rate and temperature. A second complication is that different aspects of reproduction may have different temperature relations. For example, the constant temperature at which Acyrtosiphon pisum attains maximum peak reproduction rate is about 22.5°C but the species' total nymph production is highest at about 15°C (Siddiqui et al., 1973). The same trend was shown in studies on Therioaphis maculata at fluctuating temperatures (Messenger, 1964) and Myzus persicae and Macrosiphum euphorbiae at constant temperatures (Barlow, 1962) with maximum peak reproduction rates achieved at higher temperatures than maximum total fecundities.

Little detailed information is available on the effects of low or fluctuating temperatures on aphid reproduction or longevity. Wood & Starks (1972) reported that at temperatures fluctuating between -1.1 and 15.6°C the greenbug Schizaphis graminum produced an average of 42.3 nymphs/adult on barley, as compared to only 10.1 nymphs/adult at a constant 10°C, suggesting that relatively low fluctuating temperatures have a considerable stimulating effect on reproduction. For Therioaphis maculata, Messenger (1964) found fluctuating temperature regimes with means between 8°C and 30°C had considerable stimulating effects on total fecundity and longevity. But for Acyrtosiphon pisum Siddiqui et al. (1973) found a constant temperature of 15°C gave higher total fecundities than fluctuating temperature regimes with the same mean temperature, and the same trend applied at 17.5°C and 20°C. Longevity was also generally shorter at fluctuating temperatures than at the corresponding constant temperature, although overall, intrinsic rates of increase were higher at fluctuating temperatures due to shorter development times. Again for A. pisum, Harrison & Barlow (1972) showed that short exposures to extreme high or low temperatures (39°C and -12°C) permanently reduced longevity and fecundity, and these effects were also evident in the offspring of the exposed aphids.

Lastly, when considering the temperature relations of development, reproduction and longevity in viviparous

aphids, it must be remembered that they feed more or less continuously, so that temperature effects may result partially or completely from temperature-related changes in the condition of the host plant, rather than from the direct action of temperature on the aphids themselves.

4.1.3 Previous work on the temperature relations of development, reproduction and longevity for *S. avenae*

The most detailed study of the temperature relations of development, reproduction and longevity of *S. avenae* on cereals was that of Dean (1974b) in which temperature relations were determined for the apterae of the three main cereal aphid species on barley under constant temperature conditions between 10° and 30°C. Some additional data for the performance of *S. avenae* on wheat at intermediate and high constant temperatures were given by Rabbinge *et al.* (1979) although the work was not reported in detail. Campbell *et al.* (1974) gave the theoretical threshold and thermal constant for development for a Vancouver clone of *S. avenae* determined from constant temperature laboratory work but no details of the data from which these parameters were calculated were presented. Markkula & Pullianen (1965), Wratten (1977), and Watt (1979b) undertook laboratory studies not specifically designed to determine temperature relationships but these provided some data on the relative performance of *S. avenae* apterae and alatae on cereals at intermediate, constant temperatures. The field studies of Chaudhary *et al.* (1969), Dean (1973) and Markkula & Myllymaki (1963) provided some additional information on performance at intermediate and high fluctuating temperatures but the temperature data recorded (usually long-term means) were not detailed enough for temperature relations to be determined. Dean's constant temperature work (Dean, 1974b) has formed the basis for temperature relationship algorithms in the two computer simulation models of *S. avenae* population

dynamics on wheat so far developed (Carter, 1978; Rabbinge et al., 1979) and, because these are models of summer populations which experience intermediate temperatures, it appears adequate. Rabbinge et al. (1979) compared S. avenae development times at constant and fluctuating temperatures around 13°C and, as expected at this intermediate temperature, they were similar.

The only work so far published on S. avenae development, reproduction and longevity under low, fluctuating temperature conditions is that of Dean (1974a). He monitored S. avenae apterae on potted wheat in the field in ten tests run over a complete year. The temperature data collected (daily maxima and minima, summarized as monthly means in his paper) were insufficient for the temperature relations to be characterized but interesting general, seasonal trends are apparent. The most striking feature of the results was a drastic decrease in total nymph production per adult (to around 10 nymphs/adult) in the two mid-winter tests and the surprisingly short reproductive period and long post-reproductive period under these conditions. Aspects of Dean's results and those of the other researchers are discussed in Section 4.4.

4.1.4 The experimental approach

As already stated, the central aim of this experiment was an applied one - to provide data on the temperature relations of development, reproduction and longevity for use in further investigations of the population dynamics of S. avenae viviparae overwintering on winter wheat. The results are presented in a way which should enable them to be incorporated easily into a deterministic computer simulation model. The approach adopted when analysing the rate/temperature relationships for development and reproduction was not to try and fit complex, curvilinear functions to the data if simple, straight-line ones seemed adequate. In deciding whether such simple relationships were adequate, statistical parameters were used to provide indices of the amount of

residual variation left unexplained and, when making these decisions on the adequacy of rate/temperature functions, the following points were borne in mind:

1. The functions determined were for use in studying changes in natural, field populations for which other processes, such as abiotic and biotic mortalities, immigration and emigration, can, inevitably, only be roughly assessed.
2. The simpler the rate/temperature relationships adopted, the easier they would be for others to use.

Special effort was put into analysing other aspects of the data which have an important bearing on their use in a simulation model. In particular, the stability of the temperature relations of development and reproduction rates from autumn to spring and on seedlings at different growth stages was investigated, as was the reproduction rate/age relationship and the possibility of sexual morph production.

In the analysis and discussion below no attention has been paid to accurately defining real 'biological' threshold temperatures for development and reproduction. The thresholds determined and referred to are, unless stated otherwise, of the theoretical type referred to above. That is, they are arrived at by assuming that the straight line which best fits the temperature/rate data continues downwards to meet the temperature axis. These theoretical thresholds are important as part of the process of finding and describing best-fit straight-line relationships but have little biological meaning in their own right. Even 'real' temperature thresholds are unimportant from an applied viewpoint as, in investigating winter population changes, it is the relationship between development or reproduction and temperature over a range of low temperatures which is important, not the exact temperature below which no reproduction or development can occur.

During the experiment data were gathered for both *alatae* and *apterae* but effort was concentrated on the latter morph because in natural winter populations on winter wheat they are usually much more common (Hand, 1982; Vorley, pers. comm.).

4.2 MATERIALS AND METHODS

The experiment was carried out at Southampton University Biology Department's field site at Chilworth. It involved monitoring the development and reproduction of *S. avenae* viviparae clip-caged on winter wheat seedlings in the field between 21 September 1980 and 29 March 1981. A Grant Temperature Recorder was used to record the temperature inside clip-cages at hourly intervals throughout this period. The aphids were checked and aphid data recorded every third day. A new cohort of first instar nymphs born since the previous recording day was set up on nearly every recording day between 24 September 1980 and 10 January 1981. Regression analysis was used to determine the relationship between the development and reproduction rates of aphids born on different dates and the temperatures they had experienced during nymphal and adult life. Other variables, such as host plant growth stage, which might also affect development and reproduction, were recorded for each aphid and their modifying effects analysed.

4.2.1 The aphids used

All the aphids were derived from a green *S. avenae* exule collected from winter wheat at South Allenford Farm (Grid Ref. SU 090175) at Damerham near Fordingbridge, Hampshire on 9 July 1980. This aphid was kept on potted winter wheat (cv. Hobbit) in a growth room at $20^{\circ} \pm 1.5^{\circ}\text{C}$ and 16 h day length until early August. The resulting clonal culture was then maintained out-of-doors for the whole of the experiment. From August to the start of the experiment in the second half of September it was kept

in Perspex and muslin cages on pots of field-grown Hobbit seedlings. In the third week of September alatifform and apteriform fourth instar nymphs were transferred into clip-cages on field-grown Hobbit on the experimental plot. Their offspring were used as the first aphids whose development and reproduction were monitored. After the first of these had reached maturity, in mid-October, their own offspring were used as the next generation of experimental animals.

The simpler procedure of taking new-born nymphs from a culture kept in a glasshouse or culture-room was not used because preliminary tests during the 1979/80 winter had shown that nymphs transferred from laboratory to winter field conditions as first instars had a very short reproductive period when adult and produced very few nymphs. It was thought this unexpected reproductive pattern resulted from the sudden change from laboratory to field conditions, and that aphids from overwintering populations kept in the field from early autumn would produce more offspring. The 1980/81 field experiment showed this to be the case.

4.2.2 The plants used

The winter wheat cultivar used throughout was Hobbit. Seed dressed with Gamma HCH and organo-mercury was broadcast sown on a bare plot treated with Growmore NPK fertiliser at $c.50g/m^2$. The seed was sown in 8 m x $\frac{1}{2}$ m strips with strips of bare ground 1 m wide between, so that all the plants could be reached easily. The plant density in the strips was approximately $320/m^2$. To reduce the range of host plant growth stages and allow some investigation of the effect of host plant sowing date on aphid development and reproduction rates, the wheat used was sown on three different dates: 21 August, 25 September and 3 November. The first sowing date was artificially early but was necessary to ensure that enough aphids were established in clip-cages before cold weather slowed development and reproduction.

The parents of the first monitored aphids were caged on August-sown wheat as were all their offspring established before 18 October. Aphids established between 18 October and 23 December were kept on September-sown wheat while those established after this were caged on November-sown wheat. All adult aphids were kept on plants sown on the same date as those they were reared on, with the exception of 50 aphids established on August-sown wheat and maturing after 24 October. These were transferred to the more realistic September-sown wheat as pre-reproductive adults.

Plants on which aphids were caged varied in growth stage from 12,20 to 19,29,30 (Zadoks et al., 1974).

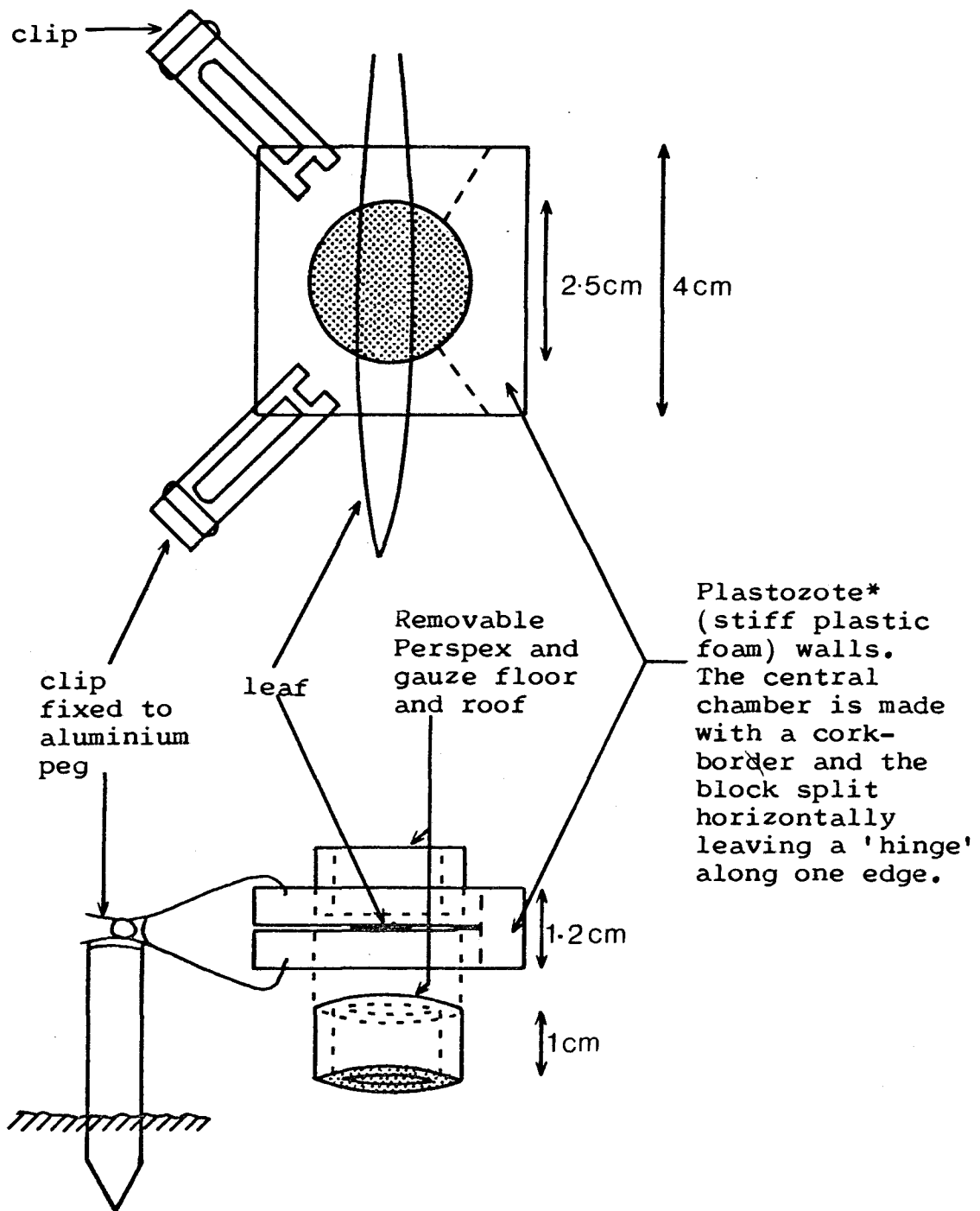
4.2.3 Clip-cage design

The clip-cages used (Fig. 4.2) were designed specifically for winter fieldwork. The Perspex and gauze roof and floor were held in place by the elasticity of the Plastozote walls gripping their bases. The design had the following advantages under winter field conditions:

1. The roof and floor could be removed and the cage examined with the Plastozote wall still clipped to the leaf. This minimized disturbance and losses.
2. The opaque Plastozote wall, although it reduced light intensity in the cage somewhat, reduced overheating on sunny days.
3. The Plastozote wall provided a good surface for the clips to grip. Consequently, the clips were not glued to the cages. This made handling the cages easier and allowed them to be used with a rigid stand when necessary.

Leaves were always positioned in the cages twisted over, so that their adaxial surfaces faced downward, not upward. With the leaves in this position, aphids nearly always settled on the adaxial surfaces, which ensured that

Fig. 4.2 The clip-cage design used in winter fieldwork.



* Plastozote suppliers: Hinders Leslie Ltd., Higham Hill road, Walthamstow E17, London. Details: po 53 unperforated white standard Plastozote, 12 mm thick.

they were protected from rain and shaded from direct sunlight. Earlier investigation (see Chapter 3) had shown that the lower side of a horizontal leaf, particularly if this was the adaxial surface, was one of the most common feeding positions for S. avenae viviparae on wheat seedlings under winter field conditions. When in use the cages were held from about 1.5 cm to 5 cm above ground level.

4.2.4 Temperature monitoring

A Grant Model D Temperature Recorder was used to record temperatures at hourly intervals on chart-rolls between 21 September 1980 and 29 March 1981. Thermistors connected to the recorder were placed under leaves in three clip-cages among those containing aphids and at the same height above the ground. Two more thermistors, not inside cages, were placed nearby 4 cm above the soil. One of these was shaded by a 7 cm x 3 cm diameter metal hemicylinder and the other was unshaded. They were used to assess the differences between temperatures inside and outside the cages.

All the thermistor probes were Grant's type CM except for one of the three in cages which was type FF. Type CM probes have the thermistor enclosed in a 2.5 cm long by 3 mm diameter steel tube. The type FF probe has no metal shield and the thermistor is embedded in a 2 mm sphere of black plastic. This design was thought more likely to record temperatures similar to those of an aphid in the same position and it was used to check the realism of the standard CM type. No consistent differences between temperature records from FF and CM probes inside the cages were found. The temperature experienced by the aphids at any time was taken as the mean of the temperatures recorded by the three thermistors inside clip-cages, rounded to the nearest whole degree Centigrade. On every ninth day the Grant Recorder's calibration and the thermistor positions were checked. On every eighteenth day the Recorder's battery was renewed. Chart rolls were changed as necessary.

4.2.5 Cageing and handling aphids and recording aphid data

Development and reproduction data were recorded on every third day between 21 September 1980 and 29 March 1981. Thus the experiment was split up into a total of 63 3-day periods separated from each other by recording days. Cohorts of about 20 first instar nymphs born since the preceding recording day were established in clip-cages on most recording days between 24 September and 10 January 1981. On some days fewer than 20 nymphs were established due to low nymph-production in the preceding 3-day period or because weather conditions were too unfavourable. On four recording days in this interval no new first instars were established. The nymphs used were always among the first 15 produced by an adult. New first instar nymphs were placed in cages on the second unfolded leaf of a plant's main shoot - a leaf was considered "unfolded" if the adaxial surface was visible over more than half of the leaf's length. All cages were given a code number written on an attached tag.

New first instar nymphs were transferred from the parent's cage to an empty cage using a fine sable brush and a small petri dish. When adults were moved this was done in the same way. The nymphs were kept two to a cage until maturity. Both nymphs in a cage were born in the same 3-day period to the same morph of parent and, in the great majority of cases, to the same parent. They were kept in pairs as it was thought initially that mortality between birth and maturity might approach 50%. In fact mortality was much lower and both nymphs reached maturity in nearly all the cages.

Nymphs were examined on every recording day only after they had reached the third or fourth instar. The results of checks on nymphs were kept on record sheets on which each nymph's birth period and the morph of its parent were also recorded. Only apterous parents were used for the two cohorts set up before 30 September, but it was soon realized that this resulted in too many nymphs becoming alatae, so subsequent cohorts had a mixture of

apterous and alate parents. The ratio of the two parent morphs varied from cohort to cohort, depending on availability, but nymphs produced by alatae were generally selected when available in order to maximize the number of apterae in the cohort.

The period in which each nymph became adult and the morph of the adult were recorded. Although both nymphs in a clip-cage usually survived to maturity one adult was often discarded to keep the number of adults whose reproduction was being monitored within manageable limits. In this case the adult to be kept was transferred to the second unfolded leaf of the shoot on which it had been reared. Often two nymphs in a cage matured in different 3-day periods. To prevent bias in the selection of the adult to be kept the following rule was applied: If the cage code number was even then the first aphid to mature was kept and if the cage number was odd the second to mature was kept. This rule was usually waived for apterae which were all kept except on one or two occasions when a large number matured in the same 3-day period. When both aphids reared in the same cage were kept as adults one was transferred to the second unfolded leaf on the main stem of a new plant. The rule given above was used to decide which one was moved to the new plant.

The cages of adults whose reproduction was being monitored were checked on every recording day until they died or until the experiment was ended in the second half of March. The number of nymphs present was recorded and as many as possible were removed without disturbing the adult. The number of nymphs too close to the adult to be removed, if any, was also recorded. Nymph production in a 3-day period was the number recorded at the end of the period minus the number left on from the previous period. If the nymphs were not required for monitoring they were removed with a spot of 'Hyvis'* sticky-trap compound on the tip of a mounted needle. This was quicker and easier than using a brush. Whenever an adult was found on the

* supplier: The Wolfson Unit, Dept. of Biology,
University of Southampton.

walls of a clip-cage this was recorded and it was returned to the leaf. An adult's death and any oddities in its appearance before death, such as discolouration or Entomophthora infection, were also recorded. The time of day at which aphid data were taken was noted on each recording day. At the end of the experiment the mean recording time was calculated and used in dividing up the hourly temperature records into 3-day period segments.

Apart from the leaf change made when an aphid became adult, aphids were not moved to new leaves at set times. But on each recording day all the leaves with aphids caged on them were examined and any aphids on leaves showing damage or chlorosis were moved to the second unfolded leaf of the same shoot. On the rare occasions when this leaf was also damaged the aphid was transferred to the first or third leaf, or to the second unfolded leaf of a side tiller, or, if the whole plant was unhealthy, to a new plant. Whenever an aphid was moved to a new leaf the number of unfolded leaves above the old leaf and the date of the move were recorded. The old leaf was always one of the five youngest unfolded leaves on the shoot. At the end of the experiment the data were checked to see whether the timing and frequency of leaf changes affected development and reproduction, but no effects could be detected. The cages appeared to have little effect on the leaves, probably largely because of the low temperatures and slow plant growth rates. On average an adult was moved about once every 25 recording days after its first transfer as a newly-moulted adult.

4.2.6 Plant growth stage recording

Details of host plant growth stage were recorded for each aphid when it was established in a cage as a first instar and again when it became adult. For aphids transferred to a new plant at maturity the growth stages of both old and new plants were recorded. All the plant growth stages recorded lie between 12,20 and 19,29,30 (Zadoks et al., 1974). Because of the relatively narrow range of growth stages used, additional details not incor-

porated in the Zadoks scale were taken. The parameters recorded were:

1. Number of live and dead leaves on the main shoot and each tiller.
2. Number of tillers.
3. Height of main shoot (soil level to tip of longest leaf when held vertical).
4. Length of longest leaf sheath on main shoot (shoot at soil level to ligules).
5. Sowing date.

4.2.7 Checking for the production of sexual morphs by the monitored adults

Because the experiment was run through the autumn and winter there was a possibility that the adults whose reproduction was being monitored would produce some oviparae and/or males. Whether or not this was occurring clearly required investigation as the production of sexual morphs, particularly if this continued through the winter, would inevitably reduce recruitment to a winter population of viviparae and reduce its chances of surviving the winter successfully.

The extent to which the viviparae being monitored were producing sexual morphs was assessed by rearing a sample of their progeny to adulthood in the field. On each recording date one of the newly moulted adults was selected and all the progeny it produced during its lifetime were reared and their morphs determined. Both apterae and alatae were used to provide the progeny sample. As far as was possible the morph of the adult selected for progeny rearing was changed from one recording date to the next. One to five progeny were reared in each clip-cage. Other rearing conditions were the same as those for the monitored aphids.

4.2.8 The duration of the experiment and the number of aphids monitored

The first aphids whose development and reproduction were monitored were established on 24 September 1980 and the last recording date was 29 March 1981. The last cohort of aphids to be established was born between 7 and 10 January 1981 and all were adult by 14 March. Monitoring of most adults stopped on 14 March but apterae which had yet to produce their twentieth nymph were checked after this until they had done so. A total of 242 apterae and 319 alatae were reared to adults and of these 235 apterae and 171 alatae were kept and their nymph production monitored. Adult mortality was low and only 59 apterae and 72 alatae had died before 14 March; these included eight killed accidentally during scoring and eight infected by Entomophthora.

4.2.9 Determining the duration of each nymphal instar

To check whether the relative durations of the nymphal instars were approximately the same under winter field conditions as those determined by Dean (1974b) at higher temperatures in the laboratory, the cohort of 18 nymphs born between 7 and 10 January was examined on every recording day between birth and maturity. Changes in siphunculi width-to-length ratio and the presence of moulted skins in the cages were used to distinguish between instars. Because third and fourth instar nymphs were large and different enough to be distinguished quickly and easily, without the need to examine the clip-cages for moulted skins, estimates of the duration of the fourth nymphal instar of each morph were based on larger samples, namely, all aphids born between 11 December and 10 January.

4.3 RESULTS AND ANALYSIS

For several reasons - most importantly the variation in the numbers of apterae and alatae in different cohorts - cohort means were not used as the basic aphid data units for analysis. Instead, the data for each individual aphid were taken as the basic units. For the most part

temperature and aphid data were handled and analysed using programs written in Basic-plus on Southampton University's PDP11/45 computer. Multiple regression analyses and the calculation of Kendall's rank correlation coefficients were done on Southampton University's ICL 2970 computer using existing S.P.S.S. programs (Nie et al., 1975). To simplify analysis all dates (such as aphid birth and maturation dates) were converted to 3-day period code numbers. The periods were numbered in time sequence from one (21 to 24 September 1980) to 63 (26 to 29 March 1981). In all parts of the analysis data for apterae and alatae were analysed separately.

Finding functions to describe the relationships between development and reproduction rates and ambient temperature was central to the analysis. In examining these relationships the approach used was to assume initially that the simple relationship between rate and temperature found to hold good at intermediate temperatures for many insects (rate increases linearly with temperature measured from a theoretical threshold temperature) was applicable. Assuming this simple relationship, the best threshold temperature could be found using regression analysis and an assessment made of whether or not a straight line adequately represented the relationship between rate and temperature measured above this best threshold. If not, the assumption of linearity would be abandoned and an attempt made to find a more complex function to describe the relationship.

In finding functions to describe the relationships between development and reproduction rates and temperature, data from as many aphids as possible were used, but, in later stages of the analysis of reproduction, data from aphids born on August-sown wheat (all those born before 18 October) were treated separately or omitted. This was for the following reasons:

1. If these data were excluded the distribution of birth dates through time was similar for apterae and alatae. Thus comparisons between the morphs

could be made using all the data for each morph, with little chance of bias due to differences in birth date. If they were included the median birth and maturation dates for alatae were well before those for apterae.

2. Analysis revealed that many aphids reared in the early autumn on August-sown wheat showed atypically low total fecundities.
3. A central aim of the analysis was to provide basic parameters for a simulation model of a natural overwintering population of S. avenae on winter wheat. Such a population would not include aphids on August-sown wheat, as commercially grown wheat is never sown this early.

No evidence was found to suggest that aphids on August-sown wheat differed from the rest in their temperature requirements for development (see section 4.3.5) and so they were not treated separately in the analysis of development data.

4.3.1 Finding the best theoretical threshold for development and assessing the linearity of the development rate/temperature relationship

To find the best theoretical threshold temperature for development a trial-and-error method was used, assuming a different threshold each time. Each trial involved calculating the regression line of 'aphid development rate' on 'mean temperature during development measured above the assumed threshold' and then calculating an index of the extent to which the regression line explained the total variation in development rate.

The development rate of each aphid was defined as the reciprocal of the number of days from birth to maturity. Although birth and maturation dates were not known exactly (as aphids were examined only every third day), it was assumed that an aphid was born or became adult half-way

through the 3-day period preceding the recording date on which birth or maturation was noted. In calculating mean development temperatures it was assumed that mean temperature was the same in the first and second halves of a 3-day period in which an aphid was born or matured. These assumptions can only have resulted in minor inaccuracies as all aphids took more than five 3-day periods to reach maturity and all but 57 more than double that time. Because of these unavoidable assumptions several aphids from the same cohort often have identical estimated development rates and mean development temperatures.

Because the assumed development threshold was changed for each trial the mean development temperature for each aphid had to be recalculated each time. This was done by having all the hourly records of the temperature inside clip-cages (rounded to the nearest $^{\circ}\text{C}$) summarized in a frequency table stored in the computer as a 63×37 array. Each row of the array held data on one of the 63 3-day periods and each column data for a particular temperature from -7°C to 29°C (the extremes recorded during the experiment). Each number in the array represented the total number of hourly records of a particular temperature in a particular 3-day period. By multiplying this array by a different 37-item 'temperature transformation' array in each trial the basic frequency data were converted to hour-degree totals for each 3-day period measured above the assumed threshold. For example, if the assumed threshold temperature was 5°C a 'transformation' array was used such that all frequencies for temperatures of 5°C or below were multiplied by zero and hence ignored; 6°C records were counted as 1°C by multiplying the appropriate frequencies by one, 7°C records were counted as 2°C by multiplying their frequencies by two, and so on. An array holding each aphid's birth period and maturation period code numbers was also stored and the mean development temperature measured above the assumed threshold was calculated for each aphid from these and the 3-day hour-degree totals.

Once development rates and mean development temperatures had been calculated for each aphid a regression line

was fitted. The line was fitted through the origin as in each trial the threshold temperature used was assumed to be the 'real' one (the temperature below which no development occurs and above which development rate is proportional to temperature measured from it). The statistic used to assess how well the line explained the total variation in development rate (y) was:

$$\frac{S_{y.x}^2}{S_y^2} = \frac{\text{Variance of deviations of } y \text{ from regression line}}{\text{Total variance of } y}$$

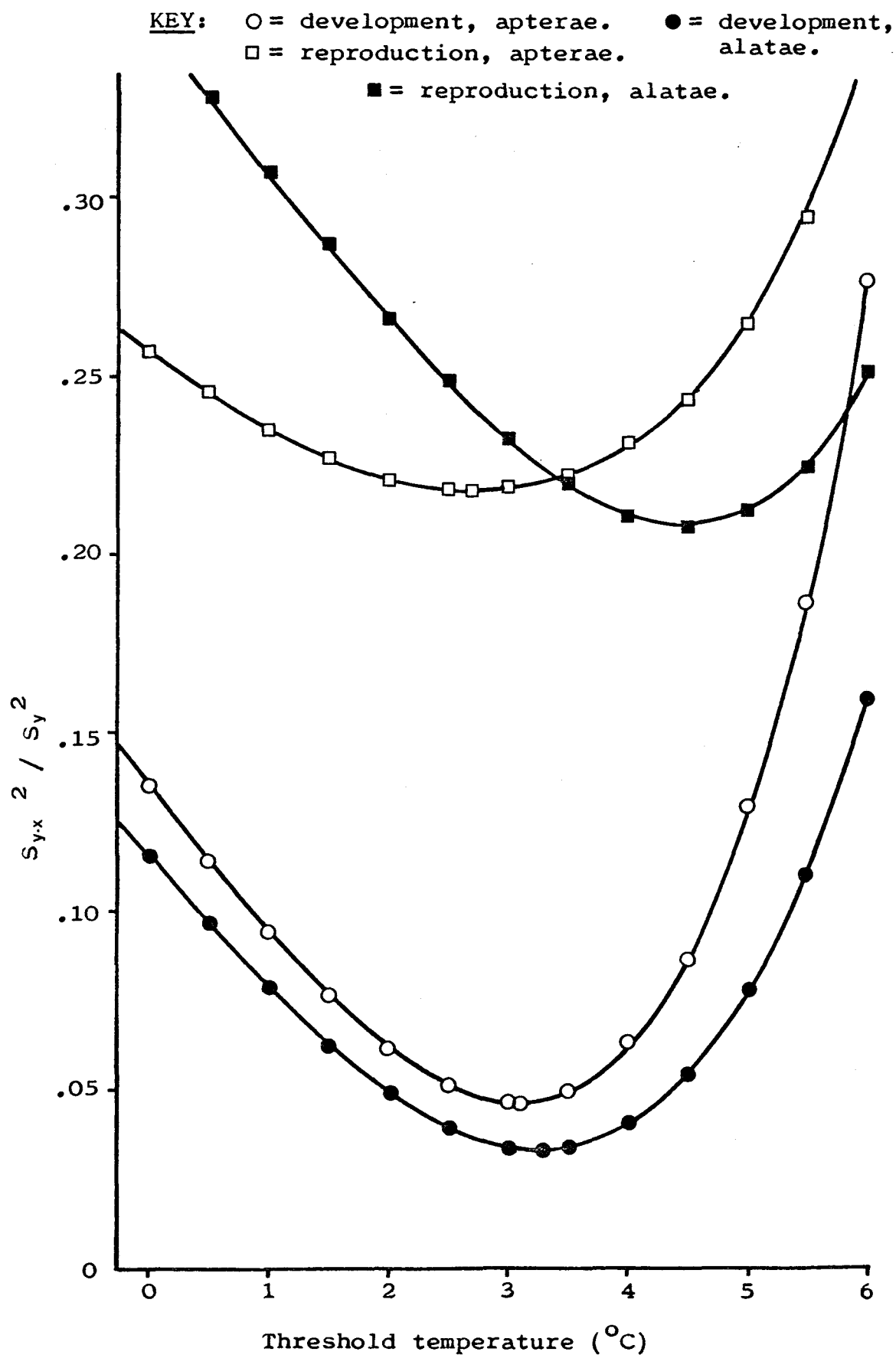
$$\text{where } S_{y.x}^2 = \left[\sum Y^2 - (\sum XY)^2 / \sum X^2 \right] / (n - 1)$$

(Snedecor & Cochran, 1967; p.166).

$S_{y.x}^2/S_y^2$ represents the approximate proportion of the total variation in y (development rate) which is independent of x (mean development temperature) (Snedecor & Cochran, 1967). Thus a low value for $S_{y.x}^2/S_y^2$ indicates a good fit of the regression line to the data. The parameter has the advantage over Pearson's correlation coefficient of not assuming bivariate normality in the data. However, it was not treated as an exact measure of 'goodness-of-fit' in this analysis because the data used here only approximately fitted the assumptions of regression analysis, due to the relatively large number of tied values and a tendency for errors in the estimates of development rate to increase as mean temperature increased (because recording was always done at 3-day intervals).

The statistic $S_{y.x}^2/S_y^2$ was quite sensitive to changes in the assumed development threshold (Fig. 4.3). The 'best' thresholds for apterae and alatae (those minimizing $S_{y.x}^2/S_y^2$) were 3.1°C and 3.3°C respectively. At and around these values the proportion of variation in development rate left unexplained by the regression line was about 0.05 or less (Fig. 4.3). Given that there were approximations in both mean temperature and development rate values, a common development threshold of 3°C for both morphs was used in later stages of the analysis. This allowed direct comparisons between the two morphs to

Fig. 4.3 Relationship between $S_{y \cdot x}^2 / S_y^2$ and assumed temperature threshold for the regressions of development and reproduction rates on temperature.



be made. There is no sign of curvilinearity in plots of development rate against mean development temperature measured above 3°C for either apterae or alatae (Figs 4.4 and 4.5). For both morphs most of the variation about the regression lines occurred at high mean development temperatures. This was an inevitable consequence of the experimental routine, as recording dates were three days apart whatever the temperature in the intervening periods. When temperatures were high the assumptions that birth and maturation occur half-way through a 3-day period were likely to result in greater errors and the birth and maturation periods represented a larger proportion of the aphids' nymphal life.

Regression lines not constrained to pass through the origin were also fitted for each assumed threshold temperature but $Sy.x^2/Sy^2$ for these was insensitive to changes in the threshold because discrepancies between the assumed and 'real' threshold could be compensated for by changes in intercept and slope. The temperature thresholds which minimized $Sy.x^2/Sy^2$ for these regression lines were 4.0°C and 3.2°C, for apterae and alatae respectively, close but not identical to best thresholds obtained when regression lines were constrained to pass through the origin. Minimum values for $Sy.x^2/Sy^2$ were 0.045 (apterae) and 0.033 (alatae) compared with 0.046 and 0.033 when regression lines were fitted through the origin.

4.3.2 Finding the best theoretical threshold for reproduction and assessing the linearity of the reproduction rate/temperature relationship

The time taken to produce the first 20 nymphs measured from the start of reproduction was used as the basic reproduction parameter. Its reciprocal gives a 'mean reproduction rate' for the early part of an adult's reproductive life. Associated with this rate is the mean temperature over the interval from the start of reproduction to the production of the twentieth nymph, the 'mean reproduction temperature'. The best reproduction threshold was found using these two parameters and the same

Fig. 4.4 Relationship between development rate and mean temperature during development measured above 3°C for apterae.

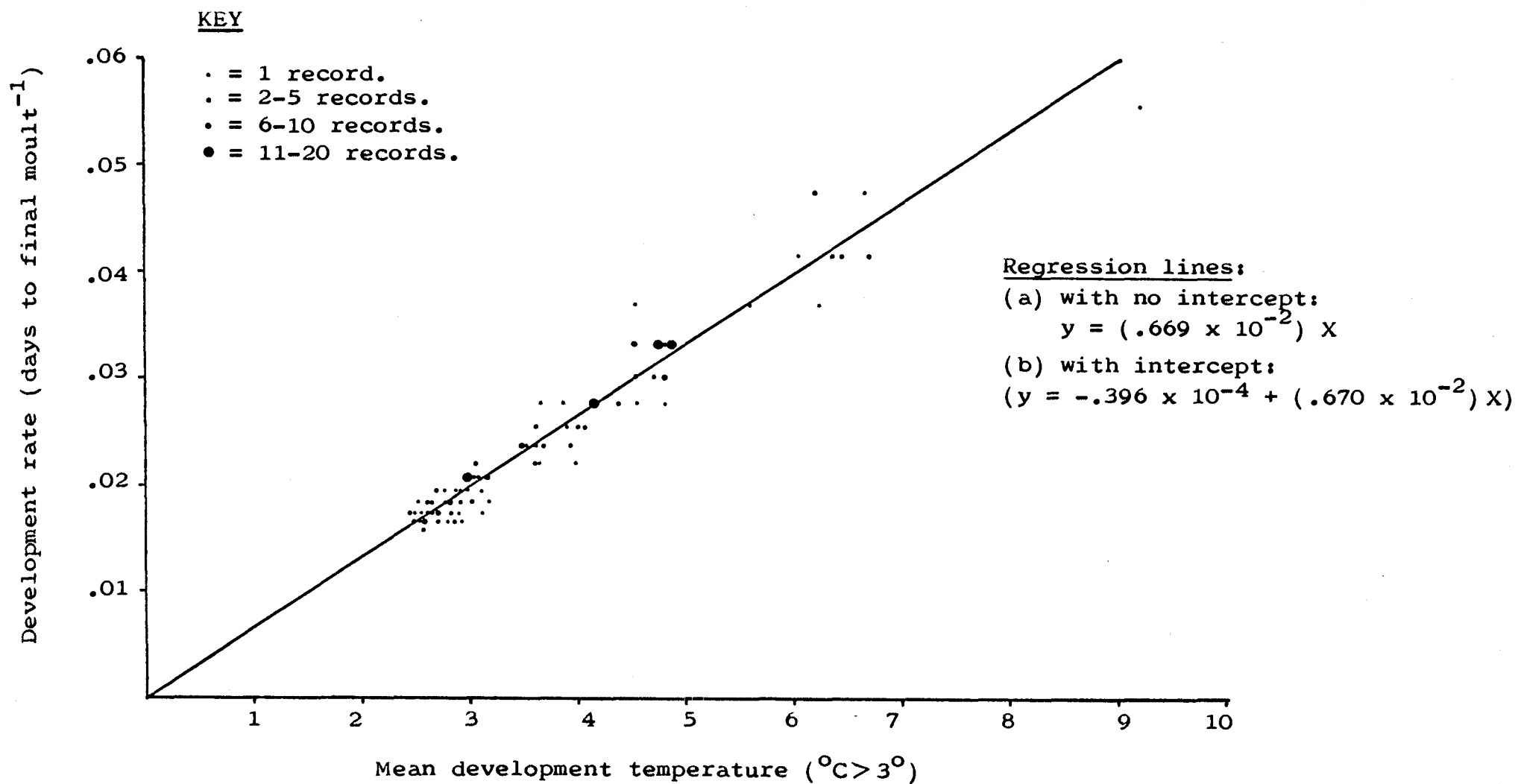
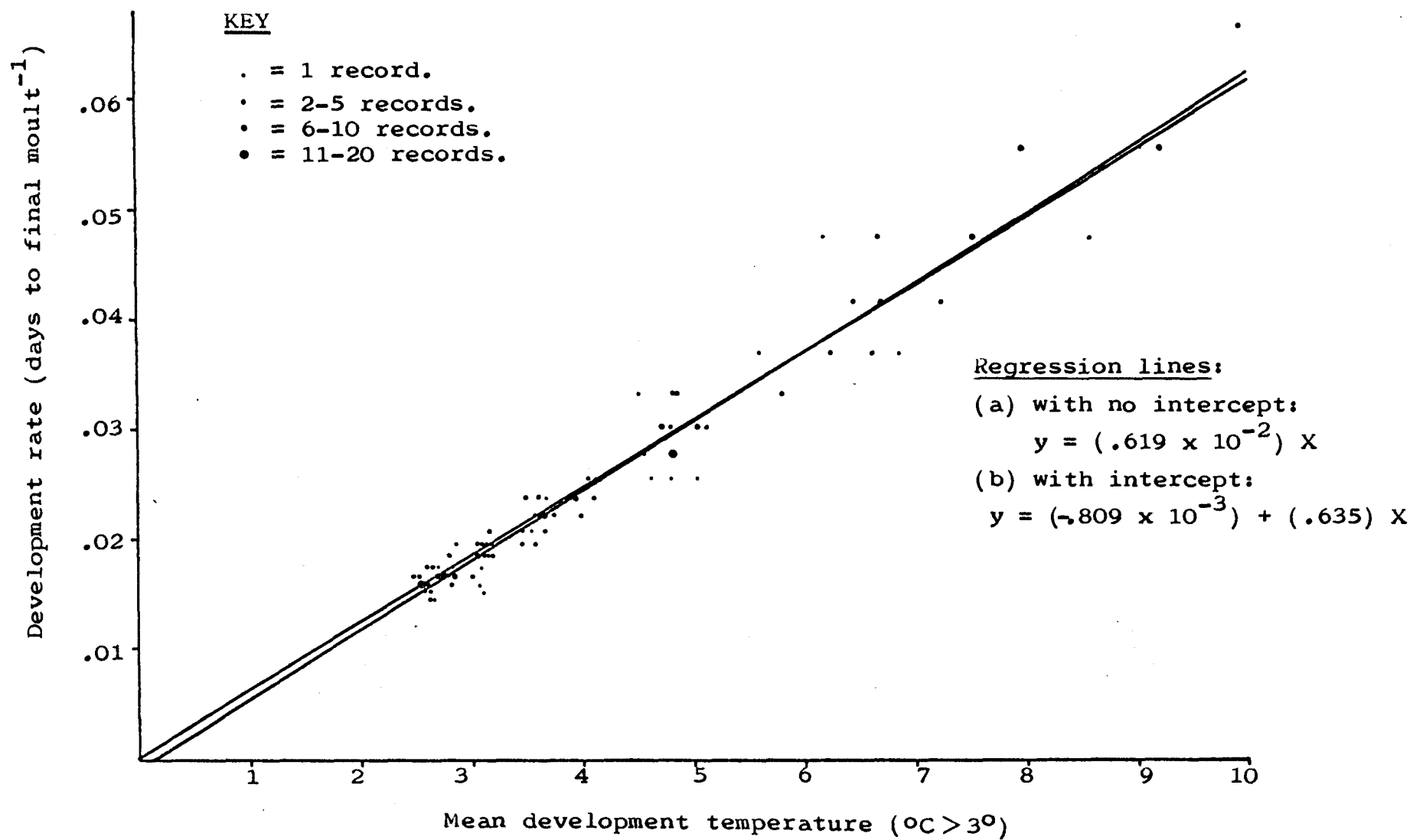


Fig. 45

Relationship between development rate and mean temperature during development measured above 3°C for alatae.



trial-and-error regression procedure used to find the best development thresholds.

The value of 20 nymphs was chosen for the reproduction parameter after examining scatter plots of nymph production by apterae and alatae in one mild 3-day period in March. In these plots the number of nymphs produced in the period by each adult was plotted against the number of nymphs it had already produced. Twenty nymphs was chosen as a compromise between a high number, which would reduce the number of adults for which the reproduction rate parameter could be calculated and would make it more likely to be affected by differences in total fecundity, and a low number, which would lead to larger inaccuracies of estimate. The value was selected with the analysis of reproduction by apterae in mind. It was a little high for alatae, whose total fecundity was lower (see section 4.3.7), but was used for both morphs in order to make direct comparisons between them possible.

In determining 'mean reproduction rate' it was assumed that for each adult:

1. the temperature in the 3-day periods in which the first nymph was produced was constant and that the first nymph was born half-way through the period.
2. the temperature in the 3-day period in which the twentieth nymph was produced was also constant and the time interval taken to produce each nymph in this period was equal. Thus, if an adult had produced 19 nymphs by a particular recording date and produced 4 more in the following 3-day period it was assumed that the twentieth nymph had been produced a quarter of the way through this period. As with the analogous assumptions used in calculating development rates and mean development temperatures these assumptions introduced errors which tended to increase as temperature increased but are relatively small because all but 20 adults took at least five 3-day periods to produce 20 nymphs.

Of the adults whose reproduction was monitored, 201 apterae and 93 alatae were used in the analyses to find the best reproduction thresholds. The remainder consisted of 34 apterae and 31 alatae which died or were lost before producing their twentieth nymph and a further 47 alatae from cohorts most members of which were still alive at the end of the experiment but had yet to produce 20 nymphs. All the 47 alatae from these cohorts (even those which had produced 20 nymphs) had to be excluded from the analysis to avoid bias. Of the apterae which died or were lost before producing their twentieth nymph all but three were either lost, or died early from causes other than old-age, such as Entomophthora infection or accidental damage during recording. Thus their omission did not bias the estimate of reproduction threshold for apterae. However, of the 31 alatae lost or dead before producing 20 nymphs, 14 were apparently healthy individuals which had very low fecundities despite surviving for a long time. Eleven of these were reared early in the experiment on August-sown wheat and they represented quite a large proportion of the alatae which experienced high mean reproduction temperatures. Thus their omission from the regression for alatae did introduce some bias, as one would expect their mean reproduction rates to be lower than average if these could be measured using some other parameter. This bias could not be removed by excluding all alatae born on August-sown wheat from the analysis as data from alatae reared on September and November sown plants showed too little variation in mean temperature during reproduction for them to be used on their own.

As in the analysis of relationships between development rates and temperature, the statistic $Sy.x^2/Sy^2$ was quite sensitive to changes in the assumed threshold temperature for reproduction (Fig. 4.3). However, the minimum values of $Sy.x^2/Sy^2$ calculated for the regressions of reproduction rate on mean temperature during reproduction (0.218 and 0.208 for apterae and alatae respectively) were well above those for the regressions of development rate on mean development remperature. But even so over

75% of variation in mean reproduction rate was associated with the regression line in both cases (Fig. 4.3). The best reproduction threshold for apterae (2.7°C) was, like development thresholds for both morphs, close to 3°C but that for alatae was 4.5°C . However, for the reasons given above, this was probably a biased estimate and so it cannot be taken to indicate a definite difference between the morphs. For both apterae and alatae, scatter plots of mean reproduction rate against mean temperature during reproduction assuming a reproduction threshold of 3°C (Figs. 4.6 and 4.7) show slight signs of curvilinearity. But in both cases the scatter of points suggests that by far the largest part of the unexplained variation was due to differences between individuals experiencing similar temperatures during reproduction, and not to a breakdown of the assumption of a linear relationship between reproduction rate and temperature.

In summary, it appears that under the conditions of this experiment the relationships between development rate and mean development temperature and between mean reproduction rate and mean reproduction temperature can be adequately described assuming that the rates increase linearly with temperature measured above a 'threshold' temperature. Best estimates of the development thresholds for both morphs and of the reproduction threshold for apterae all lay within 0.5°C of 3°C . The best estimate of the reproduction threshold for alatae was 4.5°C but was probably an over-estimate because the regression line for alatae was largely determined by data from a few early autumn aphids with atypically low fecundities. Consequently, a common threshold of 3°C for the development and reproduction of both morphs seems acceptable. This threshold was used when calculating mean temperatures and accumulated day-degrees in the rest of the analysis. The abbreviations used for these are ' $^{\circ}\text{C} > 3^{\circ}$ ' and 'day $^{\circ}\text{C} > 3^{\circ}$ '.

Fig. 4.6 Relationship between aptera reproduction rate (1/time to produce 1st 20 nymphs) and mean temperature during the production of the 1st 20 nymphs, measured above 3°C.

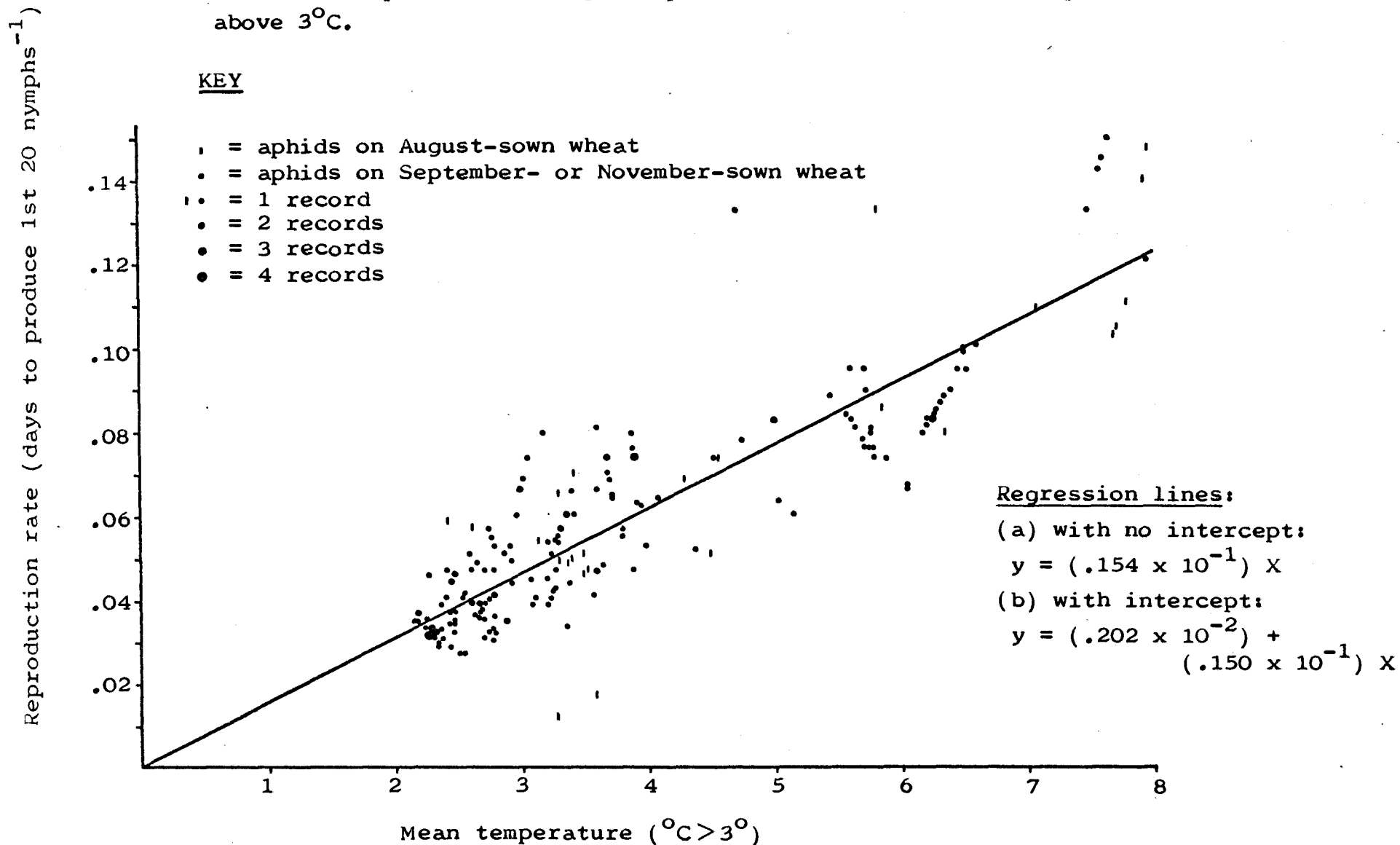
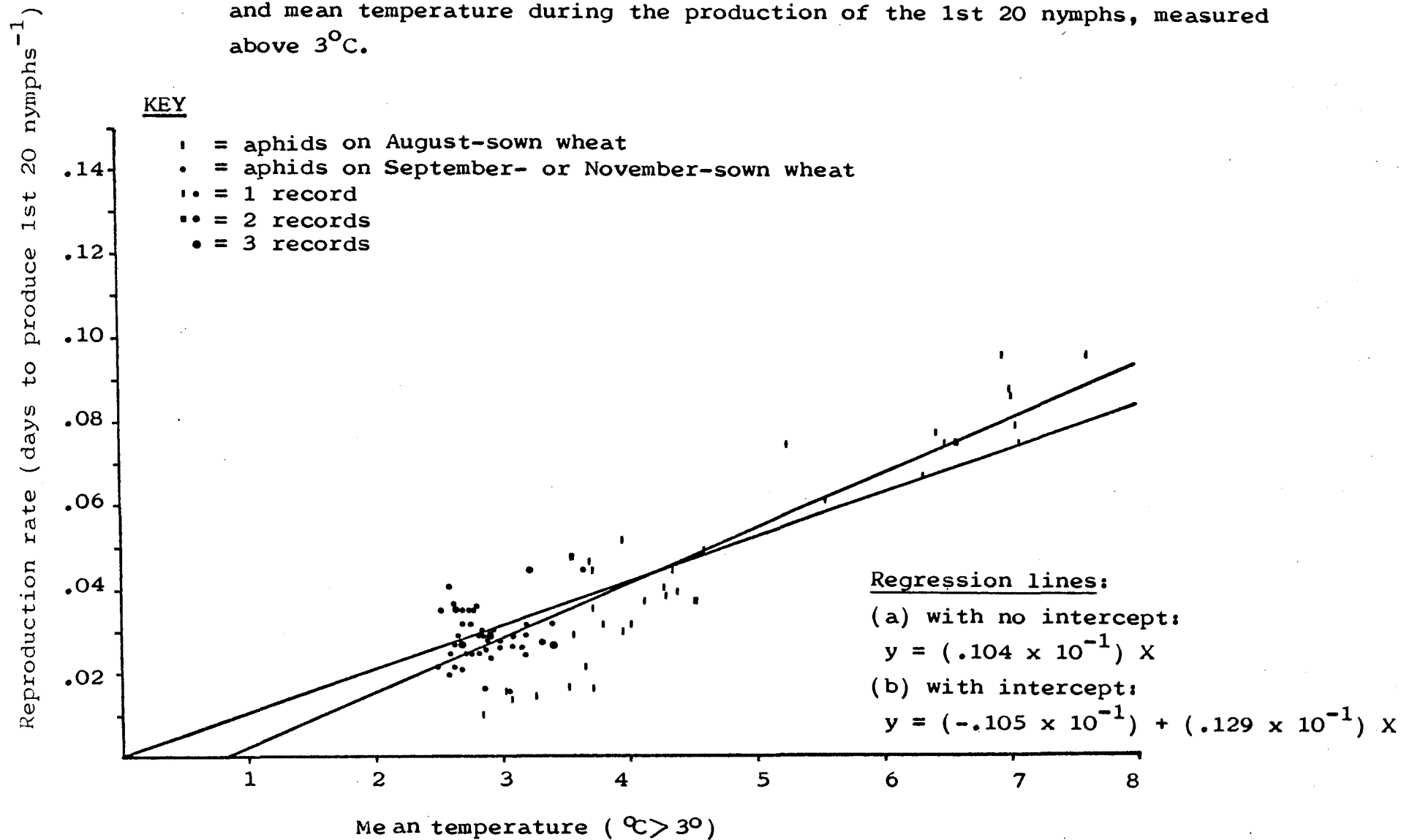


Fig. 4.7 Relationship between alata reproduction rate (1/time to produce 1st 20 nymphs) and mean temperature during the production of the 1st 20 nymphs, measured above 3°C.



4.3.3 Average day-degree requirements* for development and reproduction

The mean day-degree requirements for the development period (birth to final moult), adults' pre-reproductive period (final moult to birth of the first offspring) and 'period to produce the first 20 nymphs from the start of reproduction' for apterae and alatae were calculated assuming a 3°C threshold temperature (Table 4.1). For the reasons given above (page 259) data for aphids reared on August-sown wheat were excluded when calculating the pre-reproductive and 'first 20 nymphs' periods. Because of their relatively short duration, estimates for the pre-reproductive periods are inevitably only approximate. The day-degree requirement for the production of the first 20 nymphs was calculated separately for adults with apterous and alate parents because the parent's morph was found to affect reproduction significantly (see section 4.3.4). Mean day-degree requirements for development, pre-reproductive period and 'first 20 nymphs' period (Table 4.1, Figs. 4.8 and 4.9) were significantly higher for alatae than for apterae (t tests not assuming sample variances equal: $P < 0.001$ in all tests). For both apterae and alatae, the mean day-degree requirement for the production of the first 20 nymphs was significantly greater for adults with alate parents than for adults with apterous parents (t tests not assuming sample variances equal: $P < 0.001$ in both cases).

4.3.4 Factors modifying the day-degree requirements for the production of the first twenty nymphs

In this section the number of day-degrees above 3°C accumulated between the birth of an adult's first and twentieth nymphs is used as a parameter of reproduction rate measured in physiological time units and is referred

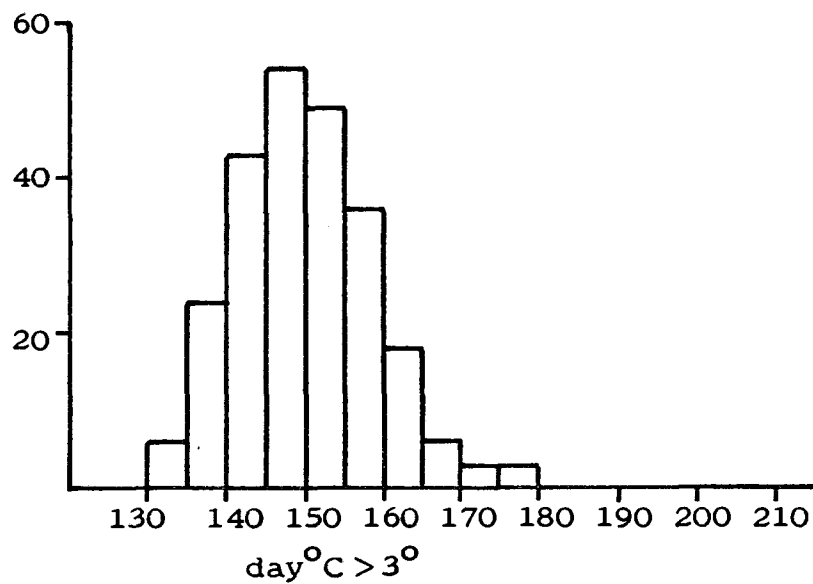
* the term 'day-degree requirement' has been used in preference to the more usual 'thermal constant' because it does not imply that the value is unchangeable.

Table 4.1: Day-degree requirements for apterae and alatae ($\text{day}^{\circ}\text{C} > 3^{\circ}$).

PERIOD	APTERAE			ALATAE		
	$\bar{x}$	S.E.	n	$\bar{x}$	S.E.	n
Nymphal development	150.1	0.58	242	163.6	0.6	319
Pre-reproductive adult life	17.4	0.77	191	31.7	1.5	119
Start of reproduction to birth of 20th nymph (all adults)	66.2	0.87	176	104.4	3.34	54
Start of reproduction to birth of 20th nymph (adults from alate parents)	69.3	0.86	140	116.5	4.68	28
Start of reproduction to birth of 20th nymph (adults from apterous parents)	54.1	1.26	36	91.5	3.27	26

Fig. 4.8 Frequency distributions for the day-degree requirements for development.

(a) Apteræ.



(b) Alatae.

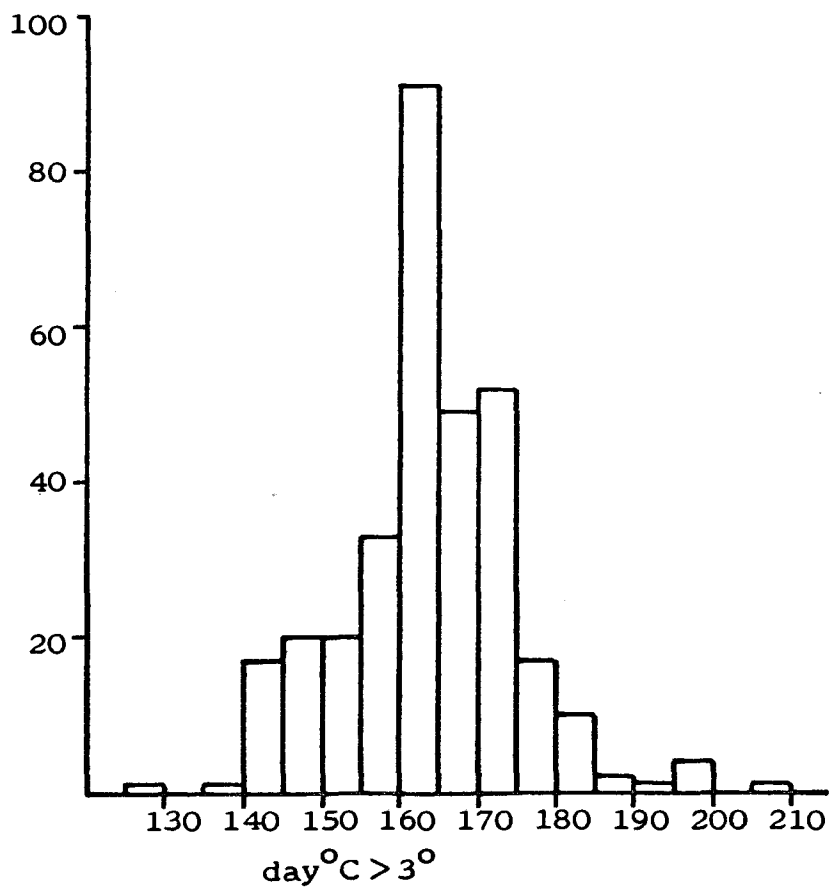
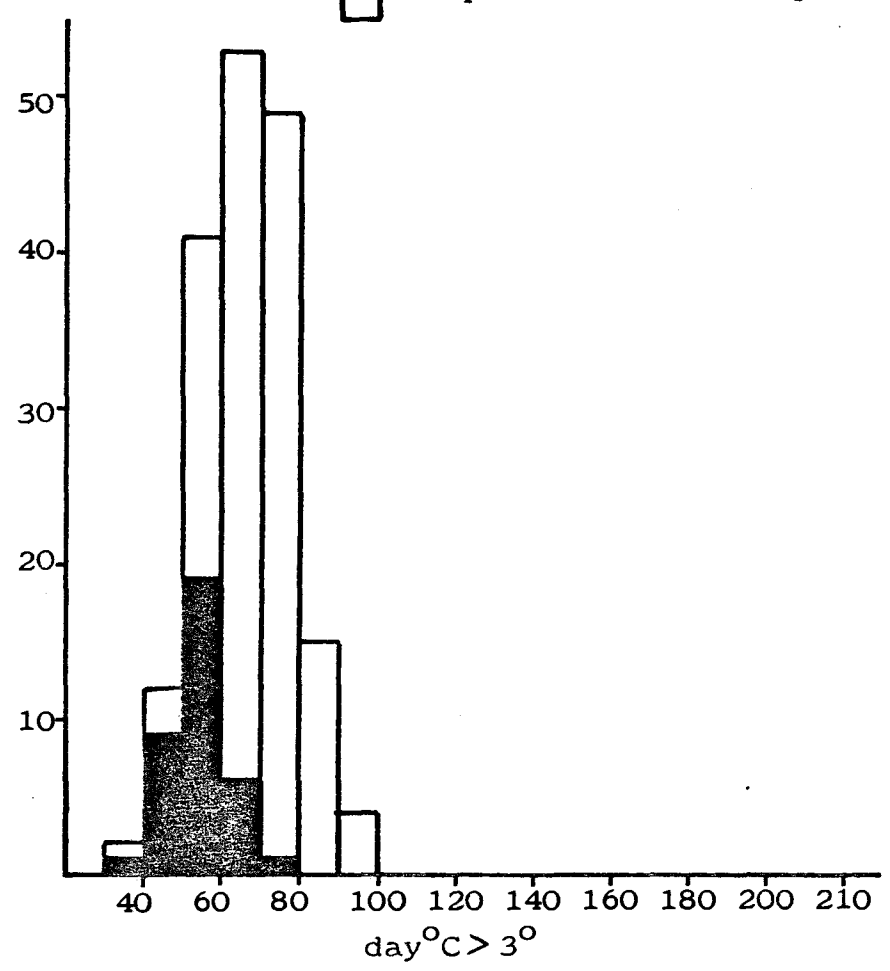
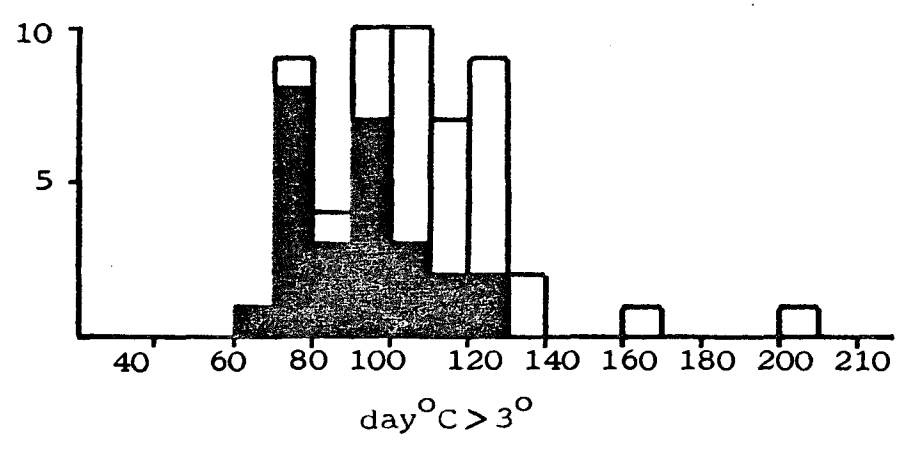


Fig. 4.9 Frequency distributions for the day-degrees required to produce the first 20 nymphs (excluding aphids reared on August-sown wheat).

 = aphids with apterous parents.
(a) Apterae.
 = aphids with alate parents.



(b) Alatae.



to as the '20 nymph day-degree requirement', abbreviated to '20 nymph d.d.r.'. It is used to assess the extent to which reproduction rate, measured in physiological time units and for adults of the same physiological age relative to the start of reproduction, varied during the experiment and to try to determine the factors producing this variation.

A Kruskal-Wallis one-way analysis of variance by ranks (Siegel, 1956) was carried out on the apterae 20 nymph d.d.r.s to determine whether they changed significantly with maturation date. This non-parametric test was used instead of normal analysis of variance because there were several reasons why the data might not be normally distributed with similar variances for all 'treatments'. For the test, 20 nymph d.d.r.s were divided into five groups, each 30 days long, according to maturation date. The test showed very significant changes in mean 20 nymph d.d.r. with maturation period ($H = 49.94$, $P < 0.001$). The same test on the smaller amount of data for alatae showed no significant effect ($H = 5.83$, $P > 0.1$). Further analysis of the data set for apterae was undertaken to investigate the pattern and possible causes of the variation. In this analysis two apterae with total fecundities just above 20, and 20 nymph d.d.r.s of over $200 \text{ day}^{\circ}\text{C} > 3^{\circ}$ (over double that of any other apterae) were excluded.

A scatter plot of the 20 nymph d.d.r.s of apterae against maturation period was drawn and Kendall's rank correlation coefficients were calculated between the 20 nymph d.d.r.s and various parameters of possibly causal factors. Multiple regression analyses were performed with the 20 nymph d.d.r.s of apterae as the dependent variable and the same set of parameters used for the calculations of correlation coefficients as the independent variables. The following parameters of possibly causal factors were used:

1. Mother's morph (1): denoted by a 'dummy variable' with a value of one if the mother was alate and zero if it was apterous.

2. Parameters of 'time since the beginning of the experiment' (2):
 - (a) Birth period (3-day period code number).
 - (b) Maturation period (3-day period code number).

3. The total number of day^{°C} > 3[°] accumulated between birth and maturity.

4. Parameters of temperatures experienced (2 x 4):
 The same four parameters were used for the temperature experienced during development and during the period in which the first 20 nymphs were produced. These were:
 - (a) Mean temperature (measured above 3^{°C}) over the period.
 - (b) Minimum temperature (^{°C}) during the period.
 - (c) Mean number of hour^{°C} at or below 0^{°C} experienced per night during the period.
 - (d) Mean number of hour^{°C} at or below -5^{°C} experienced per night during the period.
 The last three parameters were included as it was thought that sub-zero temperatures might inhibit or have damaging sub-lethal effects on reproduction.

5. Parameters of host plant growth stage (2 x 5):
 The same five parameters were used for plant growth stage recorded at birth and when the aphid became adult:
 - (a) Total number of live, unfolded leaves.
 - (b) Total number of leaves on the main shoot.
 - (c) Number of tillers.
 - (d) Length of the longest leaf sheath.
 - (e) Height.

6. Parameters of date on which the host plant's seed was sown (2 x 2):
 The three possible sowing dates were denoted by two 'dummy' variables, the first with a value of one if the wheat was sown in September and zero otherwise, the second with a value of one if the wheat was sown

in November and zero otherwise. These were used for the plant on which the aphid was reared and the plant on which it was kept as an adult. For all aphids, except a few reared on August-sown wheat, the sowing dates were the same in both cases.

A full list of the Kendall's rank correlation coefficients between the 20 nymph d.d.r.s of apterae and these parameters is given in Appendix 4.1 with their associated probabilities.

From the scatter plot of the 20 nymph d.d.r.s of apterae against maturation period (Fig. 4.10) and the Kendall's rank correlation coefficients calculated, the following points are apparent:

1. Apterae with apterous parents had significantly lower 20 nymph d.d.r.s than those with alate parents. In other words their reproductive rate, measured in physiological time units, was faster during the period in which the first 20 nymphs were produced. The correlation between mother's morph and 20 nymph d.d.r.s was positive and highly significant ($P \leq 0.001$).
2. There was a gradual increase in the 20 nymph d.d.r.s from autumn to early spring. The correlations between birth period or maturation period and 20 nymph d.d.r. were highly significant ($P \leq 0.001$ in both cases). This was true whether or not aphids on August-sown wheat were excluded and whether or not the 'mother's morph effect' was excluded by calculating rank correlations based only on the data for apterae with alate parents.

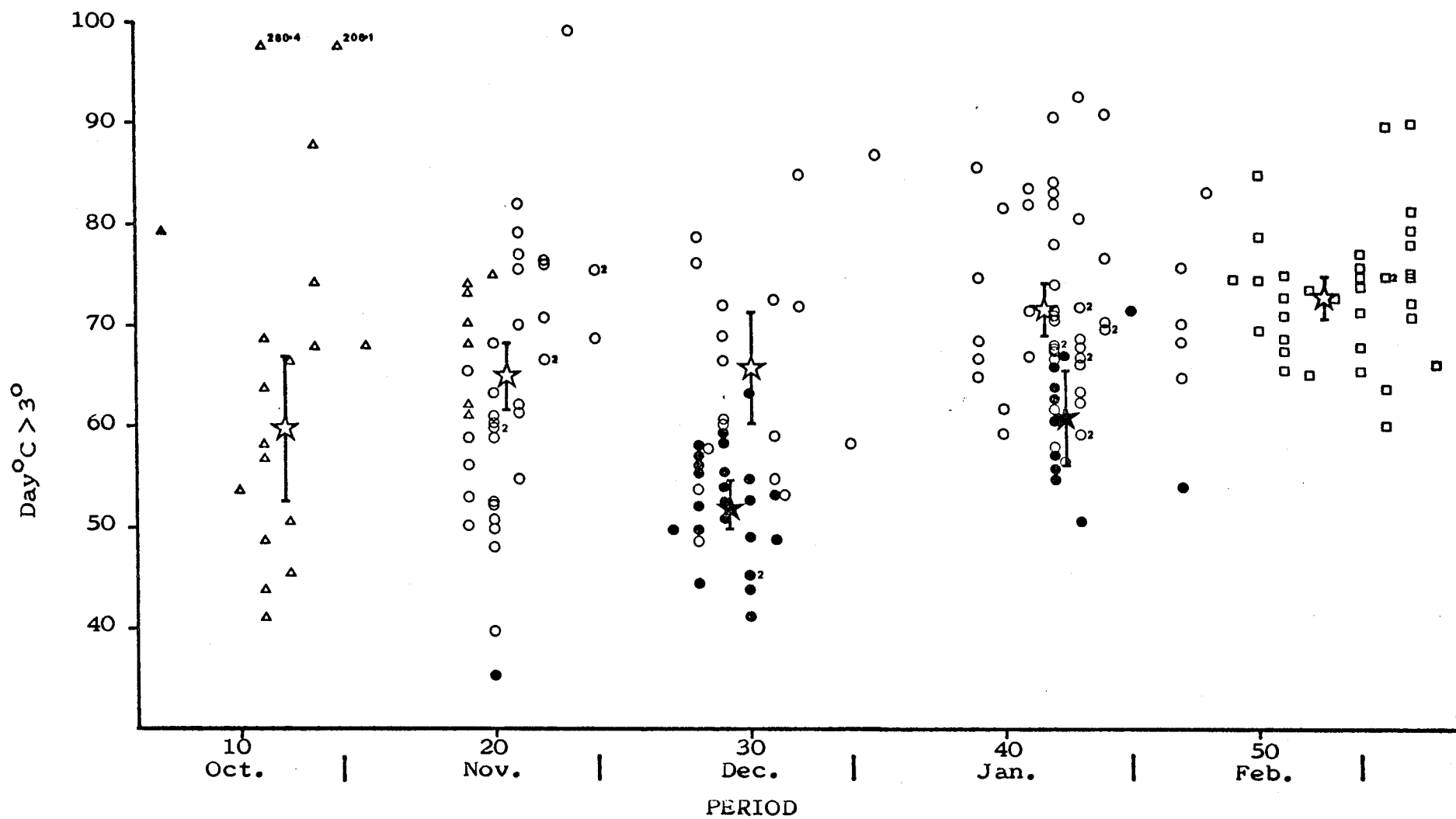
This 'time-of-year effect' may have been a direct seasonal influence (for example some direct effect of daylength on the aphids' physiology), a similar direct physiological effect on the host plant, or an 'indirect' effect via some factor which is usually related to season, such as temperature or host plant growth stage. Because

KEY: $\Delta \blacktriangle$ = aphids reared on August-sown wheat.
 $\bigcirc \bullet$ = aphids reared on September-sown wheat.
 $\square \blacksquare$ = aphids reared on November-sown wheat.

Solid symbols denote aphids with apterous parents and
hollow symbols aphids with alate parents.

$\begin{array}{c} | \\ \star \\ | \end{array} \quad \begin{array}{c} | \\ \blackstar \\ | \end{array}$ 30-day period means, with 95% confidence
interval, for aphids with alate and
apterous parents.

Fig. 4.10 Relationship between day-degrees required to produce 20 nymphs and maturation period, for apterae.



several of the parameters used in the analysis are highly correlated with date measured from the beginning of the experiment, and because a host of other, unmeasured, factors are also likely to be correlated with time of year, it is impossible to be certain of the cause of the time-of-year effect but an attempt was made to reduce the number of possible causes. To do this, Kendall's rank correlation coefficients were recalculated using data only from apterae with alate parents, to exclude the effect of the parent's morph (see Appendix 4.1 for details).

When this was done, birth and maturation date had significant correlations ($P < 0.05$) as did the four parameters for temperature during development, two of the dummy variables for sowing date, the day-degree requirement for development and the minimum temperature experienced during reproduction. None of the plant growth stage parameters gave significant correlations nor did the three other parameters for the temperature during reproduction, which suggested that these were not important factors in producing the variation shown in 20 nymph d.d.r.s with time of year. The number of day-degrees accumulated during development is an intrinsic rather than an extrinsic factor and variation in it must be ultimately produced by some environmental factor. The significant positive correlation between it and 20 nymph d.d.r.s shows that there was a tendency for aphids with low development rates (measured in physiological time) to have low reproduction rates also but does not help to identify the ultimate cause of the changes in reproduction rate, apart from suggesting that it was some factor acting during the aphids' development.

If the variation in 20 nymph d.d.r.s with time of year were caused by differences in sowing date or plant growth stage, one would expect sudden changes in 20 nymph d.d.r.s between the last aphids to mature on August-sown wheat and the first to mature on September-sown wheat, and between the last to mature on September-sown wheat and the first on November-sown wheat, as these were points at which sowing date changes and large and abrupt changes in plant growth stage occurred. But no such changes in

d.d.r.s are apparent (Fig. 4.10), so that sowing date and plant growth stage can be ruled out as important causal factors. It also appears that the temperature experienced during reproduction was unimportant in modifying 20 nymph d.d.r.s as, if this were important, one would expect 20 nymph d.d.r.s to fall again towards the end of the experiment and this did not occur (Fig. 4.10). The possible causal factors remaining are time-of-year itself, acting directly on the aphid or its host, an unmeasured factor correlated with time since the beginning of the experiment, or the temperature experienced during development. If the increase in 20 nymph d.d.r. from autumn to spring was produced by a direct effect this cannot have been a simple response to daylength as, if this were so, 20 nymph d.d.r.s would have started to fall in early spring. The temperature experienced during development seems a likely causal factor. Aphids experiencing low temperatures during development might be expected to have slower reproduction rates in the early part of their adult lives as a result, or perhaps a by-product, of physiological acclimatization to cold.

The multiple regression analysis performed using the 20 nymph d.d.r.s of apterae as the dependent variable and the set of parameters listed above as the independent variables added parameters to the regression equation in a stepwise fashion. At each step the parameter added was the one which would give the most significant partial regression coefficient when included in the equation. Only parameters with significant ($P < 0.05$) partial regression coefficients were included. In addition to the variables listed above a set of nine 'dummy' variables was included in the set of possible independent variables. Each represented a different 15-day period. If an aphid matured at some time in this period the dummy variable was given a value of one, and if not it was given a value of zero. These period variables were included to take account of variation linked to maturation date in some non-linear way. They were given a lower 'inclusion level' than the other variables in the analysis, which meant they were only considered for inclusion at each

step if all the other variables not yet in the equation had non-significant partial regression coefficients. The analysis was performed three times, first for all the apterae and then for two subsets of data: those apterae reared on September- and November-sown wheat and those reared on September-sown wheat only - too few aphids were reared on August-sown and November-sown wheat for their data to be analysed separately. In all three analyses plots of standardized* residuals against maturation date and against predicted standardized* 20 nymph d.d.r.s were examined to check that they were approximately normally distributed.

Details of the results of these analyses are shown in Appendix 4.2. They confirm the importance of mother's morph as a predictive variable - it was included first in all three regression equations. The importance of time-of-year, or some variable correlated with time measured from the beginning of the experiment, is also confirmed. In two of the analyses 'maturation date' was the second variable to be included in the equation and in the analysis for aphids reared on September-sown wheat a plant growth stage parameter highly correlated with maturation and birth dates for this subset of data was entered second. Despite the conclusion reached (from examinations of Fig. 4.10 and Kendall's rank correlation coefficients) that development temperature was a likely cause of the time-of-year effect, a development temperature parameter was only included in one regression equation. However, this is not surprising as once one of a set of inter-correlated variables is included in a multiple regression equation the others are unlikely to be, even if they are causal, because a measure of their effect is already in the equation in the form of the first, correlated variable.

A high degree of inter-correlation among the independent variables also produces an unstable regression equation whose independent variables and partial-regression coefficients change dramatically depending on the data set used. Thus, despite each of the three equations' having

*'standardized' data were transformed so that they have a mean of zero and standard deviation of one.

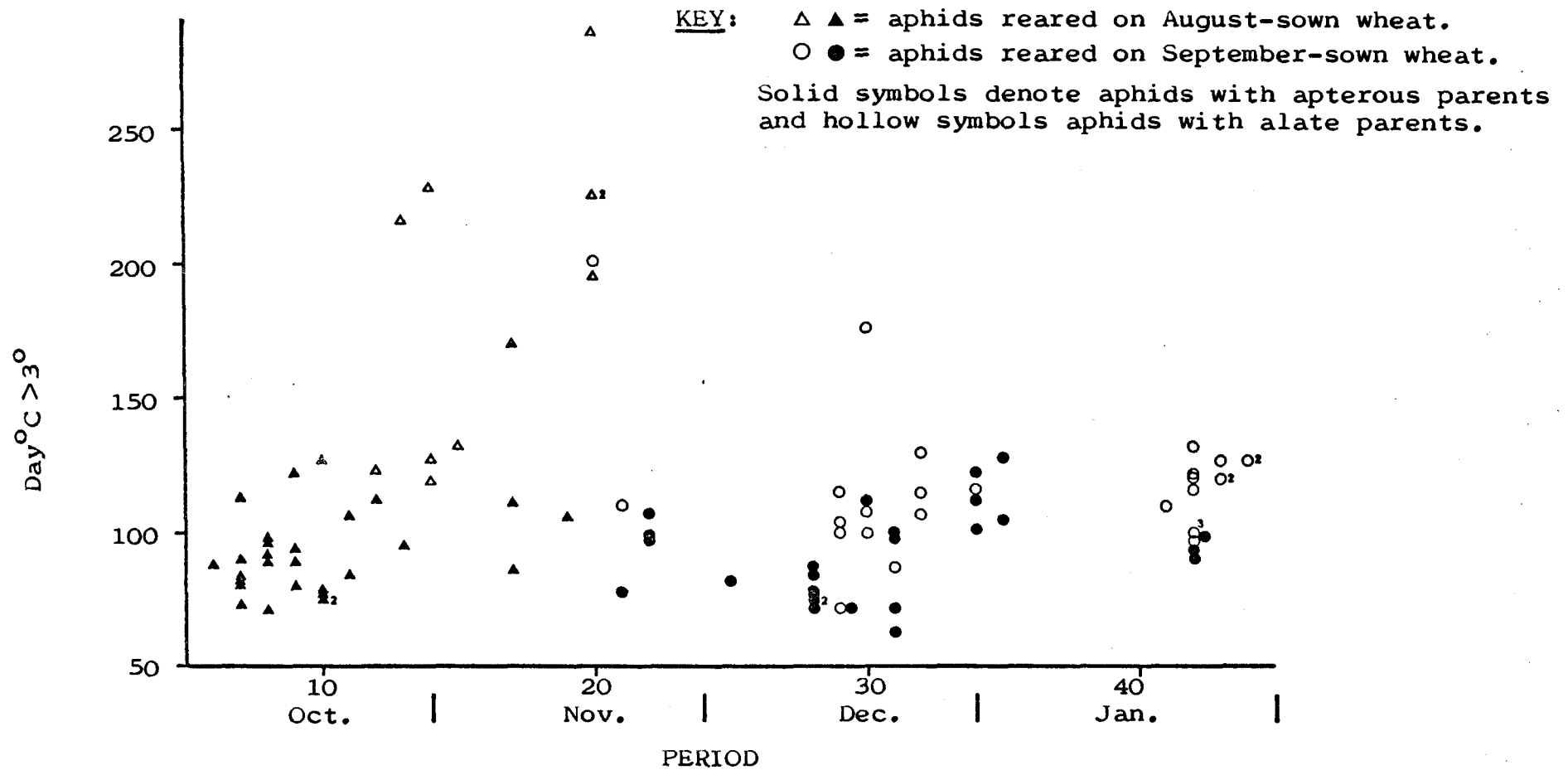
seven or eight independent variables in them, only two variables were common to all three, 'mother's morph' and a 'dummy' period variable. Pearson's r^2 (which approximates to the proportion of the total variation in the dependent variable explained by the equation) was less than 0.6 for all three equations, showing that, despite the large number of independent variables in the equations, they only explained about 60% of the total variation in 20 nymph d.d.r.s. For this reason, and because of their instability, the equations are inadequate as predictors of variation in 20 nymph day-degree requirement.

As with the apterae, alatae with apterous parents had lower 20 nymph d.d.r.s than those with alate parents (Fig. 4.11). The effect was less clear cut than for apterae but still highly significant, whether or not alatae reared on August-sown wheat were excluded (Kendall's rank correlation coefficient: $\tau \geq 0.47$, D.F. ≥ 53 , $P < 0.01$ in both cases). But, if the influence of the parents' morph was controlled by calculating Kendall's rank correlation coefficients for aphids with apterous and alate parents separately, there was no significant tendency for 20 nymph d.d.r.s to increase or decrease with maturation date, whether or not alatae reared on August-sown wheat were excluded (Kendall's rank correlation coefficients: $|\tau| \leq 0.23$, D.F. ≥ 25 , $P > 0.1$). The main feature of variation in 20 nymph d.d.r.s for alatae was the occurrence of several alatae with very high 20 nymph d.d.r.s during the last week of October and the first three weeks of November (Fig. 4.11). These aphids had low total fecundities of just over 20 nymphs and several other alatae in this period had total fecundities of less than 20 nymphs. Seasonal changes in total fecundity are discussed further below (section 4.3.7).

4.3.5 Factors modifying the day-degree requirements for development

Kruskal-Wallis one-way analysis of variance by ranks was carried out on the day-degree requirements for development for apterae and alatae to determine whether these

Fig. 4.11 Relationship between day-degrees required to produce 20 nymphs and maturation period, for alatae.



changed significantly with birth date. For each test the d.d.r.s were split into four groups, each 30 days long, according to birth date. Neither test showed a tendency for development d.d.r.s to change with birth date (apterae: $H = 5.22$, D.F. = 3, $P > 0.1$; alatae: $H = 1.45$, D.F. = 3, $P > 0.5$).

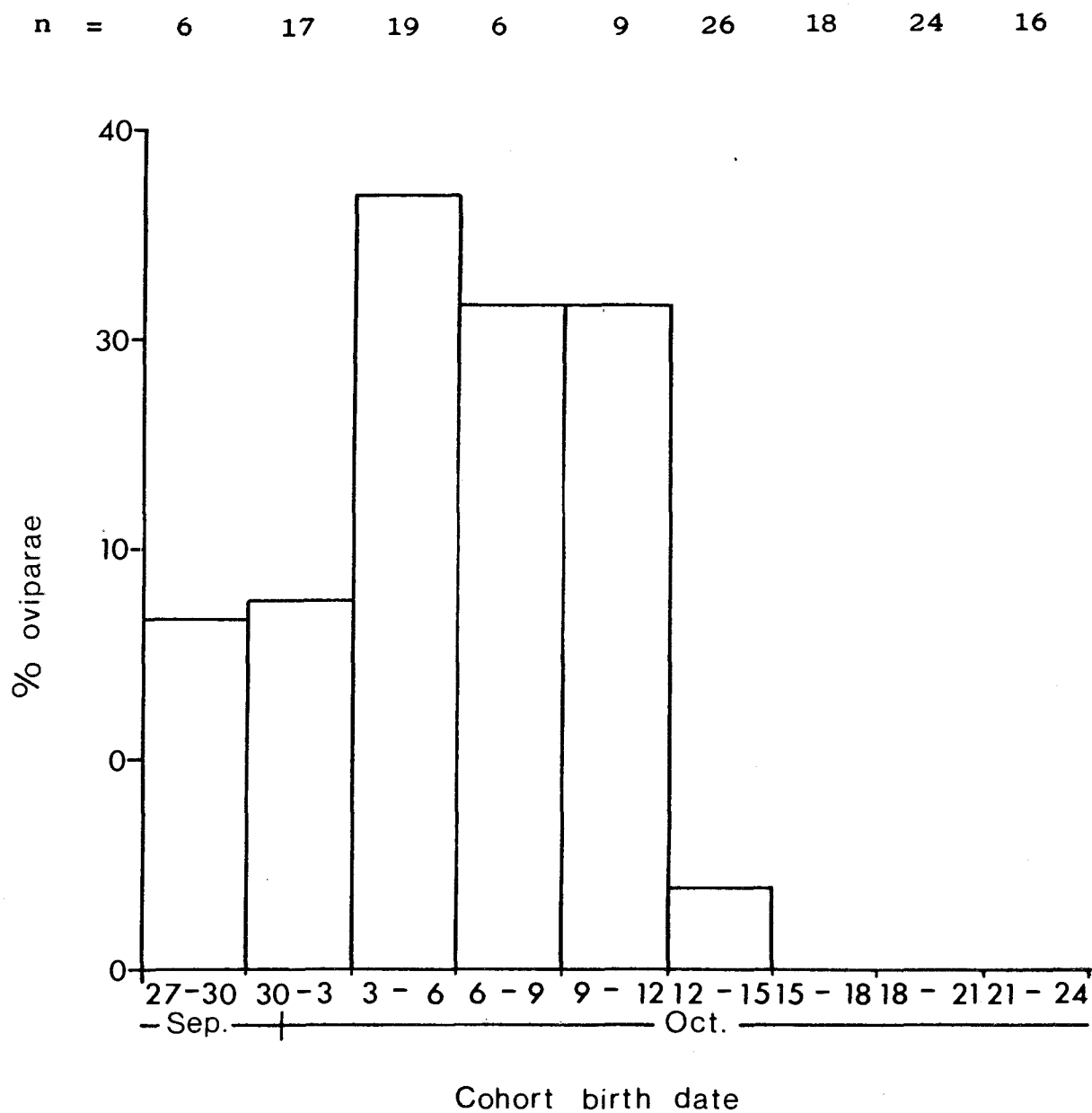
Kendall's rank correlation coefficients were calculated between the development d.d.r.s for apterae and a set of 25 parameters of possibly causal factors. This was a subset of those used in the analysis of 20 nymph d.d.r.s, with those parameters only relevant to adult life omitted. A list of the parameters used, the Kendall's τ associated with each and its probability is given in Appendix 4.3. None of the correlations, which included those with birth date and parent's morph, were significant ($P > 0.05$ in all cases). Thus it appears that the day-degree requirements for development recorded during this experiment can be regarded as coming from single populations for each morph.

4.3.6 The production of sexual morphs

It appears that apterous and alate viviparae of the experimental clone born after 21 September were producing viviparae exclusively as none of the subsample of their progeny reared to adulthood (533 aphids) were oviparae or males. However, 17 of the first generation of aphids reared for monitoring were oviparae. All of these were born in the period 27 September to 15 October. In this interval a total of 40 nymphs born to apterae and 83 born to alatae were established in clip-cages, but all 17 oviparae were born to alatae. This difference in the numbers of oviparae produced by the two morphs is significant ($\chi^2 = 7.87$, D.F. = 1, $P < 0.01$). This supports the conclusion reached by Watt (1979b) and Hand (1982) after laboratory studies, that oviparae of S. avenae are usually produced by alatae.

As aphids in the first two cohorts (those born before 27 September) were all born to apterae, ovipara production by alate gynoparae of the experimental clone

Fig. 4.12 The proportion of oviparae in early autumn cohorts expressed as the percentage of total nymphs born to alatae in each cohort.



may start before 27 September. The proportion of the offspring of alatae which became oviparae reached a peak in early October (Fig. 4.12). It is not surprising that no males were produced along with the oviparae as all the nymphs reared for monitoring were among the first 15 produced by an adult and males are only produced later in reproductive life (Watt, 1979b; Hand, 1982).

4.3.7 Changes in total nymph production with aphid birth date

As most aphids did not have a well-defined post-reproductive period, unbiased estimates of total fecundity could only be obtained from cohorts in which all or nearly all the aphids had died before monitoring was stopped in mid-March. For this reason, and because the aphids, particularly the apterae, survived extremely well, data on total fecundities are available only for apterae in the first 11 cohorts and alatae in the first 13, that is, individuals born before 24 October and 30 October respectively.

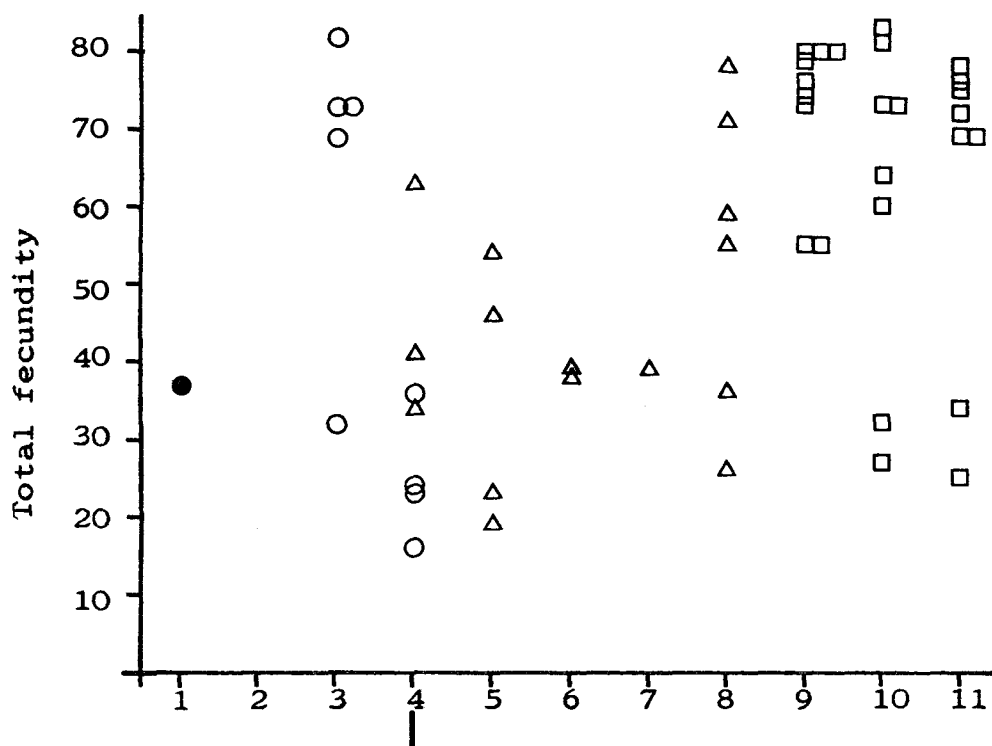
Despite the small number of aphids for which data on total fecundity are available and the limited range of birth dates which these aphids represent, obvious changes in total fecundity with birth date are apparent for both apterae and alatae (Fig. 4.13). In both cases there was a decline in total fecundity from aphids born in late September to those born in early to mid-October, followed by a recovery. The two morphs differed in that for apterae the trend was less marked, the lowest fecundities were reached slightly earlier, the recovery was more abrupt and complete, and total fecundities were usually higher than for alatae born at the same time, particularly for aphids born after mid-October. The trend in total fecundities cannot be attributed simply to aphids with low nymph production dying younger than the others and hence not having time to produce their full complement of nymphs, as aphids with low fecundities were atypical in usually having a long post-reproductive period. Also, all the apterae and all but four of the alatae represented in

Fig. 4.13 Changes in total fecundity with birth date.

KEY ○ ● = aphids kept on August-sown wheat.
 □ ■ = aphids kept on September-sown wheat.
 △ ▲ = aphids reared on August-sown wheat but transferred to September-sown wheat as teneral adults.

Solid symbols denote aphids with apterous parents and hollow symbols aphids with alate parents.

(a) Apteræ.



(b) Alatae.

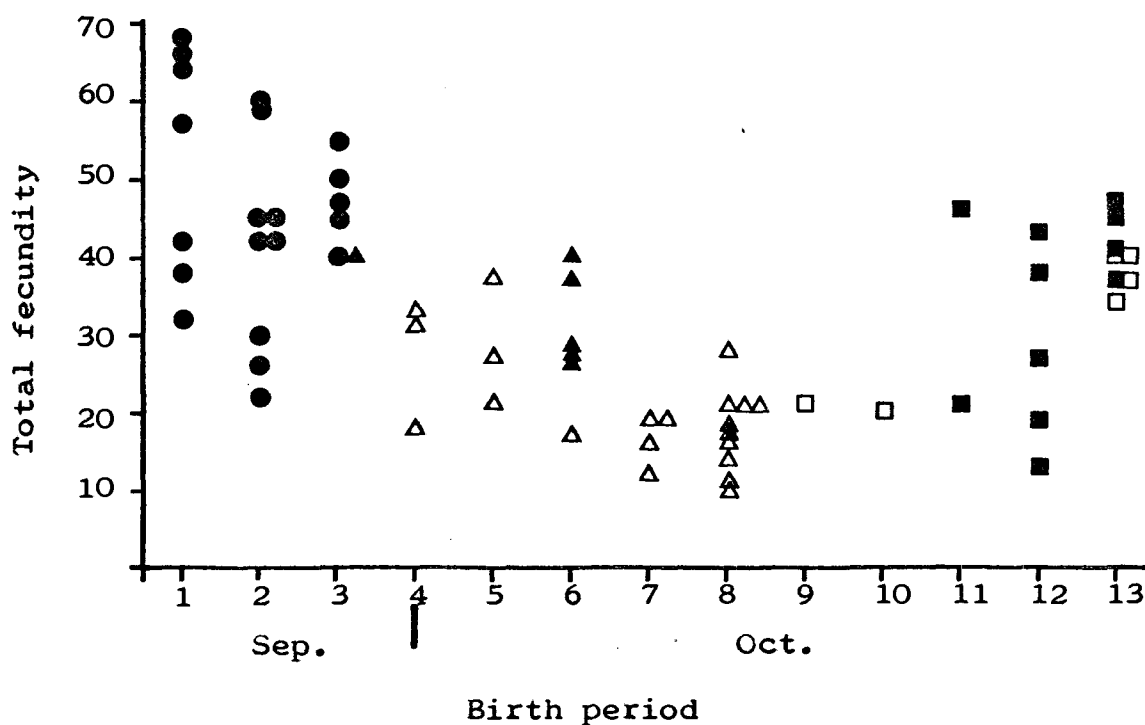


Fig. 4.13 had adult longevities of over 200 day^{°C}>3[°], by which time an average aptera born after mid-October should have produced over 55 nymphs and an average alata over 35. Mean adult longevities for the aphids represented in Fig. 4.13 were 344 day^{°C}>3[°] (apterae) and 297 day^{°C}>3[°] (alatae). However, the relatively small amount of data available, its variability and the large number of other possible causal factors correlated with each other and time since the start of the experiment, made isolation of the causal factors using regression analysis or correlation coefficients impossible. For both apterae and alatae Kendall's rank correlation coefficients were calculated between total fecundity and a set of parameters for possibly causal factors (similar to that used in the analysis of variation in 20 nymph day-degree requirements above). But nearly all the correlation coefficients were statistically significant ($P < 0.05$) and their size ranking changed drastically from one morph to the other.

However, examination of the data (Fig. 4.13) provides some evidence as to the causes of trends in total fecundity. Differences in the parent's morph can have had no effect on the pattern of total fecundities for apterae as all but one were born to alatae. The tendency for alatae with apterous parents to have birth dates in late September and late October did exaggerate the trend in total fecundities for this morph as individuals with apterous parents appeared to have slightly higher fecundities than those with alate parents and the same birthdate. However, the same trend in total fecundities is still shown by alatae if only individuals with the same morph of parent are considered (Fig. 4.13). The pattern of variation (Fig. 4.13) also suggests that the tendency for aphids transferred from August- to September-sown wheat to have low total fecundities was a coincidence of their birth dates and that changes in host plant were not the cause.

If changes in total fecundities were directly related to changes in temperature during development or reproduction one would not expect them to have declined to a minimum in mid-October and then recovered. But temperature changes might have produced this pattern if

a fall in temperature during development or early adult life resulted in low total nymph production in an aphid which had experienced relatively high temperatures earlier in its life. Aphids born later in the autumn might have been capable of producing more nymphs because they had experienced low temperatures throughout their lives and were thus acclimatized. An alternative possibility is that the changes in total fecundity were related to day-length or some other factor irrevocably linked to time of year. Gynoparae of *S. avenae* have lower total fecundities than virginoparae (Watt, 1979b) but, from the sample of progeny reared in the present study, no aphids born after 21 September were producing sexual morphs. However, reduced total fecundity might be a physiological 'by-product' of an autumn switch to sexual morph production which persists for a few weeks after this has ceased.

4.3.8 The relationship between reproduction rate and physiological age

The relationship between reproduction rate and physiological age measured from the final moult was determined using all the records of 3-day period nymph production by adults born on September and November-sown wheat. A computer program was used to convert each record for nymph production by a single adult in a single 3-day period into a reproduction rate expressed in physiological time (nymphs produced per $\text{day}^{\circ\text{C}} > 3^{\circ}$) and then to calculate the physiological age of the adult concerned, defined as the total $\text{day}^{\circ\text{C}} > 3^{\circ}$ accumulated between the final moult and the mid-point of the 3-day period in question. The 3-day period reproduction rates were then grouped according to the physiological age of the adults and mean reproduction rates for each physiological age-class were calculated. An age-class interval of 25 $\text{day}^{\circ\text{C}} > 3^{\circ}$ was used, so that mean reproduction rates were obtained for adults of 0 to 25 $\text{day}^{\circ\text{C}} > 3^{\circ}$, 25 to 50 $\text{day}^{\circ\text{C}} > 3^{\circ}$ and so on. Data for alatae and apterae were analysed separately and, within each morph, data for aphids with apterous parents and alate parents were separated. The reproduction rates are listed in

Appendix 4.4 together with the number of 3-day period records on which each is based. It should be remembered that physiological age^{was} measured from the final moult, not the start of reproduction.

As expected, reproduction rates for apterae were higher than those for alatae of the same physiological age (Fig. 4.14) except for adults older than $200 \text{ day}^{\circ}\text{C} > 3^{\circ}$. The reproduction rate/age pattern of the alatae is unusual in that this morph showed a late second peak in reproduction rate as high as the initial peak (Fig. 4.14). Possible reasons for this are discussed in section 4.4.10. For both apterae and alatae, individuals with apterous parents showed slightly higher reproduction rates early in adult life than those with alate parents, as expected from the analysis of 20 nymph day-degree requirements (section 4.3.4). This trend was more marked for apterae than for alatae but in alatae individuals with apterous parents showed a larger second peak in reproduction rates (Fig. 4.14).

Two types of estimate of total fecundity were obtained from the reproduction rate/age data. Firstly, the average total nymph production of an aphid surviving to the end of its potential reproductive life was obtained by summing mean reproduction rates for all ages of a particular morph and multiplying the result by 25 (the age-class interval in $\text{day}^{\circ}\text{C} > 3^{\circ}$). Secondly, an estimate of the average total nymph production actually realized by each morph (taking adult mortality into account) was calculated. This was done by multiplying reproduction rates for each age class by adult survival for the age class (predicted from probit curves, see section 4.3.10) before summing the results for all the different age classes and multiplying by 25. Because most adults, particularly apterae, survived until the end of potential reproductive life, these two types of fecundity estimate differ only slightly (Table 4.2).

One of the central aims of the present experiment was to provide parameters needed for a simulation model of overwintering by S. avenae viviparae on winter wheat. However, the initial population data input into such a

Fig. 4.14 Relationships between reproduction rate and physiological age.

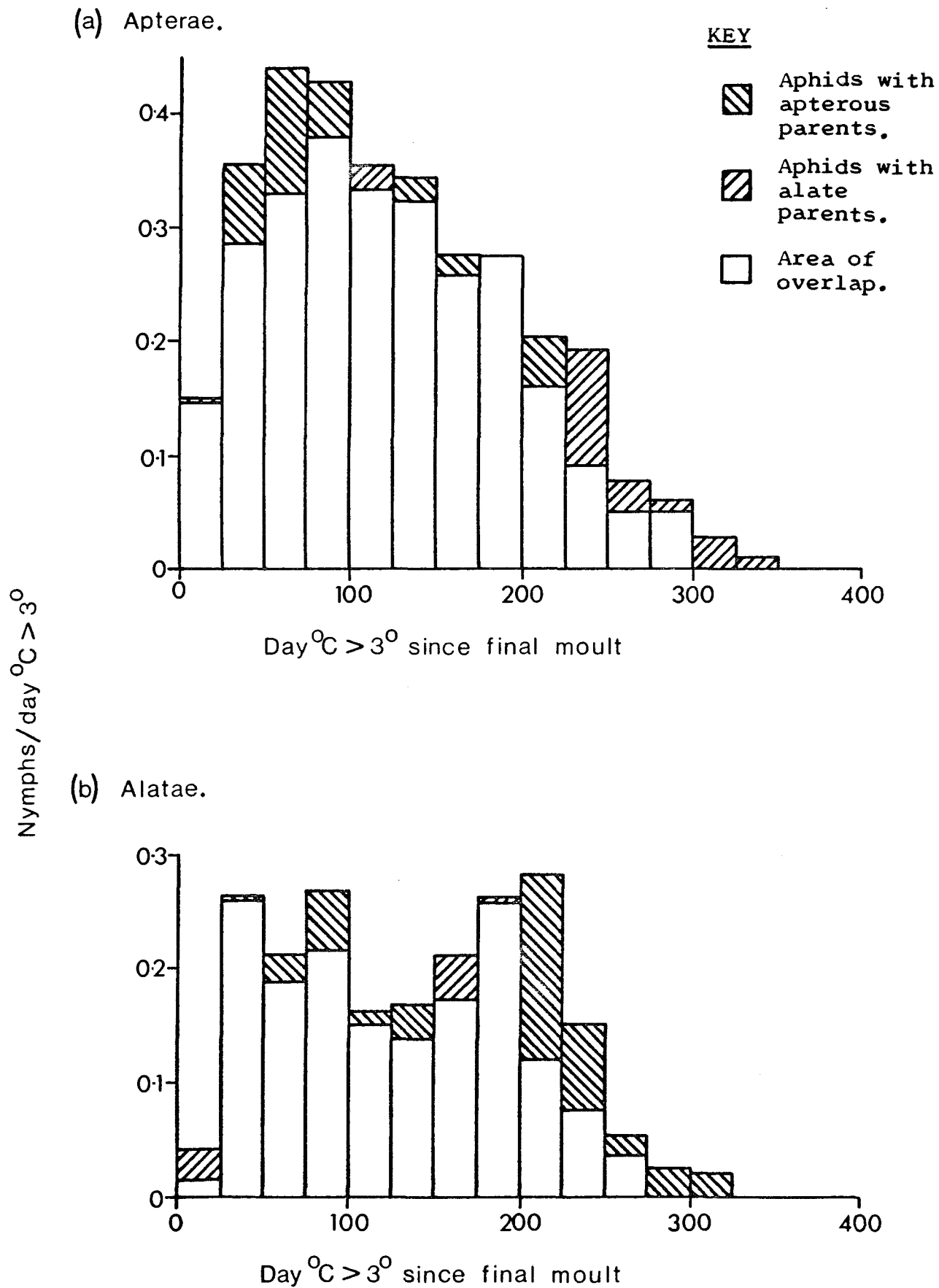


Table 4.2: Estimated total fecundities (to the nearest whole nymph).

	APTERAE		ALATAE	
Assumptions on survival	Apterous parents	Alate parents	Apterous parents	Alate parents
Survival until end of reproduction	75	72	51	42
Average adult survival	74	70	45	39

model is likely to be based on estimates of population density derived from destructive sampling. If so, the physiological age of the adult aphids sampled will not be known. Consequently all adults may well have to be assigned a single 'mean' reproduction rate and it will probably be impossible to obtain an accurate estimate of mean adult age. The mean reproduction rate of the population of adults present at any one time obviously depends on the mean physiological age, which in turn depends on the level of more or less continuously acting abiotic and biotic mortality factors. If this continuously acting mortality is at a very low level, so that most adults survive until near the end of their potential reproductive lives, then the mean reproduction rate will be close to the mean for all age classes of adults from zero to over 300 day^{°C} > 3[°]. But if continuously-acting mortality is high then mean reproduction rate will be determined only by reproduction rates of the first few age classes.

The feasibility of using a mean reproduction rate for all adults depends on two factors:

1. The extent of knowledge of mortality factors
- the more detailed the information the easier it is to estimate mean adult age, and hence mean reproduction rate, accurately.
2. The extent to which mean reproduction rate changes with mean adult age - the more sensitive the mean rate is to changes in mean age the more likely it is that important errors of estimate will be made.

The second factor was investigated using the reproduction rate patterns determined above. To do this mean reproduction rates were calculated with the age-structure of the adult population altered by the introduction of a non-age-specific mortality which acted by removing a fixed proportion of the adults between one physiological age-class and the next. Thus the mortality was in effect a mortality rate per 25 day^{°C} > 3[°]. The

Table 4.3: Mean reproduction rates calculated
assuming different levels of continuously
acting mortality.

% mortality per 25 day °C > 3°	Mean reproduction rate (nymphs/day °C > 3°)	
	Apterae	Alatae
0	.206	.120
10	.246	.141
20	.270	.151
30	.274	.151
40	.265	.142
50	.247	.128
60	.227	.111
70	.206	.093
80	.185	.072
90	.166	.051
100	.148	.029

proportion of adults removed between age classes was changed in each trial. Apart from this changeable non-age-specific mortality, mortality due to 'old age' was assumed to follow the smoothed curves determined by probit analysis on adult survival data (see section 4.3.10). The reproduction rates used for each age-class of each morph were the means of the values for aphids with apterous parents and those with alate parents. The mean reproduction rates of both morphs were surprisingly insensitive to changes in mortality rate in the range 10 to 50% per $25 \text{ day}^{\circ}\text{C} > 3^{\circ}$ (Table 4.3). This was a consequence of their reproductive patterns.

4.3.9 Examination of the relationship between reproduction and temperature using three-day period nymph production data

In calculating reproduction thresholds, reproduction rate and mean temperature were estimated over relatively long time intervals (the time taken to produce the first 20 nymphs). It was realised that this might have resulted in the degree of curvilinearity in the relationship between reproduction rate and temperature being masked and so this was re-examined using 3-day period nymph production data to provide a more 'instantaneous' measure of reproduction. Apteræ and alatae were considered separately and, to control for the effect of physiological age on reproduction, only data for aphids in narrow physiological age ranges were considered. The age ranges were chosen using the reproductive patterns determined above (section 4.3.8). They were $50 \text{ to } 125 \text{ day}^{\circ}\text{C} > 3^{\circ}$ and $25 \text{ to } 100 \text{ day}^{\circ}\text{C} > 3^{\circ}$ since final moult, for apteræ and alatae respectively, which coincide with the initial peaks in reproduction rate for each morph.

For each 3-day period the mean number of nymphs produced per adult was calculated for adults whose physiological ages fell within the appropriate range. The mean reproduction rates for different 3-day periods were based on samples varying in size from 10 adults upwards. Means based on data from fewer than 10 adults were discarded.

The mean sample sizes were 34.9 for apterae and 23.2 for alatae. First-order ($y = a + b x$) and second-order ($y = a + b_1 x + b_2 x^2$) regression equations were then calculated for the relationships between mean nymph production in each 3-day period and mean temperature during each period, and Pearson's correlation coefficients (r) and r^2 calculated as an approximate estimate of 'goodness-of-fit'. The 3-day period mean temperatures were calculated from hourly records. For each morph the analysis was carried out first with mean temperatures calculated in conventional $^{\circ}\text{C}$ and then with them calculated above 3°C (by counting hourly temperature records of 3°C and below as zero and those above as the difference between the recorded temperature and 3°C). The regressions are based on data from 39 3-day periods for apterae and 27 for alatae.

Differences in the way mean temperature was calculated and in the type of regression equation fitted produced little change in r^2 for either apterae or alatae (Table 4.4). This suggests that most of the unexplained variation was due to sample error and/or heterogeneity in the adult populations sampled unrelated to temperature conditions during the 3-day periods over which reproduction rate was estimated, though it might have been related to temperatures experienced earlier in the aphids' lives. The data for apterae fits the regression lines well (Fig. 4.15) but data for alatae shows much more unexplained variation (Fig. 4.16). This must be at least partly a result of the smaller sample sizes for alatae. But from casual observations during the experiment, it appeared that the alatae tended to produce nymphs in a more uneven temporal pattern than apterae, with relatively concentrated bouts of nymph production followed by lulls. This unevenness may well have contributed to heterogeneity, as the samples were small and reproduction was measured over a short time interval. As expected, the data for both morphs fits first-order equations better if temperature is measured in $^{\circ}\text{C} > 3^{\circ}$ rather than conventional $^{\circ}\text{C}$ (Table 4.4) but the difference is surprisingly small. Considering the second-order equations, the data for apterae fit slightly

Table 4.4: Regression statistics for relationship between reproduction rate (nymphs/adult/3-day period) and 3-day period mean temperature ($^{\circ}\text{C}$)

(a) Apterae (physiological age 50 to 125 day $^{\circ}\text{C} > 3^{\circ}$ since maturity)

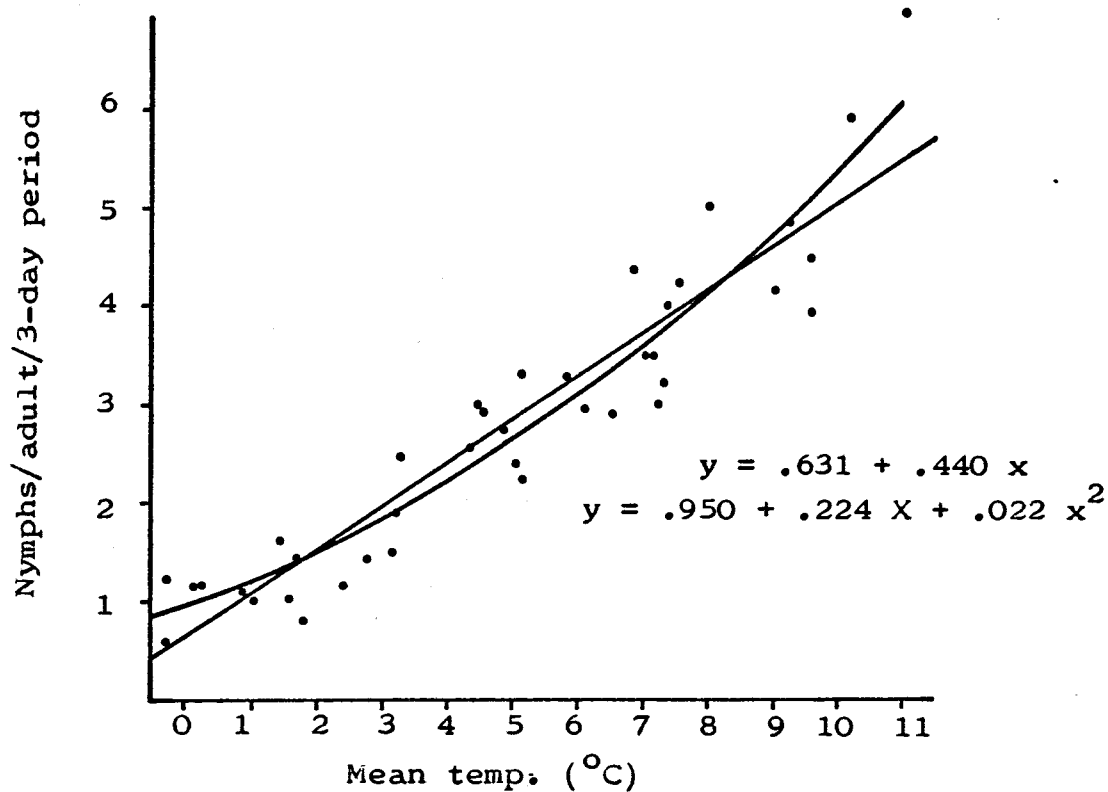
Temperature measure	Regression equation	Goodness-of-fit (r^2)
Conventional $^{\circ}\text{C}$	1st order: $y = .631 + .440 X$	.878
Conventional $^{\circ}\text{C}$	2nd order: $y = .950 + .224 X + (.218 \times 10^{-1}) X^2$	.898
$^{\circ}\text{C}$ above 3°C	1st order: $y = .657 + .678 X$	.890
$^{\circ}\text{C}$ above 3°C	2nd order: $y = .642 + .690 X - (.163 \times 10^{-2}) X^2$	.890

(b) Alatae (physiological age 25 to 100 day $^{\circ}\text{C} > 3^{\circ}$ since maturity)

Temperature measure	Regression equation	Goodness-of-fit (r^2)
Conventional $^{\circ}\text{C}$	1st order: $y = .575 + .200 X$	.562
Conventional $^{\circ}\text{C}$	2nd order: $y = .630 + .164 X + (.363 \times 10^{-2}) X^2$	.564
$^{\circ}\text{C}$ above 3°C	1st order: $y = .600 + .311 X$	.579
$^{\circ}\text{C}$ above 3°C	2nd order: $y = .403 + .465 X - (.204 \times 10^{-1}) X^2$	.592

Fig. 4.15 Relationship between reproduction rate and 3-day period mean temperature for apterae of physiological age 50 to 125 day^{°C} > 3° since maturity.

(a) Temperature in conventional °C.



(b) Temperature in °C measured above . 3°C.

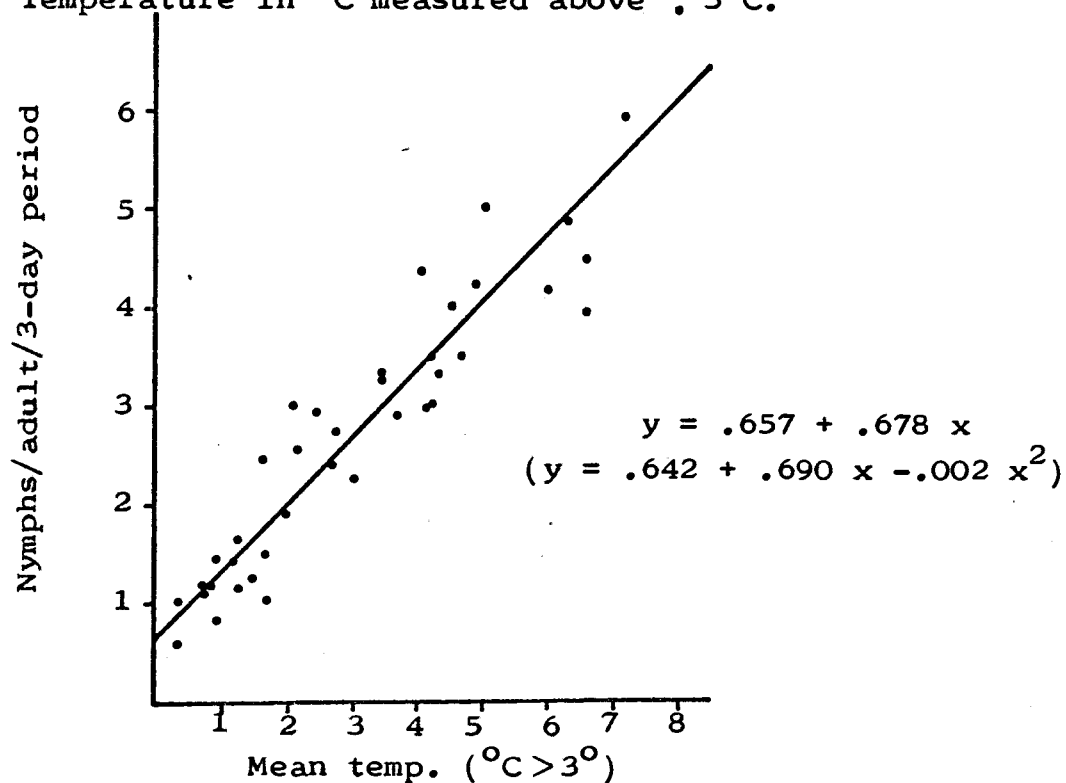
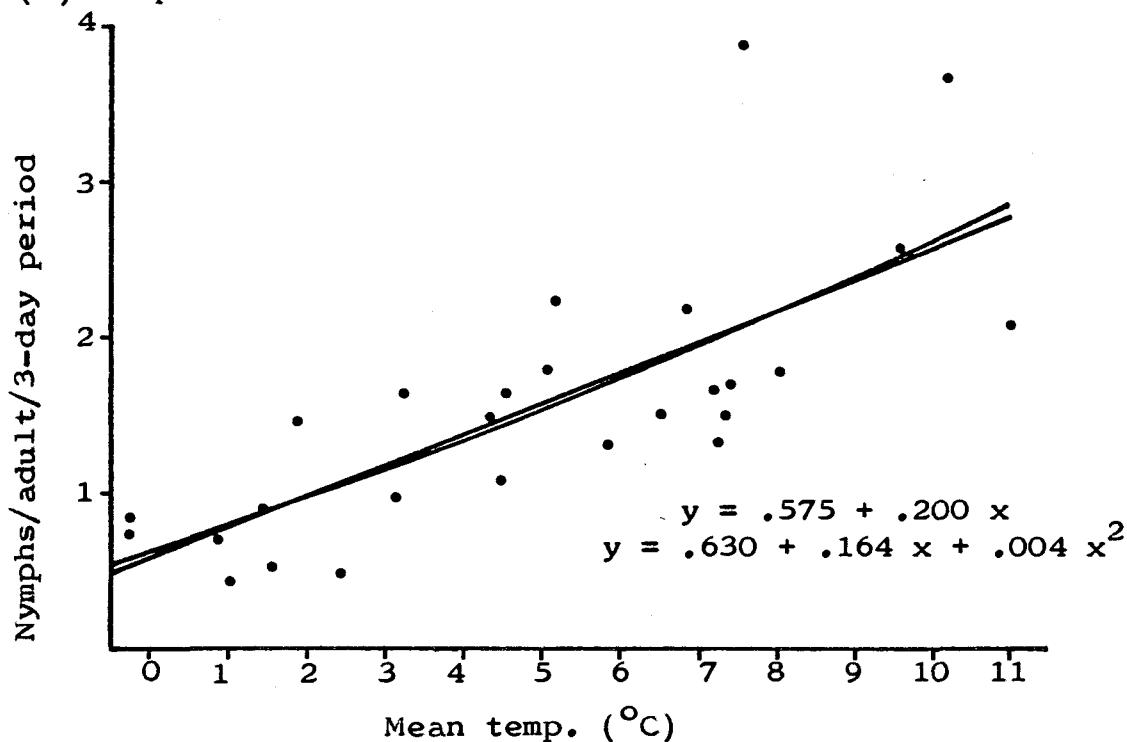
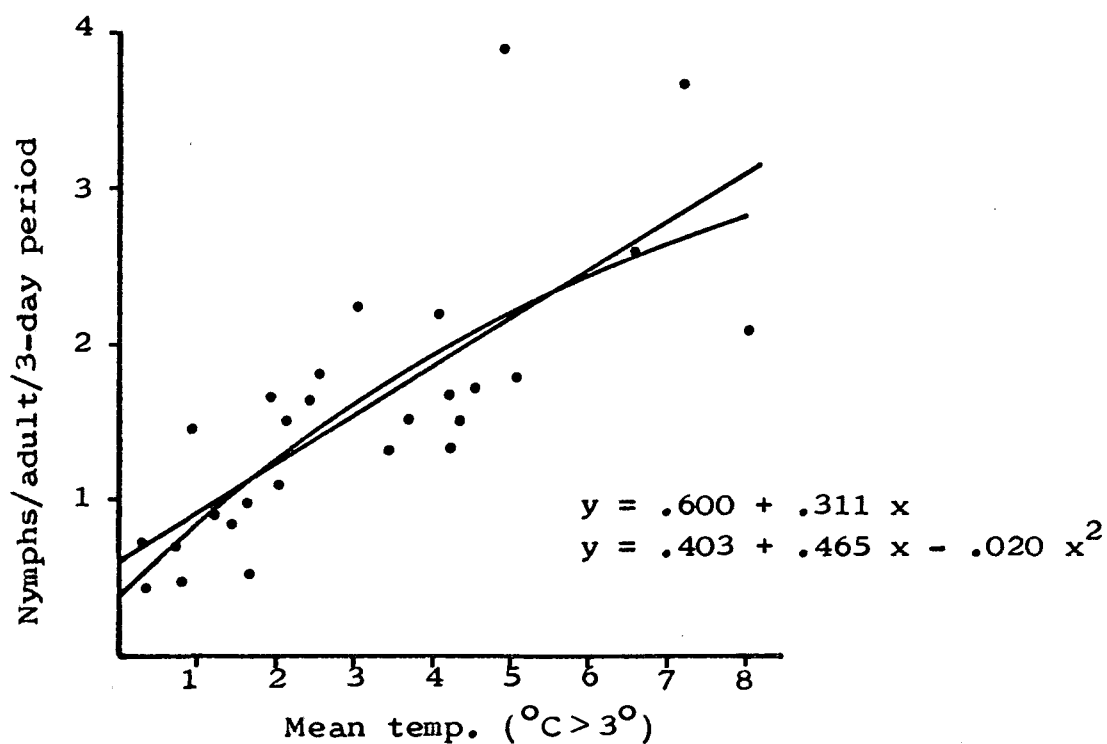


Fig. 4.16 Relationship between reproduction rate and 3-day period mean temperature for alatae of physiological age 25 to 100 day^{°C} > 3[°] since maturity.

(a) Temperature in conventional °C.



(b) Temperature in °C measured above 3°C.



better when temperature is measured in conventional °C but the reverse is true for alatae (Table 4.4).

The intercepts of regression lines for both morphs were significantly positive even when mean temperature was measured above the 3°C 'threshold'. This apparent discrepancy was probably caused firstly because the 3°C threshold was selected as a compromise between reproduction and development thresholds for both morphs and secondly because it was determined assuming a linear relationship between reproduction/development rate and temperature, and using rate and mean temperature estimates measured over relatively long time intervals. As such it is the correct 'threshold' for use in a model predicting population growth on a physiological time scale, but is inevitably an overestimate of the temperature below which it is impossible for an aphid to give birth, if the reproduction rate/temperature relationship is at all curvilinear at low temperatures. Also, 'reproduction' is not a single physiological process and 'reproduction' rates measured over long or short time intervals probably do not measure each of its component processes to the same extent. The 3-day period reproduction rates examined here, because they are estimates made over short time periods, are more measures of the 'rate of birth of nymphs' than of the 'rate of embryogenesis'. These are two distinct though related processes which may have different temperature relations. The relationship between rate of embryogenesis and temperature will play a more dominant role in determining the parameters obtained from regression analyses of 'reproduction rate' on temperature if rates and mean temperatures are measured over long time intervals. In this case the 'reproduction' rate will be at least partly determined by an aphid's ability to replenish its supply of fully-developed embryos under the temperature conditions measured, as well as its ability to give birth to them.

When mean temperature was measured in conventional °C the relationship between reproduction rate and temperature for apterae (Fig. 4.15(a)) showed some curvilinearity. But the extent of this curvilinearity was quite

small and so r^2 s for first- and second-order equations are similar (Table 4.4(a)). From the scatter of points it seems that pronounced curvilinearity is confined to mean temperatures below about 3°C and that the relationship could be well represented by two straight lines of different slopes joined at 2°C, with the line between 0° and 2°C nearly horizontal. When mean temperature was measured above 3°C positive curvilinearity was completely removed so that lines for first- and second-order equations are nearly exactly coincident (Fig. 4.15(b)).

The relationship between reproduction rate and temperature for alatae showed very little sign of curvilinearity even when temperature was measured in conventional °C, though neither first- or second-order equations fit the data very well (Fig. 4.16, Table 4.4). When mean temperatures were calculated above 3°C, positive curvilinearity was over-compensated for so that the second-order curve has a decreasing slope (Fig. 4.16(b)). The reproduction rates of apterae and alatae were quite dissimilar for the warmer 3-day periods but were similar for periods with low temperatures. This suggests that the minimum temperature at which birth could occur was similar for the two morphs.

4.3.10 Survival and longevity

The aphids survived extremely well and the great majority were still alive when monitoring was stopped in mid-March. Many of them were over three months old at this date and the oldest aptera was over five months old. Because of their extremely good survival, seasonal changes in longevity could not be investigated but an approximate survival curve and median longevity for each morph was obtained by analysing the relationship between % survival on 14 March (the last date on which most aphids were checked) and physiological age on that date. In order to increase sample sizes for each physiological age-group, cohorts were grouped together in pairs, so that each age-group consisted of aphids with a six-day range in birth dates. The oldest age group was an exception and

contained the first three cohorts. Eight aphids which became infected with Entomophthora and a few lost or damaged during recording were excluded from this analysis. Because some of each cohort were discarded as newly moulted adults, 'whole-life' survival (between birth and 14 March) could not be calculated directly but was obtained by multiplying the proportion of monitored adults surviving to 14 March (adult survival) by the proportion of nymphs which had survived to adulthood in the same age-group. Survival by apteriform and alati-form nymphs was assumed equal.

Nymphal survival was very high throughout (minimum = 91.67%, mean = 96.96%) and so adult and 'whole-life' survival estimates are very similar (Table 4.5). A Wilcoxon's signed rank sum test on the difference between adult survival for apterae and alatae for age groups in which the survival of at least one morph lay between 0 and 100% showed that apterae survived significantly longer than alatae ($T = 63$, $n = 11$, $P < 0.02$). Smoothed curves of 'whole-life' survival plotted against physiological age on 14 March (Fig. 4.17) were determined using the probit analysis program referred to in Chapter 2, section 2.2.4, and listed in White & White (1981). The analysis was done by taking the log of the difference between physiological age and $700 \text{ day}^{\circ}\text{C} > 3^{\circ}$ as the 'dose' variable, because examination of the scatter of points showed the survival curves were not symmetrical sigmoids. Smoothed curves were also fitted to adult survival data and were used in the analysis of the effect of different continually acting mortalities on mean reproduction rate described in section 4.3.8 above. The estimated median longevities determined for both morphs (Table 4.6) are very similar whether or not nymphal mortality was taken into account but the shapes of the curves differ somewhat.

From the laboratory and field tests on low temperature mortality described in Chapters 2 and 3, the extreme minimum temperature recorded during this experiment (-7°C) was not expected to produce significant mortality in acclimatized S. avenae which were protected from hoar

Table 4.5: Survival of cohorts up to 14 March 1981.

Nymphal survival = birth to maturity.

Adult survival = maturity to 14/3/81.

'Whole-life' survival = birth to 14/3/81.

Birth Period	Nymphal survival % (n)	Adult Survival		'Whole-life' Survival		Physiological age on 14/3 (day ^o C > 3 ^o since birth)
		Apterae % (n)	Alatae % (n)	Apterae %	Alatae %	
21-30/9	91.67 (60)	0.0 (6)	0.0 (22)	0.0	0.0	698
30/9-6/10	94.74 (38)	0.0 (14)	0.0 (7)	0.0	0.0	621
6-12/10	100 (25)	33.3 (3)	0.0 (10)	33.3	0.0	575
12-18/10	100 (48)	33.3 (15)	9.1 (11)	33.1	9.1	546
18-24/10	100 (44)	31.3 (16)	0.0 (5)	31.3	0.0	515
24-30/10	100 (38)	75.0 (12)	46.2 (13)	75.0	46.2	466
30/10-5/11	96.87 (32)	91.7 (12)	66.7 (9)	88.8	64.6	435
5-11/11	96.43 (28)	93.8 (16)	75.0 (4)	90.5	72.3	432
11-17/11	95.00 (20)	85.7 (10)	100 (4)	81.4	95.0	418
17-23/11	94.44 (18)	100 (3)	66.7 (9)	94.4	63.0	371
23-29/11	100 (41)	100 (20)	80.0 (20)	100	80.0	325
29/11-5/12	95.00 (20)	100 (12)	80.0 (5)	95.0	76.0	320
5-11/12	94.59 (37)	90.0 (20)	100 (7)	85.1	94.6	312
11-17/12	100 (20)	100 (8)	100 (6)	100	100	284
17-23/12	100 (20)	100 (6)	100 (4)	100	100	271
23-29/12	97.22 (36)	100 (15)	100 (9)	97.2	97.2	242
29/12-4/1	92.50 (40)	100 (10)	100 (17)	92.5	92.5	223
4-10/1	100 (28)	100 (15)	100 (6)	100	100	208
	$\bar{x} = 96.96\%$					

Fig. 4.17 Relationship between cohort age and proportion surviving on 14 March.

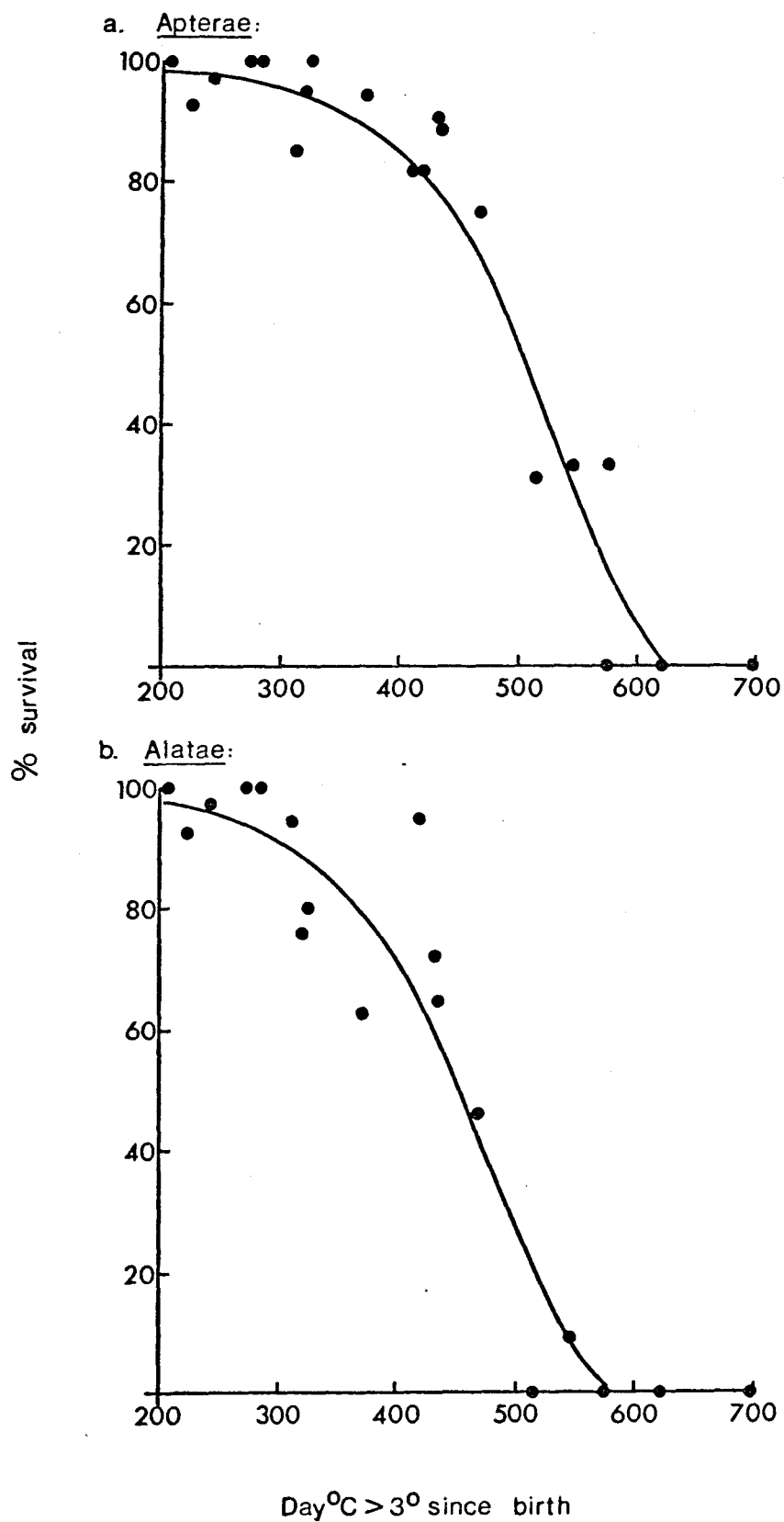


Table 4.6: Results of probit analyses on survival data.

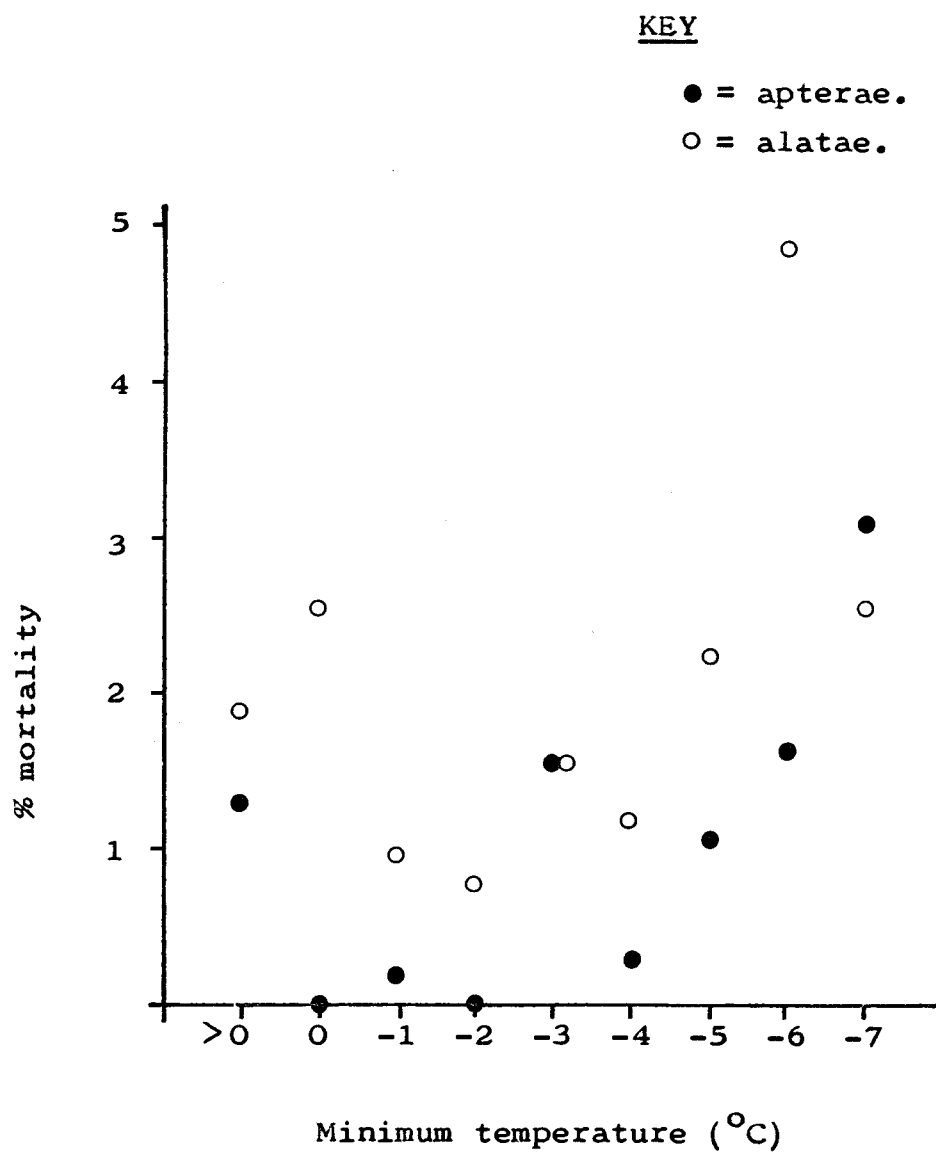
	Probit line*		Median longevity (day °C > 3°) (95% C.I.)	Goodness-of-fit χ^2
	Intercept	Slope ±S.E.		
<u>Whole-life survival</u>				
Apterae	-12.3603	5.4304 ±0.7182	511.1 (488.9-537.5)	7.47, D.F.=5 P > 0.1
Alatae	-16.2651	6.7861 ±0.9711	450.6 (425.4-479.5)	5.78, D.F.=4 P > 0.2
<u>Adult Survival</u>				
Apterae	-15.0344	6.6212 ±0.9220	513.5 (493.8-537.0)	3.98, D.F.=3 P > 0.2
Alatae	-17.5802	7.3399 ±1.0548	451.6 (427.5-479.3)	6.77, D.F.=3 P > 0.05

*the 'dose' is $\log (700 \text{ day}^\circ\text{C} > 3^\circ - \text{physiological age in day}^\circ\text{C} > 3^\circ)$

frost by clip-cages, and so it is not surprising that the survival curves showed that nearly all the mortality in this experiment could be attributed to age. To check whether there was any relationship between mortality and temperature the proportion of adults dying in a 3-day period was related to the minimum temperature reached in the period. Mortalities of apterae and alatae were analysed separately. For each morph all periods in which less than 20 adults were alive at the start were ignored. The % mortalities were calculated for the remainder and grouped according to the minimum temperature recorded in the period, measured to the nearest $^{\circ}\text{C}$. All periods in which the minimum temperature was above 0°C were grouped together. A mean mortality for each minimum temperature was calculated with all 3-day periods in the group given equal weight. The analysis revealed a weak relationship between mortality and minimum temperature (Fig. 4.18) but all mortalities for both morphs were extremely low even though the data include very old adults, which might be expected to be especially susceptible to low temperatures. Kendall's rank correlation coefficients between % mortality and minimum temperature are 0.28 ($n = 9$, $P > 0.1$) for alatae and 0.54 ($n = 9$, $P < 0.05$) for apterae. This difference between the morphs was not due to the mortality of apterae being higher than that for alatae during cold periods, but to it being lower during warmer ones (Fig. 4.18). The slightly higher, more erratic mortality of alatae was probably just a result of more individuals of this morph dying of old age before the end of the experiment, due to their slightly lower potential longevity and the fact that a greater proportion of the total were established in the first few weeks of the experiment on August-sown wheat.

For a few adults on September- and November-sown wheat (six apterae and seven alatae) there was some evidence of low temperatures reducing reproduction without killing the aphid. These individuals had atypically low fecundities (zero to six nymphs for the alatae, two to 34 for the apterae). Four of the apterae stopped producing

Fig. 4.18 Relationship between adult mortality in 3-day periods and minimum temperature.



nymphs abruptly at the same time. One of the apterae and six of the alatae developed discoloured, brownish abdomens, suggesting that their embryos had been killed. However, there is no clear relationship between the timing of these reproductive anomalies and particularly cold periods, though all occurred in the three months with the lowest minimum temperatures (December, January and February).

4.3.11 The duration of each nymphal instar

Estimates of the durations of the first three nymphal instars (in $\text{day}^{\circ}\text{C} > 3^{\circ}$) were based on the final cohort of nymphs only (Table 4.7) while those for the fourth nymphal instar (Table 4.8) were based on a larger sample (as explained in section 4.2.9). Day-degree requirements for the different instars were calculated assuming nymphs were born and moulted half-way through a 3-day period. Both nymphs in a clip-cage usually moulted in the same 3-day period and when they did not they could often be distinguished because one was apterous and the other alatiform. Occasionally they could not be separated in this way, in which case it was assumed that the first nymph to moult into a particular instar was also the first to moult out of it. Definitive instar duration estimates (Table 4.9) were made by correcting for small discrepancies between the total day-degree requirements for development of those aphids whose nymphal moults were recorded and the mean day-degree requirements calculated for all the apterae and alatae reared (150.1 and 163.6 $\text{day}^{\circ}\text{C} > 3^{\circ}$ respectively).

Comparing the development of the two morphs, the day-degrees accumulated between birth and the end of the third instar was slightly but significantly lower for alatae than for apterae (Table 4.8) (significance test based on normal distribution: $d = 2.36$, $P < 0.05$) and the day-degree requirement for the fourth instar was significantly higher (Table 4.8) ($d = 11.27$, $P < 0.001$). From the data of Dean (1974b) the relative durations of instars one to four for apterae reared at 10°C in the laboratory are 26.5, 22.2, 24.8 and 26.5% of total development time. These proport-

Table 4.7: Duration of all four nymphal instars based
on data from nymphs born between 7 and 10/1/81.

Instar	Apterae day ^o C > 3 ^o $\bar{x} \pm \text{S.E.}$ (n)	%*	Alatae day ^o C > 3 ^o $\bar{x} \pm \text{S.E.}$ (n)	%*
I	40.1 $\pm$ 2.673 (8)	26.1	37.7 $\pm$ 1.633 (10)	23.1
II	34.0 $\pm$ 1.808 (8)	22.1	32.1 $\pm$ 1.679 (10)	19.7
III	39.0 $\pm$ 1.476 (8)	25.4	38.5 $\pm$ 1.434 (10)	23.7
IV	40.6 $\pm$ 2.615 (8)	26.4	54.6 $\pm$ 3.976 (10)	33.5
I-IV	153.8		162.9	

Table 4.8: Duration of the fourth nymphal instar based
on data from nymphs born between 11/12/80
and 10/1/81.

Instar	Apterae day ^o C > 3 ^o $\bar{x} \pm \text{S.E.}$ (n)	%*	Alatae day ^o C > 3 ^o $\bar{x} \pm \text{S.E.}$ (n)	%*
I-III	109.8 $\pm$ 1.20 (59)	71.6	106.2 $\pm$ 0.98 (81)	64.4
IV	43.5 $\pm$ 0.97 (59)	28.4	58.6 $\pm$ 0.93 (81)	35.6
I-IV	153.3		164.8	

* % of total development.

Table 4.9: Corrected durations of each nymphal instar assuming mean development requirements.

Instar	Apterae		Alatae	
	day ⁰ C > 3 ⁰	%*	day ⁰ C > 3 ⁰	%*
I	38.1	25.4	36.7	22.4
II	32.3	21.5	31.2	19.1
III	37.1	24.7	37.5	22.9
IV	42.6	28.4	58.2	35.6
I-IV	150.1		163.6	

* % of total development.

ions agree well with those determined here. No comparable laboratory data exist for alatae.

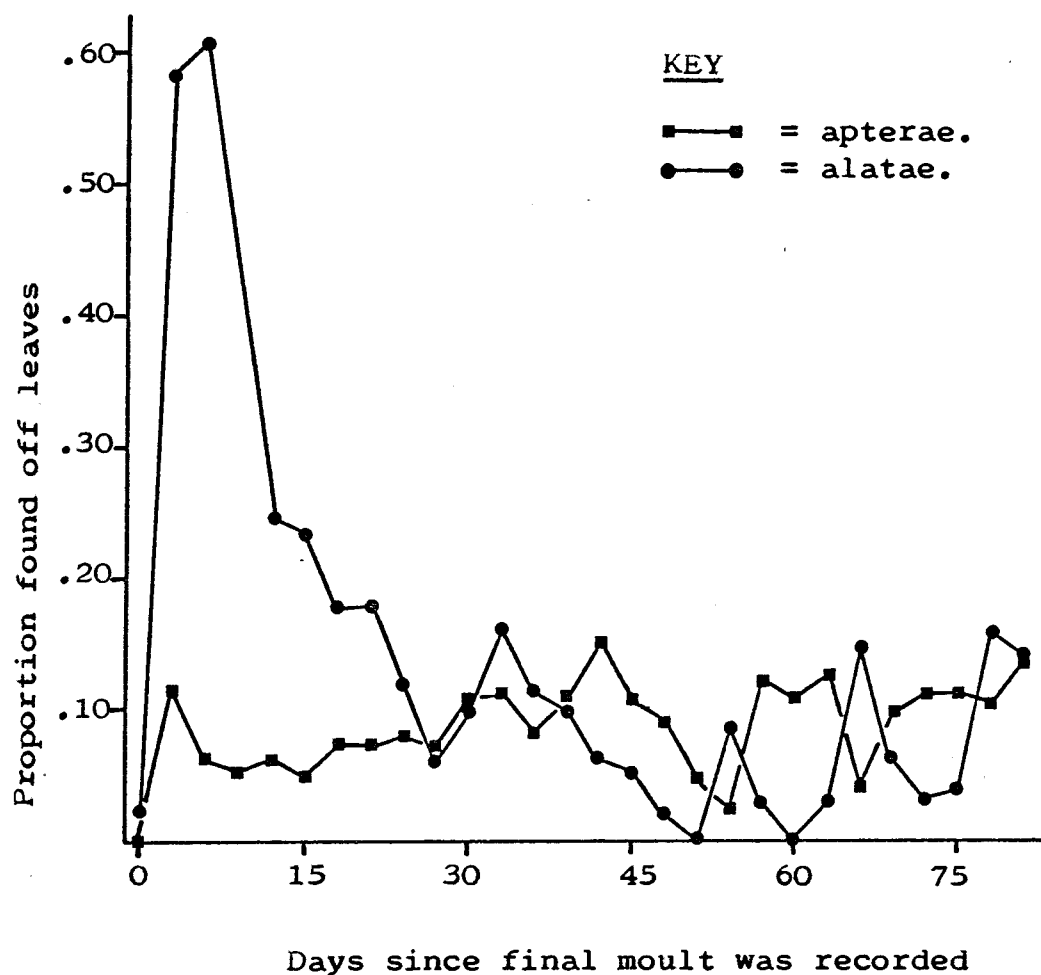
4.3.12 Adult restlessness

The probability of finding an adult wandering on the clip-cage walls rather than feeding varied greatly with aphid age and morph (Fig. 4.19). For alatae there was a period of restlessness soon after the final moult during which they spent much of their time off the leaves. Nearly all alatae found off leaves were wandering on the Perspex or gauze in the top half of a clip-cage, and appeared to be in a state of migratory readiness despite the low mean temperatures and the fact that maximum temperatures in the cages rarely reached 15°C (see Appendix 4.5). The alatae's ability to fly at field temperatures was not tested but those from the sample of progeny reared to check for the production of sexuales, which were examined as young adults in the laboratory, could certainly fly at laboratory temperatures (c.20°C). Alate restlessness was most intense in the period between the final moult and the start of reproduction but continued into the reproductive period. After about four weeks alatae were just as sedentary as apterae. But even apterae were slightly more restless than one would expect from laboratory clip-cage work. This was probably mainly due to the fluctuating temperatures in the field. It is unlikely to have been due to the leaves being in an unfavourable condition, as the fecundities and longevities achieved by the apterae were high compared to those determined in laboratory studies.

4.3.13 Differences between temperatures inside and outside clip-cages

As relationships between aphid development or reproduction and temperature were determined using temperatures measured actually inside clip-cages, differences between the temperatures experienced inside clip-cages and at crop-level outside were of no consequence as long as

Fig. 4.19 Relationship between the probability of an adult being found wandering on the clip-cage walls and adult age.



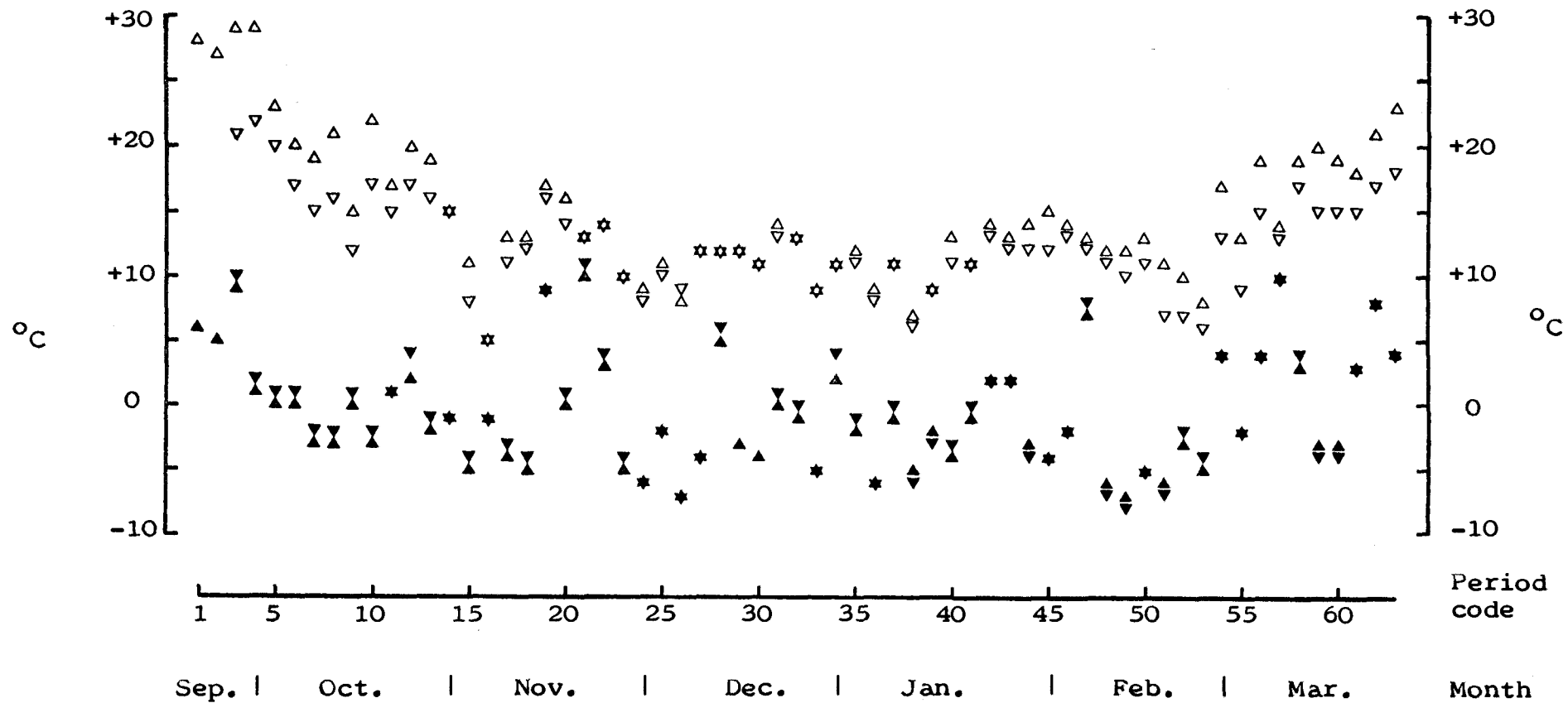
Sample sizes: Apterae: mean = 112, range = 148 - 66.
 Alatae: mean = 60, range = 88 - 21.

they were not so great as to make inside-cage temperatures unrepresentative of a normal winter temperature regime. To check that this was not the case maximum and minimum temperatures and $\text{day}^{\circ}\text{C} > 3^{\circ}$ totals measured inside and outside clip-cages were compared. Two measures of air temperature outside the cages at crop-level were available, recorded by two type CM thermistor probes 4 cm above the ground, one shaded and the other not.

Between 16 February and 13 March 1981 the temperature at 2.5 cm above ground-level on the underside of a wheat leaf was measured with a 2 mm type FF probe without a metal casing, to obtain as realistic an estimate as possible of the temperature experienced by an uncaged aphid. Daily $\text{day}^{\circ}\text{C} > 3^{\circ}$ totals were calculated from these records and compared with those calculated from temperatures measured by shaded and unshaded type CM probes to determine which CM probe estimated 'uncaged-aphid temperature' best. Both the type CM probes overestimated slightly, the unshaded one by an average of 5.3%, the shaded one by 1.4%. Thus records from the shaded probe were taken as the more realistic measure of uncaged-aphid temperature.

Records of 3-day period maxima and minima (Fig. 4.20) show that temperatures inside and outside clip-cages were quite similar. On average, 3-day period maximum temperatures were 2.0°C higher inside clip-cages (S.E. = 0.243, $n = 61$); the differences ranged from $+8^{\circ}\text{C}$ to -1°C . From November to mid-February maxima rarely differed by 2°C or more. Earlier and later than this the difference was larger and was greater than 5°C in the two periods in September for which there are outside clip-cage records. Differences were associated with sunny weather and were therefore greater in spring and autumn, when days were longer and the sun higher in the sky. In the four September 3-day periods maxima inside the cages approached 30°C but this is unlikely to have been damaging to the aphids as temperatures above 25°C were only shown by a total of eight hourly temperature recorder records. No mortality associated with the high temperatures was observed and only 60 of the monitored aphids were established by the end of September.

Fig. 4.20 Three-day period maximum and minimum temperatures recorded: (a) inside clip-cages (Δ and $\blacktriangle$) and (b) by a shaded thermistor at crop level (∇ and $\blacktriangledown$). Hollow symbols denote maxima, solid symbols minima.



Three-day period minima inside clip-cages were very similar to those recorded outside and were on average slightly lower (mean difference -0.4°C , S.E. = 0.096, $n = 61$), with differences ranging from $+1^{\circ}\text{C}$ to -2°C . The consistent 1°C difference shown until the end of November was probably just the result of the shaded crop-level thermistor being slightly higher above ground level than those in clip-cages during this interval (clip-cage thermistors were moved occasionally if the leaves they were under showed signs of senescence).

A further comparison was made using daily day-degree totals measured above 3°C (as estimated from the hourly records of thermistors inside and outside cages). Day-degree totals inside and outside the cages were very similar (Fig. 4.21) despite the differences in maximum temperatures on sunny days. The close agreement was a result of the short day lengths during most of the experiment. On average, daily day-degree totals measured inside clip-cages were very slightly higher than those measured outside but this difference was not significant (mean difference = $+0.057$; 95% confidence limits for $\mu = +0.116$ to -0.002). The difference also remained non-significant when the data was grouped in five, 10, 20 or 40 day intervals before analysis ($t < 1$, $P > 0.1$ in all cases).

In summary, the effect of the clip-cages was to increase the temperature range slightly on both a seasonal and a daily basis and increase mean temperatures a little; but the temperature regime experienced inside the cages was very similar to that which would have been experienced by an uncaged aphid overwintering on a winter wheat seedling in a typical sheltered feeding position.

4.3.14 Temperature conditions during the experiment

All the results on the temperature relations of development and reproduction by S. avenae viviparae overwintering on winter wheat presented here should be regarded as more or less specific to the type of temperature regime experienced during the experiment, which was that of a mild

Fig. 4.21 Relationship between daily day-degrees above 3°C recorded inside clip-cages, and by a shaded thermistor at crop level.

● = 2 records.

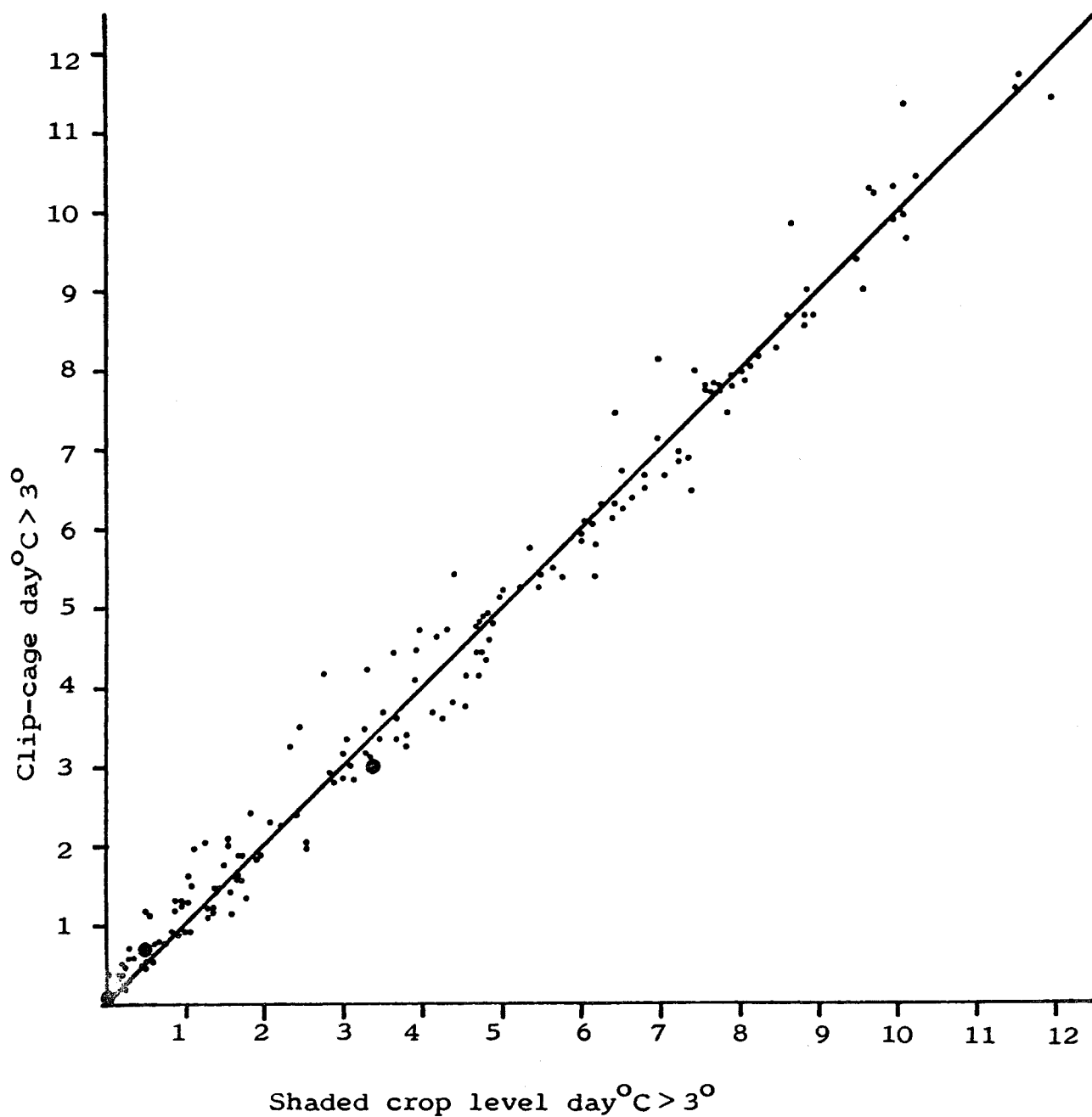


Table 4.10 A summary of maximum and minimum temperatures at Chilworth recorded in a Stevenson's screen at 1.2m and by a shaded thermistor 4 cm above ground level, measured to the nearest °C.

Period	Stevenson screen temperature				Crop-level shade temperature			
	Maximum		Minimum		Maximum		Minimum	
	mean	range	mean	range	mean	range	mean	range
28-30 Sep. + Oct. '80	13.7	18, 9	6.6	14, 0	15.3	25, 9	4.9	14, -2
Nov. '80	9.6	15, 3	4.7	13, -2	10.2	16, 3	3.5	13, -6
Dec. '80	9.1	13, 4	3.5	11, -5	9.2	13, 5	1.7	10, -7
Jan. '81	8.4	12, 4	2.4	9, -3	8.8	13, 4	0.9	9, -6
Feb. '81	6.9	13, 3	1.2	10, -4	8.3	13, 3	-0.6	10, -8
1-28 Mar. '81	11.2	15, 4	6.5	11, -1	12.9	18, 7	5.5	11, -4

winter in southern England. Table 4.10 gives a summary of temperature conditions in the form of mean and extreme maximum and minimum temperatures for each month measured to the nearest $^{\circ}\text{C}$ in a Stevenson's screen 1.2 m above ground level and by the shaded thermistor at crop-level (4 cm above the soil). Maxima and minima in each 3-day period measured inside clip-cages and by the shaded crop-level thermistor are also shown in Fig. 4.20. Appendix 4.5 gives a fuller summary of temperature conditions inside the clip-cages in each 3-day period. Over the whole experimental period mean temperature inside the cages was 6.5°C .

4.4 DISCUSSION

4.4.1 The nature of the relationship between development or reproduction rates and temperature

There is good evidence, based mainly on laboratory studies, that the development rate/temperature relationship for insects is significantly curvilinear at low temperatures (see section 4.1.1). Despite this, there was very little sign of curvilinearity in the rate-temperature relationships determined in the present study, in which the overall mean temperature, at 6.5°C , was only 3.5°C above the theoretical threshold for development and reproduction and in which mean temperatures experienced by individual aphids must have been very close to this threshold.

Straight lines fitted the data from the present experiment well, probably for two related reasons. Firstly, the rate/temperature relationships for S. avenae (if they could be measured over very short time periods in a fluctuating temperature regime) must be markedly curved only over a narrow range of temperatures close to the theoretical threshold. Secondly, during the winter, temperatures (particularly at crop-level) fluctuate widely between day and night. The consequence is that even if the average temperature experienced during an aphid's

development or reproduction lies in the range within which the rate/temperature relationship is significantly curved, the actual proportion of time during which the ambient temperature lies in this range is likely to be small. Day-night fluctuations must ensure that temperatures are usually either above this range (so that rate is proportional to temperature) or below it (so that rate is at, or very close to, zero). Moreover, the temperature range in which the rate/temperature relationship is significantly curved lies below that in which rate and temperature are linearly related. Consequently, an equal amount of time spent in these two ranges results in a much smaller proportion of total development or reproduction occurring in the range of significant curvilinearity, so that the amount of error produced is small.

Work done at constant temperatures is likely to overestimate the significance of curvilinearity in rate/temperature relationships, not only because temperatures can be held constant in the range of significant curvilinearity, but also because low, constant temperature, being a condition very rarely encountered by natural populations of insects (and thus one to which they are unlikely to be physiologically adapted) may produce development or reproduction rate estimates quite different from those which would result during a few hours' exposure to the same temperature in a fluctuating temperature regime.

4.4.2 The role of embryogenesis and eclosion in determining reproduction rates

It was suggested above (section 4.3.9) that a reproduction rate/temperature relationship determined at fluctuating temperatures by assessing rate and temperature over long time intervals is likely to be dominated by the relationship between embryo development and temperature, but if a rate/temperature relationship is determined using a short time interval, the rate at which adults can give birth to fully-developed embryos might be the limiting factor at low temperatures. However, from results presented in section 4.3.9 it seems that for S. avenae

viviparae under winter field conditions low temperature always exerts its limiting effect on reproduction by slowing embryo development rather than by limiting eclosion, as nymph production continued during 3-day periods with mean temperatures well below the theoretical threshold (3°C) determined for the relationship between reproduction rate and temperature during the production of the first 20 nymphs (Figs 4.15 and 4.16). This is clearly adaptive as if eclosion rather than embryogenesis were limiting, the situation could arise in which an adult was unable to give birth despite containing several fully-developed embryos.

If Figs 4.15 and 4.16 are compared it is clear that both apterae and alatae were capable of producing nymphs during the coldest 3-day periods and there is no evidence that alatae have a higher temperature threshold for eclosion than apterae. In fact nymph production by alatae appears to have been higher relative to that of apterae during the coldest periods. The faster reproduction of apterae was mainly due to their producing more nymphs during the milder periods, presumably due to faster embryogenesis.

4.4.3 Adult restlessness

From data in the literature, alatae of S. avenae and other aphid species do not take off from host plants at temperatures below about 14°C and the median lower temperature thresholds for take-off in the species so far studied are usually several degrees above this temperature (Johnson & Taylor, 1957; Heathcote & Cockbain, 1966; Dry & Taylor, 1970). Consequently, S. avenae alatae reaching maturity during the winter are unlikely to experience temperatures high enough for take-off unless their low-temperature experience during development results in their having take-off thresholds several degrees lower than alatae reared at intermediate temperatures. Nevertheless, in the present experiment alatae exhibited typical restless, migratory behaviour as young adults under winter field conditions (see section 4.3.12 and Fig. 4.19) and the

few tested at laboratory temperatures could fly.

Winter alatae thus appear to behave similarly to alatae produced during the summer, even though it is likely that their degree of restlessness is non-adaptive under winter conditions, in view of the following facts:

1. Their chances of successful flight are very low (unless they are 'flight-acclimatized').
2. Wide dispersal is probably unnecessary in winter as, due to low development and reproduction rates, overcrowding, if it occurs, is likely to be on a small scale and could be avoided just by walking onto another part of the host plant, or onto a neighbouring plant. This degree of dispersal is probably quite adequately achieved by the much less intensive wandering of apterae.
3. The highly restless behaviour of young alatae increases their chances of being knocked off the host by wind or rain.
4. It also results in their spending a considerable part of early adult life not feeding, which must slow embryo development, and may well be responsible for the non-adaptive two-peaked reproductive pattern (see section 4.4.10 below).

However, one consequence of restless behaviour not being 'switched-off' by low temperatures is that alatae can disperse widely as soon as spring temperatures rise above the flight-threshold in early spring, even if they have developed under cold, winter conditions. This capacity for early spring dispersal is probably one of the main advantages of an anholocyclic overwintering strategy, as it enables new host plants to be colonised at the onset of rapid spring growth, when their nutritional status is very high. This advantage is probably important enough to ensure that migratory behaviour is retained at low temperatures. The apparently non-adaptive, restless

behaviour of alatae in late autumn and winter is likely to have little impact on the success of natural overwintering populations simply because apterae are much the commoner morph in the winter (Hand, 1982; Vorley, pers. comm.). The relatively high proportion of alatae in the present study is artificial and a result of experimental conditions (see section 4.4./2 below).

4.4.4 The development of apterae

As there is good evidence in the literature that development/temperature relationships determined from constant, intermediate temperature laboratory studies may not hold under fluctuating, low temperature conditions (see section 4.1.1) it is interesting to compare the relationship found for apterae in the present study (development from birth to adult requires 150.1 day-degrees above a threshold of 3°C) with two relationships derived from data obtained at intermediate, constant temperatures by Dean (1974b) and Campbell et al. (1974). Campbell et al. fitted a straight line to their data which gives a threshold of 4.8°C and a "thermal constant" for development (birth to adult) of 117 day-degrees above the threshold. Unfortunately their raw data are not given and very little information is provided on the conditions under which they were collected, apart from the fact that they were obtained at three or four constant, intermediate temperatures using a clone of S. avenae from Vancouver, Canada.

Dean (1974b) fitted a quadratic function to development data obtained at seven constant temperatures at 2.5°C intervals between 10°C and 27.5°C using an English clone reared on excised leaf sections from barley seedlings, floating on a nutrient solution. His quadratic gives development times for the period from birth to the start of reproduction, not birth to adult, and, to make direct comparisons with the present study and that of Campbell et al. (1974), a new regression line was calculated using birth-to-adult times given in his paper. In fitting this line, data from high temperatures (above 20°C) at which the development rate-temperature relationship becomes

significantly curvilinear, were excluded. Over the range 10° to 20°C a straight line fits the data very well ($r^2 = 0.98$).

A comparison of the three development rate/temperature relationships (Fig. 4.22) clearly shows that no firm statement on the confidence with which laboratory-derived relationships can be applied to winter field conditions can be made, because although the relationship of Campbell et al. (1974) agrees quite well with that from the present study, that based on Dean's data does not. The discrepancy between the relationship of Campbell et al. and that in the current study is in the expected direction as, from evidence in the literature and theoretical considerations (see section 4.1.1) one would expect that over some range of relatively low temperatures the development rate/temperature relationship should be significantly curvilinear with increasing slope as temperature increases (see Fig. 4.1). Consequently, a straight line fitted to data obtained at intermediate temperatures should give a higher theoretical threshold and have a steeper slope than one fitted to low temperature data. In contrast, Dean's data give a threshold lower than that determined from winter field data (-1.75° as compared to 3°C) and also well below the range (1.7° to 8.3°C) found in laboratory assessments on 11 clones of four other aphid species (Campbell et al., 1974). Furthermore, if a straight line is fitted to rates determined by Dean for the interval 'birth to the start of reproduction' over the temperature range 10° to 22.5°C , the threshold predicted is even lower (Carter, 1978), at -3.6°C .

The result of the differences between the development rate/temperature relationships is that there are large differences in the development times predicted from Dean's data compared to those based on the present study or Campbell et al. (1974) at temperatures below about 13°C , despite this being an intermediate temperature for which all three relationships ought to give reasonable predictions (Fig. 4.23). At 10°C the discrepancy between the prediction of Campbell et al. (1974) and that from the present study is small but the straight line fitted to

Fig. 4.22 Linear regression lines fitted to development time (birth to adult) - temperature data from:

- (a) Dean (1974b). Simple regression equation fitted to birth to adult data over the range 10° - 20° C.
- (b) Campbell et al. (1974).
- (c) Present study.

Lines are shown unbroken over the approximate temperature range of the data used.

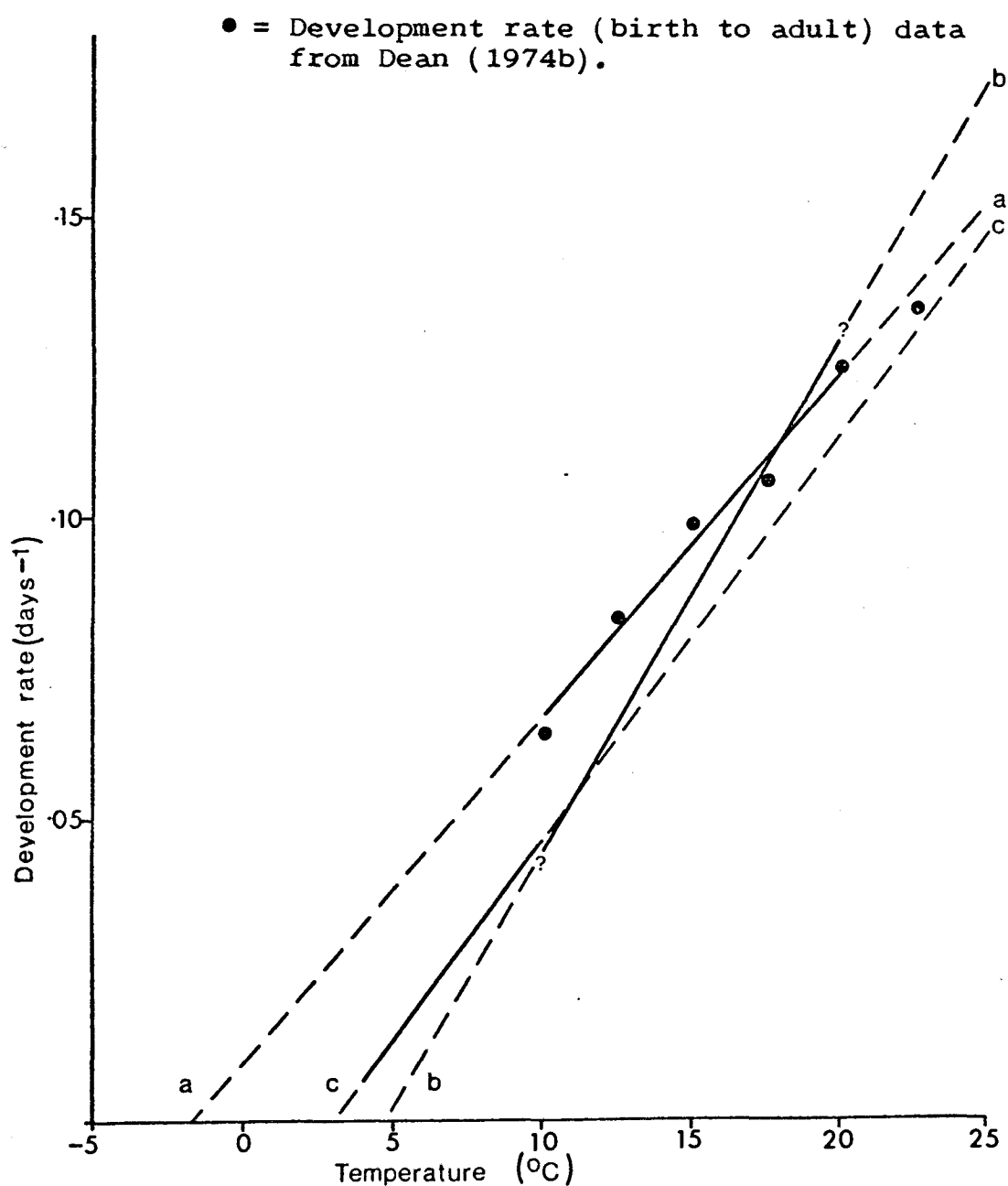


Fig. 4.23

Predicted development times derived from:

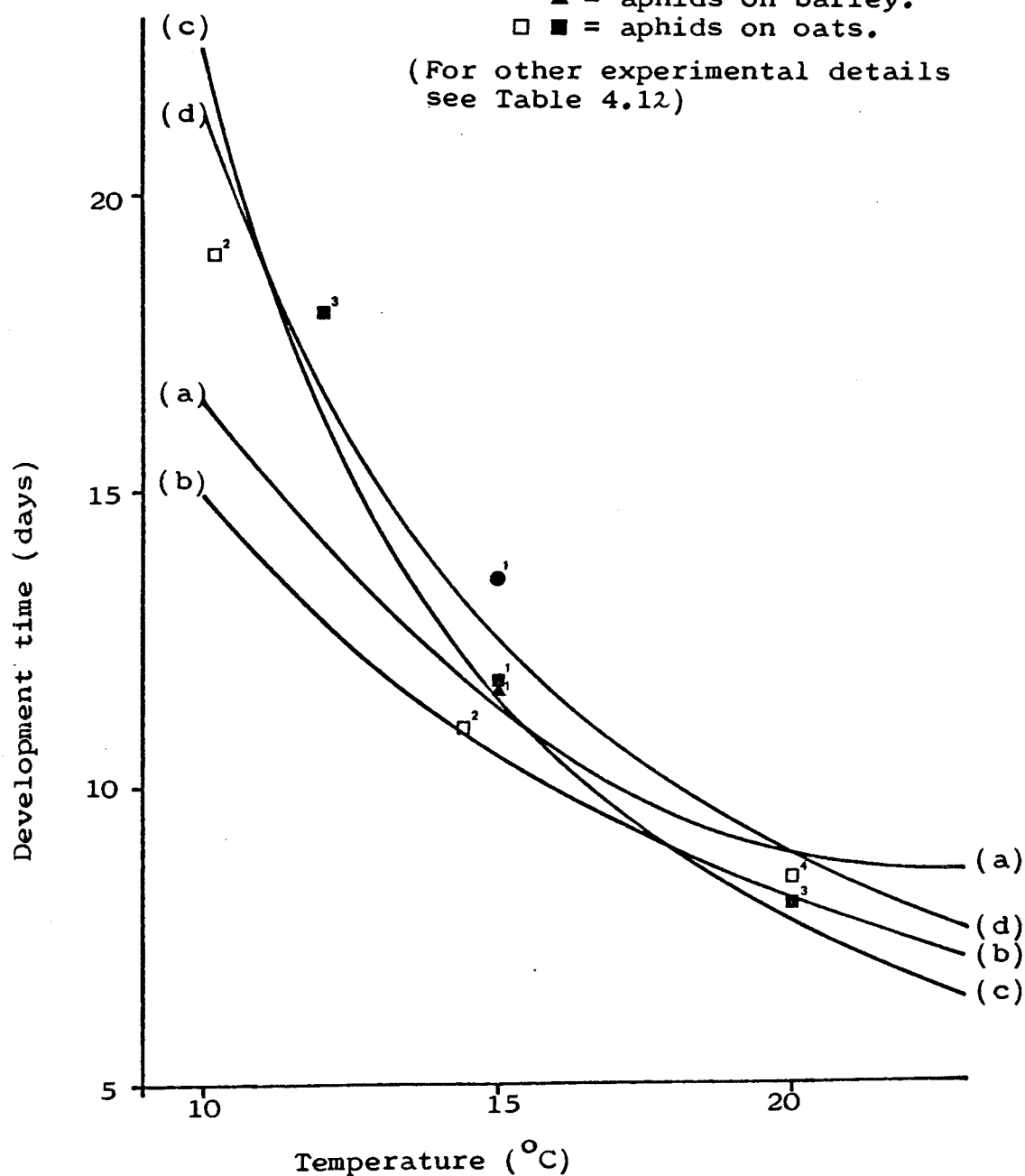
- (a) Dean, 1974b (quadratic equation for birth to reproduction).
 - (b) Linear regression equation for birth to adult using Dean's data for 10°- 20°C.
 - (c) Campbell *et al.* (1974) (birth to adult).
 - (d) Present study (birth to adult).
- Independent data from Dean (1973) (1), Markkula and Myllymaki (1963) (2), Markkula and Pullianen (1965) (3), Watt (1979b) (4).

KEY

Solid symbols = time from birth to start of reproduction. Hollow symbols = time from birth to adult.

- = aphids on wheat.
- ▲ = aphids on barley.
- ■ = aphids on oats.

(For other experimental details see Table 4.12)



Dean's development rate data between 10° and 20°C predicts a development time about 30% shorter. Even Dean's original quadratic (which, unlike the other curves shown, includes the adult's pre-reproductive period) predicts a development time over 20% shorter.

Rabbinge et al. (1979) mention that tests they did on wheat at 11°C gave much lower development rates than Dean's 10°C test on barley and, as Dean (1973) found that at 15°C development on wheat took nearly two days longer than on barley or oats, part of the difference between the curves shown in Fig. 4.23 is probably the result of host plant differences. But, unless development rates on different host plants change markedly relative to each other with small temperature shifts, it would appear that host plant differences are not a large contributing factor. The evidence for this is that the only independent data found for temperatures near 10°C (Markkula & Myllymaki, 1963; Markkula & Pullianen, 1965) agree much more closely with predictions from the present study and Campbell et al. (1974) than with those from Dean (1974b) (see Fig. 4.23) despite these data being for development on oats, which, at 15°C , is very similar to that on barley (Dean, 1973).

The reason for the discrepancy between the three curves shown in Fig. 4.23, which is large enough to produce important errors around and below 10°C , is impossible to pinpoint, particularly because of the lack of information on Campbell's study. Because the two laboratory studies differ so much, the discrepancy is unlikely to be due to differences between laboratory and field conditions. A possible reason is Dean's use of short lengths of leaf floating on nutrient solution rather than whole plants. Adams & van Emden (1972) discouraged the use of detached leaves or leaf discs as "excision of plant tissue results in rapid and very considerable physiological changes". Furthermore, Dean changed his leaf sections every two days, so that, unless the leaves were actually grown at the test temperature, their metabolism may well have been unrepresentative of plants grown continuously at 10°C .

Two other possible causes are errors in temperature recording or, as mentioned above, a strong interaction between temperature and host plant suitability - with barley becoming much more favourable than both wheat and oats at temperatures around 10°C , despite development times on barley and oats being very similar at 15°C (Dean, 1973). The latter possibility makes the use of development/temperature relations determined on one host to predict development times on another particularly dangerous. If discrepancies between predictions from Dean's data and those from the present study are due to Dean's use of excised plant tissue and/or to host plant differences, and if the results of Campbell *et al.* (1974) are representative of laboratory studies, then it appears that the constant temperature laboratory method for determining development/temperature relationships is quite robust, though, from Fig. 4.22, predictions from Campbell *et al.* (1974) would lead to rather large errors under cold conditions in which a substantial fraction of total development was achieved at temperatures below about 7° or 8°C .

The amount of independent data on development times is small (see Fig. 4.23). The only independent data for *S. avenae* on wheat are those of Dean (1973) at 15°C . When 16 h (the adult pre-reproductive period at 15°C (Dean, 1974b)) is subtracted from this development time, it is found to lie only 8 h from that predicted by the present study. This suggests that the relationship derived in the present study from low temperature data, gives good predictions at intermediate temperatures also.

Unfortunately, temperature data from Dean's winter fieldwork (Dean, 1974a) are not detailed enough to allow accurate comparison with the present study, but the range of development times (9 to 12 days during the summer and autumn, rising to nearly 60 days in mid-winter) agrees well with those found here. This also suggests that the theoretical threshold and day-degree requirement for development predicted from his laboratory study on excised barley leaves (Dean, 1974b) is unrealistic for development on wheat at low, fluctuating temperatures. The straight line fitted through Dean's laboratory data between 10° and

20°C predicts development should require only 176 day-degrees above a threshold of -1.75°C. If this held under winter conditions the average number of day-degrees above -1.75°C accumulated during the development period would have to be less than three per day for an aphid to take 60 days to develop. Weather this cold over such a long period seems very unlikely in southern England.

4.4.5 The development of alatae

Surprisingly little data are available in the literature on the development of alatae but in studies in which alate development times have been determined (Markkula & Myllymaki, 1963; Markkula & Pullianen, 1965; Chaudhary *et al.*, 1969) those for apterae are also given, so that a comparison of the duration of development for the two morphs is possible even when accurate temperature data are not available. This enables an examination of the data for a possible relationship between the relative duration of development for the two morphs and mean development temperature.

From the data available (Table 4.11) there is no suggestion that alate development times change relative to those of apterae with changes in temperature. (The relatively large difference between the morphs shown for 20°C and 28°C were derived from means read from graphs in Markkula & Pullianen (1965) which could only be read off to the nearest °C.) The fact that the differences between the morphs are not statistically significant except for data from the present study (Table 4.11) is probably just a reflection of the fact that in this study sample sizes were large and temperatures so low that a 9% difference in development time represented several days' difference, and was thus easily detectable.

4.4.6 Survival and longevity

Data from Dean's laboratory study (Dean, 1974b) suggest that the longevity of apterae (measured as the period between birth and 50% mortality) remains approximately

Table 4.11: Development times of alatae at a range of temperatures expressed as % of apterae development times.

Sources: 1= Markkula and Myllymaki (1963)
2= Markkula and Pullianen (1965)
3= Chaudhary et al. (1969)

Source	Mean temp.	<u>Alate dev. time</u> <u>Aptera dev. time</u>	Statistical significance
Present Study	6.5°C overall	109%*	P < 0.001
1	10.2°C	105%	P > 0.05**
2	12°C	106%+	P > 0.05
1	14.4°C	109%	P > 0.05**
3	17.7°C	103%	P > 0.05
2	20°C	113%+	P > 0.05
2	28°C	120%+	P > 0.05

* Based on the ratio of mean day-degree requirements.

** Significance levels are not quoted in Markkula and Myllymaki (1963) but given the small sample sizes (n always < 9) and widely overlapping ranges the means cannot be significantly different at the 5% level if the data are approximately normally distributed.

+ Development time = birth to start of reproduction, data taken from graphs.

constant as temperature drops from 20° to 10°C . Results from his field study (Dean, 1974a) however, showed mean longevity increasing from about 25 days in July to over 100 days in the two mid-winter tests. Markkula & Pullianen (1965) also found that mean longevity was greater at lower temperatures, being about 42 days at 20°C but 73 days at 12°C . But in their study aphids were reared at 20°C and only transferred to 12°C as teneral adults. In Dean's winter field tests maximum longevity recorded for an individual was about four and a half months and the longevities found seem in agreement with those observed in the present study.

When estimates of mean longevities for apterae determined at intermediate, constant temperatures are converted to day-degrees above 3°C to allow comparison with those determined in the present study (see Table 4.6) they are found to vary quite widely, from $238 \text{ day}^{\circ}\text{C} > 3^{\circ}$ (aphids on barley at 10°C and 16 h day length, Dean (1974b)) to $714 \text{ day}^{\circ}\text{C} > 3^{\circ}$ (aphids on oats at 20°C and 24 h day length, Markkula & Pullianen (1965)). Two summer field studies give longevities for apterae of $473 \text{ day}^{\circ}\text{C} > 3^{\circ}$ (mean temperature of 17.7°C , aphids on wheat, Chaudhary *et al.* (1969)), $489 \text{ day}^{\circ}\text{C} > 3^{\circ}$ and $676 \text{ day}^{\circ}\text{C} > 3^{\circ}$ (mean temperatures of 13.2° and 13.9°C , aphids on oats, Markkula & Myllymaki (1963)).

In summary, it appears that lower temperatures usually result in greater longevity, though mean longevity expressed in day-degrees cannot be treated as a 'thermal constant'. From the present experiment, longevities on wheat under winter field conditions (expressed as $\text{day}^{\circ}\text{C} > 3^{\circ}$) lie near the middle of the range found at intermediate temperatures in both laboratory and field studies, provided the temperature does not drop to the lower lethal zone.

All three intermediate temperature studies in which the longevities of apterae and alatae have been compared have shown no significant difference between the morphs (Markkula & Myllymaki, 1963; Markkula & Pullianen, 1965; Chaudhary *et al.*, 1969) and alate longevity did not vary much - from 97% to 119% that of apterae. In the present study, alate median longevity was 88% of apterae's and

significantly different from it, suggesting that alatae are less well able to cope with low temperature stress.

4.4.7 Total nymph production by apterae

From data available in the literature (Table 4.12) total fecundities for apterae of S. avenae at temperatures between 10° and 20°C generally lie between 35 and 50 nymphs. The only exception is the data of Dean (1974b) which show a higher fecundity (61 nymphs) at 20°C. Dean's field and laboratory studies (Dean, 1974a, 1974b) provide the only reasonably complete sets of data for a range of temperatures. Both show that fewer nymphs are produced at lower temperatures. The lowest fecundity recorded in these studies was an average of about 10 nymphs per parent in the two mid-winter field tests. In the laboratory study the adults' mean reproductive life declined slightly from 18 days at 15°C to 15 days at 10°C. In the field study this trend was shown much more strongly between autumn and winter. Aphids in the mid-October test had the longest reproductive period, at around 50 days, but those born in November and January had reproductive periods of only about 25 and 13 days respectively followed by post reproductive periods of 20 to 30 days.

Thus, from data in Table 4.12, in particular Dean's field and laboratory studies, one would predict that the apterae monitored in the present study born after early October (by which time 3-day period mean temperatures were nearly always below 10°C and often below 5°C) should have had total fecundities well below 40 nymphs and quite short reproductive lives, followed by long post-reproductive periods. The actual results were in stark contrast to this. Mean total fecundities for apterae on September- and November-sown wheat, estimated indirectly from the reproduction rate/age relationship, were 70 or 74 nymphs depending on the parent's morph (Table 4.2). The aphids survived for so long that direct measurements of total fecundity were only possible for apterae born before 24 October (Fig. 4.13(a)). Of these, apterae born between 14 and 24 October (3-day periods 8 to 11) had a mean

Table 4.12: A summary of data on the total fecundity of S. avenae on cereals.

Unless stated otherwise the following points apply:

1. Laboratory studies were undertaken at constant temperature (maximum variation 2°C or less) with 16h D.L.*
2. Host plants were young, potted seedlings which were changed every week or 10 days.
3. Aphids were confined in cylindrical, transparent cages with muslin vents.
4. Studies were done in England on English clones.

The data from Wratten (1977) are means for nymph production in the first 20 days of adult life, by which time reproduction and survival were low. Unlike the other data, they control for size differences and longevity differences between the morphs as they are means for size-classes in which both apterae and alatae are represented and each size-class was given equal weight. The means shown were obtained by summing means for 3 consecutive periods of adult life (days 1-5, 6-10 and 11-20), each with different sample sizes but sample size was always greater than 30. Only data for aphids surviving through each period were used in calculating each period's mean.

Dean's aphids were all the offspring of apterae; the parent's morph is not known for the other studies.

Data from Dean (1974a) and Markkula and Pullianen (1965) were taken from graphs.

Data from Markkula and Pullianen (1965) shown are for a green clone.

* day-length

Table 4.12(a): Data for apterae only.

Source	Host plant	Other experimental conditions	Temperature (°C)	Total nymph production (apterae) ($\bar{x} \pm \text{S.E.}$)
Dean (1973)	Wheat (cv. Capella) Barley (cv. Proctor) Oats (cv. Blenda)	LAB.	15°	40.8 $\pm$ 4.6 43.9 $\pm$ 4.4 36.8 $\pm$ 3.1
Dean (1973)	Wheat (cv. Capella) Barley (cv. Proctor) Oats (cv. Blenda)	FIELD. June-August	no data	38.0 $\pm$ 4.5 49.2 $\pm$ 4.2 48.8 $\pm$ 3.8
Dean (1974a)	Barley	FIELD. Winter '70/'71 n = 20 in each test. Aphids taken out of glasshouse on plants as 1st instars. Test 1. Nymphs born in Sept. Test 2. " " mid-Oct. Test 3. " " mid-Nov. Test 4. " " mid-Jan. Test 5. " " mid-Mar.	Monthly means during development and reproduction: c.12.5° - c.13.5° c. 2.5° - c.12.5° c. 2.5° - c. 4.0° c. 1.0° - c. 3.0° c. 1.0° - c.12.5°	c.44 c.40 c.8 c.10 c.31
Dean (1974b)	Barley	LAB. Stock culture at 15°C, aphids born at test temp.	10° 15° 20° 25°	33 $\pm$ 3.6 46 $\pm$ 4.6 61 $\pm$ 3.6 33 $\pm$ 1.9

Table 4.12(b): Data for apterae and alatae.

Source	Host Plant	Other experimental conditions	Temperature (°C)	Total nymph production ($\bar{x} \pm \text{S.E.}$)		
				Apterae	Alatae	Al./Ap. x 100
Chaudhary et al. (1969)	Wheat	FIELD N.India (Punjab) Feb.-Apr. '67. Details of plant changes unknown.	Mn.temp: 17.7° Mn.max: 25.5° Mn.min: 9.8°	42.2 $\pm$ 2.26	37.9 $\pm$ 1.57	89.8%
Markkula and Myllymaki (1963)	Oats (cv.Sisu)	Insectary, temp. conds. like FIELD. Finland (nr.Helsinki) 1st test: aphids born 28 May '62. n = 8 (ap.) & 7 (al.) 2nd test: aphids born 28 June '62. n = 4 (ap. & al.)	Mn.temp during reproduction: 14.7°	35 (17-54)	36 (27-52)	102.9%
			Mn.temp during reproduction: 14.3°	43 (30-49)	35 (26-47)	81.4%
Markkula and Pullianen (1965)	Oats (cv.Sisu)	LAB. Finland (Tikkurila). Aphids kept at 20°C till adult. 24 h D.L. n > 45 (ap. & al. combined) for 12° test. n > 60 (ap. & al. combined) for 20° & 28° tests.	12° 20° 28°	c.35 c.43 c.8	c.49 c.36 c.19	140 % 83.7% 237.5%
Watt (1979b)	Oats (cv.Aster)	LAB. Aphids clip-caged. Details of plant changes unknown.	15°	40.69 $\pm$ 1.53	31.53 $\pm$ 1.73	77.5%
Wratten (1977)	Barley (cv.Proctor)	LAB.	20°	46.11	33.33	72.3%

fecundity of 63.5 nymphs. Even during the brief drop in fecundity earlier in October (possible reasons for which were discussed in section 4.3.7) mean fecundities only fell to around 30 nymphs. Furthermore, most apterae had reproductive periods lasting several months and no distinct post-reproductive period before death.

It is impossible to be certain of the reason for the large difference between Dean's field data and those of the present study. There is no doubt from Dean's temperature data that the 1970/71 winter was colder than the 1980/81 winter, but the difference does not appear nearly large enough to produce such a large difference in reproductive performance, particularly as the long mean post-reproductive periods of the aphids in Dean's two mid-winter tests show that temperatures were not low enough to kill many adults before they had completed reproduction. The reproductive pattern shown by these aphids (short reproductive period, low total fecundity and long post-reproductive period) was in fact very similar to that shown by apterae born at 20°C and put out in clip-cages at Chilworth during the 1979/80 winter as a trial run for the present experiment. As Dean also kept his aphids in a glasshouse and took them out as first instars it seems likely that their low fecundities were due to this artificiality in his experimental design.

Aphids produced by unacclimatized parents probably suffer temperature shock when transferred suddenly to winter field conditions, so that embryogenesis is disrupted and only those embryos which are well developed at the time of the transfer to the field are able to complete development. Consequently, the experimental animals' reproductive pattern is quite unrepresentative of that in natural winter populations. This hypothesis is supported by the fact that, in the present study, aphids born in early to mid-October showed distinctly lower fecundities than those born earlier or later (Fig. 4.13). This dip in fecundities seems to correspond to aphids which experienced relatively warm conditions during development but cold conditions later in life (see section 4.3.7). The differences in total fecundity by overwintering viviparae shown in Dean's

study and the present one highlight the point that it should not be assumed that a parent's temperature-experience does not affect the performance of its offspring.

4.4.8 Total nymph production by alatae

Few data are available in the literature on total fecundities of alatae, and none for low temperature conditions. From the data available for intermediate temperatures (Table 4.12) total nymph production seems generally to vary from 30 to 40 nymphs and is usually below that of apterae, although the difference is relatively small (30% or less). The data from the 12°C test by Markkula & Pullianen (1965), in which alatae were considerably more fecund than apterae, are the only exception. But these data are suspect because aphids were transferred from 20°C to 12°C as teneral adults. The mean total fecundities of alatae at low fluctuating temperatures determined in the present study were a little higher than those found at intermediate temperatures (39 or 45 nymphs depending on the parent's morph). But, because the total fecundities of apterae were considerably increased at low temperatures, alatae produced many fewer nymphs relative to apterae. Their mean total fecundity was only 58% that of apterae and, even when the effect of their shorter potential longevity was excluded, it was still only 63%. Moreover, the shape of the reproductive pattern for alatae (with a relatively long pre-reproductive delay and a marked late peak in nymph production) was such that if an additional non-age specific mortality were to act on the population, the difference between the fecundities of the two morphs would increase further. If a mortality of 50% per 25 day°C > 3°C acts, winter alatae can be expected to produce only half as many nymphs as apterae (see Table 4.3).

4.4.9 The reproductive pattern of apterae

Reports of most studies of reproduction by S. avenae

either do not show the relationship between reproduction rate and adult age, or, in doing so, use relatively long time units so that detail is obscured. The exceptions are studies by Wratten (1977) and Watt (1979b) who determined the relationship at constant, intermediate temperatures for both apterae and alatae with days as the time unit. This gives sufficient detail for useful comparisons to be made with the results of the present study (Fig. 4.14). Their results are for aphids at 15°C on oats (Watt, 1979b) and at 20°C on wheat (Wratten, 1977). Although peak daily reproduction rates were as expected, higher at 20°C, the reproductive patterns shown in Watt and Wratten's work were similar, and the small differences can be attributed to the inclusion of the adult's pre-reproductive period and exclusion of adult mortality in Wratten's data. This makes it directly comparable with data shown in Fig. 4.14(a) and results in the reproduction rate on the first day being lower and subsequent reproduction more sustained than in Watt's data.

Both Watt's and Wratten's data show daily nymph production highest on the second day of reproduction, declining slowly over the next two weeks and then more rapidly thereafter. The relatively flat reproductive pattern during early adult life with a small, early peak, contrasts with that determined under low temperature field conditions and illustrated in Fig. 4.14(a). This shows reproduction rate rising gradually to a peak which is only reached after about 20% of reproductive life has elapsed and is followed by a steady, comparatively rapid decline.

Although, as expected, reproduction proceeded for much longer under cold conditions than at relatively high temperatures, if the high temperature patterns determined by Watt (1979b) and Wratten (1977) are expressed in day-degrees above 3°C, to make direct comparisons with the winter field data possible, it can be seen that reproduction at low temperatures was spread over a shorter period measured on this physiological time scale. For example, in winter conditions reproduction by apterae had ceased about 350 day°C > 3° after the final moult, whereas at 20°C

apterae surviving to 340 day $^{\circ}\text{C} > 3^{\circ}$ after the final moult (20 days) were still producing 0.09 nymphs/day $^{\circ}\text{C} > 3^{\circ}$ (Wratten, 1977). And from Watt's data reproduction stops at about 430 day $^{\circ}\text{C} > 3^{\circ}$ after the final moult at 15 $^{\circ}\text{C}$.

But, although reproductive life seems relatively short at low temperatures on this physiological time scale, this appears to be more than compensated for by the fact that, on the same scale, reproduction rates are much higher. The maximum daily nymph productions found in Watt's and Wratten's studies were around 0.18 nymphs/day $^{\circ}\text{C} > 3^{\circ}$, compared to maxima of about 0.4 nymphs/day $^{\circ}\text{C} > 3^{\circ}$ found in the present study under winter field conditions. If the peak reproduction rates determined in Dean's laboratory study (Dean, 1974b) are converted from nymphs/week to nymphs/day $^{\circ}\text{C} > 3^{\circ}$ it can be seen that, measured in this way, they also increased as temperature decreased, from about 0.17 at 20 $^{\circ}\text{C}$ to about 0.35 at 10 $^{\circ}\text{C}$. But, because of the relatively short adult survival at 10 $^{\circ}\text{C}$, total nymph production was low at this temperature.

From the examination of the day-degree requirement to produce 20 nymphs (section 4.3.4) it seems that the reproduction rate of young apterae remained fairly constant on a physiological time scale over the range of temperature and other conditions experienced during the present experiment. However, from the discussion above it is obvious that this "thermal constant" does not hold under higher temperature regimes. For example, from Wratten's data, an aptera at 20 $^{\circ}\text{C}$ would take over 120 day $^{\circ}\text{C} > 3^{\circ}$ to produce 20 nymphs, nearly double the requirement determined here for aphids under winter field conditions. Thus, unlike development rates, reproduction rates cannot be regarded as approximately constant when expressed on a physiological time scale except over relatively narrow ranges of temperature and other conditions.

The relationship between peak nymph production per day $^{\circ}\text{C} > 3^{\circ}$ and temperature shown by Dean's data suggests that the differences between the present study and those at higher temperatures are the result of a real temperature-related effect and not due to other, unrelated, differences between laboratory and field conditions. This is supported by Watt's summer field data (Watt, 1979b) which show that

reproduction rates by young adult apterae of S. avenae on wheat leaves at mean daily temperatures between 10° and 18°C varied from about 0.15 to 0.25 nymphs/day $^{\circ}\text{C} > 3^{\circ}$. The fact that reproduction rates expressed in day $^{\circ}\text{C} > 3^{\circ}$ are higher at lower temperatures suggests an adaptive shift in the temperature relations of reproduction to maximize nymph production at low temperatures when these prevail.

The reproductive pattern for apterae determined in the present study suggests they are well adapted to reproduce at low temperatures. Firstly, potential longevity and the reproductive pattern seem well matched, so that an aptera has produced nearly all of its full complement of nymphs by the age of median longevity but does not have a long post-reproductive period (compare Fig. 4.14(a) and Fig. 4.17(a) bearing in mind that apterae take 150 day $^{\circ}\text{C} > 3^{\circ}$ to develop). This close matching is reflected in the constancy of the total fecundity for apterae whether or not adult survival is taken into account (Table 4.2).

In Aphis fabae, which forms dense colonies, it has been found that a nymph developing on a leaf with a colony on it is heavier and more fecund as an adult than one reared on a leaf by itself (Dixon & Wratten, 1971). Thus, in this species, a post-reproductive adult might still contribute to the survival of its clone as its mere presence could help to increase the fecundity of its offspring. But in S. avenae, which does not usually form dense colonies, there is no evidence for such an effect. Hence the post-reproductive period is essentially wasteful, it is a period during which no contribution is made to the clone's survival and in which it may compete with younger clone members for space and food.

It is thus adaptive for S. avenae to match ageing and reproductive processes so that the end of potential reproductive life and death due to 'old age' occur close together. This was just what was found in the present study, but in Dean's mid-winter fieldwork (Dean, 1974a) apterae had short reproductive periods in which few nymphs were produced, followed by long post-reproductive periods.

This is a wasteful reproductive pattern and suggests an animal responding to environmental conditions in a non-adaptive way because it is close to the limits of its range of tolerance.

As was mentioned above, the poor reproductive performance of the aphids in Dean's mid-winter field tests was probably due to the sudden transfer of aphids from glasshouse to field conditions and it is felt that the performance of those used in the present study is much closer to that in natural populations. In Dean's laboratory test at 10°C (Dean, 1974b) longevity was much shorter, rather than longer, than potential reproductive life, so much so that adults had a less than 50% chance of surviving to the age when reproduction rate was highest. Again this is a wasteful pattern. In this case the unadaptive response at a quite moderate temperature was probably due to the aphids not being adapted to unnatural, constant temperature conditions.

It could be argued that it would be better for apterae to achieve peak reproduction rates earlier in adult life, as this would increase the intrinsic rate of increase (r_m). However, as Watt (1979b) has pointed out, r_m may be an inappropriate measure of evolutionary fitness in aphids as this parameter assumes a constant age structure in the population. Cereal aphids rarely attain a constant age structure (Carter, Aikman & Dixon, 1978). Furthermore, this assumption implies a population increasing in a constant environment, whereas the life-history strategy of aphids depends for its success on spatial and temporal heterogeneity in the environment.

Watt has suggested that under most conditions each nymph produced has an equal and high 'value' to the clone's survival because of heavy mortality during the frequent migrations by alatae to new hosts. Consequently, R_0 the number of offspring contributed to the next generation, is probably a better measure of fitness than r_m . In winter long distance migration between hosts is unlikely to be either necessary or possible but, because of the low development and reproduction rates under cold conditions, Watt's argument still applies. An earlier peak in

reproduction rate would increase R_0 as well as r_m , but only if it could be achieved without a significant reduction in total fecundity. It seems likely, given the inevitable intrinsic constraints on an aphid's reproductive system, that this is not possible and the winter reproductive pattern shown by apterae in this experiment represents the best compromise between an early reproductive peak and a high total fecundity.

When reproduction rates attained at summer and winter temperatures were compared above in terms of nymphs produced per day $^{\circ}\text{C} > 3^{\circ}$ it was found that they rose as temperature fell, suggesting an adaptive response. However, an examination of the number of day $^{\circ}\text{C} > 3^{\circ}$ required by apterae to produce 20 nymphs in the present study revealed a slight but statistically significant tendency for apterae experiencing lower temperatures during development to require more day-degrees to produce 20 nymphs, suggesting a shift in the temperature relations of reproduction in a non-adaptive direction. But there is a possibility that this is due to the reproductive peak, which has been shown to be later under cold conditions, being pushed even further back to keep total fecundity high and R_0 maximal. However, in considering the evolutionary fitness of the reproductive pattern at low temperatures it should be remembered that the reproductive system of viviparae has to function under a wide range of temperature, host plant and other conditions and it is likely that some fitness under cold conditions has been sacrificed in order to increase that under summer conditions when the survival of the species is entirely dependent on viviparae, unlike the situation in winter in which an alternative strategy, holocyclic overwintering, is available.

4.4.10 The reproductive pattern of alatae

The reproductive pattern shown by alatae in the present study had two peaks separated by a reproductive lull about half-way through adult life (Fig. 4.14(b)). But alatae studied at intermediate, constant temperatures showed no sign of this unusual pattern (Wratten, 1977;

Watt, 1979b) and it seems it was a direct or indirect effect of winter temperature conditions. Taylor (1975) found alatae of Aphis fabae had two separate reproductive peaks, though the reproductive pattern differed from that shown in the present study in that the initial peak was a short rapid burst of nymph production on the first day of reproductive life and the second a lower but much more sustained one. Moreover, Dixon & Wratten (1971), also working on A. fabae found the second peak much less prominent.

A possible reason for a two-peaked reproductive pattern in alatae is that the second peak is due to the release of nutrients, particularly nitrogen, when the wing muscles autolyse soon after the alate settles. Johnson (1957) found that, in the alatae of several aphid species, embryo development was arrested soon after the final moult and was resumed after the aphids had settled down to feed, at the same time as wing muscle autolysis started. Although he considered that breakdown products from the muscles would probably become available for embryo development, he thought it unlikely that developing embryos depended in any way on them, as a parent aphid would already be settled and feeding when embryo development resumed. Dixon (1973) also considered wing muscle autolysis unimportant in embryo development, quoting the example of Aphis fabae, in which the wing muscles weigh only about twice as much as a single nymph at birth. It seems more likely that the synchrony between resumed embryo development and wing muscle autolysis is due to both processes having a common, possibly hormonal, controlling agent (Johnson, 1957, 1959).

However, Johnson's finding that embryogenesis in alatae was usually halted between the final moult and settling provides the basis for an alternative explanation. If, as was suggested above (section 4.4.2), it is the rate of embryo development rather than the rate of eclosion which limits reproduction at low temperatures, then a complete or partial suppression of embryogenesis in alatae between the final moult and the time when they become sedentary would result in a two-peaked reproductive pattern,

provided that those embryos which were most advanced at the time of the final moult could be born while embryo development was still partially or completely at a standstill. This was found to be the case in several of the species studied by Johnson (1957).

On average $13.5 \text{ day}^{\circ}\text{C} > 3^{\circ}$ were accumulated in each 3-day period during the present study (Appendix 4.5). Thus the period of alate restlessness (c.9 days, see Fig. 4.19) represented approximately $120 \text{ day}^{\circ}\text{C} > 3^{\circ}$ and it ended late in the initial reproductive peak, or at the beginning of the reproductive lull (see Fig. 4.14(b)). The reproductive lull can thus be interpreted as the period during which the settled alatae, now feeding more or less continuously, resumed embryo development and replenished their stock of well-developed embryos, which had been depleted as a result of sporadic deposition of nymphs in the "restless" period, during which further embryo development was at least partially suppressed. After a time-lag the second peak of nymph production occurred, probably as a result of the resumption of rapid embryogenesis. Only partial suppression of embryogenesis during early adult life would be necessary for such a reproductive pattern to occur and even if embryo development was not suppressed directly by some intrinsic factor, the fact that young restless adult alatae spent a substantial proportion of their time not feeding must inevitably have slowed embryo development to some extent.

At intermediate temperatures, the lower total fecundity of alatae compared to apterae is to some extent compensated for because they produce a greater proportion of their nymphs early in adult life (Wratten, 1977; Watt, 1979b). However, from the results of the present study the reverse seems true at low temperatures. This difference is due largely to the two-peaked reproductive pattern shown by the alatae studied here, which gave them an added reproductive disadvantage relative to apterae. The two-peaked pattern would ensure that as the level of non-age specific mortality acting on a natural winter population increased, the difference in the realized fecundities of apterae and alatae would also increase.

Another characteristic of the reproductive pattern of the alatae in the present study was that, when expressed as nymphs/day $^{\circ}\text{C} > 3^{\circ}$, reproduction rates were, like those of apterae, considerably higher than those shown at intermediate temperatures (Wratten, 1977; Watt, 1979b). Like apterae, alatae at low temperatures showed close synchrony between potential longevity and the end of potential reproductive life, so that estimated fecundities were similar whether or not the effect of adult mortality was excluded (Table 4.2).

4.4.11 Low temperature adaptation in the reproduction of apterae

From the comparisons above between reproductive performance observed at low temperatures in the present study and that observed at intermediate temperatures by earlier workers there is circumstantial evidence for several types of cold adaptation in apterae. These are summarized below:

1. Slower reproduction rates at low temperatures are offset by an increase in total fecundity.
2. Apterae born to adults which have experienced low temperatures and which experience low temperatures themselves, appear to be able to avoid sub-lethal cold-stress which otherwise reduces fecundity (as shown by apterae in Dean's winter field tests, the 1979/80 winter trial at Chilworth and by apterae born in the first half of October in the present study).
3. When reproduction rates are expressed as nymphs/day $^{\circ}\text{C} > 3^{\circ}$ they are higher under winter conditions, which suggests an adaptive shift in the temperature relations of reproduction to maximize nymph production at low temperatures.
4. Under winter conditions potential longevity and the

reproductive pattern are well matched so that apterae have produced nearly their full complement of nymphs by the time median longevity is reached but do not have a long, wasteful post-reproductive period.

It appears that, particularly for apterae, the characteristics of reproduction are very malleable and can be adjusted to suit a wide range of temperature and other conditions. In this respect reproduction contrasts with development, for development times can be quite accurately predicted over a wide range of temperatures, using a single, simple relationship. Why this contrast exists is an interesting theoretical question but one which cannot be answered conclusively without detailed information on the physiological processes involved.

4.4.12 The disadvantages of alatae under winter conditions

The alatae studied here showed the potentially adaptive changes in reproduction listed above, but to a lesser extent than did the apterae. The morph's only advantage over apterae is its ability to fly, which is probably of very little benefit in late autumn and winter, although it may well be important during mild weather in early spring (see section 4.4.3). From the present study it seems that alatae show several disadvantages when compared with apterae under winter conditions:

1. Development time is longer (although there is no evidence that its duration is longer relative to that of apterae when compared with development times at intermediate temperatures).
2. Inappropriate, restless, migratory behaviour is shown by young adult alatae which probably reduces their reproductive fitness (see sections 4.4.3 and 4.4.10).

3. Alate total fecundity is lower and represents a smaller proportion of apterae's total fecundity than at intermediate temperatures.
4. The two-peaked reproductive pattern results in alatae producing more of their nymphs later in reproductive life than do apterae (the reverse is true at intermediate temperatures).
5. Potential longevity is shorter (though there is no significant difference between the morphs at intermediate temperatures).
6. The offspring of alatae (whether apterae or alatae) have slightly slower reproduction rates as young adults than the same morph produced by apterae.

In view of these disadvantages and the usually low densities of winter populations, it is not surprising that in natural overwintering populations of S. avenae viviparae, apterae are usually much the commoner morph (Hand, 1982; Vorley, pers.comm.). It seems that the high proportion produced in the present study was unnatural and probably a result of rearing two nymphs in each cage. Although, superficially, this would not appear to constitute the crowded conditions usually needed to stimulate alate production, two nymphs per cm length of leaf is an extremely high average density under winter conditions. If a natural winter population of nymphs reached this average density, their subsequent maturation and reproduction would probably result in disastrous overcrowding given that plant growth is very slow during the winter. Thus, if the main way in which crowding stimulates alate production in S. avenae is a direct one rather than via detrimental effects on the nutritional state of the host, it was quite appropriate that a relatively high proportion of alatae were produced in the present study. That this degree of crowding during development did not in fact produce harmful effects can be seen from the extremely good survival and high fecundities of the monitored aphids.

4.4.13 The stability of the relationships between development, reproduction and temperature determined in the present study

From the lack of any significant relationship between development day-degree requirement and birth date and the reasonably good agreement at intermediate temperatures with relationships derived by Campbell *et al.* (1974) and Dean (1974b), it appears that the temperature/development rate relationship for *S. avenae* on young cereals holds quite well over a range of laboratory and field temperature conditions.

An examination of the day-degree requirements for the production of the first 20 nymphs showed significant correlations with parent's morph and time of year. Possible causes for the 'time of year' effect have already been discussed (see section 4.3.4). Of those suggested, temperature during development, acting either directly or via the host plant, is probably the most likely. It is perhaps surprising that a 'parent's morph' effect has not been reported for *S. avenae* before, in view of the number of studies on the species' biology. But the lack of previous evidence is easily explained as in earlier work either the experimental animals all had the same morph of parent (Dean, 1973, 1974a, 1974b) or the parent's morph was not determined (Markkula & Myllymaki, 1963; Markkula & Pullianen, 1965; Chaudhary *et al.*, 1969; Wratten, 1977; Watt, 1979b). Dixon (1976b) found that, in *Rhopalosiphum padi*, alatae were heavier as newly-born nymphs and as adults if their parents were apterae rather than alatae. As adult weight and fecundity are positively correlated in *R. padi* it seems that parent's morph affects fecundity in the same way as found here for *S. avenae*. A relationship between adult weight and fecundity has also been shown for *S. avenae* (Wratten, 1977; Watt, 1979b) and for several other aphid species (Dixon, 1971; Dixon & Wratten, 1971; Taylor, 1975; Wratten, 1977; Kempton, Lowe & Bintcliffe, 1980) and it is likely that the variations in 20 nymph day-degree requirements found in the present study are correlated with adult size differences. In considering the relationship between fecundity and adult size it has often

been stated or implied that size differences cause fecundity differences. However, correlation does not prove causation, and as size is a characteristic of the aphid, not its environment, it cannot be the ultimate cause of fecundity differences, indeed it may not even be the immediate cause. It could be argued that it is a result, not a cause, of greater fecundity, fecund adults being large because of the number of embryos they contain. Although size and fecundity are closely linked it is probably better to think of them not as cause and effect but as two aphid characteristics reacting similarly to a similar set of environmental variables.

Although the 'parent's morph' and 'time of year' effects on 20 nymph day-degree requirement were highly significant ($P < 0.001$), the use of mean reproduction rates for each morph ignoring these factors would not lead to very large errors. For example, for both apterae and alatae, the mean 20 nymph day-degree requirements for aphids produced by apterae and alatae (shown in Table 4.1) differed from the means for both parent morphs combined by about 12%. And, when apterae produced by alatae were grouped according to 30-day maturation periods the mean 20 nymph day-degree requirements for each period (shown in Fig. 4.10) differed from the grand mean for all periods by 11% or less. If aphids maturing during October were excluded, the difference was less than 6%. Hence the relationship between reproduction rate and temperature, as assessed by 20 nymph day-degree requirements, appears stable enough for predictive purposes over the range of conditions experienced in the experimental period.

A similarly extensive investigation of variation in total fecundity could not be undertaken because of the aphids' extreme longevity, and so total fecundity and reproduction rates later in adult life may change more drastically than first 20 nymph day-degree requirements. However, changes in reproductive performance late in adult life have relatively little effect on population growth, as reproduction rates and survivorship must be low for older adults. The reduction in total fecundities shown by aphids born in early autumn (see Fig. 4.13) may be

large enough to have important effects on autumn population dynamics if it is shown by natural populations.

Apterae would seem unlikely to be greatly affected in most years as in the present study they recovered high total fecundities rapidly and early. If the timing of the recovery shown by the monitored apterae was typical (those born soon after mid-October had normal, high fecundities) then it is very unlikely that many apterae with low fecundities would be born on commercially grown winter wheat, which is usually sown from late September onwards. However, if, as suspected, the changes in total fecundities were a result of damaging sub-lethal effects of low temperature on incompletely acclimatized aphids, then a late, warm autumn changing suddenly to a harsh winter might produce a later and more important drop in the total fecundities of apterae.

For alatae, an autumn drop in total fecundities might well be more important, unless the size and timing of the drop shown by the alatae monitored in the present study were not typical. If they were, many of the immigrants settling on newly emerged winter cereal crops could have low fecundities. However, these autumn immigrants develop on grasses, not cereals, and thus their reproductive characteristics may be quite different from those of alatae in the present study. It would seem advisable for anyone attempting to simulate S. avenae population dynamics on winter wheat to begin the field season with relatively frequent sampling so that any large discrepancies between the immigrants' reproduction and that predicted for them from the present study could be determined.

Although the reproduction rates of adults, expressed in physiological time units, were reasonably constant over the range of conditions experienced in the present study, it was shown above that they were very different from those determined at higher temperatures by earlier researchers. Thus the reproduction/temperature relationships determined here for the period October to late March should not be used to predict population growth much later in the spring or in summer, unless independent reproduction rate determinations are undertaken to check their accuracy under the warmer conditions.

4.4.14 The production of oviparae

Though the present study was not designed to investigate morph determination, the monitoring revealed the interesting fact that the clone used, although capable of producing oviparae from late September to mid-October, switched back to producing just viviparae after this. For the large majority of aphid species studied, exposure of viviparae to short day-lengths is the prime factor producing the switch from viviparae to sexual morph production (Hille Ris Lambers, 1966; Lees, 1966). The second main environmental factor is temperature. In several aphid species the critical photoperiod needed to trigger sexual morph production is lengthened as the temperature is lowered. Laboratory studies by Hand (1982) and Watt (1979b) have shown that these factors determine sexual morph production in S. avenae.

Given this basic mechanism, the finding in the present study that an S. avenae clone produced some oviparae in the autumn but stopped later in the winter when photoperiods were shorter and temperatures lower would appear to imply additional influences acting under field conditions. Three possible explanations are:

1. Changes in host plant condition are able to switch off sexual morph production even under light and temperature regimes which, according to laboratory results, would otherwise lead to many sexual morphs being produced. The oviparae in the present study were all born to mothers reared on potted early-sown wheat seedlings, whereas aphids born after this were all produced by mothers reared on later-sown, unpotted, field-grown plants. If sexual morph production is only stimulated when the host plant's condition is relatively poor then the pattern of oviparae production found might result.
2. S. avenae viviparae do not simply react to photoperiod but also to changes in photoperiod from one day to the next and are stimulated to produce sexual morphs by short photoperiod combined with a rapid

decrease in photoperiod. The photoperiod only decreases rapidly enough to stimulate sexual-morph production during a relatively brief period in the autumn, after which it is switched off again.

3. S. avenae has an 'interval timer' which switches off sexual morph production in late autumn a certain length of time or number of generations after it has started.

However, there appears to be no good experimental evidence for any of these effects. With respect to possible host plant effects, some root aphids, which, being subterranean, cannot detect photoperiod, have been shown to use physiological changes in the host to initiate sexual-morph production (e.g. Schwartz, 1932; Sethi & Swenson, 1967) as has Dysaphis devectora which produces sexual forms in mid-summer and thus requires a non-photoperiod cue (Forrest, 1970). Bonnemaison (1951) reported that Myzus persicae reared in a physical environment that induced gynoparae was switched back to parthenogenetic reproduction by transferring the aphids from cabbage to drier peach foliage. But this appears to be the only instance of an important host plant effect in a species in which the primary sexual morph determining cue is photoperiod and, unlike the situation in the present study, it involved a change of host plant species. Lees (1966) stated that nutrition seldom seems of importance in sexual morph determination. In several species of aphid an 'interval timer' has been found which suppresses sexual morph production in the fundatrix and the first few spring generations and therefore prevents oviparae and males being produced under short photoperiod conditions in early spring (Hille Ris Lambers, 1966; Lees, 1966). However, no such mechanism has ever been shown to switch off sexual morph production once it has started.

Usually the persistence of viviparae through the winter has been attributed to the existence of clones which are incapable of producing sexual morphs and/or to the fact that although sexual morph production continues

through the winter some viviparae are always produced with them, so that a population of winter viviparae is maintained. There appears to be no data in the literature to explain the pattern of ovipara production found in the present study but this is probably because work on morph determination has rarely been done under field conditions and because past researchers have been primarily concerned with the physiology of sexual morph determination, rather than the implications for winter population dynamics. It appears that field work on sexual morph production, though arduous, is an area which deserves more attention and, furthermore, might enable the principles determined in the mass of laboratory studies to be used in an applied way.

In S. avenae, which does not switch completely to sexual morph production under short photoperiods (Watt, 1979b; Hand 1982) and which is well suited to surviving the winter as parthenogenetic viviparae, some mechanism for switching off sexual morph production in late autumn (provided the aphid is on a host which does not die back in the winter and is of reasonable nutritional status) would have great advantages for the clone's survival. Without it males and oviparae would continue to be produced until early spring, despite the fact that under winter field conditions the chances of successful mate-finding are remote and any resultant eggs might well be unable to complete diapause.

4.4.15 Incorporating the relationships for winter development, reproduction and longevity into a simulation model

Several characteristics of the relationships determined here make their incorporation into a simulation model easier than expected and, although a few others lead to complications, the overall impression is that the use of the relationships in an iterative, deterministic, computer model of population dynamics is quite feasible. The relevant characteristics are summarized below:

1. Relationships between development or reproduction rates and temperature: Both were well described by straight lines. Consequently, both processes can be expressed on a physiological time scale and reasonably accurate estimates of development and reproduction rates are easy to calculate. The best estimates of theoretical thresholds for development and reproduction were so close that both processes could be assumed to have a common threshold of 3°C in both apterae and alatae. Examination of development and 'first 20 nymph' day-degree requirements showed that both development rate and reproduction rate/temperature relationships were quite stable over the range of conditions experienced in the experiment. There was no evidence that the relationships were affected by growth stage within the range 12, 20 to 19, 29, 30 (Zadoks' scale). But the reproduction rate, as assessed by 20 nymph day-degree requirement, was affected by both parent's morph and some factor associated with time of year. However, if mean reproduction rates ignoring these effects are used, the additional error would not be great relative to those inevitable in estimating other population processes.
2. The relationship between reproduction rate and age: Earlier studies have shown that at intermediate temperatures the reproduction rates of both apterae and alatae are relatively little affected by age during early adult life, so that in summer population models the assumption of a constant reproduction rate for all young adults is unlikely to lead to large errors (Carter, 1978). But under winter conditions the reproduction rates of young adults were markedly affected by adult age. Thus it would be worth defining age-specific reproduction rates in a winter population model, using a physiological time scale for age. However, the shapes of the winter reproductive patterns shown in the present study result in

mean reproduction rates being relatively insensitive to changes in mean adult age produced by continually acting non-age-specific mortality, and so all individuals in the adult population could be given the same mean reproduction rate without very large errors occurring. This would probably be necessary for adults in the population at the beginning of the period over which the simulation was being run.

3. **Total fecundity:** The present study revealed that fecundities of unacclimatized aphids might be reduced by the onset of cold weather in autumn. However, in apterae this effect only occurred early in the autumn, before populations on commercially grown winter cereals could have become established, and the recovery of normal fecundity was rapid. The effect may be significant for the immigrant alatae but their reproductive performance is unlikely to be known at all as their source is unknown. From November onwards very few aphids showed evidence of reduced fecundity due to low temperature stress. For both morphs, reproductive periods of clip-caged adults were usually so long that potential total fecundity is very unlikely to be achieved by an adult in a natural population.
4. **Survival and longevity:** Longevity of the clip-caged aphids was so great that in a natural population the huge majority of aphids must die from the effects of extrinsic mortality factors rather than old age. There was no evidence that S. avenae on winter wheat suffers mortality due to prolonged exposure to low temperature above the lower lethal zone determined from short-term laboratory and field experiments (see Chapters 2 and 3).
5. **Sexual morph production:** If the experimental clone is typical, production of sexual morphs would have ceased by the time a population was established on winter wheat sown after September.

It should be stressed that the characteristics given above and the relationships determined only apply to S. avenae on winter wheat. Results presented in Chapter 5 clearly show that their use for other species or other host plants is unjustified. The relationships could also change if temperatures fell much below -7°C (the minimum experienced at crop-level in the present study). Moreover, the changeable nature of reproductive characteristics means that application of the reproduction data from this study to populations much later in the spring might lead to significant errors. In particular, the algorithms for calculating reproduction and development times must incorporate the curvilinearity of the temperature-rate relationship at temperatures around and above the optimum if temperatures reach these levels for a significant proportion of the time. And the optimum may not be as high under field conditions as at constant temperatures (c. 22.5°C from Dean (1974b)). Possible differences between clones should also be borne in mind. However, if the relationships determined here are applied to S. avenae on winter wheat in conditions similar to the experimental ones, the errors produced are likely to be small relative to those which are inevitable in the measurement of mortality effects.

Lastly, though the relationships between development, reproduction and temperature determined in this experiment were expressed in day-degrees, it was realized that some workers wishing to use the data may be unable to measure day-degrees directly. For this reason, the accuracy of two methods for estimating day-degrees using daily maximum and minimum temperatures were tested using temperature data collected during the 1980/81 winter. The results of these tests are given in Appendix 4.6.

CHAPTER FIVE

CHAPTER FIVE

DEVELOPMENT, REPRODUCTION AND SURVIVAL OF S. AVENAE
AND R. PADI ON WINTER WHEAT AND PERENNIAL RYE GRASS
IN FIELD PLOTS5.1 INTRODUCTION

The winter sampling of Gramineae in Hampshire by Hand (1982) showed that viviparae of both S. avenae and R. padi overwinter on wheat, barley and grasses in southern England. Thus a complete study of anholocyclic overwintering by cereal aphids should, ideally, involve detailed investigations (similar to those described above for S. avenae on winter wheat) of both aphid species on several cereal and grass hosts. Insufficient time was available for this, but the development, reproduction and survival of single batches of S. avenae and R. padi on winter wheat and perennial rye grass (Lolium perenne L.) were monitored between November 1980 and March 1981 to assess the magnitude of between-species and between-host differences.

Perennial rye grass was chosen as the grass host because it is the most widely sown herbage grass, both in the west Hampshire study area (Hand, 1982) and in Britain as a whole (Hubbard, 1968). Some information on the development and reproduction of S. avenae and R. padi on cereals other than wheat and on perennial rye grass and other grasses is available (eg. Coon, 1959; Villaneuva & Strong, 1964; Rautapää, 1970; Markkula & Roukka, 1972; Dean, 1973; Watt, 1979b; Leather, 1980b). However, all these studies were undertaken at intermediate or high temperatures.

5.2 MATERIALS AND METHODS

Clones of S. avenae and R. padi were derived from single viviparae collected from winter wheat in west Hampshire in July 1980. Cultures were kept in the field continuously from early August until the beginning of the experiment in November 1980, caged on field plots of winter

wheat seedlings (cv. Hobbit). The experiment involved four batches of aphids, one for each aphid/host combination (S. avenae on wheat, S. avenae on rye grass, R. padi on wheat and R. padi on rye grass).

All the aphids were born between 24 and 29 November. Their survival, development and reproduction were monitored until 29 March 1981, by which time all R. padi and all but 22 S. avenae had died.

The parents of the monitored aphids were taken from the stock cultures as alatiiform fourth instar nymphs or newly moulted adult alatae on 23 November. They were transferred to clip-cages on field plots of winter wheat (cv. Hobbit) sown on 25 September, or perennial rye grass (cv. S23) sown on 21 August, and checked daily for the next 6 days. The nymphs they produced each day were placed, two to a clip-cage, on the second unrolled leaf on main stems of the appropriate host.

Only two of the 40 R. padi surviving to adulthood were alatae. Because of this only the apterae of both species were monitored through adult life, the alatae being discarded as newly moulted adults. The total numbers of nymphs (apterous and alatiiform) present in each treatment initially and the numbers of apterae reaching adulthood are given in Table 5.1. Average plant densities were: winter wheat: c. 300/m², perennial rye grass: c. 4,000/m². Average growth stages (Zadoks' scale) in early December were: winter wheat: 15, 22, perennial rye grass: 14, 21.

As first to third instars, nymphs were checked at 3- to 12-day intervals and the number surviving in each cage was recorded. When they reached the fourth instar they were checked at 3-day intervals. The few alatae were discarded as newly moulted adults, while newly moulted adult apterae were moved to new leaves (the second unfolded one of a main stem), caged singly and examined at 3-day intervals. Adults were also moved to new leaves if those they were on showed signs of damage or chlorosis. On each recording date the number of nymphs produced by each adult was noted and the nymphs were then removed. A Grant Temperature Recorder with thermistor probes placed

Table 5.1 Numbers of nymphs initially present and numbers of apterae* reaching adulthood in each treatment.

Treatment	nymphs	adult apterae
<u>S. avenae</u> on winter wheat	41	20
<u>S. avenae</u> on perennial rye grass	45	15**
<u>R. padi</u> on winter wheat	63	36
<u>R. padi</u> on perennial rye grass	52	2

* alatae were discarded as newly moulted adults.

** one lost soon after last moult.

inside clip-cages was used to record temperatures at hourly intervals. Other details of monitoring, clip-cage design and temperature recording are as given in Chapter 4. Details of temperature conditions are given in Appendix 4.5.

5.3 RESULTS

5.3.1 Mortality

As with many of the other batches of S. avenae reared on winter wheat through the 1980-81 winter (see Chapter 4) those in this experiment showed no nymphal mortality (Fig. 5.1). Adult apterae were still producing nymphs when monitoring stopped on 29 March and only 10% of adults had died by this date - low mortality before the end of reproductive life was expected from the more detailed investigation of S. avenae on wheat described previously (Chapter 4).

The survival of S. avenae on perennial rye grass was markedly different to that on wheat. On the former host the species suffered steady mortality throughout nymphal and adult life (Fig. 5.2) and over 70% of the apterae reaching adulthood were dead before the end of March. In laboratory experiment 2.4.6, S. avenae reared on perennial rye grass showed slightly lower cold-hardiness than those reared on winter wheat, even when the aphids were tested after removal from their host plants. However, the differences in mortality observed in the off-plant laboratory tests were much smaller than those revealed in the present field experiment. In the laboratory tests, the mortality of acclimatized S. avenae reared on perennial rye grass was only 20% after exposure to -10°C for 90 min. Reasons for discrepancies between laboratory and field experiments are discussed below.

To investigate the relationship between the mortality of S. avenae on perennial rye grass and low temperature in the field Wilcoxon's rank correlation coefficients were determined between the percentage of S. avenae dying in periods between successive recording dates and three low temperature parameters. The parameters used were:

Fig. 5.1 Survival of S. avenae on winter wheat.

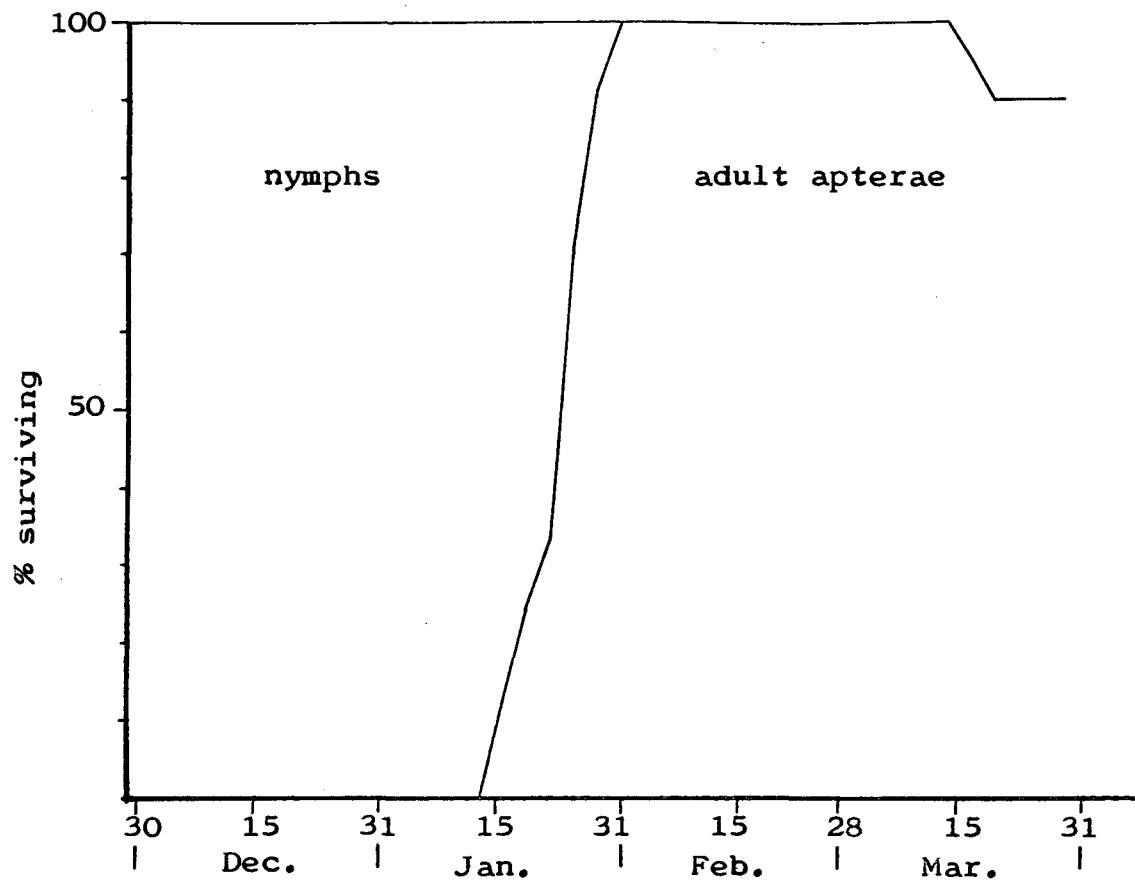


Fig. 5.2 Survival of S. avenae on perennial rye grass.

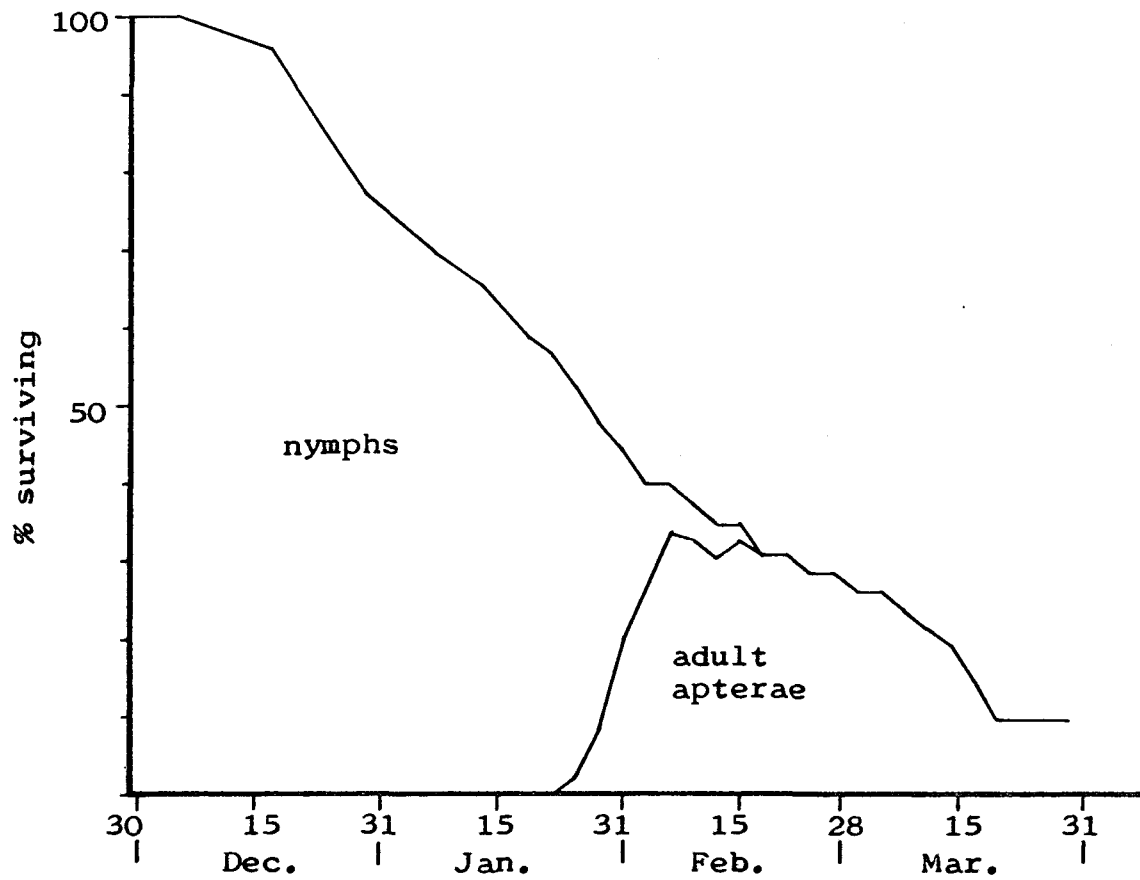


Fig. 5.3 Survival of R. padi on winter wheat.

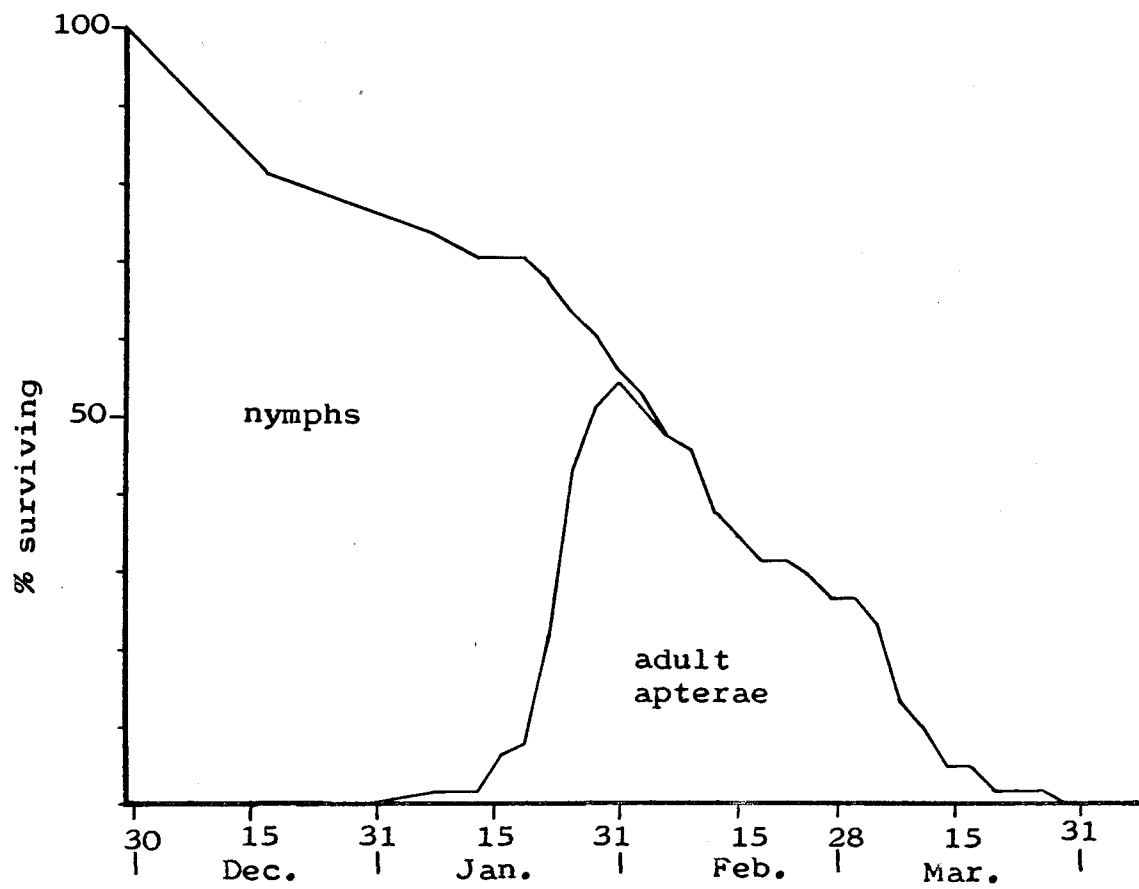
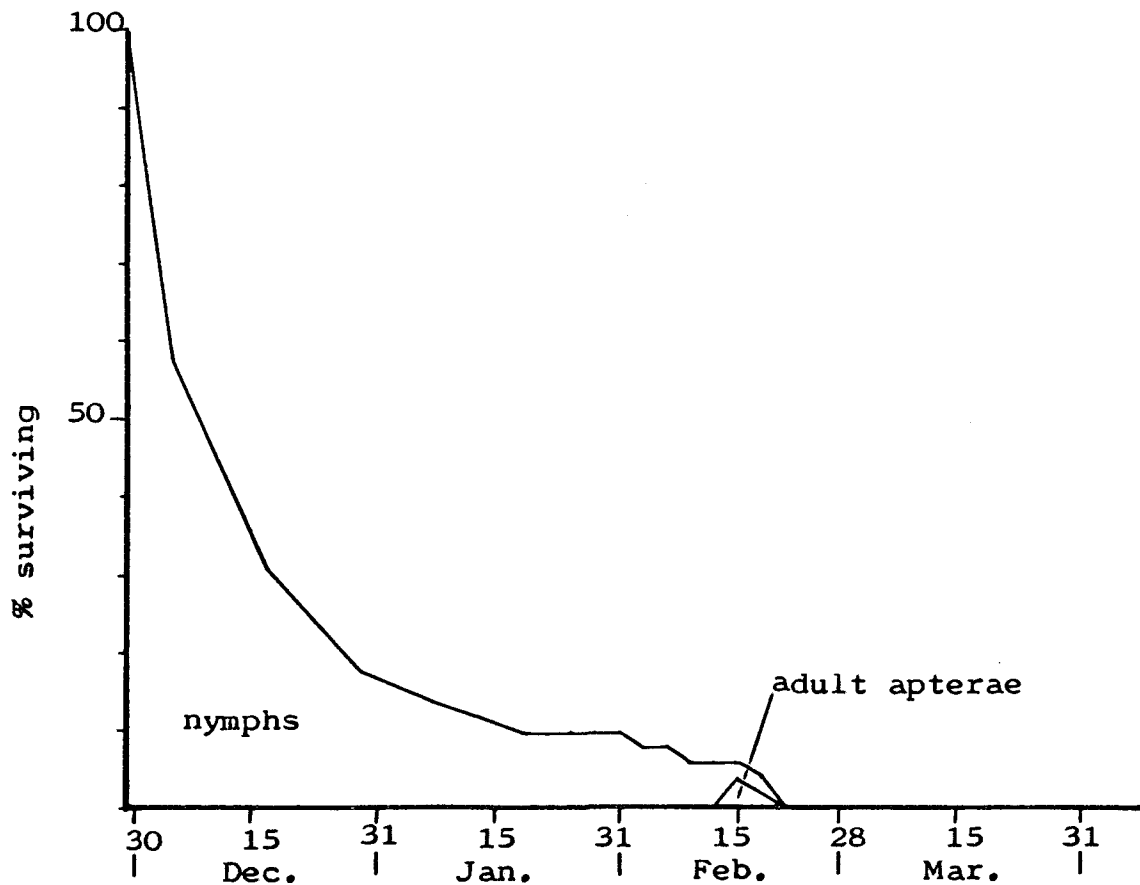


Fig. 5.4 Survival of R. padi on perennial rye grass.



minimum temperature, hour-degrees below 0°C , and hour-degrees below -5°C . Periods in which less than 20 aphids were alive initially were ignored. None of the correlation coefficients were significant and there was no sign that mortality was higher in colder periods (Table 5.2, Fig. 5.5). Indeed, there was a tendency for more aphids to die in the warmer periods. Also mortality continued even in periods when the temperature did not fall below 2°C .

R. padi on winter wheat suffered much greater mortality than S. avenae on the same host and all adult apterae were dead by the end of March. As with S. avenae on perennial rye grass, the mortality occurred steadily through nymphal and adult life (Fig. 5.3). However, Wilcoxon's rank correlations between percentage mortality in each period and the same three low temperature parameters used in the analysis of mortality of S. avenae on perennial rye grass showed that, for R. padi on wheat, there was a significant tendency for higher mortality to occur in colder periods (Table 5.2, Fig. 5.6). Even so, the relationship between mortality and low temperature was not as expected from earlier laboratory experiments (Chapter 2, sections 2.4.2 & 2.4.3) and fieldwork (Chapter 3), and was not as clear cut. The discrepancies are discussed below.

As with S. avenae, the survival of R. padi was much less on perennial rye grass than on winter wheat (Fig. 5.4). Only two of 52 aphids survived to maturity and all were dead by the last week in February. The observed mortality, like that of R. padi on wheat, was likely to have been related to low temperatures, but correlations between mortality and low temperature could not be calculated because the aphids were depleted so rapidly. The mortality rate was very high early on but declined subsequently. This pattern was not due to milder weather during January and February. It was probably due to the initially heavy mortality leaving only the most hardy aphids alive.

The very different mortality rates of the four treatments produced marked between-aphid species and

Table 5.2 Wilcoxon's rank correlation coefficients
between percentage mortality and three
low temperature parameters.

Aphid and host plant	Low temperature parameters		
	minimum temp.(°C)	hour-degrees < 0°C	hour-degrees < -5°C
<u>S. avenae</u> on winter wheat (n = 11) †	0.35	-0.11	-0.46
<u>R. padi</u> on rye grass (n = 18) †	-0.40*	0.34*	0.62***

*: $P < 0.05$ ***: $P < 0.001$

† periods with less than 20 aphids alive initially
have been ignored.

Table 5.3 Percentage survival to adulthood by apterous
and alatiform nymphs.

Aphid species	Host plant	
	wheat	rye grass
<u>S. avenae</u>	100.0 (41/41)	40.0 (18/45)
<u>R. padi</u>	60.3 (38/63)	3.9 (2/52)

Fig. 5.5 Relationship between percentage mortality of S. avenae on perennial rye grass and minimum temperature.

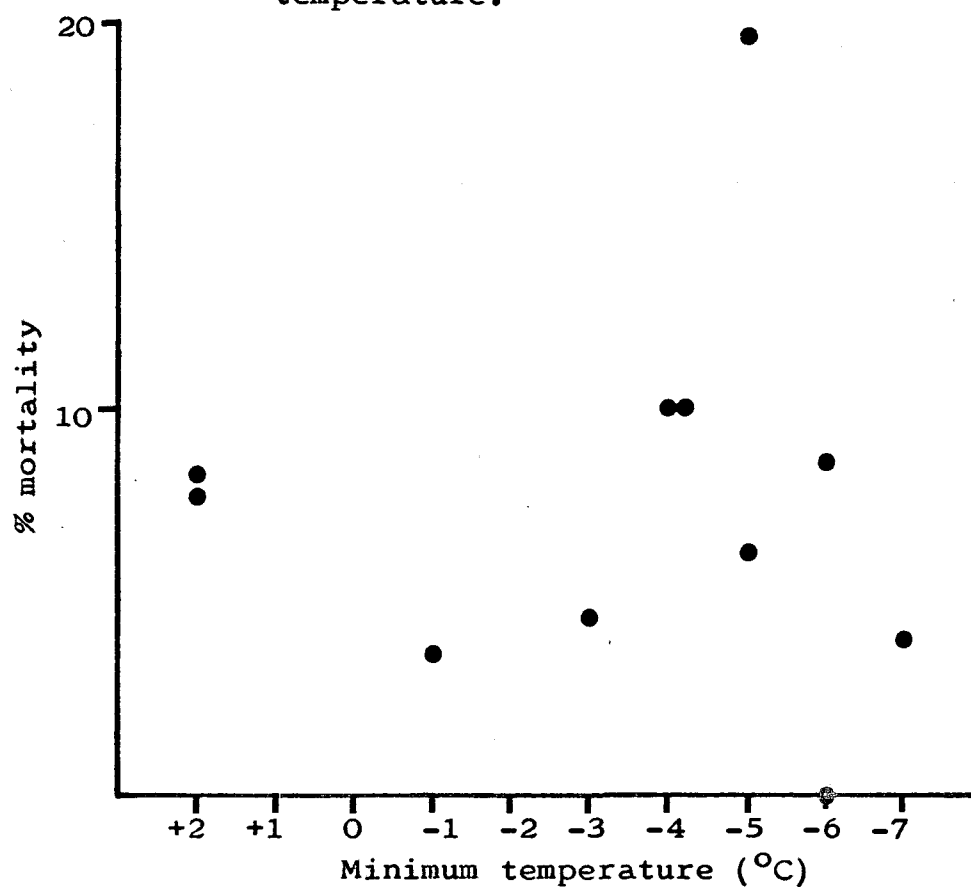
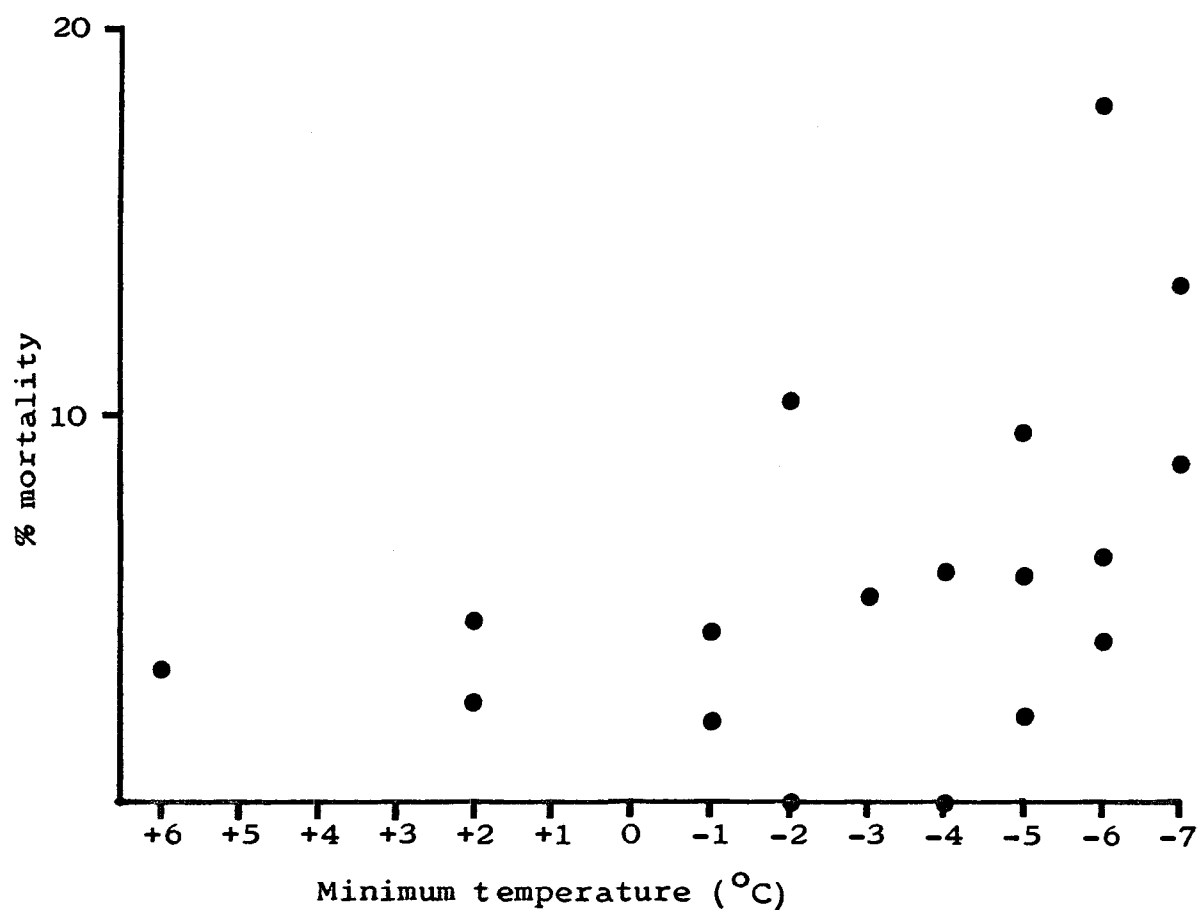


Fig. 5.6 Relationship between percentage mortality of R. padi on winter wheat and minimum temperature.



between-host species differences in the proportion of aphids reaching adulthood (Table 5.3), all of which were significant (comparison of percentages based on two large samples (Bailey, 1981, p.186): $P < 0.05$ in all cases).

5.3.2 Development time and adult longevity

Development times were measured on a physiological time scale (day-degrees) because temperatures fluctuated and aphids were born over a 6-day period and they matured on different days. In measuring day-degrees, the threshold temperature for development was assumed to be the same for both aphid species on both hosts, so that direct comparisons could be made. The threshold temperature used was 3°C , as this had been found to be the best threshold to use for S. avenae on winter wheat (see Chapter 4). Adult longevity was also measured in day-degrees above 3°C .

Both aphid species took longer to develop on perennial rye grass than on winter wheat and on both hosts R. padi took longer to develop than S. avenae (Table 5.4). Mann-Whitney U tests on these differences showed that all were significant ($P < 0.05$) (Table 5.5). However, differences in development time were relatively small compared with those in reproduction and survival. Mean adult longevities reflect the different mortality patterns described above. Both aphid species showed greater adult longevity on winter wheat than on perennial rye grass and on both hosts S. avenae had greater longevity than R. padi (Table 5.4). These differences between treatments were underestimates because 18 S. avenae on wheat and four on rye grass were still alive when monitoring stopped. Despite this, Mann-Whitney U tests showed that all differences were significant ($P < 0.05$) (Table 5.5).

5.3.3 Reproduction

Whether reproduction was measured as total fecundity or as mean reproductive rate through adult life, both aphid species showed greater reproduction on wheat than on

Table 5.4 Development and reproduction of S. avenae and R. padi apterae on winter wheat and perennial rye grass. $\bar{X} \pm \text{S.E. (n)}$

	<u>S. avenae</u> on wheat	<u>R. padi</u> on wheat	<u>S. avenae</u> on rye grass	<u>R. padi</u> on rye grass
Birth to adult (day ^{°C} > 3)	146.9 $\pm$ 1.91 (20)	158.7 $\pm$ 2.43 (36)	188.8 $\pm$ 4.67 (15)	226.2 $\pm$ 0 (2)
Adult life (day ^{°C} > 3)	268.4 $\pm$ 5.91 (20)*	98.5 $\pm$ 8.32 (36)	138.1 $\pm$ 20.88 (14)*	6.5 $\pm$ 1.81 (2)
Mean nymphs/adult	59.4 $\pm$ 3.49 (20)*	8.6 $\pm$ 0.61 (36)	13.3 $\pm$ 2.30 (14)*	0 (2)
R ₀ (survival to adult x mean fecundity)	59.4 (20)*	5.19 (36)	5.32 (14)*	0 (2)
Mean reproduction rate (nymphs/day ^{°C} > 3)	.221 $\pm$.012 (20)	.098 $\pm$.0053 (36)	.101 $\pm$.0076 (14)	0 (2)

* = underestimates as 18 S. avenae on wheat and 4 on grass were still alive when monitoring stopped.

Table 5.5 Mann-Whitney U tests comparing the performance of S. avenae (S.a.) and R. padi (R.p.) on winter wheat (w.w.) and perennial rye grass (p.r.g.)

Performance parameter and comparison	n ₁	n ₂	U	P
DAY DEGREES > 3°C TO MATURITY				
S.a. on w.w. <u>v</u> S.a. on p.r.g.	15	20	5	<0.001
R.p. on w.w. <u>v</u> R.p. on p.r.g.	2	36	0	<0.05
S.a. on w.w. <u>v</u> R.p. on w.w.	20	36	185	<0.01
S.a. on p.r.g. <u>v</u> R.p. on p.r.g.	2	15	1	<0.05
DAY DEGREES > 3°C OF ADULT LIFE				
S.a. on w.w. <u>v</u> S.a. on p.r.g.	14	20	7	<0.001
R.p. on w.w. <u>v</u> R.p. on p.r.g.	2	36	0	<0.01
S.a. on w.w. <u>v</u> R.p. on w.w.	20	36	6	<0.001
S.a. on p.r.g. <u>v</u> R.p. on p.r.g.	2	14	0	<0.05
TOTAL FECUNDITY				
S.a. on w.w. <u>v</u> S.a. on p.r.g.	15	20	11	<0.001
R.p. on w.w. <u>v</u> R.p. on p.r.g.	2	36	0	<0.05
S.a. on w.w. <u>v</u> R.p. on w.w.	20	36	28	<0.001
S.a. on p.r.g. <u>v</u> R.p. on p.r.g.	2	15	0	<0.05
MEAN REPRODUCTION RATE (nymphs/day-degrees > 3°C)				
S.a. on w.w. <u>v</u> S.a. on p.r.g.	15	20	15	<0.001
R.p. on w.w. <u>v</u> R.p. on w.w.	2	36	0	<0.01
S.a. on w.w. <u>v</u> R.p. on w.w.	20	36	36	<0.001
S.a. on p.r.g. <u>v</u> R.p. on p.r.g.	2	15	0	<0.05

rye grass and on both hosts S. avenae showed greater reproduction than R. padi (Table 5.4). Mann-Whitney U tests showed that all the differences were significant ($P < 0.05$) (Table 5.5). The net reproductive rate (R_0) (= proportion of aphids surviving to adult $\times$ mean number of nymphs produced per adult) is the potential population growth between one generation and the next. Because treatments showing high nymphal mortality also showed low reproduction per adult, the differences between R_0 for each treatment were even more pronounced than differences between survival or reproduction parameters considered separately.

5.3.4 Plant growth rates

Plant growth rates were not monitored in detail, but, for all aphids surviving to maturity, the number of new leaves emerging on the host plant's main stem between the aphid's birth and final moult was recorded. The mean numbers of leaves emerging in this period were: winter wheat: 2.5, perennial rye grass: 1.1, showing that the wheat was growing much faster than the rye grass.

5.4 DISCUSSION

5.4.1 Limitations of the experimental design

Before discussing the results of this experiment, some limitations of its design should be pointed out. These were tolerated because the experiment was seen as a preliminary assessment of between-aphid species and between-host differences, not as an in-depth study. Nor was the experiment specifically designed to investigate temperature/mortality relationships.

The most important limitation was that though aphids monitored on perennial rye grass were born on that host, their parents had been reared on winter wheat. Switching the parents of experimental animals to new host varieties or species as young adults is common practice when assessing host resistance to cereal aphids (eg. Rautapää, 1970;

Markkula & Roukka, 1972; Lowe, 1978, 1980). However, this is not ideal, as physiological adjustment to a new host may take more than one generation. Evidence for this comes from experiments with M. dirhodum (Kay, Wratten & Stokes, 1981) which revealed that when aphids were reared experimentally on the same species of plant as that on which their parents were cultured (wheat seedlings) the weight and embryo complement achieved were greater than those of animals whose parents were cultured on barley. Thus, in the present experiment, there was a possibility that the poor survival and reproduction of both aphid species on rye grass may have been partially due to the fact that they had been given insufficient time to adapt to the new host after several generations on wheat. It is unlikely that this was more than a modifying influence on performance but results from perennial rye grass presented here should be treated with caution and there is a need for further work in which aphids are kept on rye grass for several generations. This need is confirmed if the results of this experiment are compared with those of Hand's field sampling of grass leys, most of which were predominantly perennial rye grass (Hand, 1982). The field sampling showed that R. padi could persist through the winter on grassland except in particularly harsh winters, whereas, from the very poor survival and reproduction of R. padi on perennial rye grass in this experiment, one would predict that the species would soon die out even in relatively mild winters.

A second limitation of the present experiment was that, because aphids were clip-caged, their choice of feeding sites was artificially restricted. Winter observations on both aphid species on winter wheat in the whole-plant shelter cages used for stock cultures revealed that, when sheltered, most aphids fed on the leaves (although when plants were unsheltered, as in the mortality experiment described in Chapter 3, most R. padi fed in more sheltered sites near the ground on the stems). On perennial rye grass, however, the aphids, particularly R. padi, may prefer feeding on stems whether or not the plants are sheltered.

For two treatments, S. avenae on perennial rye grass and R. padi on winter wheat, discrepancies were evident between the mortality/temperature relationships determined here and those determined from earlier laboratory and fieldwork. To some extent these differences were due to differences in experimental conditions. Conditions specific to this field experiment are likely to have biased the mortality/temperature relationships shown in two important ways:

1. The results were obtained from a single batch of aphids subjected to a series of low temperature exposures one after the other. Under these conditions the average cold-hardiness of the batch must have changed with time because the less cold-hardy aphids would be eliminated first. This effect was most clear cut for R. padi on rye grass but must have affected other treatments also.
2. In laboratory experiments some aphids died as a result of low temperature exposure over 24 h after testing. Thus in this field experiment some aphids may have suffered lethal cold-injury in one period but may not have died until the next.

Both these factors would tend to blur and distort mortality/temperature relationships. In addition, some of the mortality on perennial rye grass may have been 'artificial' - due to the fact that aphids may not have been given long enough to adapt fully to the new host. Nevertheless, though the effects listed above make close agreement between mortality/temperature relationships determined here and those determined in earlier experiments unlikely, the size of the discrepancies observed suggest that other, less trivial, factors were also involved. These are discussed below.

5.4.2 Differences in development and reproduction

Differences between treatments observed here under

winter field conditions are at variance with results of work at intermediate and high temperatures in several respects.

On wheat the performance of R. padi was markedly worse than that of S. avenae - development time was longer and reproduction much lower (Table 5.4). But laboratory and field experiments at summer temperatures show that R. padi on wheat and other cereals develops and reproduces faster than S. avenae and has similar or higher total nymph production (Dean, 1973; Dean, 1974b). Thus temperature appears to strongly affect the performance of the two species relative to each other. S. avenae seems well adapted to low temperatures, while R. padi is not. The very high development and reproduction rates of R. padi at intermediate and high temperatures suggest that the species has a physiology adapted specifically for optimum performance under summer conditions. Perhaps as an inevitable consequence it has only a limited ability to adjust to winter conditions, which require enzyme systems capable of operating efficiently at lower temperatures and at much slower rates. Low temperature probably exerts its limiting effects on development and reproduction not only by affecting the aphids' physiology directly but, perhaps more importantly, by slowing the host plant's metabolism and growth; in particular, by reducing the rates of photosynthesis and translocation.

Apteræ from Finnish clones of S. avenae and R. padi showed similar fecundity on perennial rye grass (30 to 40 nymphs/adult) when kept at intermediate temperatures in a glasshouse (Markkula & Roukka, 1972). Leather (1980b) also found that nymph production by R. padi apteræ on perennial rye grass at 20°C varied around 30/adult but was modified by plant growth stage. But in the present experiment, the performance of both species on perennial rye grass, especially that of R. padi, was much poorer than expected from the above results. Thus it seems that low temperatures, or perhaps some other aspect of winter field conditions, had a greater detrimental effect when the aphids were reared on perennial rye grass rather than on winter wheat. This may well be due to the fact that

perennial rye grass was growing at only half the rate of winter wheat (as assessed by the rate of new leaf emergence). The much slower growth of the grass, and the lower rates of translocation associated with it, probably resulted in greater limitations on aphid development and reproduction. It is not surprising that the detrimental interaction between low temperature and host was shown most markedly by R. padi, which even on winter wheat (a relatively fast growing host) performed poorly under winter conditions. Thus, differences in the developmental and reproductive performance between all four treatments in this experiment probably resulted from differences in the two aphid species' ability to overcome physiological stress imposed by long exposure to low winter temperatures, which probably acted directly on the aphids themselves and also by influencing the host plant, in particular by limiting growth rate.

5.4.3 Mortality

The mortality of S. avenae on perennial rye grass and R. padi on both hosts was greater than that shown in experiments at intermediate and high temperatures (Dean, 1973, 1974b; Leather, 1980b). It was thus probably linked to low temperature. Moreover, there was a clear association between poor developmental and reproductive performance by a particular aphid species on a particular host and high mortality. This suggests that all three aspects of performance were causally linked, implying that at least some of the mortality observed was due to physiological stress resulting from rearing under low temperature conditions on a slow-growing host, rather than being the type of 'direct' low temperature mortality caused by short exposure to relatively extreme low temperatures, which was demonstrated in earlier laboratory and field tests (Chapters 2 and 3). This view is supported by the fact that the mortality of S. avenae on perennial rye grass was not correlated with exceptionally cold periods. Though more R. padi died in the colder periods, the relationship between mortality and temperature was

less clear than that revealed in the laboratory (Chapter 2, experiments 2.4.2 and 2.4.3) and some died even in the mildest periods. The occurrence of mortality due to continuous exposure to low temperatures in the field on a slow-growing host would account for discrepancies between laboratory and field results mentioned above, as this type of mortality could not show up in the short-duration laboratory and field tests described earlier (Chapters 2 and 3).

There is some circumstantial evidence in the literature for this type of 'chronic' low temperature mortality associated with prolonged exposure to cold rather than with short periods of extreme low temperature. Heavy mortality of fundatrices of the potato aphid Macrosiphum solanifolii and the buckthorn aphid Aphis abbreviata in Maine, U.S.A., was ascribed by Shands, Simpson & Wave (1958) to several weeks with exceptionally low mean temperatures but a minimum of only 25°F (-4°C). Powell & Parry (1976) recorded reductions of anholocyclic winter populations of Elatobium abietinum viviparae in Scotland after periods of two to three weeks during which temperatures never rose above 5 or 6°C but never fell below -7°C. They suggested that the mortality was due to starvation as the aphids must have spent considerable time in chill coma, unable to feed. Adams (1962) also suggested starvation as the cause of slow steady mortality of five species of aphids kept in the laboratory at 28°F (-2°C) for many weeks, though mortality observed under this artificially uniform temperature regime is difficult to relate to natural populations. Way & Banks (1968) suggested that mortality of fundatrices of Aphis fabae when temperatures remained low for long periods may have been due to dessication by dry wind in conditions too cold for the aphids to feed and thus compensate for water loss.

5.4.4 Seasonal changes in aphid performance and the role of the host plant

Results of this experiment demonstrated even more clearly than those of the last (Chapter 4) that the devel-

opment, reproduction and longevity of viviparae can undergo large seasonal changes, so that winter performance cannot be extrapolated from the results of experiments at higher temperatures. The poor performance of both species on perennial rye grass was probably exaggerated in this experiment because the parents of all the monitored aphids were reared on wheat and because seedlings were used, which are a relatively unfavourable growth stage of this host (Leather, 1980b). Nevertheless, it is clear that the aphids' performance on perennial rye grass was influenced much more severely by winter conditions than was their performance on wheat. This strongly suggests that seasonal changes in aphid performance are produced by season-related environmental factors (temperature in particular) which act not only on the aphids themselves but also on their host plants.

Though attention has been focussed recently on the importance of host plant growth stage to cereal aphid population dynamics (eg. Watt, 1979b; Leather, 1980b) the effect of seasonal changes in host plant physiology, not necessarily linked to differences in growth stage, have been very little studied. The results of this experiment suggest such changes may play a dominant role in autumn, winter and spring population dynamics. This view is supported by results of botanical studies which clearly imply that large seasonal changes in the nutritional status of grasses and cereals are commonplace. The rate of growth of grasses varies enormously through the year (Williams, 1980). The seasonal changes in growth rate, which must affect a plant's nutritional status, are largely controlled by temperature, light intensity and daylength (Alberda, 1966; Keatinge, Garrett & Stewart, 1980; Williams, 1980), which affect different grass species and cultivars differently (Langer, 1972; Keatinge *et al.*, 1980). As well as governing growth rates, temperature and light have more subtle physiological effects on Gramineae which are probably important to aphid nutrition. For example, they alter patterns of translocation (Friend, 1966; Langridge & McWilliam, 1967) and soluble carbo-

hydrate content (Alberda, 1966) and affect mineral uptake (Langridge & McWilliam, 1967).

5.4.5 Implications of the results for the modelling of anholocyclic winter populations

Results of this and earlier field experiments suggest that the effect of temperature on the winter population dynamics of S. avenae viviparae on winter wheat could be modelled reasonably accurately by combining information on development, reproduction and potential longevity (Chapter 4) with that on the relationship between low temperature mortality and minimum temperature (Chapter 3). However, results of this experiment strongly suggest that for S. avenae on perennial rye grass and probably for R. padi on both wheat and grass, a significant amount of 'chronic' low temperature mortality occurs due to long exposure to relatively low temperatures, rather than to occasional short exposures to extreme cold. This type of low temperature mortality complicates modelling because it is not well correlated with minimum temperatures. Before it can be built into a population model more work is needed to determine temperature parameters which can be used to predict it, and, as it is greatly affected by the host plant aphids are feeding on, such studies would need to be undertaken on a range of different hosts. It should also be pointed out that in exceptionally severe winters in England, or in regions with lower mean winter temperatures, S. avenae viviparae on winter wheat may suffer 'chronic' low temperature mortality, even though they did not show it in this experiment.

Though good winter field data on the development, reproduction and potential longevity of S. avenae on winter wheat are now available (Chapter 4) the present experiment demonstrated that winter development and reproduction varies greatly according to host and aphid species, and cannot be predicted from laboratory data obtained at higher temperatures. Several other Gramineae, in particular winter barley and the common herbage grasses,

may be of equal or greater importance than winter wheat as hosts to overwintering cereal aphid viviparae (Hand, 1982). Consequently, for a reasonably complete understanding of temperature effects on anholocyclic populations, more field data on several different hosts are required. In particular, parameters which can be used to predict the seasonal changes in aphid performance must be identified.

CHAPTER SIX

CHAPTER SIX

HOLOCYCLIC OVERWINTERING BY M. dirhodum ON ROSE6.1 GENERAL INTRODUCTION

Reasons for the choice of M. dirhodum for work on holocyclic overwintering are given in Chapter 1, section 1.4. The holocyclic life-cycle of the species is summarized in Fig. 6.1 and more detail is given in Chapter 1, section 1.3, and in the glossary of aphidological terms (Appendix 1.1).

Other aphid species whose holocyclic life-cycles have been studied in detail in the field, such as Aphis fabae (eg. Way & Banks, 1964, 1968) and R. padi (eg. Dixon, 1971 ; Leather, 1980b) are usually found at relatively high population density on rather uncommon primary hosts. By contrast, Rosa spp, the primary hosts of M. dirhodum, are abundant in southern England and the average population density on rose bushes is much lower. In the present study this low population density governed decisions on feasible objectives and methods to a large extent. As the direction of further analysis was often determined by hypotheses developed after initial examination of the data, some interpretation of the results has had to be included in 'results' rather than 'discussion' sections in this chapter.

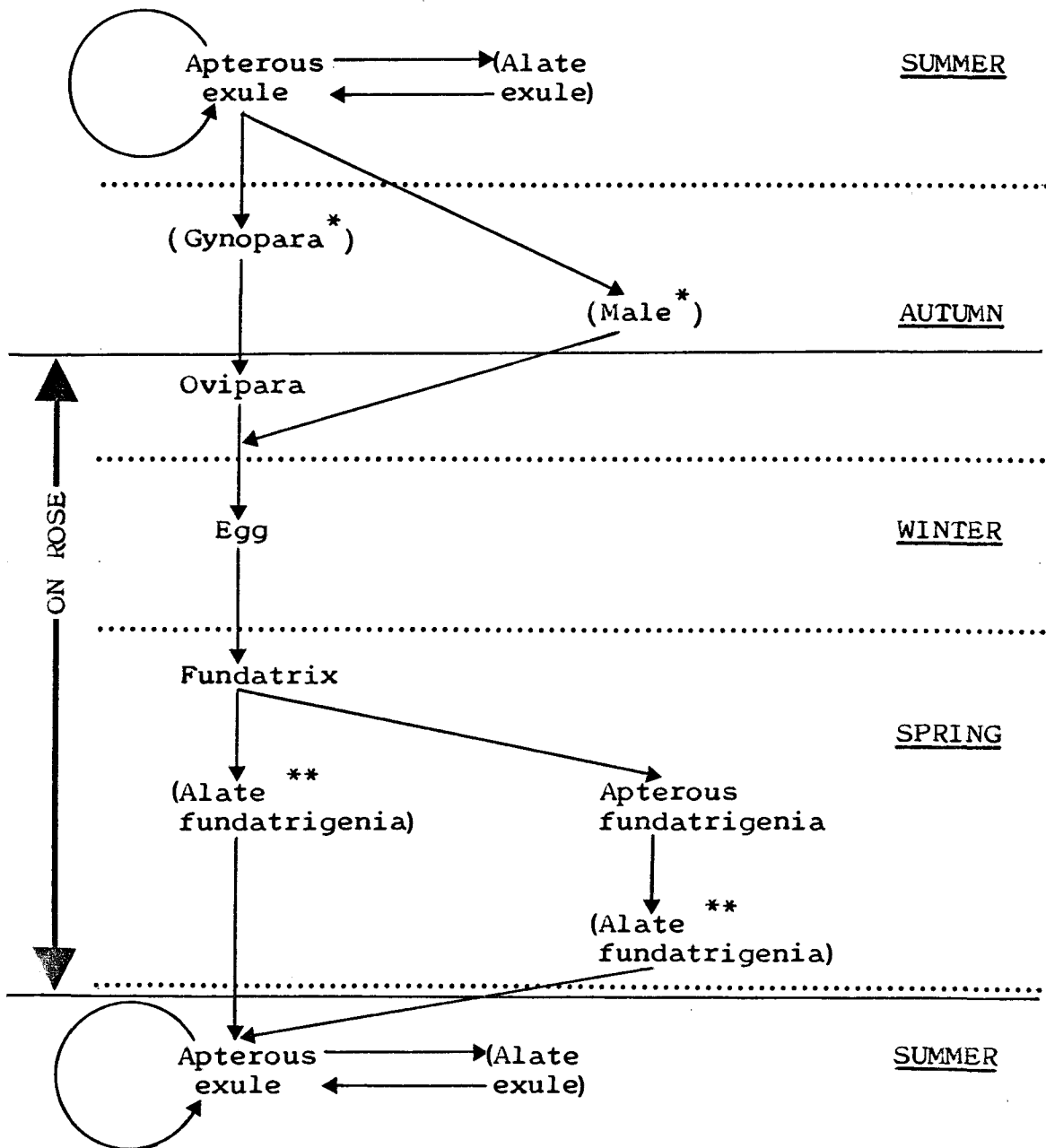
6.1.1 The work undertaken

The study, which was undertaken on a farm in west Hampshire, consisted of two main types of work:

1. Population surveys of M. dirhodum on wild rose in spring and autumn (section 6.2).
2. Monitoring of colonies on selected rose bushes (sections 6.4 and 6.5).

In addition, a small amount of laboratory work on oviparae was undertaken (section 6.3) to answer some questions

Fig. 6.1 The holocyclic life-cycle of M. dirhodum.
(winged morphs in brackets)



* = morphs undertaking autumn migration to rose.

** = morphs undertaking spring migration from rose.
The proportion of alatae in the first generation of fundatrigeniae is variable (Hille Ris Lambers, 1947).

relevant to the colony monitoring.

(a) The surveys

These were planned and carried out in collaboration with S.C. Hand. The main aims of the work were:

1. To determine what types of rose bushes and rose bush habitats were most favourable for colonization and/or survival by holocyclic M. dirhodum.
2. To obtain estimates of the numbers of M. dirhodum on rose in the different habitats and an overall estimate of the holocyclic population in the study area at the times of the surveys. By comparing these estimates with the results of Hand's extensive winter 'D-vac' sampling of Gramineae in the study area, the relative importance of holocyclic and anholocyclic overwintering in the years in question could be assessed.

In addition, some data on the timing of the holocyclic cycle and survival between autumn and spring were obtained.

Four surveys were undertaken, in autumn 1978 and spring 1979 and in autumn 1979 and spring 1980, timed to coincide with population peaks of oviparae (autumn) and fundatrigeniae (spring). Although the planning and field-work were carried out jointly, Hand undertook the data collation and the planning and execution of statistical analysis and results presentation, as well as detailed aphid identification work (see below). The surveys are dealt with thoroughly in Hand (1982) and the main points were presented in a joint paper (Hand & Williams, 1981). Because of this, the surveys are dealt with more briefly here than in Hand's thesis. Although organization and results presentation have been altered somewhat, the population survey section below (section 6.2) is closely based on Hand's presentation and little new analysis has been undertaken. But an attempt has been made to minimize overlap with Hand's thesis in the discussion of the results.

(b) Colony monitoring

This was undertaken with the following aims:

1. To determine the timing of the different stages of the holocyclic cycle. In particular, knowledge of the timing of spring migration from rose could be used with data on Rothamsted Insect Survey suction-trap catches to provide more information on the relative importance of holocyclic and anholocyclic winter populations as initiators of summer infestations.
2. To obtain information on levels of mortality at different stages in the holocyclic cycle and the influence of different mortality agents and other factors on colony success.

Monitoring was carried out in spring 1979 and from autumn 1979 to spring 1980. It is dealt with in two parts. The first (section 6.4) covers monitoring from the production of oviparae in autumn 1979 to the maturation of fundatrices in spring 1980. The second (section 6.5) covers the period from the maturation of fundatrices to the end of spring migration, for which data are available for 1980 and 1979.

6.1.2 The study area

The work was carried out on South Allenford Farm (Grid Ref. SU 090 175) at Damerham, near Fordingbridge, Hampshire (Fig. 6.2). This was a mixed arable-livestock farm of c. 800 ha on rolling chalk downland with c. 12.5 ha of woodland and c. 4 ha of open scrubland. About 250 ha of woodland adjoins the farm to the south but was not used in the study. The fields were large (mean c. 20 ha). Most were separated by hedgerows but some were bounded by wood edges or post and wire fences.

Wild rose was common in field boundaries (hedges and wood edges) and in the woodland and open scrubland. The hedges varied in height, width, cutting regime and species

Fig. 6.2 The study area.

(Only sites referred to specifically in the text are labelled.)

KEY:



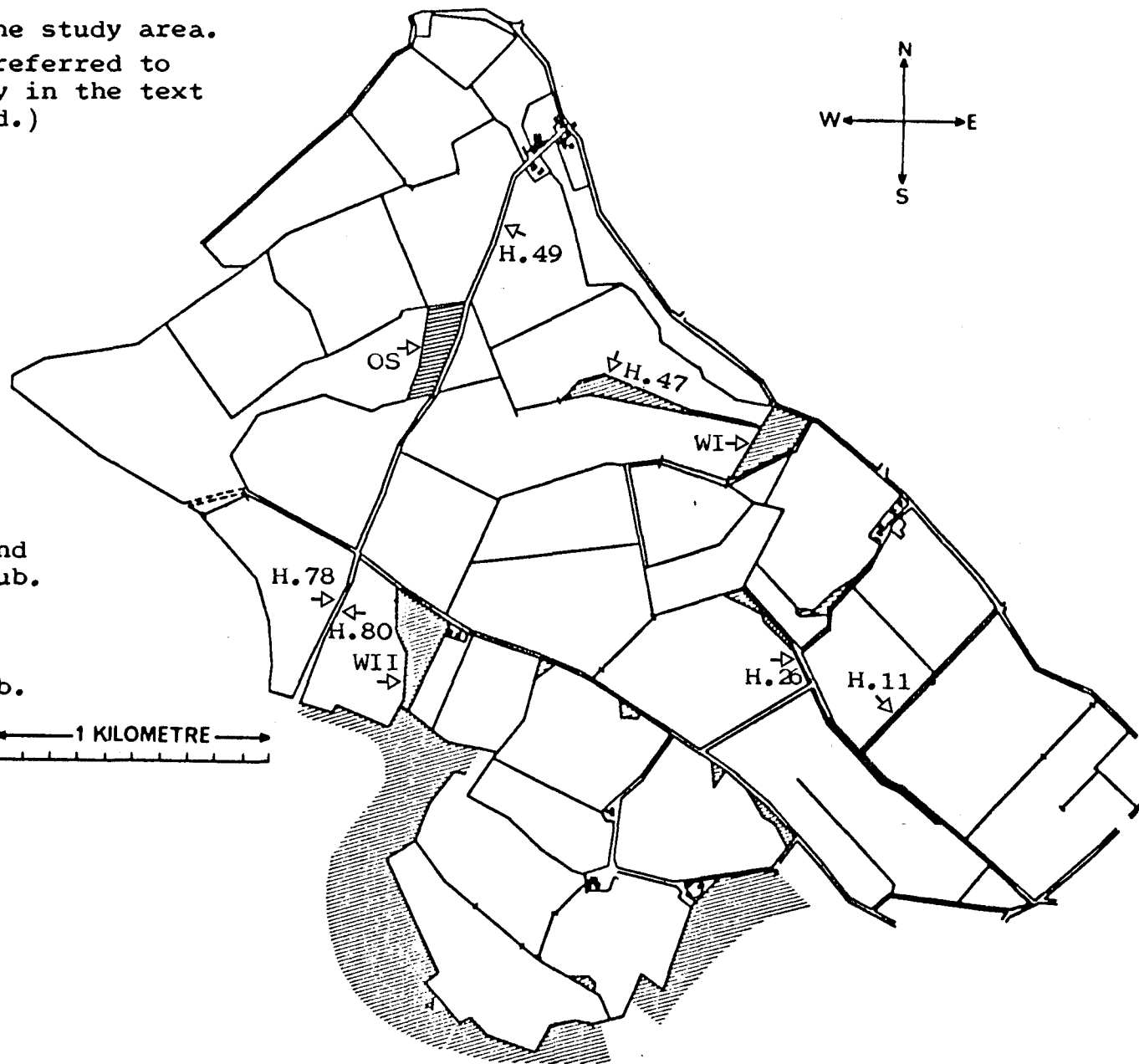
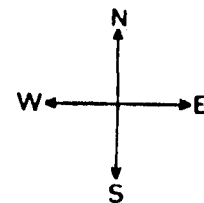
= woodland
or scrub.

H = hedge.

W = wood.

OS = open scrub.

← 1 KILOMETRE →



composition. Most were dominated by hawthorn (Crataegus monogyna Jacq.), blackthorn (Prunus spinosa L.) or hazel (Corylus avellana L.) but a wide variety of other species such as bramble (Rubus fruticosus agg.) and elder (Sambucus nigra L.) were also present. Dog rose (Rosa canina agg.) was the commonest rose species but some sweet briar (R. rubiginosa agg.) was present and one bush of field rose (R. arvensis Hudson) was found. The woodland was predominantly deciduous and contained a wide variety of trees and bushes, including some introduced species. Much of it was periodically thinned to encourage a dense shrub layer for pheasant breeding. The woodland could be divided into 'dense' and 'open' areas. Dense wood consisted of thickets of young trees with a dense shrub layer or areas with a more open shrub layer but with more mature trees forming a closed canopy. Open wood comprised grassy woodland clearings and recently-thinned areas with a sparse shrub layer and the trees well separated. R. canina and R. rubiginosa were common in both dense and open areas. The open scrubland consisted of small bushes of R. canina agg., R. rubiginosa agg., P. spinosa and C. monogyna scattered thinly over an area of uncultivated rank grassland on one of the farm's highest points.

Weather data were obtained from Hurn Airport (Grid Ref. SZ 118 978), Bournemouth, 20 km south of the study area.

6.1.3 The field seasons

The study covered holocyclic overwintering in the 1978/79 and 1979/80 winters, which had contrasting weather and were followed by very different summer populations of M. dirhodum. Monthly temperature and rainfall data for the relevant years are given in Table 6.1. Some further details of weather during the monitoring periods are given in sections 6.4 and 6.5.

October and November 1978 were particularly dry and a little warmer than average and December was also quite mild but very wet. January 1979, however, was very severe, the coldest since at least 1963 in most parts of

Table 6.1 Monthly mean temperatures and total rainfall for 1978/79
and 1979/80 recorded at Hurn Airport.

	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
<u>MEAN TEMP.</u> (°C)												
1978-79	13.8	11.85	8.75	5.85	0.45	2.05	5.25	8.25	9.7	13.85	16.75	14.95
1979-80	12.95	11.8	6.75	6.35	1.95	6.5	5.25	9.05	11.2	14.05	14.7	16.35
long term means*	14.2	11.2	7.4	5.3	4.2	4.4	6.3	8.9	11.5	14.7	16.4	16.2
<u>TOTAL RAIN</u> (mm)												
1978-79	20	3	19	201	98	67	94	56	114	35	32	48
1979-80	11	63	38	148	54	77	95	31	32	109	39	72
long term means*	75	85	95	85	85	55	56	49	58	47	49	67

* for 1941-1970.

Britain, with temperatures an average of 3°C below normal in the south of England and frequent snowfalls. At Hurn the extreme minimum recorded was -12.9°C and the extreme grass minimum -16.7°C. Most of February was also cold and snowy. The spring was cold and unsettled, with above average rainfall, particularly during May but the summer was relatively dry from June to August. M. dirhodum reached record abundance on cereals in summer 1979 with a four-weekly catch total by British Rothamsted Insect Survey suction traps of 355,048 for the period 15 July to 12 August.

The autumn of 1979 was wetter than that of 1978, particularly during October. As in 1978 there was heavy rain in December but January 1980, although colder than average, was considerably milder than January 1979 (extreme minimum at Hurn -6.8°C; extreme grass minimum -10.5°C). February 1980 was exceptionally mild, but was followed by a cold March. After this the spring was drier and warmer than in 1979 but June was exceptionally wet. In contrast to 1979, M. dirhodum was rare in summer 1980 with the peak four-weekly catch for British suction traps only 2,889.

6.1.4 Identification

In the field, the genus Metopolophium could usually be separated quite easily from other genera on rose using a hand-lens. The species identification of M. dirhodum, however, was complicated by the presence of a recently discovered species, M. fasciatum sp. nov. (Stroyan, 1982) which also alternates between Rosa and Gramineae.

M. fasciatum closely resembles M. dirhodum and the two cannot be separated without detailed microscopic examination of dead specimens. The relative abundance of the two species was estimated by S.C. Hand, who prepared slides of samples of the Metopolophium found on rose and used multivariate analysis of morphometric characteristics to separate the species, after type specimens had been identified by Stroyan (Hand, 1982).

Descriptions of the various holocyclic morphs of M. dirhodum were given by Hille Ris Lambers (1947).

These cannot be used to separate M. dirhodum from M. fasciatum but are useful for separating Metopolophium from other rose aphid genera. The nymphal fundatrix was not described by Hille Ris Lambers however. First and second instars are much darker than adults and similar to nymphal fundatrices of Macrosiphum rosae, being quite dark green with an olive tinge and slightly darker siphunculi and appendages. Distal flanges of the siphunculi and small areas round leg joints and towards the end of the antennae are blackish. Third instars are paler and easily distinguished from M. rosae. They are bright green (the colour of newly burst rose buds) and only slightly darker than young adults but they often have more extensive dusky areas on siphunculi and other appendages. Older nymphs and adult fundatrices have relatively short appendages and look unusually squat and 'bull-necked' for M. dirhodum. The mid-dorsal stripe, if shown, is much narrower and less distinct than in exules. Another point worth mentioning is that oviparae, although easily distinguished from other rose aphids as nymphs and young adults by their strikingly pale, yellowish-white colour, become darker and yellowish or yellow-orange with age.

6.2 SURVEYS OF THE HOLOCYCLIC POPULATIONS IN SPRING AND AUTUMN

6.2.1 Introduction

The four population surveys described below, which cover the period autumn 1978 to spring 1980, were preceded by an attempted survey of the egg population in the 1977/78 winter and a small-scale survey for fundatrigeniae in spring 1978. Neither of these were successful but they were important in developing ideas and obtaining a rough estimate of population density. The results of this preliminary work are given in Hand (1982). Egg surveys were abandoned due to the inconspicuousness and low population density of eggs. In addition, M. dirhodum eggs could not be distinguished from those of other rose aphids, even using a scanning electron microscope (Hand, 1982), so

that fundatrices had to be hatched and reared for identification. The spring 1978 survey was timed too late to give a good population estimate but was useful in refining sampling procedure.

6.2.2 Methods

The periods of the four surveys described were:

Autumn 1978: 23 October to 10 November.

Spring 1979: 27 April to 11 May.

Autumn 1979: 25 October to 11 November.

Spring 1980: 21 to 30 April.

They were timed to coincide as closely as possible with the peak populations of oviparae (autumn) and fundatrigeniae (spring) and so each involved a relatively short but intensive period of fieldwork. Data from monitoring of selected colonies (see sections 6.4 and 6.5) were used to ensure accurate timing.

In autumn 1978 population estimates were obtained for Metopolophium on field boundaries (hedges and wood edges) only. In the remaining three surveys dense and open woodland and open scrubland were also sampled. Different sampling methods and sample units were required for field boundaries and the other habitats because field boundaries were linear and the rose in them was often not separated into distinct bushes.

(a) Field boundaries

All the field boundaries on the farms were numbered before the autumn 1978 survey and those to be examined were selected using random number tables. Before a boundary was sampled a record was made of its orientation, average height and width, dominant plant species and whether it was cut back on top as well as at the sides. The adjacent crops were also noted. The basic sample unit used was a metre length of field boundary, because individual rose bushes in a hedge were often very difficult to distinguish. The sampled metre lengths were spaced at 15 paces (c. 15 m)

intervals and their positions marked with a dab of paint. The same sample spacing and the same field boundaries were used in subsequent surveys but the positions of sampled metres were shifted so that no metre was sampled more than once.

Any rose in a sampled metre was cut and examined in a sorting tray and the numbers and morphs of the Metopolophium found on each leaf and leaflet were recorded, as was the presence of other rose aphids. The amount of rose in each metre was estimated using four broad classes (0-100, 101-500, 501-1000 and >1000 leaves) and the height and the species of the rose was recorded. If, as occasionally happened, some rose in a sampled metre was inaccessible, the amount left unexamined was estimated and used to estimate the total Metopolophium in the metre. With practice, Metopolophium could be separated from other rose aphids in the field but aphids were brought back to the laboratory for identification when necessary and Hand undertook detailed morphometric analysis of a sample of the Metopolophium found in each survey, to estimate the proportion which were M. dirhodum (see section 6.1.4).

In the autumn 1979 survey the hedges sampled in autumn 1978 were re-examined using the same method but the number of rose leaves in each sampled metre was counted and data were taken on the proportion of senescent leaves, the height above the ground of the Metopolophium found and the side of the hedge they were on. Also, field boundaries with much rose were sub-sampled (only every second or third metre with rose was examined) to allow additional field boundaries to be included in the survey. In both spring surveys the same method was used but only boundaries having a high proportion of rose infested with Metopolophium the previous autumn were sampled. Buds (which usually consisted of several expanding leaves) were used instead of leaves as the units of foliage because one bud develops in the axil of each of the previous year's leaves. Buds were classed as unburst, burst or fully open and counted for each metre with rose. Sample sizes in each survey are summarized in Table 6.2.

Table 6.2 Sample sizes in surveys of Metopolophium populations on rose in field boundaries.

Sample size	Autumn 1978	Spring 1979	Autumn 1979	Spring 1980
field boundaries	16	9	20	6
metres*	414	299	539	220
metres with rose examined	104	48	103	35
rose leaves/buds examined	>44,000	9,082	21,663	10,759

*excluding bare fences.

Table 6.3 Numbers of bushes sampled and number of leaves or buds examined (in brackets) in surveys of Metopolophium populations on rose in woodland and scrubland.

Habitat	Autumn 1978	Spring 1979	Autumn 1979	Spring 1980
Dense wood	11 (760)	10 (1,590)	20 (2,256)	50 (3,508)
Open wood	-	2 (522)	31 (1,455)	37 (2,417)
Open scrub	-	6 (1,321)	15 (2,323)	-

(b) Woods and open scrubland*

In autumn 1978 an attempt was made to estimate the population of Metopolophium in wood I (see Fig. 6.2) using a transect method but this failed due to the difficulty of running a transect through dense thickets and due to the late sampling date. However, it revealed high numbers of Metopolophium in dense woodland. The wood was revisited on 22 November, 12 days after the end of the survey, and leaves on 11 bushes in dense areas were examined. This again revealed high numbers of aphids, and, although a good population density estimate could not be made due to the late sampling date, it was decided to concentrate more effort on woodland sampling in future surveys. In all these, individual rose bushes were used as the basic sampling unit. It should be noted that 'bushes' varied from large, much-branched bushes to small stems with only a few leaves.

Bushes to be sampled were selected by choosing a starting point near the centre of an area of woodland or scrub and working away from this point in several different directions, examining the first two bushes encountered in each direction. When a close group of small bushes was encountered all were examined. In spring 1979 only 12 bushes in wood I were sampled but in autumn 1979 and spring 1980 a larger number were examined in woods I and II (Table 6.3) and the balance between bushes sampled in dense and open woodland was improved by selecting centres in dense and open areas in each wood. A maximum of 250 leaves or buds were examined on each sampled bush and the amount of unsampled foliage on large bushes was assessed. The species and height of the bushes and details of surrounding vegetation were also recorded, as were the numbers and morphs of the Metopolophium found on each leaf or bud and their height above the ground. Some Metopolophium were taken back to the laboratory and were identified to species by Hand. Bushes in scrubland I (see Fig. 6.2) were sampled in the same way in spring 1979 but in autumn 1979 all the bushes not sampled in the spring were examined.

*see section 6.1.2 for descriptions of habitats.

To enable estimates of total Metopolophium populations in dense and open woodland to be made, Hand estimated the proportion of dense and open wood and the mean number of bushes per m^2 using large random quadrats (Hand, 1982). He found that dense and open areas each comprised about 50% of the total woodland and that rose bush density was very similar in both (mean = 0.0705 bushes/ m^2), although bushes in dense woodland were larger.

6.2.3 Results

(a) The species and morphs found

Hand's laboratory identification of samples collected during each survey revealed that the large majority of Metopolophium on rose were M. dirhodum and not M. fasciatum. From the samples, the estimated proportions of Metopolophium gynoparae which were not M. dirhodum were 35% and 28% (autumn 1978 and autumn 1979 respectively) and the proportions of oviparae were 28% and 27%. All the males and spring morphs identified to species were M. dirhodum. As the M. fasciatum represented only a small proportion of the total Metopolophium population its presence does not affect conclusions on the overwintering of M. dirhodum drawn from the survey results but it was taken into account in the calculation of total farm populations of M. dirhodum on rose. The other species found commonly on rose were, in order of decreasing abundance, Macrosiphum rosae (L.), Myzaphis rosarum (Kaltenbach) and Macrosiphum euphorbiae (Thomas). M. rosae was often as or more common than Metopolophium. Hand also identified a further 12 species from rose but all these were found only occasionally.

In spring the great majority of the Metopolophium found were alatiform, nymphal first-generation fundatrigeniae. Fundatrices and fundatrigeniae were usually found between unfolding leaflets and on and between stipules. During the autumn surveys the large majority of Metopolophium were nymphal oviparae. Oviparae, gynoparae and males were found on the abaxial surfaces of the leaves, including those almost ready to fall, but not on unfolding leaflets. Both gynoparae and fundatrices

(which produce oviparae and fundatrigeniae respectively) tend to give birth to several nymphs on the same leaf. Consequently, the Metopolophium found in spring and autumn showed a strongly aggregated distribution on leaves. Males were very closely associated with oviparae: of the 27 found, 15 (56%) were on the same leaves as oviparae despite the fact that oviparae were found only on 438 (0.9%) of the c. 51,000 leaves examined during autumn surveys. Nine males were found on leaves with adult oviparae only, three with nymphal oviparae only and three with both adult and nymphal oviparae. A comparison of this ratio with the relative abundance of leaves with adult oviparae, nymphal oviparae and both ages together showed that there was a significant tendency for males to be found with adult rather than nymphal oviparae ($\chi^2 = 15.04$, D.F. = 1, $P < 0.001$). This could simply have been due to the fact that adult oviparae had been alive longer than nymphs and had thus had more time to attract males, but may have been due to adults having a greater ability than nymphs to attract and/or arrest males. The latter seems likely in view of the fact that oviparae of a species of Schizaphis have been found to emit a chemical sex attractant but only during the 'active copulatory period' of their lives (Pettersson, 1970b).

Several unexpected morphs of Metopolophium were found during the surveys, as well as the expected gynoparae, oviparae and males (autumn) and fundatrigeniae and fundatrices (spring). In autumn 1978 three alatiform nymphs were found and Hand reared two of these to adults. One proved to be a normal alate vivipara and the other an alate ovipara. In spring 1980 a gynopara was found giving birth to oviparae and four others were found during colony monitoring (section 6.5). All these gynoparae had presumably been produced by viviparae persisting through the winter on Gramineae.

(b) Effect of habitat type on the distribution of Metopolophium

In autumn 1978 only one habitat, field boundaries, was sampled. In the three later surveys, in which wood -

land and open scrubland were included, significant differences were found between the proportions of leaves infested with Metopolophium in the different habitats (Table 6.4). In spring 1979 dense and open woodland were not sampled separately but 10 of the 12 woodland bushes sampled were in dense areas. Woodland rose had a significantly higher proportion of leaves infested than field boundary and scrubland rose ($P < 0.001$). No Metopolophium were found on scrubland rose but the spring population on field boundaries was too low for the difference between these two habitats to be statistically significant ($P > 0.05$).

In autumn 1979 open and dense woodland were separated and sample sizes were larger so that habitat differences showed up more clearly. Again dense woodland rose had a greater proportion of infested leaves than other habitats ($P < 0.001$). Rose in open woodland and field boundaries had similar levels of infestation but there was a significantly lower proportion of infested leaves on scrubland ($P < 0.05$). Populations in spring 1980 were much lower than in spring 1979 and scrubland rose was not sampled in this survey due to the low infestation found the previous autumn. Again dense woodland had a greater proportion of infested leaves than open woodland and field boundaries but the population level was too low for the difference to be significant ($P > 0.05$).

(c) Effects of field boundary characteristics on the distribution of Metopolophium

Two types of characteristics pertaining to individual field boundaries (rather than to field boundaries as a whole or to particular metre lengths with rose) were thought likely to affect Metopolophium distribution:

1. Height: high field boundaries might be expected to collect more gynoparae than low ones, whether or not migrants had much control over their flight direction. Survival and reproductive success might also be related to hedge height because of its influence on shelter.

Table 6.4 Percentage of rose leaves infested with
Metopolophium in different habitats.

Survey and habitat	% leaves/buds infested	leaves/buds examined	significant differences*
<u>SPRING 1979:</u>			
Wood (dense + open)	4.45	2,112	A
Field boundaries	0.25	9,082	b
Scrub	0	1,321	b
<u>AUTUMN 1979:</u>			
Dense wood	2.39	2,256	A
Open wood	0.41	1,455	b
Field boundaries	0.37	21,663	b
Scrub	0.09	2,323	c
<u>SPRING 1980:</u>			
Dense wood	0.17	3,508	A
Open wood	0	2,417	A
Field boundaries	0.065	10,759	A

*Within each survey, habitats with different letters differ at the 5% significance level and habitats with different letters of different cases differ at the 1% level. No comparisons were made between surveys.

2. Orientation: if, as the evidence suggests (Johnson, 1954) *alatae* are largely wind-delivered, field boundaries facing the predominant wind might be expected to collect more gynoparae than those running parallel to it.

When testing for differences in infestation levels between field boundaries of different heights or orientation, only autumn survey data could be used because in spring surveys boundaries were not selected at random. The proportions of metre lengths with rose which were infested with Metopolophium were compared, rather than the proportions of rose leaves which were infested, because the latter was more susceptible to bias due to differences in sampling date. If a boundary consisted of two or more parts with different heights or orientation, each part was treated as a separate hedge for the purposes of this analysis.

Height: field boundaries on the farm were clearly divisible into low (≤ 4 m) and high (> 4 m) types. Low boundaries were usually under 3 m high and were kept short by regular cutting back on top. High ones were usually well over 5 m and were only cut back regularly at the sides (often only on one side). High field boundaries comprised high hedges and all wood edges, both of which tended to have a greater diversity of woody plants than low hedges. Also, high hedges were usually wider than low ones. In autumn 1978 whole boundaries were classified as high or low but in autumn 1979 the hedge height in each sampled metre was recorded, as very occasionally metre lengths where the vegetation was less than 4 m tall were found in otherwise high hedges, and vice versa.

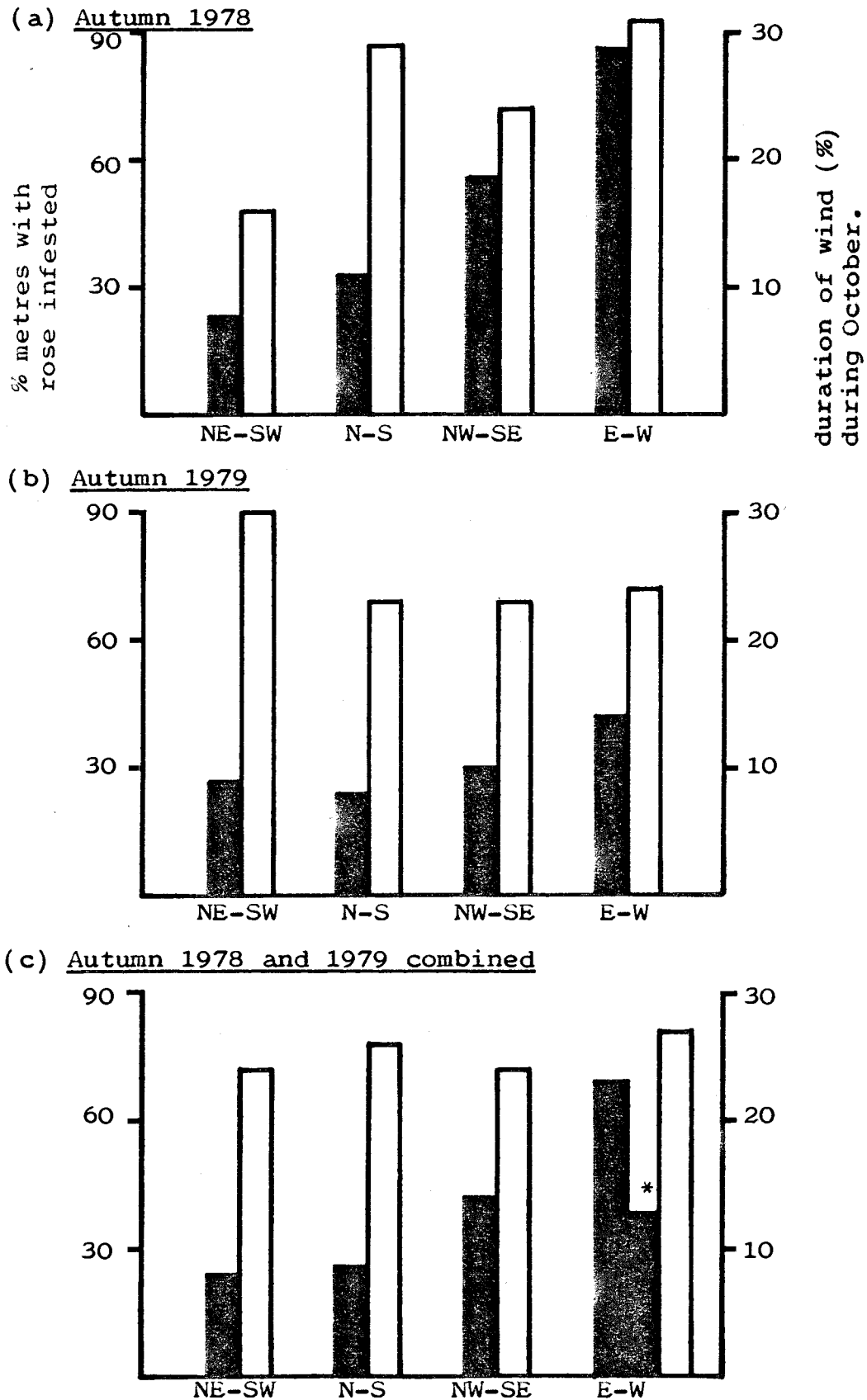
In both autumns the proportion of metre lengths with rose which were infested with Metopolophium was significantly greater in high field boundaries than in low ones (1978: $\chi^2 = 24.6$, D.F. = 1, $P < 0.001$; 1979: $\chi^2 = 8.7$, D.F. = 1, $P < 0.01$). However, high boundaries had significantly fewer metres with rose per unit length than low ones ($\chi^2 = 6.77$, D.F. = 1, $P < 0.01$). Because of this, one might expect a greater proportion of the rose in high

boundaries to have been colonized even if the same number of gynoparae arrived per unit length on high and low boundaries, and survival and reproductive success were similar on the two types of boundary. This possibility was investigated by comparing the proportion of all metre lengths sampled (including those with no rose) which were infested in the two boundary types. In autumn 1978 there was still a significantly higher level of infestation in high boundaries, even when metres with no rose were taken into account ($\chi^2 = 4.1$, D.F. = 1, $P < 0.05$) showing that either more gynoparae settled per unit length of high boundaries or that survival and/or reproductive success were better. The proportions of total metre lengths infested on the two boundary types were not significantly different in 1979 but this was probably due to the lower numbers of Metopolophium found. The comparisons above are all based on the proportion of metre lengths with rose which were infested. Infestation levels in autumn 1979 can also be compared using the proportion of all rose leaves infested. Using this criterion high boundaries still had a significantly greater level of infestation than low ones ($\chi^2 = 25.4$, D.F. = 1, $P < 0.001$).

High field boundaries were of two kinds: high hedges and wood edges. In autumn 1978 wood edges had a significantly lower proportion of metres with rose infested with Metopolophium than high hedges ($\chi^2 = 6.8$, D.F. = 1, $P < 0.01$) but this difference was due to a single hedge (hedge 49) with a large amount of rose and large numbers of Metopolophium. There was no significant difference between high hedges and wood edges in autumn 1979, and in both years wood edges had significantly higher levels of infestation than low hedges (1978: $\chi^2 = 5.2$, D.F. = 1, $P < 0.05$; 1979: $\chi^2 = 11.3$, D.F. = 1, $P < 0.001$).

Orientation: there was no significant difference in the proportion of high and low field boundaries facing in different directions ($\chi^2 = 5.12$, D.F. = 3, $P > 0.05$). But in neither autumn was there much sign that boundaries facing the commonest wind direction during the migration period were more heavily infested with Metopolophium (Fig. 6.3). In 1978, or when both years' data were combined,

Fig. 6.3 Relationship between proportions of metres with rose infested with *Metopolophium* (■), wind direction during autumn migration (□) and the direction field boundaries faced.



*excluding hedge 49.

there was the suggestion of a trend, in that the wind most often blew from the east or west and boundaries facing these directions had high infestation levels, but this result was due to a single high hedge (hedge 49) with much rose and large numbers of Metopolophium. If the two years' data were taken together, there was a significant tendency for boundaries facing different directions to have different proportions of metres with rose infested with Metopolophium ($\chi^2 = 27.3$, D.F. = 3, $P < 0.001$) but if hedge 49 was excluded differences between boundaries with different orientations were no longer significant ($\chi^2 = 5.6$, D.F. = 3, $P > 0.05$). However, the negative results of this analysis were by no means conclusive, as they were based on relatively few boundaries in an area of rolling downland where local topography must have played an important role in modifying wind patterns and may thus have masked the effect of boundary orientation.

(d) Effects of bush characteristics on the distribution of Metopolophium

Data on several characteristics pertaining to individual rose bushes (wood and scrub) or metre lengths with rose (field boundaries) were collected. Of these, the three thought most likely to affect the distribution of Metopolophium were:

1. Rose species.
2. Amount of rose foliage.
3. Rose height.

Reasons for selecting these factors are discussed below.

It was thought that physiological or structural differences between rose species may have affected how acceptable a bush was to gynoparae as well as their subsequent reproduction and survival and that of succeeding generations. It was also thought that taller bushes and/or those with more foliage might have collected a greater number of gynoparae per bush if migration was largely a passive process, simply because wind-blown aphids would be more likely to hit larger bushes. If, on the

other hand, gynoparae were actively selecting bushes from a distance, larger bushes might still be favoured because they provided larger visual or olfactory 'targets'. There might also have been nutritional differences between large and small bushes linked to bush age. It was realized, however, that separating the possible effects of bush height and amount of foliage would be very difficult as these two characteristics were inter-related (taller bushes tend to have more foliage) and because their effects were likely to be altered according to the height and amount of surrounding vegetation.

In the description below of the analysis of bush characteristic effects, metres with rose in field boundaries are referred to as 'bushes' for syntactical simplicity. Wherever possible bushes in different habitat types and in high and low hedges have been separated in the analysis. As with the analysis of field boundary characteristics above, only data from autumn surveys (when all habitats were sampled randomly and population levels were reasonably high) have been used.

Rose species: The autumn 1979 survey was the only one in which the number of sampled bushes of species other than R. canina was sufficient to make rose species comparisons worthwhile. Even in this survey only R. canina and R. rubiginosa were encountered often enough for a reasonable comparison to be made. Within each habitat the proportion of bushes of these two species which were infested with Metopolophium were similar (Table 6.5). In dense and open woodland, the two habitats in which infestation levels and numbers of R. rubiginosa sampled were sufficient for a significance test to be performed, no significant differences were found between the two species (Fisher exact probability test: $P > 0.1$ in both habitats). However, in dense wood the proportion of R. canina leaves which were infested was significantly greater than that for R. rubiginosa ($\chi^2 = 5.6$, D.F. = 1, $P < 0.05$). Only one bush of R. arvensis was found and this had Metopolophium. It should also be noted that several wild rose species which are common in other parts of Britain (such as R. villosa agg. and R. pimpinelli-

Table 6.5 Number of sampled bushes of different rose species which were infested with Metopolophium in autumn 1979.

	<u>R. canina</u>	<u>R. rubiginosa</u>	<u>R. arvensis</u>
<u>FIELD BOUNDARIES:</u>			
total bushes	100*	4*	1
infested bushes	31	0	1
<u>DENSE WOOD:**</u>			
total bushes	13	6	0
infested bushes	7	3	-
<u>OPEN WOOD:</u>			
total bushes	7	9	0
infested bushes	2	2	-
<u>SCRUBLAND:</u>			
total bushes	6	9	0
infested bushes	0	1	-

*two metres had both R. canina and R. rubiginosa.

**includes three bushes of R. rubiginosa not in the survey.

folia L.) and may well be important primary hosts of M. dirhodum were not found on the study area.

Amount of foliage: Unless gynoparae show a tendency to settle preferentially (or survive and reproduce better) on bushes with a particular amount of foliage, then, on average, one might expect the proportion of leaves infested on bushes with different amounts of foliage to be equal. Consequently, the probability of a bush being infested should be proportional to the amount of foliage on it. However, data from autumn surveys showed that this was not the case.

When bushes in field boundaries were divided into the four broad leaf-number categories used in the autumn 1978 survey (Table 6.6) it appeared that larger bushes were not infested as frequently as one would expect if the probability of infestation were proportional to leaf number. The same trend was shown in other habitats. However, comparing the proportion of bushes with different amounts of foliage which were infested was not strictly valid because large bushes were sub-sampled. To avoid this bias, the proportion of leaves which were infested on different bushes was compared for the autumn 1979 survey. (This was not possible for the autumn 1978 data because the numbers of leaves examined were not counted accurately). This analysis confirmed that the probability of a bush being infested was not strictly proportional to its leaf number, as in all three habitats in which sample sizes and infestation levels were high enough for comparisons to be made, significantly higher proportions of leaves were infested on bushes with relatively little foliage (Table 6.7). Possible reasons for this are discussed below (section 6.2.4).

Bush height: In autumn 1978 there was a significant difference between the heights of infested and uninfested bushes, with infested bushes in both high and low field boundaries being higher than uninfested ones (Mann-Whitney U tests: high boundaries: $U = 241$, $n_1 = 9$, $n_2 = 33$, $P < 0.001$; low boundaries: $U = 230$, $n_1 = 20$, $n_2 = 42$, $P < 0.001$). (Sample sizes and infestation levels were too

Table 6.6 Relationships between the presence of Metopolophium and the amount of rose foliage in metre lengths of field boundary.

Rose leaves present	Autumn 1978		Autumn 1979	
	% of metres infested	total metres examined	% of metres infested	total metres examined
1-100	33	21	17	46
101-500	50	32	35	48
501-1000	57	21	78	9
>1000	57	30		

Table 6.7 Percentage of rose leaves infested with
Metopolophium on bushes with different
amounts of foliage in autumn 1979.

(Bush size classes vary between habitats to allow χ^2
tests to be performed).

Habitat and amount of foliage	Percent of leaves infested	Results of χ^2 tests on differences between size classes
<u>HIGH HEDGES:</u> ≤100 leaves 101-500 leaves >500 leaves	 1.13 0.70 0.37	 $\chi^2 = 8.20$ D.F. = 2 $P < 0.05$
<u>LOW HEDGES:</u> ≤250 leaves >250 leaves	 0.39 0.04	 $\chi^2 = 12.25$ D.F. = 1 $P < 0.001$
<u>DENSE WOOD:</u> ≤100 leaves >100 leaves	 6.18 1.69	 $\chi^2 = 23.87$ D.F. = 1 $P < 0.001$

low for significance tests to be performed on the data for other habitats.) This relationship between infestation and bush height within a single habitat type could be partly explained by the fact that shorter bushes tended to have smaller amounts of foliage but the relationship was still significant even when bushes with less than one hundred leaves were ignored (Mann-Whitney U tests: high boundaries: $U = 10.5$, $n_1 = 3$, $n_2 = 29$, $P < 0.05$; low boundaries: $U = 196.5$, $n_1 = 19$, $n_2 = 32$, $P < 0.05$).

In autumn 1979 however, though there was a suggestion of the same trend in high field boundaries, this was not statistically significant. And in low hedges and dense woodland there was very little sign of any relationship (Mann-Whitney U tests: high boundaries: $U = 136.5$, $n_1 = 19$, $n_2 = 20$, $0.1 > P > 0.05$; low boundaries: $U = 254$, $n_1 = 13$, $n_2 = 51$, $P > 0.1$; dense wood: $U = 29$, $n_1 = 8$, $n_2 = 8$, $P > 0.1$). The results are thus inconclusive. Possibly this is because rose bush height was not the causative factor involved but was correlated with it in some field boundaries. Hedge height is an obvious candidate but unfortunately this was not recorded for each metre length with rose in 1978.

(e) The proportion of leaves infested at different heights above the ground

When all bushes in a habitat were considered together a tendency for foliage near the ground to have a greater proportion of leaves infested than that higher up was revealed (Table 6.8). The trend was not so clear for high field boundaries because an unexpectedly high proportion of leaves over 3 m above the ground were infested. However, this discrepancy was caused by data from a single hedge (hedge 47) running along the bottom of a steep, north-facing bank of scrub which may well have been exceptionally sheltered from prevailing winds. If this hedge was excluded the same pattern shown in other habitats was revealed for high field boundaries.

Bushes with little foliage tended to have a greater proportion of their leaves low down. This, together with the apparent tendency for low leaves to be more heavily

Table 6.8 The percentage of leaves infested with Metopolophium at different heights in autumn 1979.

Habitat and bush size	Height (sample size in brackets)			
	0-1m	1-2 m	2-3 m	>3 m
<u>Dense wood:</u>				
All bushes	6.67 (45)	4.06 (961)	.69 (720)	1.34 (522)
Small bushes (<100 leaves)	6.67 (45)	7.51 (253)	0 (58)	- (0)
Large bushes (>100 leaves)	- (0)	2.82 (708)	.76 (662)	1.34 (522)
<u>Low boundaries:</u>				
All bushes	.21 (2,349)	.18 (6,722)	.08 (2,557)	
Small bushes (<250 leaves)	.41 (1,230)	.38 (2,622)	.39 (256)	
Large bushes (>250 leaves)	0 (1,119)	.05 (4,100)	.04 (2,301)	
<u>High boundaries:</u>				
All bushes	.88 (1,599)	.65 (3,549)	.24 (3,361)	.79 (2,021)
<u>Excluding hedge 47:</u>				
All bushes	.74 (1,477)	.60 (3,325)	.20 (2,997)	.26 (1,143)
Small bushes (<500 leaves)	.95 (1,154)	.64 (1,711)	.35 (1,413)	.12 (851)
Large bushes (>500 leaves)	0 (323)	.56 (1,614)	.06 (1,584)	.68 (292)

infested, might have explained the fact that leaves on bushes with little foliage were relatively heavily infested. However, when leaves at each level were considered separately, bushes with little foliage still had a greater proportion of their leaves infested than large ones (Table 6.8). And, looking at just one size category of bush at a time, there was little sign of a relationship between foliage level and the proportion of leaves infested. Thus it seems that some factor other than differences in foliage height produced the heavier than expected infestation level on bushes with few leaves, and that the relationship between the proportion of leaves infested and leaf height was largely an effect of the difference between bushes with different amounts of foliage, rather than its cause.

(f) Population estimates

The total populations of Metopolophium in dense and open woodland in the study area (Table 6.9) were estimated for each survey period from the mean number of Metopolophium per bush (taking aphids missed due to sub-sampling into account), the mean density of bushes in woodland ($0.0705/\text{m}^2$) and the total area of each type of woodland (6.25 ha). Total populations of Metopolophium in field boundaries (Table 6.10) were estimated from the mean number of Metopolophium per metre of boundary (again taking aphids missed due to sub-sampling into account) and the total length of field boundary on the farm (51.2 km). The scrubland area was only sampled in 1979. In the spring no Metopolophium were found and in the autumn seven were found on c. 70% of the bushes, giving an estimated total population on scrubland of only 10 aphids.

Estimates of the total Metopolophium population on rose in the study area were calculated for the last three surveys, but not for autumn 1978 because no woodland estimate is available for this period. All the spring morphs of Metopolophium identified to species by Hand were M. dirhodum but in autumn 1979 some M. fasciatum were present. The total population of M. dirhodum at this time (Table 6.11) was estimated using the proportion of

Table 6.9 Estimates of the total farm population of Metopolophium on rose in dense and open woodland.

Period	Spring 1979		Autumn 1979		Spring 1980	
Wood type	Dense	Open	Dense	Open	Dense	Open
Bushes sampled	10	2	20	31	50	37
Bushes with aphids	5	0	8	4	2	0
Total aphids on sampled bushes*	496	0	206.5	24	30	0
Aphids/bush	49.6	0	10.33	0.774	0.600	0
Aphids/m ²	3.50	0	0.728	0.055	0.042	0
Estimated total population	218,600	0	45,500	3,400	2,600	0

*includes an estimate of those missed due to subsampling of larger bushes.

Table 6.10 Estimates of the total farm population of Metopolophium on rose in field boundaries.

Period:	Autumn 1978	Spring 1979	Autumn 1979	Spring 1980
Aphids found*	688	198 (216)	254 (319)	29 (36)
Mean number of aphids:				
(i) per m with rose	6.62	2.08	2.25	0.25
(ii) per m of boundary	1.38	0.433	0.504	0.057
Estimated total population	70,700	22,200	25,800	2,900

*numbers in brackets are estimates of numbers found if no subsampling had been done.

Table 6.11 Summary of total farm population estimates for Metopolophium and M. dirhodum.

Period	Autumn 1978	Spring 1979	Autumn 1979	Spring 1980
<u>Metopolophium</u> population:				
(i) in woods	(>140,000*)	218,600	48,900	2,600
(ii) in field boundaries	70,700	22,200	25,800	2,900
(iii) total	-	240,800	74,700	5,500
Total population of <u>M. dirhodum</u>		240,800	54,500**	5,500
Equivalent population density on Gramineae		1/33 m ²	1/147 m ²	1/1,455 m ²

*rough estimate from 11 bushes sampled on 20/11.

**estimated from the sample of Metopolophium oviparae identified to species.

Metopolophium which were M. dirhodum in the sample of oviparae identified to species (73%).

Population estimates clearly show that open woodland and open scrub held insignificant numbers of holocyclic Metopolophium but dense woodland and field boundaries were both important (Tables 6.9 and 6.10). In all but the last survey period woodland appears to have held a substantially greater proportion of the total population than field boundaries (Table 6.11). It should be remembered, however, that the numbers shown are only rough estimates which, particularly for woodland in spring 1979, are based on relatively few samples.

Survival between autumn and spring surveys could be compared very roughly for the two winters by comparing the estimated autumn and spring populations (Table 6.11). However, a comparison based on the proportion of sample units which were infested was better as it was more amenable to statistical analysis and less subject to bias due to small differences in sampling date. When only field boundaries sampled in both autumn and spring were considered, the proportion of metres with rose which were infested dropped between autumn 1978 and spring 1979 from 41 out of 60 examined to 6 out of 48 (a 'survival' of infested sample units of 18%). Between autumn 1979 and spring 1980 the corresponding drop was from 24 out of 25 to 2 out of 35. This gave a 'survival' of infested sample units of 6%, which suggests greater mortality through the second winter. Both the autumn to spring declines were highly significant ($\chi^2 = 7.5$, D.F. = 1, $P < 0.001$ in both cases). A similar survival estimate for dense woodland could not be made for the 1978/79 winter but between autumn 1979 and spring 1980 the number of infested sample-units (bushes) declined significantly ($\chi^2 = 14.8$, D.F. = 1, $P < 0.001$) from 8 out of 20 to 2 out of 50 (a survival of 10%). This suggested slightly better survival in dense woods than in field boundaries but the sample sizes are small and the reverse was suggested from the estimated populations in each habitat (Table 6.11). Colony monitoring (section 6.4) provides better data with which to compare survival in the two habitats.

Despite the huge summer population which developed

in 1979, the proportion of metres with rose in field boundaries which were infested with Metopolophium was significantly greater in autumn 1978 than in autumn 1979 ($\chi^2 = 7.6$, D.F. = 1, $P < 0.001$). From the population estimates for field boundaries (Table 6.11) there were nearly three times as many Metopolophium on rose in autumn 1978. This between-year difference was even more pronounced when the spring numbers were compared, probably due to greater mortality through the 1979-80 winter.

6.2.4 Discussion

(a) The relative importance of holocyclic and anholocyclic overwintering in different years

A very precise assessment of the relative importance of holocyclic and anholocyclic overwintering in the two winters under study is impossible because Gramineae cover nearly the entire study area. Consequently only very low population densities on the secondary hosts are required to give spring populations of anholocyclic M. dirhodum equal in size to the holocyclic populations estimated from the spring surveys. Even in spring 1979, when the holocyclic population was much larger than in 1980, a population density on Gramineae of less than one exule per 30 m² would have sufficed (Table 6.11). However, Hand (1982) sampled about 150 m² of Gramineae between January and April 1979 and found no overwintering exules, so it seems that aphids on rose represented at least a substantial proportion of the total species population in the study area which had survived the 1978/79 winter successfully, and they probably played an important role in initiating summer colonies on secondary hosts.

This view is strongly supported by two types of evidence. Firstly, M. dirhodum overwintering on Gramineae in the cold 1978/79 winter were likely to have suffered very heavy low temperature mortality as grass minimum temperatures even in southern England dropped as low as -16.7°C (see section 6.1.3), a temperature which, from laboratory and field experiments (see Chapters 2 and 3) is low enough to kill a large majority, if not all, over-

wintering exules. Secondly, the relative timing of the migration from monitored colonies on rose, the first appearance of M. dirhodum alatae in R.I.S.* suction-trap catches and the summer population build-up on Gramineae suggests very strongly that summer infestations were initiated mainly by migrants from rose (see section 6.5.4 for full discussion).

Although no M. dirhodum exules were found on Gramineae by Hand during the 1979-80 winter, the much smaller population on rose in the spring (Table 6.11) suggests that holocyclic overwintering was much less important than in the previous winter and this is supported by three different kinds of evidence. Firstly, the 1979/80 winter was warmer than the previous one. The lowest grass minimum recorded near the study area was only -10.5°C , which from laboratory and field experiments should not have killed many acclimatized exules. Secondly, several gynoparae were found during survey and monitoring work in spring 1980 and some males were caught in R.I.S. traps, both suggesting that some exules had overwintered successfully. Lastly, the relative timing of the spring migration from monitored colonies, the first catches of alatae by R.I.S. traps and the build-up of summer infestations clearly shows that spring migrants cannot have been important in initiating summer colonies (see section 6.5.4).

Thus, even from just two winters' data, it is clear that the relative importance of holocyclic and anholocyclic overwintering can vary considerably from year to year. Variation in the size of autumn populations on rose must be a major factor governing this. From the surveys, the autumn population on rose in field boundaries in 1978 was nearly three times greater than that in 1979, despite the huge numbers present in summer 1979 (see section 6.1.3). This suggests that very heavy mortality between the cereal harvest and autumn migration can occur and clearly shows that the size of the autumn population on rose need not be at all closely linked to the size of the summer population. It is likely that weather conditions during the autumn migration period are important in determining the success

*Rothamsted Insect Survey

of primary host colonization. This view is supported by the fact that the relatively large population in autumn 1978 occurred after particularly calm, dry weather at the time of migration (see section 6.1.3).

A second major factor governing the relative importance of holocyclic and anholocyclic overwintering is survival between autumn and spring. Cold winters must favour holocycly because eggs are more cold-hardy than viviparae. The unimportance of low temperature mortality to overwintering eggs was confirmed by the rose survey results which showed lower mortality in the cold 1978/79 winter than in the relatively mild 1979/80 winter. The extra mortality in the milder winter may have been partly caused by increased egg predation but colony monitoring (section 6.4) revealed that egg mortality, even in the 1979/80 winter, was low compared to that of oviparae and fundatrices. It seems likely that the decisive factor which caused the greater overall mortality in 1979/80 was heavy mortality of nymphal fundatrices in early spring (see section 6.5.3). This was caused by mild February weather - causing early egg-hatch - followed by a cold March, which inhibited bud burst and thus forced developing fundatrices to feed in very exposed positions.

(b) Characteristics of the distribution
of Metopolophium on rose

Results of these surveys showed that the proportion of rose leaves infested with Metopolophium (mainly M. dirhodum) were highest in dense woodland, approximately equal in open woodland and field boundaries, and lowest in exposed, open scrub. Within field boundaries, the population density on rose was greater in high hedges and wood edges than in low hedges. These relationships between aphid distribution and habitat agree well with evidence in the literature on the flight behaviour of aphids and the effect of shelter on the distribution of small flying insects.

The zone of air close to the ground within which wind speed is lower than an insect's flight speed, so that it can control its direction of movement, is known as the

'boundary layer' (Taylor, 1960; Lewis, 1967). The depth of an insect's boundary layer varies with wind speed and also depends on the insect's maximum flight speed. For alate aphids, which have flight speeds below 1 m/s (Kennedy, Booth & Kershaw, 1959b; Taylor, 1960; Kring, 1972; Kennedy & Thomas, 1974) the boundary layer is usually well under 2 m high (Taylor, 1960). Although aphid take-off is generally inhibited at wind speeds above about 0.7 m/s (Johnson, 1969), alatae, particularly gynoparae, will still take off when wind speeds are well above the maximum flight speed (Haine, 1955) and after take off migrants show persistent, upward and object-avoiding flight into the sky (Johnson, 1954, 1969; Kring, 1972). This behaviour, coupled with thermals and changes in wind conditions, ensures that the majority of alatae fly or are carried out of the boundary layer and are then wind-transported with no control over their direction (Johnson, 1954).

Alate aphids and other small flying insects have been found to accumulate in the lee of artificial wind-breaks (Lewis, 1965), hedges (Lewis, 1969) and windbreaks of tall trees (Lewis, 1970). These accumulations are not produced by insect flight behaviour and are largely simple physical phenomena: the reduction of wind speed behind barriers causes the rate of wind-delivery into this sheltered zone to exceed the rate of loss from it. Consequently, the patterns of accumulation shown by small flying insects are very similar to those for small wind-borne spiders and experimental paper discs (Lewis, 1965). Accumulations increase with barrier height (Lewis, 1967) and impermeability (Lewis & Stephenson, 1966) because both these factors increase the degree of wind speed reduction and the extent (in both height and width) of the sheltered zone. Thus, it is expected that accumulations of Metopolophium alatae (and other small wind-delivered flying insects) should be greatest in areas with the greatest height and density of vegetation. Consequently, they should be most marked in dense woodland, less so in open woodland and in the lee of hedges and least marked in open scrubland; and they should also be greater near high hedges than low ones.

Alatae of Aphis fabae, Brevicoryne brassicae and Myzus persicae (including gynoparae) tend to fly into the wind (Kennedy et al., 1959a,b; Kennedy & Thomas, 1974). If, as is likely, this behaviour is shown by other aphids, including M. dirhodum gynoparae and males, it would tend to keep them in the sheltered areas inside woodland and in the lee of hedges. Air flows through permeable barriers as well as being deflected over them. Thus some alatae will be blown into hedges and may have the chance of landing on their way through, particularly in wider and denser hedges and in relatively calm conditions. Others will either be blown right through hedges or over them and will enter the sheltered zone on the lee side. Maximum reduction of wind speed and accumulation of insects are not found immediately behind a hedge, but at a distance of two to four times the hedge's height to leeward (Lewis, 1969). However, the tendency of alatae to fly upwind would ensure that, providing winds are not too strong, alatae will make their way into a hedge from the area of maximum shelter and can then land.

Although the great majority of alatae are probably wind-delivered to their approximate place of landing, reduced wind speed in the landing area is probably important to successful landing. Field observations on gynoparae of M. persicae and A. fabae (Kennedy et al., 1959a,b) have shown that landing is an active process - the aphids are not simply blown onto leaves. Indeed it is difficult to envisage how a gynopara could land successfully on foliage well above ground level in wind speeds much faster than its flight speed. Thus the probability of gynoparae landing would probably be greater in a sheltered situation even without the accumulation effect described above.

However, even when they are in the enlarged boundary layer associated with a wood or hedge and have control over their flight direction and landing, M. dirhodum gynoparae and males are unlikely to be able to distinguish Rosa spp from other foliage by olfactory or visual cues before landing. Gynoparae of M. persicae and A. fabae (Kennedy et al., 1959a,b) and alatae of other species (Kring, 1972; Blackman, 1974a) have not been found to land

preferentially on hosts. Although surfaces very different from the host in colour or structure may be ignored (Kennedy et al., 1959b), accumulation on hosts tends to result from reduced rejections after tasting, and even on the correct species the rejection rate is high (Kennedy et al., 1959a,b). However, an olfactory response to the primary host has been demonstrated for R. padi (Pettersen, 1970a) and it is possible that olfaction might play a role in sheltered areas close to the host. Olfactory responses by males to pheromones given off by oviparae have also been demonstrated (Pettersen, 1970b; Marsh, 1975) but these are only effective over a few centimetres.

The finding that rose bushes with less foliage had a greater proportion of their foliage infested with Metopolophium suggests that attraction from a distance was unimportant, as both the visual and olfactory "conspicuousness" of rose should increase with bush size. The observed difference in infestation levels on large and small bushes may have been caused by the trial-and-error searching strategy of gynoparae. Even a small area of woodland or hedgerow contains a huge amount of 'potential host surface' (that is, tree and shrub foliage which cannot be rejected as 'non-host' until a gynopara has tasted it) and also provides the sheltered conditions in which a gynopara can make the many short, controlled flights needed to test the available foliage. Thus, although no field data are available, it seems likely that a gynopara's chance of successfully locating rose foliage would be optimized if, having arrived in a particular wood or hedge, it restricted its search to the immediate area for a relatively long time before embarking on another long flight likely to take it above the boundary layer and perhaps carry it to a totally unsuitable habitat. Such an apparently optimal, aggregated search strategy would tend to increase the proportion of rose leaves colonized in areas of a particular habitat where relatively little rose was available.

However, it is possible that the observed differences between bushes with different amounts of foliage were produced by differences in nutritional quality, perhaps

linked to differences in growth rates and/or age. Similarly, although the observed distribution of Metopolophium between habitats can be explained solely in terms of differential settling due to shelter effects, nutritional differences between rose in different habitats may have contributed. In particular, rose in field boundaries in the study area (especially that in short hedges) was cut back regularly while woodland rose was not. Cutting back usually leads to a greater amount of growth late in the growing season which, from casual observation, is unsuitable for autumn colonization.

Differential settling by males in different habitats and differential survival and/or reproduction by all stages from gynoparae to fundatrigeniae would either counteract or accentuate the primary pattern of distribution between habitats initially produced by differential settling by gynoparae. Evidence for these effects, obtained by monitoring colonies in a woodland and an exposed hedgerow site, is discussed in section 6.4.4.

Finally, it should be noted that although the surveys revealed that differences in population density on rose in different habitats are likely to be a significant influence on the habitats' relative importance as sources of spring migrants, the prime factors controlling this must be the relative areas of each habitat type and the abundance of rose in each. Both of these vary considerably with geographical area, soil type and management practices. In particular, it should be borne in mind that both soil type and woodland management for shooting (regular canopy thinning and ride clearance) encouraged woodland rose in the study area used here. On non-calcareous soils rose may be less abundant in all habitats or different species, more or less suitable for M. dirhodum, may be present.

6.3 LABORATORY WORK ON OVIPARAE

6.3.1 Introduction

A small amount of laboratory work on oviparae was undertaken to provide some information useful in the

interpretation of data from the monitoring of autumn colonies on rose. In autumn 1978 observations on mating and subsequent egg laying were made and during autumn 1979 data on potential fecundity and the laying of infertile eggs by unmated oviparae were obtained.

6.3.2 Methods

(a) Observations on mating and egg laying

The mating of oviparae and males collected from Rosa canina during October 1978 was observed in the laboratory at 22°C. Eighteen mated oviparae were kept on leaves of R. canina in petri dishes with damp filter paper at $15^{\circ} \pm 1\frac{1}{2}^{\circ}\text{C}$ and 16 h daylength and the number of eggs they laid was recorded.

(b) Potential fecundity and the laying of eggs by unmated oviparae

Mating oviparae in the laboratory and recording the number of eggs laid subsequently proved a poor way of estimating their potential fecundity (see results section). Therefore in autumn 1979 potential fecundity was determined by dissecting adult oviparae. Forty-five oviparae were collected from R. canina as fourth instars and kept in petri dishes with damp filter paper and rose leaves (replaced every week) at $15^{\circ} \pm 1\frac{1}{2}^{\circ}\text{C}$ and 16 h daylength. They had no chance to mate and thus any eggs laid were infertile. The duration of adult life and number of eggs laid were recorded. Immediately after death 40 were dissected and the eggs they contained were counted.

6.3.3 Results and discussion

(a) Observations on mating and egg laying

There was a large amount of variation in the number of eggs laid by oviparae mated in the laboratory (Table 6.12). The maximum number laid was eight but the mean was only 2.6. This strongly suggested that most oviparae failed to lay their full complement of eggs. This may have been because an ovipara usually needs to be mated

Table 6.12 The mating and egg-laying of
18 M. dirhodum oviparae.

Number of eggs laid	Time taken mating (min.)	Previous matings by male that day
8	5	0
7	19	4
5	6	2
4	9	0
4	11	1
3	11	0
3	8	0
3	10	1
3	9	2
3	6	0
1	5	3
1	2	5
1	24	0
0	?	0
0	1	0
0	17	2
0	5	0
0	8	1

several times before all its eggs are fertilized, but might also have been due to the unnatural conditions under which mating took place here, in particular the atypically high temperature experienced in the laboratory. A single male can mate with several oviparae in quick succession and at least some of these multiple matings appeared to be successful (Table 6.12). Although males sometimes remained in the mating position for over 20 min, 4 to 10 min was more usual.

(b) Potential fecundity and the laying of eggs by unmated oviparae

Unmated oviparae did lay some eggs, although the mean number laid before death was only 0.42 (Table 6.13) and adult oviparae which survived 10 days or less did not lay any. This delay is liable to be longer in the field due to the lower temperatures experienced. Fertile eggs are pale green when laid and darken to glossy black within a few days or weeks, but the infertile eggs laid by unmated oviparae collapsed before they had blackened. These results strongly suggested that few if any of the eggs found on monitored bushes (see section 6.4) were infertile ones laid by unmated oviparae. Unmated oviparae of Aphis fabae also lay very few eggs (Way & Banks, 1964) and in R. padi infertile eggs collapse before blackening (Leather & Lehti, 1981).

The total number of eggs developed by unmated oviparae of M. dirhodum, as well as the number they lay, increased with adult age (Table 6.13). The mean potential fecundity of the oviparae, 8.9 eggs, was similar to that found for other aphid species. For example, oviparae of S. avenae lay an average of 13.06 eggs (Watt, 1979b) and those of Myzus persicae between four and 13 (Tamaki, 1974). The mean adult life span of M. dirhodum oviparae was 16.6 days (S.E. = 0.98) at 15°C. This compares with 22 days for viviparae kept on barley leaves at the same temperature (Dean, 1974b). However, potential longevity of oviparae is probably considerably longer at the much lower temperatures experienced in the field during October and November.

Table 6.13 Number of eggs laid and number of eggs developed
by unmated M. dirhodum oviparae.

Adult age class (days)	No. dying in age class	Eggs laid before death			Eggs present in body at death			Total eggs developed		
		$\bar{X}$	S.E.	n	$\bar{X}$	S.E.	n	$\bar{X}$	S.E.	n
1-5	4	0	0	4	-	-	0	-	-	0
6-10	4	0	0	4	8.25	1.109	4	8.25	1.109	4
11-15	8	0.63	0.375	8	7.50	0.906	8	8.13	0.789	8
16-20	15	0.20	0.145	15	8.40	0.653	15	8.60	0.567	15
21-25	14	0.79	0.206	14	9.23	0.601	13	9.92	0.582	13
Total	45	0.42	0.112	45	8.48	0.377	40	8.90	0.351	40

6.4 MONITORING COLONIES FROM OCTOBER 1979 TO APRIL 1980

6.4.1 Introduction

Colonies of M. dirhodum on 13 rose bushes were monitored from the production of oviparae in October 1979 to the maturation of fundatrices in April 1980. The weather during this period was described previously (section 6.1.3). The bushes used were grouped in two sites, one a sheltered woodland and the other an exposed hedgerow, to allow some assessment of the importance of shelter to colony survival.

It was originally intended to carry out some natural enemy exclusion experiments on the holocyclic colonies but the low population density of M. dirhodum on its primary host meant that not enough reasonably large colonies in similar sites could be found for this to be undertaken. Even for the basic monitoring work described here and in section 6.5, extensive searching was required to locate the minimum number of aphids needed, particularly because a number of the colonized bushes found were too tall, dense or tangled to be suitable for monitoring. Without knowledge of nutritional differences between bushes it was considered unwise to move aphids onto previously uncolonized bushes.

M. dirhodum on rose cannot be distinguished in the field from the recently described species Metopolophium fasciatum (see section 6.1.4). In the autumn, aphids were not collected off the monitored bushes for identification but Hand (1982) estimated from samples taken from other bushes during the population survey that 89% of gynoparae, 73% of oviparae and all males were M. dirhodum in autumn 1979. Fundatrices survived to produce spring colonies on three of the monitored bushes. Alate fundatrigeniae collected from these were all identified as M. dirhodum by Hand.

6.4.2 Methods

Four of the monitored bushes were in wood II and the

remaining nine in hedge 26 (see Fig. 6.2). Hedge 26 was one of the most exposed sites on the study farm, running SE to NW along the top of a ridge. It was not typical of other hedges on the farm, but was more suitable for monitoring and provided an exposed site to contrast with the sheltered woodland. It consisted of a trackside shelter belt, about 4 m wide, of mature beech (Fagus sylvatica L.) with a thin understorey of scattered Rosa canina agg. and Rubus fruticosus L. bushes, bounded on its field side by a thin hawthorn (Crataegus monogyna L.) hedge. Rose bushes under the beech trees were relatively small and well separated and much easier to examine than those in typical hedges. The monitored bushes varied from 15 cm to 1.5 m high (mean = 0.75 m) and had 12 to 62 leaves (mean = 37) when monitoring started on 24 October. Wood II was dense and protected by higher ground to SW and NE. The four bushes used in this site were larger than those in the hedgerow. They varied from 1 to 2 m high, with 33 to 217 leaves (mean = 121.5) on 24 October. The two smaller bushes were on the edge of a clearing and the two larger ones under a closed oak (Quercus robur L.) canopy. One of these was sweet briar (Rosa rubiginosa agg.) but the remainder and all those in hedge 26 were dog rose (Rosa canina agg.). None of the monitored bushes were in contact with other rose bushes.

During the autumn the bushes were visited five times. The recording sessions took 2 or 3 days and each is referred to below by the mean date. These were: 24 October, 1, 8 and 14 November and 1 December. Intervals between recording dates were irregular because a survey of the holocyclic population was being conducted during the same period (see section 6.2) and because recording was sometimes delayed by rain or high winds which made counting aphids without disturbing them difficult. On each recording date, leaves and stems of each bush were examined for M. dirhodum gynoparae, males and oviparae and for natural enemies. Oviparae were divided into four instar categories: 1st or small 2nd, large 2nd or 3rd, 4th, adult. The positions of aphids were recorded according to branch (each given a number) and leaf position,

and colonized leaves were marked using wire-stiffened paper tags attached to the stem nearby. A note was also made of which leaflets of a colonized leaf were present and which had aphids.

Brief descriptions of natural enemies were taken to aid identification but they were not removed. The positions of natural enemies were recorded as was their presence on the same leaves or leaflets as aphids and attacks on aphids. The number of leaves on each bush was also recorded on each visit. (Leaves tended to shed leaflets individually but a leaf was not recorded as 'fallen' until none of its leaflets remained.) Four gynoparae and 21 oviparae of other rose aphid species (mainly Macrosiphum rosae L.) were removed from the monitored bushes during the autumn because M. dirhodum eggs cannot be distinguished from those of other species. On 14 November and 12 December the stems were examined thoroughly for M. dirhodum eggs and the positions of those present were marked with a spot of enamel paint on the stem nearby.

In 1980 the bushes were visited on five occasions between egg-hatch and the maturation of the last fundatrix. The dates were 25 February, 14 March, 2, 9 and 20 April. Eggs which had obviously collapsed or hatched were collected and examined under a binocular microscope in the laboratory. Those which still appeared viable were left on the bushes until late April and then collected. Monitoring from 10 April, which involved 10 new bushes, is described in section 6.5.

6.4.3 Results

(a) Population change in autumn colonies

When monitoring started on 24 October 1979 gynoparae had already arrived on the rose bushes (Table 6.14). The 217 oviparae already produced were predominantly first to third instars on this date but nine were adults and six males were present on woodland bushes. Although at least 54 more oviparae were born after 24 October (Table 6.15) numbers declined rapidly throughout the monitoring period

Table 6.14 Population change in monitored colonies
of M. dirhodum on rose in autumn 1979.

	24/10	1/11	8/11	14/11	1/12
<u>HEDGE 26:</u>					
<u>Oviparae:</u> (instars)					
* small 1st-3rd:	29	12	1		
* large 1st-3rd:	37	15	5	1	
4th:	16	14	10	4	
adult:	5	18	13	12	3
total:	87	59	29	17	3
<u>Gynoparae:</u>	2	3			
<u>Males:</u>		6	1	1	
<u>WOOD II:</u>					
<u>Oviparae:</u> (instars)					
* small 1st-3rd:	49	20		8	
* large 1st-3rd:	59	3	8	2	
4th:	18	41		3	
adult:	4	18	24	8	2
total:	130	82	32	21	2
<u>Gynoparae:</u>	5	2		1	
<u>Males:</u>	6	11	5	3	

*'small 1st-3rd' instars = 1st instars and small 2nd instars;
'large 1st-3rd' instars = large 2nd instars and 3rd instars.

Table 6.15 Numbers of M. dirhodum oviparae produced in monitored colonies on rose bushes after 24 October 1979.

Period	24/10-1/11	1/11-8/11	8/11-14/11	Total as % of total population
Hedge 26	24	0	0	21.1
Wood II	20	2	9	19.3

Table 6.16 Percentage of M. dirhodum oviparae which moved between recording dates on rose bushes monitored in autumn 1979.

Period	24/10-1/11	1/11-8/11	8/11-14/11	14/11-1/12
% missing	62.6	56.1	96.2	100.0

Table 6.17 Percentage of M. dirhodum oviparae wandering on stems on rose bushes monitored in autumn 1979. (Numbers of aphids on bushes are in brackets.)

Date	24/10	1/11	8/11	14/11	1/12
Nymphs	0 (208)	1.0 (105)	4.2 (24)	5.6 (18)	- (0)
Adults	0 (9)	5.6 (36)	10.8 (37)	50.0 (20)	20.0 (5)
All stages	0 (217)	2.1 (141)	8.2 (61)	28.9 (38)	20.0 (5)

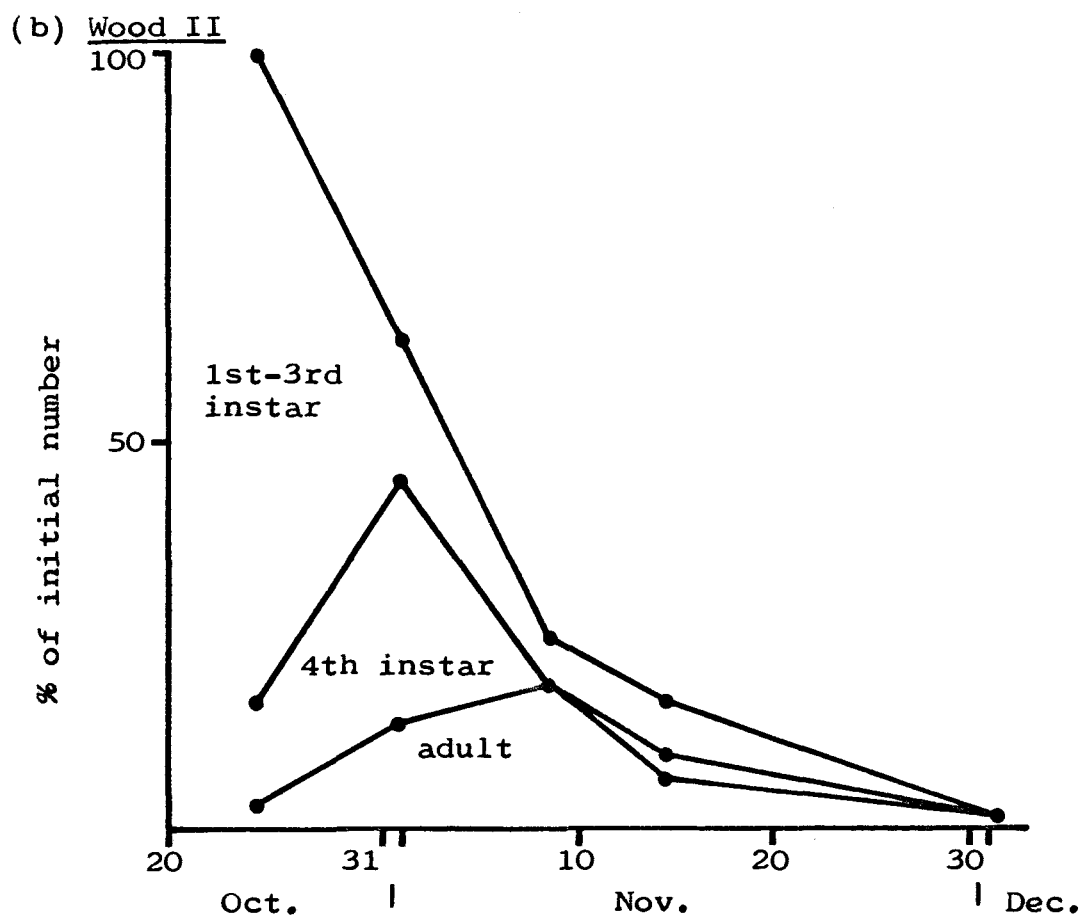
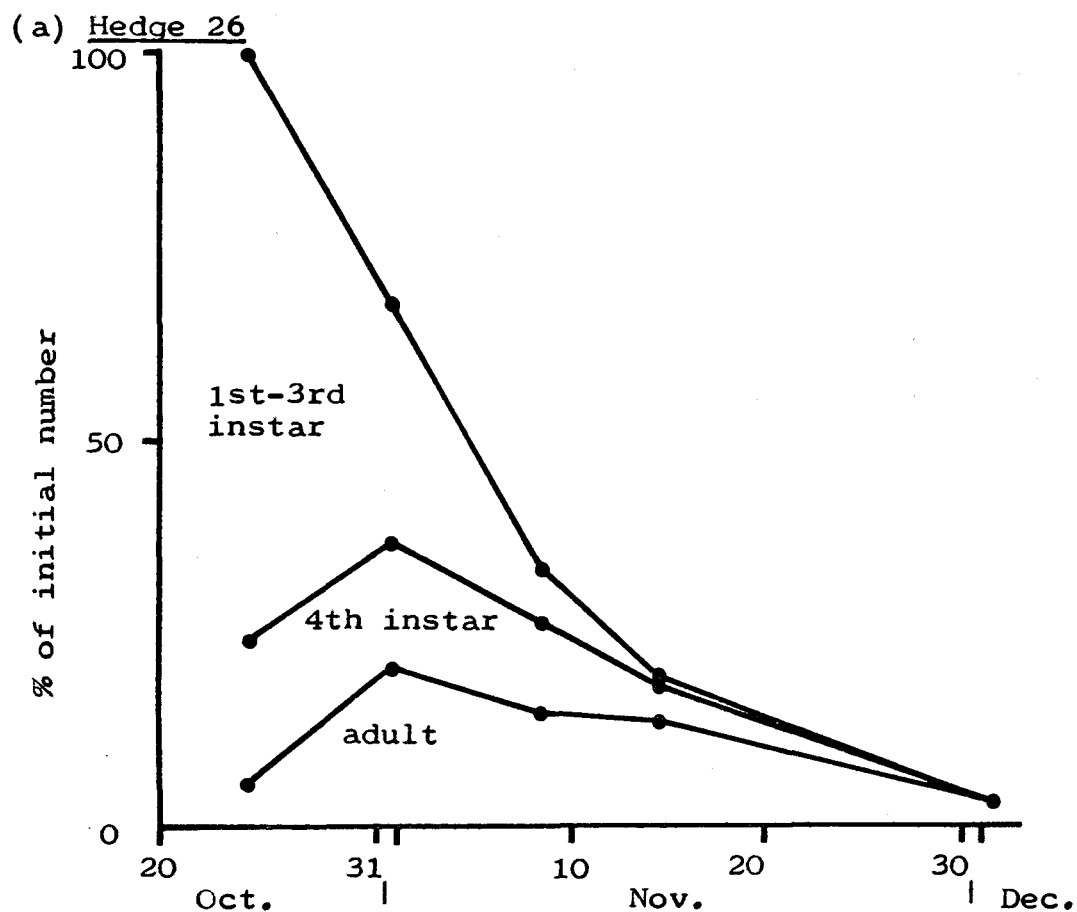
(Fig. 6.4). However, the population's age structure on 24 October suggested it had only recently reached its peak. The overall population density of oviparae declined from 0.26 per leaf on 24 October, the loss of aphids outstripping leaf fall. Very few oviparae were produced during November and only one gynopara was found after the first of the month. Peak numbers of males were found on 1 November and peak numbers of adult oviparae on 1 November (hedgerow bushes) and 8 November (woodland bushes). Oviposition took place during November, with just over 50% of the eggs laid by 14 November. By 1 December the only aphids remaining were five adult oviparae. Population change followed the same pattern on woodland and hedgerow bushes although the decline in numbers of oviparae between 24 October and 8 November was slightly faster on the latter, which also had more males, probably due to their more sheltered position.

If it is assumed that an aphid's chances of survival are the same whether or not it moves to a different leaflet, the proportion of the total population which moves between one recording date and the next can be estimated from the proportion of survivors not on the same leaflet on the two recording dates. Using this method of estimation showed that oviparae moved a great deal, even as young nymphs, with over 50% moving between recording dates (Table 6.16). Although nymphal oviparae were rarely found off the leaves (Table 6.17) the proportion of adults wandering on stems increased to 50% by mid-November. These were probably oviparae which had already mated and were ready to oviposit.

(b) Natural enemies

Only three syrphid eggs were recorded on the monitored bushes during the autumn and no larvae were found. No other predators or parasites were recorded apart from Araneae and adults of Anthocoris nemorum (L.), both of which were found feeding on aphids. A. nemorum was found on only two bushes, both in wood II. On one bush two adults were recorded on 24 October and two on 1 November. Between them they were seen attacking four oviparae (Table

Fig. 6.4 Changes in the numbers of *M. dirhodum* oviparae on monitored rose bushes in autumn 1979.



6.18) and may well have been responsible for the early disappearance of the small colony of aphids on this bush. On the second bush with A. nemorum two adults were found on 1 November.

Araneae were much more numerous and were found on all bushes (Table 6.19). The majority were less than 3 mm long. From notes taken on the spiders and their webs and identification of samples collected in similar sites elsewhere on the study farm, the great majority of the small spiders on the woodland bushes were Theridiidae (mainly Theridion spp.) (Locket & Millidge, 1951, 1953). On hedgerow bushes small Theridiidae were less common but in late September and early October there were more small Linyphiidae, which appeared to have blown off neighbouring fields. Larger (≥ 4 mm) spiders were mainly Theridiidae and Argiopidae with one Thomisidae and possibly a few Linyphiidae. The density of spiders (numbers/leaf) was slightly higher on hedgerow bushes than on woodland bushes while the Linyphiidae were common, and slightly lower thereafter.

It is clear from records of attacks on M. dirhodum (Table 6.18) that natural enemies were responsible for greater mortality on woodland bushes than in the hedgerow and that this was mainly due to greater predation by spiders up to early November in woodland. Spiders were actually at slightly higher density on hedgerow bushes in this period but a smaller proportion of the hedgerow spiders were small Theridiidae and, with one exception, all the spiders seen attacking aphids were of this type. Many of the Linyphiidae on the hedgerow were probably grassland species deposited during a seasonal aerial migration and so may not have attempted to feed while on the bushes.

Spiders were probably more effective than A. nemorum in reducing autumn M. dirhodum colonies not only because of their greater abundance but also because of their ability to catch gynoparae and males and because their webs continued to snare aphids even when the spiders themselves had left.

Table 6.18 Records of attacks on M. dirhodum by natural enemies on monitored rose bushes in autumn 1979.

Date	Site	Natural enemy	Aphid morph
24/10	Wood II	spider	ovipara (1st-3rd)
	"	spider	ovipara (1st-3rd)
	"	spider*	ovipara (1st-3rd)
	"	spider*	ovipara (1st-3rd)
	"	<u>Anthocoris</u>	ovipara (1st-3rd)
1/11	Wood II	spider	ovipara (1st-3rd)
	"	spider	ovipara (1st-3rd)
	"	spider	ovipara (1st-3rd)
	"	spider	ovipara (4th)
	"	spider	gynopara
	"	spider	male
	"	spider*	male
	"	<u>Anthocoris</u>	ovipara (1st-3rd)
	"	<u>Anthocoris</u>	ovipara (1st-3rd)
	"	<u>Anthocoris</u>	ovipara (4th)
8/11	Hedge 26	spider*	ovipara (1st-3rd)
	Wood II	spider	ovipara (adult)
	Wood II	spider*	gynopara
	Wood II	spider*	male
14/11	Wood II	<u>Entomophthora</u>	ovipara (1st-3rd)

*aphids found dead in web but no spider present.

Table 6.19 Numbers of spiders found on monitored rose bushes in autumn 1979.

Date	24/10	1/11	8/11	14/11	1/12
<u>HEDGE 26</u>					
Small (≤ 3 mm) Theridiidae	5	3	3	1	2
Small (≤ 3 mm) Linyphiidae	9	9	1	0	0
Others	2	3	3	0	1
Total	16	15	7	1	3
<u>WOOD II</u>					
Small (≤ 3 mm) Theridiidae	14	14	11	3	0
Small (≤ 3 mm) Linyphiidae	2	0	0	1	0
Others	5	7	10	4	0
Total	21	21	21	8	0

(c) Leaf fall and other mortality factors

Leaf fall occurred earlier on woodland bushes than in the hedgerow (Fig. 6.5). Considering the greater exposure to wind of hedgerow bushes this suggested that bush age and/or light intensity were more important than exposure in determining the timing of leaf fall. The difference in the timing of leaf fall must have ensured that more oviparae were lost from woodland bushes because the leaves they were feeding on fell, unless the aphids tended to select leaves which were not due to drop. The latter possibility was not supported by the evidence available as, firstly, leaves with aphids on them on a particular recording day were not significantly more or less likely to have fallen by the next recording day than were uncolonized leaves (χ^2 tests for the periods 24/10 - 1/11, 1 - 8/11 and 8 - 14/11* all give $\chi^2 = 0.8$, D.F. = 1, $P > 0.25$). Secondly, from the data for leaves with oviparae, for which the fall of individual leaflets was recorded, the probabilities of colonized and uncolonized leaflets falling between recording dates were also not significantly different (χ^2 tests for the periods 24/10 - 1/11, 1 - 8/11 and 8 - 14/11* all give $\chi^2 = 3.1$, D.F. = 1, $P > 0.05$).

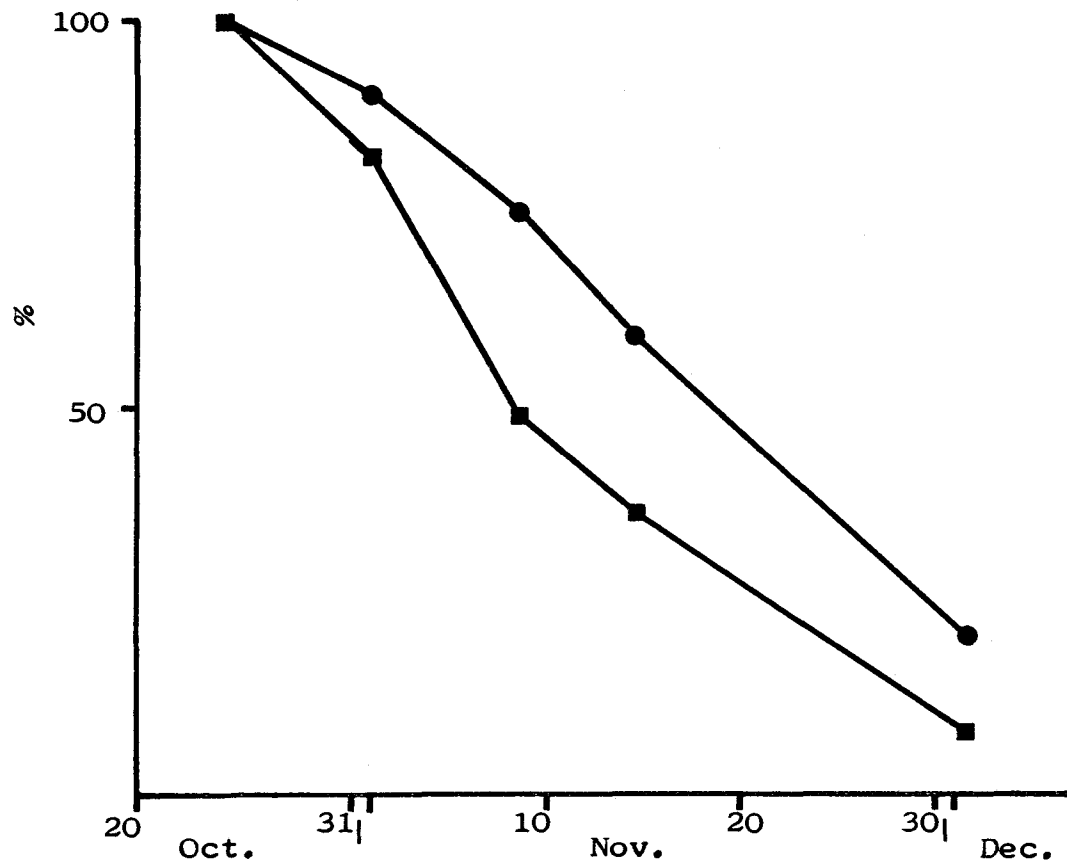
As colonized and uncolonized leaves and leaflets had approximately equal probability of falling, the proportion of oviparae lost due to leaf fall could be estimated despite their high mobility. The proportion lost between one recording date and the next was approximately:

$$\frac{\text{(Oviparae present on the first recording day, on leaflets which fell between the two recording days)}}{\text{(Total oviparae present on the first recording day)}}$$

As total loss due to all factors could also be calculated for the period between recording dates, loss due to factors other than leaf fall (natural enemies, weather, emigration) could be estimated by subtraction, and the relative importance of leaf fall as a mortality factor

*too few oviparae were present between 14/11 and 1/12 for a similar χ^2 test to be performed on the data.

Fig. 6.5 The percentage of leaves present on 24 October remaining on each subsequent recording date on monitored rose bushes in hedge 26 (●) and wood II (■) in autumn 1979.



assessed. In this assessment it was assumed that very few of the oviparae which fell from bushes were able to regain the same or any other bush, as the bushes were well separated. Being winged, males and gynoparae were probably able to relocate rose bushes much more readily. It was also assumed that emigration by oviparae was insignificant as this behaviour would seem to be extremely non-adaptive.

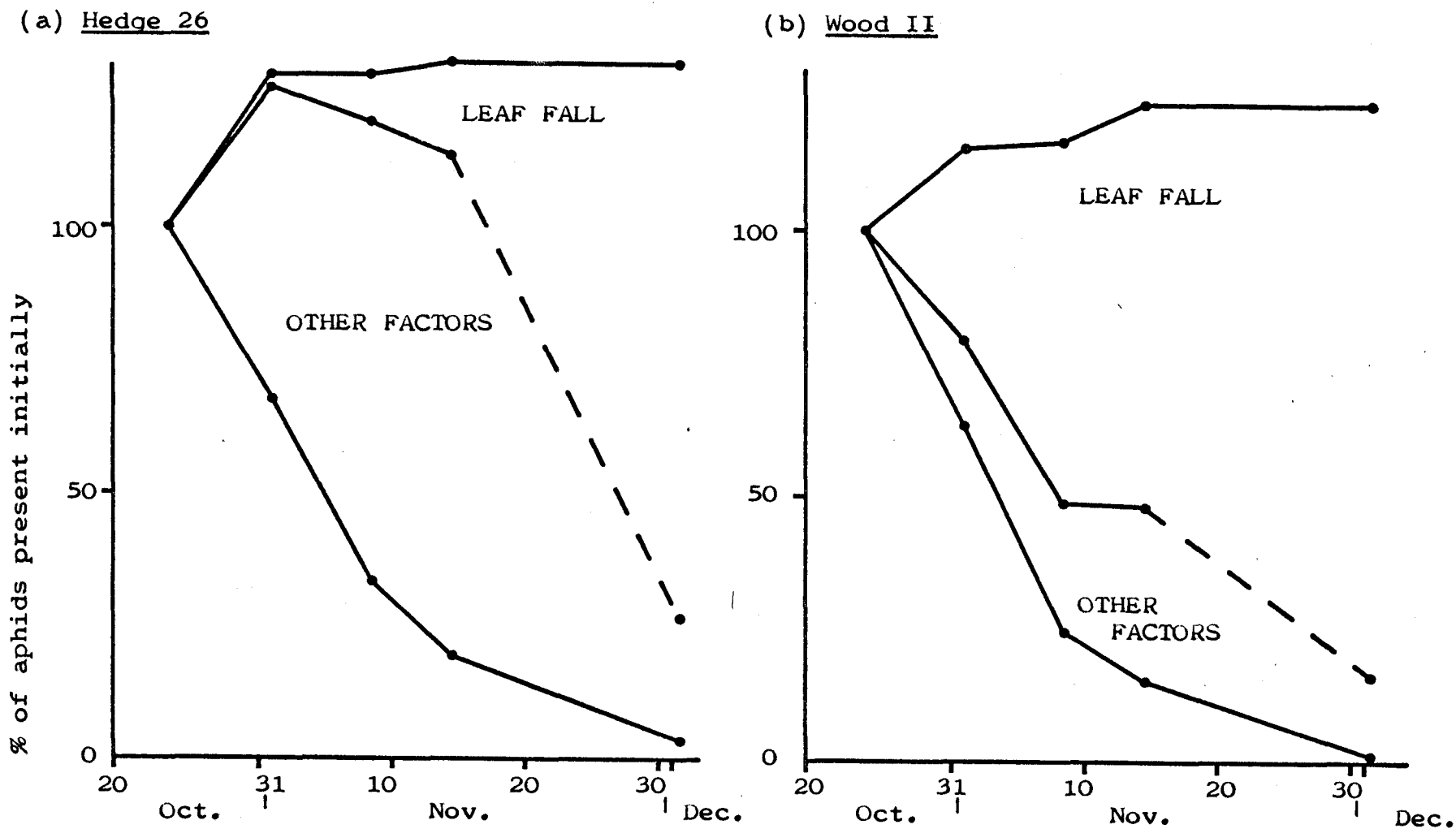
The mortality estimates (Table 6.20 and Fig. 6.6) showed that between 24 October and 8 November (the period during which the large majority of aphids were lost) leaf fall was a more important cause of mortality than all other factors combined for oviparae on woodland bushes but was unimportant for those on hedgerow bushes. The extent of the difference was dramatic, more so than expected from examining Fig. 6.5, but it was compensated for by the fact that aphids on the woodland bushes suffered much less mortality from other factors, so that overall population decline in the two sites was similar. As natural enemies had a greater impact on populations on woodland bushes most of the 'other factor' mortality on hedgerow bushes was probably due to weather factors.

Temperatures fell below -3°C on only one night before December (Fig. 6.7) and wood II was, if anything, subject to lower temperatures than hedge 26, due to its position in a frost hollow. Consequently low temperature mortality was unlikely to have been important in creating the difference in 'other factor' mortality between sites. Thus the difference appeared to be due to the greater exposure of the hedgerow bushes to wind and rain. Wind probably played the dominant role as late October and November 1979 were unusually dry (Fig. 6.8) and oviparae always occupied the undersides of leaves, although adults wandering on stems would be more vulnerable to rain. As few if any partially unfolded leaves were available on rose bushes in October and November, oviparae had to occupy feeding positions on the flat undersides of leaves. They could be knocked off these, even in a light breeze, when leaves and stems brushed against each other, so that even in an autumn with only moderate wind speeds (Fig. 6.9)

Table 6.20 Percentage mortality of M. dirhodum oviparae attributed to leaf fall and other factors in autumn 1979. (Numbers in brackets are percentage mortality/day.)

Period	Total	Leaf fall	Other factors	N
<u>HEDGE 26:</u>				
24/10-1/11	60.9 (8.12)	2.3 (0.31)	58.6 (7.82)	87
1/11-8/11	50.9 (6.78)	5.1 (0.68)	45.8 (6.10)	59
8/11-14/11	48.3 (8.05)	6.9 (1.15)	41.4 (6.90)	29
14/11-1/12	82.4 (4.84)	76.5 (4.50)	5.9 (0.35)	17
<u>WOOD II:</u>				
24/10-1/11	52.3 (6.97)	36.2 (4.82)	16.2 (2.15)	130
1/11-8/11	63.2 (8.43)	40.2 (5.37)	23.0 (3.06)	82
8/11-14/11	62.5 (10.42)	15.6 (2.61)	46.9 (7.81)	32
14/11-1/12	90.5 (5.32)	66.7 (3.92)	23.8 (1.40)	21

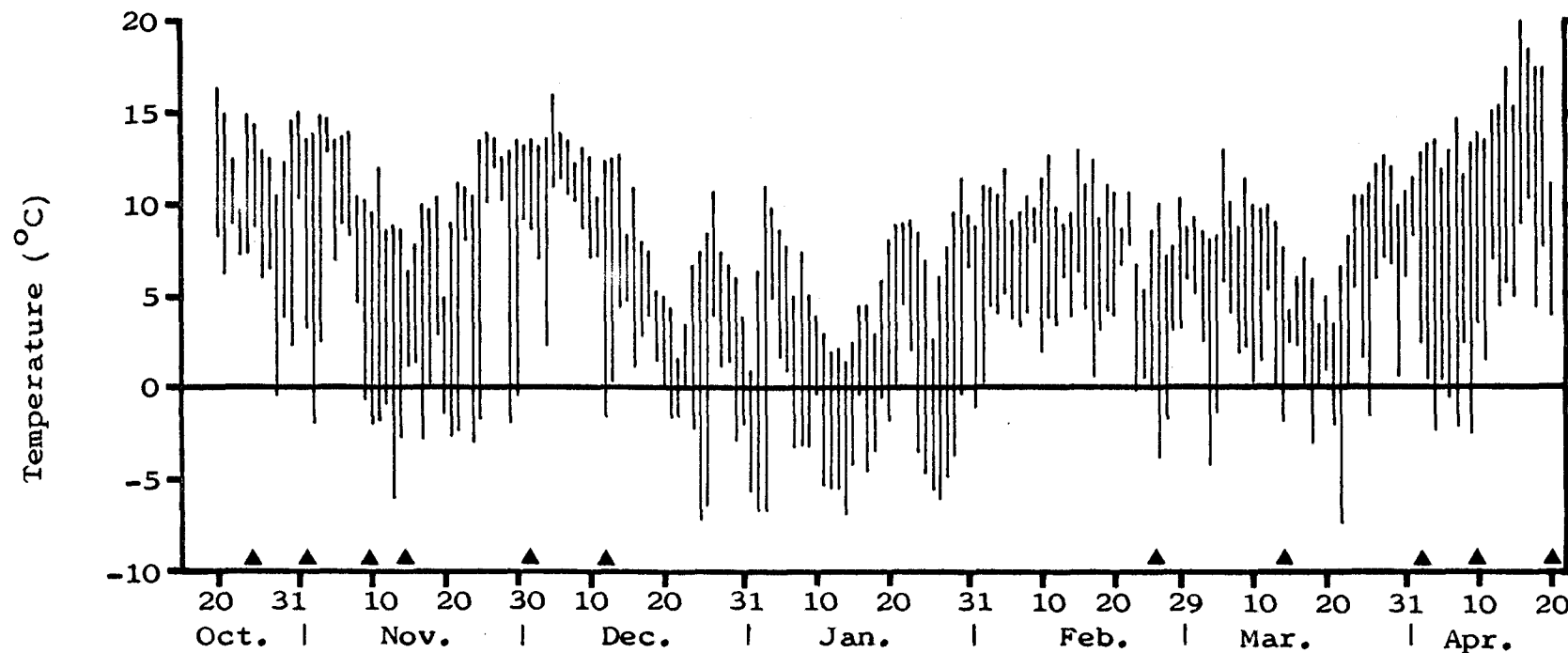
Fig. 6.6 The role of leaf fall in the loss of *M. dirhodum* oviparae from monitored rose bushes in autumn 1979.



Survival was calculated assuming oviparae born after 24 October were born on the day on which they were first recorded.

Fig. 6.7 Daily maximum and minimum temperatures ($^{\circ}\text{C}$) from October 1979 to April 1980, recorded at Hurn Airport.

▲ = aphid recording dates.



Monthly means ($^{\circ}\text{C}$)

Maximum:	15.8	11.3	9.6	6.2	9.9	8.8	14.1
Mean:	11.8	6.8	6.4	2.0	6.5	5.3	9.1
Minimum:	7.8	2.2	3.1	-2.3	3.1	1.7	4.0

Fig. 6.8 Daily rainfall from October 1979 to April 1980, recorded at Hurn Airport.

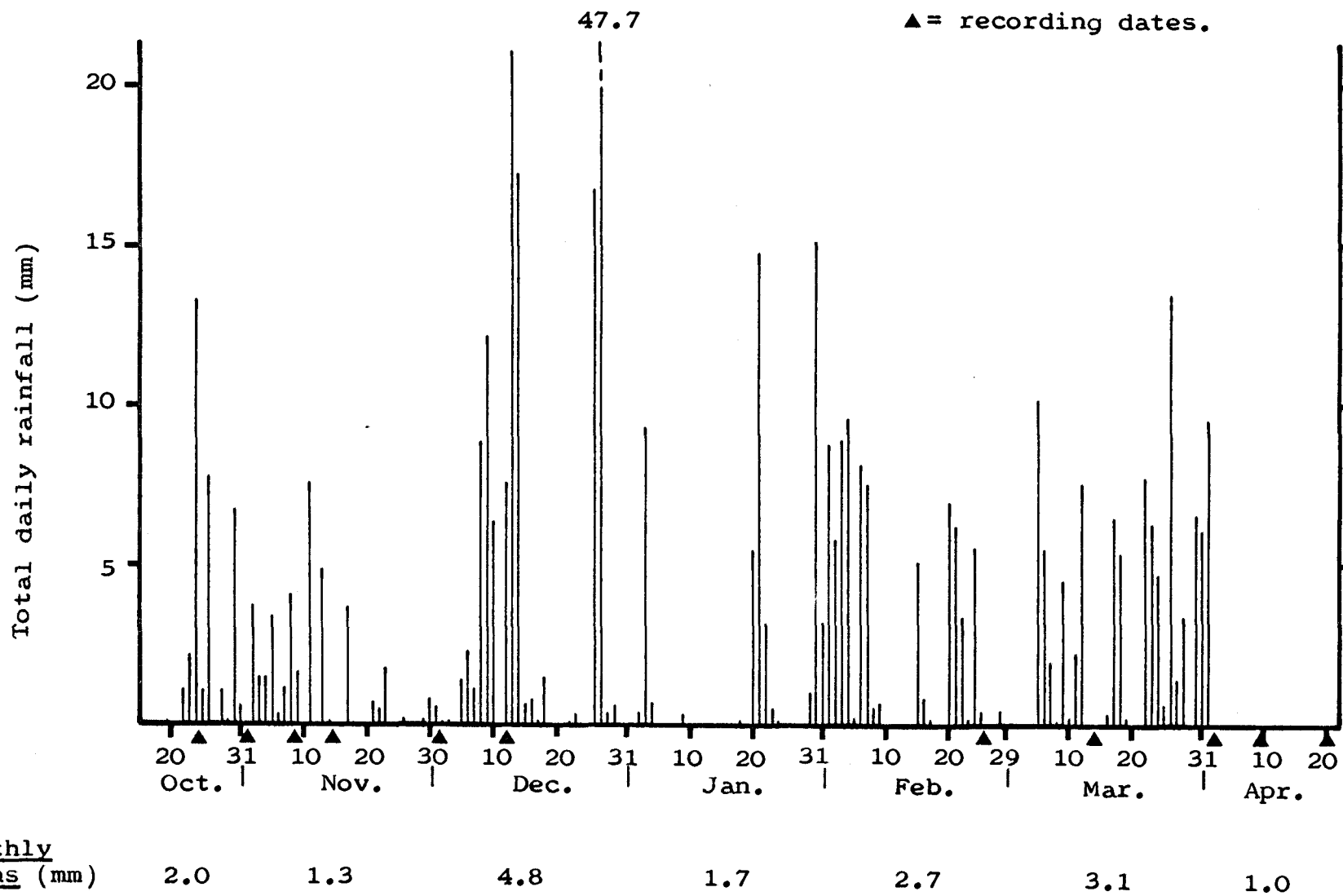
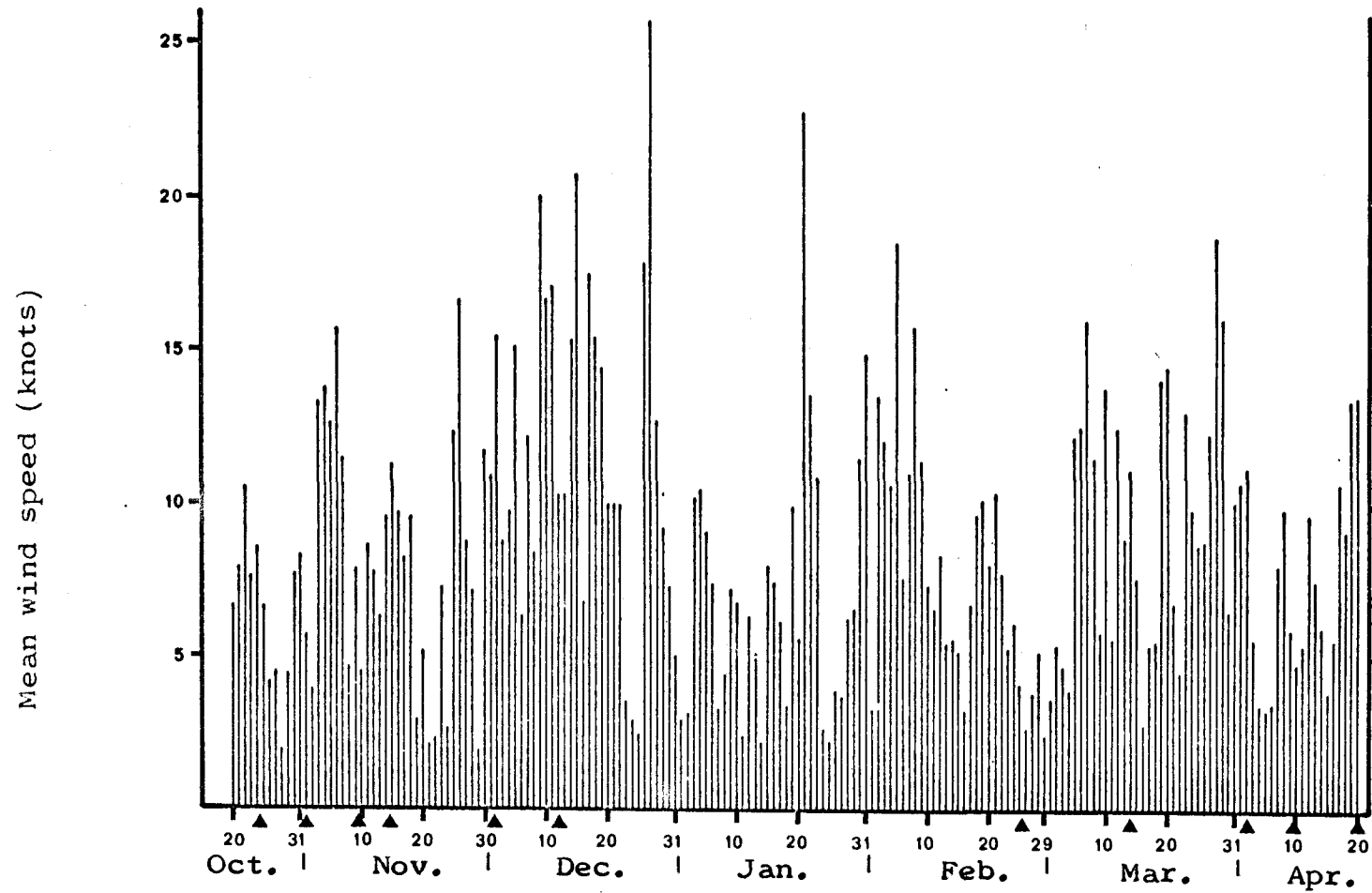


Fig. 6.9 Daily mean wind speeds from October 1979 to April 1980, recorded at Hurn Airport.

▲ = aphid recording dates.



Monthly
means (knots)

5.9

8.2

11.9

7.1

7.9

9.4

7.2

much mortality could result in relatively exposed hedgerow sites.

When bushes were considered individually, the difference in the importance of leaf fall in woodland and hedgerow sites was not as clear-cut as suggested by overall mortality estimates for each site. The two smaller woodland bushes at the edge of a clearing, which contributed relatively little to the overall estimates for woodland, showed leaf fall patterns similar to the hedgerow bushes. This was probably because they experienced higher light intensities than the two larger bushes, which were under a closed canopy, and/or because they were younger.

(d) Egg laying and colony success

Some oviparae reached adulthood on 11 of the 13 monitored bushes and eggs were laid on eight bushes. On these the number of eggs varied from one to 39 and the number of eggs per adult ovipara from 0.5 to 4.3. Sixty-one eggs had been laid by 15 November and a further 57 by 12 December. Only one egg was produced after this. As with other aphid species the eggs adhered firmly to the stems and were pale yellow-green when laid, changing to glossy black within a few weeks. The majority were laid on thorns or against thorn bases (Table 6.22) and only a small number were laid in bud axils. Six eggs, all produced before 15 November, were laid on leaves and were lost at leaf fall. Eggs on the stems were nearly invariably laid singly and were scattered widely over the bushes on both old and new wood, sometimes 15 cm or more from the nearest bud. They were not in clusters except on one small hedgerow bush with 24 eggs, on which three close groups (of five, four and two) were found.

The success of an autumn colony is best expressed as the number of eggs laid on stems per ovipara. Measured in this way the overall success of colonies on woodland and hedgerow bushes was similar (Table 6.21). In the wood, survival of oviparae to adulthood was lower but this was compensated for by the higher number of eggs laid per adult ovipara. However, there was considerable variation between bushes in each site so that these overall differ-

Table 6.21 Estimates of the success of holocyclic
M. dirhodum colonies in autumn 1979.

	Hedge 26	Wood II
Number of eggs* laid per nymphal ovipara:	0.41	0.44
Proportion of nymphal oviparae reaching maturity:	0.30	0.20
Number of eggs* per adult ovipara:	1.35	2.23

*6 eggs laid on leaves omitted

Table 6.22 The positions in which M. dirhodum eggs were laid on the rose bushes monitored in autumn 1979.

Position	Number of eggs	%
Thorns	44	37.0
Thorn bases	26	21.8
Crevices in rough bark	24	20.2
Smooth bark	15	12.6
Bud axils	4	3.4
Leaf petioles/stipules	5	4.2
Leaf lamina	1	0.8
Total	119	

Table 6.23 The fate of M. dirhodum eggs laid on the stems of monitored rose bushes in autumn 1979.

	Hatched	Missing	Collapsed not split	Collapsed and split	Total
HEDGE 26					
number	25	14	5	1	45
%	55.6	31.1	11.1	2.2	
WOOD II					
number	30	10	14	9	63
%	47.6	15.9	22.2	14.3	
TOTAL					
number	55	24	19	10	108*
%	50.9	22.2	17.6	9.3	

*Five of the 113 eggs laid on stems were either lost due to hedge cutting or could not be definitely assigned to one category and so have been omitted.

ences were not statistically significant when bushes were considered separately (Mann-Whitney U-tests give $P > 0.05$ in both cases). The numbers of oviparae per bush on 24 October varied from six to 83, giving densities of 0.12 to 1.50 oviparae per leaf. But colony success, measured as the number of eggs laid per ovipara, was not correlated with either population size (Kendall's rank correlation coefficient = 0.08, $n = 13$, $P > 0.2$) or population density (Kendall's rank correlation coefficient = 0.17, $n = 13$, $P > 0.2$). This suggested that density related effects (such as competition for feeding sites or responses of natural enemies to prey density) were unimportant.

As expected, the number of eggs laid on a bush was correlated with the peak number of adult oviparae recorded (Fig. 6.10) (Kendall's rank correlation coefficient = 0.52, $n = 11$, $P < 0.05$). But there was a considerably improved correlation with the number of eggs laid if the total number of adult oviparae recorded on all recording dates after 2 November was used as the index of oviparae abundance (Fig. 6.11) (Kendall's rank correlation coefficient = 0.78, $n = 11$, $P < 0.001$). This was probably because, after reaching adulthood, oviparae needed at least a week for mating, fertilization and oviposition. The arrival of males is obviously essential to a colony's success. Males definitely visited eight of the 11 bushes on which oviparae reached adulthood but from the numbers of eggs laid it is clear that a large majority of adult oviparae failed to mate or were killed before they could oviposit. The number of eggs laid per ovipara on each bush was not related to the number of males recorded but this was probably because of the relatively long intervals between recording dates.

(e) Egg survival

The great majority of eggs which hatched did so a little before 25 February 1980 and the fundatrices were first instar nymphs on that date, with the exception of one second instar. Five eggs were in the process of hatching but no eggs which did not show signs of hatching in late February hatched subsequently. The hatching date

Fig. 6.10 Relationship between the number of M. dirhodum eggs laid on each rose bush monitored in autumn 1979 and the peak number of adult oviparae recorded on each bush.

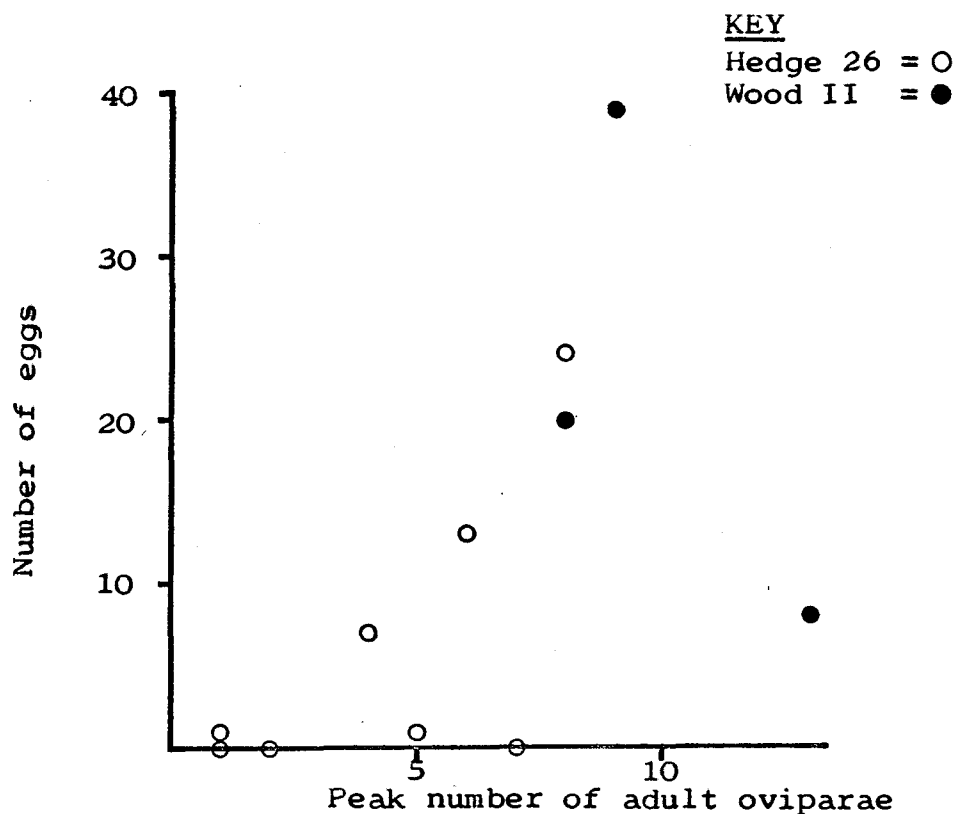
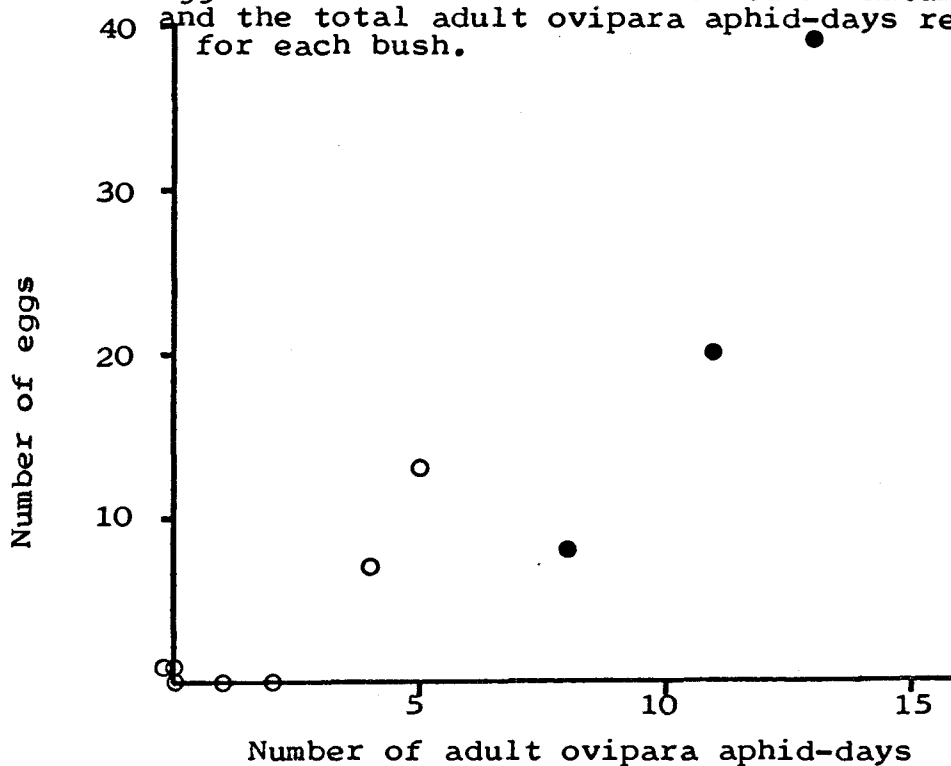


Fig. 6.11 Relationship between the number of M. dirhodum eggs on each rose bush monitored in autumn 1979 and the total adult ovipara aphid-days recorded for each bush.



was probably earlier than in most other years due to exceptionally mild weather during February (Fig. 6.7). Eggs hatched on six of the monitored bushes, three from each site, and overall hatching success was just over 50% (Table 6.23). There was no significant difference in the proportion hatching on hedgerow and woodland bushes ($\chi^2 = 0.38$, D.F. = 1, $P > 0.25$). A greater proportion of eggs laid before 12 December failed to hatch than of those laid after this date but the difference was not significant ($\chi^2 = 3.39$, D.F. = 1, $P > 0.05$).

The eggs which failed to hatch could be divided into three categories: missing, collapsed with a split in the shell and collapsed without a split in the shell (see Table 6.23). Eggs which were missing may have been dislodged by rain or may have been removed by predators. Eggs which had collapsed and were split had probably been attacked by predators. They could be distinguished from hatched eggs firstly because the split in the chorion was jagged and irregular and often too small to allow a nymph to emerge, and secondly because they sometimes contained dried remains of their contents. Hatched eggs showed a straight, longitudinal, dorsal split running from one end for at least half their length and the inside of the empty shell was clean. Collapsed eggs showing no signs of damage to the shell could have succumbed to cold but this seems very unlikely given that no temperatures below -7°C were recorded (Fig. 6.7). More probably they were laid without being fertilized, a possibility revealed by earlier observations in the laboratory (section 6.3), or failed to break diapause for some reason. The former appeared to be the major cause as 13 of the eggs which collapsed had done so by mid-December and most of these had failed to darken completely. A greater proportion of eggs on hedgerow bushes disappeared between mid-December and late February than on woodland bushes, perhaps because of their greater exposure to heavy rainfall during the second half of December (Fig. 6.8). Only one egg in the hedgerow site was collapsed and split, compared to nine in woodland, suggesting that predation may have been more important in the latter situation. However, given the

small number of eggs involved, no firm conclusions can be drawn from the observed differences between sites.

(f) Survival of fundatrices

Although 55 eggs hatched only eight fundatrices on three bushes reached maturity (Table 6.24). The high mortality was probably due to the fact that mild weather during February, which probably stimulated hatching and the first stages of bud burst, was followed by a cold March, which slowed the fundatrices' development and retarded leaf expansion so that few sheltered feeding sites between expanding leaflets were available until mid-April. The higher mortality on the hedgerow bushes (Table 6.24) was probably due to their greater exposure to wind and rain. Four nymphs, two from each site, were found dead with their stylets still inserted. These had probably been killed by inoculative freezing from hoar frost. No natural enemies were found on hedgerow bushes but a total of 10 spiders were found on woodland bushes between 25 February and 2 April, one of which was seen feeding on a fundatrix. Visits to the bushes on 9 April and 20 April represent the first two recording dates of the spring 1980 monitoring period, which is described in section 6.5. In this period the three bushes monitored through the 1979/80 winter were visited together with 10 new bushes with spring colonies.

6.4.4 Discussion

(a) A comparison of winter survival estimates based on the results of colony monitoring and population surveys

From the holocyclic population surveys (section 6.2) it was estimated that the population peak in spring 1980 was only about 7% of that in autumn 1979 (see Table 6.11). However, the numbers of aphids on the bushes monitored through the same period were 163 at the median date of the autumn 1979 survey (1 November) compared to 332 at the median date of the spring 1980 survey (26 April) - that is, spring numbers on monitored bushes were approximately 200%

Table 6.24 Survival of M. dirhodum fundatrices on monitored rose bushes in spring 1980.

(a) Hedge 26: (25 eggs hatched)

INSTAR	DATE				
	25/2	14/3	2/4	9/4	20/4
1st	23				
2nd	1	9			
3rd		4	1		
4th			1	1	
adult					1
Total	24	13	2	1	1

(b) Wood II: (30 eggs hatched)

INSTAR	DATE				
	25/2	14/3	2/4	9/4	20/4
1st	18				
2nd		7			
3rd		9			
4th			10	2	
adult			1	7	6
Total	18	16	11	9	6

of those in the autumn. The same discrepancy is shown when survival of whole colonies is considered: aphids survived to produce spring colonies on three of the 13 monitored bushes (23%) but survival of infested sample units between autumn 1979 and spring 1980 was estimated from survey results as only 10% in dense woodland and 6% in field boundaries (see section 6.2.3). It is clear, therefore, that the results of colony monitoring suggest considerably better survival between autumn and spring colony peaks than do the results from the population surveys. Possible reasons for this discrepancy were explored.

Monitored bushes were similar in size to infested bushes sampled in the autumn survey and had similar aphid population densities. The process of monitoring itself cannot have caused the discrepancy because, if anything, it should have increased mortality, due to disturbance. However, the oviparae found during the autumn survey were younger than those on monitored bushes at the survey's median date (1 November). Of the oviparae found during the survey 66% were first to third instar nymphs, 17% fourth instar nymphs and 17% adults, but on monitored bushes on 1 November 35% were first to third instars, 39% fourth instars and 26% adults. This difference in age structure was almost certainly at least partially responsible for the discrepancy between survey and monitoring results. It occurred because colonies to be monitored had to be selected by searching bushes before the autumn population survey started. Consequently, only those bushes which were colonized relatively early were used. Thus oviparae on the monitored bushes were generally older than those in the rest of the population and were ready to mate and lay eggs earlier. Consequently, they probably had a greater chance of mating successfully and laying eggs before the population of males dwindled and leaf fall eliminated unmated oviparae. This hypothesis is supported by the fact that the discrepancy between survey and monitoring results was greatest in woodland, where leaf fall was earlier than in hedgerows. Thus the results of colony monitoring probably underestimated both the mortality of

oviparae before they could lay eggs and the importance of leaf fall as a mortality factor. But the mortality shown by eggs and fundatrices is likely to have been typical of the rest of the holocyclic population.

The discrepancy between colony monitoring and population surveys may also have been partly due to some sampling error in the population surveys. Population estimates were based on relatively small samples in spring and were therefore very approximate. From experience of searching for fundatrices in early spring 1979 and 1980 (for spring colony monitoring) I consider that the survey in spring 1980 underestimated the population and that it actually represented well over 2% of the spring population in 1979, although there was no doubt it was considerably smaller.

(b) Autumn populations

The timing of autumn populations of M. dirhodum on rose in Hampshire was similar to that found in Holland (Hille Ris Lambers, 1947) with the arrival of gynoparae and the build-up in numbers of oviparae being rather later than for other heteroecious species such as R. padi (Dixon, 1971 ; Leather, 1981b; Leather & Lehti, 1982), R. insertum (Gair, 1953), Aphis fabae (Way & Banks, 1968) and Myzus persicae (Tamaki, Landis & Weeks, 1967). If population density is expressed as the proportion of host plants or host leaves infested, autumn colonies of M. dirhodum on the primary host occurred at much lower densities than did those of the other species listed above. This was probably because rose was much more abundant in the study area used here than were the primary hosts of the other species in the places where they were studied.

The number of eggs laid per ovipara present at the autumn population's peak can be used as a crude measure of autumn colony success. The M. dirhodum colonies studied here produced on average 0.45 eggs per ovipara. Although this is probably an overestimate of average colony success (see above) it is well below the potential fecundity of an ovipara (8.9 eggs, see section 6.3).

However, it is much better than the egg productions found for R. padi (Leather & Lehti, 1982) and M. persicae (Tamaki, 1974). From data for autumn populations of R. padi at three sites in southern Finland in 1981, the number of eggs per ovipara varied from 0.029 to 0.077. For M. persicae in Washington state, U.S.A. in autumn 1971 the equivalent figures for two varieties of peach tree were 0.011 and 0.007. The peak autumn population densities of M. persicae were 34.4 and 78.1 oviparae/leaf (Tamaki, 1974) compared to 2.5 to 9.0 oviparae/leaf for R. padi (Leather & Lehti, 1982) and only 0.26 oviparae/leaf for M. dirhodum in the present study. This suggests that greater success may be linked to lower population density, possibly because populations at high densities may suffer from increased natural enemy attack and greater competition for food and sheltered feeding sites. As a consequence M. dirhodum, with its common primary host, would have an inherent advantage compared to the other heteroecious species studied.

However, it is clear from studies on A. fabae (Way & Banks, 1968) that differences between species unconnected with population density are also important. Although oviparae of A. fabae were most successful when autumn populations were not at extremely high density, even when this was the case (84,600 aphids on three 2 m spindle bushes) 0.4 eggs were laid per aphid, a comparable success to M. dirhodum in the present study. And in the two years when autumn populations of A. fabae were moderate (between 1,000 and 2,000 on the three bushes) the numbers of eggs produced per aphid were considerably higher (1.7 and 3.3).

Colony monitoring clearly showed that leaf fall could be a very important mortality factor but there was little evidence that natural enemies were important. Spiders and anthocorids were the only types found. Although these were more abundant and were recorded attacking M. dirhodum more often on woodland than on hedgerow bushes, mortality caused by factors other than leaf fall (weather factors and/or predation) was much higher in the hedgerow site. This fact, together with the relatively

low numbers recorded, suggests that predators were not important in causing the population decline in either site; but without predator exclusion experiments it is impossible to be certain. Although studies on autumn populations of other species, such as R. padi (Dixon, 1971 ; Leather & Lehti, 1982), R. insertum (Evenhuis, 1968), A. fabae (Way & Banks, 1968) and M. persicae (Tamaki, 1974) have generally revealed a greater diversity of natural enemies (probably because of these species' earlier arrival on their primary hosts) researchers have usually concluded, from the low numbers found, that natural enemies are less important than abiotic factors in reducing aphid numbers.

The generally small impact of natural enemies on holocyclic autumn colonies is probably largely due to the time of year (after most natural enemies have stopped reproducing) and the very transient nature of the aphid population. An exception to the general rule is M. persicae, whose very crowded autumn colonies on peach in Washington, U.S.A., were predated to a significant extent by syrphid larvae after the population had reached its peak (Tamaki et al., 1967; Tamaki, 1974). But even for this species the authors concluded that weather factors were more important early on, and largely determined the peak populations achieved.

(c) Eggs

The overall mortality of the M. dirhodum eggs monitored in the present study (49.1%) was lower than that found for most other aphid species. For example, Dunn & Wright (1955b) estimated egg mortality of Acyrtosiphon pisum eggs on hop trefoil as 83% while Bronson (1935) estimated that of another species of pea aphid, Illinoia pisi, in Wisconsin, U.S.A., as 69.7% when eggs were exposed in small wire mesh cages. Egg mortalities of 70% and 81% have been recorded for R. padi in England and Finland respectively (Leather, 1980a, 1981a) and Gillette & Taylor (1908) record mortalities of up to 99% for aphid eggs on apple. However, Hafez (1961) found only 30% mortality in eggs of Brevicoryne brassicae and for A. fabae

Way & Banks (1964) found that egg mortality was less than 40% on most trees, although the maximum recorded was 73%.

Laboratory experiments described previously (section 2.5.4) showed that eggs of S. avenae and Metopolophium fasciatum can survive low temperatures rarely experienced in a British winter and for other species field and laboratory evidence exists to support the view that few aphid eggs are killed by low temperature in the field (Bronson, 1935; Way & Banks, 1964; Leather & Lehti, 1981).

Peterson (1920) presented laboratory and field evidence suggesting that hatching success of the eggs of two apple aphid species was largely dependent on humidity in the period immediately before hatching. But Way & Banks (1964) found that eggs of A. fabae were unlikely to be killed by low humidity in the field. Dunn & Wright (1955b) considered that rainfall may have been an important factor in reducing the number of eggs of A. pisum on hop trefoil but Way & Banks (1964) appear not to have considered it in their study on A. fabae.

Attempts to determine the impact of natural enemies on egg populations have been made for only two species. Way & Banks (1964) concluded from a predator exclusion experiment in the 1958/59 winter that predation by birds and arthropods was not important in that winter, although Anthocoris nemorum was seen feeding on eggs before the end of October. In the following winter cages which excluded birds but not arthropods were used and it was found that birds did cause significant losses at some sites, by feeding on eggs and by pecking off dormant buds. Way & Banks (1964) also noted heavy mortality apparently due to a fungal infection on one bush. Leather (1981a) used predator exclusion and shelter cages to separate losses due to birds, arthropod predators and rain, and concluded that predation by arthropods was the most important mortality factor and that rain was insignificant. However his conclusion rests on the assumption that a muslin predator exclusion cage had no effect on rainfall, whereas in fact it is likely to have considerably reduced the speed of raindrops and altered the pattern of rainfall and may thus have mitigated the effect of rain. In the present

study 22.2% of M. dirhodum eggs disappeared during the winter. As the proportion of these removed by predators is unknown it is difficult to assess the relative importance of predators and rainfall. However, predation probably caused the loss of between 9.3% and 31.5% of the total eggs laid on stems as most of the 9.3% of eggs found collapsed with the egg-shell split had presumably been attacked by predators.

Oviparae of M. dirhodum, like those of R. insertum (Gair, 1953) and Drepanosiphum platanoides (Dixon, 1976a) but unlike those of R. padi (Rogerson, 1947; Dixon, 1971) and A. fabae (Way & Banks, 1964) do not lay eggs around buds. The tendency to lay eggs singly and scattered over both new and old wood may make them less vulnerable to predation and probably minimizes competition between fundatrices in the spring but has the disadvantage that fundatrices may have to migrate quite long distances to find feeding sites. Thorns, thorn bases and crevices in rough bark (the favoured egg-laying sites) may be positions in which eggs are less likely to be encountered by predators or washed off than if they were laid on smooth bark.

Only one or two of the eggs monitored here were lost due to hedge cutting but it should be pointed out that mortality of this kind may be much higher in narrower, lower, and more closely managed hedges.

(d) Nymphal fundatrices

Between hatching in late February and becoming adult in April, 77% of M. dirhodum fundatrices died on woodland bushes and 96% died on hedgerow bushes. The aphids were particularly vulnerable at this time because few buds had opened, so that sheltered feeding sites were not available. Also, first instar fundatrices had to migrate for quite long distances (over 50 cm in some cases) along exposed stems to reach the buds from their hatching sites. Although the fundatrix mortality recorded may have been above average for M. dirhodum because egg hatch was induced by warm weather in February 1980, followed by a cold March which retarded bud burst, similarly high early spring mortality of nymphal fundatrices before bud burst has been

recorded for R. padi (Dixon, 1971), R. insertum (Evenhuis, 1968), D. platanoides (Dixon, 1976a), A. fabae (Way & Banks, 1968), Macrosiphum solanifolii and Aphis abbreviata (Shands, Simpson & Wave, 1958).

Although Peterson (1918) observed mortality of Rhopalosiphum fitchii fundatrices when they were subjected to temperatures low enough to frost young apple leaves, Evenhuis (1968), Shands et al. (1958) and Way & Banks (1968) found nymphal fundatrices were able to survive sudden drops in temperature to well below 0°C. However, Evenhuis (1968) showed that fundatrices of R. insertum were killed by ice on the body surface at -7.5°C, despite surviving this temperature when exposed dry. At least four of the young M. dirhodum fundatrices monitored in the present study probably died from inoculative freezing from surface ice. Fundatrices are probably particularly susceptible because of their very exposed feeding sites. Way & Banks (1968) suggested that A. fabae fundatrices may have been killed by dessication by dry wind in conditions too cold for nymphs to feed and make up for lost body-water, while Shands et al. (1958) suggested that many days of below average temperatures during the development of M. solanifolii and A. abbreviata fundatrices caused heavy mortality. Evenhuis (1968) also suggested rain, hail and wind as important mortality factors at this time of year, while Dixon (1971 , 1976a) thought both rainfall and predation by birds were important for fundatrices of R. padi and D. platanoides. Authors of the studies mentioned above generally considered that predators killed few nymphal fundatrices although their impact increased later in the spring.

Dixon (1976a) provided an explanation for the fact that fundatrices may hatch before bud burst and suffer heavy and apparently unnecessary mortality as a result. For D. platanoides he showed that fundatrices able to start feeding at bud burst could achieve twice the adult weight (and hence greater fecundity) and their offspring could mature more quickly and also achieve greater size than fundatrices that emerged after bud burst because younger leaves were of higher nutritional quality (Dixon,

1963). The timing of bud burst varied between trees and between years, so that egg hatch could not be exactly synchronized with it. Early egg hatch ensured fundatrices were able to take advantage of the better nutritional quality of the youngest leaves, a benefit which probably outweighed the extra mortality caused by premature hatching of eggs on some occasions.

(e) Loss of potential between the production of oviparae and the maturation of fundatrices

Estimating loss of potential for the different stages between the production of oviparae in October and maturation of fundatrices in April (Table 6.25) shows that egg mortality was relatively unimportant as a cause of loss, compared to mortality of oviparae, the failure of adult oviparae to lay eggs and mortality of fundatrices, particularly when it is borne in mind that losses of potential calculated for the first two stages were likely to be underestimates (see section 6.4.4 (a) above). The overall loss of potential between nymphal oviparae and adult fundatrices was slightly greater in the hedgerow site. The difference was due to greater mortality of nymphal fundatrices, probably due to lack of shelter in the hedgerow site when the fundatrices were forced to feed in exposed positions on the outside of unopen buds. This effect further concentrated the holocyclic population in sheltered woodland areas and thus accentuated the pattern of distribution between habitats which was observed in autumn population surveys and was probably established due to differential settling by gynoparae (see section 6.2.4).

Differences in mortality and/or reproduction between sites between the arrival of gynoparae and egg hatch were small. Although there was greater mortality of nymphal oviparae due to wind and rain in the unsheltered hedgerow this was more than compensated for by extra losses in woodland due to early leaf fall. The slightly lower production of eggs by adult oviparae on the hedgerow bushes may have been associated with the lack of shelter as males, like gynoparae, probably tend to settle in sheltered sites (see section 6.2.4) and adult oviparae

Table 6.25 Loss of reproductive potential (%) in
M. dirhodum colonies on rose monitored
 from autumn 1979 to spring 1980.

Stage	Hedge 26	Wood II
nymphal oviparae † - adult oviparae	69.4	81.4
adult oviparae * † - eggs laid	82.8	74.9
eggs laid ** - eggs hatched	51.0	52.4
eggs hatched - adult fundatrices	96.0	76.7
nymphal oviparae † - adult fundatrices	99.90	99.51

* potential egg production = number of adult oviparae
 x mean potential fecundity
 (8.9 eggs).

** includes eggs laid on leaves.

† probably underestimates of loss of potential in the
 holocyclic population as a whole, because oviparae on
 monitored bushes were produced earlier than the
 average ovipara.

wandering on stems before egg laying would be more vulnerable to wind and rain in unsheltered sites. Egg survival in the two sites was very similar.

As both the number of gynoparae which landed on the monitored bushes and their potential fecundity were unknown, loss of reproductive potential by gynoparae could not be calculated. Two gynoparae were found on leaflets with 18 and 19 first to second instar oviparae, while several others were found with 10 or more. This suggests that the potential fecundity of gynoparae lies between 10 and 20, which is similar to fecundities determined in the laboratory for R. padi (Dixon, 1976b) and S. avenae (Watt, 1979b).

6.5 MONITORING COLONIES FROM APRIL TO JUNE IN 1979 AND 1980

6.5.1 Introduction

The work described below covers spring colony development in 1979 and 1980, from the maturation of fundatrices to the end of the migration from rose by alate fundatrigeniae. In spring 1980 colonies on three of the monitored bushes had been followed since the previous autumn but the remainder and all those used in spring 1979 were found when fundatrices were in their fourth nymphal instar. Only one of the bushes used in spring 1979 was monitored again in spring 1980. However, the colonies studied were of similar size and population density in the two years, and were in the same or similar sites. They were thus broadly comparable. Summer infestations of M. dirhodum were much larger in 1979 than in 1980 (see section 6.1.3) and this made between-year comparisons of spring colony development and success and the timing of migration from rose particularly interesting.

M. dirhodum on rose could not be distinguished in the field from M. fasciatum (see section 6.1.4) so in both years a few alate fundatrigeniae were collected from most of the monitored bushes for laboratory identification. These were identified by Hand and were all found to be

M. dirhodum. Others collected during the spring population surveys were also all found to be M. dirhodum and so it was assumed that M. dirhodum was the only species of Metopolophium present in the spring.

6.5.2 Methods

Thirteen bushes in four or five different sites (Table 6.26) were monitored in each year. Hedges 78 and 80 were the main hedgerow sites used. Both were relatively open and unmanaged roadside hedges, 5 m or more tall and between 4 and 8 m wide. They consisted mainly of hazel (Corylus avellana L.) and hawthorn (Crataegus monogyna Jacq.) but contained a wide variety of other shrubs as well as frequent tall ashes (Fraxinus excelsior L.), some other deciduous trees and occasional large yews (Taxus baccata L.). Hedge 11 was a shelter belt of tall scots pines (Pinus sylvestris L.) about 15 m wide with a mixed understorey. Hedges 78, 80 and 11 all provided much greater shelter than hedge 26, which was described in section 6.4.2. The woodland sites were described previously (section 6.1.2). Some of the woodland bushes were in dense areas under a closed canopy while others were in open areas or at the edges of clearings. Large bushes and those densely tangled with other vegetation could not be monitored successfully without disturbance and so the bushes used were relatively low and small (Table 6.27). One of the bushes monitored in 1980 and two in 1979 were Rosa rubiginosa agg. All the others were R. canina agg. There was no indication that the performance of M. dirhodum was different on the two species.

In both years, bushes were visited at 6 to 10 day intervals between the maturation of fundatrices and the end of spring migration, with the exception of an unavoidable gap of 14 days in May 1980. The recording procedure was similar to that described in section 6.4.2, but colonized leaves were not tagged. Special care was needed as the nymphs occupied sheltered feeding positions between still-folded leaflets. Consequently recording was postponed during periods of rain or high winds. Thus some recording sessions were spread over 3 or 4 days. In

Table 6.26 Numbers of rose bushes monitored
in different sites.

Site	Spring 1979	Spring 1980
hedge 78	6	5
hedge 80	2	1
hedge 11	1	
hedge 26		1
wood II	4	3
wood I		3

Table 6.27 Height and size of monitored bushes.

	Spring 1979:			Spring 1980:		
	mean	range	total	mean	range	total
Height	1.6 m	.6-3.5 m	-	1.1 m	.25-2 m	-
No. of buds	39	11-85	510	52	17-189	680

referring to them, mean dates are used.

It was possible to distinguish adult fundatrices from adult apterous fundatrigeniae in the field because the former became gradually darker with age so that there was an obvious colour difference between the two generations by the time the first fundatrigeniae reached adulthood. The two types of apterae also differed slightly in their proportions, the fundatrices being stouter with slightly shorter appendages. First and second generation fundatrigeniae could not be separated however.

Young fundatrigeniae of M. dirhodum can be distinguished from nymphs of other rose aphids but this sometimes requires careful examination which causes disturbance. Consequently, other aphid species were removed when found, to minimize this problem. Aphid-parasite mummies were collected and the parasites hatched out and identified in the laboratory. Only a few mummies were found and these all appeared so late that nearly all M. dirhodum would have left the bushes by the time the adult parasites emerged. Consequently, this removal is unlikely to have altered the course of aphid population change. Other natural enemies were not collected from the monitored bushes except at the last visit in each year but some were collected earlier from other rose bushes.

Population densities of aphids and natural enemies are expressed below as numbers per bud. It should be noted that there is an element of bias in this density parameter as early on buds consisted of only a few unexpanded leaves but by June some had developed into quite large shoots. In 1980 the state of the buds was recorded at the start of monitoring (9 April) and near the end of the spring migration period (28 May). For this, leaves were classified as unopen (with their leaflets completely folded and packed closely together), partially open (with their leaflets partially unfolded and separated, with crevices between) and fully open (with their leaflets well separated and more or less flat). The number of leaves in each category was recorded for each bud.

6.5.3 Results

(a) Population build-up and the timing of spring migration

1. Spring 1979

Population change in spring 1979 is summarized in Table 6.28 and Fig. 6.12 and weather conditions are summarized in Table 6.30 and Fig. 6.14.

When monitoring started on 20 April half the fundatrices were adult. The remainder matured by 1 May. After this the number of fundatrices declined steadily and the last survivor was recorded on 31 May. The 41 fundatrices which reached adulthood reproduced extremely rapidly through the first half of May producing a peak population of over 1,500 aphids by 16 May. The great majority of first generation fundatrigeniae were alatform. As recording dates were about a week apart many fundatrigeniae recorded as alatform fourth instars on one visit must have moulted to alatae and flown by the next visit. Because of this, numbers of alatform fourth instars as well as of adult alatae were taken into account when estimating the timing of spring migration. In 1979 it was estimated that migration started on about 12 May and continued until about 8 June, though the large bulk of migrants had probably left before the end of May.

On six bushes some first generation fundatrigeniae developed into apterous adults in mid-May - on three bushes the number per fundatrix was as high as four. An estimated total of 38 adult apterous fundatrigeniae were produced, only three less than the number of adult fundatrices. But these second generation apterae were evidently much less successful at producing progeny than their parents, as there was no sign of a second peak of migrants at the end of May. However, some of the alatae migrating in early June were probably their offspring. Possible reasons for the reproductive failure of apterous fundatrigeniae are discussed below (section 6.5.4). Changes in the numbers of apterous fourth instars and adults suggest that although most of the apterous fundatrigeniae reaching adulthood were produced quite early on in the reproductive life of fundatrices, production of

nymphs destined to be apterae continued after this, possibly even into a third generation.

2. Spring 1980

Population change in spring 1980 is summarized in Table 6.29 and Fig. 6.13 and weather conditions are summarized in Table 6.30 and Fig. 6.15.

On 9 April 87% of the buds had burst. On average there were 0.9 unopen leaves, 0.8 partially open leaves and 0.7 fully open leaves per bud (including unburst buds). There was little variation between bushes, the number of partially and fully open leaves per bud varying from 0.7 to 2.6. By 28 May there were on average 0.3 unopen leaves, 0.4 partially open leaves and 4.1 fully open leaves per bud. The number of partially and fully open leaves per bud varied from 3.2 to 8.7 on different bushes. Partially open leaves provided the most shelter and were the preferred feeding sites. They were probably also nutritionally superior to fully open leaves. On some bushes unopen and partially open leaves on several buds were eaten by Lepidoptera larvae in late May.

Due to early egg hatch during a mild February (see section 6.4) fundatrices matured about 10 days earlier in 1980 than in 1979. Lower initial numbers of fundatrices and greater loss between the first and second recording dates meant that only 21 fundatrices reached adulthood on monitored bushes in 1980 compared to 41 in 1979. Colony development proceeded even more rapidly than in 1979, probably due to warmer weather during April, and the first alatae were recorded on 26 April, about two weeks before the estimated start of migration in 1979. Despite higher May temperatures spring migration was much more sustained than in 1979 with the numbers of alatae and alatform fourth instars staying more or less constant throughout May with two shallow peaks at the beginning and end of the month, followed by a rapid decline in early June.

This difference in the pattern of migration was the result of greater production of apterous fundatrigeniae in 1980, and possibly greater reproductive success by these apterae, resulting in the production of a significant number of second generation fundatrigeniae. These probably

Table 6.28 Population change in spring colonies of
M. dirhodum on rose in 1979.

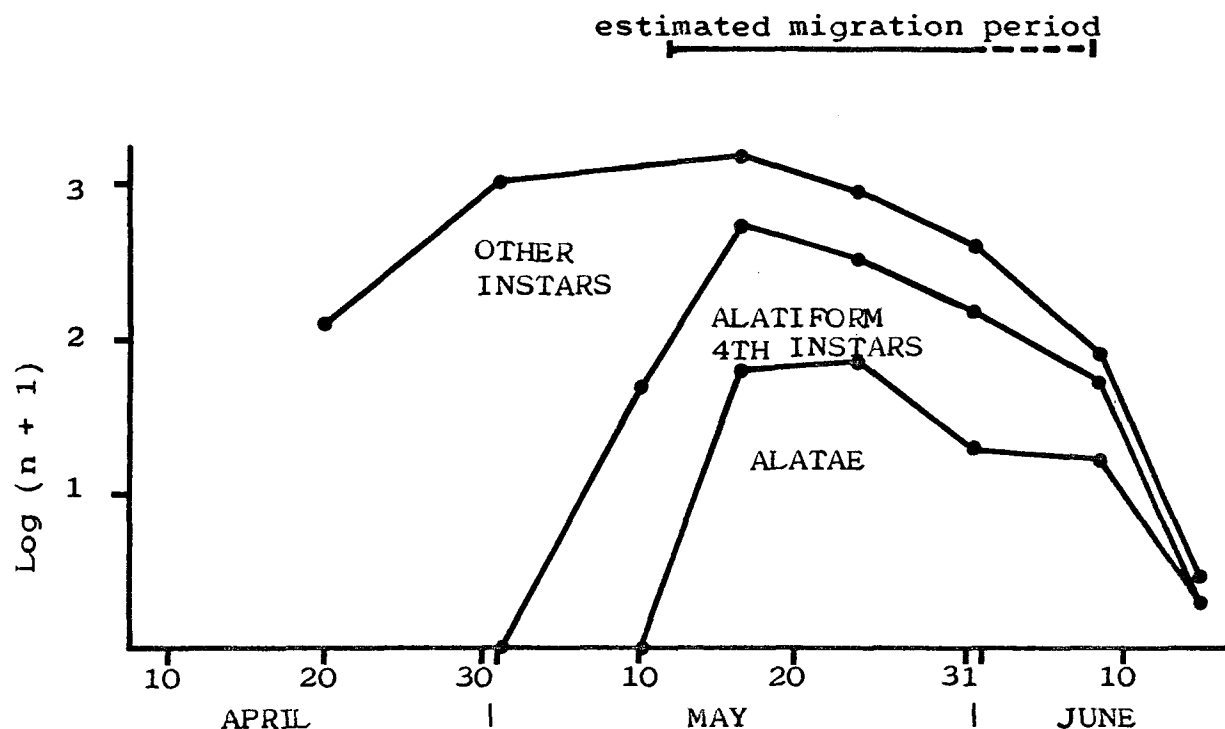
Morphs and instars	Dates 20/4	1/5	10/5	16/5	24/5	31/5	8/6	15/6
<u>Fundatrices:</u>								
4ths	21							
adults	21	38	27	14	3	1		
<u>Fundatrigeniae:</u>								
* small 1st-3rds	119	1,010	540	461	304	88	8	
* large 1st-3rds			727	484	269	150	19	1
apterous 4ths			7	5	6	10	1	
adult apterae				34	11	9		
alatiform 4ths			49	483	254	133	38	
adult alatae				64	69	18	16	1
<u>Total:</u>	161	1,048	1,350	1,545	916	409	82	2

* 'small 1st-3rd' instars = 1st instars and small 2nd instars.

'large 1st-3rd' instars = large 2nd instars and 3rd instars.

Fig. 6.12 Population change in the *M. dirhodum* colonies on rose monitored in spring 1979.

(a) Total numbers, numbers of alatae and alatiform 4th instars.



(b) Numbers of adult apterae and apterous 4th instars.

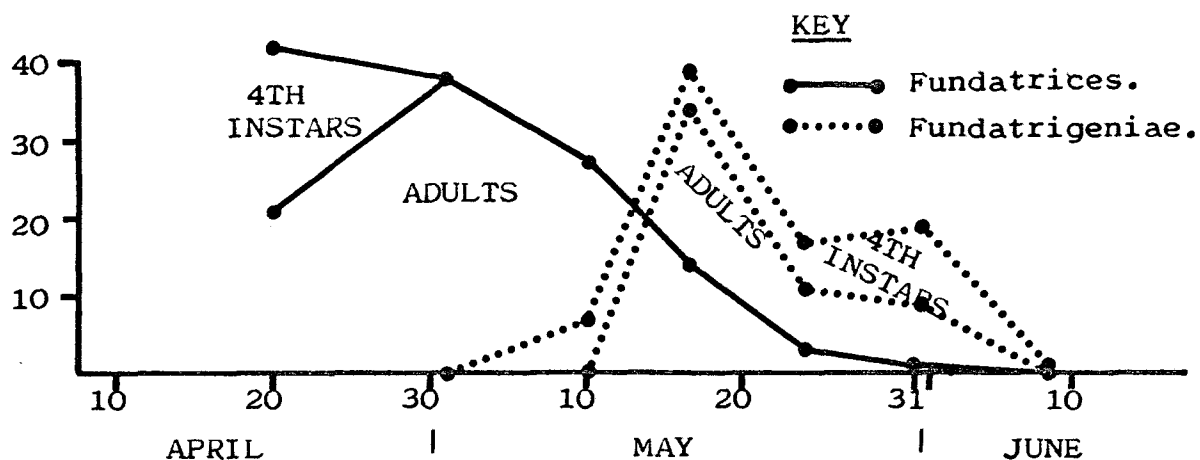


Table 6.29 Population change in spring colonies of
M. dirhodum on rose in 1980.

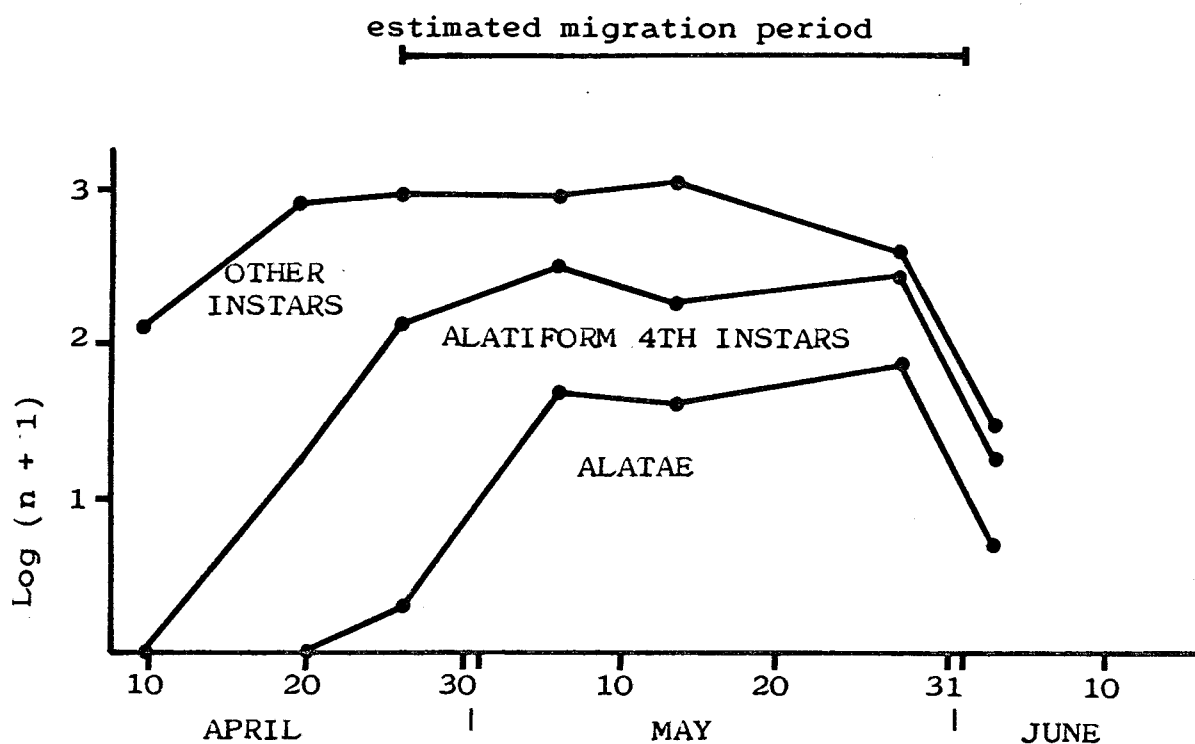
Morphs and instars	Dates						
	9/4	19/4	26/4	6/5	13/5	28/5	3/6
<u>Fundatrices:</u>							
4ths	17						
adults	13	17	11	1			
<u>Fundatrigeniae:</u>							
* small 1st-3rds	82	264	232	321	574	35	4
* large 1st-3rds	15	489	507	214	296	70	7
apterous 4ths		19	38	7			
adult apterae			16	48	30	5	1
alatiform 4ths		16	132	270	142	210	13
adult alatae			1	48	41	72	4
<u>Total:</u>	127	805	937	909	1,083	392	29

*'small 1st-3rd' instars = 1st instars and small 2nd instars.

'large 1st-3rd' instars = large 2nd instars and 3rd instars.

Fig. 6.13 Population change in the M. dirhodum colonies on rose monitored in spring 1980.

(a) Total numbers, numbers of alatae and alatiform 4th instars.



(b) Numbers of adult apterae and apterous 4th instars.

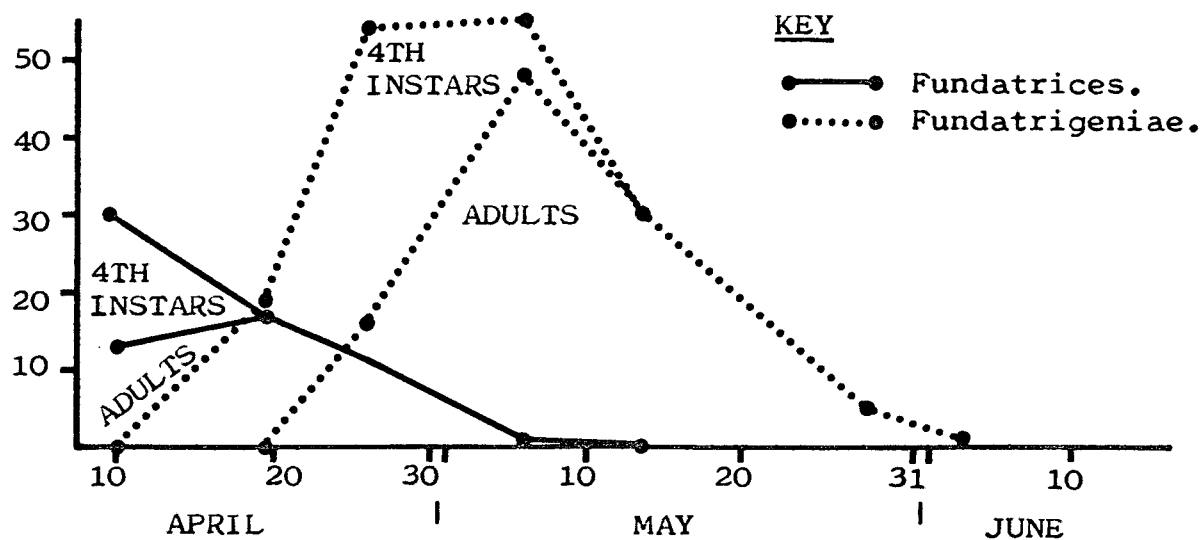


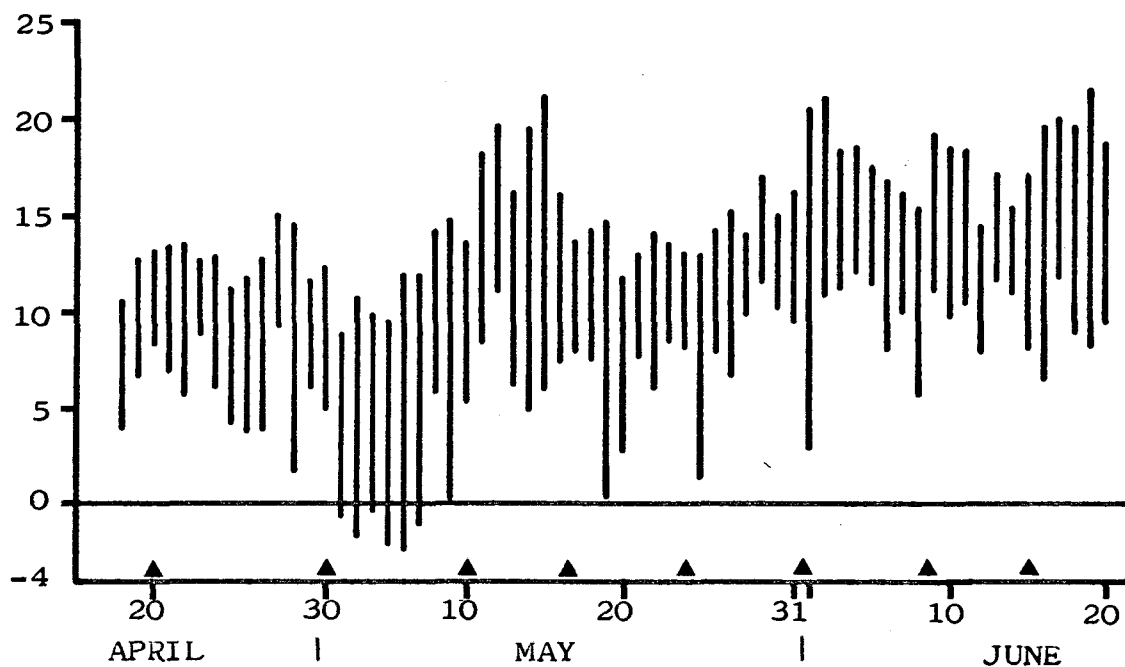
Table 6.30 Monthly weather data for spring 1979 and spring 1980, recorded at Hurn Airport.

	February	March	April	May	June
Mean temperature (°C)					
1979	2.05	5.25	8.25	9.70	13.85
1980	6.50	5.25	9.05	11.2	14.05
Total rain (mm)					
1979	67.3	93.9	56.1	113.7	35.4
1980	77.0	94.6	30.7	32.4	109.0
Mean wind speed (knots)					
1979	8.1	11.4	8.5	8.4	6.6
1980	7.9	9.4	7.2	7.7	7.9

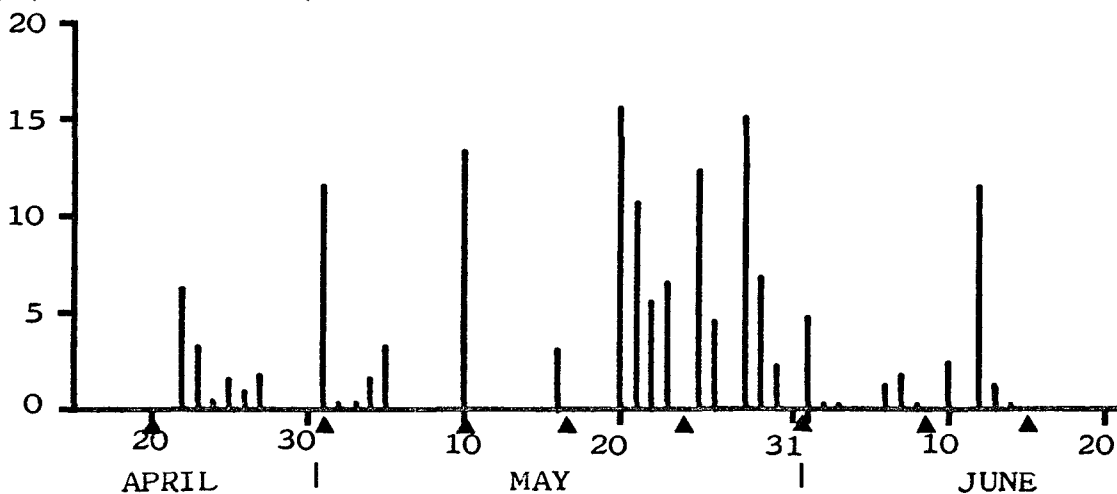
Fig. 6.14 Weather data for spring 1979 recorded at Hurn Airport.

▲ = aphid recording dates.

(a) Temperature ($^{\circ}\text{C}$)



(b) Rainfall (mm)



(c) Mean wind speed (knots)

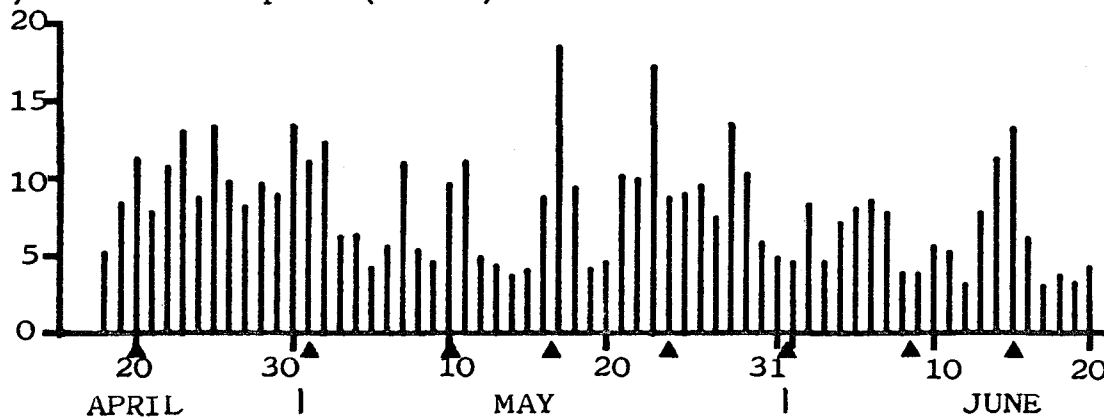
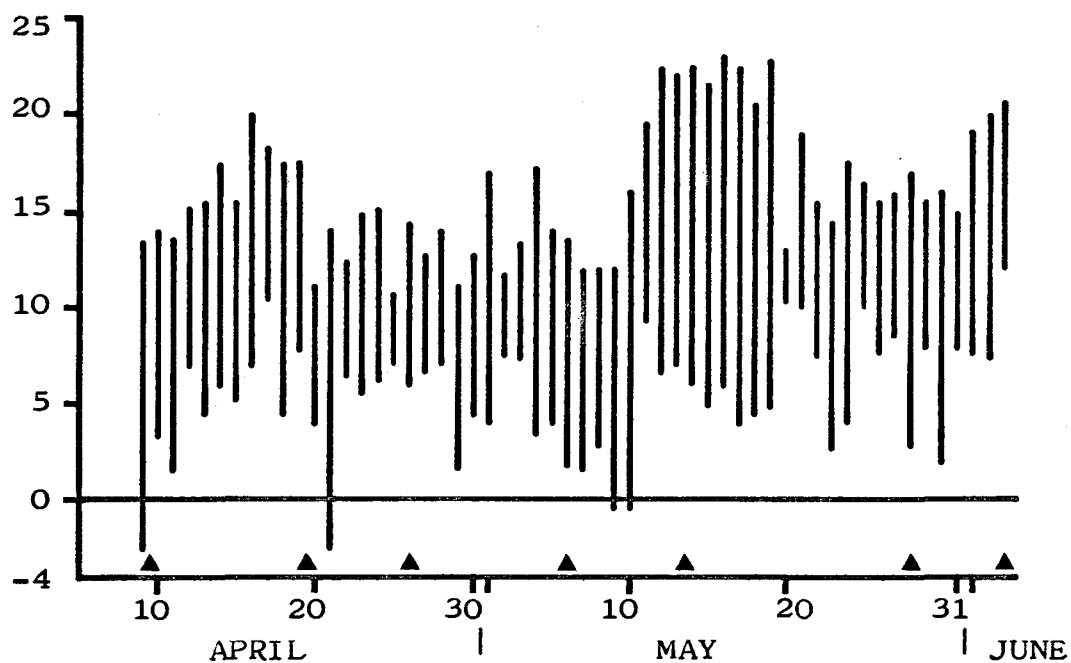


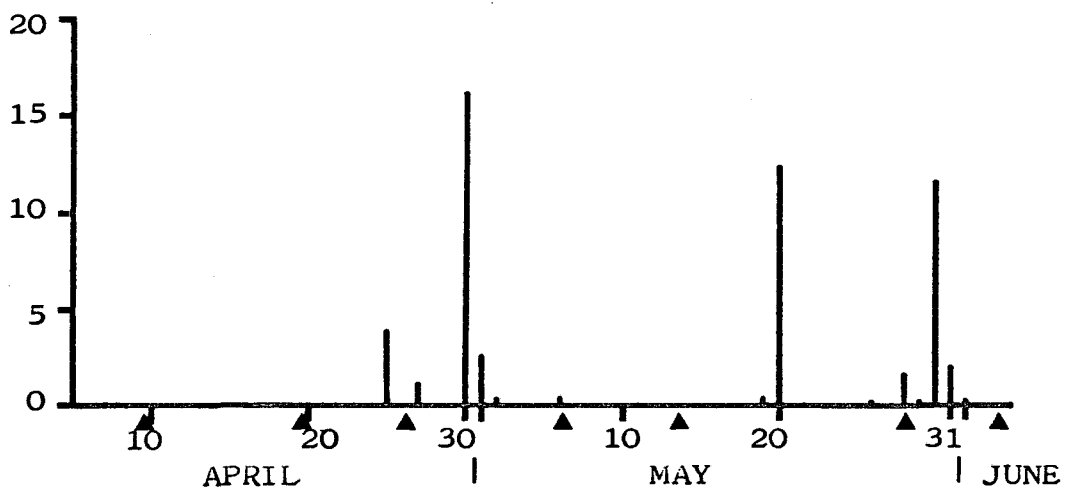
Fig. 6.15 Weather data for spring 1980 recorded at Hurn Airport.

▲ = aphid recording dates.

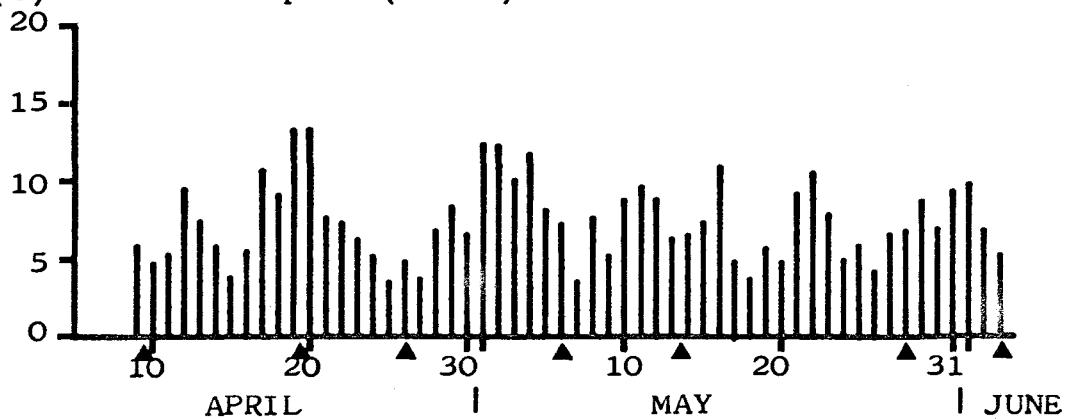
(a) Temperature ($^{\circ}\text{C}$)



(b) Rainfall (mm)



(c) Mean wind speed (knots)



constituted the majority of alatae and alatiform fourth instars recorded in the second half of May. But, as in 1979, it appears that apterous fundatrigeniae were much less successful at producing migrants than were the fundatrices, as despite 53 apterous fundatrigeniae reaching adulthood compared to only 21 fundatrices, the second peak of alatae and alatiform fourth instars at the end of May was only slightly larger than the first peak, which must have been produced exclusively by fundatrices.

Single gynoparae appeared on four of the monitored bushes during spring 1980. The first of these was recorded on 26 April and was collected for identification with the 10 oviparae it had produced. The second was found on 6 May and was left undisturbed. It had produced 20 oviparae before monitoring was stopped, 18 of which reached adulthood, though no males arrived. The last two gynoparae were found with one or two nymphal oviparae on 3 June. These gynoparae had presumably been produced by M. dirhodum viviparae surviving on Gramineae through the mild 1979/80 winter which had continued to respond to short daylength by producing some gynoparae through the winter and spring.

(b) Natural enemies

In both 1979 and 1980 spiders (Araneae) were by far the most abundant predators on the monitored bushes (Tables 6.31 and 6.32 and Fig. 6.16). They also appeared earlier than other predators. After the hard winter of 1978/79 they were scarce until mid-May, by which time M. dirhodum's migration from rose had already started. With milder winter and spring weather in 1979/1980 spiders appeared earlier and were rather more abundant, with a mean population density over all bushes and all visits of 4.5 per 100 buds, compared to 3.5 per 100 buds in 1979*. However, the peak population density achieved was similar in the two years and in both was less than 6% of the peak density of aphids. Because of this, and because the spider pop-

*In these calculations the 8th visit in 1979, when only 2 aphids were present, has been ignored to make the two years comparable.

Table 6.31 Predators found on monitored rose bushes in spring 1979.

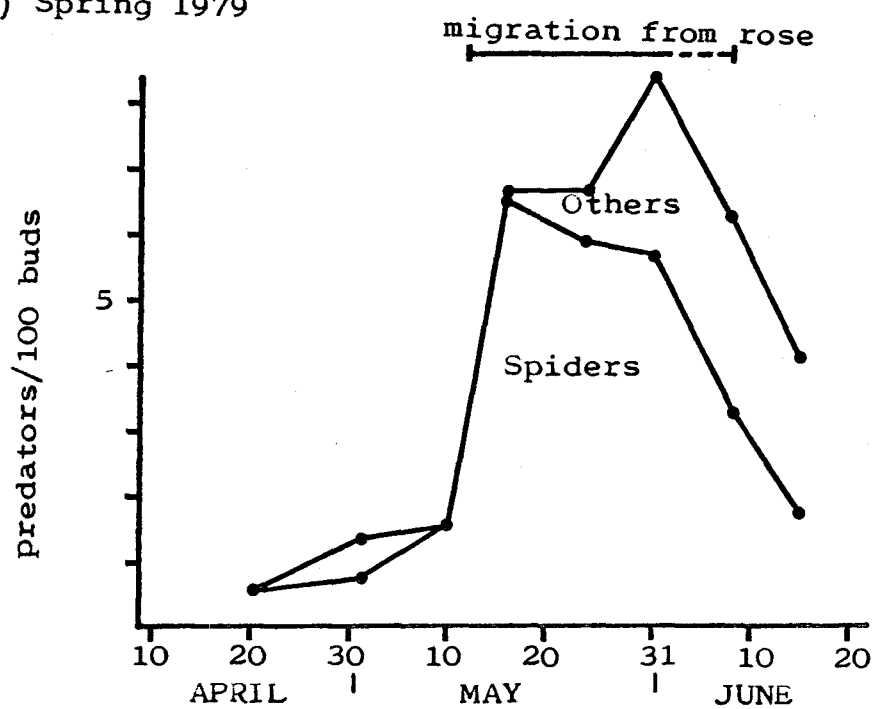
	20/4	1/5	10/5	16/5	24/5	31/5	8/6	15/6	Total
Araneae	3	4	8	33	30	29	16	9	132
Syrphidae unhatched eggs				12	23	18	13	11	(77)
larvae					3	8	5	4	20
Miridae (nymphs)					1	1	8	7	17
Coccinellidae adults						1			1
larvae							1		1
Staphylinidae						2			2
Cantharidae						1	1		2
Formicidae		3							3
Chrysopidae						1			1
Hemerobiidae				1					1
Tettigonioidae								1	1
Forficulidae							1		1

Table 6.32 Predators found on monitored rose bushes in spring 1980.

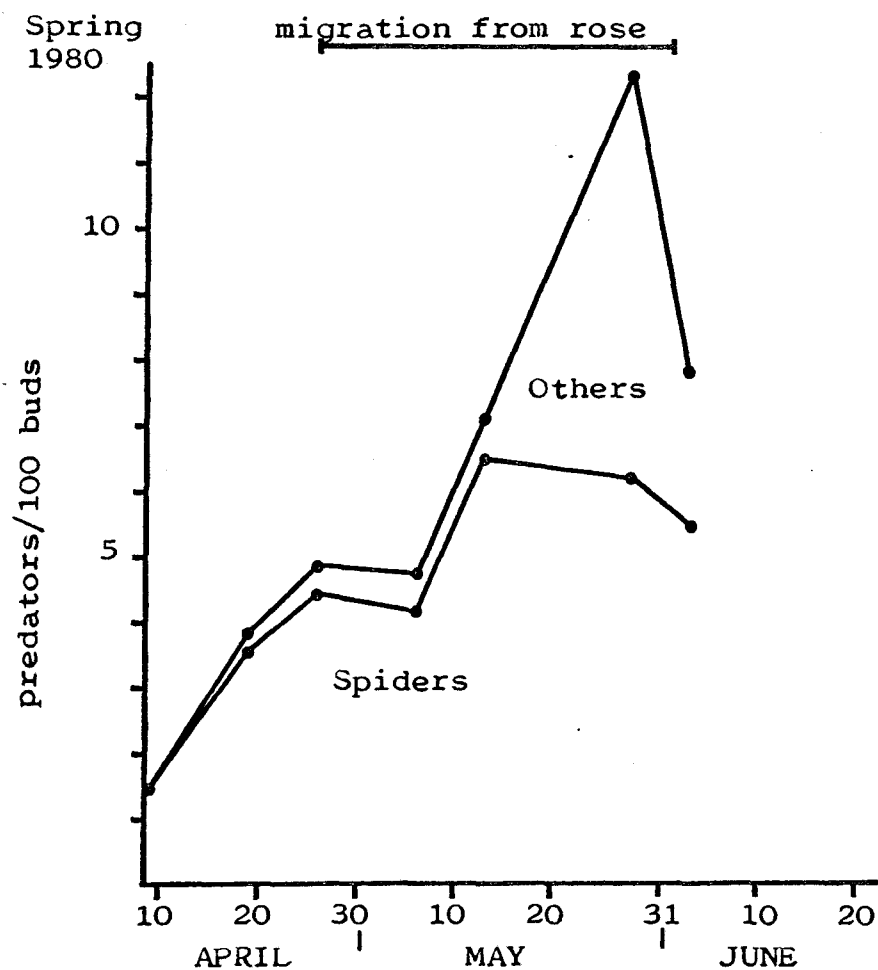
	9/4	19/4	26/4	6/5	13/5	28/5	3/6	Total
Araneae	10	24	30	28	44	42	37	215
Syrphidae								
unhatched eggs		3	1	3	5	56	17	(85)
larvae			2	2	2	36	3	45
Miridae (nymphs)					1	5	10	16
Anthocoridae (adults)		1	1	1	1			4
Coccinellidae (eggs)						26	20	(46)
Staphylinidae		1					1	2
Cantharidae							1	1
Hemerobiidae				1				1
Forficulidae							1	1
Opiliones						1		1

Fig. 6.16 Population densities of predators on rose.

(a) Spring 1979



(b) Spring 1980



ulation did not peak until after spring migration had begun, it is unlikely that spiders had more than a relatively small modifying influence on the numbers of alatae migrating from rose, although without predator-exclusion studies their effect is impossible to determine accurately.

In both years the large majority of spiders recorded were 1 to 3 mm long (over 90% in 1979 and over 75% in 1980). Nearly all those found feeding on aphids were in this size range. From notes on the spiders and their webs and identification of the samples collected from similar sites, the great majority of the small spiders were Theridiidae (mainly Theridion spp.). The larger spiders (≥ 4 mm) were mainly a mixture of Theridiidae, Argiopidae and Lynphiidae with some Clubionidae and one or two Thomisidae and Anyphaenidae (Locket & Millidge, 1951, 1953). The diversity was greater in 1980 than in 1979. In both years there was a tendency for spiders to appear earlier and be more numerous on woodland bushes compared to those in hedgerows. But there was much variation between bushes and a significance test on mean population density on different bushes shows this trend was not statistically significant (Mann-Whitney U test: 1979: $U = 10$, $n_1 = 4$, $n_2 = 9$, $P > 0.1$; 1980: $U = 10$, $n_1 = 6$, $n_2 = 7$, $P > 0.05$).

Apart from one adult coccinellid beetle, the only predators other than spiders seen feeding on aphids were syrphid larvae. In 1979 too few were present to have had any significant impact (Table 6.31) but in 1980 one hedgerow bush was quite heavily infested on 28 May, with 31 larvae present. However, the late date of this infestation meant that despite heavy predation the M. dirhodum colony on this bush was not particularly unsuccessful in producing migrants. From the few larvae collected from rose and reared to adults, the commonest syrphid present was Melanostoma scalare (Fab.) with Baccha obscuripennis (Meigen) (Ver^rall, 1901) also present. No anthocorids were found in 1979 and only four (all adults) in 1980. All the other Heteroptera found were mirid nymphs. From notes taken in the field and the subsequent rearing of a sample, the only potential aphid predator present was

Plagiognathus arbustorum (Fab.), a species which took aphids when reared in the laboratory and is known to be both a plant-feeder and a predator (Southwood & Leston, 1959). The only other common species of mirid found was the common green capsid Lygocoris pabulinus (L.) which is apparently entirely phytophagous (Southwood & Leston, 1959). All other types of predators appeared late and were only present in ones and twos (Tables 6.31 and 6.32).

No hymenopteran aphid parasites were seen on the bushes but a few mummies were found in late spring in both years (Tables 6.33 and 6.34). The presence of five mummies of Macrosiphum sp. was due to some alate viviparae, mainly of M. rosae, depositing young on several of the monitored bushes in late spring. More mummies were found in 1979 than in 1980 which suggests that aphid parasites were well able to survive the harsh 1978/79 winter. Adult parasites were hatched out in the laboratory and identified using Powell (1982) and Stary (1966). Interestingly, only one Aphidius sp. was found, while the large majority of parasites were Praon volucre, a species which is found attacking cereal aphids less commonly than Aphidius sp. when the aphids are on Gramineae. As with the predators other than spiders, hymenopteran parasites appeared to arrive too late and in too low numbers to have had more than a very minor effect on the numbers of M. dirhodum migrating from rose. However, it may be that some of the migrating alatae contained parasite eggs or young larvae. If this was so, pre-migration parasitism might have been important, particularly after a harsh winter, in providing a means by which parasites could be carried from hedgerow and woodland habitats with their hosts onto the aphids' summer host plant. However, a large proportion of the M. dirhodum mummies found in the spring were those of apterous fourth instars and adults (Tables 6.33 and 6.34). This suggests that the development of a parasite larva inside an aphid inhibits the development of wings by the host.

(c) Aphids found dead on the monitored bushes

The corpses of aphids dying from different causes

Table 6.33 Parasite mummies on monitored rose bushes in spring 1979.

Date	Site	Parasite	Host	Host morph & instar
1/6	Wood	<u>Praon volucre</u>	<u>M. dirhodum</u>	apterous 4th
"	"	" "	" "	alatiform 4th
8/6	Hedge	<u>Praon volucre</u>	<u>Macrosiphum</u> sp.	alatiform 4th
"	"	" "	<u>M. dirhodum</u>	" " "
"	"	" "	" "	" " "
"	"	" "	" "	apterous 4th
"	"	" "	" "	" " "
"	"	<u>Ephedrus plagiator</u>	" "	" " "
"	"	" "	" "	" " "
"	Wood	<u>Praon volucre</u>	<u>Macrosiphum</u> sp.	" " "
"	"	" "	<u>M. dirhodum</u>	adult aptera
"	"	<u>Ephedrus plagiator</u>	" "	adult aptera
15/6	Hedge	<u>Praon volucre</u>	<u>M. dirhodum</u>	apterous 4th
"	"	" "	" "	apterous 4th
"	Wood	" "	<u>Macrosiphum</u> sp.	adult aptera

Table 6.34 Parasite mummies on monitored rose bushes in spring 1980.

Date	Site	Parasite	Host	Host morph & instar
28/5	Hedge	<u>Praon volucre</u>	<u>M. dirhodum</u>	alatiform 4th
"	Wood	" "	" "	apterous 4th
"	"	" "	" "	alatiform 4th
"	"	" "	" "	" " "
"	"	<u>Aphelinus</u> sp.	" "	apterous 4th
"	"	<u>Aphidius ervi</u>	<u>Macrosiphum</u> sp.	adult aptera
3/6	Wood	<u>Praon</u> sp.*	<u>M. dirhodum</u>	apterous 4th
"	"	<u>Praon volucre</u>	" "	" " "
"	"	" "	<u>Macrosiphum</u> sp.	" " "

* hyperparasitised by Alloxysta sp.

have different probabilities of remaining on the bushes. For example, a relatively large proportion of those killed by spiders will be found because they remain attached to the leaves by webs. Aphids killed by other predators will be much more likely to drop off, but nearly all parasitized aphids should be found as mummies, unless they migrate from the host plant before mummification. (This behaviour is usual for aphids parasitized by Toxares del-tiger (Powell, 1982) but not for those parasitized by the other British cereal aphid parasites.) Aphids removed by wind will obviously not be found, but those killed by rain or low temperature may be. Despite these differences, the numbers of corpses found on the bushes (Table 6.35) may be used to very roughly assess the relative importance of certain mortality factors on different visits in the same year and between years. Records of the positions of the corpses show that very few remained from one recording date to the next.

Although the numbers of aphids found being eaten by spiders were similar in 1979 and 1980, spiders probably had greater impact on M. dirhodum colonies in the second year as over twice as many corpses were found dead in webs in 1980. It is impossible to tell what proportion of these were actually caught by spiders and what proportion stumbled into unoccupied webs. The 11 aphids found being eaten by syrphid larvae in 1980 were all on the one heavily infested hedgerow bush. Forty-two shrivelled corpses found on this bush at the same time had probably also been killed by syrphids. A number of other aphids whose cause of death was uncertain were probably killed by predators but in 1979 many aphids in this category appeared to have drowned when heavy rain during May (Fig. 6.14 (b)) filled the crevices between folded leaflets with water. It is not surprising, therefore, that the numbers of dead aphids whose cause of death was uncertain were lower during the dry spring of 1980 (Fig. 6.15 (b)). In neither spring did temperatures fall low enough to cause low temperature mortality (Figs 6.14 (a) and 6.15 (a)).

The proportion of the M. dirhodum population found dead on each recording date tended to increase through the

Table 6.35 M. dirhodum found dead on monitored rose bushes in spring 1979 and spring 1980.

(Percentages are of the arithmetic mean of aphids present at the recording date in question and the previous one - a crude estimate of the mean number of live aphids present in the interval between recording dates.)

	being eaten by spiders	dead in spider's web	being eaten by syrphids	para- sitized	cause of death uncer- tain	total
	no. %	no. %	no. %	no. %	no. %	no. %
<u>1979:</u>						
20/4						
1/5						
10/5		1 (.08)			19 (1.58)	20 (1.67)
16/5	3 (.21)	3 (.21)			18 (1.24)	24 (1.66)
24/5	4 (.33)	13 (1.06)			19 (1.54)	36 (2.93)
31/5*	3 (.45)	6 (.91)	1 (.15)	2 (.30)	25 (3.77)	37 (5.58)
8/6			1 (.41)	8 (3.26)	5 (2.04)	14 (5.70)
15/6						
<u>1980:</u>						
9/4						
19/4		11 (2.36)			7 (1.50)	18 (3.86)
26/4	2 (.23)	10 (1.15)			3 (.34)	15 (1.72)
6/5	5 (.54)	10 (1.08)			1 (.11)	16 (1.73)
13/5		14 (1.41)			20 (2.01)	34 (3.41)
28/5	1 (.14)	8 (1.08)	11 (1.49)	5 (.68)	42 (5.69)	67 (9.08)
3/6		1 (.48)		2 (.95)	2 (.95)	5 (2.38)

* one aphid being eaten by adult coccinellid.

spring, particularly in 1979. This suggests that increased mortality was partially responsible for the eventual disappearance of the aphids from rose. But the relatively low numbers of dead aphids found and the low numbers of natural enemies present, even in late May and June, suggests that reduced fecundity of apterous fundatrigeniae played an important role, perhaps the dominant one.

(d) The success of colonies

The best measure of a spring colony's success is the total number of alatae which migrate from it, divided by the number of fundatrices present initially. Alatae probably stay only a few days on rose before migrating and so in the present study, in which recording dates were six to 10 days apart, many must have gone unrecorded. Because of this the numbers of alatiform fourth instars as well as alatae were taken into consideration when calculating an index of success for each monitored colony. The 'success index' calculated for the colony on each bush was:

$$\frac{\text{total number of alatae and alatiform fourths recorded on all visits}}{\text{peak number of adult fundatrices}}$$

Considering all 13 colonies monitored in each year together, the overall success indices for spring 1979 and spring 1980 colonies were 27.4 and 45.2 respectively. However, there was much variation between bushes and when the success indices for each were considered separately a Mann-Whitney U test showed that the difference between years was not statistically significant ($U = 61$, $n_1 = n_2 = 13$, $P > 0.1$).

Treating the two years separately, Kendall's rank correlation coefficients between colony success indices and several variables which might have influenced colony success were calculated (Table 6.36). These variables, and the reasons for their choice, are given below:

1. Density dependent effects: The initial population density of different colonies varied from 1.6 to 25.0 adult fundatrices per 100 buds in 1979 and from 0.5 to 8.2 in 1980. If some density dependent

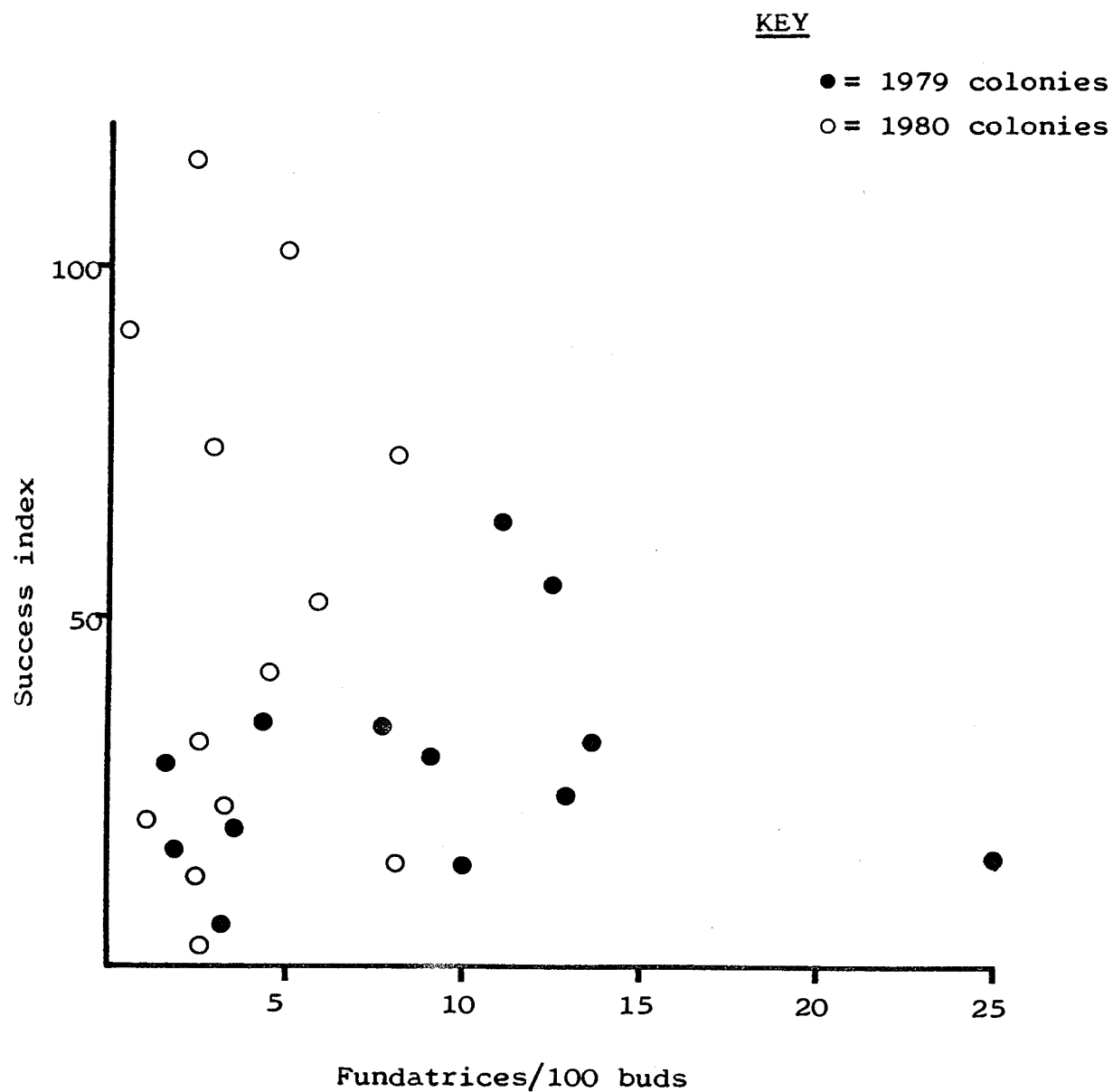
Table 6.36 Kendall's rank correlation coefficients
between the success index of M. dirhodum
spring colonies on rose and some variables
thought to affect colony success.

Other variable	1979		1980	
	τ	P	τ	P
Initial population density (adult fundatrices/bud)	.12	.29	-.15	.24
Apterous fundatrigeniae production (apterous fundatrigeniae/ fundatrix)	.15	.23	.34	.053
Shelter (ranked on scale 1 to 6)	.29	.08	-.11	.30
Spider abundance (total spiders/bud)	-.19	.18	0	.50
Bud growth stage on 9 April (mean partially and fully open leaves per bud)	-	-	-.13	.27
Bud growth stage on 28 May (mean partially and fully open leaves per bud)	-	-	-.06	.42
Index of mean duration of adult life for fundatrices (fundatrix aphid-days/ number of fundatrices)	.51	.008	.37	.040

factor - such as competition for food or sheltered feeding positions, or an aggregative response by natural enemies - limited colony success, success indices should have been inversely correlated with initial population density, but there was no sign that this was so (Table 6.36, Fig. 6.17).

2. The production of apterous fundatrigeniae: This provides an obvious way of boosting the colony's potential for producing migrants. If apterous fundatrigeniae were reasonably successful at producing migrants colony success should be correlated with the number of adult apterous fundatrigeniae produced per fundatrix. In 1979 some apterous fundatrigeniae reached adulthood on six bushes and on these the number per fundatrix varied from 0.5 to 4.0. But there was no sign of a significant correlation with colony success (Table 6.36). In 1980 apterous fundatrigeniae reached adulthood on nine bushes, with the number per fundatrix varying from 1.7 to 7.3. The correlation with colony success was close to statistical significance ($P = 0.053$) (Table 6.36) which suggests apterous fundatrigeniae played a more important role in the production of migrants than in 1979. This is also suggested by the pattern of population change (Fig. 6.13). In viviparae of many aphid species crowding is an important stimulus to the production of alatae. From this, one might expect fundatrices on the less crowded bushes to produce the most apterous fundatrigeniae. This supposition was supported by the fact that in 1980, when average population density on monitored bushes was lower than in 1979, significantly more apterous fundatrigeniae were produced per fundatrix (Mann-Whitney U test: $U = 42.5$, $n_1 = n_2 = 13$, $P < 0.05$). However, when the relationship between the production of apterous fundatrigeniae per fundatrix and a colony's peak population density was investigated for colonies in the same year, there was no suggestion that the

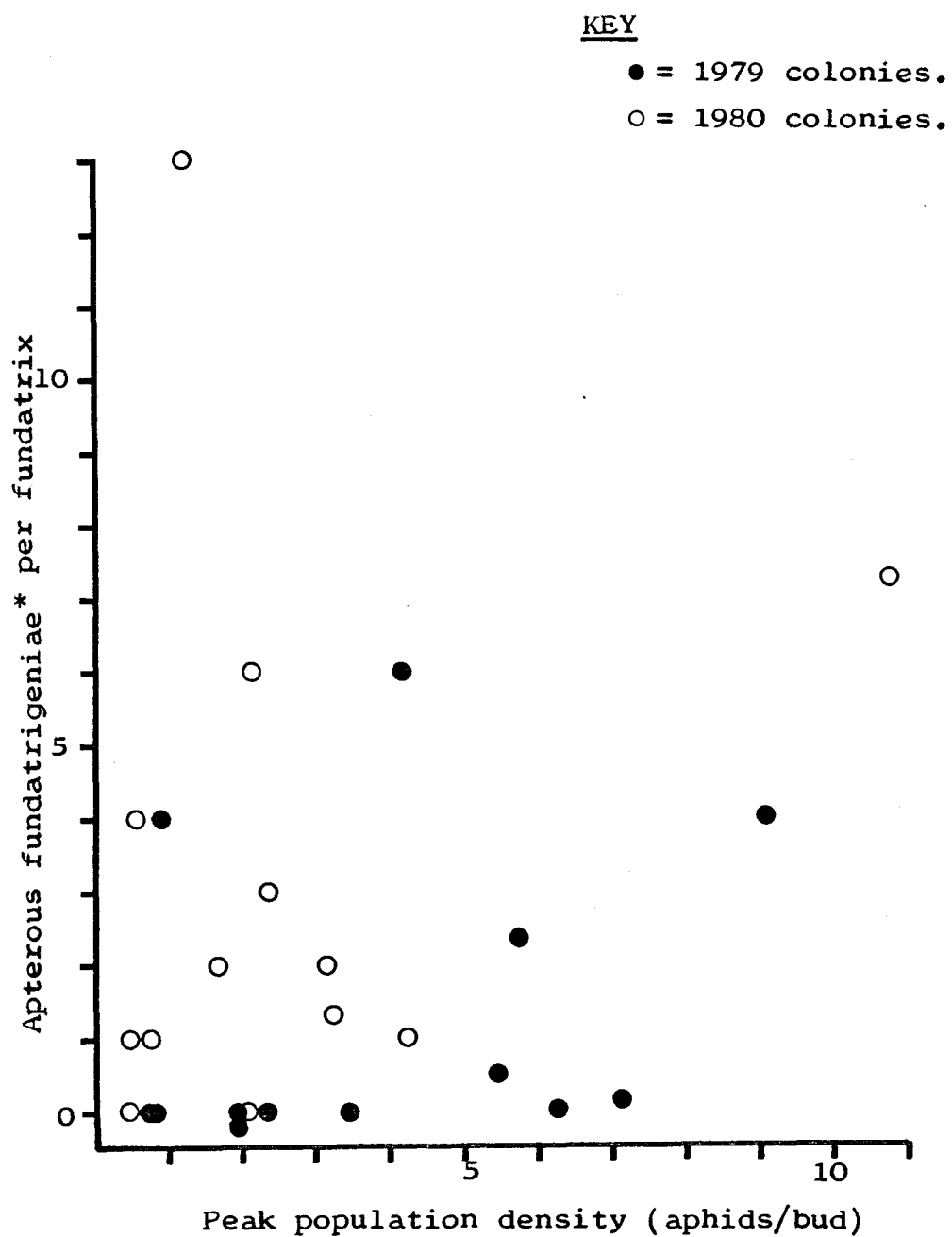
Fig. 6.17 Scatter plot of the success index for spring colonies of *M. dirhodum* on rose against the initial population density of 4th instar/adult fundatrices.



production of apterous fundatrigeniae was inhibited by crowding (Fig. 6.18). In 1980 there was no relationship between the number of apterous fundatrigeniae produced per fundatrix and peak colony density (Kendall's rank correlation coefficient = 0.14, $n = 13$, $P = 0.25$). In 1979 there was a tendency for the more crowded colonies to produce more, rather than fewer, apterous fundatrigeniae per fundatrix (Kendall's rank correlation coefficient = 0.35, $n = 13$, $P = 0.051$). Thus, although significantly more apterous fundatrigeniae were produced in 1980 than in 1979 this difference appears to be unrelated to crowding. The causative factor may well have been linked to time of year, as fundatrices matured about 10 days earlier in 1980 than in 1979.

3. Shelter: In each year bushes were given a shelter ranking on a scale from one to six, based on the degree of cover around and above them. In neither year was there a significant correlation between colony success and shelter ranking (Table 6.36), although in 1979 the correlation was nearly significant ($P = 0.08$). The lack of significant relationships was probably more a reflection of the inadequacy of the shelter ranking than of the unimportance of mortality due to wind and rain. Mortality due to wind, in particular, was probably more closely linked to small-scale differences in bush growth-forms and the positions of adjacent vegetation liable to brush against colonized leaves (factors which are very difficult to assess) than it was to large-scale differences in the amount of cover around and above bushes.
4. Spider abundance: Mean spider density on different bushes over all visits varied from 1.6 to 16.1 per 100 buds in 1979 and from 0.8 to 26.0 in 1980. If predation by spiders was of primary importance in reducing colony success a negative correlation

Fig. 6.18 Scatter plot of the number of apterous fundatrigeniae produced per fundatrix in spring colonies of M. dirhodum on rose against the peak population density of each colony.



* includes apterous 4th instars which failed to reach adulthood.

between this variable and colony success is expected. However, no such relationship was apparent from the data (Table 6.36) suggesting, as proposed earlier, that predation by spiders was a relatively small modifying influence on colony success.

5. Bud growth stage: In 1980 data on the state of the buds on monitored bushes were collected on 9 April and 28 May. The mean number of partially and fully open leaves per bud was calculated and used as an index of bud growth stage. If the rate of growth of rose was important in producing differences in success between colonies a correlation between the bud growth stage index and colony success is expected. There was no sign of such a correlation for either date (Table 6.36) although this was not surprising given the small amount of variation between bushes.
6. Duration of adult life of fundatrices: An index of the mean adult longevity of fundatrices on each bush was obtained by dividing the adult fundatrix aphid-day total (the sum of all records of adult fundatrices on all visits) by the number of fundatrices which became adult on the bush. In both years there was a significant correlation between this rough index of longevity and the colony success index. Bushes which produced most migrants were those on which adult fundatrices survived a long time and were thus able to produce all, or a large proportion, of their full complement of nymphs. That this correlation was significant while those with other colony variables were not demonstrated that few if any fundatrices survived to the end of reproductive life and that mortality factors which acted on the very small number of pre-reproductive and reproducing fundatrices determined colony success to a much larger extent than did those acting later in the spring on apterous and alatiform fundatrigeniae. It is noticeable, however, that the correlation between fundatrix longevity and colony success was

better in 1979 than in 1980. This was probably because more apterous fundatrigeniae were produced in 1980 and they played a greater role in the production of migrants.

Given that adult fundatrix longevity played a dominant role in colony success, the failure to find statistically significant relationships between success and the other variables above is not surprising. If the migrant production of a colony depends largely on the continued survival of a very few individuals (the fundatrices) random chance alone will produce very large differences in success between colonies. Consequently a much greater number of bushes must be monitored (and at more frequent intervals) if the effects of the various factors affecting fundatrices and fundatrigeniae are to be quantified.

6.5.4 Discussion

(a) Factors which may affect colony success

Particularly in 1979, natural enemies were present in low numbers on rose until after the spring migration of M. dirhodum had started and even at their peak density they were much less numerous than M. dirhodum. Moreover, the great majority were small *Therid*iid spiders, which do not usually search actively for prey (unlike other predators such as anthocorids, coccinellids and syrphid larvae), probably take longer to consume their prey and are not aphid-specific. Thus, although natural enemies probably had a greater impact in 1980 than in 1979, it seems unlikely that they had more than a relatively small modifying influence on the successful production of migrants in either year. This view is supported by the lack of a relationship between colony success on different bushes and the abundance of spiders but more detailed monitoring and predator-exclusion experiments are needed before a firm statement on the importance of predators can be made. However, from the small number of aphid mummies found, it is clear that parasites were not important in the two years of the study.

From studies of R. padi on Prunus padus in England (Dixon, 1971), Finland (Leather & Lehti, 1982) and Germany (Bode, 1980) it appears that natural enemies were not of primary importance to spring colonies of this species because, as in the present study, they were rare until after the spring migration had started. They increased in early summer and may have hastened the decline of aphid populations but even in the complete absence of natural enemies R. padi disappeared from the primary host during June (Dixon, 1971). Evenhuis (1968) working on spring colonies of R. insertum on apple considered that "predators generally are not so very important except perhaps for ladybird beetles" but the parasite Monoctonus cerasi, which appeared in March, sometimes eliminated a considerable number of aphids.

After several years of monitoring and predator-exclusion experiments Way & Banks (1968) concluded that natural enemies were not responsible for halting the growth of large spring populations of A. fabae on spindle (Euonymus europaeus L.) although they increased later and accelerated the population decline. Unlike the situation with R. padi, colonies of A. fabae did persist through the summer on the primary host if natural enemies were excluded. Relatively small spring populations of A. fabae that developed from few overwintering eggs were at greater risk than large ones and very few migrants were produced before the aphids were exterminated by predators and parasites (Way & Banks, 1968). However, it appears that even these small populations of A. fabae were at considerably higher population densities than the M. dirhodum colonies studied here. Fundatrigeniae of A. fabae are probably at particular risk from natural enemies because, unlike M. dirhodum, they remain in close groups even when less crowded feeding sites are available (Way & Banks, 1968). Fundatrigeniae of R. padi also tend to stay in tight colonies (Rogerson, 1947) but these are protected by the formation of a pseudo-gall.

The impact of natural enemies on holocyclic spring colonies is probably limited because the aphids increase and then migrate rapidly at a time of year when many

predators and parasites are only just starting to become active. Also, the relatively low temperatures limit the speed of aggregative and numeric responses to the sudden appearance of pockets of high prey density. Furthermore, the microhabitat involved is unfavourable for the overwintering of natural enemies and provides very little prey of any type until the fundatrices start to reproduce, but it offers the aphids cryptic feeding sites between folded leaflets once the buds have burst. Nevertheless, from the small amount of information available, it appears that holocyclic spring colonies of several aphid species suffer greater losses from natural enemies than do autumn colonies.

Very little information is available on the impact of weather on spring colonies feeding on open buds but mortality must be less severe than that suffered by nymphal fundatrices before but burst (see section 6.4.4). Considering differences between the feeding sites of spring and autumn generations it seems that spring colonies are less likely to be blown or brushed off by wind but are more likely to be washed off by rain or drowned, because crevices between folded leaflets (the normal feeding sites in spring) are often vertical and thus easily flooded. This view is born out by the numbers of aphids found apparently drowned in spring 1979.

Although there was no sign that M. dirhodum colonies with higher initial population densities of fundatrices were less successful at producing migrants, this is not surprising because the peak population densities on the most crowded bushes were less than 11 aphids/bud and the mean peak densities were only 3.4 and 2.2 aphids/bud for 1979 and 1980 respectively. Spring colonies of R. padi (Dixon, 1971) and particularly A. fabae (Way & Banks, 1968) may occur at much higher densities and competition for food and sheltered feeding sites may be important in limiting colony success, though inverse relationships between migrant production per fundatrix and population density have not been demonstrated.

The production of apterous fundatrigeniae by fundatrices is, at least potentially, a way in which production

of migrants can be boosted but in both 1980 and, particularly, 1979 apterous fundatrigeniae contributed little to the production of M. dirhodum migrants. The increase in natural enemies in May must have helped to reduce the reproductive success of apterous fundatrigeniae and in 1979 heavy rain during late May probably also played a role, but the difference in migrant production by fundatrices and fundatrigeniae was too large to be accounted for by these factors alone.

Soluble nitrogen is the component of phloem sap which probably limits aphid development and reproduction rates, and it has been demonstrated for the woody hosts of R. padi (Dixon, 1971) and Drepanosiphum platanoides (Dixon, 1963) that soluble nitrogen levels are highest at bud burst and decline through the spring as growth slows and leaves mature. D. platanoides, which does not alternate between primary and secondary hosts, adapts to this decrease in food quality by ceasing to reproduce during the summer, while R. padi migrates to secondary hosts before soluble nitrogen drops to its minimum (Dixon, 1971) and is incapable of surviving on mature leaves of the primary host (Leather & Dixon, 1981). A. fabae can survive on the primary host in the summer in the absence of natural enemies, but the fecundity of fundatrices is higher than that of succeeding generations (Way & Banks, 1968). In A. fabae (Dixon & Dharma, 1980) and M. dirhodum (Wellings, Leather & Dixon, 1980) fundatrices have a higher number of ovarioles than later generations, a characteristic which is associated with high fecundity and reproduction rates.

It seems likely that the nutritional quality of rose leaves, like that of other woody hosts, declines through the spring and that, as in other species, the high ovariole number of fundatrices of M. dirhodum is an adaptation allowing them to take maximal advantage of the very high nutritional quality of the primary host early in the year (Wellings, Leather & Dixon, 1980). Thus, both innate differences in fecundity associated with ovariole number and differences in the quality of food available were probably important causes of the much greater reprod-

uctive success of M. dirhodum fundatrices compared to apterous fundatrigeniae.

In Holland the first generation of M. dirhodum fundatrigeniae may consist entirely of apterae (Hille Ris Lambers, 1947) but in Hampshire even on the least crowded bushes, on which single fundatrices produced only a few nymphs before disappearing, the great majority of first generation fundatrigeniae were alatae and there was no sign of an inverse relationship between the production of apterous fundatrigeniae and population density. This suggests that English M. dirhodum fundatrigeniae are very sensitive to crowding in early life, so that contact with only a few other nymphs triggers wing production, and/or that factors other than crowding, such as food quality or some other factor partially linked to time of year play an important role. The latter is suggested by the fact that significantly more apterous fundatrigeniae were produced in 1980, when fundatrices matured earlier. In A. fabae crowding is important in the morph determination of first generation fundatrigeniae, which are mainly apterous when fundatrices are only moderately abundant but mainly alatiform when fundatrices are crowded (Way & Banks, 1968). In R. padi both crowding and food quality are important in the morph determination of first generation fundatrigeniae and subsequent generations on the primary host (Dixon & Glen, 1971; Leather & Lehti, 1982).

(b) The role of spring migrants from rose in initiating summer infestations of Gramineae in 1979 and 1980

Surveys in spring and autumn (section 6.2) showed that holocyclic M. dirhodum colonies on rose could represent a substantial proportion of the total species population surviving the winter successfully, especially after cold winters when the greater cold-hardiness of eggs must put holocyclic populations at a distinct advantage compared to viviparae persisting on Gramineae. More information on the importance of holocyclic populations to the initiation of summer infestations on Gramineae in 1979 and 1980 can be obtained by comparing the timing of spring migration from rose with early catches of M. dirhodum alatae in

Britain by R.I.S.* suction-traps in these years. The conclusions reached are especially interesting because although M. dirhodum were not numerous in 1980, they reached plague proportions in summer 1979. It was originally suggested in the media that the huge numbers of alatae seen in 1979 were migrants from continental Europe but subsequent analysis of meteorological data strongly suggested that few if any were of continental origin (Cochrane, 1980).

Spring 1979 followed an unusually cold winter (section 6.1.3), with temperatures low enough to kill most if not all M. dirhodum exules (Chapters 2 and 3). Hand (1982) found no M. dirhodum on Gramineae during extensive winter sampling but the spring population on rose was relatively large (section 6.2). These facts suggest holocyclic populations were important in initiating summer colonies on Gramineae in 1979 and this view is confirmed when R.I.S. suction trap catches for this year are examined. Both the timing and pattern of suction trap catches in spring and early summer 1979 are as expected if the summer population was derived from spring migrants from rose. Migration from rose should be more synchronous than early flights by winter survivors from Gramineae because of the more or less synchronized hatching of fundatrices. Hence the small but abrupt initial peak in alate numbers which coincided closely with the start of migration from rose (Fig. 6.19) in mid-May. This was followed by a lull until about mid-June after which weekly catches increased more or less exponentially through the rest of June and July. The lull is expected because the spring migrants were alatae and were spread thinly over fast growing graminaceous hosts of high nutritional quality, so that the large majority of first generation exules must have been apterae.

During the abnormally cold winter of 1978/79 many natural enemies must have been killed, as well as anholocyclic aphid populations and other prey. Moreover, the spring was cold, which probably delayed natural enemy emergence and further limited the alternative prey avail-

Fig. 6.19 Total weekly catches of M. dirhodum alatae for all British Rothamsted Insect Survey suction-traps in spring 1979.

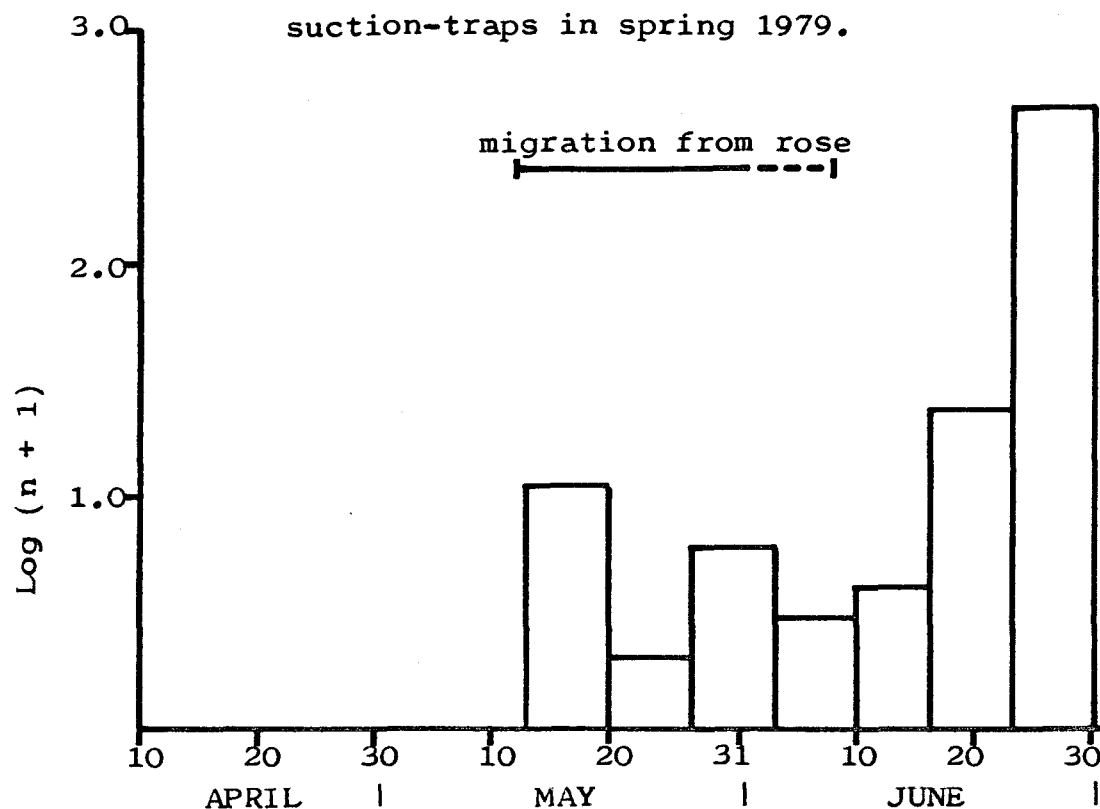
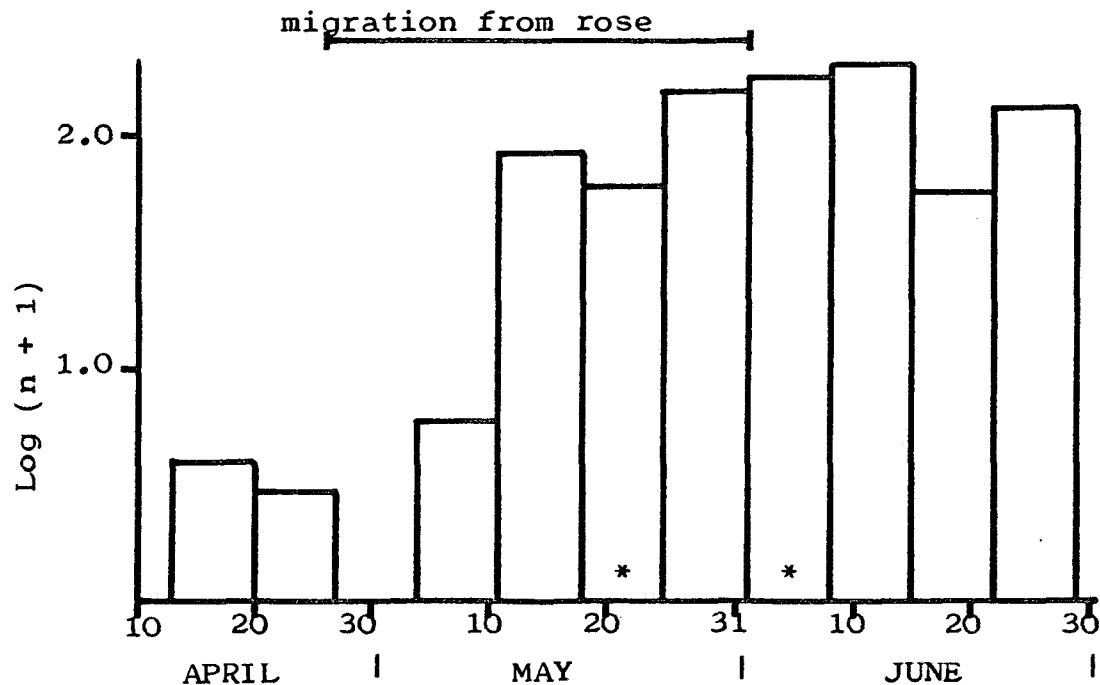


Fig. 6.20 Total weekly catches of M. dirhodum alatae for all British Rothamsted Insect Survey suction-traps in spring 1980.



*catch for 4 days only used to estimate full week's catch.

able to them so that they could not recover before holocyclic aphids arrived on Gramineae (Vickerman, 1977). In addition the weather during July was dry (Table 6.30). All these factors probably led to unusually high reproductive success by the spring migrants. The crowded colonies of first generation exules which resulted would have ensured that the second generation of exules were predominantly alatae, and the lull in suction trap catches would be over. After this the now unsynchronized subsequent generations of exules apparently continued to reproduce very successfully, at a more or less exponential rate close to the species' intrinsic rate of increase (Cochrane, 1980). This was probably due to the lack of natural enemies, continued dry weather in July and the delayed growth of cereals resulting from the cold spring, which probably prolonged the period during which the crops provided good nutritional conditions (Dean, Dewar, Powell & Wilding, 1980). During the lull between the first M. dirhodum catches and the onset of the rapid increase in the numbers of alatae in mid-June, the few alatae flying were presumably a mixture of later migrants from rose and the first few alatae from Gramineae, possibly first generation exules produced late in the reproductive lives of spring migrants which by this time were experiencing crowded conditions. Weekly mean temperatures near the study area during the lull (mid-May to mid-June) ranged from 9° to 14°C with an overall mean of about 12°C. From the temperature/development time relationship determined by Dean (1974b), apterous M. dirhodum viviparae should have taken about 12 days to develop at this temperature. Thus first generation exules should have started to become adult from roughly 24 May onwards and the first of their alate offspring should have matured near the end of the first week in June, so that an increase in the number of alatae flying would be expected soon after this. This corresponds well with the end of the lull in the weekly catches of alatae and provides further support for the interpretation of events given above.

The 1979/80 winter was mild (section 6.1.3 and Fig. 6.7) and low temperature was probably unimportant as a

mortality factor acting on aphid populations. The spring was also warmer than in the previous year (Table 6.30), although still colder than average. The density of the holocyclic population on rose was estimated at about 2% of that present in spring 1979 (see section 6.2) and during surveys and monitoring several gynoparae were found, which had presumably originated from populations surviving on Gramineae. All these facts suggest that holocyclic populations were probably less important in initiating colonies on Gramineae than in 1979 and this view is born out by examining the early R.I.S. suction-trap catches (Fig. 6.20). Firstly, although migration from rose started about two weeks earlier than in 1979, the first alatae were caught a month earlier and so are much more likely to have originated from anholocyclic populations on Gramineae than from rose. Furthermore, weekly catches of alatae increased suddenly to 84 in mid-May, half way through the migration period. These alatae were far too numerous to have been spring migrants from rose and cannot have been the offspring produced by these migrants because the large majority of first generation exules must have been apterae, having been produced in uncrowded, nutritionally favourable conditions. From the temperature/development time relationship determined by Dean (1974b) and the temperatures experienced from the start of spring migration from rose, no second generation exules derived from migrants from the primary host should have matured before the third week in May. Moreover, in 1980 considerably fewer second generation exules derived from spring migrants were expected than in 1979 because of the lower populations on rose. Thus the large increase in alatae in the week 12 to 18 May must have been derived from anholocyclic populations overwintering on Gramineae. Thus all the evidence suggests that, unlike the situation in 1979, spring migrants from rose were not important in initiating the summer population of exules. Four-weekly catches from mid-May never rose above 3,000, possibly because of higher numbers of natural enemies than in 1979 and a particularly wet June (Table 6.30).

Consideration of M. dirhodum populations in 1979 and 1980 highlights the fact that a holocyclic life cycle enables aphids to take advantage of growing seasons following unusually cold winters which, for the reasons given above, may provide conditions particularly favourable to cereal aphid outbreaks. It should be noted that successful exploitation of such conditions need not involve either early production of migrants by the holocyclic population or extraordinary reproductive success by fundatrices. Fundatrices were if anything less successful in 1979 than in 1980 and migration started a fortnight earlier in 1980. The holocyclic population was certainly considerably larger in spring 1979 than in 1980 but how exceptional it was is impossible to gauge as no quantitative data for other years are available.

CHAPTER SEVEN

CHAPTER SEVEN

GENERAL DISCUSSION

7.1 REASONS FOR BETWEEN-SPECIES DIFFERENCES IN
THE ABUNDANCE OF OVERWINTERING VIVIPARAE7.1.1 Differences between *M. dirhodum* and *S. avenae*

Hand (1982) found *S. avenae* viviparae on cereals and grasses in his west Hampshire study area through all three winters in which he sampled but found no overwintering viviparae of *M. dirhodum*. Winter sampling by Vorley (1983) in the same study area confirmed the much greater abundance of *S. avenae* viviparae (though *M. dirhodum* was found on one field) and from the winter surveys by George (1974) the situation observed in Hampshire seems typical of the country as a whole.

Possible reasons for differences in the abundance of the two species on Gramineae during the winter can be divided into three categories:

1. Reduced development and reproduction rates and/or survival during the winter due to low temperature or other season-related factors.
2. Reduced recruitment into the anholocyclic population from early autumn onwards due to viviparae switching to the production of sexual morphs.
3. Reduced initial input into the anholocyclic population in the autumn due to low survival and/or development and reproduction rates in late summer and early autumn.

The relative importance of these three types of cause is discussed below.

In both laboratory experiments (Chapter 2, experiment 2.4.2) and field experiments (Chapter 3) cold-hardened *M. dirhodum* viviparae were found to be more susceptible to low temperature mortality than *S. avenae*.

However this difference was relatively small and in the field only resulted in significant differences in mortality between the species in periods when grass minimum temperatures dropped below -8°C (Chapter 3, section 3.3.4). Thus it cannot be used to explain the very great difference in the two species' winter abundance on Gramineae and further results of the winter mortality experiment (Chapter 3), showed that other weather factors did not affect M. dirhodum more than S. avenae.

Unfortunately, no data were obtained during the present study on the low temperature development and reproduction of M. dirhodum or on the possible occurrence of 'chronic' low temperature mortality due to prolonged exposure to winter temperatures. However, Smith (1981) found that artificially established colonies of M. dirhodum on wheat in shelter cages actually increased through the 1977/78 winter while those of S. avenae declined. He suggested that this was due to faster reproduction by M. dirhodum. This was supported by the laboratory studies by Dean (1974b), using constant temperatures from 10°C upwards, which showed that M. dirhodum developed and reproduced faster than S. avenae at lower temperatures. Though the artificially established colonies of M. dirhodum monitored by Dean (1974a) through the 1970/71 winter declined more rapidly than those of S. avenae the species difference was not great and M. dirhodum survived better than R. padi. Thus it seems clear that, at least on cereals, heavy mortality due to temperature or other weather factors, or low development and reproduction rates, cannot be used to explain the rarity of anholocyclic M. dirhodum compared to S. avenae.

Even during October and November, Hand (1982) did not find M. dirhodum on cereals or grasses. This strongly suggests that the species' rarity on Gramineae during the winter was primarily the result of a low initial input into the anholocyclic population compared to S. avenae. One reason for this lower input was probably greater production of gynoparae and males by M. dirhodum during early autumn. Evidence for this comes from autumn monitoring of artificially established

colonies on wheat (Dean, 1978) during which M. dirhodum switched to the production of large numbers of males and consequently died out, while S. avenae produced no males and very few oviparae. Further support for the low production of sexual morphs by English S. avenae is provided by the finding that some clones were completely anholocyclic and others, though holocyclic, continued to produce some viviparae under short daylength conditions (Singer *et al.*, 1977; Watt, 1979b; Hand, 1982). Moreover, Hand (1982) found only one male and no oviparae of S. avenae during his winter sampling.

The second possible reason for reduced input into the anholocyclic population of M. dirhodum, apart from a switch to sexual morph production, is a large population decline during late summer or early autumn due to low survival and/or low reproduction and development rates. Cereal aphid populations have received very little attention at this time of year. Moreover, in this period (between harvest and the emergence of winter cereals) the aphids must all be on grasses and their performance on these hosts has not been studied in any detail. It may be that M. dirhodum shows low development and reproduction rates on the commoner grass species under late summer field conditions. Alternatively, the species may suffer more heavily than S. avenae from natural enemy attack at this time. Natural enemies likely to have relatively species-specific biotypes, such as hymenopteran parasites or Entomophthora spp., are most likely to be important in producing differential mortality in the two species. It has been suggested that in late summer 1979 the very large population of M. dirhodum was severely reduced due to Entomophthora attack (Vickerman, pers. comm.). Whatever the cause, it is clear that a drastic population crash did occur before October, as the numbers of gynoparae and males colonizing roses were lower in autumn 1979 than in autumn 1978, despite the much higher summer population (Chapter 6, section 6.2.3). This tends to support the view that large population declines during late summer and early autumn are important in reducing the input into anholocyclic winter populations of M. dirhodum.

7.1.2 Differences between R. padi and S. avenae

Hand (1982) generally found R. padi viviparae more numerous than S. avenae up to late December, particularly on mature grasses. The species declined more rapidly than S. avenae after this but still overwintered successfully on Gramineae in at least two of the three winters studied. George (1974) also found R. padi viviparae surviving through until early spring in 1972 and 1973, while more rapid declines through the winter compared to S. avenae were observed in the artificially established populations monitored by Dean (1974a).

From Rothamsted Insect Survey suction-trap catches, R. padi appears much more abundant than S. avenae in late summer and early autumn. This is probably due to the species' rapid rate of increase at high temperatures (Dean, 1974b) and its preference for grasses (Leather, 1980b). The large numbers of gynoparae colonizing Prunus padus and frequent records of holocyclic overwintering in Britain (see Chapter 1, section 1.5.3) suggest that R. padi switches more strongly to the production of holocyclic morphs than does S. avenae. Nevertheless, the species' great autumn abundance and good performance on grasses ensures that the numbers of R. padi viviparae remain higher than those of S. avenae through late autumn and early winter and often into the New Year (Hand, 1982).

The subsequent rapid declines of R. padi populations observed by Hand (1982) are unlikely to have been caused by the continued production of many gynoparae and males through the winter as he did not report finding sexual morphs in the winter. Also, the pattern of population change usually shown was not the steady decline expected if continued sexual morph production was the cause. Instead, numbers often increased considerably in November and December before declining sharply. Also, the R. padi used in mid-winter experiments in the present study produced apterous viviparae rather than gynoparae and males and neither Dean (1974a) nor Leather (1980b) mentioned finding gynoparae or males during their winter monitoring of R. padi colonies on Gramineae. It thus seems possible that, as Leather (1980b) has suggested,

holocyclic clones of R. padi are able to switch off sexual morph production after the autumn. The evolutionary implications of this are discussed below (section 7.2).

The relatively high low-temperature mortality of R. padi viviparae, demonstrated in laboratory and field experiments on acclimatized aphids (Chapter 2, experiment 2.4.3, and Chapter 3), must have been an important cause of the species' faster decline during mid- and late winter compared to S. avenae. But the results of the winter field experiment on survival, reproduction and development on wheat and perennial rye grass (Chapter 5) strongly suggest that low reproduction rates and steady mortality (both of which were probably linked to prolonged exposure to winter temperatures) were probably even more important than the 'acute' low temperature mortality due to short periods of exposure to extreme cold demonstrated in the earlier experiments. These effects were more marked when aphids were on rye grass rather than wheat, and this probably accentuated their heavier impact on natural R. padi populations compared to those of S. avenae, because a greater proportion of R. padi were found on grasses rather than cereals (Hand, 1982).

As the reduced reproduction and 'chronic' low temperature mortality observed were likely to have been caused by prolonged exposure to winter temperatures, their full effects on population growth should only have been felt after a time lag. This would explain why R. padi populations survived well in early winter, crashed surprisingly late, and then appeared incapable of responding to warmer weather in March and April. It would also explain why artificially established populations of R. padi viviparae monitored by Dean (1974a) and Leather (1980b) died out in spring after the worst of the winter weather was over.

In the present study it was found that R. padi viviparae were poorly adapted to low temperature survival and observations by other workers support this view (Dean, 1974a; Leather, 1980b; Hand, 1982). However, there are suggestions that in colder regions more cold-hardy clones of the species exist as Griffiths & Wratten (1979)

found that the laboratory determined LT50 of a Finnish clone was -12.3°C , while in Oregon, U.S.A., Greene (1966) found R. padi overwintering on a variety of Gramineae and this anholocyclic population was not exterminated by a sudden drop in temperature to 15°F (-10°C). Much more surprisingly, Andre and Tate (1933) reported finding alatae, apterae and nymphs of Rhopalosiphum prunifoliae (Fitch) (an old synonym for R. padi (Richards, 1960)) in Iowa which had apparently survived minimum temperatures around -20°F (-29°C) without snow cover. However, given the large degree of taxonomic confusion over the genus Rhopalosiphum in North America it is possible that the species involved here was not R. padi.

7.2 THE BALANCE BETWEEN HOLOCYCLIC AND ANHOLOCYCLIC OVERWINTERING STRATEGIES

The simultaneous use of both holocyclic and anholocyclic overwintering strategies by British populations of all three of the cereal aphids studied here (see Chapter 1, section 1.5) is a reflection of the fact that both strategies have advantages and disadvantages and the relative importance of these varies through time as well as space.

For a heteroecious species, such as M. dirhodum or R. padi, holocyclic overwintering, which involves host-alternation, has the following advantages:

1. Holocycly involves the production of diapausing eggs which provide a very cold-hardy overwintering stage (see Chapter 2, experiment 2.5.4) which does not need to feed and may also be better than viviparae at withstanding winter mortality produced by wind, rain or snow. A very cold-hardy overwintering stage may be particularly important because it enables holocyclic populations to take advantage of particularly favourable summer conditions following harsh winters. These conditions may include delayed and reduced natural enemy attack

(Vickerman, 1977) and a prolonged period during which secondary hosts are nutritionally favourable (Dean et al., 1980) (see also Chapter 6, section 6.5.4 (b)).

2. Host-alternation allows the aphids to exploit the favourable nutritional conditions provided by woody plants in spring and autumn as well as the favourable nutritional conditions provided by non-woody plants during the summer (a time when woody hosts are unfavourable) (Mordvilko, 1928; Kennedy & Stroyan, 1959; Dixon, 1971, 1973). The consistently high nutritional status of woody hosts in early spring is fully exploited because it is 'anticipated': spring generations, particularly fundatrices, have more ovarioles, and consequently greater reproductive potential, than later morphs (Wellings, Leather & Dixon, 1980). The result is a very rapid population build-up before the return to secondary hosts (see Chapter 6, section 6.5).

3. Host-alternation allows holocyclic aphids to escape from natural enemy populations (Way & Banks, 1968). In M. dirhodum (see Chapter 6, section 6.5.3 (b) and 6.5.4 (a)) and R. padi (Dixon, 1971) natural enemy attack does not appear to be important in causing the final disappearance of spring generations from the primary host, as it is with Aphis fabae (Way & Banks, 1968). Nevertheless, switching hosts and life-stages (from aphids to aphid eggs) must help to throw off natural enemies and is probably particularly important in autumn when it provides a way of escaping from those natural enemies which can overwinter in a dormant state alongside populations of viviparae and so are well placed to attack them early in the following year (for example resting spores of Entomophthora spp. and diapausing mummies of aphid parasites). Because Rosa spp. are relatively common, populations of M. dirhodum on the primary host are at low density. Other

rose aphids are also thinly spread during autumn and spring, so that aphid-specific natural enemies are unlikely to be attracted. As there is also little alternative non-aphid prey available, polyphagous predators are also scarce (see Chapter 6, sections 6.4.3 (b) and 6.5.3 (b)). This contrasts with the situation on grass or cereal fields where alternative prey is usually available for natural enemies.

4. Holocycly involves sexual reproduction which, as in other organisms, ensures increased genetic variability in the population and therefore allows the species as a whole to be more responsive to changing environmental conditions (Blackman, 1974b; Maynard Smith, 1971). However, a degree of genetic variation could arise in a parthenogenetic aphid line due to gene mutation and, possibly, endomeiosis (Cognetti, 1961). The high reproductive potential and short generation times of parthenogenetic aphids help to maximize the frequency with which mutations occur (Bunting, 1981) but without a mechanism to allow genetic recombination and thus bring adaptive mutations together, evolutionary progress would be limited. Cognetti (1961) provided some cytological evidence for recombination during 'endomeiosis' in the first stages of aphid parthenogenesis. However, as there is much evidence that parthenogenetic lines are genetically stable (Blackman, 1971), Cognetti's findings require confirmation and it appears that sexual reproduction during the holocycle does provide the main mechanism for increasing genetic variability in aphid populations. This is apparently the only advantage of holocycly which is associated with sexual reproduction itself rather than with egg-production or host-alternation and, unlike the others, it would appear to produce benefits only in the long-term. Nevertheless, no Aphididae produce parthenogenetic eggs (though some Adelgidae and Phylloxeridae do) and almost all

species have retained the genetic potential for holocycly (Blackman, 1974b). This suggests that the long-term advantage of greater genetic variation has been successful in preventing the uncoupling of sexual reproduction from ovipary, despite the obviously greater egg production possible if sexual reproduction was not an essential part of the process.

To offset the advantages, holocycly also has the following disadvantages for a heteroecious species:

1. Host-alternation involves migration between primary and secondary hosts which must involve heavy mortality given the apparent trial-and-error nature of aphid host- and mate-finding. In cereal aphids spring migration is likely to involve relatively low mortality because of the ubiquity of graminaceous hosts but autumn migration is probably much more risky, particularly for R. padi with its rare primary host.
2. Holocycly involves a large loss of reproductive potential as first gynoparae with low fecundity are produced (Dixon, 1976b; Watt, 1979b and see Chapter 6, section 6.4.4 (e)) followed by males which are completely unproductive and oviparae which have even lower fecundity than gynoparae (see Chapter 6, section 6.3) and must mate before laying eggs. Results of autumn colony monitoring suggest that failure to mate was an important cause of loss of reproductive potential in holocyclic M. dirhodum populations (Chapter 6, sections 6.4.3 (d) and 6.4.4 (e)). For at least four months, from the laying of eggs to the maturation of fundatrices, no reproduction is possible, whereas in a mild winter and in sheltered conditions reproduction in an anholocyclic population during this interval could more than offset mortality (Smith, 1981, and see Chapter 4).

3. Holocyclic aphids may be exposed to greater mortality on primary hosts than on secondary hosts due to early leaf fall (Chapter 6, sections 6.4.3 (c) and 6.4.4 (b)) and more exposed feeding sites, particularly in early spring before the buds open (Chapter 6, sections 6.4.3 (f) and 6.4.4 (d)).

For monoecious species, like S. avenae, the advantages and disadvantages associated with host-alternation obviously do not apply (advantages 2 and 3, and disadvantages 1 and 3 above) but the main advantages of holocycly - the production of a cold-hardy egg, and sexual reproduction conferring greater genetic variation - are still effective, as is its main disadvantage - the loss of reproductive potential compared to continued parthenogenetic reproduction in mild winters.

Despite the small amount of information on the relative importance of holocyclic and anholocyclic overwintering in cereal aphid populations outside southern Britain there are signs, for S. avenae at least, of large scale regional variations in the relative importance of the two strategies, linked to the severity of winter climate (see Chapter 1, section 1.5.1). In Myzus persicae, for which much more information is available, it is clear that this is the case. Blackman (1974b) explained the occurrence of holocycly, anholocycly or both in M. persicae populations in different parts of the world largely in terms of winter climate, dividing the globe into six zones delimited by selected isotherms. Britain falls in his 'medium temperate' zone (with mean temperatures in January between 0° and 10°C) in which both holocycly and anholocycly occur, as they do in cereal aphid populations.

Short-term, year-to-year variations in the numbers of cereal aphids which overwinter successfully as viviparae rather than as eggs in Britain are probably mainly determined by the direct effects of winter temperatures: low temperatures reduce development and reproduction and increase mortality in anholocyclic populations while having little or no effect on overwintering eggs. More subtle effects may also affect the balance. For example low

temperatures or heavy rain and strong winds during the migrations between secondary and primary hosts or soon after egg hatch would reduce the success of holocycly in heteroecious species, while mild winter weather before egg hatch might have the same effect by allowing increased egg-predation.

Long-term shifts in the balance between holocycly and anholocycly in Europe are also likely to be produced primarily by temperature changes which would again have direct effects on the survival of overwintering viviparae and more subtle, indirect ones via natural enemies or the distribution and condition of host plants. The appearance of new mutations and gene combinations in the aphid populations which increase the success of one or other overwintering strategy (for example evolution of more cold-hardy viviparae) would also be important.

For heteroecious species a large decrease in the abundance of the primary host, whether produced by climatic changes, man's action or some other factor, would tend to strongly favour either anholocycly or the evolution of monoecious holocycly. The fundatrix of a species of Sitobion closely resembling S. avenae was found on bog whortleberry (Vaccinium uliginosum L.) the alate progeny of which colonized grasses (Müller, 1969, quoted in Dean, 1978) and it has therefore been suggested that S. avenae or an ancestral species was originally heteroecious with bog whortleberry as its primary host. Hand (1982) pointed out that if this was so the very restricted distribution of this plant would have provided strong selection pressure for monoecy in S. avenae. Whether or not bog whortleberry was the ancestral primary host, there is general consensus that S. avenae, and other species with a monoecious, holocyclic life-cycle on perennial herbaceous hosts, are descended from heteroecious species (Mordvilko, 1928; Kennedy & Stroyan, 1959; Blackman, 1974a).

Superficially, one might think that having made the rather dangerous evolutionary step from heteroecious to monoecious holocycly S. avenae would be in an advantageous position, reaping the benefits of holocycly without having to undertake dangerous migrations between secondary and

primary hosts. However, by adopting monoecy the advantages of holocycly associated with host-alternation are lost (see above). Also, the autumn migration of heteroecious species back to the primary host, though it involves heavy mortality, is beneficial in that it ensures very thorough mixing of the population so that matings between members of the same or closely related clones are minimized and the increase in genetic variability following sexual reproduction is maximized. Thorough mixing could be ensured in monoecious species if males were inhibited from mating before flying. But, if this were so males of a species like S. avenae, which has a very widespread, low-growing host, might find it particularly difficult to locate oviparae because they would have to search on any suitable looking surface near the ground, where vegetation is more or less continuous. In contrast, males of heteroecious species with woody hosts could be 'programmed' to concentrate their searching on suitable looking surfaces at bush or tree height, where the oviparae would also be concentrated. Thus S. avenae's monoecy may be one reason for its apparently heavy reliance on an anholocyclic overwintering strategy in England. The ability of the species' viviparae to withstand low temperatures better than M. dirhodum and R. padi (Chapters 2 and 3) as well as its good development and reproduction at low temperatures (Chapters 4 and 5) may have pre-adapted S. avenae to anholocycly. Alternatively these characteristics may have evolved as a result of strong selection pressure after anholocycly was established as an important mode of overwintering.

Just as the rarity of the ancestral primary host of S. avenae may have played a role in its present heavy reliance on anholocycly in Britain, so the relative abundance of the primary hosts of M. dirhodum and R. padi may be important in determining their level of commitment to sexual morph production in the autumn. As suggested above (section 7.1.1) it seems likely that one reason why M. dirhodum is very rare on Gramineae even early in the winter is because the autumn population puts a larger part of its reproductive effort into the production of sexual morphs than the other species do. This seems adaptive

as the primary host of M. dirhodum is common and so the autumn migration to it is likely to involve comparatively low mortality. If roses become less common, winters become milder or some other change occurs which increases selection pressure in favour of anholocyclic overwintering, M. dirhodum would probably be able to adapt readily as its viviparae seem pre-adapted to low temperatures to an extent which would ensure successful overwintering in most English winters. The primary host of R. padi is very rare in southern England and this is likely to have led to strong selection pressure for anholocyclic overwintering in the region, resulting in a proportionally greater commitment to continued viviparae production in the autumn even though, compared to M. dirhodum, the low temperature performance of the species' viviparae is poor.

Considerations of how the abundance of primary hosts and the winter climate affect the balance between holocycly and anholocycly leads one to ask the question: 'Are the holocyclic and anholocyclic overwinterers in the species population linked genetically?' The traditional view is that they are genetically completely isolated from each other. Thus, after a particularly harsh winter in which the anholocyclic overwinterers are completely exterminated, the species population entirely loses the genetic potential for anholocycly but it is subsequently regained by mutation (Lees, 1966). If winters are consistently mild natural selection favours anholocyclic clones so they increasingly dominate the population until sexual reproduction by holocyclic aphids throws up a new, more adaptive gene combination which, as it is only present in the holocyclic part of the population, reverses the swing towards anholocycly. Eventually genotypes with the new character become anholocyclic by mutation and the trend towards anholocycly starts again (Watt, 1979b).

However, this situation, in which all genotypes are totally committed either to holocycly or to anholocycly is not found in Myzus persicae, the only aphid whose population genetics have been studied thoroughly (Blackman, 1971, 1972, 1974b). In Britain, anholocyclic clones of this species (which are completely genetically isolated

and have chromosome abnormalities) are rare and the summer population consists mainly of androcyclic clones (producing males but not oviparae) and holocyclic clones (producing both sexes). All females of the androcyclic clones are parthenogenetic and thus overwinter anholocyclically but the males from these clones mate with oviparae from holocyclic clones, so that the genes from androcyclic clones overwinter both as eggs and as viviparae. Androcycly is a recessive character and even when a harsh winter completely exterminates the anholocyclic overwinterers the genetic potential for anholocyclic overwintering is present in those fundatrices that emerge the following spring which are heterozygous for androcycly. Thus anholocyclic overwintering can be re-established at the next sexual phase when two heterozygous individuals mate. This genetic link between anholocyclic and holocyclic overwinterers is very adaptive as it minimizes the risk that one or other overwintering method is lost completely, and maintains a very flexible 'mixed' overwintering strategy capable of coping with a wide range of winter conditions.

Information on the population genetics of morph determination in S. avenae is much more limited. Nevertheless, studies so far have not revealed androcyclic clones and the summer population seems to be divided between holocyclic clones and fairly numerous anholocyclic clones (Singer et al., 1977; Watt, 1979b; Hand, 1982). It is thus unlikely that the situation described for M. persicae applies. However, it is still quite possible that, though anholocyclic clones must be completely genetically isolated and rely only on anholocyclic overwintering (as they produce no sexual morphs), the 'holocyclic' clones could survive from one year to the next by holocyclic or anholocyclic overwintering (ie. as viviparae). This apparent contradiction is possible because all the holocyclic clones tested so far have not switched completely to the production of sexual morphs under short daylength conditions (Singer et al., 1977; Watt, 1979b; Hand, 1982). Also there is some evidence from the field experiment described in Chapter 4 (section 4.4.14) and

from the autumn monitoring of S. avenae by Dean (1978) that holocyclic clones which produce some sexual morphs in autumn switch back to producing only viviparae during the winter.

It could be that holocyclic S. avenae clones in Britain vary in a continuous way from those which are strongly holocyclic (producing many sexual morphs and few viviparae under short daylength conditions) to those which are very weakly holocyclic (producing only a few males and oviparae for a short period in the autumn). Strongly holocyclic clones would contribute more to the gene pool after a harsh winter and so a greater proportion of the population would start the following winter as eggs. After a mild winter the genetic influence of the weakly holocyclic clones would be larger and more of the population would start the following winter as viviparae. In parts of the species' range, like Britain, where the winter climate is mild enough to allow successful anholocyclic overwintering by members of a weakly holocyclic clone through many winters, anholocyclic clones might be formed by the accumulation of gene mutations and chromosomal abnormalities in the long-running parthenogenetic lines, as these changes would ultimately make delicate mechanisms such as sexual morph determination and gametogenesis impossible (Blackman, 1971) and clones genetically incompatible.

The scheme outlined above allows a simpler genetic link between holocyclic and anholocyclic overwinterers than is found in M. persicae and fits the small amount of data available so far. It provides a theoretically viable basis for a flexible 'mixed' overwintering strategy which would allow rapid fine tuning to local climatic conditions. The most contentious point about the hypothesis is that it is largely, though by no means completely, dependent on the existence in some clones of a mechanism for at least partially switching off sexual morph production in winter, a phenomenon which, though highly adaptive (see Chapter 4, section 4.4.14) has not been demonstrated. However, as already mentioned, there is some good circumstantial evidence to support its

existence and it is not surprising that more evidence is not available as no thorough studies on sexual morph determination have been done in the field. In winter, sexual morphs have not been found in natural populations of *R. padi* (Hand, 1982) or in artificially established colonies of this species (Dean, 1974a; Leather, 1980b; and see Chapter 5). Similarly, *M. dirhodum* has been found to produce sexual morphs in autumn (Dean, 1978) but not in winter (Dean, 1974a; Smith, 1981). Thus it may be that an autumn off-switch for sexual morph production exists in these two species as well.

In the discussion above temperature variation in successive winters has been considered as the main factor producing the variable selection pressure which, in the short-term, maintains the presence of both holocyclic and anholocyclic overwintering in British cereal aphids. However, seasonal temperature variation between winter and summer may also be important. It could have two types of effect:

1. During the summer high temperature adaptations are likely to be selected for (such as rapid high temperature development and reproduction). These may well be incompatible with, and thus negate, low temperature adaptations acquired due to selection pressure on overwintering viviparae.
2. A tendency for holocycly or anholocycly might be genetically linked to a characteristic selected for under summer conditions.

The great fecundity and short generation times of aphids during the summer as well as the possibility of severe inter-clonal competition would probably allow strong season-related selection pressures to produce significant seasonal shifts in the gene pool.

Lastly, though in all the Aphididae so far investigated eggs are more cold-hardy than viviparae, so that severe winters favour holocycly, this is not necessarily true of related families. For example, species of

Adelgidae such as Adelges cooleyi (Parry, 1980) and Adelges piceae (Amman, 1967; Greenbank, 1970) overwinter on conifers as viviparae called sistens, which hatch from parthenogenetic eggs in autumn, enter diapause as first instars before starting to feed, and can survive below -30°C .

7.3 PROSPECTS FOR LONG-TERM FORECASTING

The present study and that of Hand (1982) have revealed that the overwintering ecology of cereal aphids is, if anything, more complex than originally thought. All three of the species studied employ both anholocyclic and holocyclic overwintering in Britain and the relative importance of the two methods probably varies markedly from year to year, depending on winter weather and other factors (see section 7.2 above and Chapter 6, section 6.5.4 (b)). Even considering anholocyclic overwintering alone, the system is extremely complex due to:

1. The abundance and diversity of Gramineae used as hosts by overwintering viviparae (Hand, 1982).
2. The existence of important differences between aphid species in their low temperature mortality (Chapters 2, 3 and 5) and low temperature development and reproduction (Chapter 5).
3. The existence of large differences in a species' performance on different host plants (Chapter 5).
4. The strong suggestion, for R. padi at least, of large seasonal shifts in the performance of a single species on a single host, probably linked to temperature and plant growth rates (Chapter 5).

Complicating factors like these make a detailed simulation model of the whole system, in which ecological processes for each species on each host are modelled real-

istically, a very remote prospect. Even if such a detailed model was eventually developed, inaccuracies in long-term weather forecasting and uncertainty about biotic and abiotic conditions during the summer would make accurate predictions of summer numbers on cereals based on winter conditions impossible.

The approach to long-term forecasting should therefore be to develop relatively simple, unrealistic models which nevertheless summarize the effects of key processes accurately enough to provide a useful preliminary assessment of the probability of an outbreak in a given year. Information supplied to growers based on such a long-term forecasting system would not include definitive advice on whether or not to apply aphicides later, but would allow them to make contingency plans in years of high outbreak risk and thus alleviate some of the problems of responding to more accurate short-term predictions at only a few days notice.

A reasonably accurate system for predicting the summer incidence of sugar beet yellow viruses from winter and spring temperature data using a simple multiple regression equation has been developed (Watson, Heathcote, Lauckner & Sowray, 1975). Alatae of Myzus persicae are primarily responsible for spreading the viruses and so the prediction is effectively a long-term aphid forecast. It is presumed that warm winter and spring temperatures are linked to high virus incidence because they promote successful aphid overwintering and early population build-up. However, the system was developed without any detailed investigations of the overwintering and low temperature biology of M. persicae, simply by undertaking regression analyses using different winter and spring temperature parameters. One might conclude from this that a similar predictive equation could be developed for cereal aphids without any need for detailed overwintering studies like the present one. However, the success of the approach adopted by Watson et al. (1975) was probably mainly due to the simplicity of the overwintering ecology of M. persicae in England, in which holocycly is unimportant (Broadbent & Heathcote, 1955). In cereal aphids,

where both holocyclic and anholocyclic populations can make significant contributions to the spring population, regression analyses without complementary studies on overwintering biology are unlikely to come up with a dependable prediction system.

Several authors have observed relationships for S. avenae and M. dirhodum between the timing of the first appearance of alatae or of summer population peaks and winter or spring temperatures (Sparrow, 1974; Robert & Rouzé-Jouan, 1976; Turl, 1980; A'brook, 1981; Hand, 1982). In all cases these appear to reflect simple, direct effects of temperature on aphid populations: low temperatures are associated with later arrival and later population peaks. But high summer numbers are not associated with early arrival (Vickerman & Wratten, 1979) and relationships determined between the size of summer populations and winter and spring weather (Vickerman, 1977; Hand, 1982) have revealed that temperature has a less simple, direct effect on summer numbers than on timing.

Vickerman (1977) found a highly significant correlation between peak numbers of cereal aphids (mainly S. avenae) on winter wheat in west Sussex and air frost days in April or deviations from the long-term April mean temperature, with the aphids being more abundant after colder Aprils. As it is very difficult to explain this relationship in terms of a direct effect of temperature on the spring aphid populations, Vickerman suggested it was due to an indirect effect via natural enemies. He proposed that warm winter and spring weather reduced the likelihood of high cereal aphid populations in the summer because natural enemies would survive well, appear early and have abundant alternative prey (including other aphid species) to support them until cereal aphids started to increase on the crop. Following cold winters and springs natural enemy populations would be reduced and delayed not only because of direct temperature effects but because alternative prey (in particular anholocyclic aphids such as Metopolophium festucae) would be scarce early in the year. Thus when migrant cereal aphids derived from

cold-hardy eggs arrived on the crop they would be able to increase rapidly.

Vickerman's theory would appear to be basically sound though it probably needs some refining. This is suggested by the fact that as originally proposed, it implies that the advantage to cereal aphids of cold weather early in the year is linked to the survival of holocyclic aphid populations as cold-hardy eggs. However, this is probably not the whole story as the temperature parameters Vickerman found most significantly correlated with summer abundance were for April, and there is little doubt that the large majority of aphid eggs would have hatched before then (in 1980, M. dirhodum eggs hatched in February). It therefore seems likely that though one reason why cold weather during the winter is advantageous to cereal aphids is because some are present as cold-hardy eggs, the advantage of low April temperatures is mainly due to the good low temperature survival, reproduction and development of S. avenae and M. dirhodum viviparae, compared to their natural enemies. At present there is very little evidence to support this view, but this is simply because characteristics of the low temperature performance of natural enemies (in particular relationships between foraging activity and temperature) are very little known. However, in support of this idea, the development thresholds determined for aphid parasites so far (Campbell et al., 1974; Vorley, 1983) are generally several degrees above the 3°C threshold determined for S. avenae (Chapter 4, section 4.3.1). Also, cool temperatures have been found to strongly suppress foraging by Coccinellid beetles (Frazer & Gilbert, 1976).

Hand (1982) developed the type of regression analyses undertaken by Vickerman (1977) further, using aphid data from M.A.F.F. monthly summaries and R.I.S. suction-trap catches. He found that both S. avenae and M. dirhodum were more abundant following more severe winters, thus supporting Vickerman's theory that relationships between cereal aphid summer populations and weather conditions the preceding winter and spring are a result of inter-

actions between the aphids, the natural enemies and their alternative prey. However, Hand also found that the number of S. avenae alatae in the autumn was a better predictor of numbers the following summer than any winter weather variable. Low summer numbers were associated with high numbers during the previous autumn, which suggests further complications due to a density dependent effect.

It has been found that Vickerman's simple prediction system does not hold in all years or in all parts of the country (Carter et al., 1981). This is not surprising given that the equation does not take account of temperature and other conditions in the winter, and that the explanation behind the equation involves subtle ecological interactions. However, development of Vickerman's theory might well lead to more consistent predictions if multiple regression analyses were used to produce more complex predictive equations. It is possible that this development may necessitate two distinct components (possibly two separate equations) for each species, so that the very different characteristics of holocyclic and anholocyclic overwinterers can be separated.

However the prediction systems develop, it is important that use is made of information from winter field experiments and sampling, so that biologically meaningful parameters for temperature and other variables can be selected. Though winter sampling is needed during the development of a predictive system it would be best to end up with one that uses the previous year's R.I.S. suction-trap data and variables for winter and spring conditions as predictor variables and does not depend on routine winter sampling, because, though this may be economically justifiable for pests like Aphis fabae with winter populations concentrated on a single relatively uncommon host species (Way, Cammell, Taylor & Woiod, 1981), it is unlikely to be so for cereal aphids in Britain.

Though the significant relationships revealed by Vickerman (1977) and Hand (1982) suggest that prospects for developing 'rough-and-ready' long-term prediction systems for S. avenae and M. dirhodum are good, the

situation with R. padi is much less promising. This is firstly because the species is economically important in Britain mainly as a virus vector, so that damage/aphid numbers relationships will be complicated by variation in the proportion of alatae carrying virus. Secondly, the distribution of the primary host makes large regional differences in the importance of holocycle inevitable. And thirdly, all attempts to find relationships between the timing or abundance of summer populations and winter or spring weather have failed (Turl, 1980; A'brook, 1981; Hand, 1982).

The conclusion that a detailed and realistic computer simulation model of overwintering cereal aphid populations on all hosts is an unrealistic goal and also of dubious value for long-term predictions (see above) does not imply that computer models should not be developed for particular species populations on particular hosts. But if they are they should generally be developed as research tools for identifying key processes or important gaps in our knowledge, and not for direct use in prediction. However there is one component of the system, S. avenae on winter wheat, for which a computer simulation model would have more direct relevance to prediction. This is not only because overwintering on the crop itself has special implications for pest status (see Chapter 1, section 1.4.1) but also because a simulation model for summer S. avenae populations on winter wheat is already being developed (Carter et al., 1981). For this model initial populations of S. avenae are calculated from spring R.I.S. suction-trap catches. But after mild winters, during which a significant number of viviparae may already be present on the crop having successfully overwintered there, using suction-trap data alone may seriously underestimate spring populations. A model to predict winter survival on winter wheat would thus be of direct use.

From consideration of the characteristics of winter development, reproduction and longevity of S. avenae viviparae on winter wheat (see Chapter 4, section 4.4.15) it was concluded that in general these facilitate simulation modelling. Characteristics of mortality due to low

temperature and other weather factors (Chapter 3) would also seem to make a simulation model feasible. For example low temperature mortality in 4-day periods could be predicted quite well from grass minimum temperatures and there was no sign of 'chronic' low temperature mortality due to prolonged exposure to winter temperatures on wheat, though there was on perennial rye grass (see Chapter 5, section 5.3.1 and 5.4.1). However, the predictive equations for weather mortality should be checked against independent data and some inaccuracy in mortality prediction seems inevitable. This is particularly likely in harsh winters when 'chronic' low temperature mortality might occur and significant increases in cold-hardiness through the winter as a result of selective mortality of less cold-hardy individuals seem likely. If such a model were to be developed additional field data need to be obtained, in particular on the effect of natural enemies and on alate determination.

7.4 PROPOSALS FOR FUTURE RESEARCH ON OVERWINTERING

These proposals are made assuming that, though there are interesting theoretical points involved, the study of aphid overwintering should be pursued primarily for applied ends. That is, it should have the central aim of improving outbreak prediction or identifying practices which could enhance non-chemical control. Results should therefore be directly relevant to natural populations and, because of the difficulties of simulating winter conditions realistically in the laboratory, as much data as possible should be obtained in the field. Results of any laboratory work should be tested under field conditions whenever possible.

The need for a field-biased approach is clear if results of some laboratory and field studies already undertaken are compared. For example:

1. Low temperature mortality: Supercooling point determinations, the previously used laboratory

assessment method, greatly overestimated cold-hardiness in the field (Chapter 2, experiment 2.3.2; Chapter 3, section 3.3.2). Laboratory-determined LT50s gave good approximations to those obtained in the field, provided the effects of cooling rates were taken into account (Chapter 3, section 3.4.1) but laboratory tests overestimated the importance of inoculative freezing by surface ice (Chapter 2, experiment 2.4.7) and field work was needed to reveal the importance of 'chronic' low temperature mortality due to prolonged exposure to cold (Chapter 5, section 5.3.1).

2. Temperature relations of development, reproduction and longevity: Relationships determined in the laboratory (Dean, 1974b) did not agree with field data (Chapter 4).
3. Sexual morph determination: Field data showed that holocyclic clones of S. avenae ceased to produce sexual morphs after the autumn (Chapter 4, section 4.3.6) but no evidence of this was found during laboratory studies (Watt, 1979b; Hand, 1982).

Suitable areas for future research are discussed below, concentrating on areas most closely related to the present study. Apart from some of the field sampling all the work outlined could be tackled as part of intensive studies running over a few years.

7.4.1 Sampling natural populations

The only long-term aphid sampling programmes established so far (the Rothamsted Insect Survey suction-trap scheme and the routine sampling of cereal crops in the Game Conservancy west Sussex study area (Vickerman, 1977)) are concerned with summer populations. Even the R.I.S. suction-traps, which run from early spring to late autumn, only provide very indirect information on overwintering populations. There is therefore a need for some long-

term winter sampling. This would greatly enhance the value of short-term, intensive studies on selected aspects of overwintering and is probably essential for the development of long-term forecasting systems.

Long-term sampling programmes should be designed so that they are simple, standardized and not labour-intensive. The most useful and feasible is routine sampling of Gramineae using a Dietrich vacuum insect net. This is probably best limited to a week or so in early spring (roughly late March to early April) to assess the population density of winter survivors on Gramineae before spring migrants arrive from primary hosts. Winter cereals and a range of grass types (both cultivated and uncultivated) in a fairly restricted but typical part of the country should be sampled. Sampling conditions and timing should be as standardized as possible from year to year. If necessary, sorting and analysis could be postponed and incorporated in a future Ph.D. project. Though this programme would exclude holocyclic populations of the heteroecious species, holocyclic S. avenae would be included (though not separately identified) because eggs would have hatched. If time allowed a similar mid-winter survey would be useful but not essential.

Long-term sampling programmes for holocyclic populations of heteroecious species are likely to be more difficult to design. As no work on holocyclic R. padi was undertaken in the present study and much has been done by Leather (1980b) only M. dirhodum will be considered here. Probably the most feasible approach for this species would be spring sampling approximately coinciding with the peak population of alate fundatrigeniae to obtain a rough comparative index of the number migrating from rose each year. From work described in Chapter 6 sampling should be along the following lines:

1. It should be undertaken in dense woodland (as population densities on rose are highest in this habitat). Preferably the woodland should be relatively small areas surrounded by typical agricultural land.

2. The same woodlands should be used each year and if possible foliage should be searched without being removed so that the sites are not altered.
3. Population densities should be expressed as aphids/leaf but bush size and other characteristics should be kept as constant as possible from year to year.
4. At least some Metopolophium should be identified to species in the laboratory to check for the occurrence of M. fasciatum. Characters for identifying M. fasciatum are given in Stroyan (1982).
5. A few bushes with M. dirhodum should be located before the spring population peak and examined at intervals to ensure the sampling is correctly timed.

Two types of sampling programme which would be particularly useful even if undertaken for just two or three field seasons would be:

1. Vacuum insect net sampling of grasses during late summer and early autumn adopting a similar approach to that used by Hand (1982) for his winter sampling, with particular care being taken to identify sexual morphs.
2. Intensive vacuum net sampling of one or a small group of early-sown winter wheat fields from emergence to late spring with an interval between samples of one or two weeks and relatively large sample sizes. This would provide the detailed data needed to develop and test a simulation model for S. avenae populations incorporating the relationships for development, reproduction and weather mortality collected in the present study. This programme should also involve other trapping methods for natural enemies.

7.4.2 Laboratory experiments on low temperature effects

Laboratory work during the present study has already established the principles of cereal aphid low temperature mortality and priority should now be given to fieldwork but some ideas for laboratory experiments are given below. Unlike the rest of the suggestions for future work, some are primarily of theoretical interest. Most involve the type of direct mortality assessments described in Chapter 2.

1. Supercooling points: Do fully field-acclimatized viviparae ever survive down to their supercooling points? This would involve cooling acclimatized aphids down to temperatures just above their supercooling points on a thermocouple and then re-warming them and assessing survival.
2. Previous low temperature exposure: To what extent do short-term temperature changes (for example between day and night temperatures) alter cold-hardiness? Do cold-hardened viviparae pass on any cold-hardiness to their offspring? How is cold-hardiness affected by repeated short exposures to low temperatures?
3. Inoculative freezing: Is R. padi more susceptible to inoculative freezing (from the host's phloem or surface ice) than S. avenae or M. dirhodum? Can S. avenae or M. dirhodum be inoculated with ice from the host's phloem when feeding on plants other than winter wheat?
4. Between-morph differences: Do alatae and apterae of each species differ in cold-hardiness? Does cold-hardiness of gynoparae, oviparae, males or fundatrices differ from that of viviparae under the same temperature conditions? Both these experiments are best done with field acclimatized aphids.

5. Between-clone differences: How do clones from different parts of Britain and Europe differ in cold-hardiness? This development of work on British S. avenae clones (Chapter 2, experiment 2.5.2) and European clones of all three species (Griffiths & Wratten, 1979) should concentrate particularly on R. padi as there are suggestions that large differences exist between clones of this species (see section 7.1.2). Are viviparae of anholocyclic clones more cold-hardy than those of holocyclic clones? This has been found for Myzus persicae (Tamaki, Annis, Fox, Gupta, Meszleny, 1982).
6. Low temperature mortality of Metopolophium festucae: This species is of particular interest because it is apparently completely anholocyclic in Britain and populations on grasses build-up early in the year (Vickerman & Wratten, 1979) and may provide food for natural enemies which later attack the species studied here (Vickerman, 1977). The species' low temperature mortality has not yet been studied.

As yet there is very little information available on temperature thresholds for chill-coma and flight in overwintering viviparae. These are probably best determined in the laboratory using field acclimatized aphids.

7.4.3 Field experiments on the performance of viviparae

There are several high priority topics which are best investigated by monitoring clip-caged aphids in the field using adaptations of the experimental methods described in Chapters 4 and 5. These are:

1. 'Chronic' low temperature mortality due to prolonged exposure to winter temperatures, concentrating particularly on what temperature parameters best predict it.

2. Sexual morph determination under natural temperature and light conditions and on grasses rather than on cereals, including investigation of whether or not sexual morph production is suppressed in winter.
3. Differences in reproduction, development and survival on different hosts, especially on grasses.
4. Seasonal changes in aphid performance from late summer/early autumn to late spring, especially on grasses.

Work on grasses is important because they are important winter hosts (Hand, 1982) on which little work was done in the present study. In addition, there is evidence for large within-species differences in the reproduction and survival of S. avenae and R. padi on winter wheat and perennial rye grass and for large seasonal shifts in the performance of R. padi on the latter (Chapter 5) which are likely to be very important influences on winter populations. Also, grasses must be the hosts on which gynoparae and males are produced as cereals are not available in late summer and early autumn. In the experiments plants should be in as natural a condition as possible so the grasses used should be grown in close swards. As well as the topics listed above monitoring clip-caged aphids in the field could be used to investigate differences in development, reproduction and survival of morphs and clones mentioned as possible subjects for laboratory studies on low temperature mortality.

Investigation of temperature and weather mortality is probably best pursued using a combination of detailed natural population monitoring (see section 7.4.1) and clip-cage studies (see above). A repeat of the type of weather mortality experiment described in Chapter 3 using the modifications suggested (see section 3.4.5) would also be worthwhile but this type of experiment is rather labour intensive. There are two further types of specialized experiments on aspects of low temperature mortality which would be worth attempting:

1. An experiment on the effect of surface ice, basically like that described in Chapter 3 but with detailed assessment of hoar frost and frozen rain or dew and with some pots in small, roofed, fine-mesh cages to exclude surface ice.
2. An experiment to investigate whether or not aphids ever die of dehydration due to prolonged low temperature exposure (as suggested by Way & Banks (1968) and Smith (1981)). This would involve determining water content before, during and after periods of low temperature.

Both experiments could be combined with laboratory studies.

7.4.4 Other topics

Some research areas not investigated in the present study are discussed briefly below:

- (a) The impact of natural enemies during autumn, winter and spring

Some exclusion-cage experiments to assess the effect of natural enemies on holocyclic populations of R. padi have been undertaken by Leather (1980b). Similar experiments on holocyclic M. dirhodum would be useful, if only to confirm the suggestion (see Chapter 6) that the impact of natural enemies is small. However, these would be difficult because of the low population density of M. dirhodum on rose. Any attempt to artificially boost aphid numbers on experimental bushes would probably alter natural enemy mortality.

Investigations of natural enemies on cereals and grasses during autumn, winter and spring are also likely to present problems due to low population densities but should nevertheless be undertaken as there is increasing evidence that they are important. On present evidence the groups most important early in the year are parasites (Gair, 1953; Powell et al., 1981; Vorley, 1983) and

polyphageous predators (Vickerman & Wratten, 1979). Information needed includes basic data on population densities and phenology, as well as data on the temperature relations of survival, reproduction, development, foraging activity, prey consumption and diapause.

(b) Analysis of the patterns of R.I.S. suction-trap catches and biometric analysis to separate holocyclic and anholocyclic spring alatae

Both these research areas were undertaken by Hand (1982) and are discussed in detail by him. Biometric analysis of spring alatae is potentially by far the most feasible and least labour-intensive way of assessing the relative importance of holocyclic and anholocyclic overwintering. The analysis of R.I.S. catch patterns and of relationships between catches and autumn, winter and spring conditions is central to the development of long-term forecasting systems. Both these research areas have the special advantage that, when fully developed, they would enable large amounts of extra information to be extracted from past years' R.I.S. catches. Hand's progress clearly shows that they are promising research areas and should have high priority.

(c) Temperature and weather effects on summer populations

With the detailed field data provided by this study and those of Hand (1982) and Smith (1981) we are now in the unlikely position of knowing more about temperature and weather effects on winter populations than we do about their effects in the summer, where information is largely limited to the results of laboratory experiments and descriptive rather than analytical field work. Detailed sampling and field experiments should be undertaken to correct the imbalance.

7.4.5 Conclusions

There are several important aspects of cereal aphid overwintering biology which require further investigation

using an applied, field-biased approach. Most of these are suitable components for Ph.D. projects for several reasons. For example:

1. They can be undertaken in a short, intensive period of study.
2. Many are of theoretical as well as applied interest.
3. They provide scope for a variety of sampling, experimental and analysis techniques.
4. Superficially at least, they are less directly 'applied' than studies of summer populations on cereals. They are thus likely to be allocated to Ph.D. students rather than permanent staff in agricultural research institutes.

However, if more Ph.D. projects wholly or partially concerned with overwintering are envisaged, then there is a real need for more long-term planning and co-ordination to sustain and direct effort, and for some long-term winter sampling (particularly of Gramineae in early spring). Neither the planning or sampling need be very time-consuming but both would greatly enhance the value of the short-term research projects.

SUMMARY

SUMMARY

CHAPTER 1 General Introduction

1.1 The three species studied were Sitobion avenae (F.), Metopolophium dirhodum (Walk.) and Rhopalosiphum padi (L.). Their pest status was summarized.

1.2 Reasons why the study of their overwintering biology may lead to more effective control were explained.

1.3 Aphid life-cycles and holocyclic and anholocyclic overwintering strategies were described.

1.4 The types of work undertaken in the present study were summarized. Reasons why particular attention was paid to certain topics were given.

The bulk of the work was concerned with anholocyclic overwintering by viviparae. Temperature plays a dominant role in the population dynamics of anholocyclic populations and so most of the experiments investigated temperature effects. An analytical rather than a descriptive approach was adopted, with effects of temperature on development, reproduction and survival being investigated and analysed separately. More work was done on S. avenae viviparae than on the other two species. Most experiments involved winter wheat as the host plant. Laboratory experiments investigated low temperature mortality while field experiments, using artificially established populations, investigated weather mortality and the effects of temperature on development, reproduction and survival. Natural anholocyclic populations were not studied as this work was being undertaken by S.C. Hand.

Nearly all the work undertaken on holocyclic overwintering was field work on natural populations of M. dirhodum on Rosa spp. The main components were population surveys and the monitoring of colonies on selected bushes. The surveys were undertaken in collaboration with S.C. Hand.

All the fieldwork was carried out in Hampshire.

1.5 Evidence in the literature (up to 1981) for holocyclic and anholocyclic overwintering by each of the three species was reviewed.

CHAPTER 2 Laboratory experiments on low temperature mortality.

2.1 This work was a broad survey of the factors likely to affect low temperature mortality in the field, undertaken to assess the nature and magnitude of their effects. It was not a detailed investigation of the physiology of low temperature mortality.

Nearly all the experiments investigated the cold-hardiness of viviparae reared on winter wheat. Some experiments omitted M. dirhodum and/or R. padi.

A review of insect cold-hardiness with special reference to aphids was given. Factors likely to affect cold-hardiness were divided into: (a) 'environmental' factors - pertaining to environmental conditions before or during low temperature exposure; (b) 'intrinsic' factors - pertaining to the aphid itself.

2.2 Supercooling points were determined for one experiment. All the others involved the direct assessment of mortality after low temperature exposure. Most of these involved exposing several batches of aphids (usually 50/batch) off host plants to a range of constant test temperatures for a standard time (usually 90 min) in a pre-cooled cold chamber or cold bath.

Mortality was assessed 3 days after testing because delayed mortality occurred. Mortality/temperature curves and LT50s were determined using probit analysis.

Stock cultures were kept on winter wheat at 20°C and 16 h daylength. In some experiments aphids were given low temperature pre-treatment before testing. Adult apterae were used in the first three experiments (section 2.3) but 2nd to 4th instar nymphs were usually used thereafter.

2.3 The relative cold-hardiness of unacclimated adult

apterae of the three species was investigated, as well as the relationship between mean supercooling points and directly assessed lower lethal temperatures.

LT50s were determined in a series of 3 h tests. Supercooling points were determined for 20 aphids of each species cooled at 1°C/min attached to a copper-constantan thermo-couple.

From the 3 h LT50s, R. padi was much less cold-hardy than the other species. All three species had LT50s at least 14°C above their mean supercooling points. (3 h LT50s: S. avenae: -8.2°C; M. dirhodum: -8.3°C; R. padi: 1.0°C. Mean supercooling points: S. avenae: -23.0°C; M. dirhodum: -24.4°C; R. padi: -25.4°C.)

This strong suggestion that the aphids could die of chill-injury well before they froze was confirmed by the finding that all aphids died after being cooled at 1°C/min to -15.5°C. Because of this supercooling points were not used to assess cold-hardiness in later experiments.

2.4 Environmental factors likely to affect cold-hardiness were investigated.

In the standard test procedure aphids were cooled and re-warmed at unnaturally fast rates (several °C/min). Single 3 h tests on each species near its 3 h LT50 showed that more natural slow cooling and re-warming rates (2°C/h) substantially increased survival, particularly for R. padi. Mean percent mortalities in slow-cooled and fast-cooled treatments respectively were: S. avenae at -8.2°C: 9%, 72%; M. dirhodum at -8.3°C: 28%, 82%; R. padi at -2.0°C: 17%, 81%.

Short-term low temperature pre-treatment in the laboratory (6°C for 6 days) substantially increased cold-hardiness. R. padi was most affected and M. dirhodum least affected. The 90 min LT50s for unacclimated and acclimated nymphs respectively were: S. avenae: -7.1°C, -10.1°C; M. dirhodum: -6.7°C, -8.6°C; R. padi: -1.1°C, -5.9°C. Even when S. avenae was given low temperature pre-treatment off the host plant (0°C for 23 h) cold-hardiness was significantly increased, showing that it had not been gained passively from the host.

Long-term low temperature pre-treatment in the field was investigated for S. avenae and R. padi. The 90 min LT50s of nymphs from cultures kept in the field from late September 1980 were determined in late October/early November, early January and mid March. The LT50s of both species had dropped by several degrees by October/November but showed little sign of further change after this. LT50s for all three series of tests combined were: S. avenae: -11.8°C ; R. padi: -7.3°C .

Increasing the duration of exposure at a constant temperature near the 3 h LT50 from 37.5 min to 19 h had little effect - the increase in mortality was equivalent to lowering the temperature by only 1° or 2°C . M. dirhodum was affected a little more than the other two species.

S. avenae and M. dirhodum nymphs exposed to -6.4°C for 21.5 h on cold-susceptible wheat which froze during the test showed no greater mortality than those tested off the plants. In similar tests in which S. avenae nymphs were exposed to temperatures from -6.8°C to -8.5°C for 12 h, aphids on wheat which froze suffered c. 10 to 20% higher mortality than those tested off the plants. It thus appeared that inoculative freezing from host plant sap was rare in S. avenae and M. dirhodum on wheat.

The cold-hardiness of S. avenae nymphs was slightly reduced when they were reared in the field on perennial rye grass rather than winter wheat, even when they were tested off the host plants. 90 min LT50s were -10.6°C and -11.7°C respectively.

S. avenae nymphs were exposed to temperatures between -1.7°C and -8.2°C for 14 h, some dry and some wetted, so that water droplets froze on the body surface. The mortality/temperature curve for wetted aphids suggested that the threshold for inoculative freezing from surface ice lay around -2° to -3°C and that below about -4°C c.70% of aphids not dying of chill-injury died of inoculative freezing. Inoculative freezing thus appeared to be important but several factors could mitigate its effect in the field.

2.5 Intrinsic factors (apart from the between-species differences already studied) were investigated.

Cold-hardiness of unacclimatized adult apterae and small, medium-sized and large nymphs was compared in 3 h tests near the 3 h LT50. Nymphs and adults of R. padi had similar cold-hardiness. In S. avenae and M. dirhodum there was a tendency for smaller nymphs to be less cold-hardy but differences were small and in similar tests on acclimatized S. avenae, nymphs were more cold-hardy than adult apterae.

Twelve British clones and a Norwegian clone of S. avenae showed little variation in cold-hardiness and no sign of a relationship between cold-hardiness and winter temperature in their place of origin. Tests on four clones after acclimation gave the same result.

A single test on field-reared S. avenae nymphs showed that fundatrices were no more cold-hardy than viviparae.

Eggs of S. avenae and Metopolophium fasciatum tested in February and March at -14° to -15°C for 90 min (conditions which would kill the great majority of overwintering viviparae) showed no low temperature mortality.

2.6 As so many factors were found to modify low temperature mortality, a laboratory determined LT50 cannot be used as an accurate predictor of low temperature mortality in a field population but the results suggested it could provide a reasonable index if the effects of cooling/re-warming rates and acclimatization were taken into account. All the LT50s determined were over 10°C above the laboratory determined mean supercooling points, which thus appeared to be a much less reliable cold-hardiness index for cereal aphids.

CHAPTER 3 Effects of low temperature and other weather conditions on the overwintering mortality of viviparae on winter wheat.

3.1 The effects on mortality of low temperatures, wind, rain and snow were investigated in the field using artific-

ially established populations on wheat. Most observations were made on S. avenae and M. dirhodum although some data on R. padi were also obtained.

Unlike earlier studies on weather mortality, many batches of nymphs were used one after the other, each monitored for only 8 days. As no reproduction took place detailed data could be obtained on relationships between mortality and weather variables.

3.2 The work was carried out at two sites in Hampshire, from 26 January to 27 March 1979 and from 5 December 1979 to 10 March 1980. After rearing and acclimation (6.5°C for 6 days) in the laboratory, second to fourth instar nymphs were exposed on pots of field-grown winter wheat sunk into the ground in a field plot. Predators were excluded from the pots using sticky-barriers.

Eight pots, each with 25 S. avenae and 25 M. dirhodum, were put out together. During the 1979/80 winter some pots of R. padi were also put out. Live and dead aphids were counted after 4 and 8 days. The experiment was thus divided into $\frac{6}{31}$ 4-day periods for which the mortality of each species was known.

Multiple regression analysis was used to determine relationships between mortality and weather variables recorded on-site and at local weather stations. The dependent variables used were arcsin transformed percent mortalities in a 4-day period. Data for R. padi were insufficient for regression analysis.

3.3 Aphid mortality after 4 days exposure could be divided between 'on-plant' mortality (aphids dead on plants) and 'off-plant' mortality (those dead on the soil or missing). There was a significant negative correlation between 'on-plant' mortality and minimum temperature but none between 'off-plant' mortality and minimum temperature. The threshold for 'on-plant' mortality for S. avenae and M. dirhodum was c. -3°C .

From stepwise multiple regressions of mortality on sets of weather variables (and interaction and quadratic

variables derived from these) predictive equations were determined incorporating variables with significant ($P < 0.05$) partial regression coefficients.

'On-plant' mortality equations for both S. avenae and M. dirhodum had (grass minimum temperature from -3°C) as the only independent variable and had $r^2 \geq 0.68$. 'Off-plant' mortality equations contained (presence/absence of snow), (maximum wind speed over 1 h) and (maximum wind speed over 1 h)² and had $r^2 \geq 0.62$.

Further regressions using the data from the 1979/1980 winter only (during which more detailed weather data were recorded) confirmed the results of earlier analyses but did not improve the regressions.

Mortalities of S. avenae and M. dirhodum differed significantly ($P < 0.05$) in nine 4-day periods. In five of these, lower mortality of S. avenae was probably due to this species' greater cold-hardiness.

S. avenae and M. dirhodum gradually occupied lower, more sheltered feeding positions when left in the field for up to 27 days.

R. padi suffered much greater mortality than the other species in two particularly cold periods. But there was no sign of this species' lower cold-hardiness if grass minimum temperatures stayed above -6°C . Below -6°C there was no clear relationship between mortality of R. padi and minimum temperature. R. padi suffered slightly less off-plant mortality than the other species, probably due to its more sheltered feeding positions.

3.4 Results strongly suggested that low temperature caused nearly all 'on-plant' mortality but little 'off-plant' mortality. Approximate LT50s derived from 'on-plant' mortality equations (S. avenae: -14.9°C , M. dirhodum: -12.5°C) lay about 4° or 5° below 90 min LT50s for acclimated nymphs determined in the laboratory (Chapter 2). But if the effect of the fast cooling and re-warming rates used in laboratory tests were taken into account laboratory LT50s were a good index of field cold-hardiness.

Inoculative freezing was found to be less important in the field than had been suggested by laboratory exper-

iments but several conclusions from the laboratory experiments on low temperature mortality were confirmed by the fieldwork.

Information in the literature suggested that rain caused more mortality than wind but the present study and that of Smith (1981) strongly suggested the opposite. The way snow killed aphids was unknown as it only occurred twice. In the experimental period low temperatures probably killed fewer S. avenae or M. dirhodum than did the other weather factors (mainly wind).

The use of the regression equations derived and features of the experiment's design which make them only approximate predictors of weather mortality in natural populations were discussed, as well as possible improvements to the experiment.

CHAPTER 4 Development and reproduction of overwintering S. avenae on winter wheat.

4.1 This field experiment was undertaken to determine relationships between development rate, reproduction rate and temperature and between reproduction rate, survival and age for S. avenae viviparae overwintering on winter wheat.

A review was given of the temperature relationships of development, reproduction and longevity in insects, with special reference to aphids. Evidence in the literature suggested that relationships determined at constant temperatures in the laboratory may well not be applicable to winter field conditions.

4.2 The development, reproduction and survival of viviparae clip-caged on winter wheat was recorded at 3-day intervals from 21 September 1980 to 29 March 1981. . Aphids were kept two to a cage as nymphs and singly as adults. They were born between 21 September and 10 January. In total 242 apterae and 319 alatae were monitored to adulthood and of these 235 apterae and 171 alatae were monitored through adult life.

Temperatures inside clip-cages were recorded every

hour by a Grant temperature recorder. Regression analysis was used to determine relationships between development or reproduction rates and temperature. Other variables which might have affected development and reproduction were recorded and their modifying effects analysed.

4.3 A trial-and-error method was used to determine best theoretical temperature thresholds for development and reproduction, assuming a linear relationship between rate and temperature above the threshold.

Using a common threshold of 3°C the rate/temperature relationships for development and reproduction (production of the first 20 nymphs) were well described by straight lines. Because of this development and reproduction could be expressed on a physiological time scale of 'day-degrees above 3°C '.

Mean day-degree requirements for development, the pre-reproductive period of adult life and production of the first 20 nymphs were calculated. Those for apterae were lower than those for alatae.

Day-degrees taken to produce the first 20 nymphs were used when assessing variables which modified the reproduction rate/temperature relationship. Whether apterae or alatae, aphids reproduced faster if they were born to apterae rather than to alatae. Reproduction rates of apterae dropped slightly from autumn to spring. No variables were found to modify the development rate/temperature relationship.

A sample of the progeny of monitored aphids were reared. None of these were sexual morphs but 17 of the monitored aphids born to alatae between 27 September and 15 October were oviparae.

Estimated mean potential fecundities were high (apterae: 72 to 75; alatae: 42 to 51). Aphids of both morphs born in early to mid-October had lower fecundities than those born in September or late October.

Age-specific reproduction rates expressed in day-degrees were calculated. Apterae and alatae had different reproductive patterns. Reproductive rates for apterae were higher than those for alatae of the same physiological

age, except for old adults. Alatae had an odd, two-peaked reproductive pattern.

Relationships between reproduction rate and temperature were re-examined using 3-day period nymph production data for adults of a narrow age range. Little curvilinearity in the relationships was confirmed.

Mean survival to adulthood was very high (97%) and adults survived very well. Many were over 3 months old when monitoring stopped. There was very little sign of mortality due to low temperatures. This was expected as the minimum temperature was only -7°C and aphids were protected from surface ice. Survivorship curves showed that median longevity was slightly longer for apterae than for alatae and coincided with the end of reproduction.

Relative durations of each nymphal instar were very similar to those determined in earlier laboratory studies.

Alatae had a period of restlessness in early adult life. They appeared to be in a state of migratory readiness despite the low temperatures.

Temperatures experienced inside clip-cages were very similar to those outside. The overall mean temperature in cages was 6.5°C .

4.4 The lack of curvilinearity in the rate/temperature relationships determined was discussed.

Comparison of the results with those of earlier studies on S. avenae revealed some large differences in development and, especially, in reproduction. This confirmed the suggestion that results obtained at high and/or constant temperatures are often inapplicable to overwintering viviparae.

Winter reproduction was better than expected from earlier studies. Characteristics of reproduction, particularly of apterae, appeared to be very malleable and could be adjusted to suit winter conditions.

In several respects alatae showed disadvantages under winter conditions when compared with apterae.

The production of some oviparae in September and October but not after this suggested that sexual morph production could be switched off in winter.

Several characteristics of the relationships determined appeared to facilitate their incorporation into a simulation model.

CHAPTER 5 Development, reproduction and survival of S. avenae and R. padi on winter wheat and perennial rye grass in field plots.

5.1 S. avenae and R. padi were monitored in clip-cages on seedling winter wheat and perennial rye grass to assess differences in winter performance.

5.2 All the aphids used were born between 24 and 29 November 1981. Four batches were monitored, one for each aphid/host combination, from birth until 29 March. They each contained 41 to 63 nymphs initially.

Methods were similar to those described in Chapter 4. Only apterae were monitored as adults. Parents of all the aphids came from cultures kept in the field on wheat since early August. The parents of those monitored on rye grass were transferred from wheat as alatiform fourth instars or newly moulted alatae.

5.3 S. avenae on rye grass suffered steady mortality throughout life which was not correlated with particularly cold periods. Only 40% reached adulthood and over 70% had died by 29 March, compared to 10% of those on wheat.

Mortality of R. padi was much greater than that of S. avenae on both hosts, and was much greater on rye grass than on wheat. Proportions reaching adulthood on the two hosts were 3.9% and 60.3% respectively. Increased mortality was correlated with low minimum temperatures, but not closely.

Development times, longevities and reproduction rates were measured in day-degrees above 3°C. For both aphid species development rate, mean reproduction rate, total fecundity and adult longevity were greater on wheat than on rye grass and on both hosts they were greater for S. avenae than R. padi. Differences in development times were small compared to those in survival and reproduction.

As assessed by rates of leaf emergence the wheat was growing over twice as fast as rye grass.

5.4 Aphids may not have been given sufficient time to fully adapt to perennial rye grass so their poor performance on this host may have been exaggerated somewhat.

Comparison of the results with data in the literature suggested large seasonal changes in performance, particularly for R. padi.

The high mortality and poor reproduction of S. avenae on rye grass and R. padi on both hosts was probably largely caused by low temperatures, which might have produced physiological stress by affecting aphids directly and by slowing the host's metabolism and growth. The latter was suggested because both aphids performed much worse on the slower growing host. S. avenae seemed much better adapted to low temperatures than R. padi.

Long exposure to winter temperatures on a slow growing host appeared to cause 'chronic' low temperature mortality in S. avenae on rye grass and R. padi on both hosts. This was not closely correlated with minimum temperatures, unlike the 'acute' low temperature mortality observed in earlier experiments (Chapters 2 and 3).

'Chronic' low temperature mortality, large seasonal shifts in performance, between-species differences and host plant effects complicate population modelling.

CHAPTER 6 Holocyclic overwintering of M. dirhodum on rose.

6.1 Principal components of the work were population surveys and colony monitoring on a west Hampshire study farm.

The surveys were carried out to determine the types of rose bush and rose bush habitat which were the most important overwintering sites, and to estimate holocyclic population densities. The estimates were used, with S.C. Hand's samples of anholocyclic populations in the same area, to assess the importance of holocycly.

The colony monitoring was undertaken to determine the timing of the holocyclic cycle (in particular the

spring migration from rose) and obtain data on mortality factors.

A recently described species of Metopolophium on rose (M. fasciatum sp. nov.) could not be separated from M. dirhodum in the field. Its abundance relative to M. dirhodum was estimated from samples identified in the laboratory by Hand.

6.2 The population surveys were carried out in collaboration with Hand, who undertook the great bulk of the results analysis. They were done in autumn (1978 and 1979) and spring (1979 and 1980) to coincide with peak populations of oviparae and fundatrigeniae.

Rose in 1m lengths at c. 15 m intervals was examined in randomly selected field boundaries (hedges and wood edges). Numbers of Metopolophium and various site and rose characteristics were recorded. In woodland and scrubland randomly selected rose bushes were sampled.

Approximately 28% of oviparae were M. fasciatum but none of this species were identified in spring.

Metopolophium were aggregated on leaves and males were closely associated with oviparae. Several unexpected morphs were found.

There were significant differences in the proportion of rose leaves infested with Metopolophium in different habitats. Dense woodland had the highest infestation levels. Infestations were lower in open woodland and field boundaries and lowest in open scrubland.

Low field boundaries (≤ 4 m) had lower infestation levels than high ones. There was no evidence that boundary orientation affected infestation.

Only Rosa canina agg. and R. rubiginosa agg. were common but rose species did not appear to affect infestation. A higher proportion of leaves were infested on bushes with less foliage. When this effect was controlled for there was little evidence that infestation was greater on taller bushes.

Estimates of the total population of M. dirhodum on rose on the study farm in spring and autumn showed that open woodland and scrub held insignificant numbers. But

field boundaries and, especially, dense woodland were important overwintering sites. Autumn to spring survival estimates were less than 20%. Despite huge numbers of M. dirhodum in summer 1979, autumn population density on rose was a third of that in 1978.

As Hand failed to find M. dirhodum overwintering on Gramineae, the reasonably large populations on rose probably formed a significant part of the total overwintering population, at least in the 1978/79 winter, which was cold enough to kill most viviparae.

The pattern of aphid distribution on rose in different habitats was probably largely due to more wind-delivered autumn migrants being deposited in sheltered habitats with large boundary layers. It may have been accentuated by the aphids' flight behaviour within boundary layers.

6.3 A small amount of laboratory work on oviparae was undertaken. Unmated oviparae laid 0.42 eggs each on average. From dissections mean potential fecundity was 8.9 eggs.

6.4 M. dirhodum colonies on 13 bushes in an exposed hedgerow and a sheltered wood were monitored from the production of oviparae to the maturation of fundatrices.

Autumn colonies declined from late October. Eggs were laid during November and very few aphids were left by December. Spiders (mainly small Theridiidae) were the only reasonably common natural enemies. Leaf fall occurred earlier in woodland and removed more oviparae but this was compensated for by greater losses due to wind or rain in the hedge.

113 eggs were laid on stems (0.5 to 4.3/adult ovipara on 8 bushes). They were widely scattered, usually on thorns or thorn bases. 51% hatched, all in mild weather in February. Unhatched eggs were missing (22%) or collapsed and/or split.

All but eight fundatrices (86% of those hatched) died as nymphs. The survivors became adults in April.

Autumn to spring survival was greater than estimated from population surveys probably because gynoparae had arrived early on monitored bushes.

Population densities were low and egg production per ovipara and egg survival high compared to other aphids studied. Leaf fall and wind or rain caused considerable mortality in autumn populations but natural enemies did not, though they probably killed c. 10 to 30% of the eggs. Overall success up to egg hatch was similar on hedge and woodland bushes but mortality of fundatrices was higher in the hedge, probably due to greater exposure to wind and rain before sheltered feeding positions became available.

6.5 In spring 1979 and 1980 M. dirhodum colonies on 13 bushes in hedges and woodland were monitored at 6 to 10 day intervals from the maturation of fundatrices to the migration of the last fundatrigeniae.

The timing and pattern of population change was different in the two years. In 1979 all fundatrices had matured by 1 May, fundatrigeniae peaked in mid-May and the migration was from c. 12 May to 8 June (the great majority before 31 May). A few apterous fundatrigeniae were produced but their reproduction was poor.

In 1980 fundatrices matured c. 10 days earlier. Migration started 2 weeks earlier but was sustained till c. 1 June because more migrants were produced by more numerous apterous fundatrigeniae.

Spiders (mainly small Theridiidae) appeared earlier and were much more abundant than other natural enemies.

'Fourth and fifth instar alatae aphid-days per fundatrix' was used as an index of the success of the colony on each bush. Variables thought to influence colony success were investigated but the only one correlated with the success index was a measure of the adult longevity of fundatrices.

Factors affecting spring colony success were discussed. Early mortality factors acting on fundatrices appeared to have much greater impact than those acting on fundatrigeniae. The poor reproductive performance of the latter accentuated this. As with holocyclic spring colonies of other aphids, natural enemies probably had relatively little effect as they increased too late.

Density dependent effects were not evident, probably due to the low population densities.

Comparison of the timing of migration from rose with R.I.S. suction-trap catches strongly suggested that holocyclic populations were important in initiating summer colonies on Gramineae in 1979 (a severe outbreak year) but not in 1980.

CHAPTER 7 General discussion.

7.1 Reasons for the large differences in the winter abundance of viviparae of S. avenae, M. dirhodum and R. padi were discussed in the light of results from the present study.

The small differences in cold-hardiness between M. dirhodum and S. avenae could not account for the much greater rarity of M. dirhodum, which was probably mainly due to a much more complete switch to sexual morph production in autumn and, possibly, to a reduced autumn population due to poor survival, development or reproduction in late summer.

Differences in patterns of winter population change between S. avenae and R. padi could be attributed to greater low temperature mortality in R. padi (particularly that due to chronic low temperature stress) and to its poor reproductive performance under winter conditions.

7.2 The balance between holocyclic and anholocyclic overwintering were discussed. Both strategies have advantages and disadvantages. The balance is probably maintained primarily by regional and temporal variation in winter temperatures but other factors also play a role.

Incomplete switching to sexual morph production by holocyclic clones in autumn (possibly involving a winter 'off-switch') would allow a flexible, mixed overwintering strategy. The adaptiveness of this and evidence for it were discussed.

7.3 Complexities of the overwintering ecology of cereal aphids were discussed. These probably make accurate long-

term forecasting based on realistic computer simulation of the whole system an unfeasible objective. But simpler, more approximate forecasting systems would be useful and feasible. More studies on overwintering biology are needed to develop these. A computer model of S. avenae overwintering on winter cereals could be of direct use in forecasting.

7.4 Suggestions for future work on cereal aphids were made. The need for an applied, field-biased approach involving some long-term field sampling and co-ordination was stressed.

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APPENDICES

APPENDIX 1.1

Glossary of aphidological terms. (After Blackman (1974a)).

ALATAE: Winged forms of aphids, usually referring to winged parthenogenetic, viviparous females.

ALATE FUNDATRIGENIAE: Winged parthenogenetic, viviparous females produced in spring on the primary host and migrating to secondary hosts. Also known as SPRING MIGRANTS or EMIGRANTS.

ANDROCYCLIC: Reproducing by parthenogenesis throughout the year, but producing some males to contribute to the sexual phase.

ANHOLOCYCLIC: Reproducing by parthenogenesis throughout the year, without producing any sexual forms or eggs.

APTERAE: Wingless forms, usually referring to wingless parthenogenetic, viviparous females.

EXULES: Heteroecious aphids on their secondary host plant. Also known as ALIENICOLAE.

FUNDATRIGENIAE: All spring generations of a heteroecious aphid on its primary host following the fundatrix. They are winged or wingless parthenogenetic, viviparous females. Some authors restrict the term to the progeny of the fundatrix only.

FUNDATRIX: Viviparous, parthenogenetic female developing from a fertilized egg.

GYNOPARAE: Parthenogenetic females which produce sexual females (the oviparae). Some authors restrict the term to females which produce only oviparae but here the oviparae-producers of S. avenae, which also produce viviparae and males, are referred to as gynoparae.

HETEROECIOUS: With an annual alternation between primary and secondary hosts.

HOLOCYCLIC: Having an annual sexual phase in which parthenogenesis is interrupted by the production of male and female sexual morphs and eggs.

MONOECIOUS: Without an annual alternation between primary and secondary hosts. Also known as AUTOECIOUS.

MUMMY: Dead aphid containing the well-grown larva or pupa of a hymenopteran parasite. Mummies are hard, swollen and discoloured and are usually cemented to the substrate.

OVIPARAE: Sexual females which mate with males and lay eggs.

PARTHENOGENESIS: Asexual reproduction by the development of unfertilized eggs.

PRIMARY HOST: The host plant of a heteroecious aphid on which the sexual phase of its life-cycle occurs.

SECONDARY HOST: The host plant of a heteroecious aphid on which only parthenogenetic reproduction takes place.

SEXUAL MORPHS: Males and oviparae. Also known as SEXUALS or SEXUALES.

VIVIPARAE: Female aphids giving birth to live young by parthenogenesis. A fundatrix is a type of vivipara but for syntactical simplicity these two terms have been used as mutually exclusive in this thesis. Some authors use the term VIRGINOPARAE as a synonym for viviparae but others restrict its use to viviparae which are not fundatrices and do not produce any sexual morphs.

APPENDIX 1.2

Observations on the production of eggs by S. avenae in the field.

Introduction and methods

This work was an attempt to produce S. avenae eggs for use in laboratory experiments. Though too few were produced for this purpose some observations on egg production were made.

A total of 160 15 cm pots of winter wheat in large field cages were infested with about ten first to second instar nymphs/pot at the one to three leaf stage in the third week of September. The old clone of S. avenae referred to in Chapter 2 was used, cultured at $20^{\circ} \pm 1.5^{\circ}\text{C}$ and 16 h daylength.

The number of aphids on the pots built up during October and November and several oviparae were noted, but few aphids were left by January. In mid-February the plants in five pots were examined carefully for eggs. Details of the appearance and position of those found were recorded.

Results and discussion

A total of 79 eggs were found on the five pots. Forty-four were black and apparently viable and 11 (25%) of these hatched when kept at 15°C , 16 h daylength and 73% relative humidity. The remaining eggs were dark green or brown and usually collapsed (21.5%), or black and indented or collapsed (22.8%). None of these hatched. It was estimated that about 1,500 viable eggs had been laid altogether but it would have taken over one week to collect enough for experiments.

The eggs found were cemented to the plants either on leaves (97.3%) or leaf sheaths. They were not laid in particularly sheltered positions, with 63.2% more than 2 cm above the soil and 44.4% not sheltered from above. Of those laid on leaves 79% were on adaxial surfaces.

Dean (1978) had monitored colonies of S. avenae established in a similar way in the autumn but had found

that no males or eggs were produced. The contrast with the present work was probably due to the different clones used. Hand (1982) also obtained many viable eggs from the clone used here, under short daylength conditions in the laboratory.

Though these results showed that a British clone of S. avenae could produce reasonable numbers of viable eggs under relatively natural field conditions, the number of oviparae and eggs produced may have been artificially high. Firstly, the sudden change to relatively cold, short daylength conditions after several years of culturing in the laboratory may have artificially stimulated sexual morph production. The plants used began to suffer from the effect of being in pots and the high aphid numbers during October. Their unnatural degeneration might also have stimulated sexual morph production.

APPENDIX 2.1

Glossary of terms concerned with low temperature survival.

CHILLING: Exposure of an organism to low temperatures without the formation of internal ice.

CHILL-INJURY: Damage to an organism resulting from low temperature exposure without the formation of internal ice.

CHILL-SUSCEPTIBLE: Killed by low temperatures even when ice does not form in the body.

COLD-ACCLIMATION: Cold-hardening which occurs under laboratory conditions - in which all factors except temperature are held more or less constant.

COLD-ACCLIMATIZATION: Cold-hardening which occurs under field conditions - in which several environmental factors vary simultaneously.

COLD-HARDINESS: The ability of an organism to survive low temperatures.

COLD-HARDENING: A physiological response in an organism resulting in an increased ability to survive low temperatures.

CRYOPROTECTANT: A substance whose presence in the body increases cold-hardiness.

FREEZING-INJURY: Damage to an organism due, directly or indirectly, to the formation of ice in the body.

FREEZING POINT (of an aqueous solution): The temperature at which water in the solution is in thermodynamic equilibrium with ice.

FREEZING-SUSCEPTIBLE: Killed by ice formation in the body but not by chilling.

FREEZING TOLERANT (= FREEZING-RESISTANT): Capable of surviving some internal ice formation.

HETEROGENEOUS NUCLEATION: The initiation of freezing by an ice crystal nucleus formed by the addition of super-cooled water molecules onto a non-aqueous surface or particle.

HOMOGENEOUS NUCLEATION: The initiation of freezing by an ice crystal nucleus formed from a random aggregation of water molecules alone and not involving a non-aqueous surface.

ICE CRYSTAL NUCLEUS: An aggregation of water molecules in an ice-like configuration from which an ice crystal can develop by the addition of more water molecules.

INOCULATIVE FREEZING: Freezing initiated by ice crystals formed outside the body.

LATENT HEAT OF FUSION: The heat required to change the state of a substance from solid to liquid without a change of temperature.

NUCLEATIVE FREEZING: Freezing initiated by an ice crystal nucleus inside the body.

NUCLEATOR (= NUCLEATING AGENT): A particle or surface whose molecular configuration tends to bring water molecules together in an ice-like structure to form an ice crystal nucleus.

SUPERCOOLING: Cooling to temperatures below the freezing point without ice formation.

SUPERCOOLING POINT: The temperature at which ice starts to form in a solution cooled below its freezing point.

THERMAL SHOCK: Damage to an organism due to rapid heating or cooling.

APPENDIX 2.2

The standard culturing technique.

Aphids were cultured on winter wheat seedlings (cv. Hobbit) grown in John Innes No.2 potting compost in 13 cm diameter plastic pots. Seed was sown at c. 30 seeds/pot and germinated in a greenhouse at c. 20°C and 16 h daylength. When seedlings were at growth stage 10 to 13 (Zadoks' scale) the soil was treated with c. 25 ml of 0.05% Milstem (a systemic fungicide) to prevent mildew. The following day the pots were transferred to perspex cages in a controlled environment room with 16 h daylength and were infested with aphids. The cages used were 60 x 54 x 46 cm high, with gauze vents and thermostatically controlled heaters maintaining the temperature at 20°C ± 1.5°C. Each was lit by six 75 to 85 W warm white fluorescent tubes giving a light intensity of 614 lumens/ft² at the cage floor and 838 lumens/ft² 25 cm above the floor (the approximate average leaf height). The cage base consisted of a metal tray in which the water depth was maintained at 0.5 to 2 cm.

The three species of aphid were cultured together in the same cages. Although each pot of wheat was initially infested with only one species, the movement of aphids between pots soon produced thorough mixing. To start the cultures fourth-instar nymphs were used, apterous ones being selected whenever possible. As the species had different rates of increase the following initial infestation rates were used: S. avenae:c. 11 nymphs/pot, M. dirhodum:c. 8 nymphs/pot, R. padi:c. 3 nymphs/pot. The number of pots infested with each species was varied according to need. Aphids were taken from the cultures 7 to 16 days after they had been set up. The aphids were gently shaken from the cut culture plants onto a tray and sorted with a fine brush. This handling technique produced very low mortality, even in first-instar nymphs, although these were not generally used in experiments. Cultures over 16 days old were discarded and the cage and its base cleaned.

APPENDIX 3.1

Weather conditions during the winter field experiment on weather mortality (Chapter 3).

(a) The 1978/79 winter. Data recorded at Hurn airport.

Period	Period code no.	Temperature (°C)		Rainfall		Wind speed (knots)	
		mean	grass min	amount (mm)	amount x rate (mm ² /100 min)	mean	max. over 1 h
26/1-30/1	1	-1.7	-12.2	13.3	31.7	5.8	25
30/1- 3/2	2	3.0	-9.0	15.2	25.2	8.7	26
3/2- 7/2	3	2.4	-3.4	23.1	74.7	7.8	15
7/2-11/2	4*	2.7	-1.6	15.6	18.2	10.4	17
11/2-15/2	5*	2.0	-5.6	15.5	17.0	11.1	21
20/2-24/2	6	3.1	-6.8	1.3	0.7	6.2	17
24/2-28/2	7	1.7	-9.0	2.3	3.2	6.2	20
1/3-5 / 3	8	6.5	-5.1	5.9	6.3	10.7	19
5/3- 9/ 3	9	6.5	-1.1	18.6	50.6	13.4	28
10/3-14/3	10	7.0	2.2	16.7	18.6	10.0	17
14/3-18/3	11	1.8	-3.9	8.5	6.4	11.0	19
19/3-23/3	12	4.4	-8.8	8.5	28.7	8.4	17
23/3-27/3	13	6.1	-7.6	24.0	63.2	14.9	25

* = periods with snow.

- (b) The 1979/80 winter. Data recorded at Chilworth Manor except for mean temperature (recorded at Southampton Weather Centre) and wind speed (recorded at Calshot).

Period	Period code no.	Temperature (°C)		Rainfall		Wind speed (knots)	
		mean	grass min	amount (mm)	amount x rate (mm ² /100 min)	mean	max. over 1 h
5/12- 9/12	14	12.1	6.1	26.5	52.2	11.7	25
9/12-13/12	15	8.9	-3.8	22.4	77.8	15.7	36
15/12-19/12	16	6.1	-1.7	3.0	2.5	15.0	28
19/12-23/12	17	2.7	-3.7	0.8	0.30	13.4	22
31/12- 4/1	18	2.2	-9.6	9.6	11.7	10.7	27
4/1 - 8/1	19	5.2	-4.8	1.2	0.4	7.8	17
10/1 -14/1	20	0.8	-7.1	0.8	0.04	8.9	14
14/1 -18/1	21	1.7	-5.9	0.2	0.13	8.6	13
20/1 -24/1	22	5.6	-4.7	13.3	41.2	15.1	37
24/1 -28/1	23	1.7	-6.5	0.9	0.5	6.6	13
30/1 - 4/2	24	6.7	-4.2	41.9	107.6	11.8	25
4/2 - 7/2	25	7.9	1.3	9.7	12.0	12.1	20
10/2 -14/2	26	8.0	-1.1	0.0	0.0	7.0	11
14/2 -18/2	27	8.4	-1.6	3.7	2.8	7.0	16
20/2 -24/2	28	6.7	-3.9	17.9	16.6	10.8	21
24/2 -28/2	29	4.6	-5.3	0.8	0.8	6.1	18
2/3 - 6/3	30	5.5	-7.2	9.4	13.3	8.5	23
6/3 -10/3	31	7.1	-1.9	9.6	18.1	12.9	25

APPENDIX 4.1

Kendall's rank correlation coefficients between the day-degree requirement for the production of the first 20 nymphs by *S. avenae* apterae and some variables which may affect 20 nymph d.d.r.

See Chapter 3, section 4.3.4 for full details of the variables. Those followed by '(dev.)' refer to the development period and those followed by '(rep.)' to the period in which the first 20 nymphs were produced.

PARAMETER	ALL APTERAE (n = 192)		APTERAE WITH ALATE PARENTS (n = 157)	
	T	P	T	P
Mother's morph	.386	.001*	—	—
Birth date	.254	.001*	.267	.001*
Maturation date	.273	.001*	.289	.001*
Development day °C > 3°	.097	.047*	.128	.018*
TEMPERATURE PARAMETERS				
Mean temp. (°C > 3°) (dev.)	-.249	.001*	-.264	.001*
Min. temp. (°C) (dev.)	-.081	.150	-.258	.001*
Hour °C ≤ 0°C/day (dev.)	.025	.607	.172	.002*
Hour °C ≤ -5°C/day (dev.)	.053	.287	.208	.001*
Mean temp. (°C > 3°) (rep.)	.032	.509	-.004	.944
Min. temp. (°C) (rep.)	-.137	.011*	-.144	.016*
Hour °C ≤ 0°C/day (rep.)	.066	.178	.046	.393
Hour °C ≤ -5°C/day (rep.)	.059	.234	.069	.214
HOST PLANT PARAMETERS				
Total live leaves (dev.)	.054	.289	.076	.184
Total leaves on main shoot (dev.)	.066	.210	.065	.263
Number of side tillers (dev.)	.019	.716	.004	.949
Leaf sheath length (dev.)	.050	.346	.055	.346
Height (dev.)	.017	.738	-.023	.696
Sowing date (Sep.) (dev.)	-.174	.003*	-.057	.388
Sowing date (Nov.) (dev.)	.253	.001*	.197	.003*
Total live leaves (rep.)	.001	.996	.015	.791
Total leaves on main shoot (rep.)	-.013	.796	.025	.663
Number of side tillers (rep.)	.026	.624	-.021	.710
Leaf sheath length (rep.)	.095	.071	.071	.222
Height (rep.)	.020	.692	-.007	.901
Sowing date (Sep.) (rep.)	-.187	.002*	-.094	.151
Sowing date (Nov.) (rep.)	.253	.001*	.197	.003*

APPENDIX 4.2

Summaries of multiple-regression analyses of 20 nymph day-degree requirements of S. avenae apterae on a set of variables which may affect 20 nymph d.d.r.

See Chapter 4, section 4.3.4, for full details of the analyses. Parameters followed by '(dev.)' refer to the development period and those followed by '(rep.)' to the period in which the first 20 nymphs were produced.

Independent variables with significant p.r.c.s ($P < 0.05$)	Partial regression coefficient	Increase in r^2
1. DATA SET: ALL APTERAE		
Mother's morph	11.870	.213
Maturation date	0.384	.121
Total live leaves (dev.)	0.606	.028
Number of side tillers (rep.)	-0.664	.022
Development day $^{\circ}\text{C} > 3^{\circ}$	0.125	.015
Hour $^{\circ}\text{C} \leq -5^{\circ}\text{C}/\text{day}$ (rep.)	0.785	.013
Maturation period dummy variable (periods 22-26)	11.888	.038
Constant = 21.473	Total $r^2 = 0.451$	
2. DATA SET: APTERAE REARED ON SEPTEMBER- AND NOVEMBER-SOWN WHEAT		
Mother's morph	12.434	.292
Maturation date	1.562	.111
Min. temp. ($^{\circ}\text{C}$) (rep.)	-2.122	.039
Birth date	-1.620	.021
Maturation period 'dummy' variable (periods 22-26)	13.074	.048
Number of side tillers (dev.)	1.500	.012
Mean temp. ($^{\circ}\text{C} > 3^{\circ}$) (rep.)	2.109	.015
Maturation period 'dummy' variable (periods 32-36)	8.434	.011
Constant = 10.992	Total $r^2 = 0.549$	
3. DATA SET: APTERAE REARED ON NOVEMBER-SOWN WHEAT		
Mother's morph	13.049	.275
Number of side tillers (dev.)	2.119	.117
Min. temp. ($^{\circ}\text{C}$) (rep.)	-3.816	.055
Mean temp. ($^{\circ}\text{C}$) (rep.)	4.260	.018
Mean temp. ($^{\circ}\text{C}$) (dev.)	-4.739	.027
Development day $^{\circ}\text{C} > 3^{\circ}$	0.151	.017
Maturation period 'dummy' variable (periods 22-26)	11.557	.047
Constant = 8.558	Total $r^2 = 0.555$	

APPENDIX 4.3

Kendall's rank correlation coefficients between day-degree requirements for the development of S. avenae apterae and a set of variables which may affect development d.d.r.

See Chapter 4, section 4.3.4, for full details of parameters. Those followed by '(dev.)' refer to the development period.

n = 194

PARAMETER	τ	P
Mother's morph.	-.025	.679
Birth date	.053	.288
TEMPERATURE PARAMETERS		
Mean temp. ($^{\circ}\text{C} > 3^{\circ}$) (dev.)	.026	.604
Min. temp. ($^{\circ}\text{C}$) (dev.)	-.082	.145
Hour $^{\circ}\text{C} \leq 0^{\circ}\text{C}/\text{day}$ (dev.)	-.086	.080
Hour $^{\circ}\text{C} \leq -5^{\circ}\text{C}/\text{day}$ (dev.)	.017	.738
HOST PLANT PARAMETERS		
Total live leaves (dev.)	.044	.396
Total leaves on main shoot (dev.)	.012	.821
Number of side tillers (dev.)	.063	.240
Leaf sheath length (dev.)	.101	.058
Height (dev.)	.061	.244
Sowing date (Sep.) (dev.)	-.110	.063
Sowing date (Nov.) (dev.)	.074	.217

APPENDIX 4.4

Mean reproduction rates for different physiological age-classes of S. avenae viviparae.

See Chapter 4, section 4.3.8, for details. Physiological age is in $\text{day}^{\circ}\text{C} > 3^{\circ}$ since final moult and mean reproduction rate in nymphs/ $\text{day}^{\circ}\text{C} > 3^{\circ}$ /live adult. n = number of 3-day period records on which mean is based.

(a)

<u>APTERAE</u> Physiological age-class	WITH APTEROUS PARENTS		WITH ALATE PARENTS	
	Rep. rate	n	Rep. rate	n
0-25	.1464	119	.1496	463
25-50	.3556	90	.2861	297
50-75	.4411	119	.3304	406
75-100	.4283	135	.3789	370
100-125	.3327	78	.3545	262
125-150	.3442	65	.3238	186
150-175	.2767	60	.2587	188
175-200	.2771	113	.2740	193
200-225	.2050	78	.1605	147
225-250	.0906	25	.1937	137
250-275	.0515	23	.0781	120
275-300	.0511	12	.0599	89
300-325	0	2	.0289	59
325-350	0	1	.0105	22
350-375			0	6
375-400			0	3

(b)

<u>ALATAE</u> Physiological age-class	WITH APTEROUS PARENTS		WITH ALATE PARENTS	
	Rep. rate	n	Rep. rate	n
0-25	.0158	196	.0422	161
25-50	.2638	123	.2605	151
50-75	.2124	123	.1867	166
75-100	.2683	115	.2160	133
100-125	.1616	84	.1500	86
125-150	.1680	75	.1376	66
150-175	.1721	46	.2099	70
175-200	.2566	53	.2628	62
200-225	.2828	54	.1195	26
225-250	.1505	27	.0773	16
250-275	.0535	14	.0360	9
275-300	.0243	8	0	1
300-325	.0208	2		

APPENDIX 4.5

A summary of temperature conditions inside clip-cages during the 1980/81 winter field experiment on S. avenae development and reproduction.

All data derived from hourly temperature records rounded to nearest °C.

3-day period	Code Number	Total day ^{°C} >3 [°]	Mean (°C)	Max. (°C)	Min. (°C)	Hour ^{°C} ≤ 0 ^{°C}	Hour ^{°C} ≤ -5 ^{°C}
21-24/9	1	40.0	16.3	28	6	0	0
24-27/9	2	32.7	13.9	27	5	0	0
27-30/9	3	34.7	14.6	29	9	0	0
30/9-3/10	4	25.8	11.5	29	1	0	0
3-6/10	5	25.8	11.3	23	0	4	0
6-9/10	6	20.3	9.5	20	0	3	0
9-12/10	7	12.9	6.6	19	-3	24	0
12-15/10	8	15.3	7.3	21	-3	30	0
15-18/10	9	13.8	7.4	15	0	2	0
18-21/10	10	17.5	8.1	22	-3	26	0
21-24/10	11	23.9	10.9	17	1	0	0
24-27/10	12	24.8	11.3	20	2	0	0
27-30/10	13	22.5	9.8	19	-2	26	0
30/10-2/11	14	8.7	5.1	15	-1	12	0
2-5/11	15	2.8	1.6	11	-5	84	1
5-8/11	16	0.7	2.0	5	-1	20	0
8-11/11	17	5.4	3.6	13	-4	38	1
11-14/11	18	8.2	3.7	13	-5	97	1
14-17/11	19	27.5	12.2	17	9	0	0
17-20/11	20	19.2	9.3	16	0	1	0
20-23/11	21	28.0	12.3	13	10	0	0
23-26/11	22	18.5	9.2	14	3	0	0
26-29/11	23	2.8	1.6	10	-5	87	1
29/11-2/12	24	2.1	0.2	9	-6	148	10
2-5/12	25	3.5	2.7	11	-2	40	0
5-8/12	26	3.7	0.2	8	-7	213	42
8-11/12	27	10.4	5.1	12	-4	40	0
11-14/12	28	19.0	9.3	12	5	0	0
14-17/12	29	8.2	4.9	12	-3	25	0
17-20/12	30	4.8	3.3	11	-4	44	0
20-23/12	31	13.6	7.4	14	0	2	0
23-26/12	32	14.7	7.5	13	-1	4	0
26-29/12	33	5.9	3.2	9	-5	73	5
29/12-1/1	34	13.0	7.3	11	2	0	0
1-4/1	35	12.2	6.8	12	-2	6	0
4-7/1	36	2.8	1.9	9	-6	75	3
7-10/1	37	7.8	5.1	11	-1	7	0
10-13/1	38	0.9	-0.3	7	-5	155	10
13-16/1	39	4.9	3.2	9	-2	41	0
16-19/1	40	6.3	4.5	13	-4	21	0
19-22/1	41	11.0	6.5	11	-1	3	0

APPENDIX 4.5 Continued

3-day period	Code Number	Total day ^{°C} >3 [°]	Mean (°C)	Max. (°C)	Min. (°C)	Hour ^{°C} ≤ 0 ^{°C}	Hour ^{°C} ≤ -5 ^{°C}
22-25/1	42	15.2	8.0	14	2	0	0
25-28/1	43	12.6	7.2	13	2	0	0
28-31/1	44	7.3	4.5	14	-3	31	0
31/1-3/2	45	10.3	5.8	15	-4	16	0
3-6/2	46	9.0	5.2	14	-2	23	0
6-9/2	47	19.8	9.6	13	7	0	0
9-12/2	48	3.7	1.4	12	-6	123	15
12-15/2	49	4.4	-0.3	12	-7	244	38
15-18/2	50	5.0	1.6	13	-5	133	10
18-21/2	51	2.3	0.9	11	-6	110	10
21-24/2	52	2.4	2.4	10	-3	39	0
24-27/2	53	1.0	1.0	8	-5	71	1
27/2-2/3	54	12.6	7.2	17	4	0	0
2-5/3	55	6.5	4.3	13	-2	22	0
5-8/3	56	19.7	9.6	19	4	0	0
8-11/3	57	24.0	11.0	14	10	0	0
11-14/3	58	21.6	10.2	19	3	0	0
14-17/3	59	12.3	6.1	20	-3	36	0
17-20/3	60	14.1	7.0	19	-3	24	0
20-23/3	61	18.0	9.0	18	3	0	0
23-26/3	62	24.2	11.1	21	8	0	0
26-29/3	63	25.3	11.4	23	4	0	0
Means over whole period:		13.5	6.5	15.0	-0.5	35.3	2.3

APPENDIX 4.6

The use of daily maximum and minimum temperatures to estimate hour-degree totals.

Introduction and methods

In Chapter 4 relationships between development, reproduction and winter temperature were expressed as day-degree requirements, with day-degrees measured above 3°C. However, workers wishing to use these relationships may not have access to hourly temperature records or any other relatively direct method of estimating day-degrees. The only temperature data available may well be daily maximum and minimum temperatures, either recorded at crop-level or in a standard 1.2 m high Stevenson's screen. Thus the accuracy of two methods for estimating hour-degree totals above 3°C (24 hour-degrees = 1 day-degree) using daily maxima and minima was assessed here. The methods assessed were:

1. A mean estimate - which assumed the daily temperature was the arithmetic mean of the maximum and minimum.
2. A sine-curve estimate - which assumed that maximum and minimum temperatures occurred 12 h apart and that temperature fluctuated between them according to a sine-curve. Calculations for this estimate were done on Southampton University's PDP11/45 computer using a program given by Hand (1982).

The accuracy of each estimate was determined for two situations:

- (a) In which maximum and minimum temperatures were recorded in the aphid's microhabitat, so that the only source of error was the estimation procedure itself.
- (b) In which maximum and minimum temperatures were recorded in a 1.2 m Stevenson's screen, so that

error was also caused by temperature differences between the screen and the aphids' position.

The raw data were recorded by a model D Grant Temperature Recorder with one thermistor probe in a 1.2 m high Stevenson's screen and another at 2 cm above the ground, shaded by a small shield. Recording continued every hour for 178 days between 28 September 1980 and 28 March 1981.

To discover the extent to which the accuracy of estimates was affected by the unit length of time over which estimates were being made, the analysis was done three times, first with hour-degree totals estimated for each day separately ($n = 178$) and then with the data grouped in 5 day and 20 day periods ($n = 35$ and $n = 8$ respectively).

Results

(a) Temperatures recorded in the aphids' microhabitat:

This situation was simulated by using daily maximum and minimum temperatures recorded by the thermistor in the Stevenson's screen to estimate hour-degree totals in the same position (Table 1). Both types of estimate were affected very little by the length of period over which estimates were being made. Both overestimated hour-degree totals because they assumed 12 h daylength. As expected, the sine-curve estimate was the more accurate, with mean deviations of slightly under 3% compared to mean deviations of around 8% for the mean estimate.

(b) Temperatures recorded in a Stevenson's screen used to estimate hour-degrees experienced in the crop:

Again both methods overestimated hour-degree totals and the accuracy of estimates was affected very little by period length. Hour-degree totals recorded at 1.2 m were a little over 4% higher than those recorded at crop-level (Table 2). Consequently the two estimation methods were less accurate at estimating crop-level hour-degrees than they were at estimating hour-degrees in the Stevenson's screen. Mean errors were around 12 to 13% for the mean

Table 1 Estimates of 1.2 m Stevenson's screen hour-degree totals
using Stevenson's screen daily max. and min. temperatures.

Type of estimate	Mean estimate			Sine-curve estimate		
Period length (days)	1	5	20	1	5	20
Mean deviation of estimates from actual hour-degree totals	8.0	39.5	155.9	2.9	13.9	29.7
Mean deviation expressed as % of mean hour-degrees per period	7.9	7.9	8.1	2.9	2.8	2.5
95% confidence limits expressed as % of mean hour-degrees per period	39.8 to -24.0	23.4 to -7.5	14.2 to 2.1	43.0 to -37.3	21.8 to -16.3	10.1 to -5.1

Table 2 Estimates of shade, crop-level hour-degree totals using
1.2 m Stevenson's screen daily max. and min. temperatures.

Type of estimate	Mean estimate			Sine-curve estimate			Screen hour-degree total		
Period length (days)	1	5	20	1	5	20	1	5	20
Mean deviation of estimates from actual crop-level hour-degree totals	12.2	61.2	253.9	7.2	35.6	146.2	4.3	21.7	98.0
Mean deviation expressed as % of mean hour-degrees per period at crop-level	12.1	12.3	13.2	7.1	7.2	7.6	4.2	4.4	4.5
95% confidence limits expressed as % of mean hour-degrees per period at crop-level	42.3 to -18.1	27.3 to -2.7	15.9 to 2.0	49.2 to -35.0	29.5 to -15.1	21.6 to -6.3	23.0 to -14.6	17.9 to -9.1	16.3 to -6.1

estimate and a little over 7% for the sine curve estimate (Table 2). Simple regression equations were calculated from which crop-level temperatures could be estimated from the corresponding Stevenson screen temperatures:

$$\text{Crop-level maximum} = (\text{screen max.} \times 1.017) + 0.783$$

$$\text{S.E. of slope} = 0.0322; \quad r = 0.92.$$

$$\text{Crop-level minimum} = (\text{screen min.} \times 1.147) - 2.139$$

$$\text{S.E. of slope} = 0.0203; \quad r = 0.97.$$

Conclusions

By using a max.-min. thermometer at crop-level and the computer program given by Hand (1982) errors in estimating day-degree totals above 3°C can be reduced to around 3%. If greater accuracy is required a more complex program, which takes seasonal variation in daylength into account, is necessary. If only standard temperature data recorded at 1.2 m in a Stevenson's screen are available the errors could be corrected for after the calculation of day-degrees using the mean deviations given in Table 2. Alternatively, the maximum and minimum temperatures could be corrected before estimating day-degrees, using the regression equations given above. It should be noted that all values given are more or less specific to the time and place in which the raw data were recorded, though they could probably be generalized for other near-normal winters in southern England.

APPENDIX 6.1

Long-term climatological data from Hurn Airport, nr. Bournemouth.

(Grid Ref: SZ117978; Altitude 10 m)

	PARAMETER	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Year
	TEMPERATURE: (°C)													
1	Mean Max.	6.9	7.5	10.4	13.6	16.6	19.7	21.3	20.9	18.8	15.3	10.7	8.1	14.1
1	Mean Min.	1.5	1.3	2.1	4.1	6.5	9.7	11.5	11.5	9.6	7.1	4.1	2.5	6.0
	Mean	4.2	4.4	6.3	8.9	11.5	14.7	16.4	16.2	14.2	11.2	7.4	5.3	10.1
2	Extreme max.	13.3	15.0	21.0	22.8	26.6	29.4	31.7	28.1	28.3	25.0	17.5	15.0	
2	Extreme min.	-13.4	-10.6	-11.1	-5.4	-3.6	0.4	2.6	2.4	-1.1	-3.3	-7.1	-10.5	
1	RAINFALL: (mm)	85	55	56	49	58	47	49	67	75	85	95	85	806
	DAYS OF:													
	Rain (0.2 mm)	15.8	12.4	11.7	15.0	14.3	9.9	10.2	12.3	13.3	13.8	15.6	17.2	161.5
3	Snow or sleet	3.8	3.8	3.1	0.6							0.4	3.9	15.6
3	Snow lying (9 am)	3.7	1.6	0.4	0.1								1.7	7.5
	Air frost	11.6	11.5	10.5	3.8	0.7				0.1	1.3	7.2	14.8	61.5
	Ground frost	14.6	14.9	15.9	9.1	5.5	0.9	0.1	0.3	1.1	4.9	11.2	16.7	95.2

Periods: 1: 1941-70

2: 1952-70

3: 1961-70