UNIVERSITY OF SOUTHAMPTON

CHEMICAL COMMUNICATION IN BRITISH SOCIAL WASPS (HYMENOPTERA : VESPIDAE)

A thesis submitted for the degree of
Doctor of Philosophy
by
James Bennett John Foster Aldiss

November 1983

To

Zoë and my parents

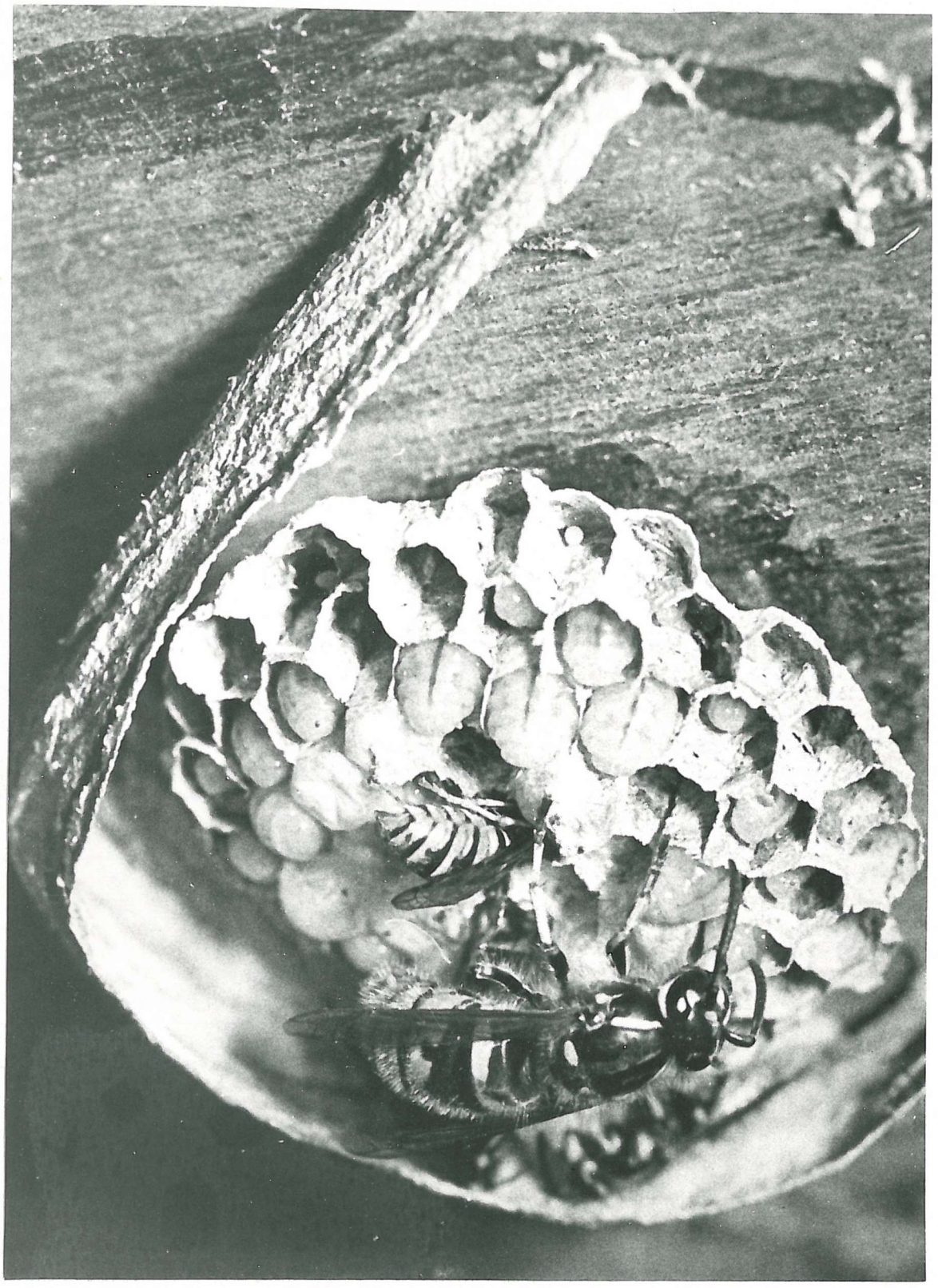


Fig. 1. A *Vespula vulgaris* queen rests on the comb of the embryo nest she has built. One of her 3 workers is cleaning out a recently vacated cell. (Courtesy: Southern Newspapers Ltd.)

CONTENTS

Page

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

BIOLOGY

Doctor of Philosophy

CHEMICAL COMMUNICATION IN BRITISH SOCIAL WASPS (HYMENOPTERA : VESPIDAE)

by James Bennett John Foster Aldiss

The alarm behaviour of *Vespula vulgaris* workers at the nest entrance, and of *vulgaris* and *germanica* workers at a foraging site, was studied. Four categories of alarm behaviour could be discerned: weak investigatory response (WIR), strong investigatory response (SIR), weak attack response (WAR) and strong attack response (SAR).

Experiments showed that workers of *Vespula vulgaris* possess an alarm pheromone which is located in the venom apparatus: one component, present in the venom sac, appears to release the stinging response, whilst another, possibly originating from the Dufour's gland, seems to have an attractant and alerting function. Worker heads contain an attractant.

The stimuli involved in the precipitation of a stinging response include: sight of nest (and environs?), movement, colour, alarm pheromone and foreign odours. None of these can elicit an attack response in isolation. The presence of reproductives at the nest entrance appears to raise the threshold of the alarm response. Reproductives do not seem to exhibit alarm behaviour.

Chemical analysis of the venom sac contents showed the probable presence of N-3-methylbutylacetamide and 4 spiroacetals.

Ginger is an effective attractant, but only at sites where wasps are familiar with it. Sources of ginger are initially located chemotactically, but visual cues and taste are important on return journeys. Concentrated extracts are more attractive than raw ginger, but different fractions of the essential oil vary in their attractiveness, the most effective being those containing a large variety of sesquiterpenes. Although equally attracted to the most effective fraction, workers of *vulgaris* and *germanica* differed in their response to others.

The saliva of *vulgaris* workers appears to contain an attractant serving to synchronize the removal of obstructions and possibly helping to co-ordinate nest building.

ACKNOWLEDGEMENTS

During the course of this research I have had help of various kinds from many people and organizations. I would particularly like to thank my supervisor, Dr. P.E. Howse, for the guidance he has given me throughout. I am also grateful to Professor R. Baker, Professor M.A. Sleigh and Dr. D.A. Evans for their advice, encouragement and wasp nests.

For the facilities provided for me at Rentokil Ltd., East Grinstead, I wish to thank Dr. P.B. Cornwell, Mr. A. Meehan, Mr. C.P. Smith and especially Mr. R. Edwards who has been particularly helpful.

Among the biologists I worked with in the Chemical Entomology Unit I wish to express my gratitude to Drs. J.W.S. Bradshaw, C. Longhurst, O.T. Jones, K. Jaffe, A. Legakis, Mrs. P. Wagner, Mr. A. Bandeira, Mr. R. Mothershaw and Mr. B. Al'Saad. I am grateful to Dr. R.J. White for his help with the statistics.

For their assistance in the production and interpretation of mass spectra I am indebted to Drs. E. Parton and W. Speed, and for their guidance with other chemical methods I wish to thank Drs. G. Jackson, C. Broomfield, R. Herbert, M. Crook, P. Briner and Mr. J. Austin.

I am very grateful to the technicians, photographers and groundsmen of Southampton University: in particular I would like to mention Mr. M. Wagner, who carried out many of the steam-distillations, and the gardeners at Chilworth Manor, who were indispensable during the setting-up of the vespiary.

I am indebted to all those who provided me with wasp nests and I would particularly like to thank Rev. J.B. Hirst, Mr. H. Carey and others who let me work in their gardens.

Many thanks are also due to the management and staff of Bendicks (Mayfair) Ltd., Winchester, for allowing me to work on their premises:

I am especially grateful to Mr. A. Cole in this respect. For the work I carried out at Spillers-French and Mother's Pride bakeries, Eastleigh, I would like to thank the management of Rank Hovis MacDougall.

Southern Newspapers Ltd., and BBC Radio Solent were kind enough to advertise my need for wasp nests and for this I am grateful.

For the spiroacetals he sent me and the mass spectra he analysed, I thank Dr. W. Francke.

This research was supported by means of a Science Research Council/Rentokil Ltd., C.A.S.E. award.

During my time in the Royal Navy, I was fortunate to be given facilities to write this thesis at R.N.A.S. Culdrose, for which I would like to thank the Commanding Officer. I am also very grateful to my colleages at Ampleforth College for the help they have given and I would particularly like to thank the headmaster, second master, Mr. John Davies, Mr. D. Smith and Mr. K. Elliott in this context.

I am grateful to Mr. C. Bailey for some of the photographs and to Mr. P. Elm who helped with the graphs.

Doctor M.E. Archer has very kindly read through the entire manuscript for me and special thanks must go to Mrs. Ann Carroll, who typed the final draft so expertly.

Finally, for her unfailing patience, encouragement and support I thank my wife, Zoë.

CHAPTER I

INTRODUCTION

I.A. Insect communication

Wilson (1971) defines biological communication as '... action on the part of one organism (or cell) that alters the probability pattern of behaviour in another organism (or cell) in an adaptive fashion'. This very broad definition encompasses most of the diverse modes of communication encountered among insects, with the exception of certain predator-prey relationships, and forms a useful basis from which to describe the interactions of social wasps.

Although all insects communicate, the number and diversity of the signals used varies from species to species, depending upon the extent to which individuals interact with one another. Thus the larvae of certain Lepidoptera, such as the Privet Hawk Moth *Sphinx ligustri*, being solitary, cryptically coloured and largely inactive, seldom have to communicate, whereas social insects are highly interactive and require a correspondingly greater number of signals with which to coordinate their behaviour. The list of recognised signals occurring within the social insects is very large and includes tactile stimulation (e.g. antennation), vibratory stimulation (e.g. stridulation and larval hunger signals), liquid food exchange (trophallaxis) and the emission and detection of chemicals (e.g. trail odours and defence secretions). Research over the past 20 years has indicated that the last category is a very important one, especially with regard to social organisation and colony cohesion among insect societies. As the present work deals largely with the responses of social wasps to chemical compounds some general principles and definitions of chemical communication will now be discussed.

I.B. Chemical communication among social insects

Communication by means of chemicals is an efficient way of disseminating information to a large number of individuals and, for reasons to be discussed later, has the added advantage that the meaning of individual messages can be adjusted relatively simply.

The chemicals used in the transmission of signals were termed 'ectohormones' by Bethe (1932), and Karlson and Butenandt (1959) coined the work 'pheromone' to describe those whose use was intraspecific. More recently interspecific ectohormones have been divided into 'allomones' (Brown *et al*, 1968), which provide the emitter with the advantage, and 'kairomones' (Brown *et al*, 1970) which are advantageous to the receiver. Examples of the former include defence secretions, and of the latter, chemicals involved in prey detection.

Pheromones are an extremely important means of communication within insect societies, not least because of their versatility, and this can be illustrated by considering the following, invoked to explain aspects of behaviour discussed in this research:

- a single signal can be detected by numerous individuals
- the ratio between the amount of the chemical released and the behavioural threshold towards it ('Q/K ratio', Bossert and Wilson 1963) can be altered, thereby adjusting the duration or effective distance of the message
- composite signals, comprising several chemicals, can be generated simultaneously
- the sense of the chemical message can be altered by emitting it under different circumstances.

I.C. Communication in social wasps

As the previous section has indicated, chemical communication is an important facet of behaviour among social insects, but in social wasps only one pheromone has so far been identified - -n-hexadecalactone, the queen pheromone of *Vespa orientalis* (Ikan *et al*, 1969). Others undoubtedly exist and in some cases their sites of secretion and the effects they have on their recipients is known. Thus Butler *et al* (1969) have shown the existence of a 'footstep pheromone' in *Vespula* species. This substance appears to confirm the presence of the nest entrance to incoming workers and may be the same compound which enables wasps to recognise their own nestmates (Francke *et al*, 1978).

Workers of *Vespula vulgaris* appear to be attracted to the saliva of other workers, a factor likely to be important in the coordination of nest-building and the removal of obstructions from the nest entrance.

Alarm pheromones are important in ensuring efficient colony defence and have been demonstrated in *Vespula vulgaris* and *V. germanica* by Maschwitz (1964).

Although pheromones undoubtedly play a major part in the organisation of wasp colonies, other forms of communication are extremely important. Certain forms of tactile stimulation and sound production seem to play a central role in the maintenance of colony cohesion among the Vespinae; in particular the antennation of the donor's mouthparts which precedes regugitation and food transfer (trophallaxis), and the rasping hunger signals made by larvae in their cells.

Communication by visual means occurs outside the nest and has been shown to be an important factor in the gathering of *Vespula* species at foraging sites (Free, 1970; MacDonald *et al*, 1974; Pflumm, 1975; Parrish and Fowler, 1983). Incoming workers appear to be attracted by the sight of others, at least in *V. vulgaris* and *V. germanica*, and this results in more efficient exploitation of food sources.

The present research investigates the communication of alarm in one British species of social wasp and examines the behaviour of this and another species in response to attractants. Both these aspects are important criteria in any projected system of wasp control and involve the study of communication, with particular reference to chemical and visual stimuli. The detailed aims of the research are as follows:

I.C.1 Alarm behaviour

(a) To provide an English translation of the paper on alarm behaviour of wasps by Maschwitz (1964).

(b) To make an accurate description of the alarm behaviour of the wasps under study viz. *Vespula vulgaris* and *V. germanica*.

(c) To demonstrate the existence of an alarm pheromone in these wasps.

(d) To locate the site of secretion of the pheromone.

(e) To investigate the possibility that parts of the body other than those containing the alarm pheromone contain substances which enhance the alarm reaction of worker wasps.

(f) To investigate the reaction of wasps challenged with varying concentrations of other possible alarm pheromones viz. 2- heptanone, 6-methyl-5-hepten-2-one and spiroacetals.

(g) To investigate the possibility that wasps avoid conflict with their nest mates by means of a colony-specific odour.

(h) To investigate the relative importance of visual and chemical stimuli in the creation of an alarm response.

(i) To compare the difference in response to alarming stimuli at the nest entrance and at a foraging site.

(j) To compare the responses of wasps to alarming stimuli at different stages of colony development, with particular reference to the effect of presence of reproductives.

(k) To analyze wasp venom in order to identify some of its constituents.

I.C.2 Wasp attractants

(a) To determine the importance of attractants in wasp control programmes.

(b) To describe modern attempts at wasp control using attractants such as heptyl butyrate and ginger syrup.

(c) To give an account of the chemistry of ginger with respect to its attractiveness to wasps.

(d) To investigate the attractiveness of concentrated extracts of ginger in comparison with ginger syrup.

(e) To investigate the attractiveness to wasps and bees of ginger extracts dispensed at different sites.

(f) To investigate the relative attractiveness of component fractions of essential oil of ginger, with particular reference to interspecific differences.

(g) To investigate the possibility that wasp saliva contains a short-range attractant.

(h) To analyze ginger in order to identify some of its constituents.

(i) To compare the efficiencies of various wasp traps.

I.D. The social wasps

I.D.1 Classification and geographical distribution

All the social wasps belong to the family Vespidae within the superfamily Vespoidea of the order Hymenoptera. Richards (1962) recognises three subfamilies, the Stenogastrinae, the Polistinae and the Vespinae, the last of which contains all the British species and is therefore of relevance to this research.

The Vespinae is an essentially tropical Asian group of wasps that have extensively invaded the temperate regions (Richards 1971). Various schemes of classification have been propounded to separate these wasps into genera, but the one adopted here is that of Edwards (1980):

Genus	Number of recognised species	Features used to separate genera
Provespa	3	1. Shape of 1st gastral segment 2. large ocelli
Vespa	22	shape of head behind the eyes
Dolichovespula	14	1. Long oculo-malar space 2. nests usually aerial
Vespula	19	1. Short oculo-malar space 2. nests usually underground or in dark cavity
TOTAL	58	

Table 1. Classification of the Vespinae

The species of *Provespa* are all nocturnal and are restricted to parts of the Oriental region. Hornets of the genus *Vespa* are especially prevalent in S.E. Asia, but extend throughout the Palaeartic and are now represented in the Nearctic by an introduced species. *Vespula* and *Dolichovespula* are Holarctic, though they are absent from most of the Oriental region. Representatives of the former genus have been accidentally introduced to several countries in the southern hemisphere.

I.D.2 The British species

(a) Introduction

The seven species of social wasp indigenous to the British Isles are listed below:

Species	Vernacular name
Vespa crabro L.	Hornet
Dolichovespula norwegica (Fab.)	Norwegian wasp
Dolichovespula sylvestris (Scopoli)	Tree wasp
Vespula austriaca (Panzer)	Cuckoo wasp
Vespula rufa (L)	Red wasp
Vespula germanica (Fab.)	German wasp
Vespula vulgaris (L)	Common wasp

Vespa crabro, the largest British wasp, is locally common in Southern England but is absent from Sussex and Kent.
Dolichovespula norwegica and *D. sylvestris* are widely distributed in Britain, though the former is commoner in the north and the latter in the south.
Vespula austriaca is the rarest British wasp and is parasitic on colonies of *V. rufa*. It is sparsely distributed over the north and west of the country but is absent from the south-east.
Vespula rufa is widely distributed, though commoner in the south.
Vespula vulgaris and *V. germanica* are the commonest British species and occur throughout most of the country, though *germanica* is absent from the north-west of Ireland and Scotland.

(b) Pest status

All social wasps can sting and are hence regarded by many people as pests. Of the British species however, only two, *Vespula vulgaris* and *V. germanica*, habitually occur in close association with man and are likely to prove a nuisance. The others are generally less common, build smaller nests and die out earlier. *Dolichovespula sylvestris* can be a nuisance as a result of its propensity for building colonies in nest-boxes and other man-made containers, whilst the hornet *Vespa crabro* occasionally causes alarm by flying into houses on moonlit nights. The other species are, however, rarely responsible for the misdeeds usually blamed on wasps in general.

Vespula vularis and *V. germanica* can undoubtedly be considered pests, particularly in the years when they are especially abundant. Their pest status is due almost entirely to food preferences and an ability to sting - two factors which can result in considerable economic losses.

As stinging insects, wasps are almost universally disliked. Whereas honeybees rarely intrude into the human environment, wasps habitually do if their nest is near enough. Moreover, foraging wasps are inquisitive and will frequently investigate human beings at close quarters. The experience of the researcher backs up those who argue that wasps will generally sting only when physically restrained or attacked, although wasps at the nest entrance have a lower 'threshold of attack' than those at foraging sites. Nevertheless, a sting from a wasp is a painful and sometimes dangerous experience which most people will attempt to avoid in the quickest way possible - remaining still as a wasp crawls across one's face is not easy.

Economically, wasps are not of primary importance in the British Isles, but in some years they can cause considerable losses, especially to fruit growers. Plums, pears, grapes and apples are the most heavily attacked fruits and evidence suggests (Kemper and Döhring, 1961) that, contrary to popular belief, wasps often inflict the initial damage.

Although wasps and hornets can cause heavy losses to beekeepers in certain countries by robbing hives of honey and killing the occupants, this is of minor importance in Great Britain, where comparatively few hives are attacked.

Wasps can be a nuisance in certain shops where fruit or meat is sold especially as, like flies, wasps frequently carry disease organisms. Manufacturers of confectionery, jams and cakes are particularly prone to the depredations of wasps, especially if attractive waste products are dumped close to the factory. Existing control methods in such situations are expensive and inadequate and have formed the basis of part of

this research in response to an increasing need for effective systems: especially urgent is the need for a means of suppressing the increasing numbers of *Vespula germanica* in New Zealand (Trought, 1982). In recent years this introduced species has become an important pest of apiaries and is badly affecting the 'outdoor leisure industry'.

No programme of wasp control is likely to be successful if the behaviour and general biology of wasps is ignored. For this reason a brief account of the biology of *Vespula vulgaris* is outlined below.

(c) Biology of Vespula vulgaris

In common with other temperate species of social wasps *Vespula vulgaris* completes its life cycle within a year (although *V. germanica* occasionally produces enormous perennial nests in New Zealand.) As the existence of the colony depends upon the presence of the queen a logical place to begin a discussion of the life cycle is with the emergence of the virgin queen in September (Fig.2 p.11).

About 1,000 males and the same number of females (queens) are produced in the average nest of *vulgaris* (Archer, 1980) towards the end of August. By the end of September most of these have left the nest for their mating flights, after which the males die and the females find a place to hibernate. The exodus of reproductives from the nest prior to mating takes place over a number of days, but only in the mornings (Arnold 1966). Hibernating queens remain quiescent for about 6 months before emerging to feed and select a nest site. Having found a suitable cavity the queen then begins to build the nest, using a material called 'carton' got by chewing wood scrapings and mixing them with saliva. The hexagonal cells of the comb hang downwards from the roof of the cavity and are protected by an umbrella of carton called the envelope. Eggs are laid in the oldest cells and the larvae which hatch from them are fed mouth-to-mouth by the queen until they have undergone 4 moults and pupated. By the middle of June the first workers (small,

sterile females) emerge and these take over from the queen the tasks of enlarging the nest and caring for the brood. A nest with a queen and 3 workers is shown in figure 1 (p iii).

The colony now expands more rapidly to produce an average of 10,300 workers (Archer, 1980) by mid-August (Fig. 2). Males and females then begin to emerge, after which the old queen dies and the remaining workers neglect the brood, with the result that the social structure of the colony breaks down and the nest begins to disintegrate.

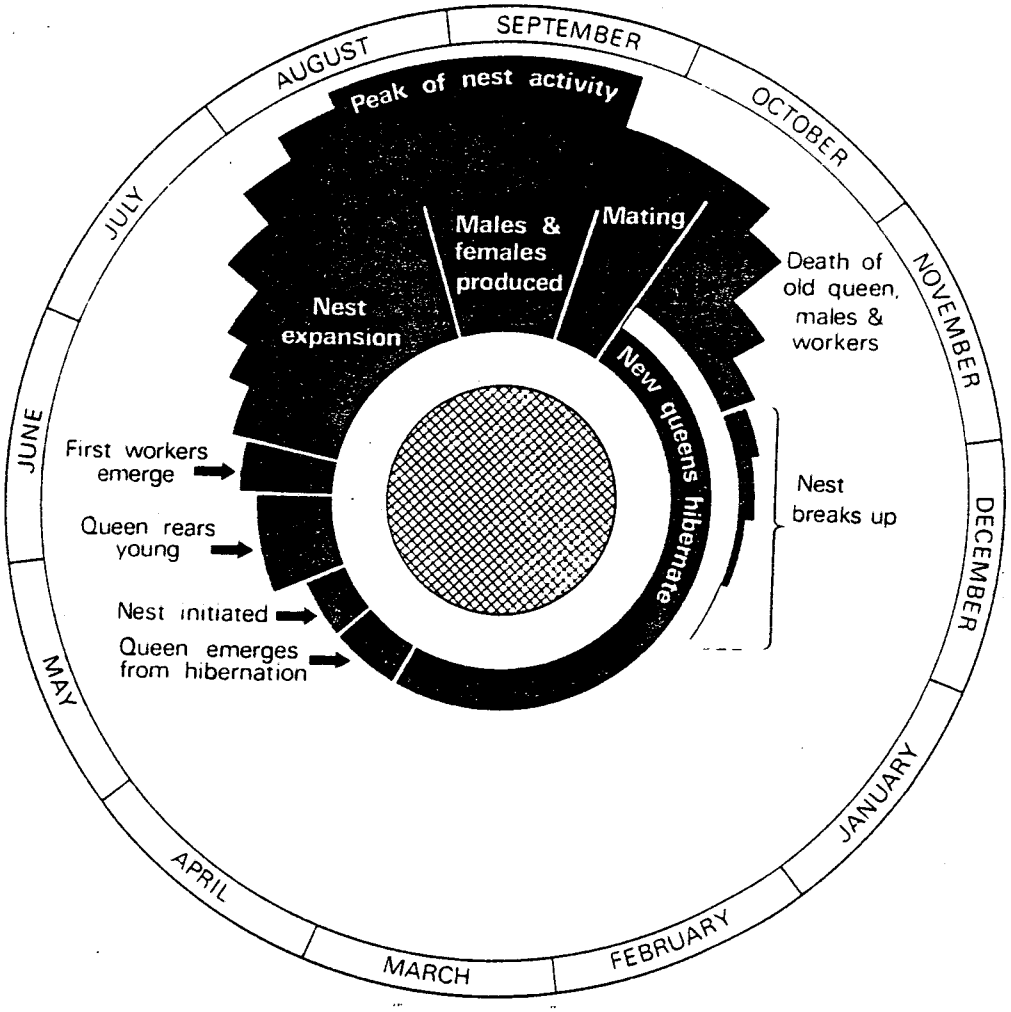


Fig. 2. The yearly life cycle of the Common Wasp, *Vespula vulgaris*. (Courtesy: Rentokil Ltd).

CHAPTER II

MATERIALS AND METHODS

II.A. Culture of wasps

II.A.1 Introduction

The study of honeybees and most species of ants is made easier by the fact that their colonies are relatively simple to culture, given certain basic facilities, but wasp nests pose special problems. In temperate climates colonies of *Vespula vulgaris* and *V. germanica* live for about 8 months before dying out at the onset of winter. Although there have been published attempts by vespinologists to keep fertile queens throughout the winter and to induce them to build nests in captivity, these have all met with failure, either because the queens have died or because they would not start a colony in confinement. For the purposes of this research nests were collected as early as possible every year and transferred to artificial nest boxes.

II.A.2 Locating nests

Looking for colonies of wasps can be extremely time-consuming, especially early in the season before streams of workers issuing from the entrance betray their presence. During this research it was necessary to investigate numerous nests, as only certain ones were suitable, either for transfer or for work *in situ*. For these reasons advertisements were put in shops and in the local newspaper, and appeals were made through radio and newspaper interviews. Some success was also had by contacting pest-control companies in the surrounding area.

The summer of 1979 was a good one for *Vespula vulgaris* in the environs of Southampton, and it proved possible to locate numerous nests within a very small area on Southampton common, simply by watching for streams of foragers. These nests, being too public to allow for uninterrupted work *in situ*, were used as sources of fresh venom for chemical analysis. A similar technique was used for locating nests in the vicinity of the chocolate factory in Winchester, but here it was a more difficult task, as the wasps coming to the ginger waste were drawn from a large area. By following the lines of

foragers, which flew between 10 and 15 feet above the ground, it was possible to discover the colonies, even if it meant negotiating obstacles such as ditches and hedges (see also p.215).

One disadvantage of advertising for nests over looking for them was that, on frequent occasions, the nests turned out to be of the wrong genus (usually *Dolichovespula sylvestris*) or even the wrong family e.g. *Bombus lapidarius* - laymen were often unable to give an accurate description over the telephone and were easily convinced by leading questions.

II.A.3 Collecting nests

Various authors have described methods of collecting wasps nests. Spradbery (1973), for example, suggests that subterranean nests be dug up and placed in a polythene bag after the occupants have been anaesthetised by pouring 200ml of ether down the entrance tunnel. Edwards (1980) describes the use of a vacuum cleaner to suck up live wasps as they emerge from their underground nest, the insects being temporarily stored in a perspex container attached to the machine 'like a giant "pooter"', thus enabling the researcher to dig up the nest in relative safety.

In the present research the first of these two methods was tried, but with little success. Large volumes of ether were generally needed to cause the colony to succumb, but this often resulted in the death of many wasps. During transfer of such colonies to artificial nest boxes it was frequently necessary to pour ether onto the nest envelope, as a result of which more wasps failed to revive and the researcher began to feel unwell. The vacuum-cleaner method was impractical because most underground nests collected were not within reach of a source of electricity.

Aerial nests pose fewer problems, except when they are inaccessible. Whereas subterranean nests are often at the end of long (and not necessarily straight) tunnels, aerial ones are usually suspended in obvious places and merely need to be cut down into a bag to be taken away. Edwards (1980) advocates this method which,

with slight adaptations, was used successfully in the present research.

(a) Subterranean nests

The methods of collection described above were tried, but were discontinued in favour of the following procedure:

The entrance tunnel of the colony was located and a long piece of rubber tubing, attached to a portable cylinder of carbon dioxide gas (BOC Ltd.) was pushed down it until it reached the nest. Gas was slowly released from the cylinder for about half to one minute, or until no more wasps emerged from the nest. The nest was then dug up carefully and placed in a large polythene bag, which itself was enclosed in another. The necks of the bags were closed with elastic bands and any remaining wasps e.g. returning foragers, were caught in a butterfly net and put in small glass jars. Among the advantages of carbon dioxide over ether are:

- no mess
- no danger of spillage during transport
- non-toxic
- safer to use
- mortality among wasps minimal
- wasps apparently suffer no harmful side-effects

(b) Aerial nests

A transparent polythene bag large enough to surround the entire nest was carefully arranged so that the colony would fall into it on being cut from its support. Usually this involved the use of a few drawing pins to fasten the bag to the support. If, as was often the case, the nest was in a roof space or attic, a torch was necessary to provide light. To prevent alarmed wasps flying up the beam to the researcher the torch was not held but was placed some distance away, its beam directed towards the nest. The colony was then cut away by means of a long knife and the neck of the bag was quickly closed and knotted. The bag was placed inside another to make sure no wasps escaped. Such nests were taken at dusk to ensure the capture of most of the workers.

(c) Protective clothing

Wasps are prone to sting when their colony is disturbed, so some form of protection for the researcher is essential. As well as being sufficiently thick to prevent penetration by stings the garment should ideally be light and well ventilated and should not inhibit movement or the performance of intricate operations.

The clothes worn by the researcher whilst collecting nests consisted of the following:

- a white overall made of heavy cotton/polyester mix with elasticated wrists and ankles and secured up the front by means of strips of 'Velcro'.
- a beekeeper's hat and veil, tied securely under the arms.
- thick socks and tight-fitting shoes.
- heavy duty rubber gloves tucked in the sleeves.

A white overall was chosen because darker shades and black especially, were known to provoke attacks by wasps more readily (Maschwitz 1964).

II.A.4 Method of transfer of colonies to nest boxes

A captured nest was transported as quickly as possible to the vespiary at Chilworth (see below), the two bags surrounding it ensuring that no wasps escaped *in transit* (especially necessary for transport by car).

At the vespiary the wasps were anaesthetised with carbon dioxide and the nest was removed from the bag. The envelope of the nest was quickly broken from the combs and discarded, whilst the insensible wasps within were swept back into the bag.

The combs were separated from one another by cutting through their suspensoria, these then being carefully removed by means of a sharp scalpel. Each comb was then prepared for fixing to the nest box: this involved cutting a flat surface on the otherwise curved roof of the comb without breaching the cells containing larvae and pupae.

This flattened surface was then smeared liberally with 'UhU' glue and the comb was pressed firmly against the roof of the nest box, until the glue had set. Care had to be taken at this stage to prevent damage to the outer cells of the comb, which were extremely fragile, whilst maintaining sufficient pressure to ensure adhesion. Other glues were tried but none were as effective as 'UhU'. Each comb was glued to the nest box roof in this way, as close to the others as possible. Food, in the form of honey solution, and water, were then placed on the floor of the box in watch-glasses and the box was shut.

The wasps were now transferred from the bag, through the entrance hole to the interior of the nest box: this was done with forceps and the wasps were re-anaesthetised if necessary to facilitate the process. To allow the wasps time to locate the combs and to prevent them escaping too soon, the entrance hole was plugged with a plastic vial stopper which exactly fitted. Twenty-four hours later the stopper was removed, by which time the queen had been reinstated, the colony had settled and wasps would not leave the nest without making orientation flights.

II.A.5 The nest box - purpose and design

(a) Purpose

There were 3 main reasons why nest boxes were used in this research:

(i) they could provide standardised colonies within easy reach of the laboratory, facilitating comparative experimentation.

(ii) they could provide convenient supplies of fresh material for chemical analysis, dissection, etc.

(iii) they could facilitate the study of wasp behaviour in the nest.

(b) Design and function

Four wooden nest boxes of a simpler type than those of Potter (1964) and similar to those of Akre (1973) were built (figure 3 p. 17).

Fig. 3. Front view of a nest box adjacent to the foraging arena in the vespiary at Chilworth. The wasps have chewed away some of the expanded polystyrene insulation and used it for nest construction.

The restricted depth of the boxes (8 cm) was an important aspect of the design, as it forced the wasps to build laterally and this enabled the observer to study the nest through the glass bottom. Each box was insulated except on the bottom with sheets of expanded polystyrene 2 cm thick, covered in aluminium foil and polythene. The bottom consisted of a double sliding floor of glass and hardboard. The insulation provided a means of maintaining the optimum temperature of 28 - 32°C (Roland 1969) within the nest without the need for thick layers of carton (the paper-like substance used by wasps). The hardboard slides maintained the interior of each box in near darkness, this being another prerequisite if the wasps were to be prevented from obscuring the combs with layers of carton. These precautions, though largely effective, did not totally prevent envelope construction, so a means of removing excess carton was required: two simple tools were devised for this purpose. By opening both sliding floors a few centimetres, a bent wire could be inserted and used to tear off the carton, which could then be scraped from the glass with an angled razor blade attached to a metal rod. The razor blade also proved useful for cleaning the glass floor without having to remove it.

II.A.6 The vespiary (fig. 4 p. 19)

The 4 nest boxes described above were firmly screwed to a weather-proofed wooden frame 4 m long, which, when embedded in the ground, stood about 2 m high. The site for the frame was a small enclosure bounded by a tall hedge of cherry laurel (*Prunus laurocerasus*) in the gardens of Chilworth Manor, Hampshire. The enclosure, which measured 10 x 6 m, was bordered on its northern end by deciduous woodland and it was here that the frame was placed, the entrances to the nest boxes facing south.

The easternmost nest box was attached to a large foraging arena or 'screenhouse' (Akre *et al* 1973) measuring 5 x 3.5 x 2 m (35 m^3) and comprising a 'Dexion' frame covered in nylon fly mesh. The arena was provided with 3 openings, each a vertical slit closed by means of Velcro strips. One opening, the largest, was at the end

Fig. 4. View of the vespiary at Chilworth looking north-east. The tubular aluminium frame of the foraging arena was later replaced by a stronger one of 'Dexion'.

farthest from the nest box and enabled the researcher to enter the arena; the other two were smaller and were positioned on either side of the cage towards the nest, and facilitated quick replenishment of food and water.

The vegetation within the foraging arena was cut at intervals to allow for easy access and observation, but flowering plants such as hogweed (*Heracleum sphondylium*) and Teasel (*Dipsacus fullonum*) were left untouched, to provide sources of nectar. A wooden 'foraging-table', painted white and measuring 2 x 0.3 x 0.3 m high, was placed within the arena near the entrance and was used in training wasps to come to sources of food dispensed in watchglasses. Supplies of insects swept from vegetation surrounding the vespiary were added to the arena from time to time to augment those already present.

Worker wasps from the 3 unrestricted colonies were trained, for certain experiments, to come to a 'foraging-table', of the type described above, on which were placed large watch glasses filled with honey solution. The table was placed 2 m beyond the boundary hedge of the vespiary on the eastern side.

II.B. Alarm behaviour experiments

These experiments were performed on wild colonies, as the captive ones in the vespiary were never active enough to provide a sufficiently high rate of exit for reliable results to be obtained.

II.B.1 Suitability of wild colonies

The following experiments were all performed at the entrance of healthy *vulgaris* nests. In order to meet the requirements of the investigation such colonies had to be chosen carefully, according to the following criteria:

(a) Accessibility

Nests close to the laboratory were preferred, but in the autumn of 1978, when wasps were scarce in Hampshire, colonies up to 25 miles distant were employed.

The majority of experiments were carried out on subterranean nests, whose entrance holes were conspicuous and within easy reach, but sites likely to be interfered with by the public were avoided. Where necessary, any vegetation obstructing the entrance hole was cut away some days before experiments began.

(b) Activity

The rate of exit of worker wasps was taken as a suitable measure of colony success. Only those nests with a rate of 8 per 30 seconds or greater were used for behavioural experiments, a rate of 10 per 30 seconds being the usual minimum. Details of the nests used are displayed in table 2:

Nest Number	Location	Position	Date of 1st Experiment	Initial Mean Rate of exit (wasps per 30 s)
1	Havant	subterranean	20.09.78	30
2	West End	aerial-under tiles	14.10.78	22
3	Chandler's Ford	subterranean	28.08.79	34
4	Bassett	subterranean	30.08.79	42
5	Moorside Rd	subterranean	16.10.79	15
6	Winnall Moor	subterranean	21.10.79	11
7	Hunworth	subterranean	09.09.80	5

Table 2. Details of the wild nests of *Vespula vulgaris* used for alarm behaviour experiments. Rates of exit are the means of 200 values recorded on the first day of experiments.

II.B.2 Materials

A short length of bamboo was bound securely to a bamboo pole 2 m in length, such that it formed the cross-piece of a 'T'. Notches were scored in the cross-piece at either end, exactly 25 cm apart, and a 25 cm length of cotton was hung from each.

A dental roll made from cotton wool and measuring 4 x 1.5 cm was attached by means of forceps to each cotton thread (figure 5 p. 23). One of the rolls, the control, was usually left blank whilst the other was treated with the substance under test. Both control and test rolls were replaced after use with fresh ones. Black dental rolls were used initially, as preliminary observations had indicated that wasps attacked dark colours more readily than white (see p. 52). The rolls were dyed according to the procedure described on page 40.

II.B.3 General Methods

Where possible, each experiment was performed at two different nests on consecutive days with ten replicates at each site. The validity of pooling results obtained on different days was checked by means of χ^2 tests. So as to minimize the possibility of habituation, at least two experiments were carried out at once, presentations for one being interspersed with those of the other, such that the wasps were exposed to the same treatment only once in every one and a quarter hours.

The replicates in each experiment were arranged in five couplets throughout the day, so as to minimise any bias due to the position of the treated roll with respect to the control. Thus, for the first presentation, the treated roll would be to the right of the control whereas for the second, fifteen minutes later, the reverse would be the case. After every couplet, the nest was left alone for 30 minutes before the other experiment took place.

The experiments consisted of repeated presentations of pairs of dental rolls at the nest entrance, the number of attacks sustained by each being recorded by means of a tally counter. Preliminary observations had shown that presentations of less than two minutes gave misleading results owing to the irregular fluctuations in numbers of insects at the nest entrance and the long reaction times in some of the experiments: by choosing a presentation time of two minutes these problems were alleviated.

Fig. 5. The apparatus used for alarm behaviour experiments, at the Havant nest.

The dental rolls were jerked up and down by means of sharp taps on the bamboo pole which was held such that the rolls were about 10 cm and equidistant from the entrance hole of the nest. Each presentation was timed with an alarm clock.

As a running check on the validity of the results and in order to monitor the general level of colony activity, the rate of exit of wasps was measured immediately before the first presentation of each couplet and also directly after the second. Rate measurements consisted of the means of ten consecutive 30-second counts, this interval being chosen for its greater sensitivity to sudden fluctuations than the rate per minute.

II.B.4 Statistical treatment of data

Within each experiment the numbers of attacks on both treated and control rolls were summed and compared. Wherever possible the data obtained from one nest were combined with those from another, the validity of such pooling being tested by χ^2 (using Yate's correction when necessary) or Fisher exact probability comparisons. The difference between the number of attacks on control and treated rolls was tested for significance by χ^2, binomial, or Fisher exact probability tests, depending on the constraints imposed by the data. Comparisons between times of first attack were made using the Mann Whitney U test.

II.B.5 General observations and classification of wasp responses

(a) Introduction

The behaviour of wasps at the nest entrance varied considerably, depending on the nature of the stimulus, but, even under the most extreme provocation, such as the presentation of a black, moving, venom-impregnated object, there were always some unresponsive insects. Among those which did respond positively there was no apparent difference in behaviour between those leaving the nest and others returning; both appeared equally likely to attack. Aggression was not restricted to unladen workers,

(b) Weak investigatory response (WIR)

The wasp hovers in front of the dental roll close enough to touch it with outstretched antennae. Initial approach is invariably upwind, but antennation can occur at any orientation and is frequently followed by alighting, but this is usually momentary and always non-aggressive. Weak investigatory responses seldom last longer than 10 seconds and are most frequently elicited by stationary dental rolls.

(c) Strong investigatory response (SIR)

With outstretched antennae the wasp hovers up to 30 cm downwind of the dental roll and continually adjusts position, to remain equidistant from it. Such behaviour may last for several minutes and is often interspersed with short bouts of fast erratic flying around the dental roll.

(d) Weak attack response (WAR)

The wasp accelerates towards the dental roll and collides with it, legs-first, then takes wing again almost instantaneously, apparently without having stung or bitten. Such a response differs markedly from the WIR in the acceleration prior to landing and the resulting force at impact. Weak attack responses are transient, lasting a few seconds at most, and, in the experiments described below, are characteristically the reaction to an 'enemy' 'in retreat', i.e. as the dental roll is removed from the nest entrance, wasps which have responded aggressively (see below) and have been stinging and biting the roll, take to the wing and bombard it, even when it is some metres from the colony. Frequently a wasp responding in this way will attack repeatedly and in quick succession before flying back to the nest. Weak attack responses usually follow strong attack responses (see below) but occasionally they may arise from SIRs.

(e) Strong attack response (SAR)

This, the most aggressive manifestation of wasp alarm behaviour, is characterised by a sudden acceleration towards the dental roll, followed by a forceful impact and protracted stinging and biting. Once a wasp has 'hit' the roll it may, as evinced by

the experiments described below, continue to cling to it for up to 15 minutes before lapsing into a WAR. The attitude of a wasp engaged in a SAR is very characteristic, and examples of it are shown in figs. 6 and 7 (p. 27). The gaster is sharply curved towards the surface of the dental roll and continuously pulsates whilst the mandibles are being used in a rhythmic and deliberate manner to 'dismember' it. The antennae, although occasionally flexed to touch the surface momentarily, are mostly held in a horizontal position with the flagellum curving back towards the thorax (fig. 7). This gives the wasp a rather 'sheepish' and distinctly aggressive mien, contrasting markedly with the 'hyper-alert' attitude characteristic of guard wasps or those hunting for prey. Wasps involved in a SAR do not relinquish their position until they are physically dislodged or the 'threat' from the 'enemy' is removed. Although apparently enervated by their efforts to succumb the 'enemy' in such attacks, the wasps take off for the nest with surprising energy when the danger is past and often lapse into a WAR whilst doing so. Very occasionally a wasp is unable to retract its sting after having attacked the 'enemy' and hence dies owing to self-evisceration. Strong attack responses often occur instantaneously where the stimulus is a powerful one. In such instances passing wasps swerve suddenly towards the 'enemy' and attack at once. More usually however, SARs arise from SIRs and, less frequently, WARs.

II.C. Attractant experiments

Apart from the preliminary work carried out at Chilworth, most of the attractant experiments involved the use of the standardized equipment described below.

Fig. 6. A wasp attacking a dental roll. The horizontally-held antennae are clearly visible.

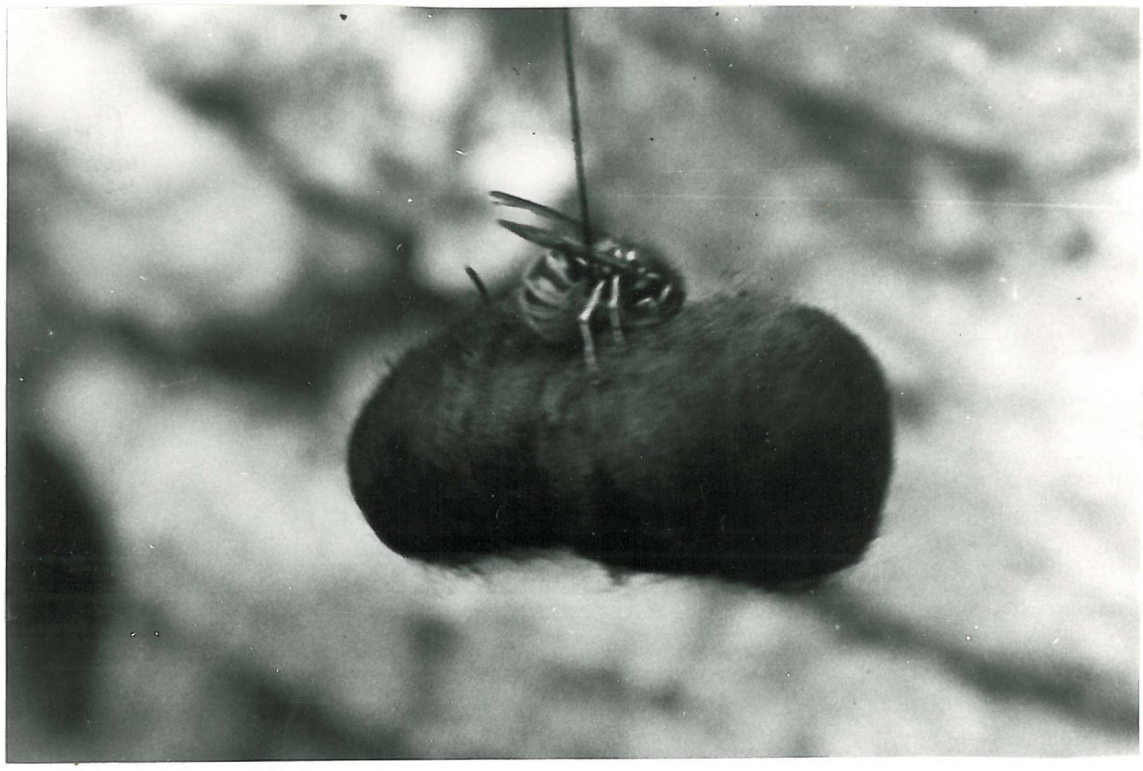


Fig. 7. A wasp attacking a dental roll. This worker is stinging and biting simultaneously.

II.C.1 Methods and Materials

Scrupulously clean, 2 ml glass vials were filled to the rim with the attractants to be tested and were then furnished with wicks cut from dental rolls. The vials were kept under refrigeration, with their phenolic, foil-lined caps screwed tightly down. Before use, each was wrapped with white tissue paper secured with an elastic band, to render it visually identical to the others. Prior to the start of the experiment each vial was opened and its wick extruded by means of a clean metal seeker. Small pieces of grey plasticine served to fix the vials to the centres of white ceramic tiles and also to secure a cone of nylon fly mesh over each one (fig. 9). The tiles were supported by means of bunsen burner tripods in a straight line (fig. 8 p. 29). During the course of the experiments continuous observations were made and every wasp or honeybee visiting the vials was recorded. A 'visit' was defined as an insect landing on the cone or changing its direction in flight to hover for 2 or more seconds in front of the cone. Every fifteen minutes the positions of the vials was altered so as to reduce directional bias. Each tile was moved along one place, the tile at the end being transferred to the beginning of the line. Fresh vials and contents were used for each replicate.

Further experiments involved the use of traps (fig. 42 p. 168 and fig. 67 p.209) to ensure that the numbers of wasps attracted to the vials were not inflated by returning insects. Each trap was placed on a ceramic tile supported by a bunsen burner tripod and a vial of attractant was placed beneath the entrance hole. The traps were filled with water to which 0.4 ml of a surfactant ('Savona' liquid detergent) were added. Various arrangements of traps were tried (see p. 180) and the insects were generally scored every 24 hours, after which the traps were cleaned and primed once more with fresh water and attractant.

Fig. 8. The factory yard at Bendicks, showing the large waste bins and ginger drums supporting vials of attractants.

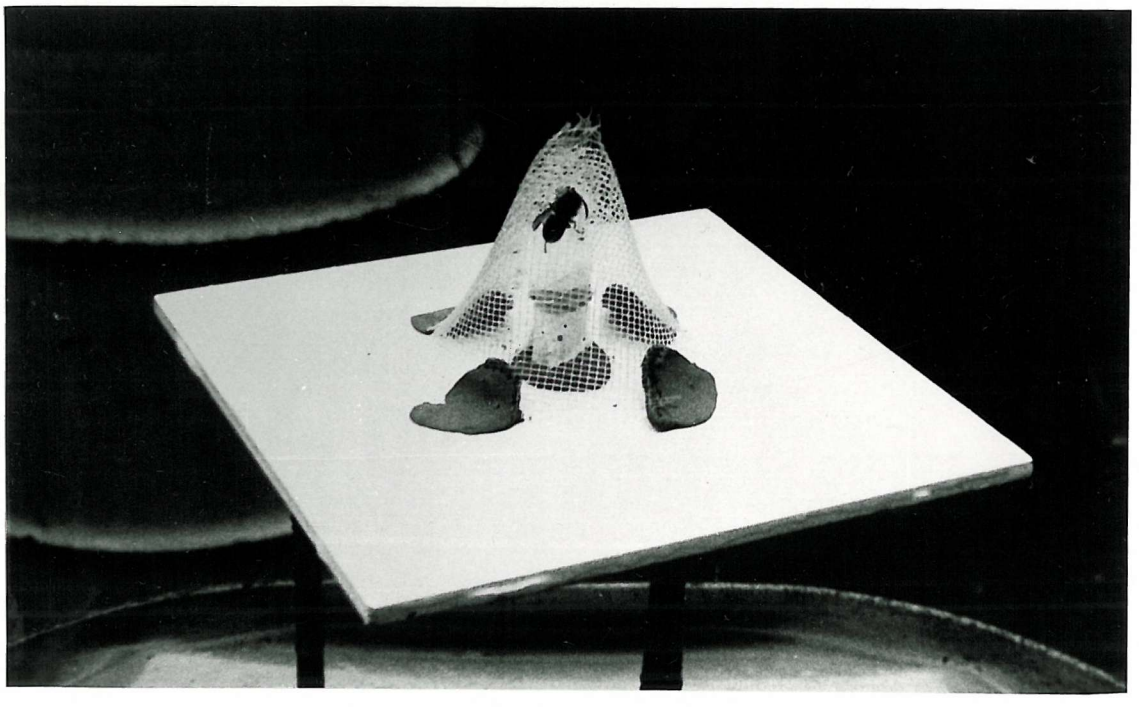


Fig. 9. A vial containing attractant protected by means of a netting cone. The wasp is trying to bite through the plastic mesh.

II.D. Chemistry

II.D.1 Extraction of the essential oil from ginger syrup

(a) Introduction

The nature and properties of ginger syrup are discussed in detail on pp.107-116. In order to remove the attractive principle from the syrup in a form likely to be useful as an attractant, a relatively non-destructive method had to be employed. Two techniques were tried: steam distillation and solvent extraction.

(b) Method 1 - Steam distillation

(i) Distillation

Ninety litres of Chinese ginger syrup, obtained from Bendicks (Mayfair) Ltd. of Winchester and stored in a large, polythene-lined drum, was kept in a cool, dark place and portions were decanted from it as required.

Three litres of ginger syrup were poured into a 5 litre capacity round-bottomed flask, which was then preheated in an electric mantle. Meanwhile an electric hotplate was used to heat the water in a steam generator to boiling point. When the steam began to come over and had heated the adjacent 500 ml separating funnel, to which it was led by means of glass tubing, the tap on the funnel was closed to force the steam through the heated syrup. When the steam entering the syrup ceased to condense and began to bubble through it the temperature of the heating mantle surrounding the flask was reduced. The steam and ginger volatiles passing out of the hot syrup were led through two large Liebig condensers connected in series to a foil-covered 5 litre separating funnel. The distillation was terminated 2 hours after the first distillate had come over, and the process was repeated twice more to give a yield of about 3 litres of distillate, most of which was water.

(ii) Extraction of the oil from the distillate

To minimise the loss of highly volatile components from the concentrated essential oil extraction and dilution were carried out on the same day. Exposure of the distillate to strong light was

avoided, to reduce the possibility of photolysis.

The solubility of the essential oil in the water was reduced by adding 200 g of vacuum - grade sodium chloride and thoroughly shaking the funnel.

Separation of the oil from the salt solution was achieved by adding 250 ml of re-distilled dichloromethane in 50 ml aliquots, the funnel being shaken vigorously for 15 seconds after each addition. As dichloromethane is slightly soluble in brine some was absorbed at the first addition, but the remainder, together with the essential oil it contained, was allowed to settle out at the bottom of the funnel, any globules adhering to the sides being tapped down. The distillate solution was then run out into a clean, dry 1-litre round-bottomed flask, and the procedure was repeated four more times, using fresh 50 ml aliquots of dichloromethane. The flask was stoppered and kept in a cool dark place.

(iii) Concentration and storage of essential oil

After 5 consecutive days of steam-distillation and extraction, the combined solutions of essential oil in dichloromethane from 45 litres of syrup were dried using anhydrous magnesium sulphate before being filtered into a clean, dry 1-litre round-bottomed flask containing anti-bumping granules. The filter paper and empty flask were rinsed with clean dichloromethane and the washings were added to the solution.

The flask containing the solution of essential oil was connected to a semi-microscale fractionating column with built-in Liebig condenser and was heated by means of a water bath set at 47-52°C. The dichloromethane, boiling off at 40°C, was collected in a 1-litre flask attached to the condenser. After 2-3 hours, when the rate of distillation had slowed, the remaining solution was transferred, with washings, to a 50 ml round-bottomed flask containing anti-bumping granules, and the distillation was continued. When no further solvent came over and the temperature at the top of the fractionating column dropped below 40°C the process was discontinued.

At this stage about 5 ml of a bright yellow liquid remained: it was transferred to 2 ml glass vials with screw tops and stored under refrigeration.

The concentration of the essential oil was found by evaporating the solvent from 1 ml of the distillate and weighing the residue:

Weight of essential oil in 45 litres of syrup = 1.72 g

therefore concentration = 38 mgl^{-1}

(iv) Discussion

Initially, each steam distillation was run until the odour of essential oil coming over with the steam became imperceptible viz. for about 5 hours. However, gas-liquid chromatography studies of successive hourly fractions indicated that 90% of the volatiles came over in the first hour. As a result of this, 2 hours was chosen as the run-length, giving time for the majority of the least volatile components to be collected.

Precautions had to be taken to prevent adulteration of the essential oil with contaminants such a phthalates. Figures 14 and 19(pp. 112,118) show the effect of replacing, with glass, the plastic tubing initally used to carry the steam to the syrup.

(c) Method 2 - Solvent Extraction

(i) Introduction

This method was tried as an alternative to steam-distillation because it precluded the possibility of thermolysis whilst enriching the essential oil with components which were not volatile in steam.

(ii) Extraction of the essential oil

Half a litre of ginger syrup was placed in a 2-litre separating funnel and an equal volume of distilled water was added. The solubility of the essential oil in the water was reduced by the addition of 100 g of vacuum-grade sodium chloride to the funnel, which was shaken vigorously. Extraction of the essential oil was effected by adding 30 ml of re-distilled dichloromethane and shaking the mixture thoroughly for 15 seconds. After allowing the two phases to separate out, the bottom layer was run off into a

clean, dry round-bottomed flask. Four more solvent additions were made in the same way and the aqueous layer was then discarded.

To clean the solution of essential oil it was put once more into the separating funnel with an equal volume of distilled water, shaken vigorously for 15 seconds and then run off. The aqueous layer was discarded and the process was repeated four more times, using fresh distilled water. The solution was then dried with anhydrous magnesium sulphate.

(iii) Concentration and storage of essential oil

The combined, dried solutions were concentrated and stored as on p. 31.

(iv) Discussion

Whereas the steam-distillate contained only the essential oil, the solvent extract consisted also of the oleoresin (see p. 108) - a non-volatile mixture of components responsible for the pungent taste of ginger, and sometimes known loosely as 'gingerin'.

(d) Preparation of essential oil for use in bioassays

(i) Introduction

Dichloromethane, as well as being extremely volatile, is repellent to wasps. For these reasons it could not be used as a diluent in the attractant bioassays and had to be replaced by another, more suitable solvent. Ethanol was chosen for this purpose, being considerably less volatile and slightly attractive to wasps.

(ii) Method

The essential oil (in dichloromethane) got from 45 litres of ginger syrup either by steam distillation or solvent extraction was transferred from the vials to a 50 ml round-bottomed flask which had been marked in water-proof ink at the 10 ml level. After the addition of 10 ml of ethanol the flask was attached to a rotary evaporator and the volume of the liquid was reduced to the level of the mark. Dichloromethane, being the more volatile of the two solvents, evaporated first. The process was repeated using another 10 ml of ethanol and the resulting solution was then transferred, in 2 ml aliquots, to clean glass vials.

II.D.2 Extraction of the essential oil and oleoresin from ground ginger

(a) Introduction

Ground ginger provided a simple means of obtaining large quantities of oleoresin relatively quickly. As it contained fewer of the more volatile components present in the essential oil derived from ginger syrup, an interesting comparison could be made of their attractiveness to wasps.

(b) Method

Ten grams of ground ginger (Schwartz Spices Ltd.) was placed in a 250 ml round-bottomed flask connected to a reflux condenser and a drying tube containing anhydrous calcium chloride. Anti-bumping granules and 150 ml of re-distilled dichloromethane were added and the flask was heated in an electric mantle. Refluxing was discontinued after 18 hours.

The extract was dried with anhydrous magnesium sulphate and was then filtered into a round-bottomed flask connected to a semi-micro scale fractionating column with built-in condenser. The flask was heated by a water-bath at 45°C until no more solvent came over, whereupon the remaining solution was transferred to a 50 ml flask and the process was continued. The dark brown solution left at the end of the distillation was transferred to vials and refrigerated. Displacement of the dichloromethane by ethanol was carried out as on p. 30.

II.D.3 Extraction of the essential oil from fresh stem ginger

(a) Introduction

The yield of essential oil from fresh stem ginger is considerably better than that from an equal volume of ginger syrup. To see whether there was a difference in attractiveness between the two and whether stem ginger would be a more economical souce of the oil, a steam-distillation of the rhizome was carried out.

(b) Method

About 112 g of fresh stem ginger was cut into small pieces before being macerated in a mortar. The ginger pulp was placed immediately in a 1-litre round-bottomed flask and covered with

distilled water to prevent loss of volatiles. After connecting the flask to steam-distillation apparatus similar to that described on p. 30 it was heated and steam was passed through the pulp for 20 hours, in accordance with the method described by Guenther (1952). Extraction, concentration and storage of the essential oil was carried out as in pp. 30-33.

II.D.4 Separation of components of essential oil of ginger using preparative thin-layer chromatography (TLC)

(a) Introduction

Although preparative gas-liquid chromatography (GLC) gave better resolution, TLC was initially chosen as the means to split the essential oil into fractions, as it obviated the possibility of thermolysis, was faster and could separate up to 100 mg of oil at a time.

(b) Method

A preparative TLC plate measuring 20 x 20 cm was coated with a layer of silica-gel 1mm thick, prepared by shaking 200 g of the gel with 100 ml of distilled water for 15 minutes. The plate was left to dry overnight and was then streaked with 50-100 mg of essential oil of ginger. The plate was developed for 15 minutes in a tank containing a mixture of benzene and diethyl ether (1:1) sufficient to cover the silica-gel beneath the streak. It was then blow-dried and was inspected under ultra-violet light, the bands thus revealed being marked lightly in pencil and scraped off into 20 ml vials to await elution.

Following a qualitative test run, after which the plate had been exposed to iodine, the position of a further 5 bands, not visible under ultra-violet light, was determined and these, too, were lightly marked off in pencil on the preparative plate.

The eluant used to wash each band off the silica-gel depended upon its 'ratio of fronts' (RF) value i.e:

$$\frac{\text{distance from initial streak to lower edge of band}}{\text{distance from streak to solvent front}}$$

Those with an RF of less than 0.5 were eluted with a mixture of 95% dichloromethane and 5% ethanol, and 100% dichloromethane was used for those with higher values. Elution was carried out by tipping the contents of each vial into a sintered crucible and washing it with 6 ml of the eluant, drawn through by means of a Venturi. Each solution was collected in a 20 ml screw-cap glass vial and was stored under refrigeration. The composition of the solutions was compared by gas-liquid chromatography (figs. 29-37 p. 148-156).

II.D.5 Separation of components of essential oil of ginger using high pressure liquid chromatography (HPLC)

(a) Introduction

Thin layer chromatography, though useful for the separation of components from a relatively small quantity of essential oil, proved too tedious to use for larger amounts and resolution was poor. High pressure liquid chromatography was a better method because it gave much improved resolution in a faster time.

(b) Method

The machine used was a Waters ALC 202.

Conditions for the main sequence of preparative runs were as follows:

column:	Li Chrosorb 10 RP 18 reverse-phase
solvent system:	10% water:- 90% re-distilled acetonitrile
flow rate:	1 ml min^{-1}
quantity of essential oil injected per run:	15 µl
type of essential oil	Chinese ginger (Treatt's Essential Oils Ltd.)

Figure 10 (p. 37) shows the trace obtained from one such run. A lag of 7 drops was allowed to compensate for the dead volume beyond the refractometer.

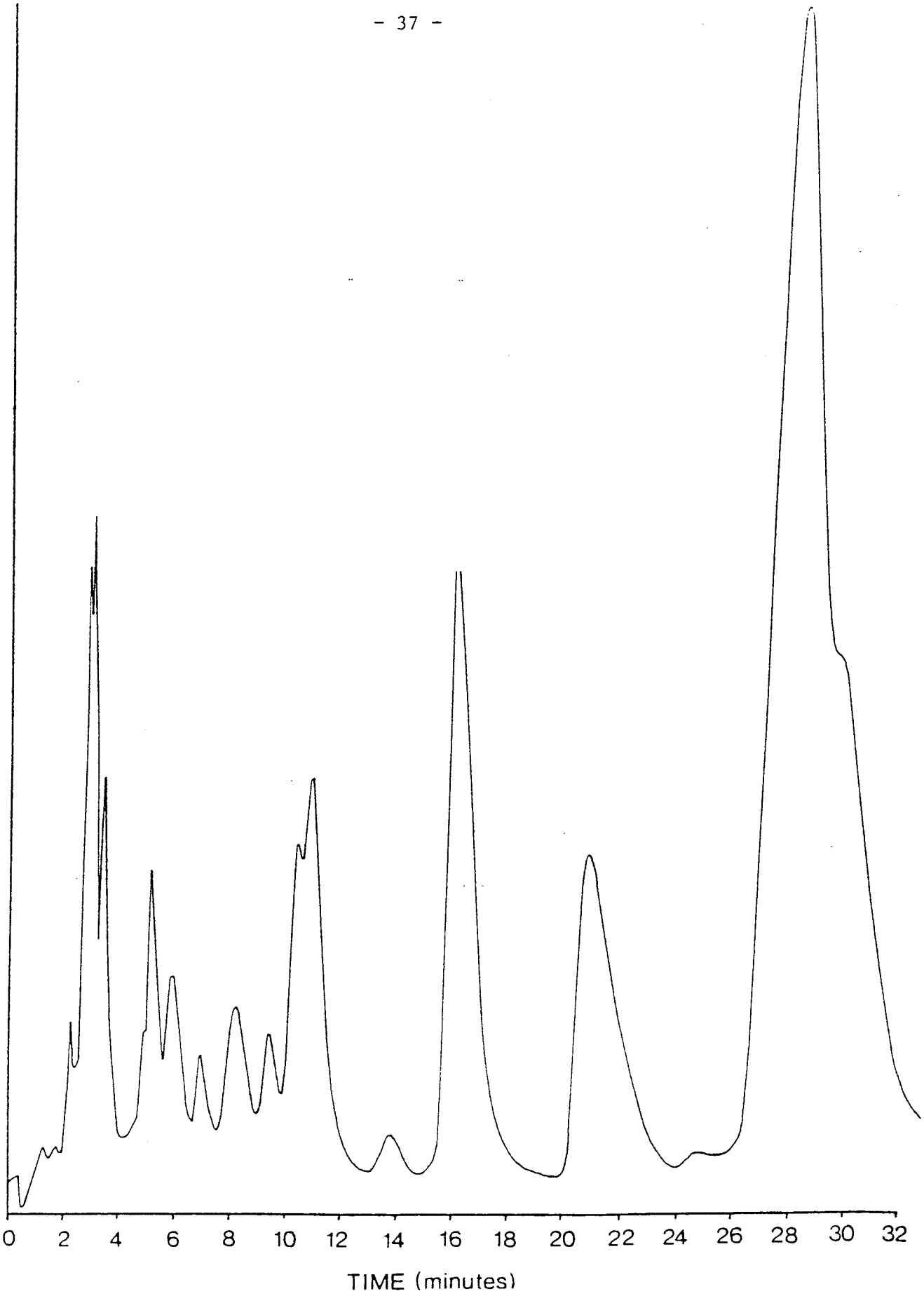


Fig. 10. HPL chromatogram of 15 µl of essential oil of ginger.

For use in the field, each fraction was concentrated and its solvent displaced by ethanol, employing methods similar to those described on p. 30.

II.D.6 Gas-liquid chromatography (GLC)

(a) Introduction

Most of the studies were done using a Pye-Unicam GCD gas chromatograph but some were carried out on a Pye-Unicam 104. The columns were all glass, with 6 mm outside diameter ground ends, connected to the detector with either a Pye-Unicam connector and viton 'O'-ring, or a modified connector and graphite 'O' ring for high temperature work (Higgs 1976). All supports and packings were supplied by J.J's (Chromatography) Ltd., Kings Lynn.

(b) Column specifications and packings

(i) 5% OV101 column

internal diameter:	2mm
length:	3m
packing:	5% OV101 silicone gum on 100-120 mesh Diatomite CLQ, acid/alkali washed, DMCS treated.
conditioning temperature:	350°C

(ii) 5% Carbowax column

internal diameter:	2mm
length:	3m
packing:	5% Carbowax 20M on 100-120 mesh Diatomite CLQ, acid/alkali washed DMCS treated.
conditioning temperature:	220°C.

(c) Column packing

All columns were packed using a Venturi to reduce the pressure at the exit and the packing was consolidated by tapping the column whilst filling.

(d) Conditioning

Those columns which required it were reconditioned with Sily-8 at 250°C.

(e) Gases

The carrier gas was oxygen-free nitrogen (BOC), passed through the column at a rate of 25 ml/min. In GC-MS studies helium (BOC) was used at the same flow rate. The ionization detector flame was fuelled by high purity hydrogen (BOC) in air (BOC).

(f) Solid-sample gas-liquid chromatography

Wasps to be studied were collected at the nest entrance in a net, which was then inverted over an open Dewar flask containing finely crushed dry ice (Drikold, ICI), to kill them quickly. Individual stings were removed by allowing each wasp to thaw to the point that the lancets protruded from the gaster and then pulling them out with forceps. The part of the sting required e.g. the venom sac, was quickly dissected out and placed in a dish of dry ice. Up to 10 frozen venom sacs were placed in a thin walled glass tube which was rapidly pushed to the end of a small glass vial (made by drawing out a pasteur pipette and sealing the end) using a clean, cold glass road. The vial was then heat-sealed and replaced in dry-ice.

A solid-sample injector (Morgan and Wadhams, 1972) was connected to the entrance of the column and the sealed vial was placed in the injector barrel. After being heated to approximately 150°C for 5 minutes the vial was then crushed by means of the plunger, allowing any volatiles present to pass through the column. The injection port heater was turned off after the GLC oven had reached 100°C, to prevent charring of the venom sacs.

(g) Gas-chromatography - mass-spectroscopy (GCMS)

Identification of compounds occurring in ginger and in the venom apparatus of wasps was carried out by means of combined gas-

chromatography and mass-spectroscopy. Mass spectra were obtained using an AEI MS 30 spectrometer and were analysed by a Data General Nova 3 data system calibrated with perfluorokerosene. The spectrometer was interfaced with a Pye-Unicam 204 gas-liquid chromatography system, via a ryhager jet separator. The carrier gas was helium, used at a flow rate of 25 ml/min and the columns employed were those described above (p. 38).

II.E. Preparation of dental rolls

For most of the experiments involving the use of dental rolls the latter were dyed black. In order to ensure uniformity of colour, various types of dye were tried, including permanent black ink (The Parker Pen Co. Ltd.) but the only one to give an homogeneous effect was Dylon ebony black (Dylon International Ltd.).

Dental rolls (Whites Dental Supplies Ltd.) were treated, according to the dye instructions and were then thoroughly rinsed in water prior to drying on blotting paper.

CHAPTER III

ALARM BEHAVIOUR

III.A. Introduction

III.A.1 Alarm communication in social insects

A characteristic feature of most social insects is their ability to react to a threatening stimulus in such a way as to alert other members of the colony. In some species this alarm communication is quite clear-cut and consists of a discrete set of signals, whereas in others a more complex situation exists, for instance in certain termites where chemical trails are used to alert others and recruit them to breaches in the nest wall possibly caused by predators. Three main types of alarm communication have so far been discovered in social insects - tactile, auditory and chemical.

Tactile stimuli communicating alarm have been demonstrated in the paper wasp *Polistes fuscatus* (Eberhard 1969) in which females alerted by the presence of the ichneumonid parasite *Pachysomoides fulvus* were seen to dart across the nest surface, causing the frail structure to vibrate. Other females reacted to this stimulus and behaved likewise. Evidence for tactile alarm communication in other social insects is generally circumstantial and in more advanced species with much larger colonies other forms of signal predominate.

Auditory signals are apparently of some importance, especially to ants and termites. The workers of many species of the former produce 'chirping' noises by raising and lowering the gaster, and Markl (1965, 1967, 1968) showed that this sound was produced by leaf-cutting ants if they were buried in soil. Other workers in the vicinity responded to the 'chirping' by digging where the sound was loudest, thus freeing the trapped ant: alarm signals like these would be of use when parts of the underground nest of such species collapse. Sound production in response to alarming stimuli has also been demonstrated in certain termites: alarmed soldiers of *Zootermopsis angusticollis* indulge in head-banging which produces vibrations in the substratum other termites can detect via their 'subgenual organs' (Howse 1964).

The most widespread and important method by which social insects communicate alarm is via chemical signals, or pheromones. Numerous alarm substances have been identified from various social insects, mainly ants and termites, and two have been found in the honeybee, but to date none have been isolated from social wasps.

III.A.2 Alarm pheromones in social insects

On the basis of the molecular configuration of alarm pheromones and the efficiency of insect chemoreceptors Bossert and Wilson (1963) predicted that most alarm substances in social insects would prove to have a molecular weight of between 100 and 200 and would contain 5-10 carbon atoms. They argued that the available diversity of molecular structure could easily allow for the limited specificity afforded by a molecule of that size. Also it was suggested that to provide an efficient alarm system, the pheromone would have to fade quite quickly and would need an intermediate threshold concentration compared to other pheromone systems. The alarm pheromones discovered since this hypothesis was proposed are consistent with the model.

A measure of the suitability of a pheromone for its role can be got from its Q/K ratio i.e.

$$Q/K = \frac{\text{No. of pheromone molecules released}}{\text{response threshold concentration (molecules per cubic centimetre)}}$$

Thus the trail odour laid by the fire ant *Solenopsis saevissima* has a Q/K value of about 1 whereas the sex attractants of moths have a Q/K of 10^{11}. Alarm pheromones have intermediate values of 10^3 - 10^4 (Wilson 1971). Thus when such a pheromone is emitted, say from the mandibular gland of an ant, into still air, it will spread outwards by diffusion forming an 'active space' (the space within which the concentration is at or above that required to initiate a response) a few centimetres in diameter before fading below the response threshold concentration some seconds or minutes later (Wilson 1971).

The alarm pheromones of most ants originate from the mandibular gland, although there are exceptions: ants of the genus *Formica*, for example, secrete alarm substances from the poison and Dufour's glands as well (Hölldobler, 1978). Some alarm substances of ants have a complex mode of action, as was shown by Bradshaw *et al* (1975) in *Oecophylla longinoda*. This species, the African weaver ant, is notoriously aggressive when defending its colony and can communicate alarm very rapidly. Secretion of the alarm pheromone from the mandibular gland results in a sequential message arising from the different active spaces of the components, the most volatile of which, hexanal, alerts other workers whilst 1-hexanol, 3-undecanone and 2-butyl-2-octenal cause attraction, attraction and biting, and biting respectively.

Two alarm pheromones, isopentyl acetate and 2-heptanone, have so far been discovered in the honeybee *Apis mellifera* (Boch *et al* 1962, Shearer and Boch 1965). Both substances release stinging behaviour (Free and Simpson 1968) although neither elicits a response as intense as that provoked by whole venom, indicating that other minor components, as yet unidentified, are acting as synergists.

III.A.3 Alarm pheromones in social wasps

(a) Introduction

No alarm pheromone has so far been identified in social wasps, and among the more primitive species studied there has been no evidnece to suggest that a chemically mediated alarm response exists (Maschwitz 1964). However, the presence of alarm pheromones in wasps of the sub-family *Vespinae* has been demonstrated by Maschwitz (1964) who did various experiments on captive and wild colonies of *Vespula vulgaris* and *V. germanica*.

(b) The work of Maschwitz

As the present research is based on his findings and, no English translation exists, a summary of Maschwitz' work is presented here.

Maschwitz did most of his research on nests of *Vespula germanica* disinterred in late summer or autumn and transferred to insulated and light-proofed, glass-walled boxes in the laboratory. Measuring 23 x 23 x 30 cm these boxes had a moveable front wall such that an entrance-slit could be made. Control experiments were performed on wild nests *in situ*, though Maschwitz did not state which of his tabulated data were from wild or captive colonies.

The basic method used for all the experiments involved holding an object (e.g. crushed wasp, extract on filter paper etc.) in front of the nest entrance and counting the number of workers flying out in 10 seconds. Control experiments were performed but no mention was made of the time allowed to elapse between successive presentations. Experiments were repeated between 3 and 10 times.

In later tests, Maschwitz made 'fabric dummies' similar to the 'stingballs' of Free (1961). Measuring 5 cm in diameter, these were hung 40 cm apart and 1.5 m away from the nest entrance, out of the mainstream of wasp activity. The dummies were only replaced after presentations during which numerous wasps flew towards them.

The experiments may be summarized as follows, all data referring to *V. germanica* unless otherwise stated:

(i) The alarm reaction

Object presented at nest entrance	Total No. of wasps flying out	Number of replicates
Empty forceps	15	10
1 lightly anaesthetized *V. germanica* worker held fast	185	10

Table 3. Maschwitz' results: the alarm reaction.

Conclusion: restrained (worker) wasps give an alarm signal.

(ii) The alarm signal is not optical

Freshly dead, crushed wasps presented at the nest entrance have the same effect as live, restrained ones, even though wasps within the light-proof nest cannot initially see them. Empty forceps and long-dead wasps from which the alarm substance has evaporated do not provoke an alarm flight.

Conclusion: the alarm signal is not an optical one.

(iii) The alarm signal is not mechanical

When males, and workers from which the stings have been romoved are held against the nest envelope no alarm flight occurs. This is contrary to the theory propounded by Weyrauch, who suggested that such signals might play a part in the alarm reaction (Weyrauch, 1935).

Conclusion: the alarm signal must be chemical in nature.

(iv) Where is the alarm substance produced? (Tables 4 and 5).

Wasps were divided into head, thorax and abdomen, and venom apparatus. Two experiments were performed:

(a) Counting the numbers of wasps emerging in 10 seconds when crushed body-parts were presented at the nest entrance.

(b) Recording the time taken for the alarm reaction to subside after presentation of the alarm substance (see (vi)).

Object presented at nest entrance	Total No. of wasps flying out *(germanica)*	Number of replicates
Plant-odour (control)	9	7
Crushed worker head	11	7
Crushed thorax + abdomen	11	7
Crushed sting + glands	72	7

Table 4. Maschwitz' results: location of the alarm substance in *V. germanica*.

Object presented at nest entrance	Total No. of wasps flying out (*vulgaris*)	Number of replicates
Untreated control paper	10	5
Crushed worker head	14	5
Crushed thorax + abdomen	12	5
Crushed sting + glands	39	5

Table 5. Maschwitz' results: location of the alarm substance in *V. vulgaris*.

Conclusion: the alarm substance is located in the venom apparatus.

(v) Accurate localization of site of secretion (Tables 6 and 7)

The component parts of the venom apparatus were separated (by dissection under water; Maschwitz, personal communication) and presented at the nest entrance.

Object presented at nest entrance	Total No. of wasps flying out (*germanica*)	Number of replicates
Untreated control paper	8	5
Crushed sting	24	5
Crushed venom sac	55	5

Table 6. Maschwitz' results: accurate localization of site of secretion in *V. germanica*.

Object presented at nest entrance	Total No. of wasps flying out (*vulgaris*)	Number of replicates
Untreated control paper	6	3
Crushed sting	14	3
Crushed venom sac	36	3

Table 7. Maschwitz' results; accurate localization of site of secretion in *V. vulgaris*.

Conclusion: the alarm substance is produced by the venom glands.

(vi) Characteristics of the alarm substance (Table 8)

To the human nose the alarm secretions of both species are indistinguishable, the smell being redolent of fermenting wine.

The venom sacs of 10 *V. germanica* workers were extracted in 0.5 ml of diethyl ether, which was then decanted and absorbed by a 2 cm^2 filter paper. The solvent was allowed to evaporate before the paper was held at the nest entrance.

Object presented at nest entrance	Total No. of wasps flying out	Number of replicates
Untreated paper	10	5
Paper + diethyl ether	12	5
Paper + venom sac extract	94	5

Table 8. Maschwitz' results: characteristics of the alarm substance.

The alarm substance was quite volatile: excitement at a *V. vulgaris* nest died down after no more than 8 minutes from the presentation of the extract from 3 venom sacs (at 23°C), whereas the smell (to a human nose) had disappeared in 3-4 minutes.

Conclusion: the venom contains a volatile alarm substance and a non-volatile toxin used for defence.

(vii) Specificity of the alarm substance

To test whether an alarm reaction could be set off by any strong-smelling substance, extracts of plant, animal and artificial origin were presented at the nest entrance. Among the odours tested were:

essential oil of *Heracleum* sp.
underarm sweat of man
sting extract of *Apis mellifera*
crushed bluebottles and locusts
formic acid
ammonium hydroxide

None produced an alarm response, whereas wasp venom controls did.

Conclusion: cause of the alarm reaction is specific to the contents of the venom sac.

(viii) Do males and queens possess alarm substances?

Object presented at nest entrance	Total No. of wasps flying out	Number of replicates
Crushed male and control odour	33	10
Alarm substance	105	10

Table 9. Maschwitz' results: test for the presence of alarm substances in males.

Object presented at nest entrance	Total No. of wasps flying out	Number of replicates
1 queen venom sac	83	5
Untreated control	11	5

Table 10. Maschwitz' results: test for the presence of alarm substances in queens.

The smell of the queen alarm substance was indistinguishable from that of workers.

Conclusion: Males have no alarm substance, but queens do.

(ix) Interspecificity of the alarm substance (Tables 11, 12, 13).

To the human nose the venom of both species smelt similar. However, of 16 *V. germanica* stings:

7 smelt strong
6 smelt intermediate
3 smelt faintly

of 15 *V. vulgaris* stings:

2 smelt strong
4 smelt intermediate
8 smelt faintly
1 did not smell

To test the reactions of each species to the other's stings the latter were presented at the nest entrances:

Test	Total No. of wasps flying out		Untreated Control	Number of replicates
	V. germanica	*V. vulgaris*		
1 *vulgaris* sting	59		12	7
1 *germanica* sting		62	6	5

Table 11. Maschwitz' results: interspecificity of the alarm substance.

The duration of the alarm flight was measured (in minutes) on presentation at the nest entrance of stings of the same or different species:

Vespula germanica

vulgaris sting (minutes)	Number of stings	*germanica* stings (minutes)	Number of stings
0.5	1	3	1
0	2	3.5	3
0	2	3.5	2
0.75	1	1	1
0.75	1	1	1

Table 12. Maschwitz' results: duration of alarm flight of *V. germanica* on presentation of stings of both species.

Vespula vulgaris

vulgaris sting (minutes)	Number of stings	*germanica* stings (minutes)	Number of stings
4	3	8	3
3.5	1	7	1
3.5	1	4	1
0.5	1	6.5	1

Table 13. Maschwitz' results: duration of alarm flight of *V. vulgaris* on presentation of stings of both species.

Conclusion: *V. germanica* stings evoke a stronger reaction in both species ($p < 0.0027$) than *V. vulgaris* stings, probably because *vulgaris* (the smaller species) has a smaller venom sac.

(x) Analysis of the alarm reaction

When alarmed, the wasp curves its abdomen towards the source of the alarming substance and sprays a stream of venom, covering the enemy in find drops. *Vespula germanica* sprays up to 1 cm, *V. vulgaris* 1.5 - 2 cm.

Out of 50 *germanica* workers observed on presentation of the alarm substance:

37 flew out of the nest
4 ran into the nest
2 flew out and then back in
6 ran about at the entrance
1 ran into the nest and flew out

Summary: Workers and queens of both species produce an alarm substance in their venom glands which they spray out when alarmed, causing other wasps to fly out of the nest and attack. The substance is interspecific.

(xi) Behaviour of alarmed wasps

When venom is placed at the nest entrance wasps inside react positively phototactically. The reaction is limited if the wasps have not already been alarmed, but if an attack has recently taken place wasps sit at the entrance and the next reaction is much more pronounced. The alarm substance also appears to act as an attractant, as wasps move up the concentration gradient in the dark and form a cluster. Wasps exhibit necrophoric behaviour when presented with corpses smelling of the alarm substance, but do not remove bodies lacking the odour.

The reaction of wasps to the alarm substnace presented at a foraging site is the same i.e. flight:of 37 wasps feeding at fondant, when presented with the alarm substance, 24 flew away, but returned within a minute. When presented with lavender scent only 3 flew away.

Conclusion: venom has a situation-specific effect i.e. 'fight or flight'.

(xii) The attack response - factors causing flight (Table 14)

Using a method similar to that devised by Free (1961) Maschwitz made 'fabric dummies' to test whether wasps reacted to colour and movement. Using black and white 'dummies', both moving and stationary, at the entrance to a nest with an exit rate of 4-6 wasps per second, he showed the following:

Dummy	No. of flights towards dummy in one hour
White, stationary	5
Black, stationary	30
White, moving	50
Black, moving	275

Table 14. Maschwitz' results: the attack response:- factors causing flight.

Conclusion: movement is the most important stimulus, followed by dark colours. If the two stimuli are combined summation of their effects ensues.

(xiii) The effect of the alarm substance on wasps flying from the nest (Tables 15 and 16)

Apart from optical stimuli, the alarm substance plays an important part. Two black dummies, one with 2 stings crushed on it, the other without, when presented at the entrance to a nest of *V. vulgaris* produced the following results:

Object presented at nest entrance	Total No. of wasps flying towards object	Number of replicates
Stung dummy	1277	28
Untreated dummy	813	28

Table 15. Maschwitz' results: effect of the alarm substance on wasps flying from the nest.

Conclusion: the alarm substance has a two-fold function:

(a) creates alarm

(b) orientates wasps towards the source of the sting.

Using a black dummy without venom paired with a white one with the alarm substance, the following results were obtained at a nest of *V. vulgaris* which had already been alarmed:

Object presented at nest entrance	Total No. of wasps flying towards object
Untreated black dummy	241
Treated white dummy	19

Table 16. Maschwitz' results: effect of the alarm substance and black and white dummies on wasps flying from the nest.

Conclusion: the initial flight is evoked by optical means.

(xiv) Summary: Wasps alarmed by a jolt, direct contact or the alarm substance fly towards dark, moving objects (usually men or animals). If the object has already been stung the attack is fiercer.

(c) This research

The techniques used by Maschwitz in the above experiments have been questioned by certain workers, and Akre (Personal communication) has been unable to duplicate any of his findings. The present research was initiated in an attempt to corroborate and enlarge upon the findings of Maschwitz, whilst using a more rigorous and reproduceable approach. The aims are described in detail on page 4.

III.B. Alarm behaviour experiments at the nest entrance

III.B.1 To show the presence of the alarm pheromone and to locate its site of secretion

(a) Reaction of workers to crushed bodies of wasps

Method

A worker wasp was captured at the nest entrance by means of a butterfly net and, by the use of clean forceps, was removed and crushed onto a black dental roll, all traces of exoskeleton being removed. A black, untreated roll was used as control. The rolls were jerked at the nest entrance for 2 minutes, immediately after crushing the wasp, and any hits viz. SARs and WARs were recorded on a tally counter. The experiment was repeated fourteen times at Havant and eight at West End.

Results

Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental roll Treated	Control	Range	Signif-icance
Havant	14	21	82	3	0-18	***
West End	8	16	5	0	0-3	*

* = $p < 0.05$ (Binomial test) & *** = $p < 0.001$ (χ^2 one sample test)

Table 17. Numbers of hits evoked by dental rolls impregnated with crushed bodies of worker wasps.

Reproductives were emerging at the West End nest, but not at Havant. Attacks on the treated roll were significantly more numerous than those on the control, especially at Havant, although results were not as conclusive at West End, and at both sites the ferocity of attack varied considerably. There were no WARs or WIRs but SIRs occurred in large numbers, especially at the treated roll.

Hits rarely occurred at the instant of presentation but when they did the ensuing attack was invariably ferocious. On the occasions when no attacks were recorded, numerous wasps exhibited a WIR, even at West End where hits were few in number.

In all cases the control was virtually ignored, the three instances of hits being sustained at Havant being readily explainable. On the first occasion (20.9.78) a wasp was accidently struck by the control roll and retaliated immediately. On the other (26.9.78) a blustery wind caused confusion by blowing across from treated roll to control with the effect that hovering wasps congregated downwind of the control roll, and re-directed their aggression accordingly.

Variation in the intensity of the response was occasionally quite marked, especially on windy days and on those mornings when males and queens were leaving the nest. Not once were reproductives seen to react to the presence of the dental rolls.

Conclusions

The bodies of worker wasps contain a substance, or substances, capable of evoking an alarm reaction in other workers of the same colony. That this response is mediated chemically is evinced by the fact that the control roll, visibly identical, was invariably ignored.

Certain discrepancies in the results demand explanation. The great variation in the number of hits sustained by the treated roll was probably due to the inherent variability of the stimulus presented, each wasp used differing in age and physiology. Although considerable care was taken to repeat the experiments exactly, some wasps were inevitably crushed more thoroughly than others, resulting in a heightened response. The weather also introduced an element of variability and it seems likely that the rooftop position of the West End nest, with its attendant convection currents and edge effects, was a major cause of the inconsistencies here.

The emergence of sexual forms coincided with a marked decrease in worker responsiveness at the West End nest. At such times the general level of activity was higher than usual but workers were less easily roused by the treated dental rolls. Why the alarm threshold should be raised in these instances is uncertain (cf. experiment 1d(ii)).

(b) Determination of the location of the alarming factor in worker wasps

Method

Three experiments, involving head, thorax and gaster respectively, were carried out in rotation to facilitate direct comparisons. Untreated, black dental rolls served as controls whilst freshly caught worker wasps were dismembered for use on the treated rolls. In each experiment the relevant part of the body was thoroughly crushed on to a black dental roll before being jerked in front of the nest for two minutes, all visible signs of exoskeleton having been removed. Hits and SIRs were recorded.

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental roll Treated	Control	Range	Signif-icance
Head	Havant	14	21	1	0		NS
	W. End	8	16	0	0		
Thorax	Havant	14	21	0	2		NS
	W. End	6	12	8	3		NS
Gaster	Havant	14	21)	60	7	0-15	***
	W. End	8	16)				

NS = not significant and *** = $p < 0.001$ (χ^2)

Table 18. Numbers of hits evoked by dental rolls impregnated with crushed heads, thoraces or gasters of worker wasps.

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental roll Treated	Control	Range	Signif-icance
Head	Havant	5	22	17	24	0-8	NS
Thorax	Havant	5	22	19	10	0-7	NS

NS = not significant (χ^2)

Table 19. Numbers of SIRs evoked by dental rolls impregnated with crushed heads or thoraces of worker wasps.

Reproductives were emerging from the West End nest, but not at Havant. There were no WARs or WIRs recorded in any of the experiments. In the course of 22 replicates at Havant and West End there was only one attack on dental rolls treated with crushed heads, compared to none on the controls. Nevertheless considerable interest was aroused in the workers at the nest entrance, both control and treated rolls evoking SIRs. However there was no significant difference between the number of SIRs recorded at both rolls in five replicates at Havant.

At the Havant nest crushed thoraces elicited no attacks, although the control was attacked twice. As with heads, thoraces attracted numerous wasps, but here again there was no significant difference between the numbers of wasps hovering at either roll. At West End there were no hits until dusk one evening when the treated roll sustained 8 hits and the control roll 3. Comparison of SIR data for heads and thoraces shows no significance at $p = 0.05$ (χ^2). Crushed gasters evoked fierce attacks at both sites, the treated rolls bearing the brunt, with 60 hits in 22 replicates at Havant and West End. In contrast the controls were hit 7 times only. There is no apparent difference between whole wasps (experiment (a)) and gasters in the proportions of attacks they precipitate on treated and control rolls ($p > 0.05$, χ^2).

Conclusions

It can be concluded from these results that the substance eliciting the alarm reaction in these wasps is located in the gaster. Volatile components of the head and thorax are probably detected by worker wasps but the sight of the black dental rolls may have been the main cause of attraction. The one occasion at West End where eleven attacks occurred on both rolls during a single presentation of a crushed thorax was a freak occurrence probably caused by an accidental hit.

(c) Localization of alarm pheromone within the gaster

Method

Gasters from which the stings had been removed were crushed singly on to black dental rolls, and ten presentations, using blank controls, were made at the Havant nest, followed by fifteen at West End. Alternating with these experiments were 25 in which the crushed gaster was replaced by a single sting, the control remaining blank. Hits and SIRs were recorded. Stings were removed from live decapitated wasps by means of fine forceps, such that the entire venom apparatus and associated glands were withdrawn without damage.

Results

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental roll Treated	Control	Range	Significance
Gaster minus sting	Havant	10	11	0	0		
	W. End	15	16	4	1		NS
Sting	Havant	10	11)				
	W. End	15	16)	27	3	0-5	***

NS = not significant (binomial test) and *** = $p < 0.001$ (χ^2)

Table 20. Numbers of hits evoked by dental rolls impregnated with crushed gasters (minus stings) or crushed stings of worker wasps.

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of SIRs at dental roll Treated	Control	Range	Signif-icance
Gaster minus sting	Havant	10	11	6	1	0-3	NS
Sting	Havant	10	11	17	0	0-4	***

NS = not significant (binomial test) and *** = $p < 0.001$ (χ^2)

Table 21. Numbers of SIRs evoked by dental rolls impregnated with crushed gasters (minus stings) or crushed stings of worker wasps.

Numerous queens and males emerged from the nest during most of the presentations. No WARs or WIRs were recorded. Gasters with their stings removed provoked only five wasps to attack, all at West End, although some workers were attracted to the rolls at Havant.

Stings, however, induced alarm behaviour resulting in attacks at both sites, most of these being directed at the treated roll (significant at $p = 0.001$). At the Havant nest 17 wasps investigated the treated roll, whereas none were attracted to the control. Stings and whole gasters produced similar effects ($p > 0.05$, χ^2), as can be seen by comparing tables 18 and 20. However, comparison of the totals (treated plus control) indicates significance at $p = 0.001$ (χ^2), the whole gasters provoking a more intense response.

Conclusion

The alarming substance in the gaster of *vulgaris* workers appears to be associated in some way with the sting, the remainder of the gaster producing, when presented alone, a slight attracting effect. If presented on the control roll, the gaster appears to act as a synergist.

(d) Determination of the source of pheromone within the sting

(i) Using stingless gasters as controls

Method

Three sets of concurrent experiments were performed, using venom glands, venom sacs and Dufour's glands of worker wasps, with stingless gasters as controls. Experiment (e) below was carried out on the same days. Wasps were caught at the nest entrance and their stings were rapidly removed. Dissection of the glands was done in a small dish of wax covered with clean water, under a binocular microscope. Fine needles were used to tease the glands apart and to smear them on to black dental rolls, the entire procedure being performed with the minimum of delay. Fresh gasters were used for each dissection. Eight 2-minute presentations of the three components were made at the Havant nest.

Results

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental roll Treated	Control	Range	Significance
Dufour's gland	Havant	8	22	19	19	0-11	NS
Venom glands	Havant	8	22	10	7	0-4	NS
Venom sac	Havant	8	22	33	7	0-20	***

NS = not significant and *** = $p < 0.001$ (χ^2)

Table 22. Number of hits evoked by dental rolls impregnated with crushed Dufour's glands, venom glands or venom sacs, using dental rolls impregnated with stingless gasters as controls.

No reproductives were emerging during the course of these experiments. There were no WARs or WIRs in any of the presentations but SIRs occurred, especially when venom sacs and Dufour's glands were

presented. There were more SIRs at the=treated roll with venom glands and sacs, but with Dufour's glands the numbers at treated and control rolls were approximately equal. Dufour's glands provoked an unusual response in 3 of the 8 replicates, in that the numbers of attacks on both rolls were effectively the same viz. 12, 13; 6, 5; 1, 1. No attacks were recorded for the other five presentations.

Venom glands, being very small and threadlike, were exceedingly difficult to dissect out cleanly and were easily ruptured. Their contents, moreover, would undoubtedly have volatilised very quickly on maceration of the glandular tissue. The inconclusive results in table 22 demonstrate this, only 17 attacks having been recorded from 8 presentations. The treated and control rolls were not significantly different in the numbers of attacks they sustained.

Venom sacs, however, evoked a highly significant response with 33 attacks on the treated roll and only 7 on the control. Twenty hits on the treated roll occurred at a single presentation, the attacks beginning almost immediately and rapidly increasing in number as the roll sustained more stings. There is no significant difference ($p > 0.05$, χ^2) between the proportions of attacks elicited by venom sacs or whole stings (experiment (e)) although the totals (treated plus control) do differ significantly ($p < 0.01$, χ^2).

Conclusion

The major alarm pheromone is probably located in the venom sac of *vulgaris* workers, and is likely to be present in the venom glands, as these drain into the sac. However, the large number of hits occurring in one presentation caused an inflated total which is therefore misleading. The Dufour's gland may contain an alerting pheromone resulting in general alarm and attraction.

(ii) Using blank controls

Method

The experiments were carried out as in d(i) above, but blank dental rolls were used instead of those impregnated with crushed gaster. Ten replicates of each were performed at Havant and six at West End.

Results

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental rolls Treated	Control	Signif-icance
Dufour's gland	Havant	10	12	0	0	NS
	W.End	6	12	0	0	
Venom glands	Havant	10	12	0	0	
	W. End	6	13	1	2	
Venom sac	Havant	10	12	0	0	NS
	W. End	6	12	0	0	

NS = not significant (Binomial test)

Table 23. Number of hits evoked by dental rolls impregnated with crushed Dufour's glands, venom sacs or venom glands, using blank controls.

Many males and females were emerging at both sites during these experiments. No WARs or WIRs occurred but SIRs were numerous, especially during presentations of Dufour's glands and venom sacs. The experiments, which were carried out late in the season, produced only three positive attacks, two of these being on the control. There were no attacks on presentation of Dufour's glands or venom sacs and only one on presentation of venom glands.

Conclusion

The fact that venom sacs elicited a stronger response when presented with crushed gasters than when offered alone might suggest that the gaster contains a synergist, perhaps an attractant, drawing wasps into the active space of the alarm pheromone.

Alternatively, and perhaps more likely, the negative reaction to venom in the latter part of the wasp season may have been due to a generalised alteration of behaviour brought about by the onset of colony breakdown and marked by an exodus of males and queens. (cf. expt. (a)).

III.B.2 To investigate the possibility that substances within the gaster and sting enhance the alarm behaviour induced by the pheromone

(a) Effect of crushed sting on *vulgaris* workers using stingless gaster as control

Experiment (c) showed that stingless gasters did not provoke attacks. They were used in this experiment as internal controls, being presented together with their own stings. If any substance in the gaster was acting as a synergist to the venom apparatus, this should be manifest in a greater number of hits, compared to stings with blank controls.

Method

The gasters of freshly caught worker wasps were removed and each sting was withdrawn by means of forceps. The gaster was then smeared on to the control roll and the sting on to the other, both rolls being cleared of any visible traces of exoskeleton. Nine 2-minute presentations were made at the Havant colony.

Results

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental rolls		Range	Significance
				Treated	Control		
Sting	Havant	9	23	69	6	2-18	***

*** = $p < 0.001$ (χ^2)

Table 24. Number of hits evoked by dental rolls impregnated with crushed stings of worker wasps using dental rolls impregnated with crushed stingless gasters as controls.

No reproductives emerged during the experiment. There were no WARs or WIRs but many SIRs occurred, especially at the treated roll. Hits were scored on the treated roll at every presentation, with 18 attacks being the greatest number sustained, whereas the control roll, smeared with gaster, was hit only 6 times. Attacks were fierce and persistent, some wasps remaining on the roll for up to 15 minutes after cessation of movement and at one stage ten workers were clinging to a roll at once. The difference between the number of attacks on the treated and control rolls is highly significant. Comparison of these results with those from experiment (c) indicates no significant difference between the proportions of hits on treated and control rolls ($p > 0.05, \chi^2$). However, the total number of hits (treated plus control) in experiment (c) was only 30 in 25 replicates compared, in this experiment, to 75 in 9. Allowing for the higher mean exit rate in the latter case this still represents an unexpectedly large number of stings where crushed gasters were used as controls.

Conclusion

These results confirm that the sting contains as alarm pheromone and imply that some factor in the gaster is acting as a synergist. However, many reproductives were emerging during the course of experiment (c) and this appeared to increase the threshold for the attack response.

(b) Comparison of the alarm behaviour elicited by crushed venom sacs and whole stings

Introduction

From the foregoing experiments it can be seen that the alarm pheromone is released from the venom sac, but there appears to be some difference between the effects of whole sting and venom sac on attacking wasps. This experiment was designed to test for any such differences.

Method

Ten replicates of each experiment were carried out alternately at the entrance to the nest of *V. vulgaris* at Bassett.

Black dental rolls were used, the control in each case being untreated. Stings were removed from freshly killed workers by means of fine forceps and the venom sacs were dissected out under water. The dental rolls were freely suspended and were jerked vigorously in front of the nest. Presentations lasted two minutes and the time of first hit was recorded by means of a stop-watch.

Results

Test	Venom sac	Whole sting
Replicates	10	10
Mean Exit rate (wasps per 30 secs.)	32	32
Number of hits (treated roll)	12	133
Number of hits (control roll)	0	11
Range	0-4	2-26
Significance	***	***
Average time for first hit (seconds)	71	29

(*** = $p < 0.001$, χ^2 test)

Table 25. Number of hits evoked by dental rolls impregnated with crushed stings or venom sacs, and average time taken for first attack to take place.

No reproductives emerged during either experiment and the prevailing weather conditions were warm, sunny and calm. No WIRs occurred during the presentation of whole stings but some were recorded at the rolls treated with venom sacs. All the hits during the sting presentations were SARs but 3 of those during venom sac replicates were WARs. The dental rolls impregnated with the contents of the venom sac were attacked 12 times in 10 presentations, the most attacks sustained by one roll being 4, and the average time for the first hit was 71 seconds after initial presentation. Although some interest was shown in the control rolls none were attacked. The difference between treatment and control is highly significant. Reaction to the rolls smeared with

whole stings was much greater, 133 hits being recorded in ten presentations, compared to 11 on the control. The average time for first hit was 29 seconds. The difference between average times for the first hit in the two experiments is not significant at $p = 0.05$ (Mann-Whitney U test). Comparison of the two experiments by means of a χ^2 two sample test shows that, at $p = 0.05$, there is no significant difference between the proportions of attacks, but the totals (treated plus control) differ significantly ($p < 0.001$, χ^2).

Conclusion

There is no significant difference between the proportions of hits on rolls treated with crushed venom sacs and whole stings when compared with the controls. However, as in experiment (d) venom sacs produced a less significant result than the whole stings, suggesting that another part of the sting contains a synergist.

(c) Effect of sting extract on workers

This experiment was tried in an attempt to minimize the effects of volatilization which were inevitable in dealing with single stings and to investigate further the apparently enhanced effect of whole stings compared to isolated venom sacs.

Method

The stings of 15 freshly-caught *vulgaris* workers were removed and macerated in 0.5 ml of liquid paraffin. As before black dental rolls were used in each presentation, the control being impregnated with a single drop of pure liquid paraffin and the other roll being treated with a drop of the extract. The latter was applied last and immediately prior to presentation in order to minimize evaporation. Six two-minute presentations were made at the Havant nest before the experiment was abandoned.

Results and Conclusion

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental rolls Treated	Control	Range	Signif-icance
Sting extract	Havant	6	27	6	0	0-3	*

* = $p < 0.05$ (Binomial test)

Table 26. Number of hits evoked by dental rolls impregnated with extract of worker stings in liquid paraffin.

No reproductives emerged from the nest during this experiment. There were no WARs or WIRs but numerous SIRs occurred, especially at the treated roll. The treated roll was attacked three times in each of the first two presentations but thereafter was effectively ignored, indicating that the alarming principle of the venom was deteriorating. There were, however, significantly more hits on the treated roll than on the control, indicating that the sting contains an alarm substance.

(d) Comparison of the alarm behaviour elicited by extracts of venom sacs and whole stings

Introduction

Comparing single venom sacs with whole stings is unsatisfactory because the former have to be dissected under water causing possible losses of the pheromone, and both stings and venom sacs are subject to volatilization on being transferred from the wasp to the dental roll. Experiment (c) at Havant (p. 66) was an attempt to standardize the results obtained with stings, by using a fixed number of them macerated in a suitable solvent and taking a small aliquot of the resulting solution with which to impregnate the dental rolls. The experiments at Bassett and Winnall Moor were an extension of this idea.

Method

Forty fresh *vulgaris* stings were macerated in 500 microlitres (40 drops) of redistilled dichloromethane and kept in

a sealed, scrupulously clean glass vial in a freezer until required. Forth fresh *vulgaris* venom sacs (+venom glands) were given the same treatment. Black dental rolls were used for the experiments, which were carried out at Bassett and Winnall Moor. Ten presentations were made at each site using controls impregnated with 12.5 µl (one drop) of dichloromethane, the other rolls being treated with 12.5 µl (one drop) of either the whole sting extract or that made from venom sacs. The rolls were jerked vigorously in front of the nest entrance for two minutes.

Results

Test	Nest	Repli-cates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental rolls Treated	Control	Range	Signif-icance
Venom sac extract	Bassett	10	25	15	4	0-6	*
	Winnall	10	20	300+	200+	15-40+	
Whole sting extract	Bassett	10	25	10	3	0-4	NS
	Winnall	10	20	300+	250+	9-50+	

NS = not significant and * = $p < 0.05$ (χ^2)

Table 27. Number of hits evoked by dental rolls impregnated with extracts in dichloromethane of worker venom sacs or whole stings.

The experiments at Bassett were carried out on 11th September, which was warm and dry but with a variable wind, fresh to strong at times, causing the dental rolls to blow about. In contrast the Winnall experiments were carried out on 30th October which was mild and overcast with drizzle and a light SE wind. At Winnall the attacks were so intense that it became impossible to keep count, as a result of which the totals given in Table 27 are approximate and cannot be statistically analysed. SIRs occurred in large numbers during both experiments, but more particularly at the Winnall Moor nest. The mean times for first hit at Winnall were 25 seconds for venom sac extract and 27 seconds for sting extract, compared to 53 and 66 seconds respectively at Bassett, both the differences

between the 2 nests being significant at p = 0.05 (Mann-Whitney U test).

Conclusion

As a result of the strong wind at Bassett the wasps, though attracted to the dental rolls, rarely attacked, presumably because the threshold concentration for release of the stinging response was seldom reached. A meaningful comparison between the effects of the two extracts cannot therefore be made. For different reasons (i.e. the high frequency of attacks) this comparison is also precluded for the Winnall data.

The enhanced effect of the extracts at Winnall was probably due to a number of factors:

(i) The air was nearly still, facilitating non-turbulent diffusion of the pheromone and the uninterrupted perception of it by the wasps.

(ii) The unfamiliar odour of dichloromethane (used on both rolls) may have lowered the response threshold (see p. 42).

(iii) Diffusion of both the solvent and pheromone would have been retarded due to the high humidity, resulting in increased duration of the active space surround the rolls.

III.B.3 To investigate the possibility that worker heads contain some form of alarm pheromone or attractant

(a) Investigation of the response of workers to crushed heads

Introduction

Experiment 1(b) showed that, although worker heads appeared to contain no alarm pheromone, they caused some attraction when crushed and presented on black dental rolls. The purpose of this experiment was to investigate once more the reaction of workers to crushed heads.

Method

Wasps at the Moorside Road and Winnall Moor nests were decapitated as required, one worker head being crushed per dental roll. Untreated black rolls were used as controls, both being jerked at the nest entrance for 2 minutes.

Results

Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits		Range	Significance
			Treated	Control		
Moorside Rd. Winnall Moor	10 10	11) 10)	106	59	0-18	***

(*** = $p < 0.001$ χ^2 test)

Table 28. Number of hits evoked by dental rolls impregnated with crushed heads of worker wasps.

Reproductives were emerging during both experiments and the weather was changeable in each case. Only 10 of the hits were WARs and no WIRs were recorded. Numerous SIRs took place mainly at the treated roll.

Conclusion

The heads of *vulgaris* workers contain a substance or mixture of substances which appear to elicit a form of alarm response when reproductives are emerging.

(b) Reaction of workers to head extract

Introduction

This experiment was performed to see whether the substances present in worker heads elicited a more extreme reaction if their rate of evaporation was reduced. Single heads, as used in experiments 1(b) and 3(a) above, being very small and rather difficult to crush uniformly, may have given somewhat erroneous results.

Method

The heads of 15 workers were crushed and macerated in 0.5 ml of liquid paraffin, one drop of the extract being applied by means of a glass rod to the treated roll. The control roll was treated with a single drop of pure liquid paraffin. Eight presentations of two minutes each were made at Havant, the number of hits and SIRs being recorded.

Results

Test	Head extract
Nest	Havant
Replicates	8
Mean exit rate (wasps per 30 seconds)	27
Number of hits (treated)	0
Number of hits (control)	0
Number of SIRs (treated)	20
Number of SIRs (control)	7
Range	0-8
Significance	*

(* = $p < 0.05$, χ^2)

Table 29. Number of hits and SIRs evoked by dental rolls impregnated with extract of worker heads in liquid paraffin.

This experiment was performed before the emergence of reproductives. Although no attacks were recorded at either roll, numerous wasps showed interest and hovered close to both, significantly more being attracted to the treated roll. There were no WARs or WIRs.

Conclusion

These results indicate that some form of attractant does exist in the head but that its function is unlikely to be that of evoking an alarm response, unless the concentration of the extract was too low for the stinging response threshold to be reached.

III.B.4 To investigate the effect of other possible alarm pheromones on worker wasps

(a) Investigation of the effect of 2-heptanone on the behaviour of workers

Introduction

Among the social insects many alarm pheromones, including 2-heptanone, are interspecific. This compound, which occurs as an alarm pheromone in honeybees, as well as some myrmicine and dolichoderine ants, was tested for activity on wasps.

Method

Moving black dental rolls were used in two series of experiments at the Moorside road and Winnall Moor nest, 10 replicates being made at each site. The control rolls were untreated, others being impregnated with 12.5 µl (one drop) of 2-heptanone. A check experiment consisting of two untreated black rolls was alternated with the 2-heptanone presentations at Winnall.

Results

Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental roll. Treated	Control	Range	Signif-icance
Moorside	10	21)	34	44	0-5	NS
Winnall	10	23)				

(NS = not significant, χ^2 test)

Table 30. Number of hits evoked by dental rolls impregnated with 2-heptanone.

Check Experiment	Replicates	Number of hits
Winnall	10	24

Table 31. Number of hits evoked by untreated dental rolls.

No reproductives emerged during the experiments. Of the 78 hits 42 were WARs, directed equally at treated and control rolls. There were no WIRs and the wasps appeared to be repelled by the treated roll. Numerous SIRs occurred, mainly at the treated roll. In the check experiment there were 24 hits of which 23 were WARs. Numerous WIRs and SIRs occurred. Table 32 compares the total number of attacks on treated and control rolls in the 2-heptanone experiment at Winnall with the total number of hits in the check experiment.

Total Number of Hits		
2-heptanone experiment	check experiment	Significance
43	24	*

(* = $p < 0.05$ χ^2test)

Table 32. Comparison of number of hits evoked by untreated dental rolls and those impregnated with 2-heptanone.

Conclusion

In the 2-heptanone experiment more wasps attacked the control than the treated rolls, indicating that the odour of 2-heptanone had a somewhat repellent effect at close range. Comparison of the heptanone experiment at Winnall with the check experiment (Table 32) shows that the 2-heptanone incited the wasps to behave aggressively. The initial concentration of 2-heptanone at its source appeared to be such that it repelled wasps at close range, causing them to redirect their attacks to the control roll until the odour had dispersed.

(b) Investigation of the effect of low concentrations of 2-heptanone on the behaviour of workers

Introduction

The 2-heptanone used in the previous experiment was undiluted and seemed to repel wasps at close range. To test whether dilute solutions of 2-heptanone would have a different

effect, concentrations similar to those found in the mandibular glands of the honeybee *Apis mellifera* (Al Saad, Pers. comm.) were used.

Method

A solution of 2-heptanone in liquid paraffin was made up to a concentration of 0.005 nanolitres (nl) per μ litre (0.1 nl. per drop) and one drop was used to impregnate a black dental roll. The control roll was untreated. The rolls were jerked in front of the Moorside nest for two minutes per presentation. A control experiment using a black roll impregnated with one drop of liquid paraffin paired with a blank roll was alternated with the other experiment. Liquid paraffin was used as the solvent, as it is odourless and reduces the rate of evaporation of substances dissolved in it, ensuring that the small amount of 2-heptanone used in each presentation did not disappear too quickly.

Results

Test	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits		Range	Significance
			Treated	Control		
2-heptanone	10	11	8	5	0-1	NS
Paraffin	10	11	2	1	0-1	NS

NS = not significant (Binomial and χ^2 tests)

Table 33. Number of hits evoked by dental rolls impregnated with 2-heptanone diluted with liquid paraffin.

Reproductives were emerging during both experiments. The attacks during the control experiment were all WARs but 8 of those during the 2-heptanone presentations were SARs. Equal numbers of WIRs took place at the roll treated with 2-heptanone and its control, but fewer occurred in the check experiment. However WIRs took place at both rolls in the latter, but there were none in the 2-heptanone presentations. The two experiments were not significantly different at $p = 0.05$ (Fisher exact probability test). Some alarm behaviour was evident, more especially in the experiment using 2-heptanone, but generally the reaction by the wasps was limited.

Conclusion

2-heptanone, at a concentration of 0.1 nl per dental roll was ineffective at inducing an alarm response in wasps at the nest entrance.

(c) Investigation of the response of workers to 6-methyl-5-hepten-2-one

Introduction

Saslavasky and Ishay (1973) showed that 6-methyl-5-hepten-2-one induced alarm behaviour in the Oriental hornet *Vespa orientalis*. This compound is also known to be an alarm pheromone of certain ants.

Method

A black dental roll was impregnated with 20 µl (one drop) of 6-methyl-5-hepten-2-one in liquid paraffin (0.1 nl per drop) and was jerked in front of the nest together with a black control roll impregnated with one drop of liquid paraffin. Ten replicates were carried out at both the Moorside Road and the Winnall Moor nests.

Results

Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits Treated	Control	Range	Significance
Moorside	8	10	24	11	0-11	*
Winnall	10	25	74	47	1-17	*

(* = $p < 0.005$ χ^2 test)

Table 34. Number of hits evoked by dental rolls impregnated with 6-methyl-5-hepten-2-one diluted with liquid paraffin.

Reproductives were emerging at the Moorside Road nest but not at Winnall. No WARs took place at Winnall, but there were 4 at Moorside Road. No WIRs occurred in either place but there were many SIRs in approximately equal numbers on treated and control rolls. The control was attacked first on 6 occasions and wasps appeared to be repelled by the treated roll during the first half of each presentation.

Conclusion

6-methyl-5-hepten-2-one elicits alarm behaviour at the nest entrance, when used in low concentrations (0.1 nl per rolls) on black moving dental rolls, but some aggression appears to be redirected towards the control.

(d) Investigation of the effect of spiroacetals on workers

Introduction

Following the discovery by Francke *et al* (1978) of methyl-1, 6-dioxaspiro [4.5] decanes (spiroacetals) in the gasters of *V. vulgaris* and the subsequent chemical investigation carried out in this research in which these compounds were isolated from the venom sac (p. 92), samples of wasp spiroacetals were applied to dental rolls and presented at the entrance to a nest of *vulgaris* to see if they induced alarm behaviour.

Method

A sample of the main spiroacetal (E)-7-methyl-1, 6-dioxaspiro 4.5 decane found in *vulgaris* (synthesized by Franke) was diluted with re-distilled pentane such that a drop applied to a dental roll would contain about 40 ng, this amount being the average quantity normally present in the venom sac of a wasp. The experiment was carried out at the entrance to a subterranean colony of *Vespula vulgaris* at Hunworth, Norfolk on 9 September 1980, the techniques used being similar to those described above (p. 22). Two black dental rolls were used, the control being treated with 12.5 µl (1 drop) of pentane and the test roll with 12.5 µl of pentane plus spiroacetal. The rolls were jerked in front of the nest entrance for 2 minutes and any hits were recorded. A control experiment in which 2 untreated, black, moving dental rolls were presented, was interposed with replicates of the spiroacetal test. Ten replicates of each experiment were made. The synthetic spiroacetal solution contained traces of the (Z)-isomer and other impurities.

Results

The rate of exit of wasps was very low, averaging 5 per 30 seconds. There were no hits recorded in either experiment, and very few wasps were attracted to the rolls.

Conclusion

These results are inconclusive owing to the moribund state of the colony. Further tests on more active nests are required.

III.B.5 Investigation of the possibility that wasps distinguish their nestmates from foreign wasps by smell

Introduction

It is a well established fact that wasps of the same species but from different nests will fight if they happen to meet in the vicinity of the other's colony. To see whether this phenomenon is brought about by differences in smell i.e. some form of colony-specific odour, the following experiment was carried out.

Method

Ten *vulgaris* workers were caught at the Moorside Road nest and were killed immediately on dry ice in a vacuum flask. They were returned to the laboratory and extracted using redistilled pentane in a Soxhlet extraction apparatus for 24 hours, after which the solvent was evaporated and the wasps dried by placing them in a clean drying-oven overnight. The insects were then placed in a dry sealed vial to prevent absorption of extraneous odours until ready for use.

The experiment was carried out at the Moorside Road nest using the dried wasps together with live, freshly caught ones as controls. A dried wasp was removed from the vial by means of a clean dry forceps and was tied round the petiole with a piece of black cotton 25 cm long, the other end of which was affixed to one end of the cross piece of the bamboo cane. To the other end was fastened a similar length of cotton, onto which was tied a freshly-caught worker wasp. In order to cancel any bias brought about by movement of the live wasp the cane was jerked vigorously

up and down before the nest entrance, such that any movements made by the wasp were disguised by the grosser motion of the apparatus. Each presentation lasted two minutes, the same procedure being adopted as for the basic dental roll experiments (see p. 22).

Results

Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on Wasps	
		Dried	Live
8	10	2	0

Table 35. Number of hits evoked by dried and live worker wasps taken from the same colony.

Reproductives were emerging from the nest during this experiment. On all occasions but one, both dried and live wasps were ignored, no WARs, WIRs or SIRs being recorded. However, 2 hits occurred at the second presentation, during which the dried wasp was fiercely attacked by two different incoming workers. Each attack was swift and unhesitating, the worker accelerating towards the dried wasp and grasping it with all six legs, whilst making an attempt to fly away with the 'victim'. Throughout the remainder of the experiment the dried and live wasps were ignored by incoming and outgoing workers.

Conclusion

Although the results are statistically inconclusive it seems likely that, had more replicates been carried out, or if the experiment could have been repeated in the absence of reproductives, there would have been further attacks on the dried wasps, as Francke *et al* have reported numerous attacks on extracted worker wasps in similar experiments carried out in Germany (1978).

The two attacks on the dried wasps at the Moorside Road nest were fierce and swift and probably arose because the 'alien' insect came into the line of sight of approaching wasps within the 'odour recognition range'. Having the visual characteristics of a wasp, but lacking the colony-specific odour, the dried insect was

recognised, not as prey, but as an enemy and hence elicited an alarm response.

The fact that only two attacks occured in eight replicates can be explained by way of 4 reasons:

(i) wasps make small visual targets

(ii) the artificial movement of the wasps may have been too rapid so that, coupled with (i), this prevented all but the closest wasps from focussing on the insects

(iii) the dried wasp, being odourless, could presumably only be distinguished as an alien at very close range. If it had had an unfamiliar smell its chances of eliciting an attack would probably have increased

(iv) reproductives were leaving the nest.

It is unlikely that the live, control wasp would be attacked however many replicates were carried out, because even if the wasps were attracted by its unusual movement they would recognise its smell and ignore it.

Further research could be done using this approach e.g.:

(i) repeat the above experiment using a fresh, but dead wasp as control - negating the need for jerking the apparatus

(ii) if motionless wasps do not provoke attack repeat (i) but with slight movement

(iii) use fresh, dead wasps from a different colony (but same species) against fresh, dead wasps from the test colony

(iv) as (iii) but replace the control by dried, extracted wasp from the test colony

(v) as (i) but impregnate extracted wasp with a small amount of an odoriferous compound e.g. ethyl acetate

(vi) as (iii) but soak the foreign wasp in an extract of the test colony.

III.B.6 To investigate the relative importance of visual and chemical stimuli in the production of an alarm response

(a) The role of visual stimuli in eliciting alarm behaviour

(i) Bassett and Chandler's Ford Nests

Method

Two alternating experiments were carried out at Chandler's Ford and Bassett, in which untreated black dental rolls were presented with untreated white ones. In one series of replicates both rolls were rigidly affixed to the bamboo crosspiece by means of 25 cm lengths of wire; in the other, cotton was used. The rolls attached to wire were presented motionless at the colony entrance, whereas those suspended from thread were jerked continuously. In both experiments the black and white rolls were interchanged at every presentation.

Results

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental rolls Black	White	Significance
Movement	Ch.Ford	10	34	3	0	NS
	Bassett	10	42	0	0	
No movement	Ch.Ford	10	34	0	0	
	Bassett	10	42	0	0	

(NS = not significant, Binomial test)

Table 36. Number of hits evoked by black or white, moving or stationary dental rolls.

No reproductives were emerging during these experiments. There were no SARs, WARs or SIRs during the stationary presentations but some WIRs were recorded at the black roll. Movement of the rolls resulted in numerous SIRs at both sites and especially at the black rolls, and 3 SARs occurred at Chandler's Ford. One of these was a direct retaliatory response after a wasp had accidentally collided with the roll, and the other two happened at dusk when

the wasps appeared to be more aggressive. Some WIRs occurred at the moving black rolls.

Conclusion

The black dental rolls used at Chandler's Ford and Bassett did not usually evoke a stinging response, whether they were in motion or not, and the white ones never did. Observations showed, however, that, whereas the white rolls were generally ignored, the black ones, especially when moving, attracted numerous wasps.

(ii) Moorside Road and Winnall Moor nests

Method

Experiment (i) above was repeated at the Moorside Road and Winnall Moor nests late in the season, using the same apparatus viz. black and white dental rolls, but omitting the stationary replicates. This experiment was alternated with one using moving black dental rolls treated with liquid paraffin and 6-methyl-5-hepten-2-one.

Results

Test	Nest	Replicates	Mean exit rate (wasps per 30 seconds)	Number of hits on dental roll		Range	Significance
				Black	White		
Movement	Moorside Road	8	10	7	0	0-3	**
	Winnall Moor	10	25	138	1	1-44	***

(** = $p < 0.01$, Binomial test, *** = $p < 0.001$, χ^2)

Table 37. Number of hits evoked by moving black or white dental rolls.

Reproductives were emerging at the Moorside Road nest but not at Winnall Moor. The weather on both occasions (consecutive days) was similar, being overcast, humid and windless. Some WIRs were recorded at the black roll during the presentation at Moorside Road but not at Winnall Moor. At both sites there were numerous

SIRs especially at the black roll. There were no WARs at Winnall Moor but 4 of the hits at Moorside Road were WARs. Attacks at Winnall Moor were very intense and occasionally the rod and myself were attacked. The difference between the times of first attack at Moorside (average 80 seconds) and Winnall (average 40 seconds) is significant ($p < 0.05$, Mann-Whitney U test).

Conclusion

An explanation for these apparently contradictory results is that the presence of reproductives at the Moorside nest somehow raised the threshold of the attack response, whilst in the absence of males and females at Winnall Moor the wasps attacked owing to the prevailing weather conditions. Research on honeybees has shown that they are more likely to attack in the presence of strange odours (Free 1961) and wasps appear to be similar in this respect (experiments 3(d), 4(b) and (c)). During the presentations of black and white rolls at Winnall Moor the air was very calm and this presumably enabled the wasps to detect the scent of the researcher. Together with the sight of the moving black object this probably released the stinging response, which then escalated as alarm pheromones were released. The active space surrounding the site of the stinging would have been slow to clear owing to the high moisture content of the air.

(b) Relation between visual and olfactory stimuli

Introduction

Crushed gasters elicit a powerful attack response at the entrance to nests of *vulgaris*. In order to investigate the role of alarm pheromones in relation to the colour and movement of dental rolls some of the latter were treated with crushed gaster before presentation.

Method

Five experiments were performed to investigate the relationship between colour, movement and odour in the alarm response.

They were:

	Control Roll	Treated Roll
(i)	White: movement	White + crushed gaster: movement
(ii)	White: stationary	White + crushed gaster: stationary
(iii)	White + crushed gaster: movement	Black: movement
(iv)	Black: movement	Black + crushed gaster: movement
(v)	Black: Stationary	Black + crushed gaster: stationary

Whole gasters rather than stings were used, as the latter had elicited variable responses in previous tests (see p. 64), were difficult to remove and were prone to evaporation in transit to the nest. Gasters were also quicker to prepare. However, where both control and treated rolls were white it was necessary to pigment the former such that both looked similar on presentation. Body fluids from the gaster could not be used for fear of contamination, so the thorax was crushed onto both rolls. To ensure uniformity and comparability throughout, thoraces were also crushed on the black rolls.

Results (Table 38)

No reproductives were emerging during any of the experiments except for number (ii) at the Moorside Road nest.

Stationary white dental rolls provoked only 2 hits at Chandler's Ford and 4 at Moorside Road, in all cases the attacks being WARs. There were no SARs at either site, but the treated white rolls evoked WIRs and SIRs.

Moving white rolls produced an intensified response, with a significant number of hits on the treated rolls at both sites. At Chandler's Ford 11 of the hits were WARs, whereas all at Moorside Road were SARs. No WIRs occurred at either nest but SIRs

	Experimental details																			
	White dental rolls								Black dental rolls								Black and white dental rolls			
	Stationary				Moving				Stationary				Moving				Moving			
	Ch.Ford		Moorside		Ch.Ford		Moorside		Ch.Ford		Moorside		Ch.Ford		Bassett		Ch.Ford		Bassett	
Replicates	8		10		8		10		8		10		10		10		10		10	
Mean exit rate (wasps per 30 seconds)	36		15		36		21		36		21		29		33		29		33	
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	B	W	B	W
Number of hits	1	1	2	2	11	2	10	2	12	1	17	6	214	51	67	5	93	5	27	1
Range					0-4		0-7		0-5		0-7		5-35	0-16	0-18	0-2	0-20	0-2	0-9	
Significance	NS		NS		*		*		**		*		***		***		***		***	

Table 38. Results of experiments using black or white, moving or stationary dental rolls. T = test roll (impregnated with crushed gaster), C = control, B = untreated black roll, W = white roll impregnated with crushed gaster. NS = not significant (binomial test), * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ (χ^2 test).

were commonplace, especially at the treated roll.

Stationary black rolls produced a similar response to moving white ones, except that the treated rolls elicited WIRs at both sites. Most of the attacks were WARs, only 4 SARs occurring at Chandler's Ford and 2 at Moorside Road, all on the treated roll. More SIRs occurred at the treated rolls than at the controls.

Moving black rolls evoked the strongest response, all the hits recorded being SARs. There were no WIRs or WARs, but numerous SIRs took place, especially at the treated roll.

When white rolls treated with crushed gasters were presented with black untreated rolls, there were numerous hits on the latter, only 3 (at Chandler's Ford) being WARs. Numerous SIRs took place, mainly at the white rolls, and these also evoked some WIRs. The experiment at Bassett took place on a windy day and this reduced the number of attacks.

Conclusion

The intensity of alarm behaviour in *Vespula vulgaris* at the nest entrance depends upon the combination of 3 main factors:

(i) colour
(ii) movement
(iii) alarm pheromone

Thus, stationary white dental rolls without the pheromone are virtually ignored, whereas black, moving, gaster-impregnated ones evoke the most intense form of alarm behaviour i.e. Strong Attack Responses (SARs), with little prior investigation. Other combinations between these two extremes produce intermediate results; thus moving, white, gaster-impregnated rolls elicit few attacks and are comparable in this respect with stationary, black, gaster-impregnated ones.

Though the alarm pheromone produces a directional response when both treated and control roll are of the same colour, this is not the case when a white roll containing the pheromone is presented with an untreated black one, in which case the black one is attacked,

but more fiercely than if it had been presented with an untreated white roll (cf. expt. 6(a)(i)).

The weaker the stimulus, the more likely it is that the wasps will investigate at close quarters; thus, stationary black rolls, both with or without the alarm pheromone, stationary gaster-impregnated white ones and moving black rolls without the pheromone were all subjected to antennation (WIRs). However, stationary white rolls without the pheromone were largely ignored. Apart from these 3 main factors the behaviour of the wasps is modified by the following influences:

(i) the emergence of reproductives (eg. expt. 1(a))
(ii) the prevailing weather (eg. expt. 2(d))
(iii) the presence of strange odours (eg. expt. 2(d))

III.C. Alarm behaviour experiments at foraging sites

1. Introduction

The series of experiments detailed above examine the alarm behaviour of *Vespula vulgaris* at the nest entrance, where the response to alerting stimuli might be expected to be most pronounced. To see how worker wasps of both *V. vulgaris* and *V. germanica* behave when presented with alarming stimuli at communal feeding sites, the following series of experiments was carried out, using similar techniques to those employed in the experiments at the nest entrance.

2. General Methods

The site chosen for the experiments was the factory yard of Bendicks (Mayfair) Limited, Winnall, Winchester in the surroundings of large bins containing waste confectionary, (fig. 8 p. 29). A forty-gallon drum of stem ginger in syrup was used to attract wasps and honeybees and the experiments were performed where the concentration of insects was the greatest, i.e. above the surface of the ginger in the drum.

Using the basic technique described on p. 22 two black dental rolls were jerked above the ginger for a presentation time of two minutes, one of the rolls having been treated according to the experimental requirements, the other acting as a control. Fresh rolls were used at each presentation, ten replicates being made for each experiment.

Most of the experiments involved the use of 'nest odour' and 'control odour'. 'Nest odour' was obtained by setting up the following apparatus: A small suction pump was attached by a length of rubber tubing via a glass 'Y' piece of two boiling tubes, each containing 10 ml of redistilled pentane. Glass tubing drew air through the solvent, one long tube being pushed into a subterranean nest of *V. vulgaris*, the other ending in the open air some metres upwind of the nest entrance. Thus any odours present in the nest were drawn into the solvent of one of the tubes, whilst the other absorbed environmental odours other than those present in the colony. The pump was switched off after 24 hours, and the boiling tubes were removed to the laboratory where their contents were blown down to 1 µl by means of jets of nitrogen. The solutions were transferred to clean screwcap vials and were maintained in a freezer until needed. The experiments were carried out between 21-24 September 1979 inclusive.

3. General observations

Numerous wasps of both species (*vulgaris* and *germanica*) foraged at the bins. Largely workers, the wasps reached their greatest numbers by noon and were joined by a few honeybees for the warmest part of the day. Both *vulgaris* and *germanica* were present in more or less equal numbers, there being an average of 100 of each at maximum.

The weather at times was cool, windy and wet, and this tended to reduce the numbers of insects present at the ginger. Additionally it caused some of the wasps, more especially those which had gorged themselves with syrup, to become extremely torpid and occasionally incapable of sustained flight.

4. Experiments

(a) Investigation of the effect of crushed gasters on the behaviour of *germanica* and *vulgaris* workers feeding at ginger

Introduction

Crushed gasters on black dental rolls evoked a powerful alarm response in workers of *vulgaris* at the nest entrance (see experiment 1(b) p. 56). This experiment was an attempt to investigate the type of response elicited at foraging sites.

Method

Two experiments, each of ten replicates, were interposed throughout one day (21 September 1979), gasters of *germanica* being used in one and those of *vulgaris* in the other. The controls in both cases were untreated black dental rolls. Gasters were taken from live wasps, caught as required, and were crushed on to black dental rolls, as described on p. 56, the treated and control rolls then being jerked above the drum of ginger for 2 minutes. Any hits were recorded.

Results

Test	Replicates	Number of hits		Significance
		Treated	Control	
germanica gaster	10	5	2	NS
vulgaris gaster	10	2	0	NS

NS = not significant (Binomial test)

Table 39. Number of hits evoked by dental rolls impregnated with crushed gaster of *vulgaris* or *germanica* at the foraging site.

In both cases the dental rolls caused some confusion and occasionally drew the attention of a few wasps, but in general they were ignored. There were 5 WARs when dental rolls impregnated with crushed gaster of *germanica* were presented and 2 on the control, but with *vulgaris* gasters there were only 2 WARs in total. Wasps were considerably

less aggressive here than at the nest entrance. Of those attracted to the rolls but not attacking, most hovered a few inches from them with legs outstretched (SIRs) before flying off again. Some WIRs were also recorded.

Conclusion

Although 7 hits were recorded when crushed gasters of *germanica* were presented, the transient attacks and general lack of interest in the rolls indicate that alarm behaviour is minimal at a foraging site, and this conclusion is supported by the results obtained with gasters of *vulgaris*.

(b) Investigation of the effect of 'nest odour' on the behaviour of workers when presented with crushed gasters

Introduction

'Nest odour' was included with the dental rolls as they were presented at the foraging site, to see if alarm behaviour became more pronounced if wasps sensed the presence of a colony.

Method

Using the same apparatus and techniques as in the previous experiment the rolls were presented above the drum of ginger, this time with 25 µl (2 drops) of 'nest odour' in pentane impregnated on treated rolls.

Results

Test	Replicates	Number of hits Treated	Control	Significance
germanica gaster + nest odour	10	1	0	NS
vulgaris gaster + nest odour	10	0	0	

NS = not significant (Binomial test)

Table 40. Number of hits evoked by dental rolls impregnated with crushed gaster and 'nest odour' in pentane.

On one occasion, as a roll treated with crushed *germanica* gaster was presented at the drum of ginger, the treated roll was attacked, but only fleetingly (WAR). Otherwise there was little alarm behaviour evident in either experiment, except for the occasional wasp hovering close to a roll for a few seconds (WIRs).

Conclusion

The *vulgaris* nest odour used did not lower the threshold of attack on black dental rolls at the foraging site. There are four possible reasons for this:

(i) Recognition of the nest entrance may be a visual process rather than an olfactory one. The combined stimuli of 'presence of an intruder' and sight of the nest entrance may be necessary to trigger an attack.

(ii) The nest odour used was from a nest in Bassett. Nest odour may be colony specific, such that wasps from other colonies will not respond to it.

(iii) The concentration of the odour may not have been sufficient to evoke a response.

(iv) Wasps may be repelled by pentane.

(c) Investigation of the effect of pentane or 'control odour' on the behaviour of workers when presented with crushed gasters

Introduction

To control for the previous experiments in which nest odour was used on both dental rolls, 3 experiments were performed in which nest odour was replaced first by control odour i.e. volatiles present in the air some distance from the nest from which the nest odour was extracted, and then by pentane, the solvent in which the odours were dissolved.

Method

Two experiments, each of ten replicates, were interposed at the foraging site, using black, moving dental rolls. In the first experiment both rolls were treated with 25 µl (2 drops) of pentane. In both cases one roll was additionally treated with a

single crushed gaster of a freshly caught wasp. A third experiment using *germanica* gasters and control odour was carried out separately.

Results

Test substance	Replicates	Number of hits Treated	Control	Significance
vulgaris gaster + pentane	10	4	2	NS
vulgaris gaster + control odour	10	0	0	
germanica gaster + control odour	10	0	0	

NS = not significant (Binomial test)

Table 41. Number of hits evoked by dental rolls impregnated with crushed gaster and control odour or pentane.

In the case of the experiment using pentane six hits were recorded, four of which were on the roll impregnated with crushed *vulgaris* gaster. One of these attacks was made by a *germanica* worker, the others being too fast to identify. All attacks were transient (WARs) and there were a number of SIRs, characterised by approach upwind with outstretched legs, but largely the dental rolls were ignored. There were no attacks in the second or third experiments, though here again a few SIRs were noted. More wasps were attracted to the treated rolls than to the controls in all experiments.

Conclusion

Although some alarm behaviour was evident during these experiments the results were not statistically significant and the few attacks which did occur were transient. The wasps were not as aggressive as at the nest entrance and in general appeared to shun the dental rolls.

III.D. Chemical analysis of *vulgaris* venom

1. Introduction

The fieldwork on alarm behaviour described above, proves that an alarm pheromone is present in the venom of *Vespula vulgaris*. Samples of venom were analysed by means of a gas-liquid chromatograph coupled with a mass-spectrometer (GLC-MS) in an effort to identify components which might play a part in chemical communication.

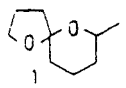
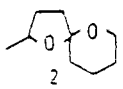
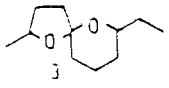
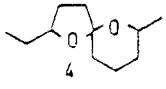
2. Methods

Stings were excised from fresh *vulgaris* workers by the method described on p. 58. Extracts were made using up to 50 whole stings macerated in re-distilled dichloromethane, whilst other stings were encapsulated in glass vials to be treated as solid samples (see p. 39). The venom sacs, with their venom glands, were dissected off some stings, to be encapsulated separately. Analysis was carried out using GLC-MS: the columns and conditions used are described on p. 38.

3. Results

(a) Venom sac and venom glands

The cross-scan report obtained from a solid sample of 2 venom sacs and their glands is shown in fig. 11 (p. 93). By programming the computer with the masses of certain major ions (base peaks) it was possible to locate probable spiroacetals. Spectra showing two base peaks separated by 3 mass units (e.g. 84, 87; 98, 101; 112, 115) are characteristic of spiroacetals. Four of these may be present in the venom sac and their structures are detailed below:

Spiroacetal	Base Peaks	Molecular weight
 1	84, 87	156
 2	98,101	156
 3	98,101	184
 4	112,115	184

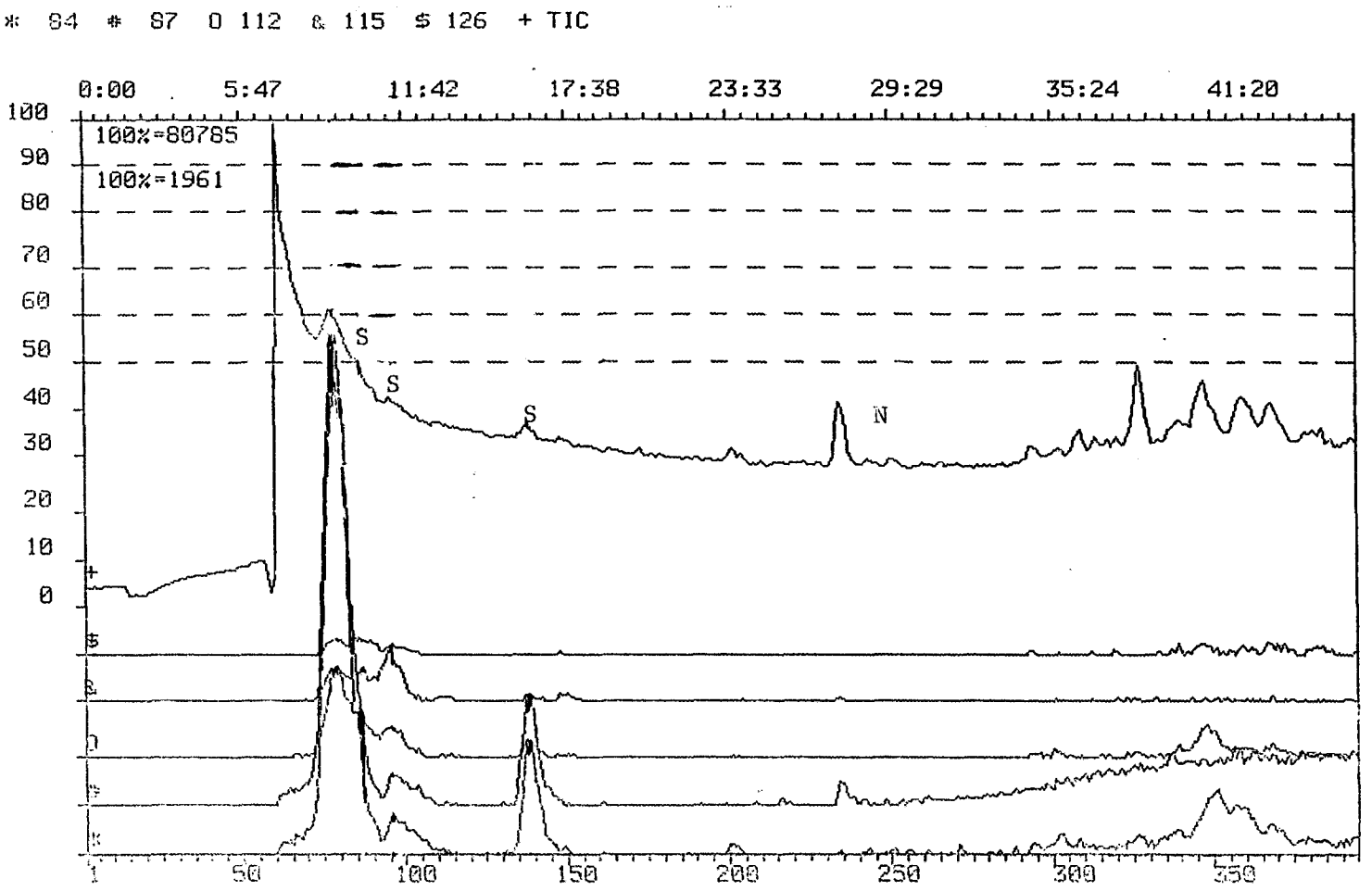


Fig. 11. Cross-scan report from GLC-MS analysis of 2 excised venom sacs (+ venom glands) from workers of *Vespula vulgaris*. Run conditions: column: OV101 (5%) Temperature program: initial temperature 50°C increasing at 8°C per minute

TIC = total ion count

84, 87, 112, 115 = base peaks of spiroacetals

N = N-3-methylbutylacetamide?

S = Spiroacetal (98,101 base peaks not shown)

The columns used in this investigation did not resolve the various spiroacetals and their diastereoisomers particularly well, but the structures detailed above were suggested by Francke (Personal communication) who analysed some of the mass-spectra obtained in this research.

Some peaks containing spiroacetals are marked on the cross-scan report in figure 11.

Another compound - N-3-methylbutylacetamide - may also be among the venom sac components. Its mass spectrum is shown in figure 71(p.234) and the peak which represents it is marked in figure 11.

(b) Whole stings

Two whole stings, treated in the same way, gave a similar cross-scan report and contained no new spiroacetals.

4. Discussion

The results of the venom analysis are discussed in the next section.

III.E. Discussion

The experiments described above have confirmed some of the findings of Maschwitz (1964) whilst bringing to light further complications in the alarm behaviour of social wasps. Owing to the scarcity of nests of *Vespula germanica* throughout the course of this research most of the following conclusions apply to *V. vulgaris*. Therefore, except when stated otherwise, the wasps referred to in this discussion are workers of *Vespula vulgaris*.

It is important in work of this nature to discuss the limitations imposed by the materials and techniques used, so that the data can be realistically analysed. Accordingly, the list which follows enumerates the major problems encountered and the restrictions which inevitably apply to the conclusions reached:

1. Colonies of *Vespula vulgaris* were rare in Hampshire in 1978, and this necessitated the use of one rather unsuitable nest (West End) and another (Havant) inconveniently far from the laboratory, as a result of which, meaningful comparisons were occasionally difficult to make.

2. Colonies were at different stages of development and some were more vigorous than others.

3. Variability of the weather sometimes precluded unambiguous comparisons.

4. Variability among the wasps used in the experiments caused large differences in response.

The alarm behaviour of wasps can be conveniently categorized according to its intensity. The four categories recognised in this research are fully described on page 24, but a resume is given here.

The weakest form of alarm behaviour in wasps is characterized by their close approach to the alien object, such that it is within antennating distance, and this has been termed a Weak Investigatory Response (WIR). It is the characteristic response to weak stimuli, such as stationary black dental rolls with no alarm pheromone.

The Strong Investigatory Response (SIR) is elicited by combinations of more powerful stimuli, especially those involving rapid movement. Wasps engaged in a SIR characteristically hover up to 30 cm downwind of the alien object, but do not usually approach it closely.

The Weak Attack Response (WAR) is the next stage in the escalation of alarm behaviour and involves acceleration towards the alien object followed by a forceful impact and instantaneous flight. No biting takes place, and stinging, when it occurs, is transient. This behaviour accompanies the presentation of objects which, though combining the majority of stimuli which precipitate attacks, do not possess the most effective

blend e.g. stationary, gaster-impregnated black dental rolls or moving, gaster-impregnated white ones. Such behaviour is also seen in response to rolls impregnated with a repellent substance.

The most intense form of alarm behaviour is called the Strong Attack Response (SAR, figs. 6 and 7) and it occurs whenever the alien object combines the most powerful stimuli. Rapidly moving black dental rolls impregnated with stings guarantee such a response.

The experiments described above have shown that wasps do possess an alarm pheromone and that it emanates from the sting, but the actual site of secretion is still open to question. Whole stings usually provoked fierce attacks, whereas venom sacs did not (p. 64); but the effect of extracts was not so clear-cut: on one occasion whole stings macerated in liquid paraffin evoked a weak and deteriorating response (p. 66), on another both stings and venom sacs (in dichloromethane) elicited attacks too numerous to count (p. 67). Tests on individual glands were difficult to do and were subject to error brought about by excessive volatilization and contamination. This was especially true of Dufour's gland which is notoriously difficult to find during dissection (Edwards 1980), and of the venom glands which are extemely small and delicate. Nevertheless, from the results of all the experiments involving the sting or its component parts and extracts the following can be deduced:

1. The venom sac (and probably venom glands) contains a pheromone which releases the stinging response (Expts. 1(d), 2(b), 3(d)).

2. This pheromone probably has a relatively low Q/K value, resulting in a localised active space; hence it will elicit few attacks except from wasps at close range (Expts. 1(d), 2(b)).

3. Another part of the sting (probably the Dufour's gland) contains an attractant of higher Q/K value than the alarm pheromone, drawing wasps into the active space of the latter and causing an increased incidence of attack (Expts. 1(d), 3(d)).

These deductions can conveniently explain some of the apparent anomalies which occurred in the above experiments. Thus the effect of liquid paraffin on the sting extract it contained (expt. 2(c)) may have been to reduce the rate of volatilization of the attractant to such a degree that its active space was severely restricted.

The still, humid air prevailing during the presentation of dental rolls impregnated with extracts of stings and venom sacs at the Winnall Moor nest (expt. 3(d)) optimized the conditions necessary for the diffusion of the attractant and alarm pheromone. The added presence of the unfamiliar odours of dichloromethane and of the researcher would have been enough to initiate an attack in the case of rolls containing the venom sac extract, after which the alarm behaviour would have escalated as a result of subsequent stinging and release of attractants.

Multicomponent alarm pheromones probably exist in most highly social insects, as they form an efficient means of mass communication resulting in cooperative defence at the site of attack. A complex system of this sort, occurring in the African Weaver ant *Oecophylla longinoda*, was described by Bradshaw *et al* (1975). In this species, the alarm pheromone consists of 4 major components (see p. 43). There is no reason to believe that wasps should not also use such a system, but because of the relatively greater importance of visual cues in their alarm behaviour it will probably be less complex chemically or more easily overridden by other factors, such as colour and movement.

To date no purpose has been found for the contents of the Dufour's gland in wasps, although in queens of the social parasite *Dolichovespula arctica* (Rohwer), in which the gland is comparatively very large (Jeanne 1977), it may serve to produce an 'appeasement' pheromone or attractant (Edwards 1980) used to pacify the workers of the host colony. However, in this research the evidence suggests that among workers of *Vespula vulgaris* the contents of the Dufour's gland are involved in the

alarm response, as the excised glands seem to evoke general alarm (p. 60). Moreover, venom sacs alone produce a lesser effect than whole stings including the Dufour's gland (p. 64). The fact that the Dufour's gland opens into the sting chamber would tend to support this hypothesis. If the Dufour's gland is not the source of this pheromone it is difficult to suggest which other structure could be involved.

Other experiments described above gave evidence to suggest that crushed gasters acted as synergists when stings were presented (p. 63). This synergism may have been due to the odour of crushed gasters being unfamiliar and attracting wasps into the active space surrounding the sting. A similar, though less pronounced effect was encountered with thoraces (p. 56).

Crushed heads were also attractive (p.56) and at the Moorside and Winnall nests provoked attacks during the emergence of males and queens, a time when most other alarming stimuli were at their least effective (see below). Notwithstanding this anomaly, the results and observations suggest that some form of attractant, and possibly alarm pheromone, does exist in the heads of worker wasps. Though probably not as powerful as the pheromones associated with the sting, such a substance could be released (possibly from a mandibular gland) during the intense biting characteristic of SARs and would serve as an extra marker for focussing the aggression of other wasps.

The colour of the alien object presented at the nest entrace was very important, black dental rolls evoking alarm responses much more readily than white (p. 80). The latter elicited comparatively few attack responses even when impregnated with gaster and moving rapidly, whilst untreated stationary white rolls were ignored.

Movement was another important stimulus provoking attacks, but it was only effective in combination with other alarming stimuli. Thus moving black rolls with no alarm pheromone generally evoked few attacks (p. 80).

An unfamiliar odour, such as that of dichloromethane, produced additive effects when presented with other alarming stimuli and at times seemed to trigger attack responses in the absence of the alarm pheromone (expt. 6(a)). The two ketones, 6-methyl-5-hepten-2-one and 2-heptenone caused alarm, but not of the sort expected of a pheromone i.e. wasps tended to be repelled at first, expecially from the concentrated 2-heptanone, and redirected their attacks to the control roll (p. 72). Such repulsion has also been noted by Francke *et al* (1978) who treated extracted wasps with mixtures of spiroacetals (see p.76) and presented them at the nest entrance. The propensity to attack when in the presence of an unfamiliar odour is not confined to wasps: Free (1961) did experiments on honeybees using 'stingballs' which had previously been soaked in human sweat and found that they were stung more frequently than odourless ones. The intense attacks on dental rolls impregnated with venom sac extract at Winnall Moor (p. 67) probably have a similar explanation. Such results suggest that Saslavasky *et al* (1973) were wrong in concluding that certain synthetic ketones (including 6-methyl-5-hepten-2-one), which cause alarm in oriental hornets, must be alarm pheromones.

Extracted wasps evoked a stinging response even in the absence of the alarm pheromone. These results agree with those obtained by Francke *et al* (1978), whose experiment differed in that the wasps were held motionless at the nest entrance, and suggest that potential enemies are recognised by sight and their lack of 'colony-specific odour'. It is interesting to note that odourless wasps were attacked on sight in Francke's experiments and the present work (p. 77), but that motionless black dental rolls in this research were ignored (p. 82).

Alarm behaviour of *Vespula vulgaris* and *V. germanica* at the foraging site was very muted (p. 86). Although some investigatory responses were noted, most wasps foraging at the chocolate factory ignored the dental rolls presented to them, whilst others appeared to be repelled, and there were only a few WARs. The

presence of 'nest-odour' made no apparent difference (p.89), although it would have had to have been extracted from the nests of the wasps present for a true comparison to have been made. Free and Simpson (1968) suggested that foraging honeybees were repelled from flowers treated with their alarm pheromone simply because it was a 'reaction to a physiological stimulus outside its normal context ..', implying that the pheromone would act like any repellent chemical. This same explanation can be invoked to explain the behaviour of wasps at a foraging site, where the essential stimulus of 'nest recognition' is lacking. The fact that wasps, when foraging for the first time, can remember the visual parameters of the nest entrance accurately enough to return to the right place, suggests that this appreciation of the nest environs forms a crucial part of their alarm behaviour. In any event it is unlikely that wasps recognize their nest by smell, as many underground colonies lie at the end of long tunnels. The stimulus of 'nest-recognition' is of adaptive significance, as it ensures effective, 'altruistic' defence of the all-important wasp colony - the reproductive 'super-organism' (Wheeler, 1928) - with comparatively little loss of its sub-units - the wasps engaged in the attack. In contrast, a mass attack on an intruder at a foraging site would be a wasteful exercise, achieving little and resulting in the loss of numerous wasps, which might otherwise have flown away to avoid danger. Further research is necessary using wasps foraging at varying distances from the nest, before this problem can be effectively resolved.

The emergence of reproductives during many of the experiments at the nest entrance had the effect, in most instances, of drastically reducing the intensity of alarm behaviour of the workers. No males or queens were seen to react to any of the dental rolls presented, and, although the exodus of reproductives only occurred in the mornings, the workers' alarm response remained subdued throughout the day. As with certain ants when swarming, the activity at the nest entrance during the emergence of reproductives was heightened, though the rate of exit of workers did

not rise appreciably. After the majority of reproductives had left the nest, the alarm behaviour of the remaining workers returned to normal or even, in some cases, became more intense than before. These observations suggest that a pheromone, or combination of pheromones, is produced whilst the reproductives are in the nest and that it has, as one of its effects, the ability to raise the threshold of the attack response in worker wasps. Presumably this pheromone is produced by the virgin queens. Although the alarm response was generally less in the presence of males and queens this was not always the case. During the presentation of crushed heads at both the Winnall and Moorside nests there were numerous attacks, but in both cases the main exodus of reproductives was over, and their influence was waning. Why the presence of adult reproductives in the nest should have this subduing effect on the alarm behaviour of workers in unclear, but the 'super-organism' concept does provide an explanation: the ultimate purpose of the 'super-organism' is to reproduce itself and until it has done so it remains vulnerable to attack - hence the need for efficient colony defence. However, once the reproductives have emerged and are leaving the nest, the colony and remaining workers become redundant and defence is no longer necessary. The fact that alarm behaviour reverts again to normal once the males and queens have left the nest is not surprising if a pheromone is the cause of the altered behaviour.

The experiment with spiroacetals was inconclusive (p. 76), but it would seem unlikely that their function is one of repellency or aggression inhibition, as was suggested by Francke *et al* (1978), because the presence of these compounds in the venom sac has been indicated (p. 92). It is interesting to note that the size of these spiroacetals conforms to the predictions of Bossert and Wilson (1963) regarding the most likely structure of alarm pheromones, i.e. 5 to 10 carbon atoms and molecular weight of 100-200.

A new compound from the venom sac, N-3-methylbutylacetamide was indicated by means of GLC-MS (p. 94). This compound occurs in other insects, notably fruit-flies of the genus *Dacus*, in which it appears to act as a pheromone (Herbert 1982). Its function in wasps is unknown.

CHAPTER IV

ATTRACTANTS

IV.A. Introduction

A wasp foraging for carbohydrates has to be able to locate a prospective source with the minimum of effort if maximum efficiency is to be achieved, and to do this it requires a means of recognising the food. There are two possible means of preliminary recognition - sight and smell.

Wasps are undoubtedly attracted to their prey by visual stimuli (Kemper, 1962) and are especially adept at catching insects rendered conspicuous either by their movement or by a contrasting background. However, sources of carbohydrate may be located by smell in the first instance, as they are usually static and inconspicuous. Evidence for chemotaxis is forthcoming from a number of sources (Brian and Brian, 1952, Gaul 1952) including this research, in which foragers of *Vespula vulgaris* and *V. germanica*, as well as honeybees, were attracted in greater numbers to certain substances than to others, even though all were dispensed in identical containers designed to disguise their colours: moreover, on days when incoming wasps were upwind of the attractants, fewer were caught (see p.129).

IV.A.1 Attractants used in wasp control

The economic importance of social wasps is such that, in certain places at the height of the wasp season, some form of control is necessary. As a consequence of this, pest-control companies and entomologists have developed an interest in the formulation of baits capable of attracting wasps to traps. Except in the Western U.S.A., where populations of *Vespula pensylvanica* have been successfully controlled using synthetic attractants (see below), no truly effective baits for use against other species have been discovered. The main problem to overcome is that, to be of any use, an attractant must be able to compete effectively with the foodstuffs to which the wasps are already attracted. Also, it must be cheap, effective in small amounts and preferably not attractive to honeybees.

IV.A.2 Wasp attractants - an historical survey

The depredations of wasps in orchards and their intrusions into the home have encouraged householders throughout the ages to find some way to dispose of them. That they have been regarded as pests for a long time is evident from writings such as those of William Cowper (1731-1800) in which he compares the 'imprisonment' of retirement to the trapping of wasps: '... like bottled wasps upon a southern wall'. Jar and bottle traps, baited with attractive substances such as preserves and honey, have evidently been in use against wasps for centuries.

More recently, other attractive substances have been employed in traps. Ranging from a broth of honey and pollen (Nesterovodsky 1947) to fermenting beer or molasses in blue-bottle traps (Thomas 1960), they have all been fairly successful on a small scale, though of limited usefulness for widespread control. Only one species of social wasp, as mentioned above, has been successfully controlled in certain areas by means of attractants: *Vespula pensylvanica*, a pest of the Pacific northwest of the U.S.A., is attracted to certain synthetic compounds such as heptyl butyrate, octyl butyrate and 2,4-hexadienyl butyrate (Davis *et al* 1967, 1968, 1972), but only under specific conditions. These compounds are relatively unattractive to other species.

The incorporation of attractants with poisons has also been practised to good effect on a localised basis. Thus Heim (1893) suggested the spraying of honey and mercuric chloride on foraging sites of hornets, and Palmer-Jones and others (1949) coated wasp foraging sites with an 'artificial honey-dew' consisting of glucose and sucrose containing DDT and dextrin (to discourage honeybees).

The idea of mixing an attractive food with a slow-acting poison so that foragers return to the nest with it and kill the occupants, raises the possibility of wasp-control on a scale suitable for commercial premises and other places were the problem is too severe for the safe and effective use of the methods described

above. Rivnay and Bytinski-Salz (1949) achieved satisfactory results using meat incorporated with thallium sulphate or lead arsenate, and Jefkins (1961) devised an effective bait composed of ginger syrup, icing sugar and dieldrin. Known as 'Waspex', this bait was commercially prepared and dispensed in plastic containers at sites of heavy infestation by Rentokil Ltd. 'Waspex', which is still used by Rentokil today, now contains a different insecticide, iodofenphos, to comply with government legislation outlawing the use of dieldrin.

As with other baits, 'Waspex', though better than most, has severe limitations. In areas where the wasps are familiar with the odour of ginger, 'Waspex' competes effectively with existing food sources, but elsewhere it is often ignored and even where the bait does lure wasps in large numbers, the attractive principle (ginger oil) evaporates after a few days (Smith, Personal Communication). Perhaps the most important drawback, however, is that the fondant base, being hygroscopic, absorbs moisture and the resulting solution becomes highly attractive to honeybees: accidental poisoning of entire colonies has been recorded (Needham and Stevenson 1966). Ennik (1973) has shown that repellent, but otherwise highly efficient, insecticides can be used effectively against *Vespula pensylvanica* if the poison is encapsulated to mask its odour. Encapsulated diazinon, mixed with cat-food and dispensed in containers hanging from trees splashed with heptylbutyrate, caused abatement of wasps from infested areas of San Francisco Bay.

The search for a 'universal' wasp attractant is still going on, but there are many problems yet to be overcome, especially with regard to the behaviour of wasps while foraging. Meanwhile, possible attractants continue to be tested, generally with negative results: researchers at Rentokil Ltd., screened 72 aromatic compounds in one season without success (Rentokil Technical Report, 1963). Of all compounds so far investigated none is more attractive to the British species of wasp than ginger syrup.

IV.A.3 Ginger as a wasp attractant

(a) Introduction

By 1960 ginger syrup had been established by workers at Rentokil Ltd., to be the best wasp attractant available, and has been incorporated in their 'Waspex' bait ever since. The syrup is a by-product derived from the preservation of fresh stem-ginger in sugar solutions of varying strengths. The ginger, having been peeled and cut into chunks, is boiled in water for 2-3 hours, sugar is added and boiling continues for a further 2 hours. The mixture is then allowed to stand for 7 days, after which more sugar and water are added and it is re-boiled (Anon 1909). Preserved ginger is exported with its syrup in large drums or barrels and is used for making confectionery, whilst the aromatic syrup can be made into ginger beer, although frequently it is discarded by manufacturers who only need the rhizome.

It is largely due to manufacturers of confectionery and ginger marmalade that the attractiveness of ginger syrup to wasps is known. Discarded syrup, usually deposited with other waste in large receptacles outside the factory to await collection, is extremely attractive to wasps and honeybees, as it has a strong aroma, high sugar content and low viscosity.

(b) Ginger syrup used in wasp control

The attractive properties of ginger syrup have led to its use by Rentokil Ltd., in bait formulations (see above). The fondant base containing ginger syrup and insecticide is dispensed in plastic bait boxes which are roofed to reduce liquefaction by rain. A small wick of absorbent material placed in the bait allows any water which collects to siphon out. The bait boxes are placed at frequent intervals around the perimeter fence of the premises to be protected and at all possible access points to the building.

Unfortunately, baiting with 'Waspex' is beset by various problems, some of which have been enumerated above (p.105), so although ginger syrup is undoubtedly a good wasp attractant, under certain circumstances its qualitites are offset by extenuating conditions.

(c) The chemistry of ginger

The attractive principle in ginger syrup is the essential oil it contains (see p.125) but, compared to fresh ginger, the percentage in the syrup is very low:

Yield from 45 litres of Chinese ginger syrup = 0.003% (This research p. 32).

Mean Yield from fresh Chinese ginger = 4.8% (various sources)

Nevertheless, what little oil is in the syrup is sufficient to attract wasps, often from considerable distances (see p.215). However, experiments carried out by Edwards and Smith (Personal Communication) indicated that after 3 days of exposure to air, ginger syrup dispensed in shallow petri dishes lost its attractiveness, suggesting that the bulk of the essential oil had evaporated.

Chemically, essential oil of ginger is an extremely complex mixture and recent investigations have suggested that it contains upwards of 600 identifiable components (Smith, Personal Communication) although some are only present in very small amounts. The following list contains some of the components of ginger oil identified to date and is taken from various sources (Guenther 1952, Pravatoroff 1967, Mathew *et al* 1973, Smith, Personal Communication, this research p.217). Figures in brackets denote the estimated yield from ginger oil (Mathew *et al* 1973).

α - bisabolene
borneol
bornyl acetate
camphene
cineole
chavicol
α - cubebene
α - curcumeme (17.7%)
para-cymone
decyl aldehyde
δ - elemene
γ - elemene
β - eudesmol
β - farnesene (9.8%)
geranial
humulene
limonene
linalool
methyl heptyl ketone
myrcene
nonyl aldehyde
α - phellandrene
γ - selinene
(-)-β-sesquiphellandrene
α - terpineol
α - Zingiberene) (35.6%)
β - Zingiberene)
Zingiberol

As well as essential oil, ginger contains certain pungent principles which are not very volatile and which give the spice its characteristic taste. Loosely called 'gingerin', this mixture of compounds consists largely of gingerol - an assortment of homologues - and shogaol, a phenyl ketone: it forms a relatively small proportion of the aromatic components of ginger syrup.

Preparation of extracts for this research involved the use of stem-ginger, ginger syrups of various types, tincture of ginger and ground ginger. Methods of preparation are described on pp. 28-30.

The composition of the various ginger formulations used in bioassays varied according to their sources and methods of extraction. Brief descriptions of each formulation follow, together with their gas-liquid chromatograms.

(i) Chinese ginger syrup (Bendicks) steam-distillate (fig. 12)

Compared with the solvent extract of the same syrup (fig. 13) the steam distillate contains a greater proportion of low-boiling volatiles and sesquiterpenes. Peaks 'p', 'q' and 'r' are artefacts originating from the tubing initially used for conveying the steam, and the polythene liners of the gingerdrums. Peak 'z' shows the main sesquiterpene constituents of ginger partially resolved into 4 peaks.

(ii) Chinese ginger syrup (Bendicks) solvent extract (fig. 13)

This chromatogram, run under identical conditions to the one in figure 12, shows a smaller proportion of zingiberene and associated sesqui- and monoterpenes. The extract also contains large amounts of high boiling components forming the oleoresin or gingerin, but these are not shown in this chromatogram.

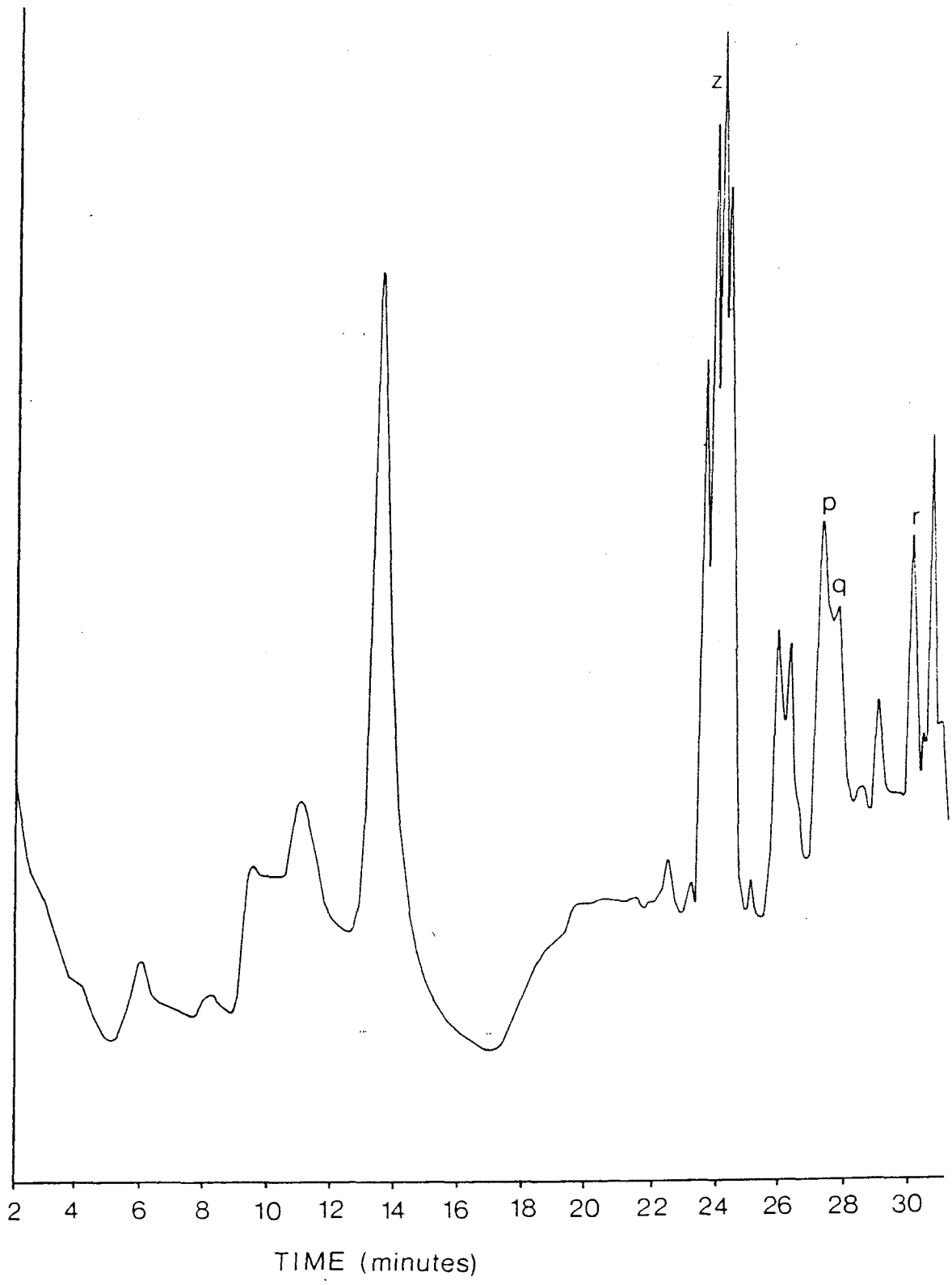


Fig. 12. Chromatogram of 2 µl of steam-distillate of Chinese ginger syrup (Bendicks) Ltd. The major sesquiterpene constituents occur in the multiple peak 'z'. Peaks 'p', 'q' and 'r' are artefacts.

Run conditions: Column: OV101 (5%)
Temperature Program: 80°C for 5 min.
2°C increase per min to 100°C; 80°C increase per min. to 250°C.
Attenuation: 80

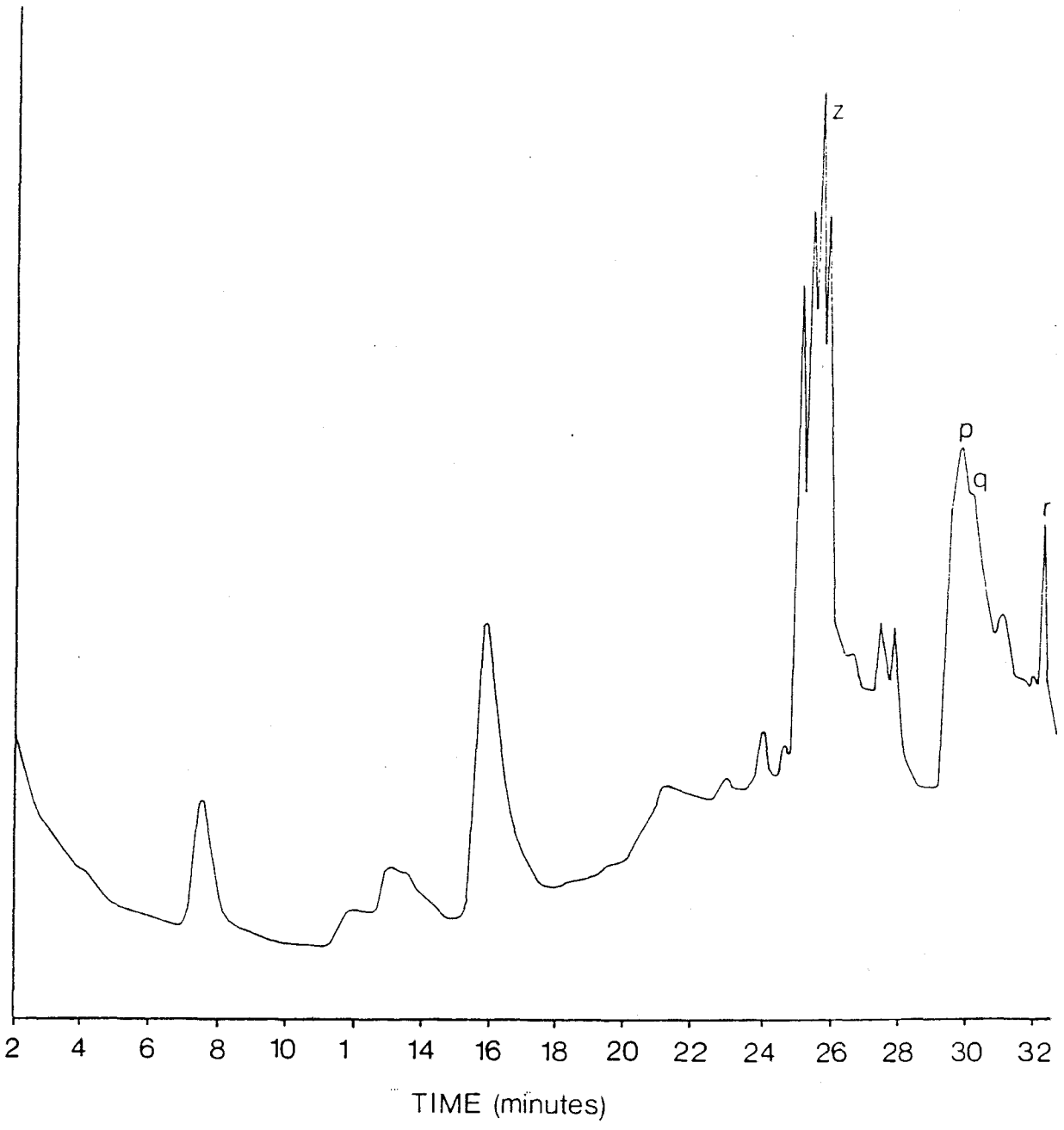


Fig. 13. Chromatogram of 2 µl of solvent extract of Chinese ginger syrup (Bendicks Ltd). The major sesquiterpene constituents occur in the multiple peak 'z'. Peaks 'p', 'q' and 'r' are artefacts.

Run conditions: as fig. 12.

(iii) Chinese ginger syrup (Bendicks) steam distillate (II) (fig. 14)

This chromatogram, run on a different programme to (i) above, shows the large multiple sesquiterpene peak 'z', this time with only 3 components visible. Peaks 'p', 'q', 'r' and 't' are plasticiser artefacts.

(iv) Chinese ginger syrup (Petty Wood) steam distillate (fig. 15)

Prepared under identical conditions to that in (iii), this distillate differs considerably from the Bendicks sample. Most noticeable is the greatly reduced triple sesquiterpene peak 'z' and the higher proportion of low boiling volatiles, indicating that the ginger had been boiled for a shorter time in the sugar solution. The peaks 'p' and 'r' are plasticiser artefacts derived from the steam-tubing.

(v) Australian ginger syrup (Petty Wood) steam distillate (fig. 16)

Prepared the same way as (iii) and (iv), this oil contains a higher percentage of low-boiling volatiles than either of them, but still considerably less sesquiterpenes than Bendicks syrup. Peaks 'p' and 'r' are plasticiser artefacts.

(vi) Ginger syrup (Rentokil, Kirkby: country of origin unknown) steam distillate (fig. 17)

This essential oil contains a very small proportion of the sesquiterpenes in peak 'z', but large amounts of low-boiling monoterpenes and other volatiles, indicating that, of the 4 syrups, this one was boiled the least. Peaks 'p' and 'r' are plasticiser artefacts.

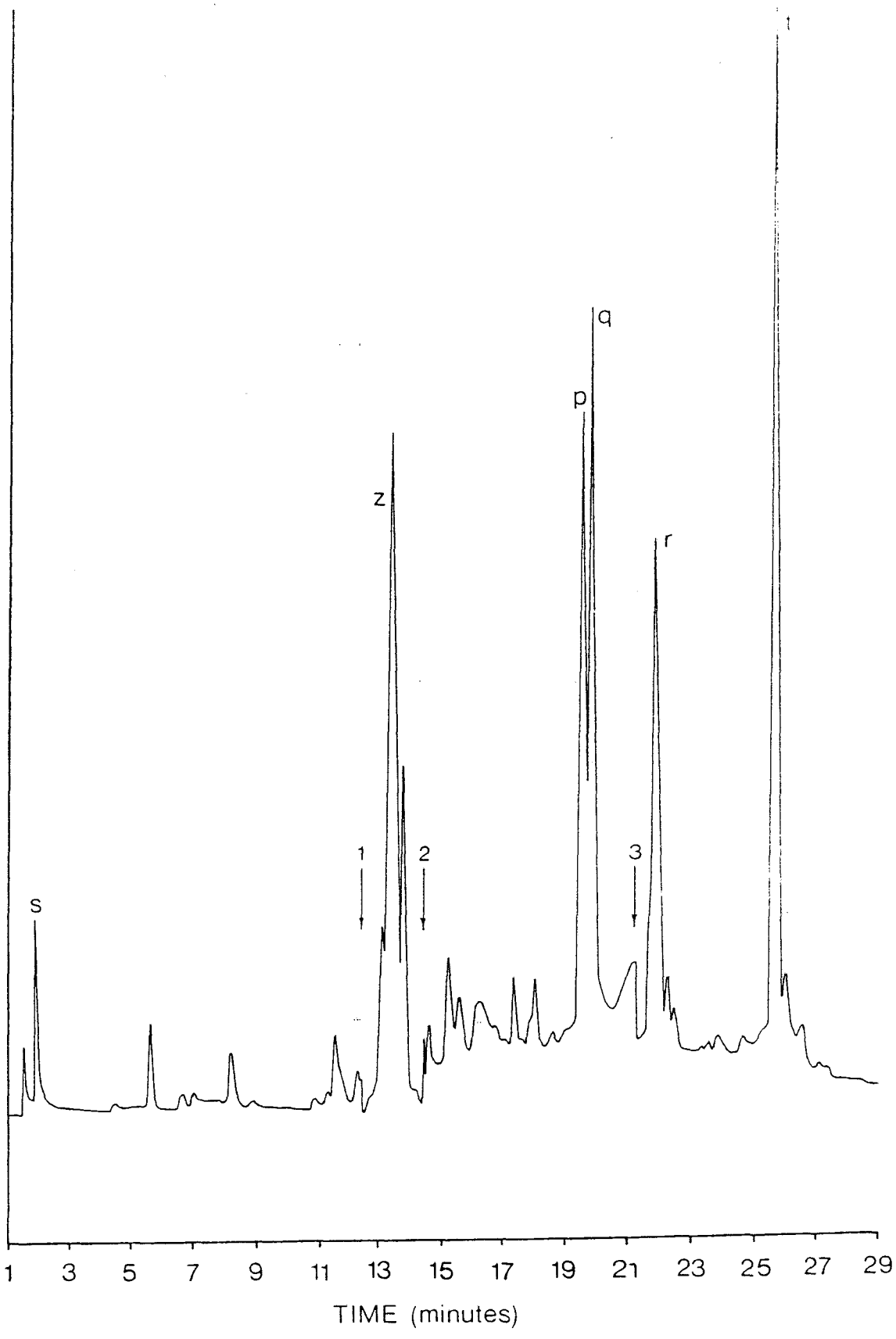


Fig. 14. Chromatogram of 1 µl of steam-distillate of Chinese ginger syrup (Bendicks Ltd.). Peaks 'p', 'q', 'r' and 'z' as in figs. 12 and 13. Peak t is an artefact. Peak s is solvent.

Run conditions: Column: OV101 (5%)
Temperature program: 80 - 300°C at 8°C per minute.

Attenuation: initial - 1,6000; 1 - 8,000; 2 - 1,600; 3 - 3,200.

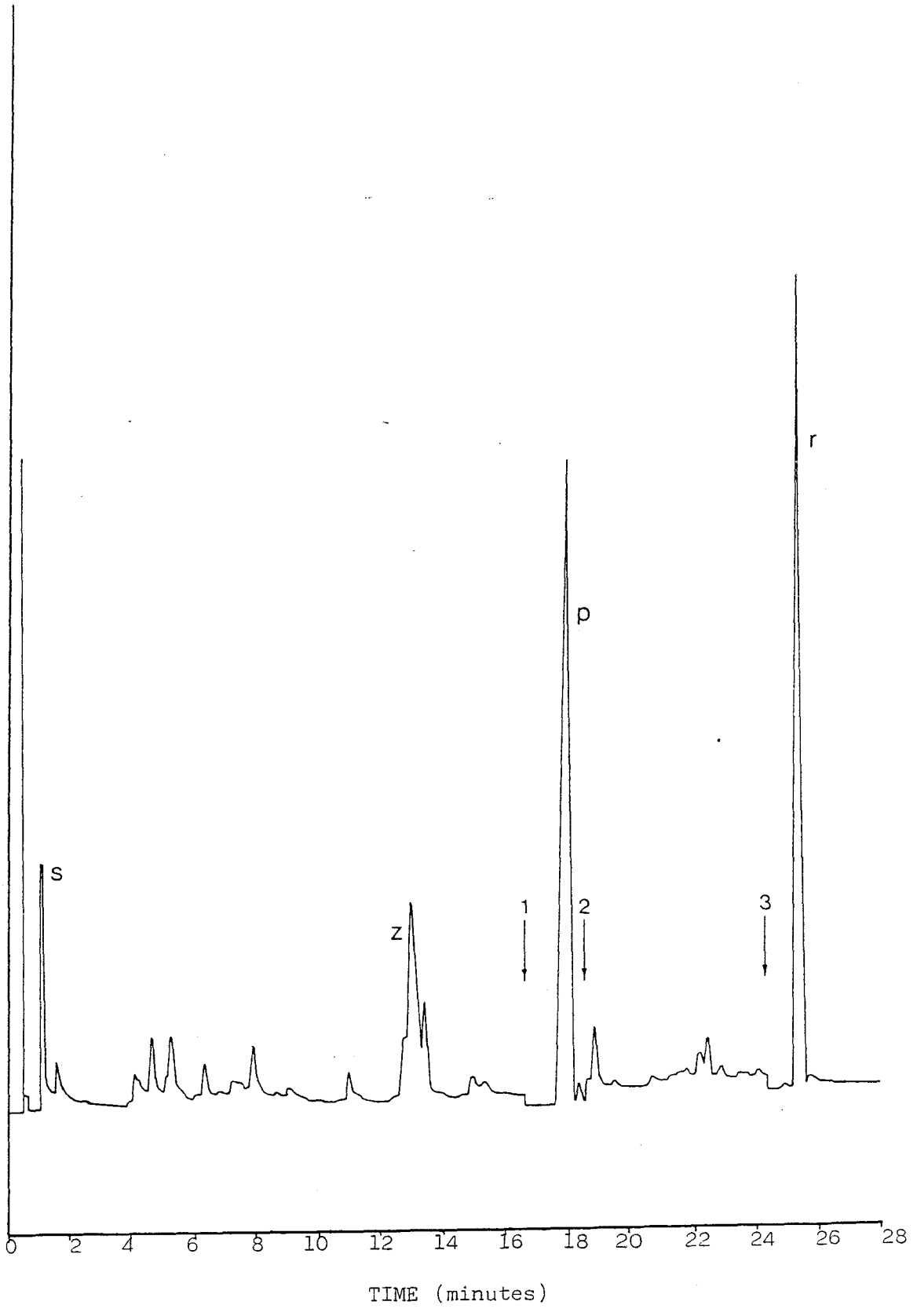


Fig. 15. Chromatogram of 1 µl of steam-distillate of Chinese ginger syrup (Petty, Wood Ltd.). Peaks as in previous figures.

Run conditions as fig. 14.

Attenuation: initial - 1,600; 1 - 32,000; 2 - 1,600; 3 - 3,200.

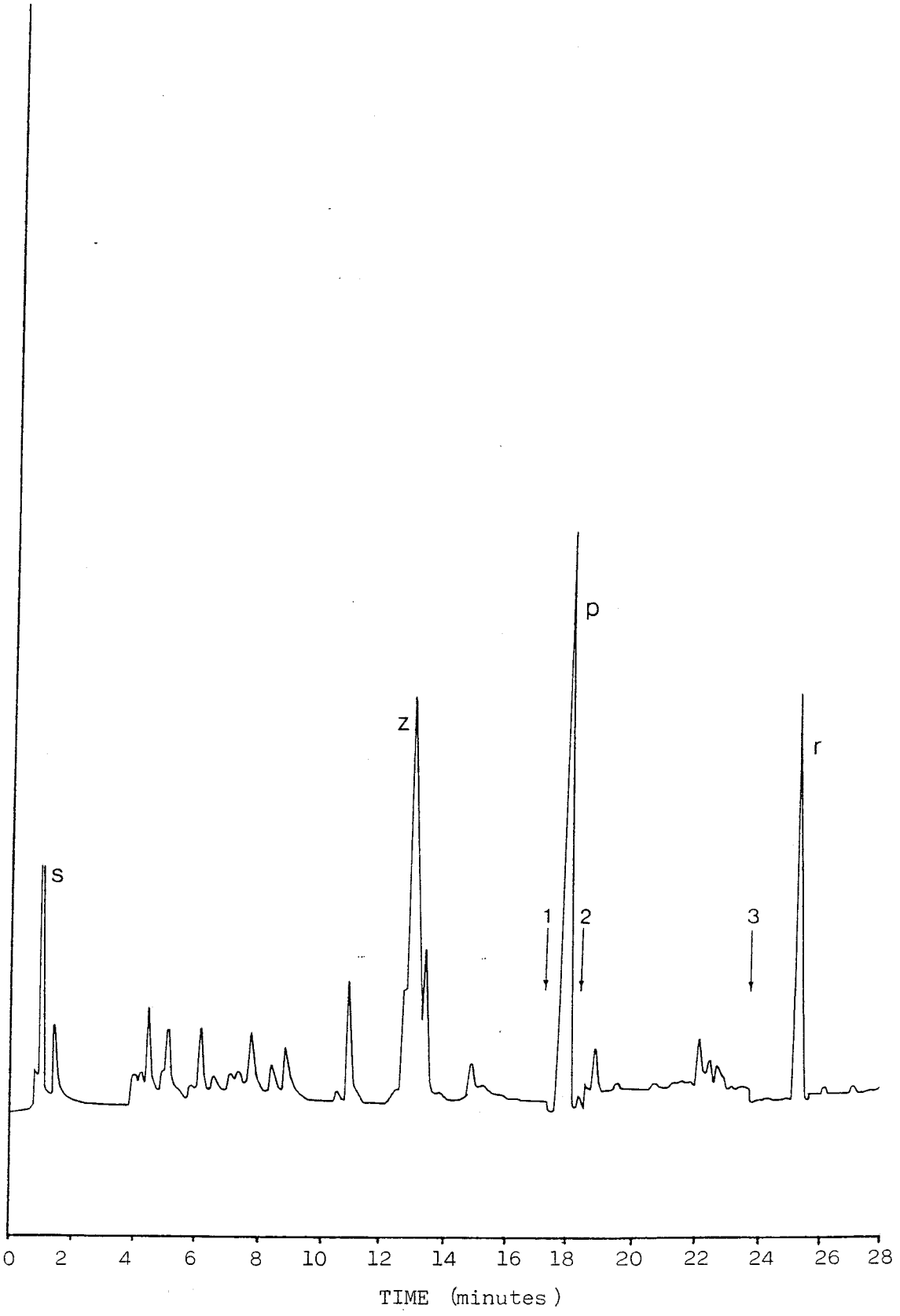


Fig. 16. Chromatogram of 1 µl of steam-distillate of Australian ginger syrup (Petty, Wood Ltd.). Peaks as in previous figures.

Run conditions as fig. 14.

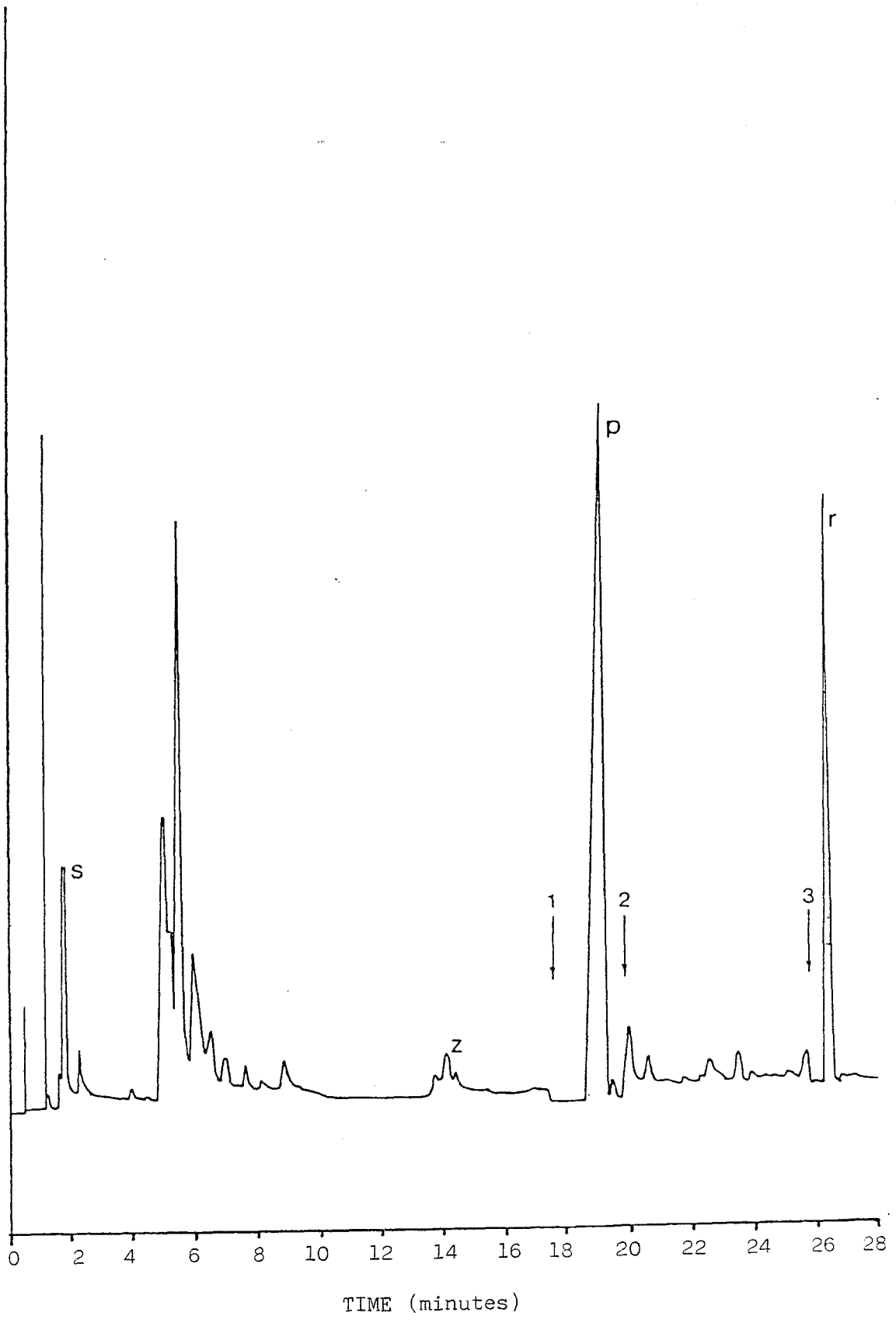


Fig. 17. Chromatogram of 1 µl of steam-distillate of ginger syrup (country of origin unknown; Rentokil Ltd.). Peaks and run conditions as in fig. 14.

(vii) Ground ginger: solvent extract (fig. 18)

Ground ginger is used to enhance the taste of various foods and consists mainly of the oleoresin ('gingerin'), most of the low boiling volatiles having evaporated. This is evident from the chromatogram, which shows a high proportion of sesquiterpenes, this time resolved into a quadruple peak 'z' which, however, differs in relative proportions to that in Bendicks ginger syrup (fig. 12). The high boiling oleoresin is represented mainly by peak 'o'.

(viii) Chinese ginger essential oil (Treatt: fig. 19)

This is commercially prepared essential oil steam distilled from fresh stem ginger. The triple sesquiterpene peak 'z' accounts for the largest part of the oil, but low boiling volatiles are also well represented, as with ginger syrup.

IV.B. Pilot surveys - Chilworth, 1977

IV.B.1 Introduction

Ginger syrup, a proven wasp attractant, was compared with other substances known to be attractive in order to gain a comparison on which to base further, more elaborate investigations. Initially the substances to be compared were dispensed in clear watch-glasses and set out on white ceramic tiles on bunsen-burner tripods at the chosen site. Six experiments were performed at the vespiary at Chilworth, either within the foraging arena (p. 18) or some distance from the free-foraging colonies, each experiment being an improved version of the one preceeding it. The table on pp.119-120 summarises these experiments and their results.

IV.B.2 Methods and results (see Table 42)

After experiments 2, 3 and 4 wasps were seen to return to the site to search for carbohydrates for up to 2 days.

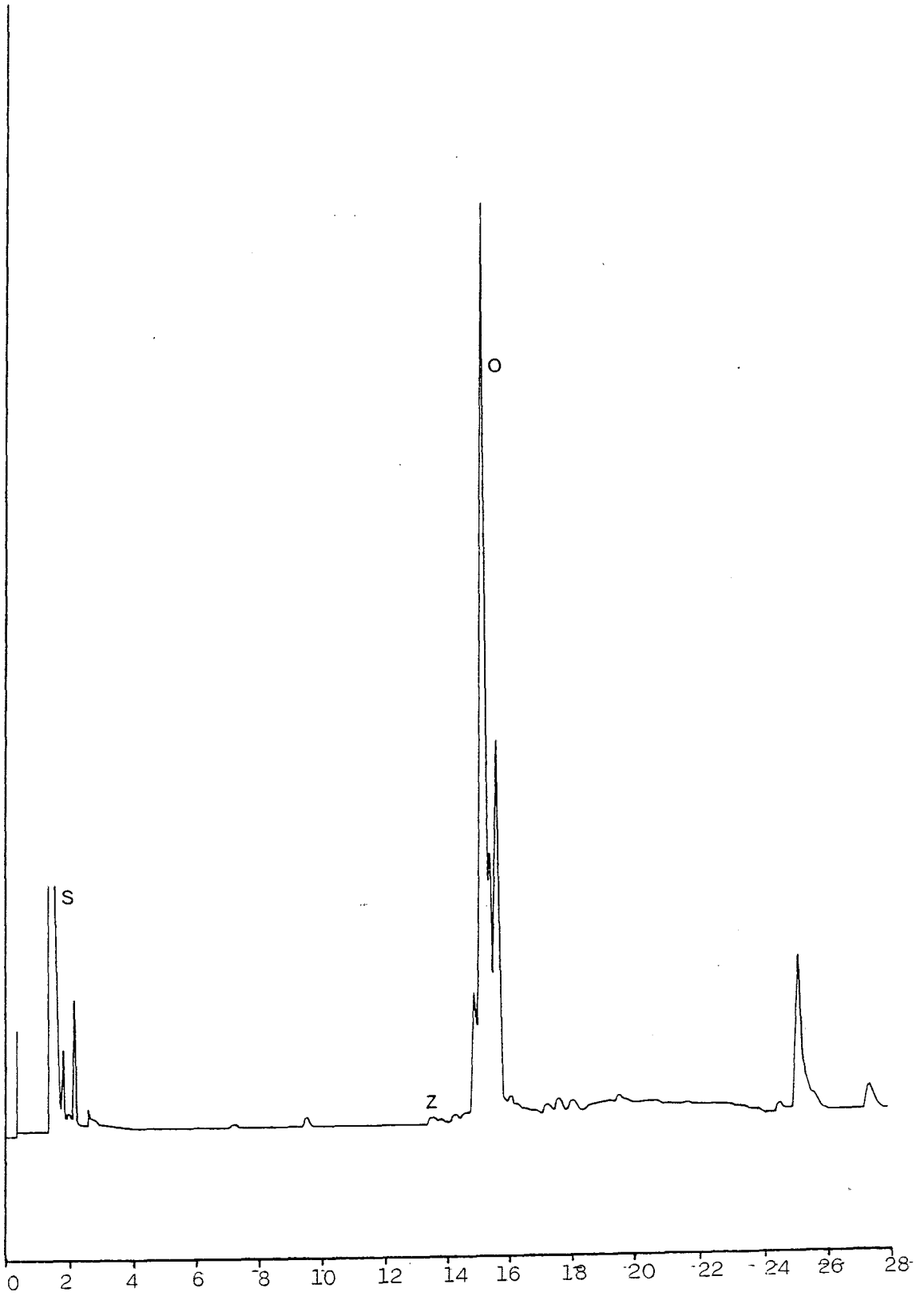


Fig. 18. Chromatogram of 1 µl extract of ground ginger. Peaks 's' and 'z' as in fig. 14. Peak 'O' is the major oleoresin component.

Run conditions: as in fig. 14.

Attenuation: 3,200

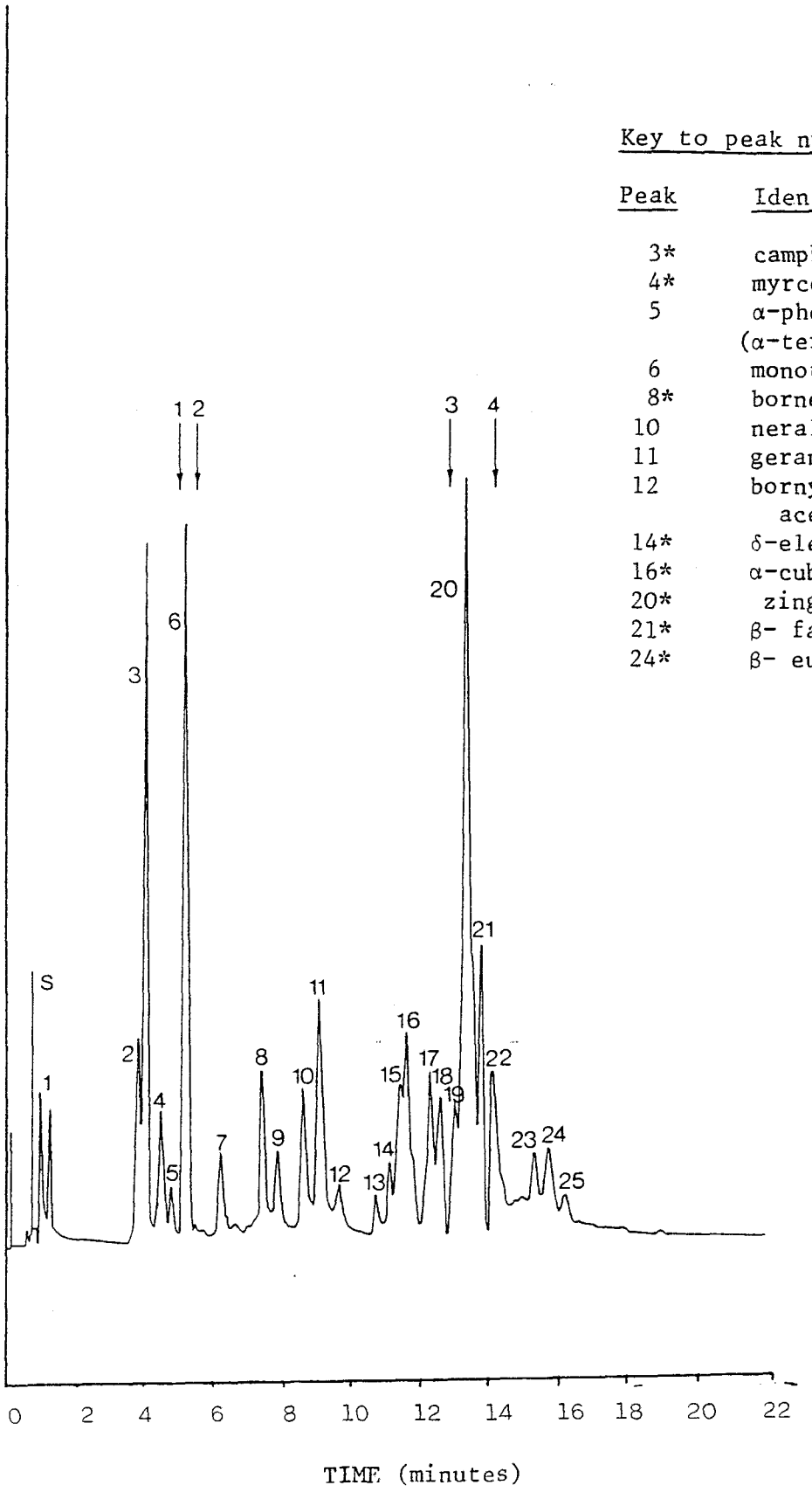


Fig. 19. Chromatogram of 1 µl essential oil of Chinese ginger (Treatt Ltd.).

Run conditions: as in fig. 14.

Attenuation: initial 1,6000; 1 - 3,200; 2 - 1,600; 3 - 16,000; 4 - 1,600.

Peak numbers marked * refer to mass spectra in Appendix C (p. 233).
See also p.217.

Table 42. Preliminary attractant experiments

Experiment	Comparisons	Experimental details	Results
1	honey, marmalade, water, blank, ginger + peppermint syrup, cat food, sucrose solution, ginger syrup, golden syrup.	a. watch-glass on tiles placed on tripods b. 1 foot apart in straight line c. in foraging arena d. observation time 2 hours	No visits
2	As experiment 1	a. as experiment 1 b. 1 metre apart c. beyond boundary hedge of vespiary d. observation time 5 1/4 hours e. each tile moved along one tripod every 15 mins. to cancel directional bias	Substance — No. of Visits: ginger + peppermint 37 sucrose 78 ginger syrup 30 honey 19 water 11 blank 1 marmalade 1 catfood 1 golden syrup 0 TOTAL: 168
3	Saturated sucrose solution: water 1:1; ginger syrup: water 1:10; blank; ginger & peppermint syrup: water 1:10 water; ginger syrup; sat. sucrose soln; ginger & pepper mint syrup; honey; ginger syrup: water 1:1	a. as experiment 1 b. as experiment 2 c. as experiment 2 d. observation time 3 ½ hours e. tiles not moved	ginger syrup 28 ginger syrup/H_2O 1:; 21 ginger & mint 10 sat. sucrose solution 6 ginger/mint H_2O 1:10 2 honey 2 water 1 sucrose soln/H_2O 1:1 0 ginger syrup/H_2O 1:10 0 blank 0 TOTAL: 70

Table 42. (Continued)

Experiments	Comparisons	Experimental Details	Results	
4	ginger & peppermint syrup; Ethanol; extract of ginger syrup in ethanol; blank; steam distillate of ginger syrup in ethanol, ginger & peppermint residue; tincture of ginger BPC; ginger residue; steam distillate of ginger & peppermint in ethanol; ginger syrup	a. 2ml glass vials on tripods with dental roll wicks attached to white ceramic tiles with grey plasticine b. ½ metre apart c. as experiment 2 d. observation time 2½ hours e. as experiment 3	ginger & peppermint syrup tincture of ginger ginger & peppermint residue ethanol ginger syrup- sol ext. steam dist. ginger residue blank ginger & mint dist. ginger syrup TOTAL:	42 23 21 15 8 8 8 4 1 1 131
5	ginger syrup; blank; ginger syrup-solvent extract; ethanol; tincture of ginger; ginger syrup-steam distillate	a. as experiment 4 b. as experiment 4 c. as experiment 2 d. observation time 3½ hours e. as experiment 3 f. vials wrapped in tissue to cancel visual bias & covered with cones of fly mesh to prevent feeding	ginger syrup ginger syrup-steam dist. tincture of ginger blank ginger syrup - solvent ext. ethanol TOTAL:	6 6 5 2 1 1 21
6	As experiment 5	a. as experiment 4 b. as experiment 4 c. 300m SE from vespiary near sycamore trees d. observation time 1½ hours e. as experiment 3 f. as experiment 5	No visits	

IV.B.3 Discussion

Experiment 1 was a failure because there were insufficient foraging wasps in the closed arena, owing to the moribund state of the attached colony. The few wasps in the cage were foraging for insects and honey, the latter being provided for them in a dish some distance from the experiments.

At the beginning of experiment 2, wasps were attracted to the watch-glass containing ginger syrup and for the first 15 minutes no other glass was visited. However, when the tiles had been moved in readiness for the next 15 minutes of observations, wasps returning to the position where formerly the ginger syrup had been, found the saturated surcrose solution and continued to visit this as well as the ginger syrup. After the next and subsequent moves of the tiles the wasps became increasingly confused, but began to search for the sucrose solution, to which they had become most attracted. The more viscous solutions (i.e. marmalade, golden syrup) proved to be much less attractive than those with a significant vapour pressure and catfood was virtually ignored, possibly because wasps foraging for carbohydrates tend to ignore protein (Free, 1970).

In Experiment 3 wasps attracted initially to the stronger ginger syrups remained faithfull to them, though, as seen from the results obtained previously, had they detected the saturated sucrose solution this would probably have been preferred. Saturated ginger syrup was the most attractive, followed closely by the 50% solution. Saturated ginger and peppermint syrup was considerably less attractive, whereas in the previous experiment more wasps collected this than the pure ginger syrup, possibly because, though the strong smell of peppermint may have been somewhat repellent, the sugar content of the syrup may have been higher. Saturated sucrose solution was even less attractive than the ginger and mint. Honey, water and the more dilute solutions of ginger syrup and sucrose were effectively unattractive.

For the first two and a quarter hours the saturated ginger syrup was attractive but thereafter this attractiveness waned. Similarly, saturated ginger and peppermint syrup was more attractive initially than in the last two and a quarter hours of observations. Conversely the 50% solution of ginger syrup attracted no wasps until $2\frac{1}{2}$ hours had elapsed, thereafter proving to be the most attractive of the remaining solutions. These observations can be explained by considering the rates of evaporation of the different solutions and their volatile constituents. Whereas the saturated ginger and mint syrups became gradually more viscous and their odours weaker in consequence, the weaker solutions lost water and became more attractive as their smell and sugar concentration became stronger.

In Experiment 4 Ginger and Peppermint syrup proved to be most attractive, followed by tincture of ginger and ginger and peppermint residue (the sugary remains left after extracting the volatiles from the syrup). Ethanol was more attractive than ginger syrup and the ginger extract and distillate. However, these results are misleading because the wasps had access to all the vials and hence returned to those containing sugar. The wick in the vial containing ginger syrup did not absorb the solution effectively, and thereby reduced the rate of evaporation of the volatiles such that wasps were not attracted. There was some indication that wasps were attracted to the vial containing tincture of ginger (sugarless) not just by its smell but by the colour it imparted to the wick.

Experiments 5 and 6 were adapted from 4 to cancel bias due to colour and taste. The results indicated that wasps were more attracted to ginger syrup, its steam-distillate and tincture of ginger, than to a solvent extract of ginger syrup, or ethanol. As no insects could reach the contents of the vials these results gave a more realistic impression of the numbers actually attracted: in previour experiments many of the observations referred to the same wasps returning again and again to obtain more food.

IV.C. Experiments with attractants at Bendicks (Mayfair) Limited, Winchester, and other sites, 1977-1979

IV.C.1 Introduction

Both *Vespula vulgaris* and *V. germanica*, together with honeybees, reach pest proportions in some localities if the conditions are suitable. Factories producing jams or confectionery on a regular basis provide sources of food which, in the course of a season, can attract wasps and honeybees in huge numbers from the surrounding area. Although firms located in cities tend to be free from honeybees, they can still have problems with wasps, as the latter, being omnivorous and capable of nesting with equal facility in holes in the ground or under roofs, are more cosmopolitan.

Bendicks (Mayfair) Limited, is a small modern factory manufacturing chocolates on the Winnall Trading Estate at Winchester in Hampshire. Built overlooking the city on the edge of Winchester Moor, the factory is plagued by wasps and honeybees in all but the worst summers and existing control measures are of comparatively little use. Waste materials, including unused fondant, sweepings from the factory floor and unsaleable chocolates are thrown into large bins near the perimeter fence outside the building (fig. 8) and it is to these that the insects are attracted, sometimes in very large numbers, especially if ginger syrup is amongst the rubbish.

In order to test the attractiveness of various substances, bioassays were set up in the factory yard during the summers of 1977, 1978 and 1979. Ginger syrup formed the basis of these investigations, being a proven wasp attractant whose constituents could be conveniently separated, analysed and tested for attractiveness.

IV.C.2 Experiments

(a) Relative attractiveness of ginger extracts to wasps familiar with ginger: Bendicks, 1977

(i) Comparison of the attractiveness of ginger syrup and its extracts (I)

Method

The apparatus consisted of 6 vials of attractant arranged under cones of nylon netting on white tiles in the factory yard at Bendicks (Mayfair) Ltd. The vials were filled as follows:

Vial + wick only (control)
Vial + wick + ethanol
Vial + wick + ginger syrup
Vial + wick + tincture of ginger BPC
Vial + wick + ethanolic solvent extract of ginger syrup
Vial + wick + ethanolic steam-distillate of ginger syrup

Preparation of the steam distillate and solvent extract is described on p. 30 and further details of the experiment appear on p. 28.

The experiment took place at Bendicks on 20-23 September 1977 over a total of 17 hours, the vials being arranged in the order:

ginger syrup	control	solvent extract	ethanol	tincture of ginger	steam distillate
A	B	C	D	E	F

Results

The results are displayed graphically in figures 20 and 21.

On 20 september 1977 the weather was sunny and warm with a fresh SE breeze. Fifty wasps were attracted to the vials, most of these (42) visiting the extracts of ginger syrup, the others being drawn to the ginger syrup and ethanol. Three honeybees were recorded, one each at the ginger extracts and one at the tincture.

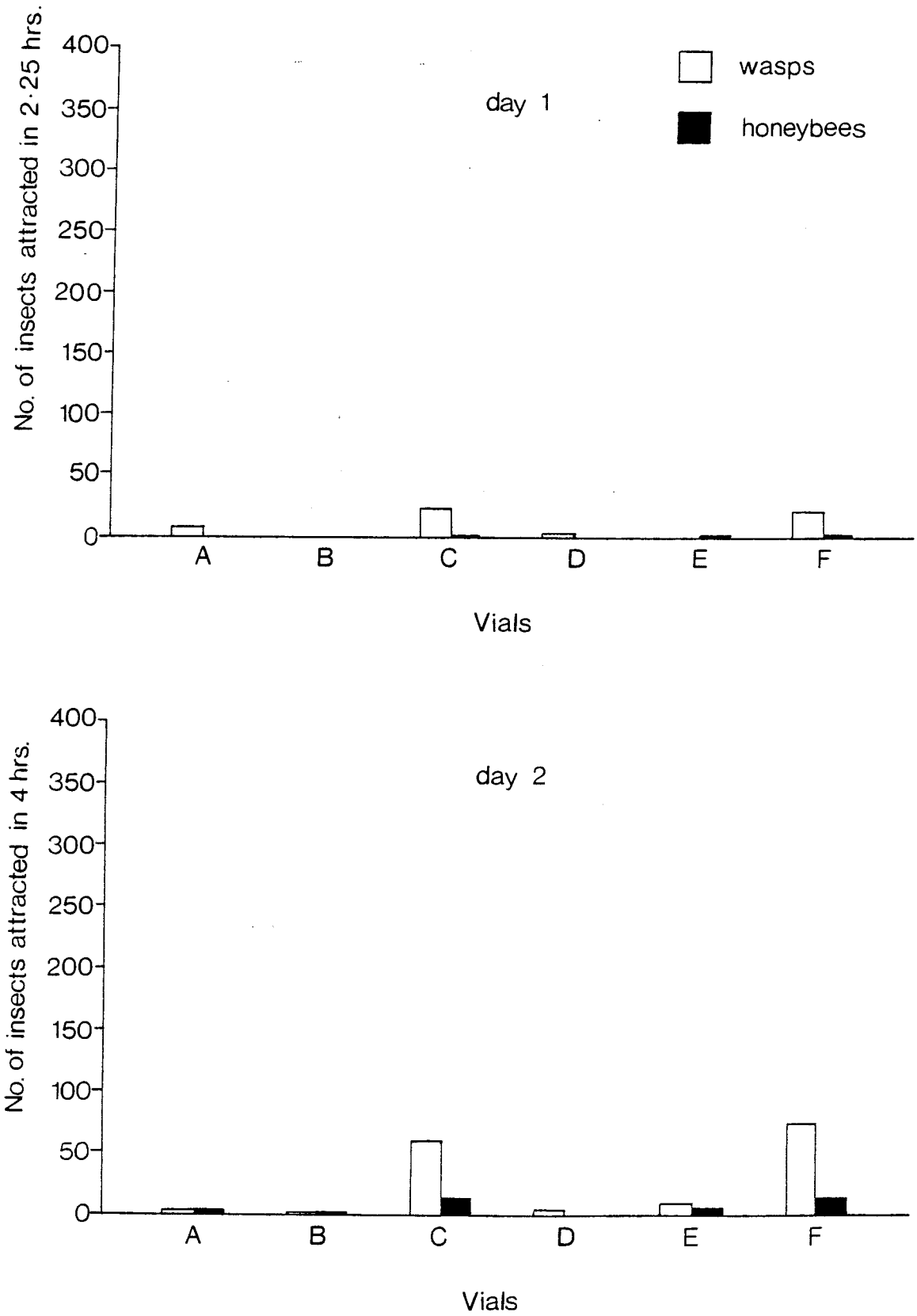


Fig. 20 Comparison of the attractiveness of ginger syrup and its extracts (I)

VIAL	CONTENTS
A	ginger syrup
B	control (wick only)
C	solvent extract of ginger syrup in ethanol
D	ethanol
E	tincture of ginger BPC
F	steam-distillate of ginger syrup in ethanol

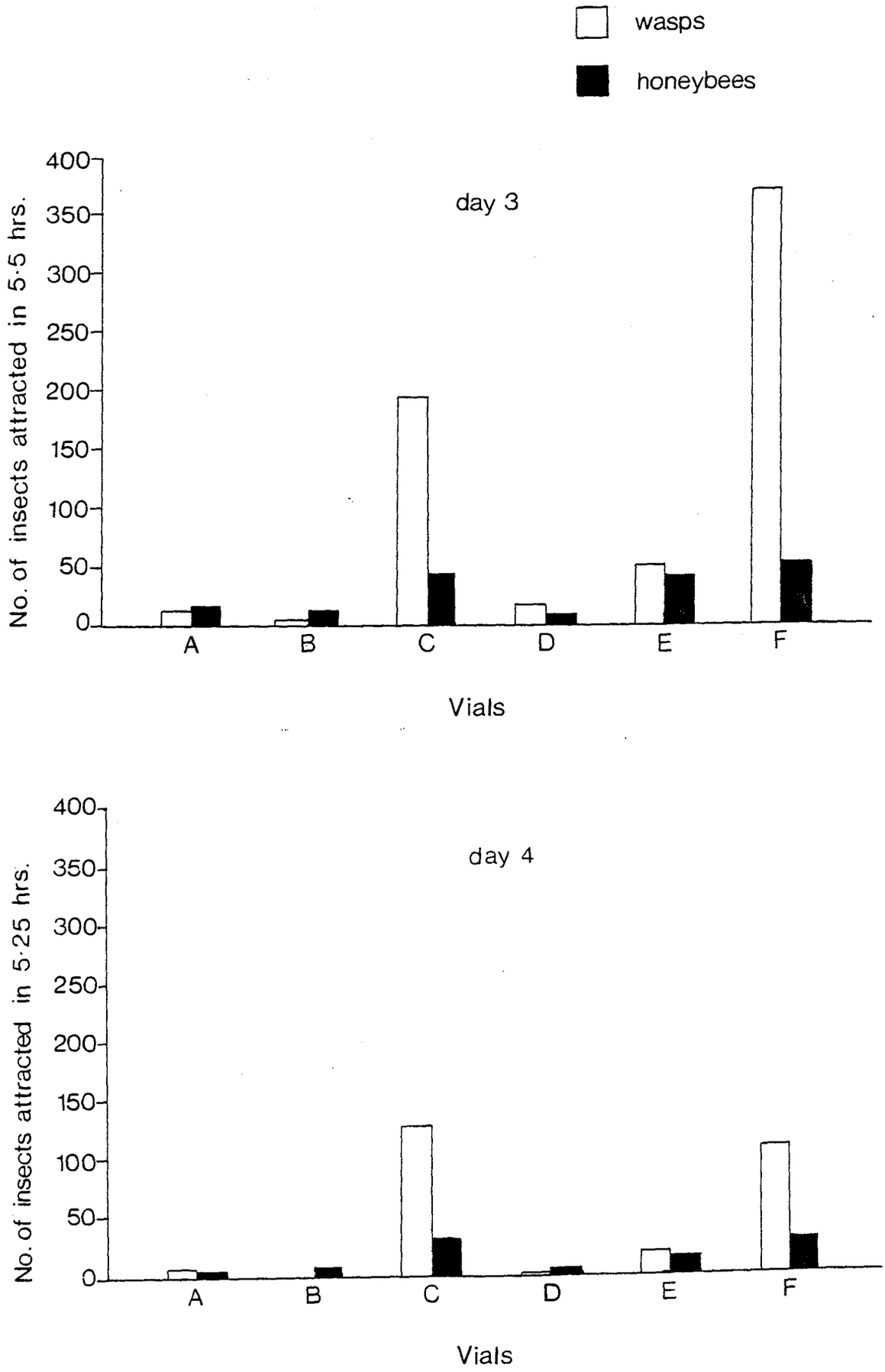


Fig. 21. Comparison of the attractiveness of ginger syrup and its extracts (I).

Vial contents as in fig. 20.

On the following day, recording took place for four hours, the number of wasps attracted to the vials being 146, three times as many as the day before, although the presentation time was less than twice as long. Ten times as many honeybees were attracted, most of these to the solvent extract and steam distillate. There is no significant difference (at $p = 0.05$) between the numbers of wasps visiting the solvent extract and the steam distillate, though between these and the other vials the difference is marked, 92% of the wasps being attracted to the two ginger syrup extracts ($p<0.001$).

On day three, observations were carried out for $5\frac{1}{2}$ hours. The weather was dull and cool, although brightening occasionally, with a light, westerly wind. There were many wasps and bees flying erratically around the large waste bins, which had been emptied that morning. Of the 637 wasps coming to the vials significantly more were attracted to the steam distillate than to the solvent extract ($p<0.001$, χ^2 test) and these numbers combined comprised 88% of all the wasps visiting the vials. Of the 151 honeybees attracted to the vials 48 came to the steam distillate and 36 and 37 to the solvent extract and tincture respectively.

On day four the weather was warm and sunny with a light easterly wind. Recording lasted five and quarter hours, during which 259 wasps and 79 honeybees were attracted to the vials. Of the wasps 91% were attracted to the ginger syrup extracts; there was no significant difference between the two. There were fewer wasps in the area than on the day before.

Discussion and Conclusions

The two extracts of ginger syrup were very attractive to wasps and, to a lesser extent, honeybees. The variation evident between the different days of the experiment indicates the importance of environmental factors on the response of bees and wasps to attractants. Disposition and design of the dispensers, coupled with availability of food and nature of the prevailing weather all contribute to affect this response. Preliminary observations had indicated that, had the vials been displayed at ground level, rather

than nearer the height of the flight-path, fewer wasps would have been attracted to them. The vast increase in the number of insects attracted on day three was due to the sudden decrease in available food as a result of the emptying of the large waste bins in the factory yard. Wasps and bees were having to search more thoroughly to find the depleted food sources, and this resulted in a build-up of insects, many of which were attracted to the powerful odour of ginger emanating from the vials.

The reduction in activity on day four was probably due to the presence of more food in the bins and the fact that many wasps would have learned that the attractants in the vials were unattainable. However, the reduction in activity was not as great as might have been expected. This can be explained by the fact that, on day four the wind was blowing from an easterly direction, towards the incoming wasps, whereas on day two the breeze was westerly, blowing the volatile consituents of the attractants away from them.

(ii) Comparison of the attractiveness of ginger syrup and its extracts (II)

Method

The apparatus was set up as in the previous experiment, this time excluding the tincture of ginger, an inferior attractant. Observations were made for $5\frac{1}{2}$ hours on day one and for 2 hours on day two.

Results

The results are depicted graphically in figure 22 (p. 130). Statistical analysis indicates that the results from both days can be combined ($p>0.05$, χ^2 test).

The control was visited by 14 wasps and the ethanol by 7. Ginger syrup attracted 11 compared with 161 to the solvent extract and 192 to the steam distillate. There was no significant differences between the distillate and the solvent extract (χ^2 test). Bees, by comparison, were far fewer, the totals being: 2 to the

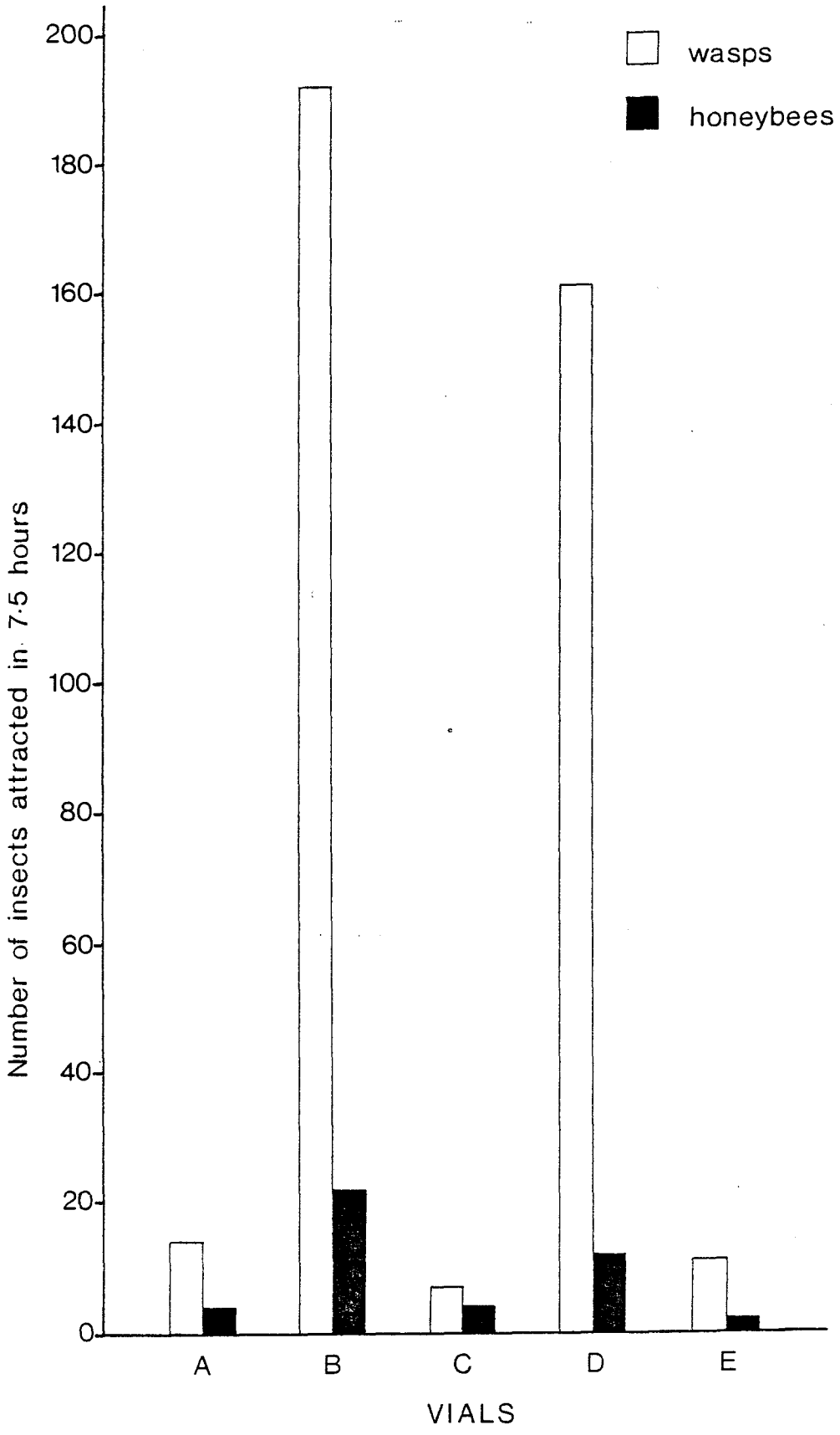


Fig. 22. Comparison of the attractiveness of ginger syrup and its extracts (II)

A - blank (control)
B - steam distillate
C - ethanol
D - solvent extract
E - ginger syrup

ginger syrup, 4 each to control and ethanol, 12 to the solvent extract and 22 to the steam distillate.

The weather during the two days was poor. On day one it was wet and cloudy, becoming sunny with showers: wind WNW, fresh to strong. On the second day weather was changeable with heavy and prolonged rain from 1300 onwards: wind WNW fresh.

Conclusion

This experiment, carried out almost a fortnight later than the previous one, produced similar results. The two extracts of ginger syrup attracted 92% of the wasps visiting the vials, ($p<0.001$, χ^2) and there was no significant difference between them. Ethanol, ginger syrup and the control were unattractive compared to the ginger extracts.

(iii) Comparison of the attractiveness of ginger syrup and its extracts (III)

Method

The apparatus was set up as before, this time with the addition of a vial containing a 50:50 mixture of steam distillate and solvent extract. Observations were continued for 1 and a quarter hours.

Results

The graph in figure 23 (p.132) portrays the results obtained. Of the 62 insects attracted, 3 were honeybees. Wasps were attracted in equal numbers to the vials containing solvent extract and the mixture of extracts, but fewer visited the steam distillate: this difference was not significant (χ^2 test). Ginger syrup, ethanol and the control were relatively unattractive. The experiment was curtailed due to heavy rain.

Conclusion

All three extracts of ginger syrup were attractive to wasps and there was no apparent synergistic effect gained by pooling solvent extract and distillate.

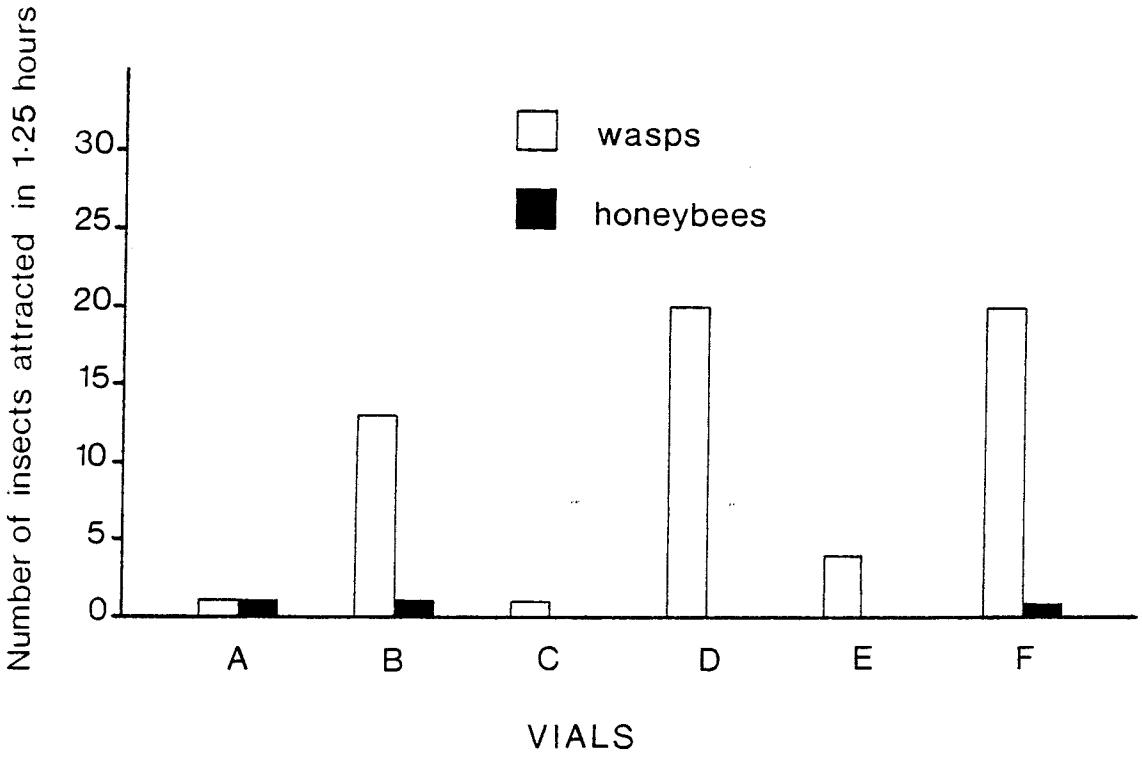


Fig. 23.

Comparison of the attractiveness of ginger syrup and its extracts (III)

A - blank (control)
B - steam distillate
C - ethanol
D - solvent extract
E - ginger syrup
F - solvent extract + distillate

(b) Relative attractiveness of ginger extracts and other attractants to wasps unfamiliar with ginger: various sites, 1978

(i) Determination of the relative attractiveness of ginger extracts to wasps and bees unfamiliar with ginger

Method

The experiment was set up as in the foregoing investigations, but the tripods were placed on a foraging table to which wasps and honeybees had been trained to come for some weeks; dishes of honey had been put out at regular intervals to encourage this. The experiment was carried out in the gardens of Chilworth Manor, Hampshire, where the insects were unfamiliar with the smell of ginger. The tripods were spaced at intervals of 1 foot and the vials were filled as follows:

A - Ethanol
B - Treatt's essential oil of Chinese ginger
C - Blank (control)
D - Extract of ground ginger
E - Steam distillate of ginger syrup

In order to ascertain what proportion of bees and wasps counted at the vials had already visited them, any insects alighting on the netting cones within the first 5 minutes of a 15 minute observation period were marked with paints of different colours (Humbrol enamel), the following 10 10 minutes of the period being used for counting visits. The experiment was continued for 4 hours.

Results

Figure 24 (p. 134) shows graphically the results obtained. Although there were numerous wasps in the vicinity, most ignored the vials and hunted for prey, especially towards late afternoon. Honeybees outnumbered the wasps, having learned to come to the honey usually put on the foraging table, though no recruitment took place during the experiment.

No wasps visited the empty, control vial and only 1 went to the ethanol. Four were attracted by the essential oil and 7 to the extract of ground ginger. The steam distillate attracted

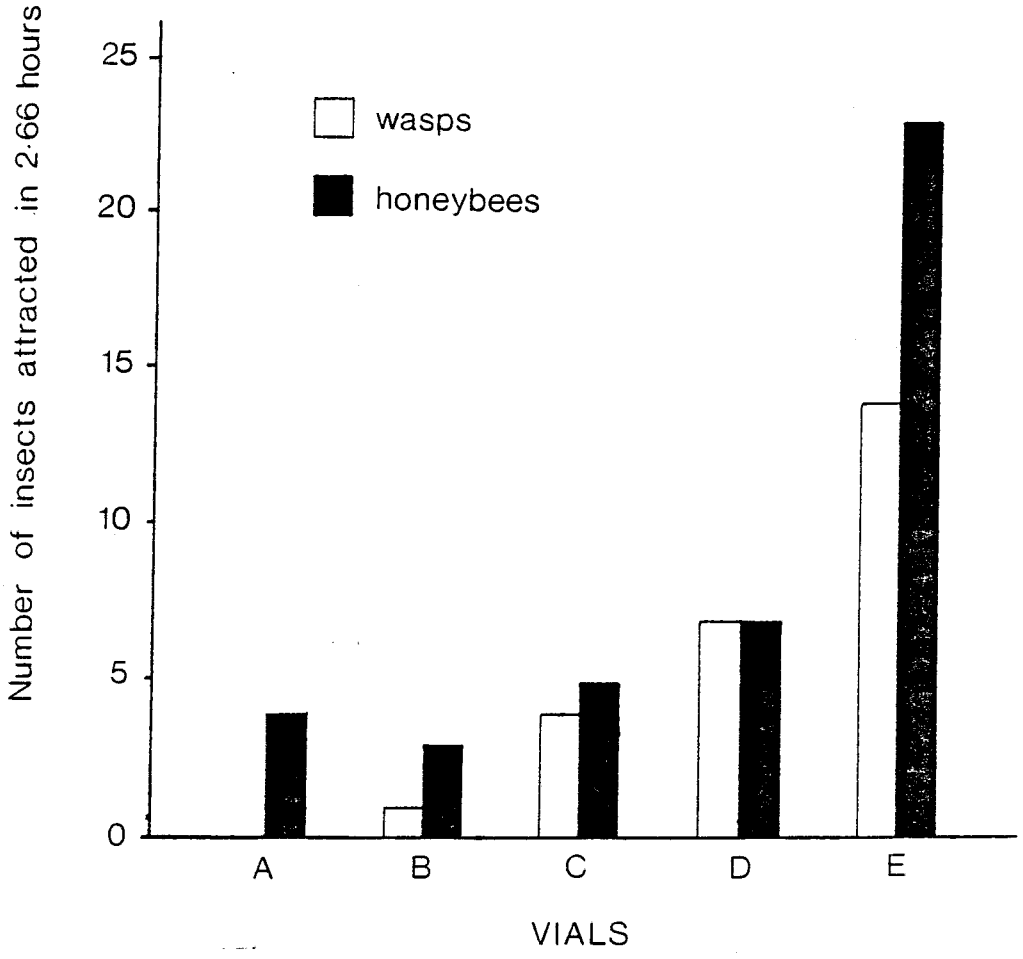


Fig. 24. Relative attractiveness of ginger extract to wasps and bees unfamiliar with ginger.

VIAL	CONTENTS
A	control (wick only)
B	ethanol
C	essential oil of ginger
D	extract of ground ginger
E	steam distillate of ginger syrup in ethanol

most (14), though this was not significant ($p > 0.05$, χ^2). During the marking phase two wasps were daubed with paint, neither returning thereafter to the vials.

Of the 42 honeybees visiting the vials, ethanol attracted the least (3), followed by the control (4), the essential oil (5), extract of ground ginger (7) and steam distillate (23), the last being significantly more attractive than the ground ginger ($p < 0.01$, χ^2). Four honeybees were marked and none returned.

Conclusions

Honeybees were more attracted to the steam distillate of ginger syrup than to any of the other attractants. The low number of wasps and bees attracted to the vials indicates that, where the smell of ginger is not associated with food, it is not exceptionally attractive.

(ii) Comparison of the attractiveness of various ginger formulations at different sites

Method

Five vials were filled as follows:

1. ethanol (control)
2. ginger syrup
3. extract of ground ginger
4. Treatt's essential oil of Chinese ginger
5. steam distillate of ginger syrup.

Each vial was placed on a white ceramic tile, as described above, the tiles being spaced at a distance of 23 inches (the diameter of 40-gallon drums used at Bendicks) on top of tripods. The tripods were placed at heights depending on the position of the food source at each site; thus at the orchard, where insects were attracted to windfall apples, the tripods were put at ground level, whereas at the chocolate factory and one of the bakeries the attractants were raised to the height of the surrounding waste receptacles.

The experiment was repeated twice at each site, using different arrangements of vials. Wasps and honeybees alighting on the vials during the first five minutes of each observation period were marked with paint, the next ten minutes being used for observation. The experiment was carried out in an apple orchard at Chilworth, Hampshire, at Bendicks (Mayfair) Limited of Winchester, and Rank Hovis MacDougall Mother's Pride Bakery (Manor Bakery) Eastleigh, Hampshire. Fresh vials and attractants were used for the presentations, each of which lasted 2 hours.

Results

The results are displayed graphically in figs. 25 and 26.

1. Bendicks. The experiment was carried out on 21 September and 20th October 1978. The weather on both days was calm and sunny with light breezes, although cloud increased on the second day to give overcast skies by 1700. Temperatures were high on the first day (22-23°C) and moderate on the second (15°C).

At the time of the first presentation honeybees outnumbered wasps by about 20:1, there being many fewer wasps this year than on the same date in 1977. By the time of the second presentation wasps had increased in number but the honeybees had disappeared.

On the first day, the waste bins had just been emptied and there were many honeybees looking for food.

As the results on the two separate days differ considerably they cannot be combined ($p< 0.05$, χ^2). Taking the first day's results alone, of the 12 wasps attracted to the vials, 6 came to the ginger syrup and 4 to the steam distillate, the other 2 being lured by the essential oil. Of the 343 honeybees attracted, 233 visited the steam distillate compared to 70 at the essential oil, this difference being highly significant ($p< 0.001$ χ^2 two sample test). Twenty-seven bees visited the extract of ground ginger, this being significantly less attractive than the essential oil

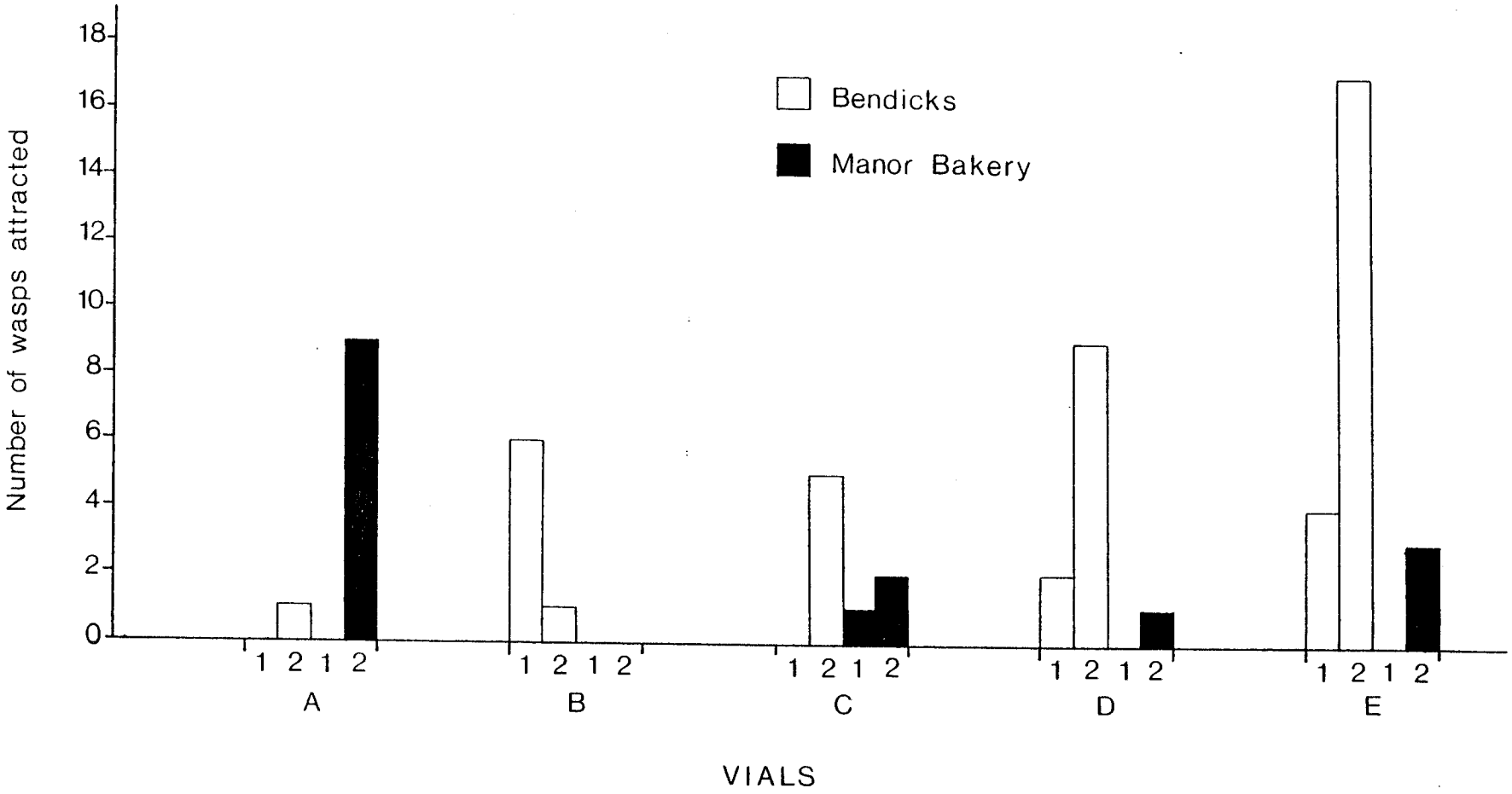


Fig.25. Comparative attractiveness of ginger extracts at different sites.

1 = day one. 2 = day two. A = ethanol (control) B = ginger syrup
C = extract of ground ginger D = essential oil E = steam distillate

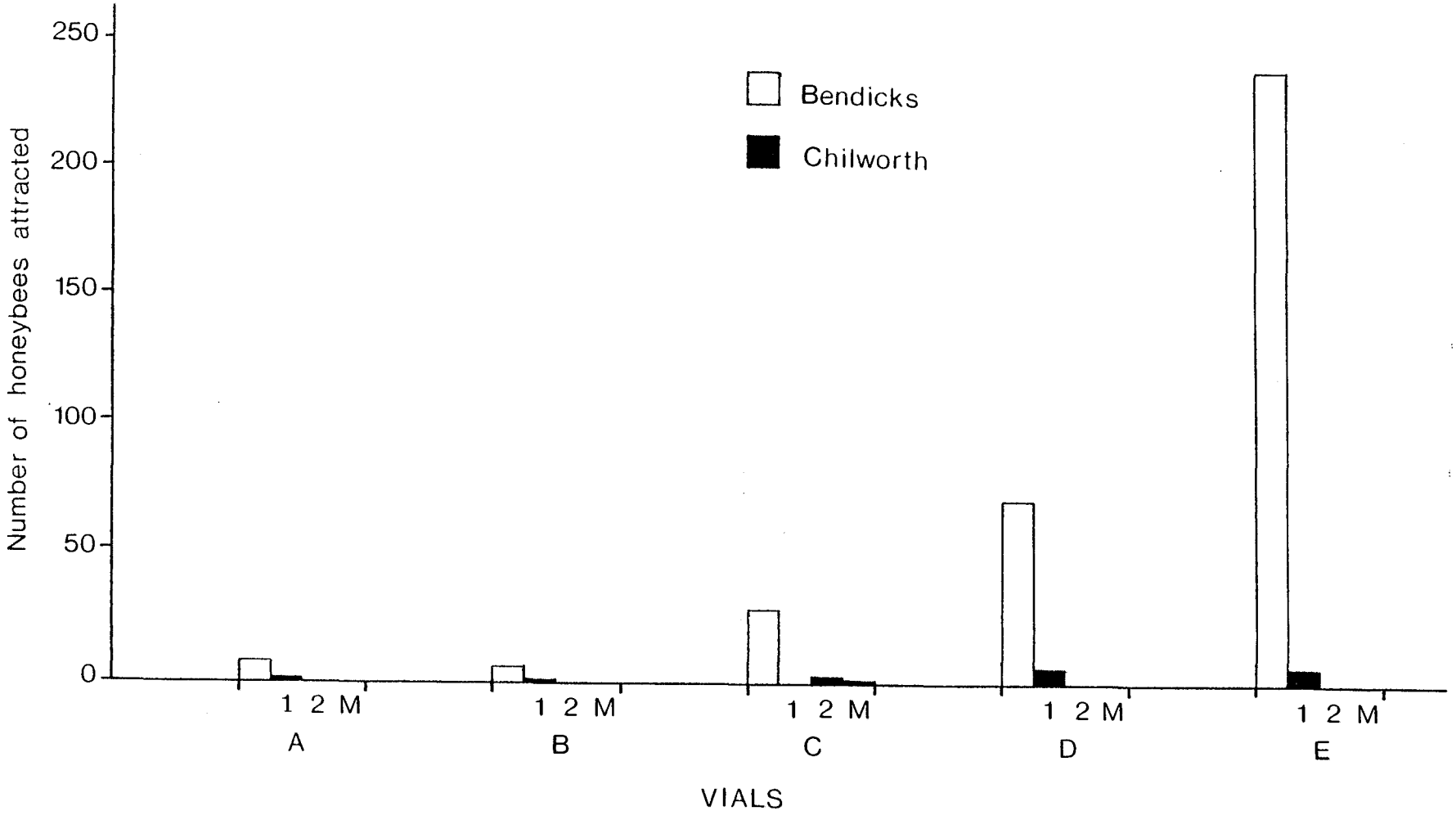


Fig. 26. Comparative attractiveness of ginger extracts at different sites.

1 = day one; 2 = day two; M = Manor bakery. A = ethanol (control)
B = ginger syrup C = extract of ground ginger D = essential oil
E = steam distillate

($p < 0.001$). Ginger syrup, attracting just 6 bees, was significantly less attractive than the ground ginger ($p < 0.001$). Ethanol, which attracted 7 bees, was similar in attractiveness to the ginger syrup.

On day two more wasps were attracted, but there were no bees. Of the 33 wasps which visited the vials 17 were attracted to the steam distillate and 9 to the essential oil, the difference between the two being insignificant at $p = 0.05$ (χ^2). The ground ginger extract attracted 5 and the syrup and control 1 each. The steam distillate was significantly more attractive than either the ground ginger ($p < 0.05$) or the syrup ($p < 0.001$). Nine bees and 5 wasps were marked, but none returned.

2. Chilworth. The experiment was carried out twice in the orchard at Chilworth, on 19 and 22 September 1978. The site of the experiment is reproduced diagrammatically in fig. 27. Both days were warm, the former especially so, with long sunny periods. The wind was light to moderate NW on both occasions. Between 50 and 100 wasps were flying in the vicinity of the vials throughout the presentations, most of them being attracted to fallen apples on the ground. Honeybees were less frequent as they were not attracted to the apples.

No wasps were attracted on either occasion, but 13 honeybees visited the vials on day 1 and 3 on day 2. The combined results are depicted graphically in figure 26 (p. 138). On the first day 6 bees were attracted to the essential oil and 5 to the steam distillate, the ethanol and syrup attracting 1 each. Those differences are not significant at $p = 0.05$ (binomial test). None came to the ground ginger. On day 2 the ground ginger extract lured 2 honeybees and the ginger syrup 1. One bee was marked and did not return.

3. Manor Bakery. The Manor Bakery site is shown diagrammatically in figure 28 (p. 141). The attractants were dispensed as usual in 2 ml. vials, but the tripods were placed on upturned empty, 1 cwt. mincemeat containers, close to the waste

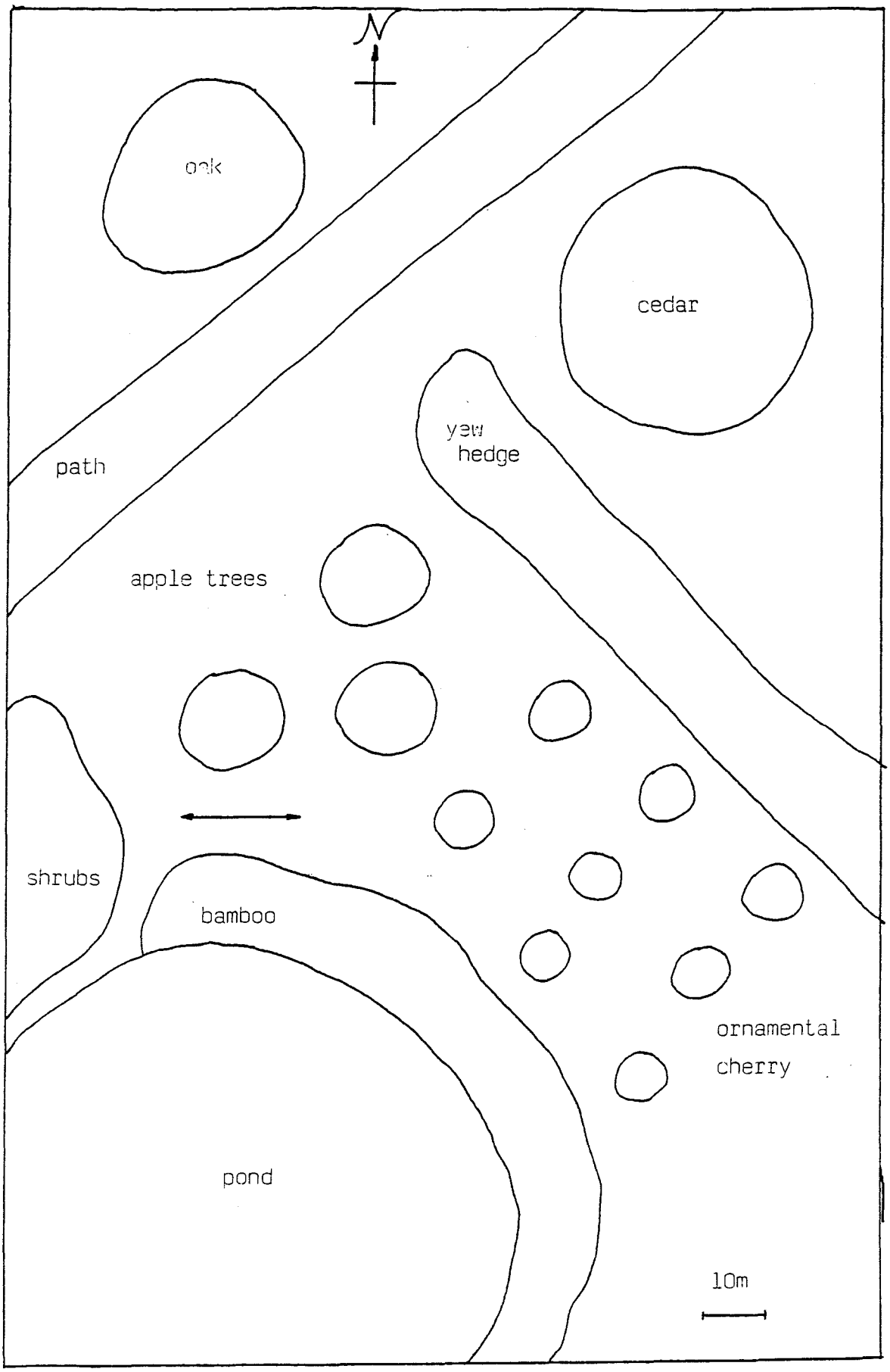


Fig. 27. The Chilworth Manor site. Attractants were set up along the line marked by the arrows.

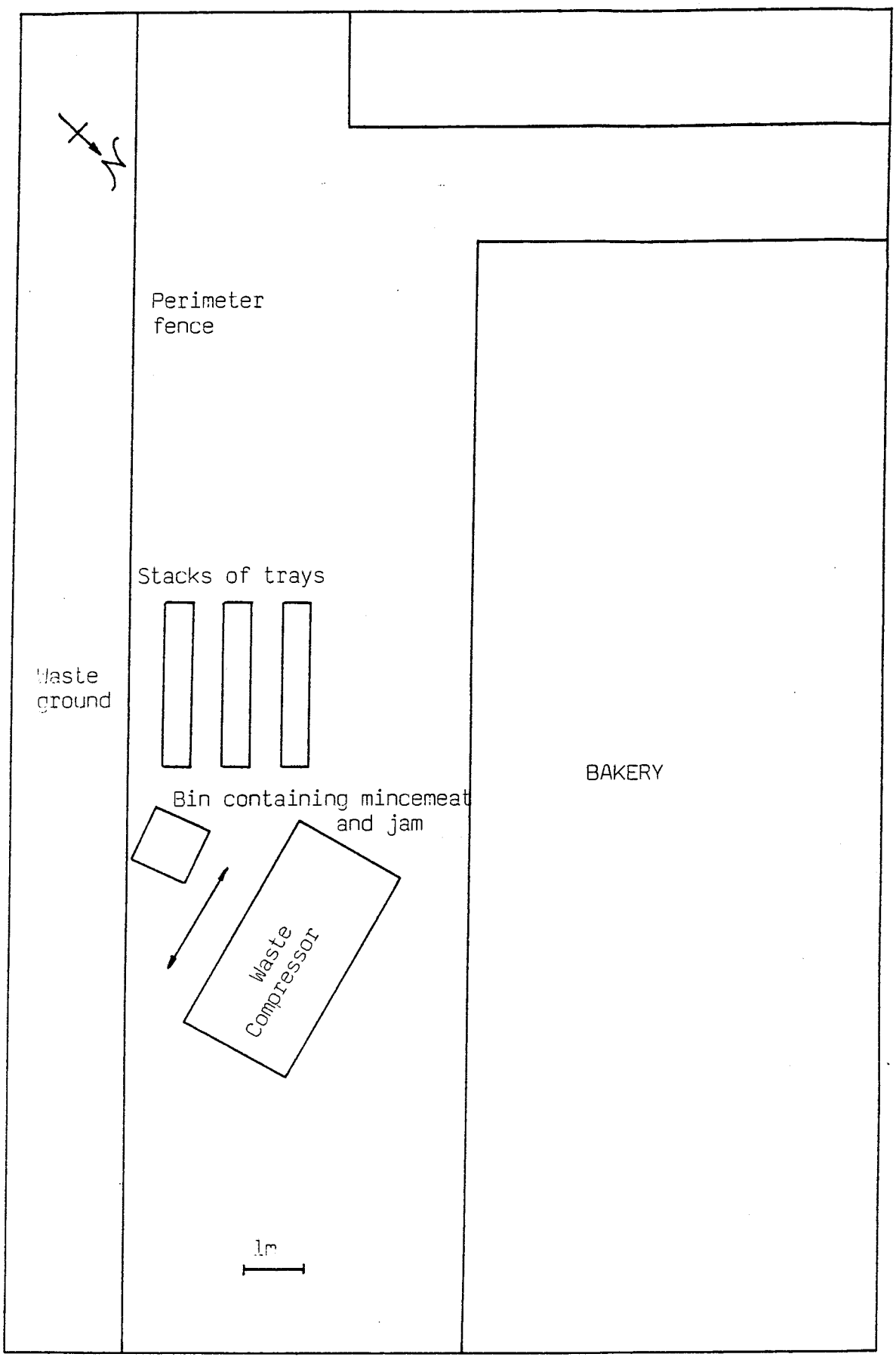


Fig. 28. The Manor Bakery site. Attractants were set up along the line marked by arrows.

compressor in the factory yard.

The first presentation of the attractants, on 25 September took place on a cool day, overcast and with occasional light rain, although becoming brighter and warmer later.

A month later, during the 2nd presentation on 24 October, the weather was calm and warm with sunny periods.

Numbers of wasps attracted to the factory waste varied considerably between the two dates. On 25 September the insects were mainly attracted by blackcurrant jam adhering to the polythene liners of empty 1 cwt. containers. On average 10-20 wasps (*vulgaris* and *germanica*) together with a few honeybees were flying in the area at any moment. In contrast, a month later, there were no honeybees or *Vespula germanica*, but the numbers of *vulgaris* had increased considerably; 50-100 being present during the experiment, this time attracted to two large waste receptacles containing artificial cream and sugar. Both containers were removed at mid-day for emptying, causing an increase in airborne wasps searching for the food source.

On day 1 of the experiment a honeybee and a wasp alighted on the vial of ground ginger extract, but no interest was shown in the other attractants (see fig. 25). On day 2, however, 15 workers of *Vespula vulgaris* visited the vials, 9 alighting on the control, 3 on the steam distillate, 2 on the ground ginger extract and one on the essential oil, most of them arriving after their food source had been removed. The difference between control and steam-distillate was not significant, but between control and ground ginger and control and essential oil it was significant at $p = 0.05$ (χ^2).

Discussion and conclusions

The results from the three sites highlight an interesting aspect in the foraging behaviour of wasps. At Bendicks, the chocolate factory, where carbohydrate waste containing ginger was a major source of food for at least four wasp colonies in the area,

(see p. 215), attractants derived from ginger proved to be more attractive. The steam distillate and essential oil were especially so, being concentrated forms of the attractive principles of ginger syrup. Extract of ground ginger and ginger syrup were less attractive, with ethanol lying in the last place. As gas-chromatographic evidence shows (see figs. 14, 18, 19) the most attractive formulations contain high proportions of sesquiterpenes e.g. zingiberene, bisabolene (peaks 'z', 20 etc).

At the orchard and the bakery, where wasps were more numerous than at the chocolate factory, the attractants were effectively ignored: at Chilworth, over the course of 4 hours of observations, not a single wasp was seen to approach the vials, even though the latter were placed among fallen apples, being visited by scores of wasps. At the bakery over the same length of time, most of the sixteen wasps visiting the vials landed at the control and then only when their major food source had been removed. Being far more numerous than wasps at Bendicks at the height of the 1978 season, honeybees came to the vials in much larger numbers, the steam distillate proving to be significantly more attractive than the others. Here again, the same hierarchy of attractiveness among the ginger formulations suggests a positive correlation between their attractiveness and the proportions of high boiling point volatiles. At Chilworth honeybees were attracted to the vials, though in small numbers, the steam distillate and essential oil being the most effective.

An explanation for the unattractiveness of ginger at the orchard and bakery sites lies in the foraging behaviour of wasps and the evolutionary advantage it undoubtedly confers on them. Unlike the honeybees, social wasps are scavengers and can exploit many varied sources of food. In early summer when wasp populations are low, their carbohydrate requirements can be met quite easily by means of nectar, both floral and extrafloral, and honeydew, especially from tree aphids, but later on as the populations reach their peak and flowers and aphids are in short supply (Wratten,

Personal communication), the need for correspondingly larger and more sustained sources of sugar has to be met by other means, largely by recourse to damaged fruits such as apples, pears and blackberries.

If an abundant supply of attractive food is discovered it is to the colony's distinct advantage that it be exploited for as long as possible. There are a number of reasons for this. First, and most important, energy is conserved, allowing the colony to develop more efficiently. As has been stated elsewhere (p.116) foraging wasps will return to a source of food for some days after it has been exhausted, and will search fruitlessly for many minutes before flying off. This wastes energy, resulting in deprivation of the dependent members of the colony. The longer-lasting the source of food, the less significant is this energy loss and colony deprivation when the food runs out.

Secondly, the risk of mortality of individual foragers is reduced if they exploit a sustained source of food, because they have no new hazards to negotiate in transit and the presence of numerous other wasps confers on them an element of 'safety in numbers'; also, being fully fed, they are likely to be stronger and more alert than wasps constantly searching for food.

How can this theory explain the fact that ginger, so attractive to wasps at one particular site, is apparently unattractive at others?

First, it is necessary to postulate a mechanism whereby news of an extensive source of food could be communicated to other members of the wasp colony. Observations strongly suggest that, contrary to current opinion, social wasps do not exploit attractive food sources purely by means of individual random searches, but that some form of communication, however rudimentary, is employed, resulting in a more efficient and concerted effort (see p. 215). Thus, taking the ginger syrup at Bendicks as an example, early in the season its presence will be detected by the first foragers as a result of random searching helped, in all probability, by a favourable wind carrying odours towards the nest.

However, subsequently, as the foragers return more frequently, having learned the position of the source of syrup, other wasps within the nest, including callows and nurses, will learn to recognise the characteristic odour as it pervades the colony. In consequence, foragers previously exploiting transient sources of carbohydrate will be likely, as these dwindle, to search for the new food, as will young workers foraging for the first time.

The results of the experiments above (p. 133) show that once worker wasps have discovered an attractive source of food, presenting them with substances apparently as attractive, if not more so, does not entice them away from it. This lends support to the above theory. By concentrating their efforts on one food source the wasps are foraging more efficiently, with less risk to themselves, whilst re-inforcing the odour-signals at the nest. This is not to say that wasps feeding on jam and cream are unattracted by ginger. The results of the experiment at the bakery (p. 139) indicate that, when their food source is removed, wasps are more receptive to other attractants.

The honeybees at Chilworth, not being attracted to apples, were unbiased and visited the vials, especially those containing steam distillate and essential oil of ginger.

(iii) Comparison of attractiveness of various ginger formulations with jam and apple juice

Introduction

This experiment was carried out to test whether wasps at the orchard and bakery would be attracted to vials containing food of the sort that they were collecting, in preference to ginger.

Method

The vials were set up as before (p. 135) in the orchard at Chilworth and at the Manor Bakery, but an extra vial was added to the line. At Chilworth it contained juice freshly extracted from a fallen apple, and at the bakery, raspberry jam. The experiment, which lasted 2 hours, was repeated twice at both sites.

Results

No wasps were attracted to any of the vials at Chilworth on either day. At the bakery, however, 11 wasps came to the attractants as follows:

	25 Oct	26 Oct
control (ethanol)	1	1
ginger syrup	1	0
ground ginger	2	0
essential oil	0	0
steam distillate	4	1
raspberry jam	1	0
Total	9	2

The weather on day 2 was cooler than day 1 and there were fewer wasps at the bakery.

No bees were recorded at the bakery but at Chilworth 7 visited the vials as follows:

	23 Sept.	22 Oct
control (ethanol	0	0
ginger syrup	0	0
ground ginger	1	0
essential oil	0	1
steam distillate	3	0
apple juice	1	1

None of the results show a significant difference between the vials (binomial test).

Conclusion

The results indicate that jam and apple juice dispensed in vials at the respective sites were unattractive, as were the ginger extracts. If, like the ginger distillate, the volatiles from the apple and jam had been concentrated, it is likely that they would have attracted wasps.

(c) Relative attractiveness of ginger extracts and fractions obtained by thin layer chromatography: various sites, 1978

(i) Attractiveness of various fractions derived from ginger syrup

Introduction

Ginger syrup is attractive to wasps and its distillate is even more so. To discover which of the many volatile components of ginger were the active principles causing this attraction 400 mg of essential oil of ginger were separated into fractions by means of thin layer chromatography and each was presented in a vial as before.

Method

Thin layer chromatography was used to separate 400 mg of Treatt's essential oil of Chinese ginger into 12 fractions. The method used is described in detail on p. 34. Each fraction was made up to a volume of 4 ml with 100% ethanol. Analysis of the fractions was carried out by means of GLC (figs. 29-37), and nine of them were subsequently used in the bioassays, all but one of the 4 fractions yielding blank GLC traces being rejected.

The vials were set up in groups of 3 together with a control (100% ethanol), each one containing ½ ml. of one of the fractions, and a wick. The vials were arranged as above (p.135) and observations were made for 10 minutes of every 15, the first 5 being used for marking any insects captured at the vials. Each experiment was repeated twice at three sites, but once only at two others. These sites were:

1.	Bendicks	chocolate factory
2.	Chilworth	apple orchard
3.	Manor bakery	
*4.	Spillers-French bakery	
*5.	Crawley	apple and plum orchard

* one run only

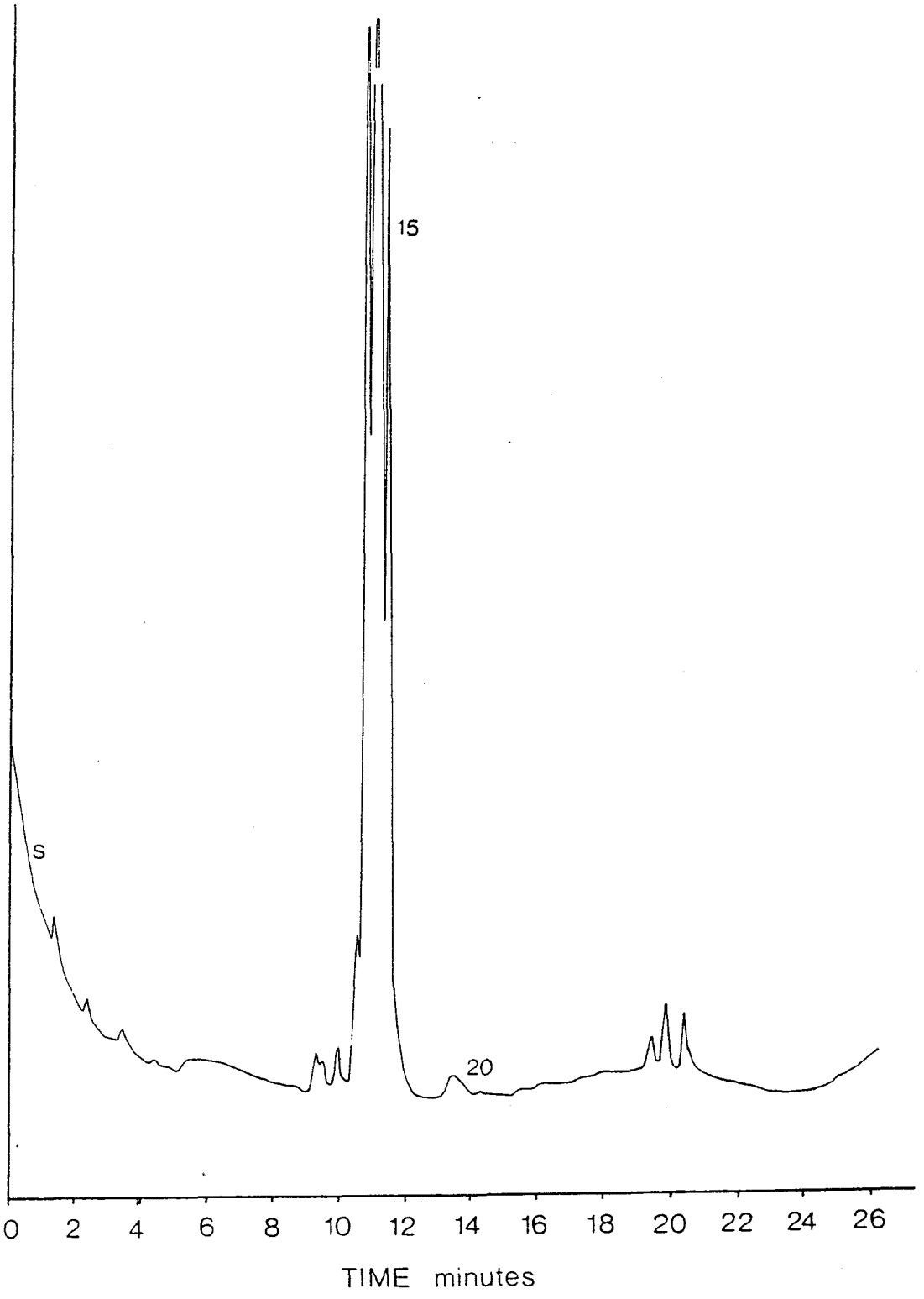


Fig. 29. Chromatogram of ginger TLC fraction 1
'S' = solvent peak. Numbers refer to positions of peaks in the whole distillate (fig. 19).

Run conditions: column: OV101 (5%)
Temperature program: initial temperature 80°C increasing at 8°C per minute.
Attenuation: 80

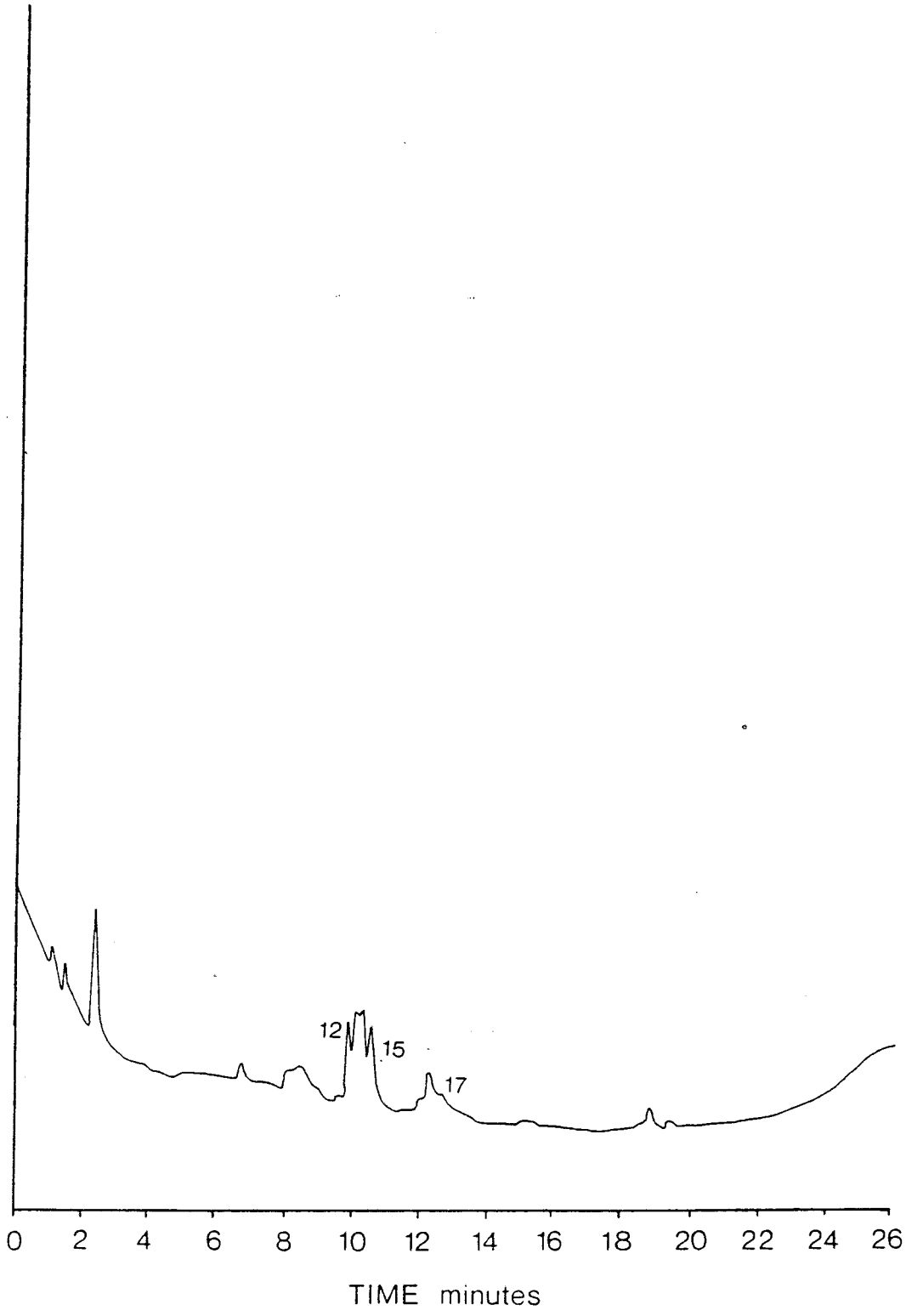


Fig.30. Chromatogram of ginger TLC fraction 2

Run conditions as fig. 29.

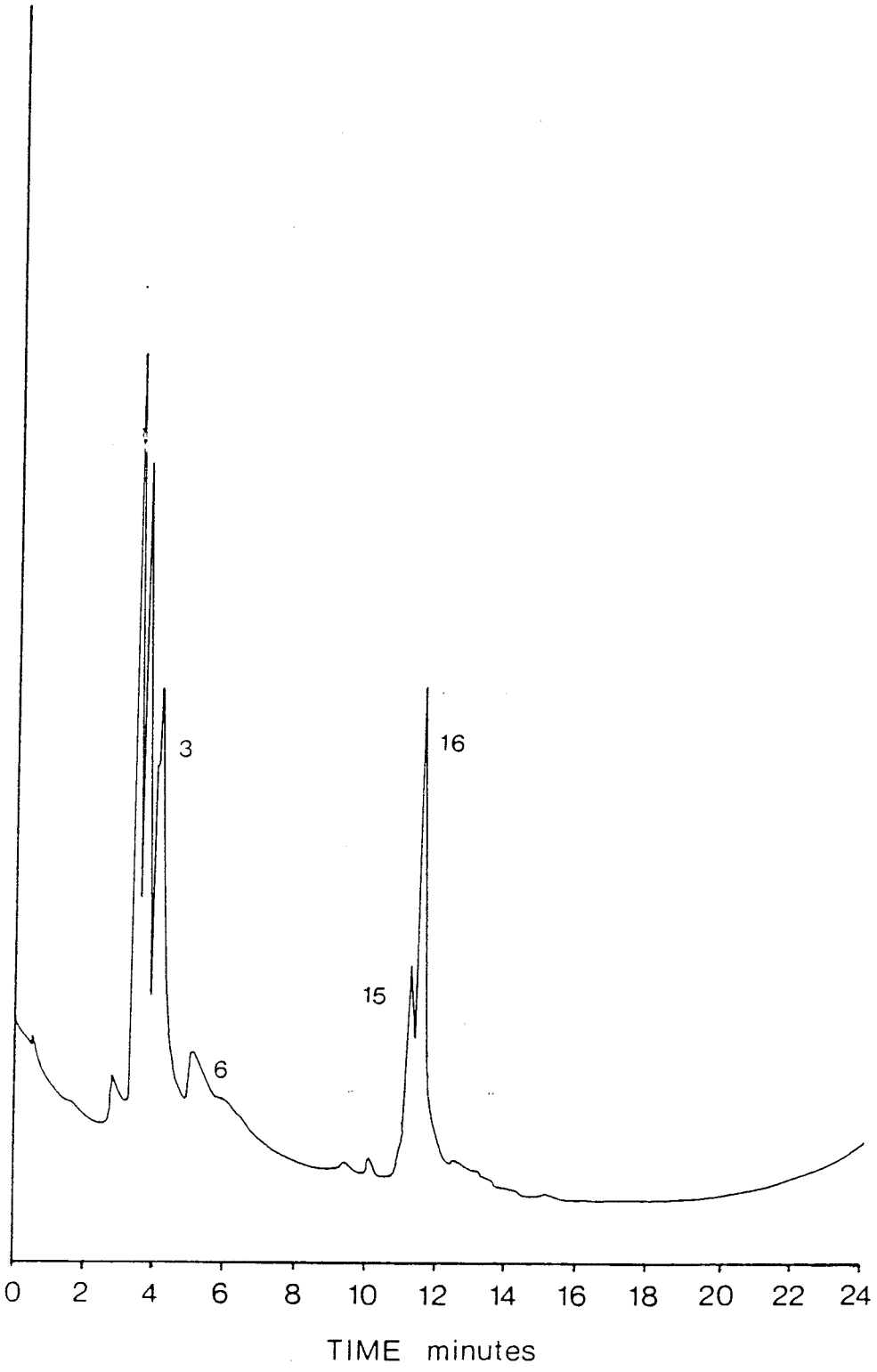


Fig.31. Chromatogram of ginger TLC fraction 3

Run conditions as fig.29.

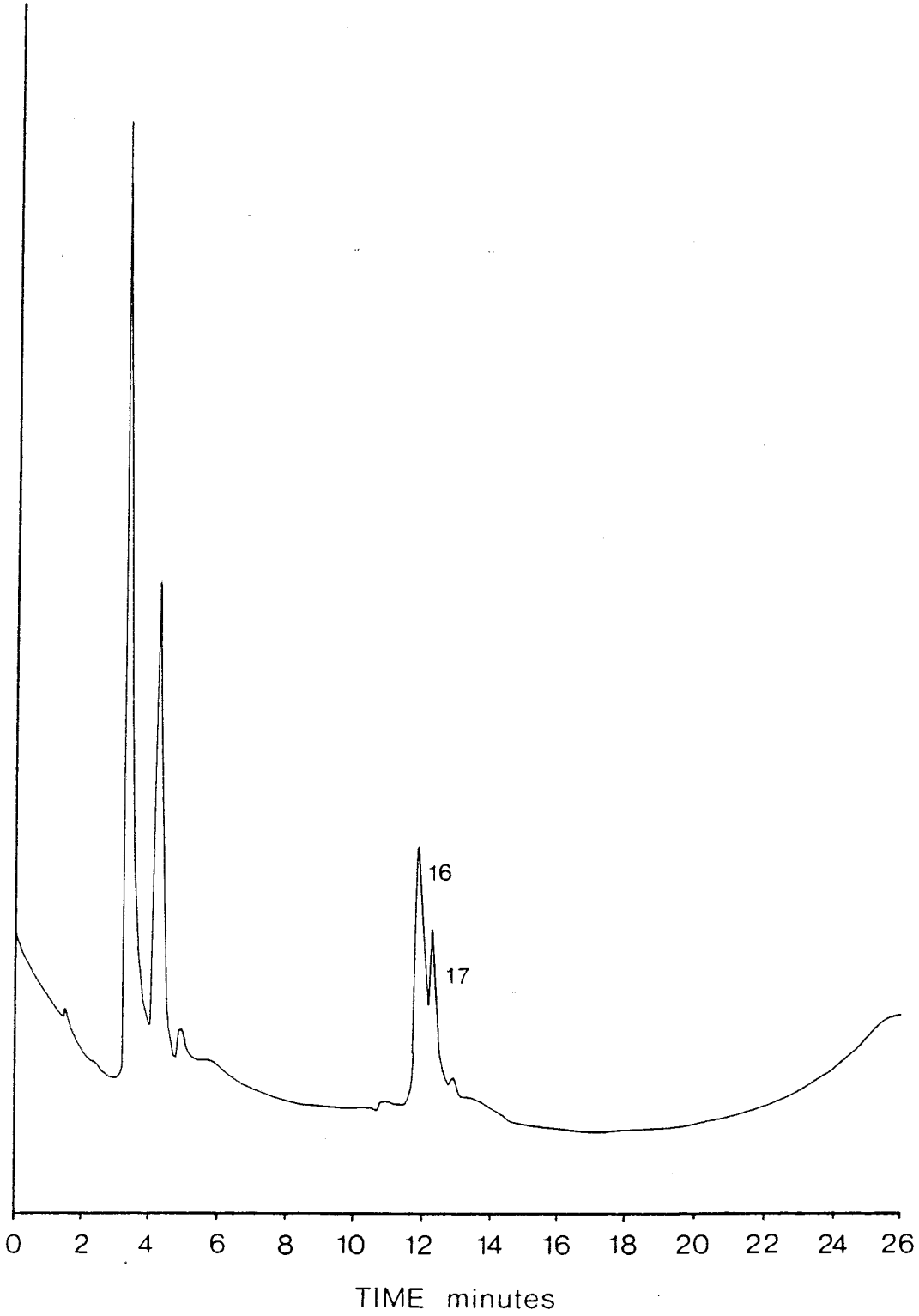


Fig. 32. Chromatogram of ginger TLC fraction 4.

Run conditions as fig. 29.

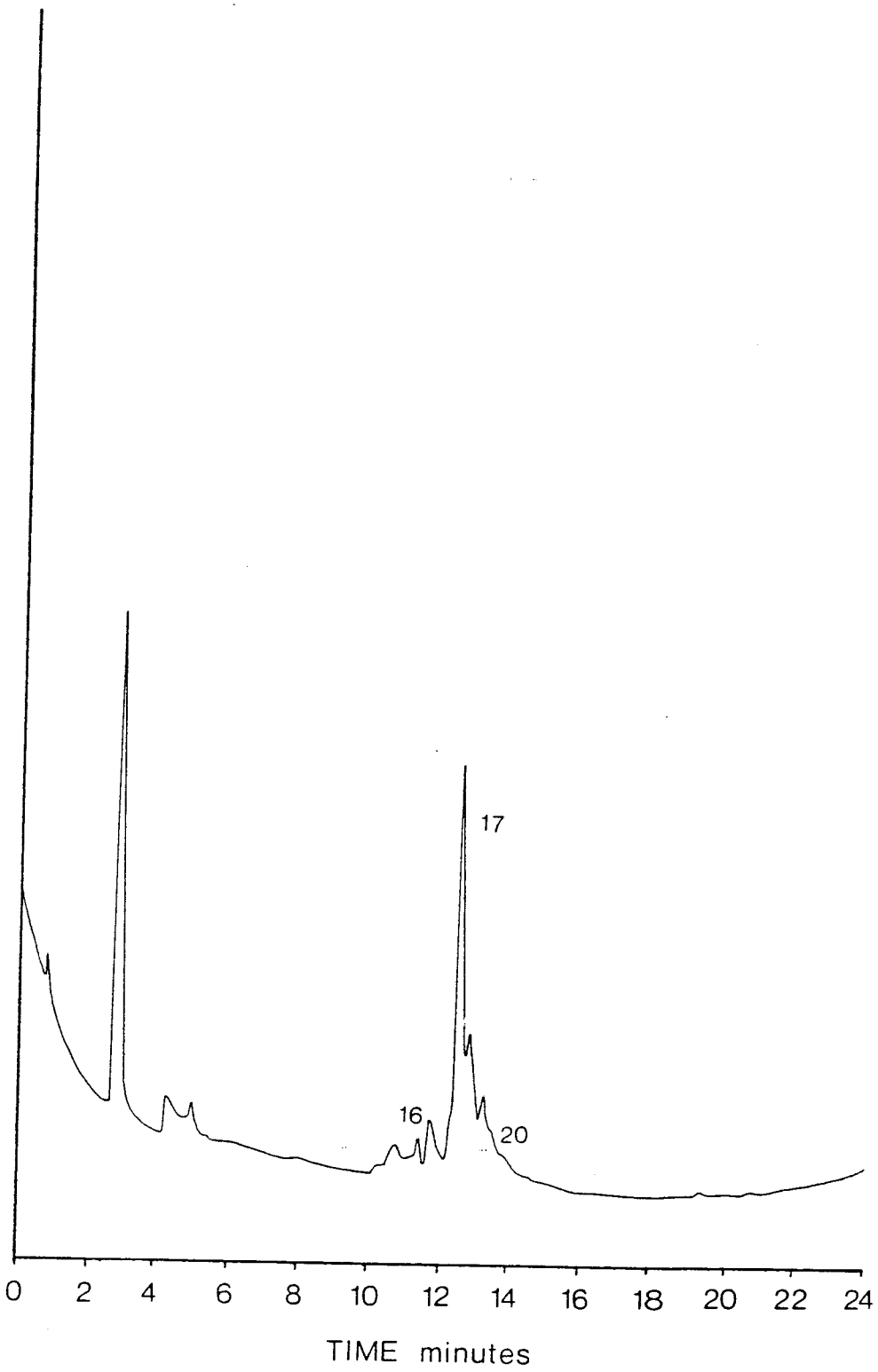


Fig.33. Chromatogram of ginger TLC fraction 5.

Run conditions as fig. 29.

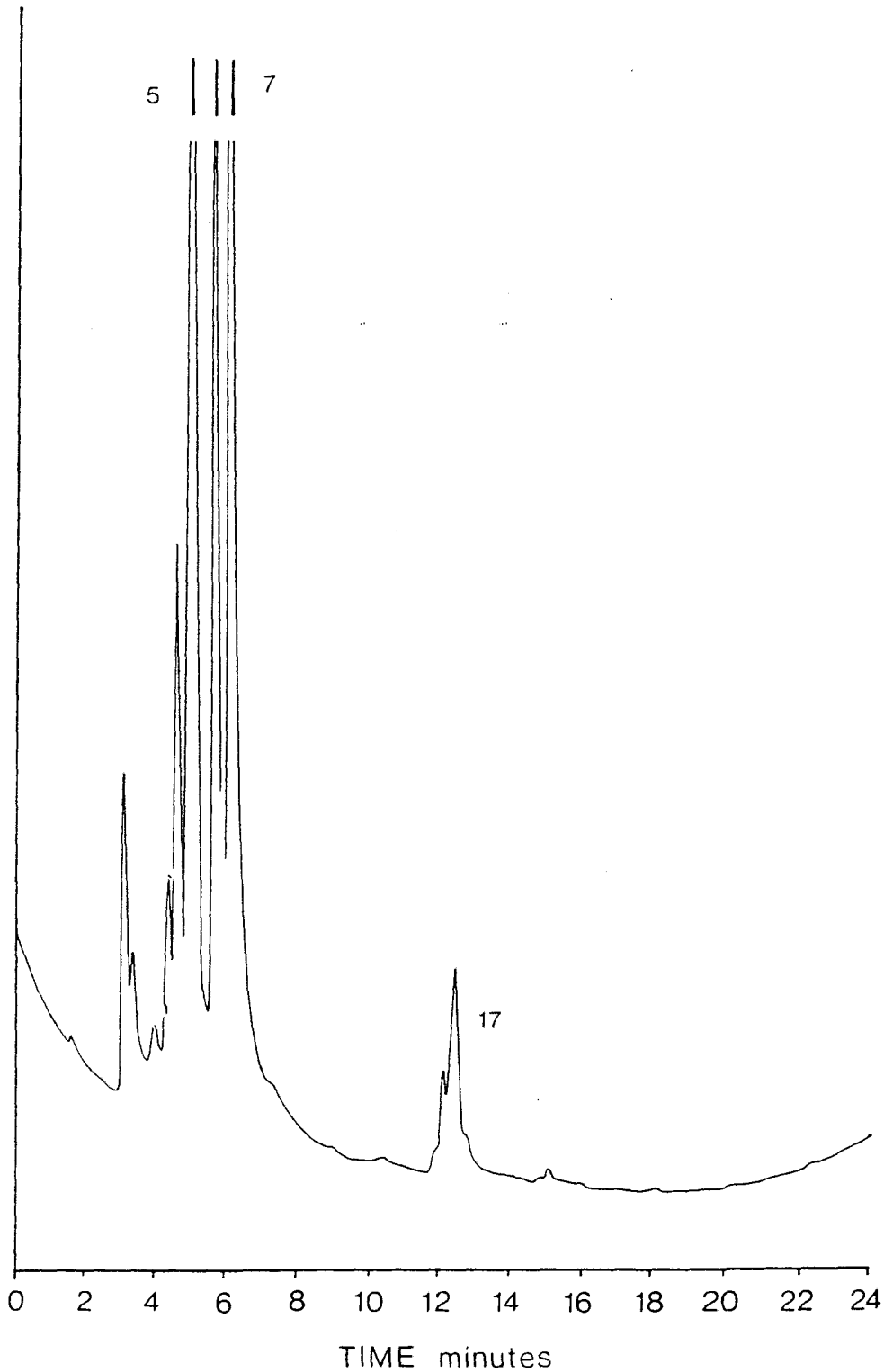


Fig.34. Chromatogram of ginger TLC fraction 6.

Run condtions as fig.29.

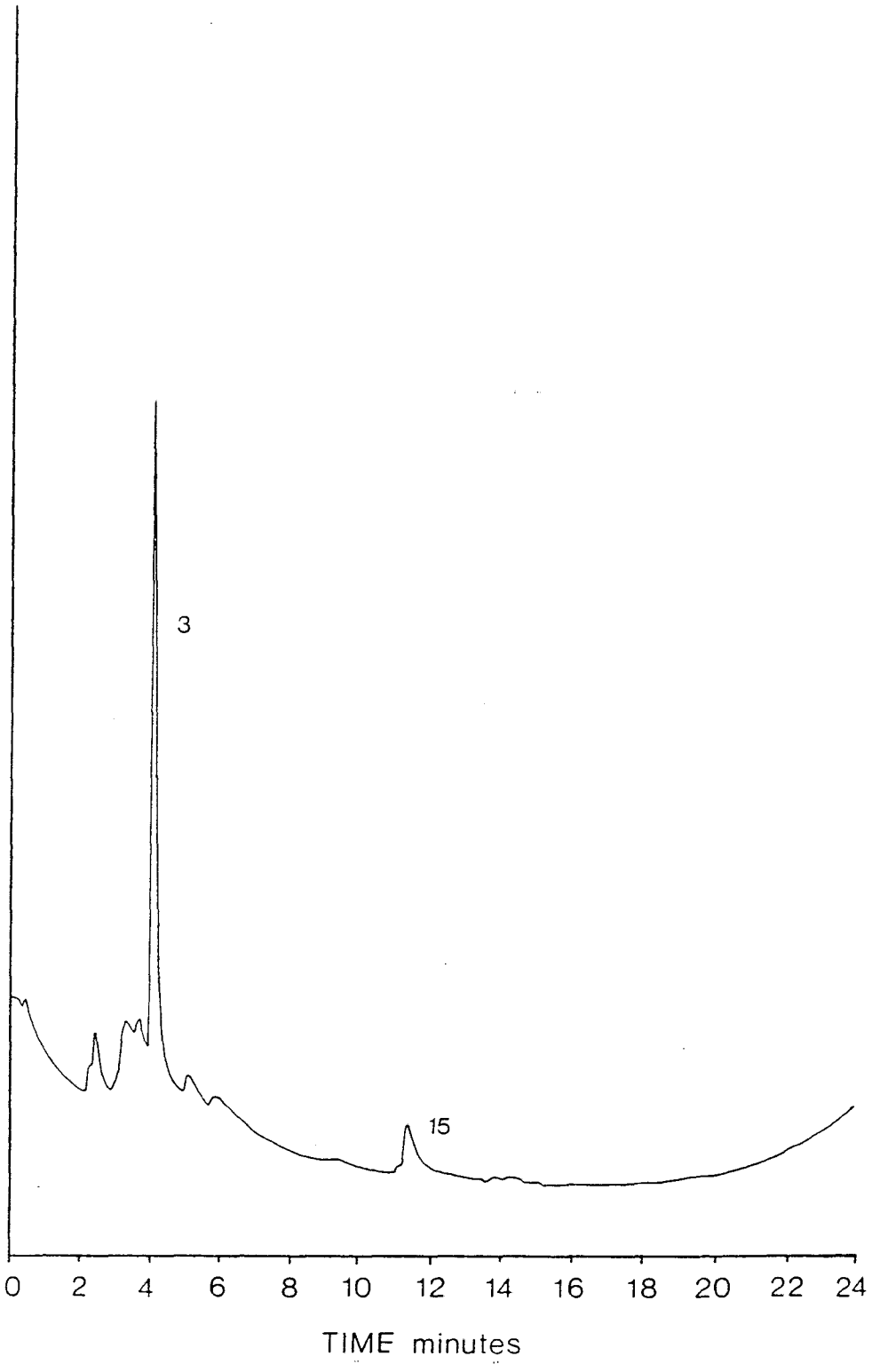


Fig.35. Chromatogram of ginger TLC fraction 7.
Run conditions as fig. 29.

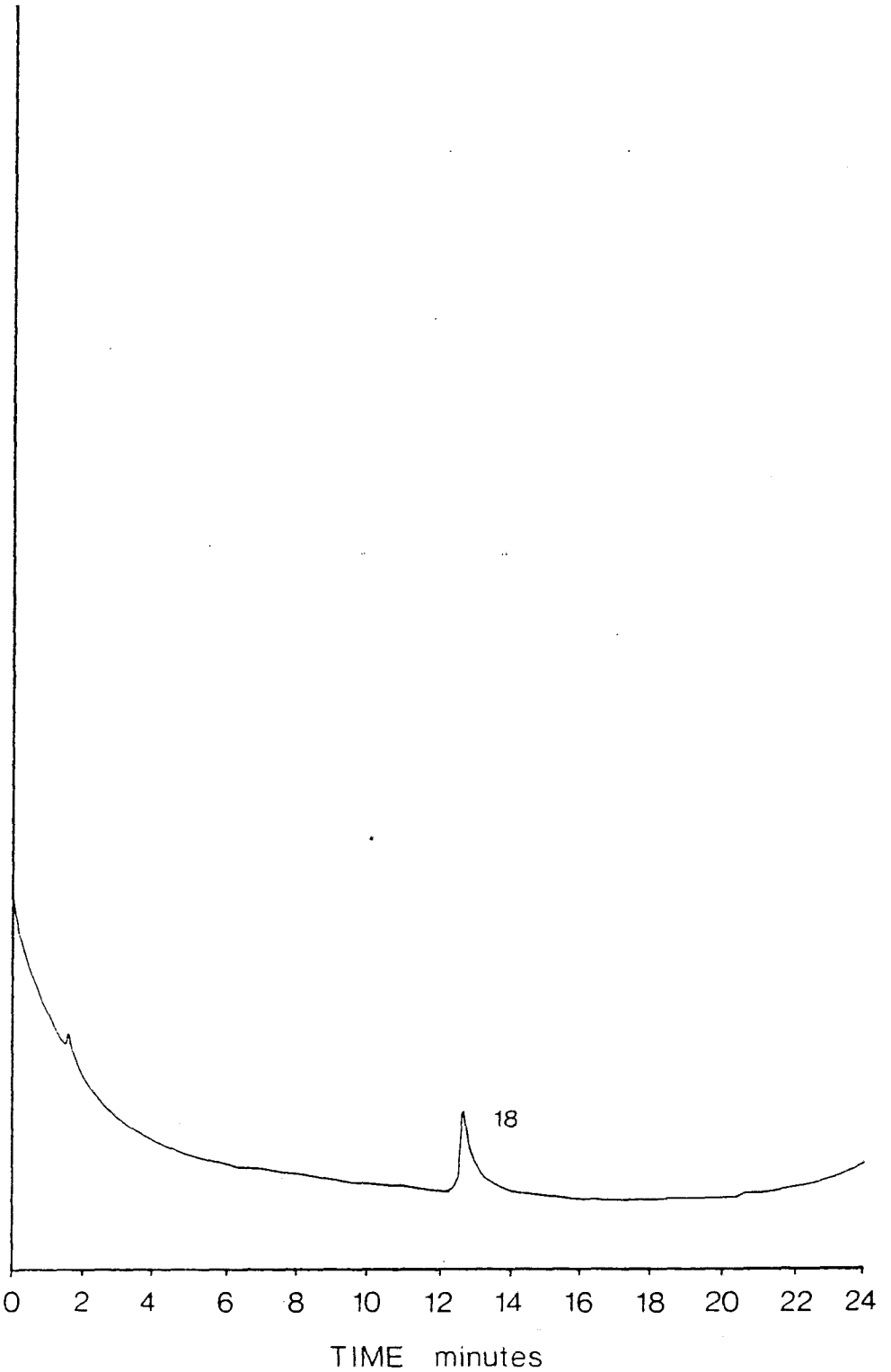


Fig.36. Chromatogram of ginger TLC fraction 8.
Run conditions as fig. 29.

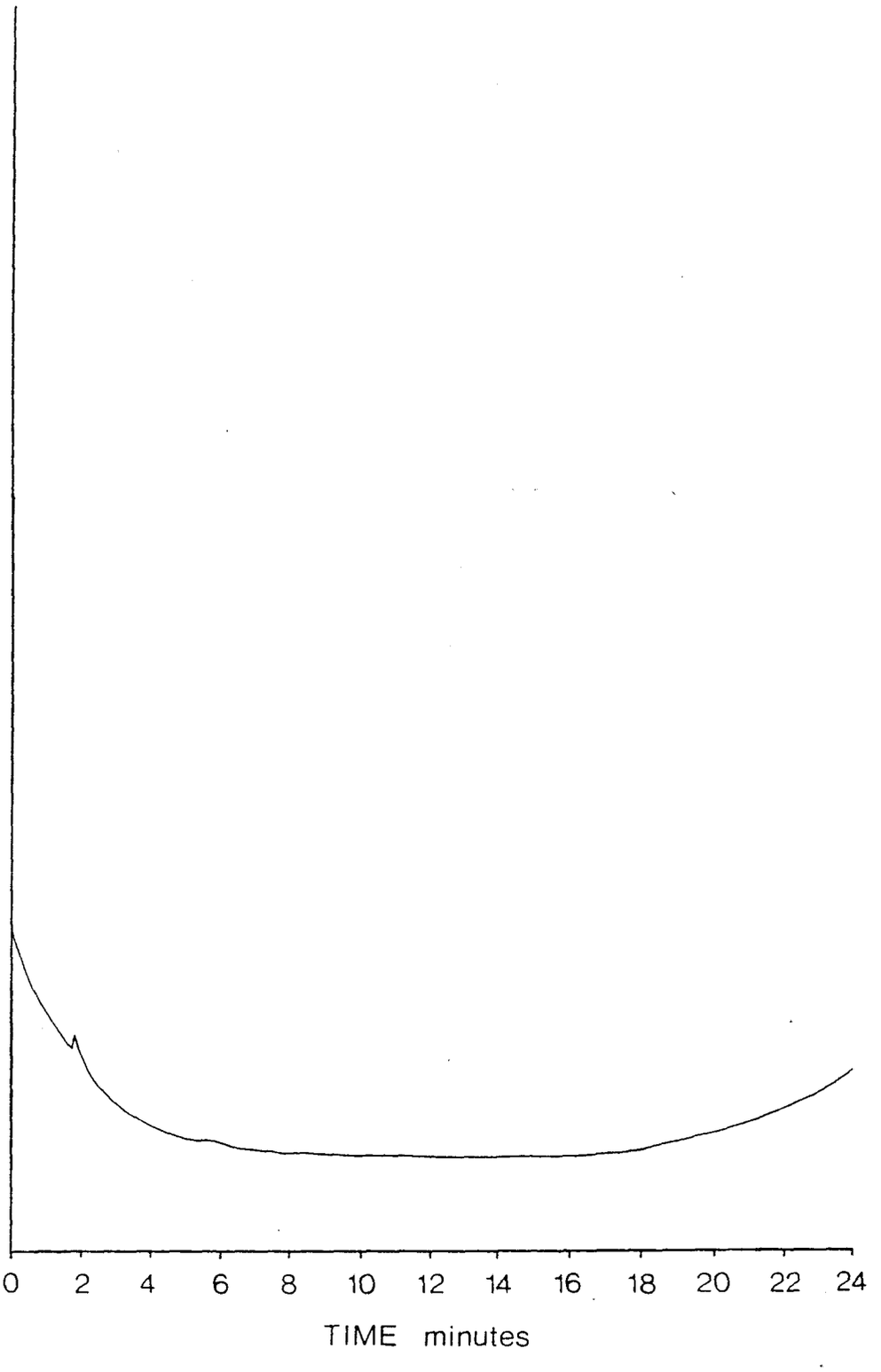


Fig.37. Chromatogram of ginger TLC fraction 9.

Run conditions as fig. 29.

Results

The major results are displayed graphically in figures 38-41 (pp. 158-161). At Spillers-French bakery and the orchard at Crawley there were very few wasps, so only one set of experiments was carried out at each site, yielding the following results:

Site	Fraction number	No. of wasps attracted
Spillers-French	1	1
	2	1
Crawley	5	1
	6	1

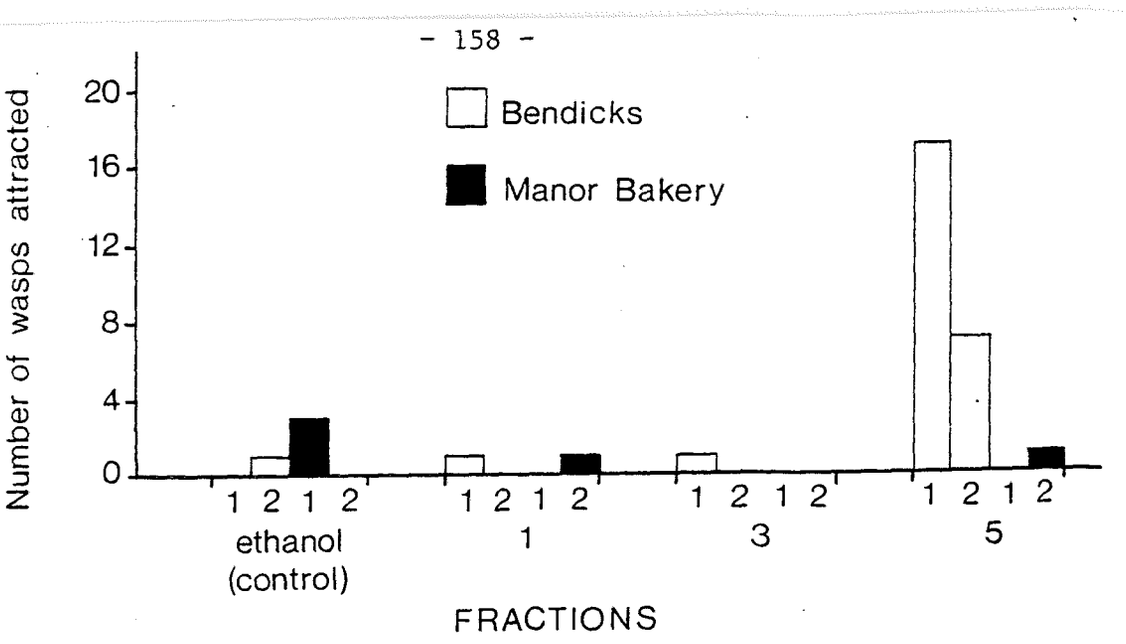
Table 43. Number of wasps attracted to vials at Spillers-French bakery and the orchard at Crawley.

No honeybees came to the vials. At Bendicks all the fractions were visited by wasps but at Chilworth they came only to fractions 6 and 7. At the Manor bakery wasps were attracted to fractions, 1, 2, 4, 5, 6 and 7, at the orchard at Crawley to 5 and 6, at Spillers-French bakery to 1 and 2. Comparison of the graphs in figures 38 , 40 and 41, shows that more wasps were attracted to the fractions at Bendicks than at the bakery and that fewer still came to the vials in the orchard.

The total numbers of wasps attracted at each site is compared below with the approximate number of wasps present during the experiments.

Site	Mean No. of wasps present at any moment during expt.	Total No. attracted to fractions in 12 hours
Bendicks	15	61
Manor bakery	150	21
Chilworth orchard	100	4

Table 44. Estimated numbers of wasps present at each site during the experiments compared to the numbers attracted to the vials.



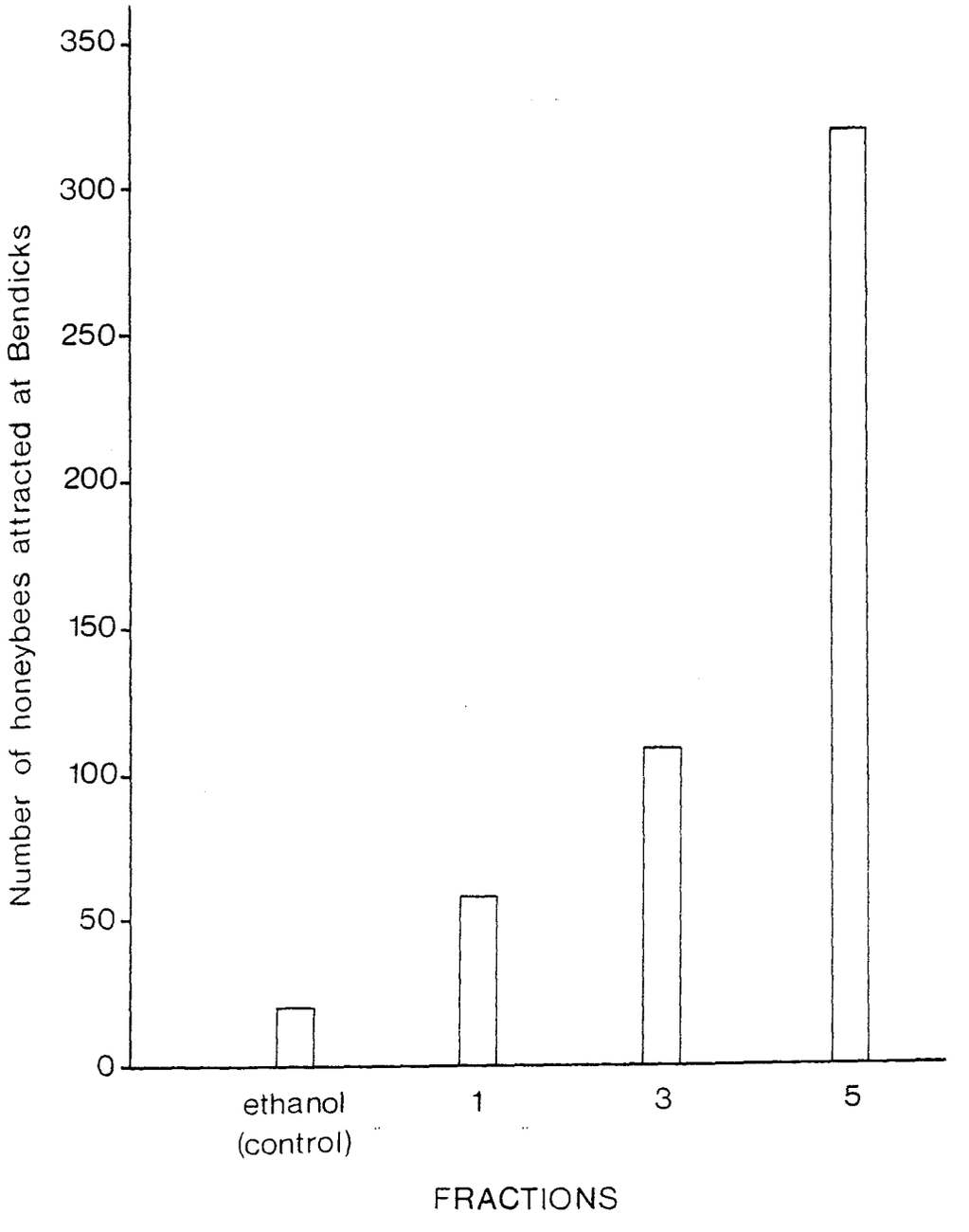


Fig. 38. Comparative attractiveness of ginger fractions at different sites

1 = day one; 2 = day two.

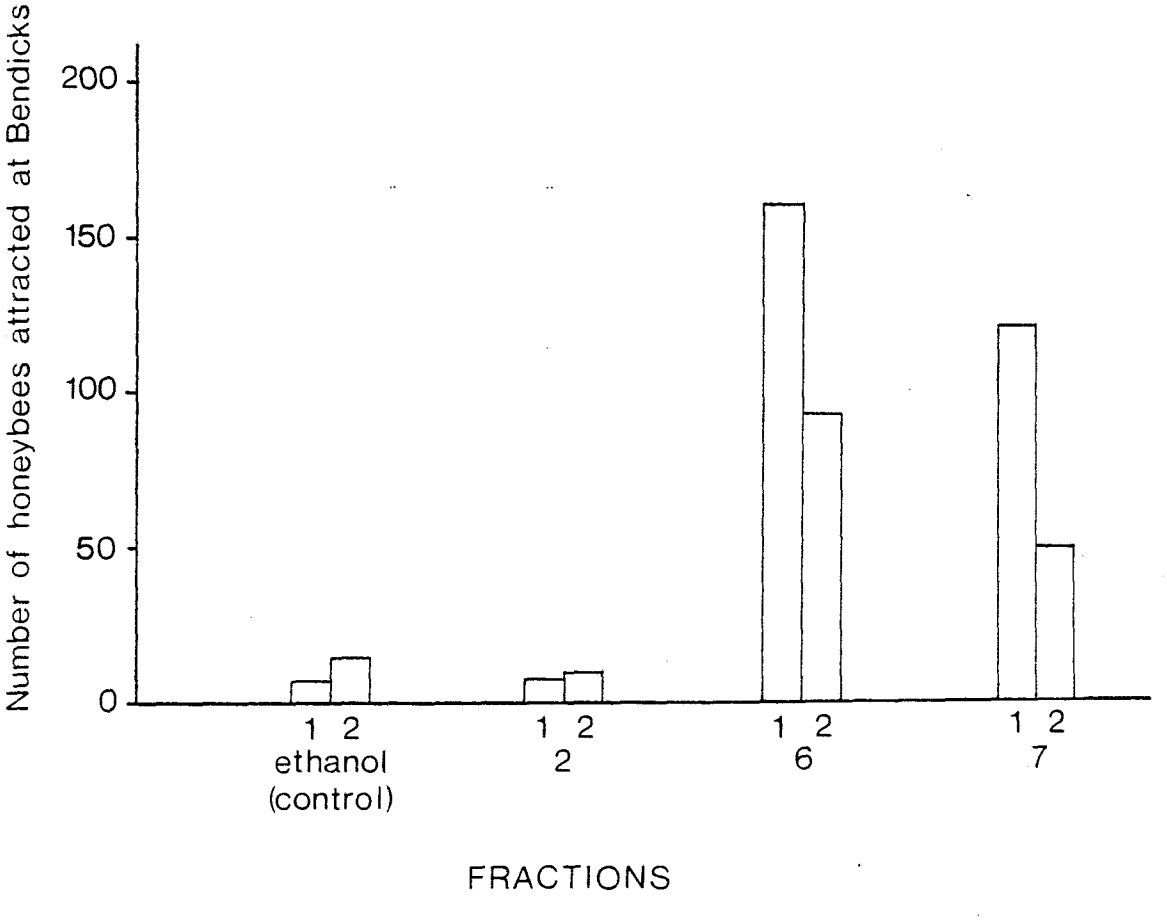


Fig. 39. Comparative attractiveness of ginger fractions at different sites: honeybees
1 = day one; 2 = day two.

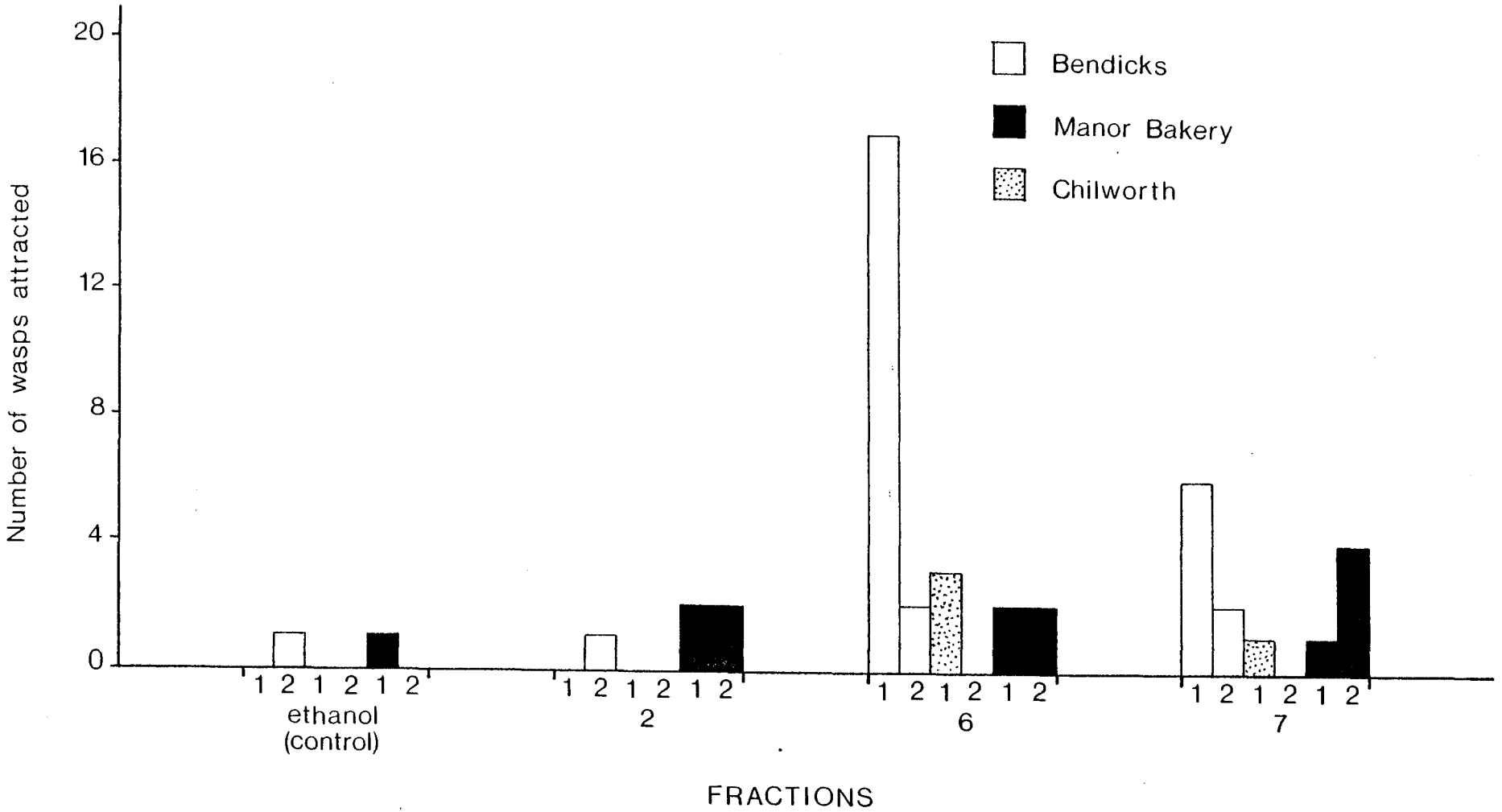


Fig. 40. Comparative attractiveness of ginger fractions at different sites: wasps 1 = day one; 2 = day two.

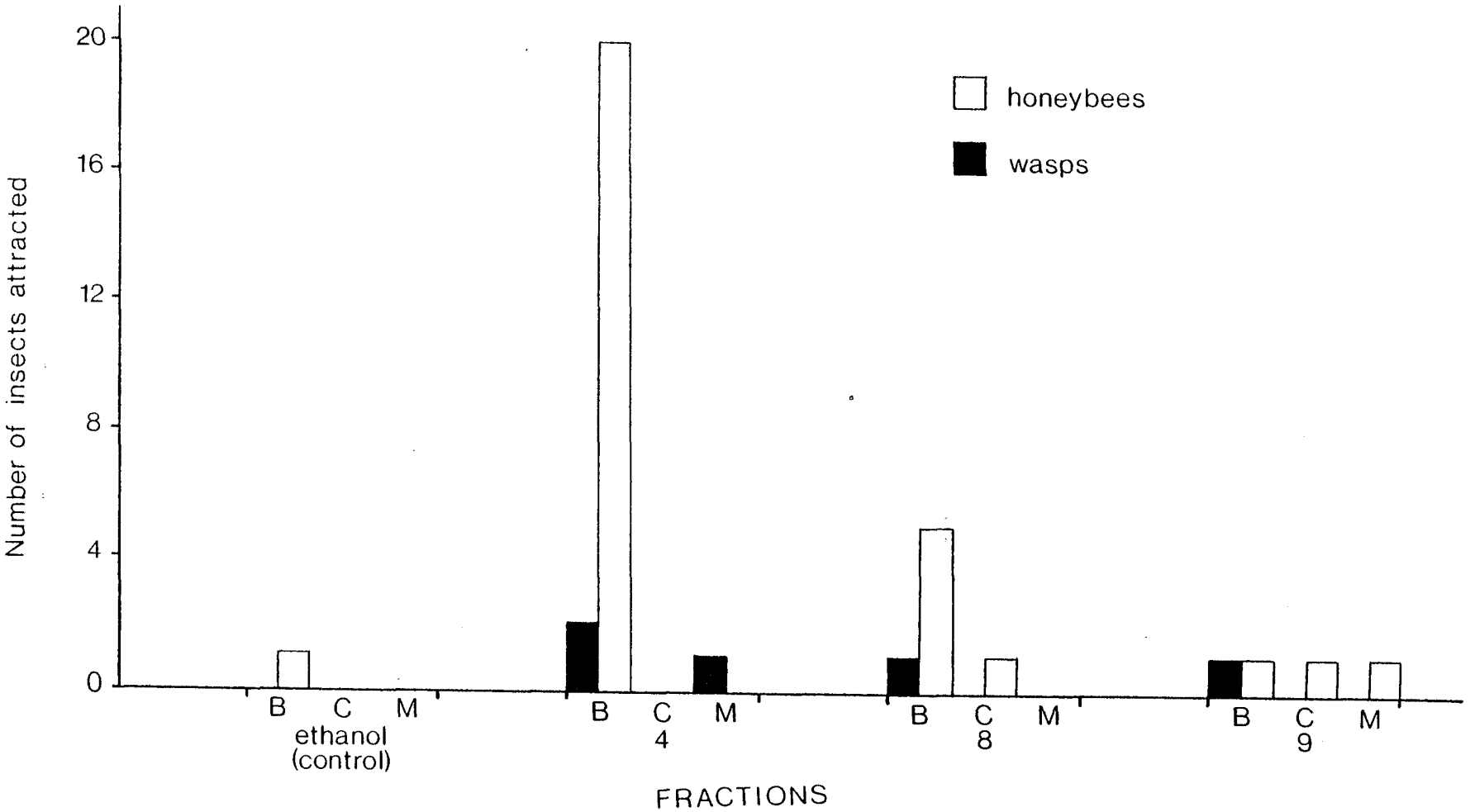


Fig. 41. Comparative attractiveness of ginger fractions at different sites.
B = Bendicks; C = Chilworth: M = Manor Bakery.

Honeybees visited all fractions at Bendicks, 5, 6, 8, 9 at Chilworth and 9 at the Manor bakery. The mean numbers of bees present at each site and the total attracted to the fractions are shown below:

Site	Mean no. of honeybees present at any moment during experiment		Total No. attracted to fractions in 12 hours
	September	October	
Bendicks	200	0	991
Manor bakery	10	0	1
Chilworth orchard	10	0	4

Table 45. Estimated numbers of honeybees present at each site during the experiments compared to the numbers attracted to the vials.

The weather throughout the experiments was of a similar type, being mainly calm and sunny with above average temperatures. In total 38 honeybees were marked and only one returned to a vial (at Bendicks). A single wasp was marked but did not return.

The experiments at the Manor bakery and Chilworth yielded too few results for a comparative analysis of fractions visited by wasps to be made, but 2, 6 and 7 were apparently more attractive than ethanol at the bakery.

At the chocolate factory on the first day fraction 5 was significantly more attractive than 1, 3 or the control ($p < 0.001$, χ^2 test), 7 in turn being more attractive than 2 ($p = 0.032$, binomial 2-tailed test) or the control ($p = 0.016$ binomial 1-tailed test). The results on day 2 are not significantly different. Fractions 4, 8 and 9 attracted too few wasps to be reliably compared.

The honeybee data can be compared using the Chi-squared one sample test. At Bendicks the results for fractions 1, 3 and 5, obtained on the two days of observation, can be combined, as they do not differ significantly (χ^2 two sample test). Of the

504 honeybees attracted in the experiment 319 alighted at fraction 5, significantly more than the 107 at fraction 3 ($p< 0.001$, χ^2). Fraction 3 was significantly more attractive than 1 ($p< 0.001$) which attracted 58 bees and this was more attractive than the control ($p< 0.001$).

Fractions 2, 6 and 7 attracted varying numbers of honeybees at Bendicks and the two replicates differ significantly ($p< 0.01$, χ^2), so cannot be combined. Taking the results separately and comparing two fractions at a time, fraction 6 is the most attractive in both cases, the difference between this and fraction 7 being highly significant ($p< 0.001$, χ^2) but 2 and the control showed no significant difference.

Fractions 4, 8 and 9 attracted honeybees on day 1 at Bendicks but on day 2 there were no bees in the area. Fractions 8 and 9 attracted one bee each at Chilworth and 9 attracted 1 at the bakery, but 4 attracted none at any sites but Bendicks.

At Bendicks fraction 4 attracted 20 bees, significantly more than fraction 8 which lured only 5 ($p< 0.01$, χ^2). However, fraction 9 and the control attracted only one bee each and did not differ significantly from fraction 8.

Conclusions

Wasps were comparatively scarce at the chocolate factory during this season, but some of the results show evidence of discrimination between fractions, numbers 5, 6 and 7 being the most attractive.

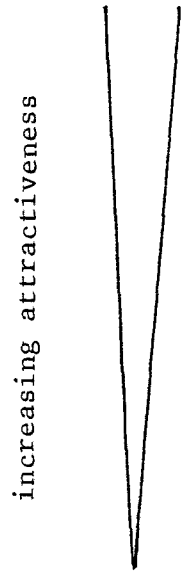
As might have been expected from previous experiments, the ginger fractions were comparatively unattractive at the bakery and orchard, though large numbers of wasps were present at both sites. More wasps visited the fractions at the bakery than the orchard, possibly because the food sources at the former site were occasionally removed.

Honeybees, being present in large numbers at the chocolate factory, also exhibited preferences when visiting the vials, any significant differences between replicates of the experiment being ascribable to lower numbers of the insects in the late afternoon and towards the end of the season. Though the wasps and bees cannot be compared reliably owing to the small numbers of the former, it is evident from the results that a similar hierarchy of attractiveness can be established, and this is discussed more fully below.

(ii) Estimation of the relative attractiveness to bees of ginger formulations and fractions

Discussion

Some of the results obtained in experiments (b)(ii) and (c)(i) above can be directly compared, to yield a hierarchy of attractiveness. On 21 September 1978, in experiment (c)(i), three separate but methodologically identical experiments were performed to determine the attractiveness of 11 solutions. On 13 September, in experiment (b)(ii), 3 of these 11 had already been compared with a further 3 others. Using the one-sample χ^2 analysis on pairs of the results gives the following order of attractiveness for the 14 solutions:



steam distillate of ginger syrup
fraction 5
fraction 6
Treatt's essential oil of Chinese ginger
fraction 7
fraction 3 + extract of ground ginger
fraction 4 + fraction 1
fraction 2
fractions 8, 9
ginger syrup + control (ethanol)

Comparing this list with the GLC traces of the relevant solutions (figs. 14, 18, 19 and 29-37) some correlation between concentration and attractiveness is evident, but this does not explain all the results. The essential oil of ginger, though diluted 1:10 with ethanol, was nonetheless highly concentrated compared to the fractions. However, both fractions 5 and 6 were more effective attractants, as was the steam distillate. Careful scrutiny of the GLC spectra shows that the group of sesquiterpenes which impart to ginger its characteristic smell (peak z) are poorly represented in ground ginger (fig. 18). The extract of ground ginger and fraction 1 are comparatively poor attractants and neither contain significant amounts of peaks 16-20. The steam distillate, however, is rich in these, as are the essential oil and fraction 6. Fraction 5 contains large amounts of one low-boiling point compound, traces of some others and a group of sesquiterpenes as major constituents, including a proportion of peak 20 (zingiberene). The table below shows the frequency of the peaks among the fractions.

PEAK NUMBER

FRACTION	3	5	6	7	9	10	11	12	13	14	15	16	17	18	20
WHOLE OIL	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲	▲
1							▲	▲	▲	▲	▲				▲
2					▲	▲		▲	▲				▲		
3	▲		▲								▲	▲			
4												▲	▲		
5												▲	▲		▲
6		▲		▲									▲		
7	▲										▲				
8														▲	
9															
TOTAL	2	1	1	1	1	1	1	2	2	1	3	3	4	1	2

Table 46. The occurrence of various peaks (numbered as in fig. 19 p. 118) in the whole essential oil and its fractions

(▲ = major constituent)

Peak 20 occurs in 2 of the nine fractions and, of the other peaks, number 17 occurs 4 times and 16 and 15, three. The hierarchy of attraction exhibited by the fractions is probably caused by a combination of factors. Some components are likely to be repellent and others attractive, such that the overall effect is dependent upon their relative concentrations in each fraction. Peaks 16-20 are likely attractants, but in fraction 1, which contains zingiberene, the preponderance of lower boiling compounds seems to counteract any attractiveness the sesquiterpene may exhibit. It may be that attraction to the fractions is a function of minor components present at concentrations too small to show on the GLC traces.

(d) Relative attractiveness of ginger fractions obtained by vacuum distillation of essential oil: Bendicks, 1979

(i) Comparative attractiveness of eight ginger fractions (I)

Method

Ten 'wasp-wizard' traps (Macrod Ltd.) were set up on 40-gallon drums at Bendicks chocolate factory as shown in fig. 42 Each trap was half-filled with water and an open vial containing a wick was taped to the roof. Apart from the control, whose vial remained empty, the others were treated with 2 drops of fractions of essential oil of Chinese ginger. The oil, obtained from Daniels Limited, had been separated into 50 fractions by means of vacuum distillation. Of these, 8 representative fractions were chosen for the bioa says: their GLC traces are shown in figures 43-50. pp. 169-174. The vial contents were as follows:

	Fraction Number
1	14
2	50
3	5
4	38
5	48
6	12
7	20
8	3
9	control
10	all fractions

After 24 hours the traps were inspected and the number of wasps and honeybees caught was recorded.

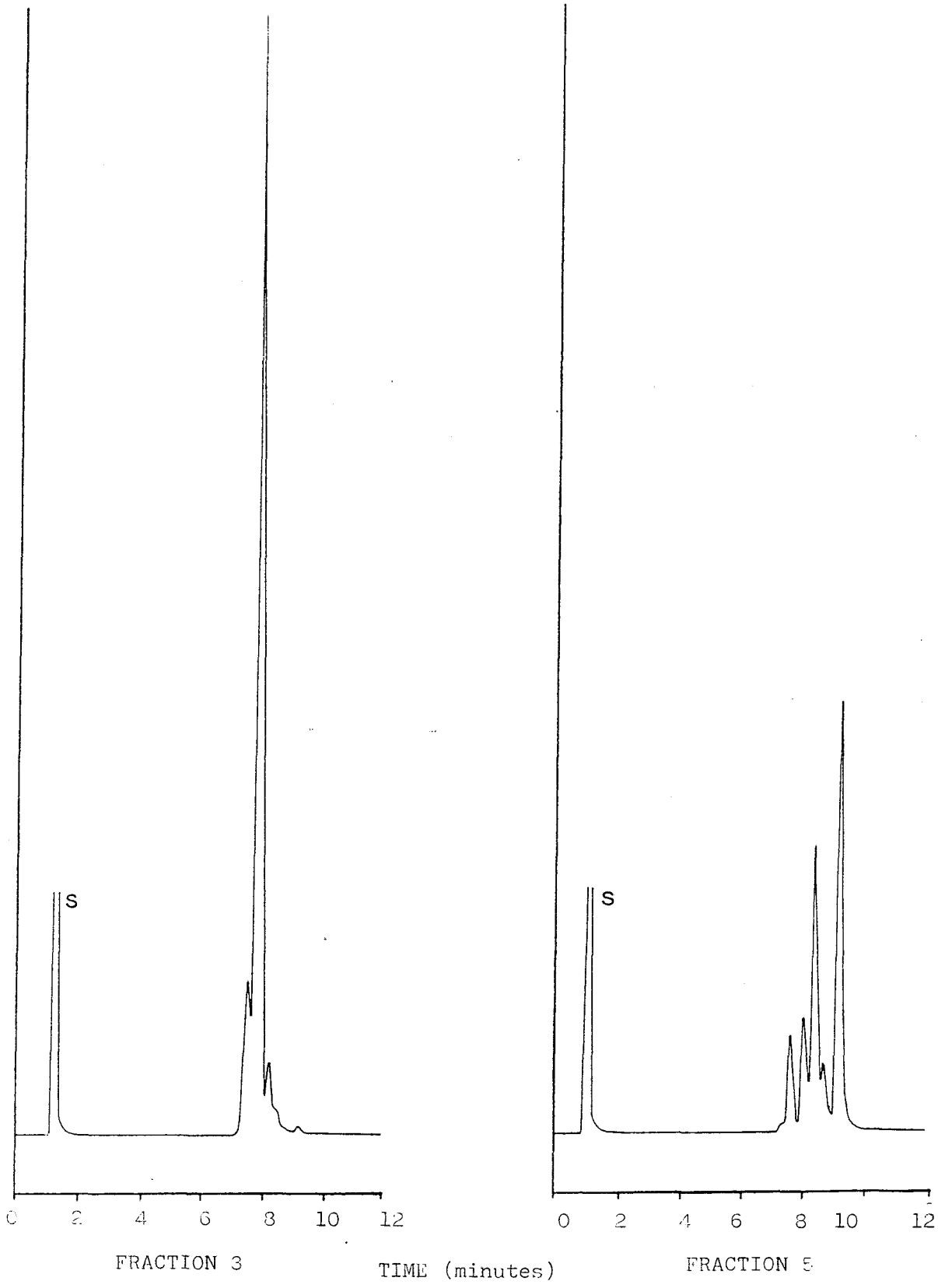
Results

Trap (fraction No.)	Number of insects trapped			
	vulgaris	*germanica*	*Apis*	Total
14	1	1	2	4
50				
5				
38				
48				
12	1			
20	4	2	2	8
3		1		1
Control				
Mixture		1		1

Table 47. Numbers of insects caught by various traps.

Observations indicated that, though numerous insects were attracted to some of the traps, the water inside was an ineffective killing agent. At one time (12.30, September 20 1979) 20 wasps and 2 bees were trapped in trap 20, but most escaped. This was a result of the water-repellent quality of their exoskeletons. The response,

Fig. 42. The 'Wasp-wizard' traps being primed with attractants at Bendicks.



Figs. 43. and 44. Chromatograms of essential oil of Chinese ginger: fractions 3 and 5. Peak 's' is solvent

Run conditions: column: OV101 (5%)
Temperature program: initial temperature 50°C increasing at 8°C per minute
Attenuation: 16,000

Figs. 45. and 46. Chromatograms of essential oil of Chinese ginger: fractions 12 and 14. Peak 's' and run conditions as in figs. 43,44.

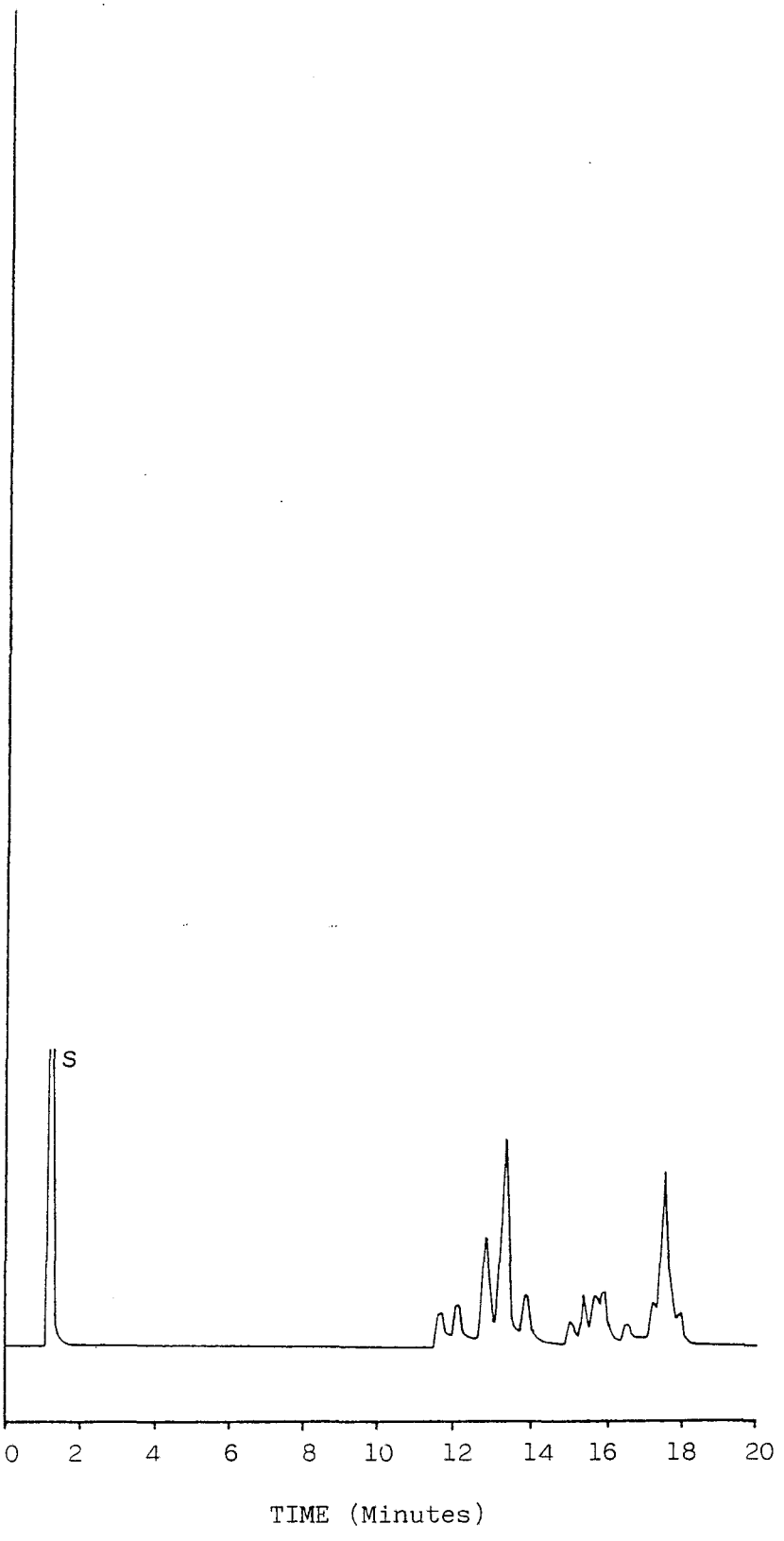


Fig. 47. Chromatogram of essential oil of Chinese ginger: fraction 20. Peak 's' and run conditions as figs. 43,44.

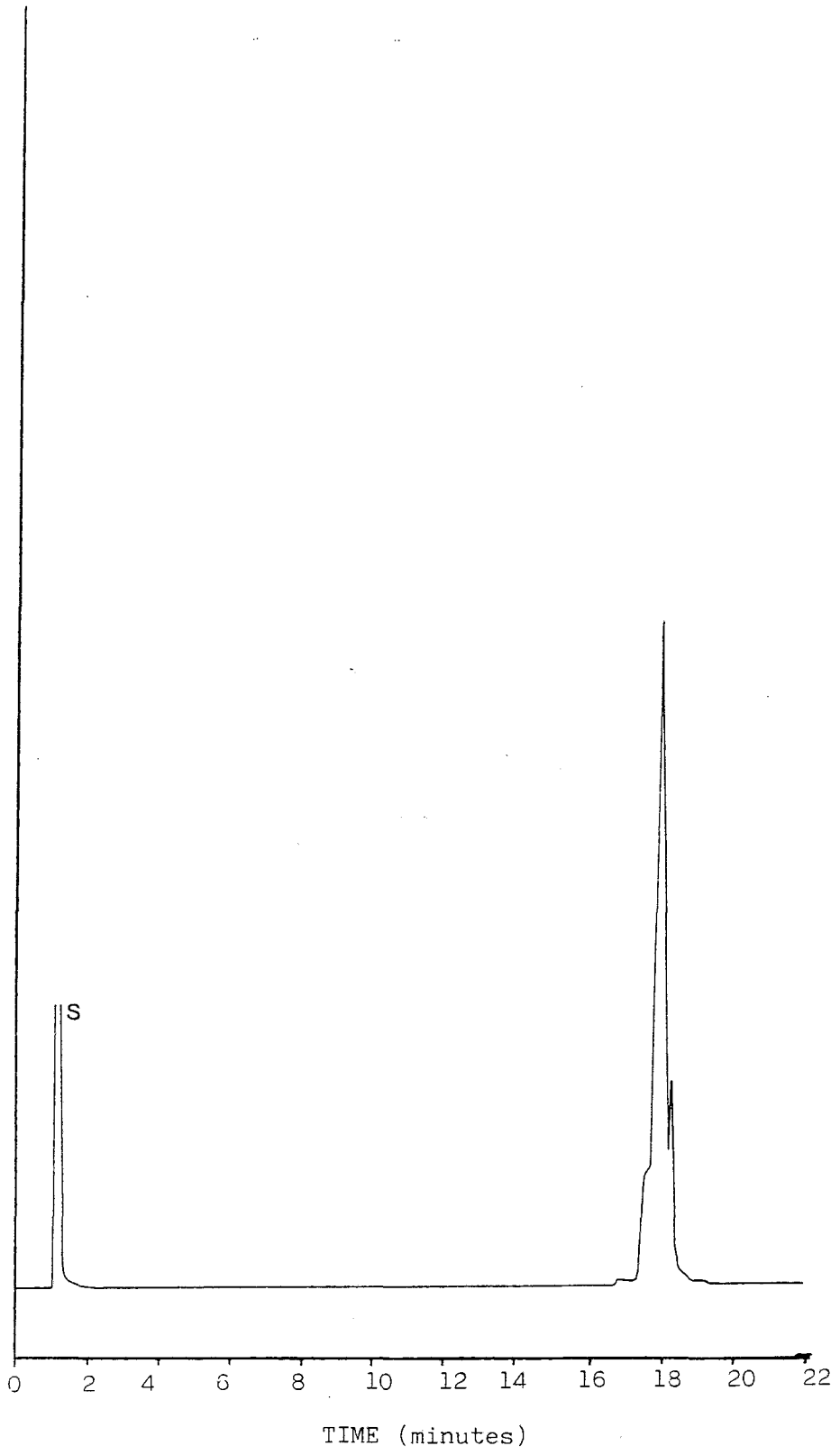


Fig. 48. Chromatogram of essential oil of Chinese ginger. Fraction 38. Peak 's' and run conditions as in figs. 43,44.

Fig. 49. Chromatogram of essential oil of Chinese ginger fraction 48. Peak 's' and run conditions as figs. 43,44.

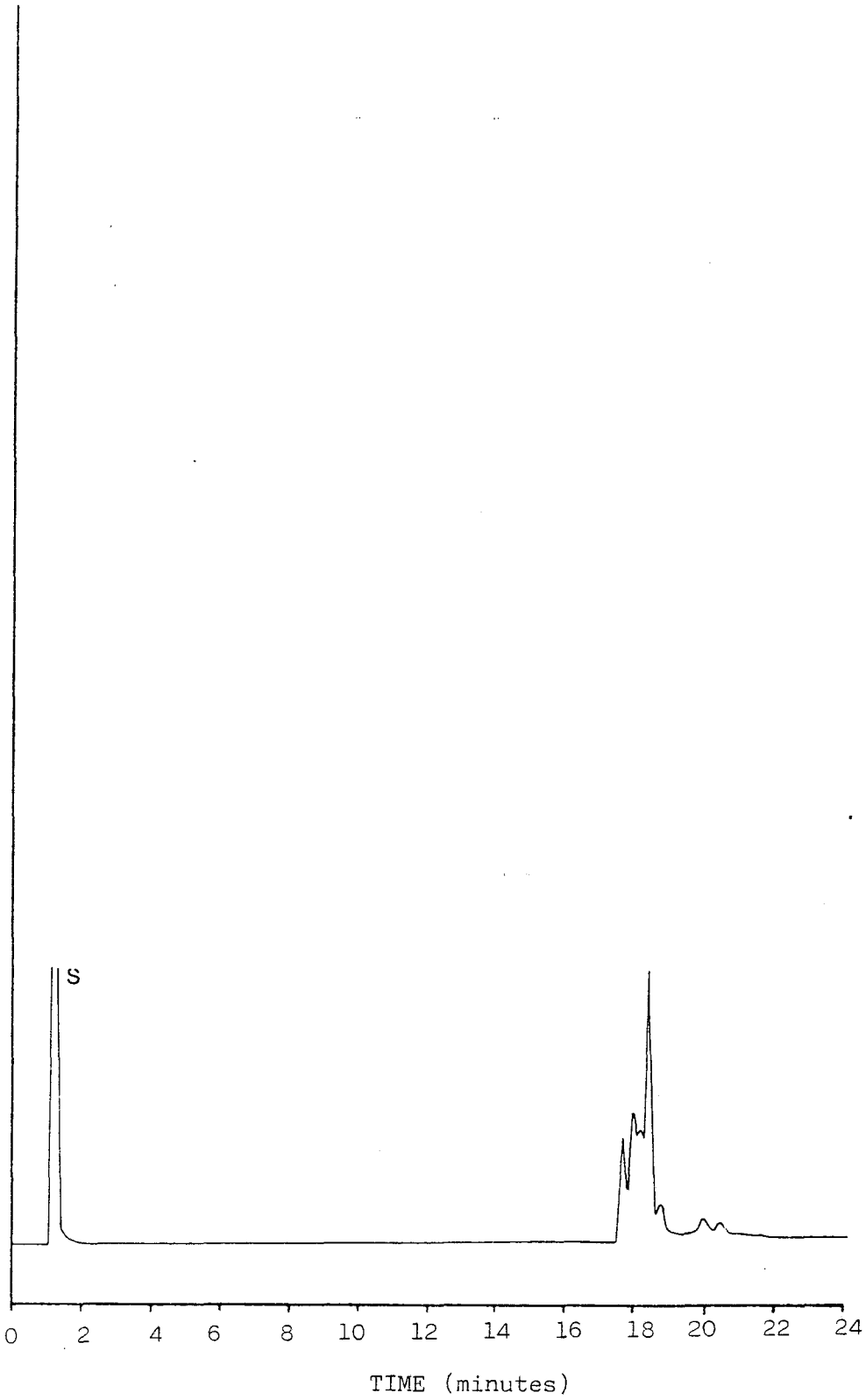


Fig. 50. Chromatogram of essential oil of Chinese ginger fraction 50. Peak 's' and run conditions as in figs. 43,44.

however, was sufficient to indicate that fractions 20 and 14 were the most attractive.

Conclusion

The traps were an effective means of capturing wasps and bees, but the water was a poor killing fluid. Fraction 20, which contains a mixture of sesquiterpenes including α-curcumene, γ-and δ-elemene and α-cubebene, was very attractive to bees and wasps, and fraction 14 was also attractive.

(ii) Comparison of various solutions used for killing wasps attracted to traps

Method

Six traps were set up as above, each containing a vial charged with 2 drops of fraction 20, but differing in the nature of its killing solutions, as shown in the table of results. The traps were moved one to the right every 15 minutes and the number of wasps trapped was recorded, together with the time each took to die.

Results

Vial No.	Nature of killing solution	No. of wasps caught in 1½ hours	Average time for death (seconds)
1	5% ethanol	0	
2	10% ethanol	7	120
3	20% ethanol	2	115
4	40% ethanol	0	
5	70% ethanol	1	
6	Water + .4ml 'Savona'	5	46

Table 48. Comparative efficiencies of various killing fluids.

Discussion

Although 10% ethanol caught the most wasps, the dilute detergent solution killed them more quickly. The higher concentrations of ethanol had an obvious repellent effect on wasps,

thus negating any benefit gained by quicker knock-down. Conversely 5% ethanol, although attracting more than the higher concentrations, was not sufficiently toxic to kill effectively, nor did it prevent insects from escaping. Wasps entering the detergent solution were rapidly wetted and submerged, to drown very quickly.

Conclusion

Savona detergent solution is an effective wetting agent and is not obviously repellent to wasps. Ethanol solutions are either too repellent or do not reduce the surface tension of water sufficiently to be useful in trapping insects.

(iii) Comparative attractiveness of eight ginger fractions (II)

Method

Ten traps were set up as described in experiment (d)(i) (p.166). To the water in each was added 0.4 ml (20 drops) of 'Savona' industrial liquid detergent. Eight vials were charged with a 10% solution of the ginger oil fractions in 100% ethanol, the other two containing a 10% solution of a mixture of the 8 fractions and 100% ethanol respectively. The vials were placed underneath the traps, attached by plasticine to plastic petri-dishes, such that the wicks protruded just below the entrance holes. Every 15 minutes the traps were moved one to the right and observations were maintained for 3 and a quarter hours, after which the contents of every trap were examined and the insects counted.

Results

The numbers of wasps caught in the traps are depicted graphically in figure 51 (p. 178). Honeybees were comparatively scarce: the numbers caught are shown below:

Trap (fraction) Number	Number of honeybees caught in 3.25 hrs.
14	2
50	1
5	2
38	1
48	1
12	5
20	5
3	4
Control	0
Mixture	0
Total	21

Table 49. Numbers of honeybees caught in various traps.

During the experiment the weather was warm and sunny and there were many wasps in the vicinity, *vulgaris* and *germanica* being present in approximately equal numbers.

The difference between the total numbers of *vulgaris* and *germanica* is significant ($p < 0.05$, χ^2 test) and an overall comparison of the trap totals of *germanica* yields a highly significant result ($p < 0.001$, χ^2 test).

Fraction 20 was the most attractive to *germanica*, the difference between this and the next most effective attractant, fraction 12, being highly significant ($p < 0.001$, χ^2). Fractions 12, 14, 5 and 3 and the mixture were not significantly different from the control, but fractions 38, 48 and 50 were less attractive (for fraction 38/control, $p < 0.001$, χ^2).

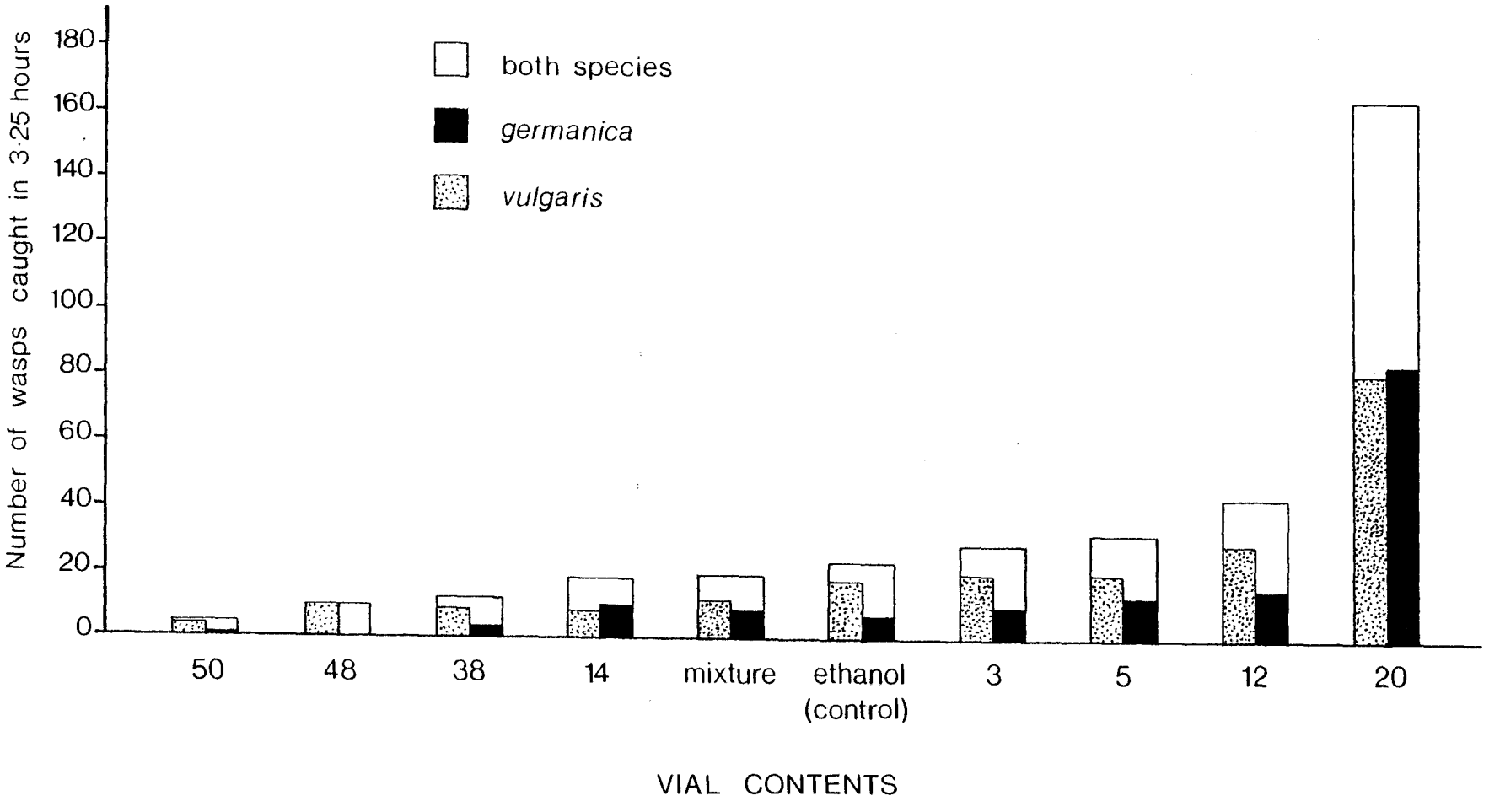


Fig. 51. Comparative attractiveness of ginger fractions (II). Total catches of wasps.

The overall comparison of the trap totals of *vulgaris* is also highly significant ($p < 0.001$, χ^2) and fraction 20 was again the most attractive ($p < 0.001$, χ^2). Apart from fraction 20 and fraction 50, which was less attractive than the control ($p < 0.001$, χ^2), all the others were not significantly different.

Fractions 20, 14, 50 and the mixture attracted similar numbers of both species of wasp, but the others lured significantly more *vulgaris*.

The total catches of wasps taken from the graph (fig. 51 p. 178) can be arranged in a hierarchy of attractiveness relative to the control:

Fraction Number	Relative attractiveness (control = 1 unit)
50	0.2
48	0.4
38	0.5
14	0.8
Mixture	0.8
Control	1
3	1.1
5	1.3
12	1.8
20	7.1

Table 50. Relative attractiveness to wasps of different ginger fractions.

Fractions 12 and 20 caught significantly more honeybees than the control ($p < 0.05$, binomial test).

Discussion

As in experiment (d)(i) (p. 166), fraction 20 caught more wasps than the others, but this time fraction 14 did less well, trapping fewer insects than the control. The fact that most of the traps caught more *vulgaris* than *germanica* indicates that these

fractions were less attractive to the latter. Fraction 20 was equally attractive to them both.

Fractions 38, 48 and 50 were less effective as lures than absolute ethanol, and fraction 48 caught no *germanica*. The mixture of fractions was comparatively unattractive, presumably because its attractive principles were counteracted by some repellent ones.

Conclusion

Fraction 20, containing α-curcumene and other sesquiterpenes, was the most attractive of those presented and it caught nearly equal numbers of *vulgaris* and *germanica*. Individuals of *vulgaris* appeared to be attracted more than *germanica* to most fractions.

(iv) Comparative attractiveness of eight ginger fractions (III)

Introduction

Following the two preliminary experiments in the series this study was designed to give an accurate idea of the relative attractiveness of each fraction and to highlight the differences between species.

Method

The traps were set up as before (experiment (d)(i) p. 166) each being half-filled with water to which was added 0.4 ml of savona liquid detergent. One vial was filled with 100% ethanol to act as the control and the others contained a 10% solution in ethanol of different ginger fractions. The traps were set out at 0800 hours daily for 10 days.

The vial contents were as follows:

	Vial Contents
1	10% solution in ethanol of fraction 14
2	10% solution in ethanol of fraction 50
3	10% solution in ethanol of fraction 5

	Vial Contents
4	10% solution in ethanol of fraction 38
5	10% solution in ethanol of fraction 48
6	10% solution in ethanol of fraction 12
7	10% solution in ethanol of fraction 20
8	10% solution in ethanol of fraction 3
9	100% ethanol
10	10% solution in ethanol of a mixture of all the above fractions.

The traps were left for 24 hours in the same positions and were then replaced by an identical set containing fresh water and wetting agent, placed over fresh vials of attractant. The used traps were then emptied, and washed ready for the next day, the insects they had caught having been identified and scored.

The experiment lasted for 10 consecutive days, during which time the traps were placed in different positions daily, according to a randomly generated Latin square design. This ensured that each trap had been in every position in the row, and that its position relative to the others had varied throughout the period of study. To ensure homogeneity of the fractions under test the solutions were made up before the experiment began and were divided into ten aliquots apiece, which were kept under refrigeration until required.

Results

1. General observations and remarks

DAY 1 (21 SEPTEMBER 1979)

Large numbers of both species flying in the area and foraging, with a few honeybees, in the waste bins. Numbers of each species of wasp approximately equal.

Weather - Starting cold (no frost), becoming warmer and sunny but with increasing cloud. Showers after 1630. Wind W, moderate.

DAY 2

Small numbers of sluggish wasps at 0800 but numbers increasing to c.100-200.

Weather - Starting cold (perhaps a frost), becoming warmer and sunny. Some cloud in afternoon. Wind cold NW, moderate.

DAY 3

Numerous wasps of both species (perhaps more *germanica*) present by midday. A few honeybees.

Weather - Overcast but quite warm. Wind SW light to moderate.

DAY 4

Large numbers of both species of wasp, but few honeybees.

Weather - Warm when sunny. Occasional cloud. No wind.

DAY 5

By 1000 over 200 wasps at drum of waste ginger which had been disturbed yesterday to reveal fresh syrup. Wasps continually flying to and from foraging site in at least 3 directions, all to west of factory.

Weather - Cool with freshening SW wind.

DAY 6

Up to 500 wasps at foraging site by midday and much activity around ginger drums and traps.

Weather - Warm but overcast. Some bright spells, becoming drizzly. Wind moderate, gusting to strong SW.

DAY 7

Rain overnight causing tops of drums to fill with water. Numerous wasps, both on ginger and around traps. Many basking in sun on tops of bins, near traps. Wasps increasing in number as more waste was put in bins.

Weather - Warm, becoming sunny by noon. Wind SE, light.

DAY 8

Hundreds of wasps on ginger and around traps.

Weather - Cold start, becoming warm and sunny. Wind SE, light.

DAY 9

Wasps as numerous as on day 8, but honeybees fewer.

Weather - Warm and sunny with very light NE wind.

DAY 10 (30 SEPTEMBER 1979)

Hundreds of wasps at foraging site, but honeybees very scarce. Numbers of *vulgaris* and *germanica* approximately equal.

Weather - Overcast but mild and calm.

2. Trapping data

(a) All insects

Apart from wasps and honeybees, the catch from each trap included various species of Diptera. For the purposes of this experiment, these were not identified, but were all included under the category of 'flies'. The totals over the 10 days, for each of the 4 categories of insect, are shown below:

Insects	Total Catch	Percentage of grand total	
V. vulgaris	962	33	49
V. germanica	477	16	
Honeybees	263	9	
Flies	1,226	42	
GRAND TOTAL	2,928	100	

Table 51. Numbers of insects caught in 10 days.

The actual numbers of insects in the vicinity of the traps were impossible to ascertain, although their relative proportions could be estimated. Thus wasps appeared, on average, to outnumber honeybees by about five to one. However, it was difficult to judge the proportions of *vulgaris* and *germanica* because of the similarity of their markings and size. Certain *germanica* workers were more

brightly coloured than their *vulgaris* counterparts and tended to be larger, but these differences were not obvious 'en masse'. On the basis of the number of nests located close to the foraging site (see p. 215) the ratio of *germanica* to *vulgaris* during the course of the experiment was estimated to be 1:2.

The proportions of Diptera compared to Hymenoptera differed according to the time of day, many of the former being trapped at night.

No Lepidoptera were caught.

The total daily catches of the different insects are shown graphically in figure 52 (p.185). The maximum catch for all categories occurred on day 1, with other maxima occurring on days 5, 7 and 9. The minima, on days 3, 6 and 10, coincided with low light intensities, the skies being overcast. The peak on day 5 coincided with a general increase in the numbers of insects at the foraging site, following the stirring-up of a barrel of waste ginger to reveal fresh syrup at the close of day 4.

The graphs for wasps and flies are very similar and have maxima and minima on the same days. Honeybees, though sharing the same peaks and troughs as the others initially, fall off in numbers earlier and do not peak on day 9.

The numbers of all insects caught in each trap are depicted in a bar graph (Fig. 53 p.186) the fractions being arranged in ascending order of attractiveness. Fractions 3, 5, 50 and 48 do not differ significantly in attractiveness from the control (χ^2 two sample test), but the rest do (for fraction 38 $p < 0.05$, χ^2 two sample test). Fraction 20 was considerably more attractive than the others, the differences between it and fraction 14 being highly significant ($p < 0.001$, χ^2 two sample test).

The relative attractiveness of each fraction to the different types of insect can be compared in table 52 (p. 187)

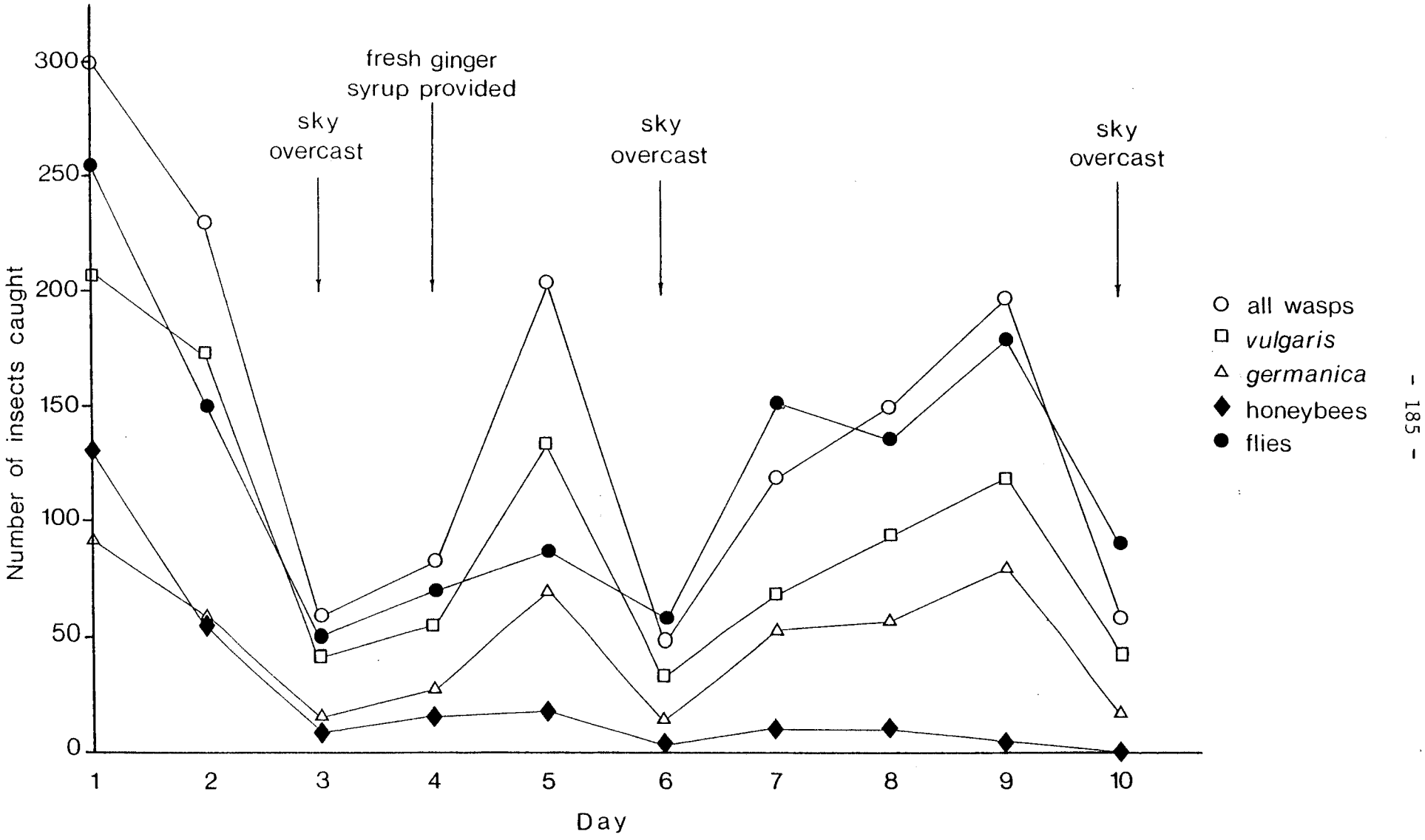


Fig. 52. Comparative attractiveness of ginger fractions (III). Total daily catches of insects.

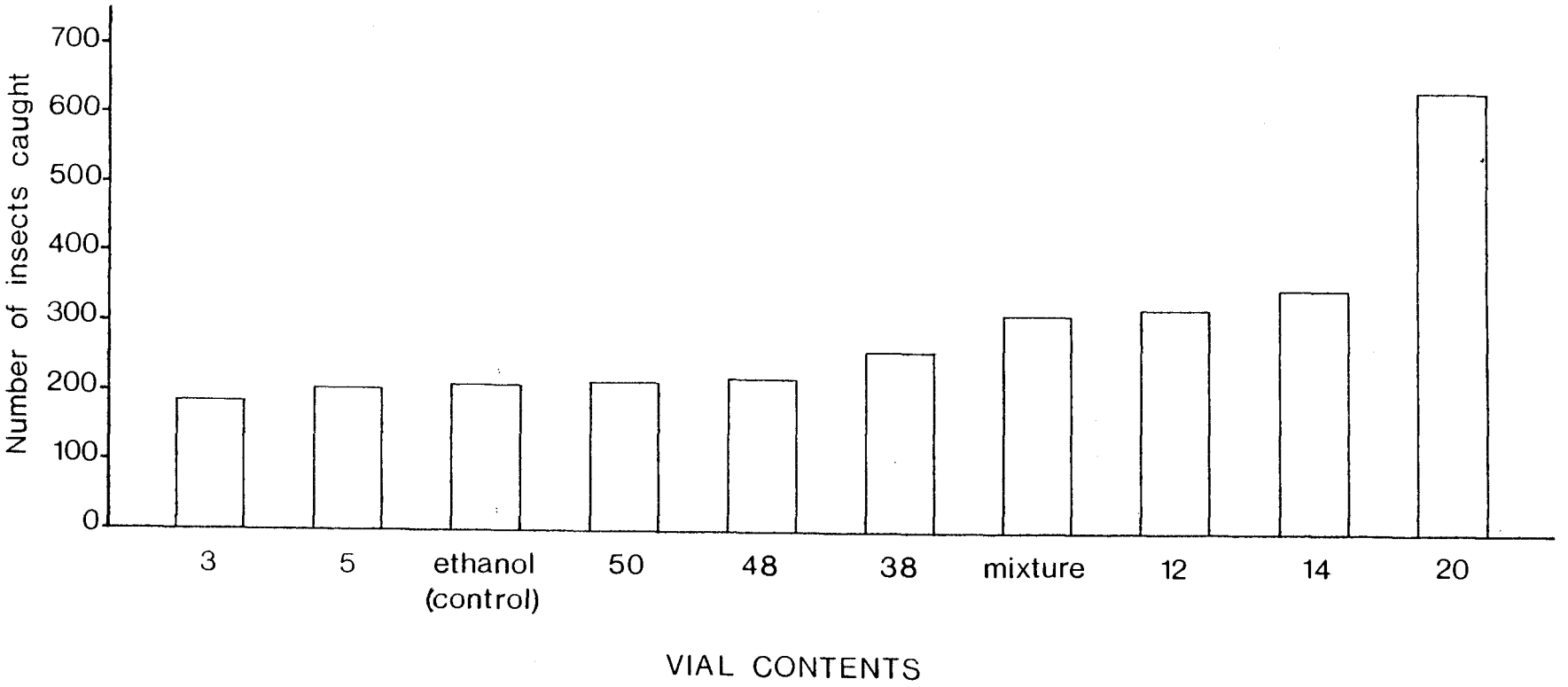


Fig. 53. Comparative attractiveness of ginger fractions (III). Total catches of insects.

	Most attractive fraction> Least attractive fraction									
Insect	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
V. vulgaris	20	mix	14	5	3	38	12	con	48	50
V. germanica	20	14	mix	12	38	48	50	5	con	3
Honeybess	14	12	20	mix	38	3	5	48	50	con
Flies	20	12	38	50	con	48	mix	14	5	3

Table 52. The relative attractiveness of the fractions. (mix = mixture of fractions; con = control).

Fraction 20 was the most attractive to wasps and flies (*germanica*, $p < 0.01$: *vulgaris* and flies, $p < 0.001$, χ^2 two sample test) but there was no significant difference between the numbers of honeybees caught in traps baited with fractions 14, 12, 20 an mixture.

(b) Wasps

These comprised nearly half the insects caught during the 10 day period (table 51 p. 183) and two-thirds of them consisted of *V. vulgaris*. The graph showing the numbers of wasps caught per day in all the traps (fig. 52 p. 185) illustrates similar trends for both species, the peaks and troughs generally coinciding. Numbers of both species were down on dull days (3, 6 and 10) but up on day 5 when fresh ginger was available.

Figure 54 (p. 189) is a bar graph showing the total numbers of wasps, and the totals of the two separate species, caught in each trap throughout the 10 day period. The fractions are arranged in order of attractiveness, based on the combined totals for both species.

Fraction 20 was the most attractive, the difference between this and the next being highly significant (*vulgaris*, $p < 0.001$: *germanica*, $p < 0.01$, χ^2 two sample test). Fraction 14 and the mixture attracted similar numbers of *vulgaris* and are significantly different from the control ($p < 0.05$, χ^2 two sample test) but none of the other fractions differ significantly from the control.

Fraction 14 attracted significantly more *germanica* than the mixture ($p < 0.05$, χ^2 two sample test), both these differing significantly from the control. The other fractions were not significantly better at attracting *germanica* than the control. Comparing the two species, differences in the relative attractiveness of the fractions are apparent in the graph and these are summarised in table 53 (p. 186). If all the fractions and their catches of wasps are compared by means of a χ^2 test, these differences are shown to be highly significant ($p < 0.001$). Figures 55 - 64 (pp. 190-199) show the daily catches of both species of wasp for each trap and highlight the differences in attractiveness.

Figure 52 (p.185) shows how the total catch varied from day to day. The minimum coincident with dull weather on days 3, 6 and 10 are especially prominent, as are the peaks on days 1, 5, 7 and 9. However, whereas wasps were caught in significantly larger numbers on day 5, the dipteran peak is considerably less pronounced.

Assuming that the wasps were normally present in the proportions 2 *vulgaris* to every 1 *germanica* and that both species were equally attracted to the different fractions, then each trap should have caught the wasps in these proportions. However, in this experiment, such was not the case. The total catch of *germanica* for each trap is expressed in table 53 below as a percentage of the total wasps caught in each trap:

Fraction	Total catch of *germanica* expressed as a % of wasps in the trap	Significance (compared to expected catch of 33.3%
3	15	**
5	18	**
Control	22	NS
38	31	NS
48	31	NS
Mixture	33	NS
50	33	NS
12	36	NS
20	39	NS
14	46	*

Table 53. Percentages of *germanica* caught in traps over 10 days. (NS = not significant; * = $p < 0.05$; ** = $p < 0.01$; χ^2 test).

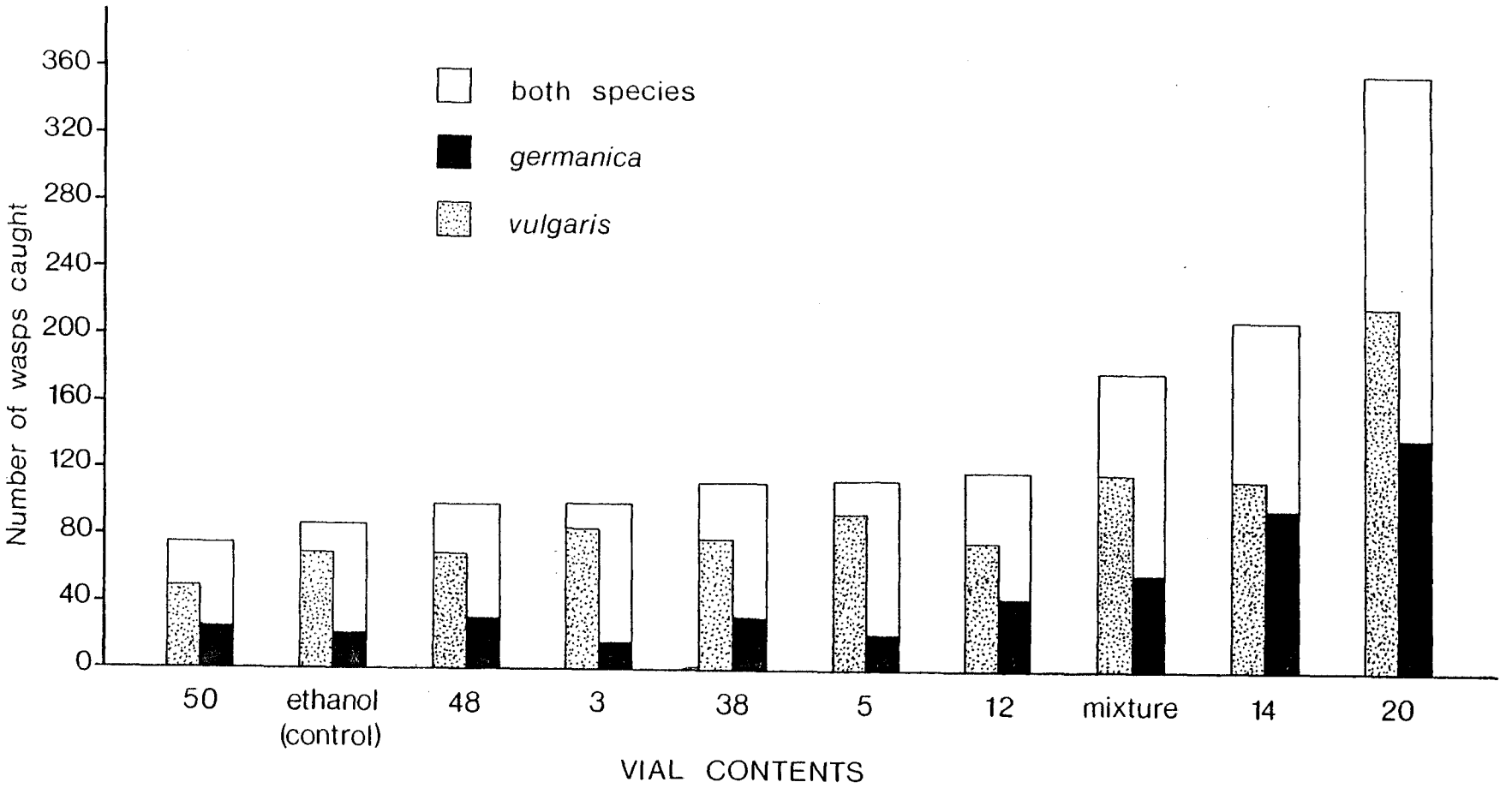


Fig. 54. Comparative attractiveness of ginger fractions (III). Total catches of wasps.

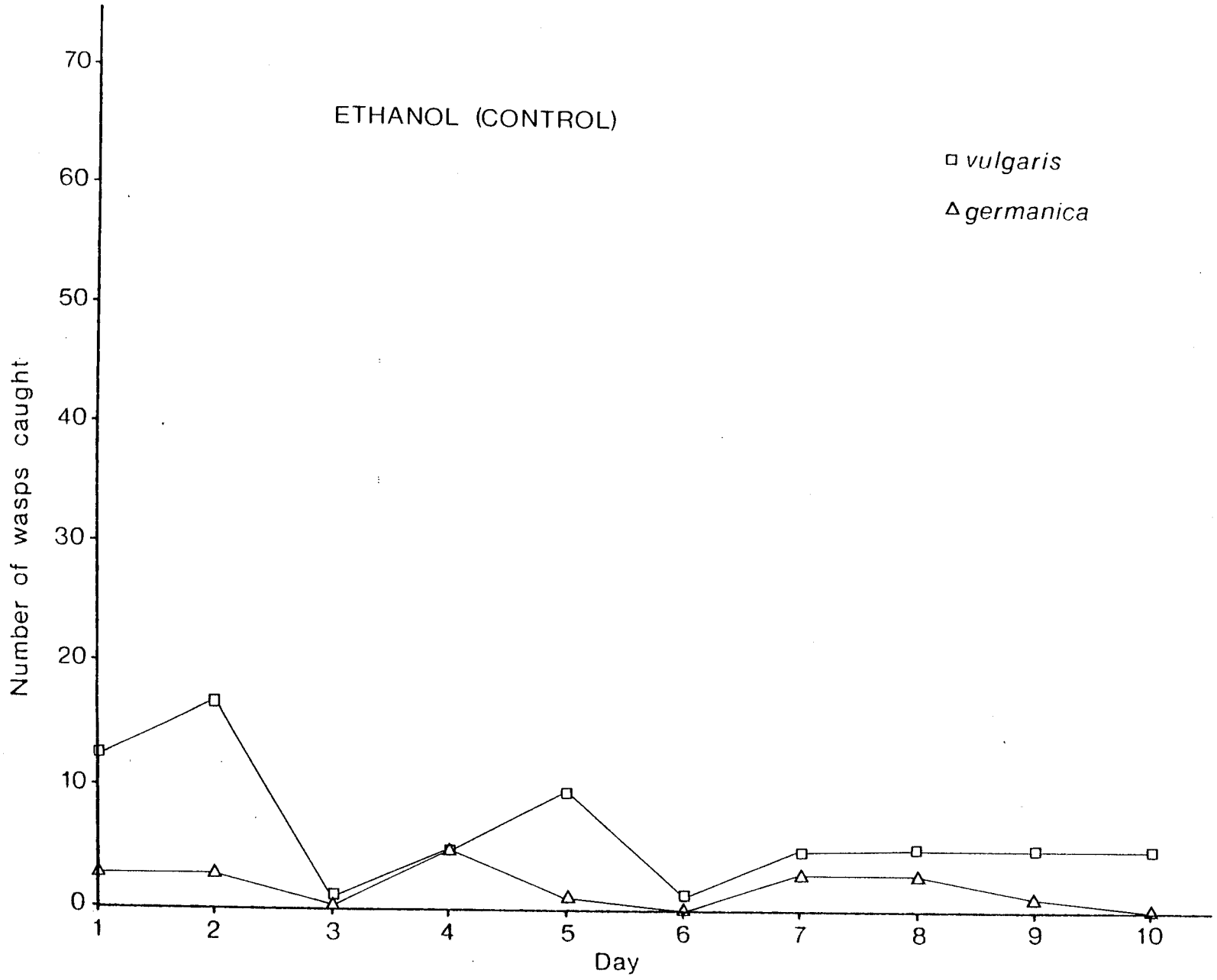


Fig. 55. Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

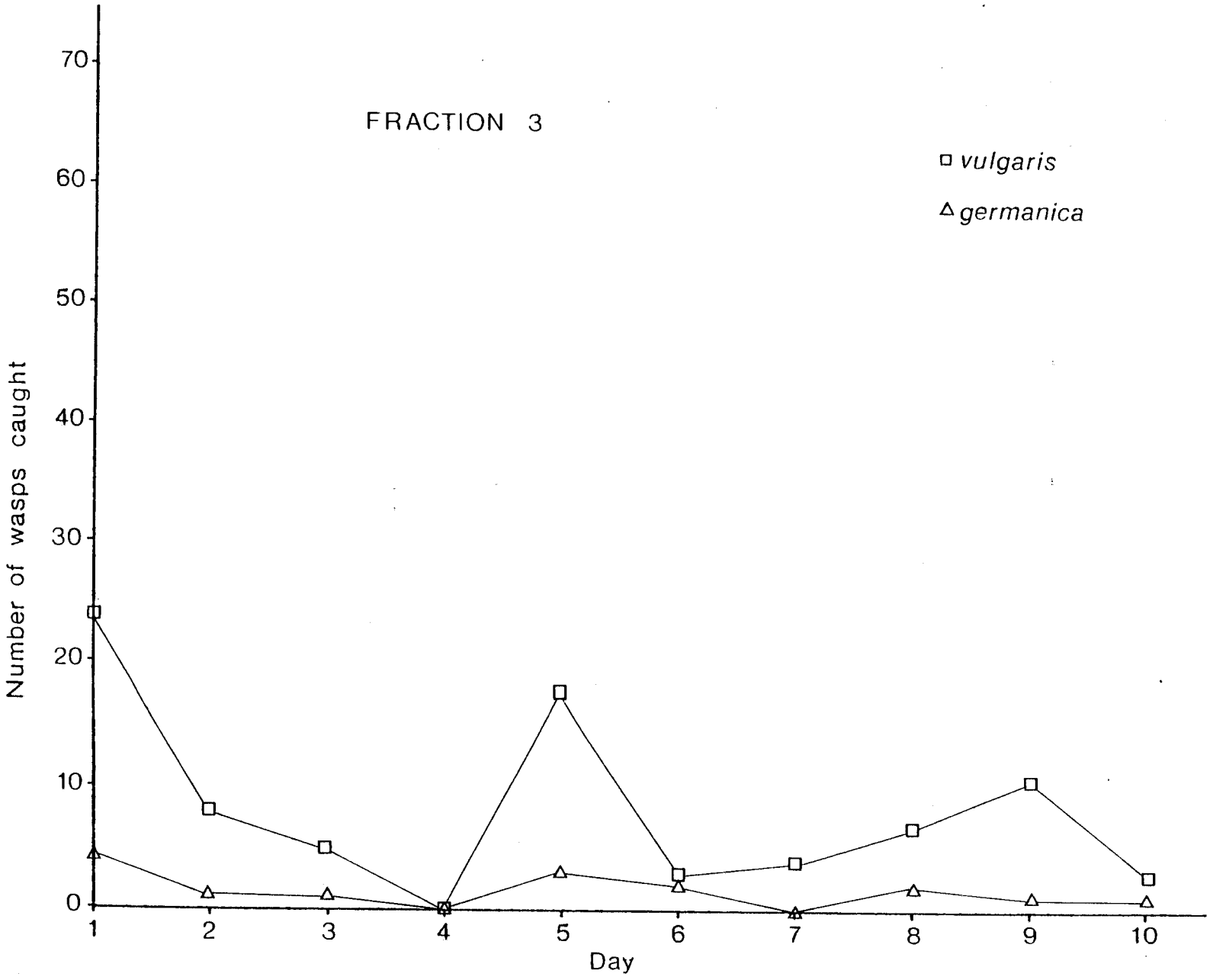


Fig. 56. Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

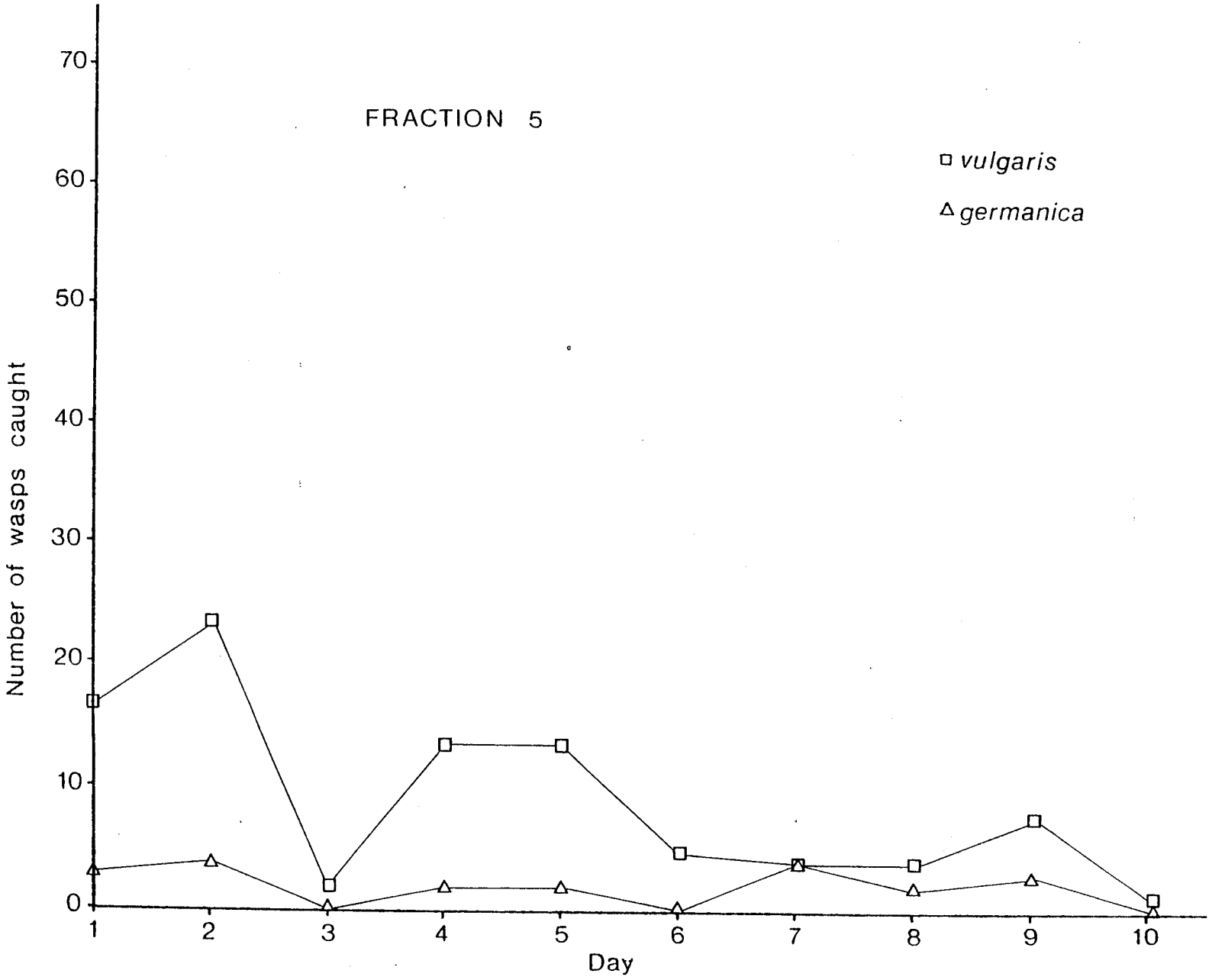


Fig. 57. Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

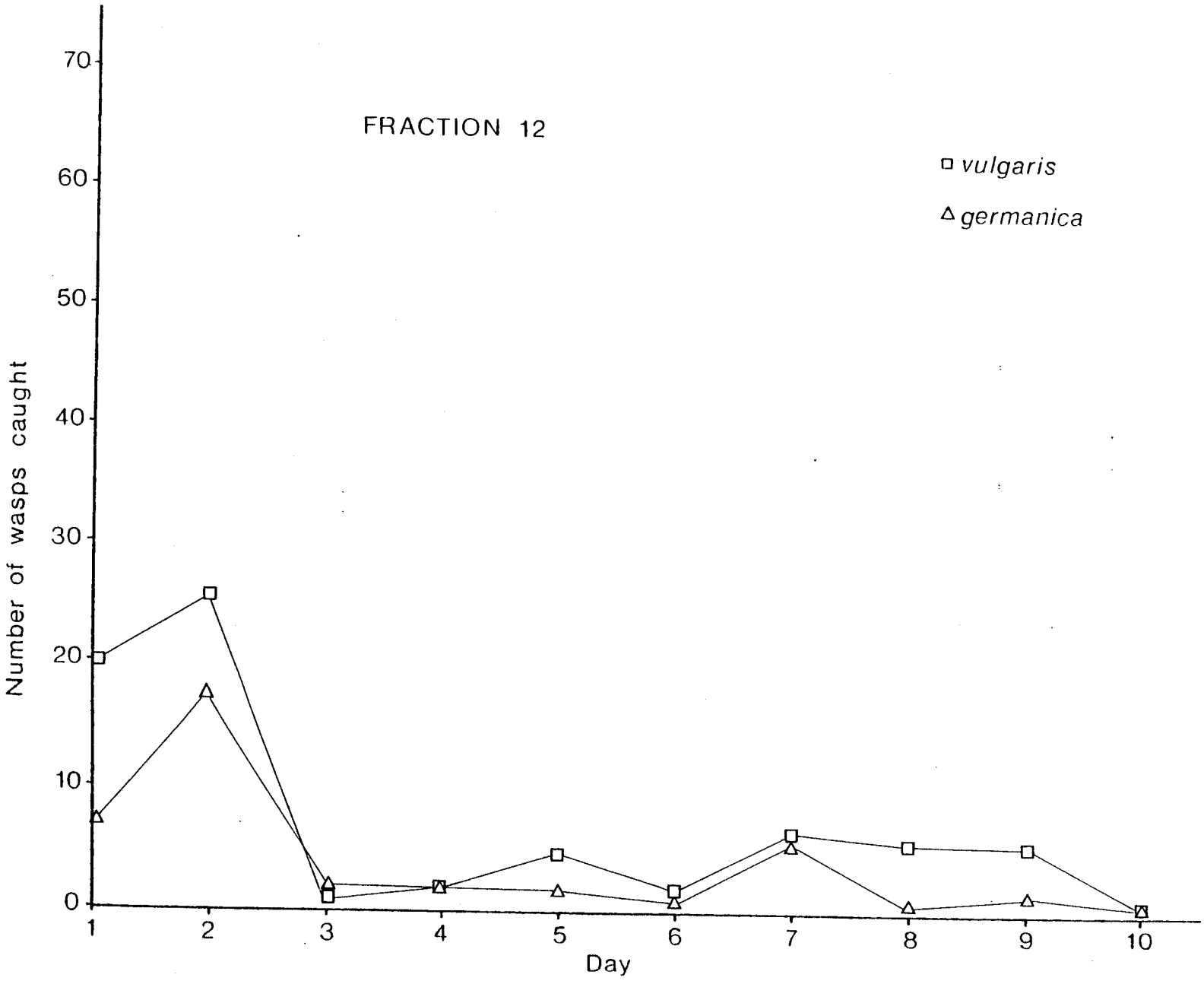


Fig. 58 Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

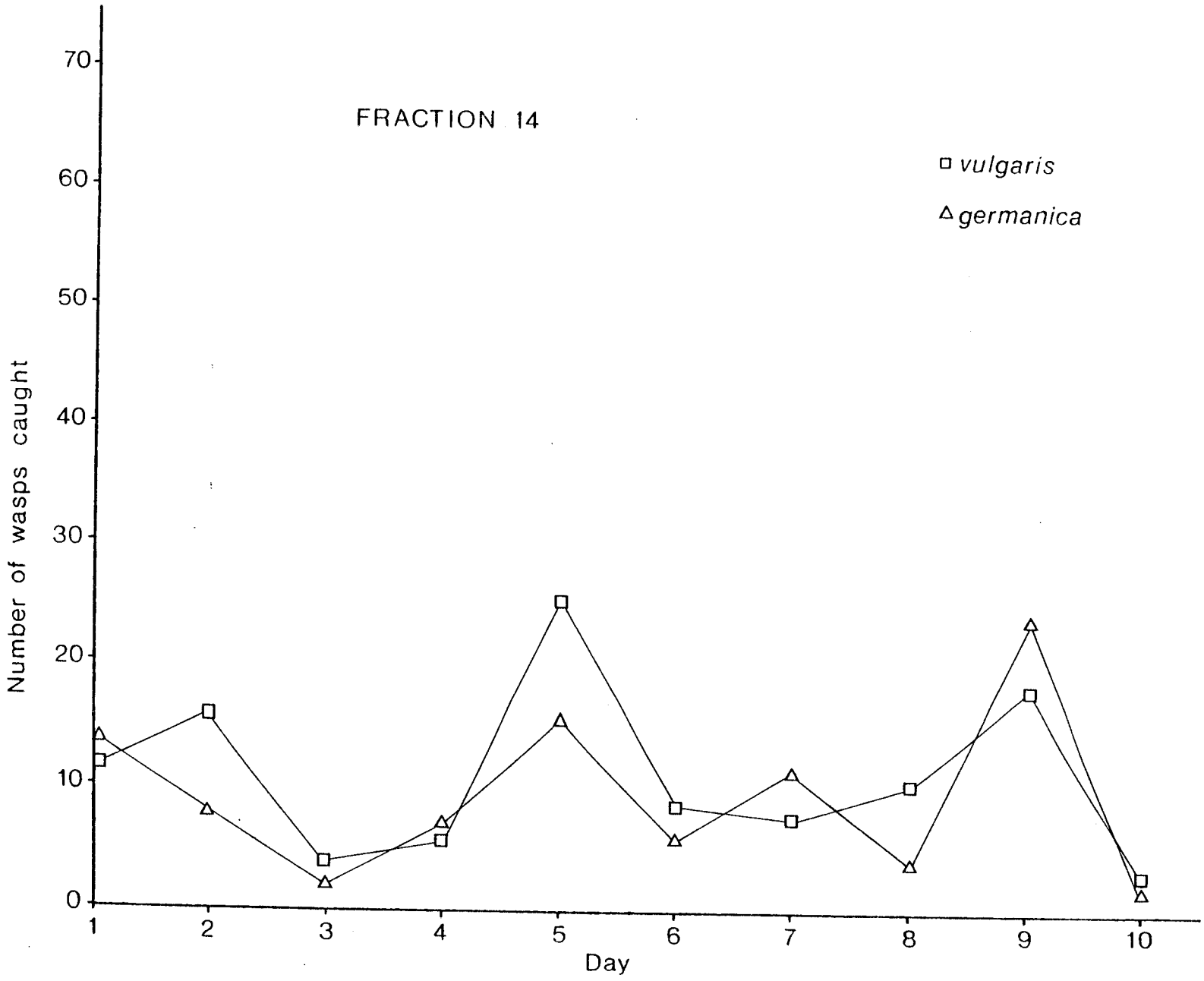


Fig. 59. Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

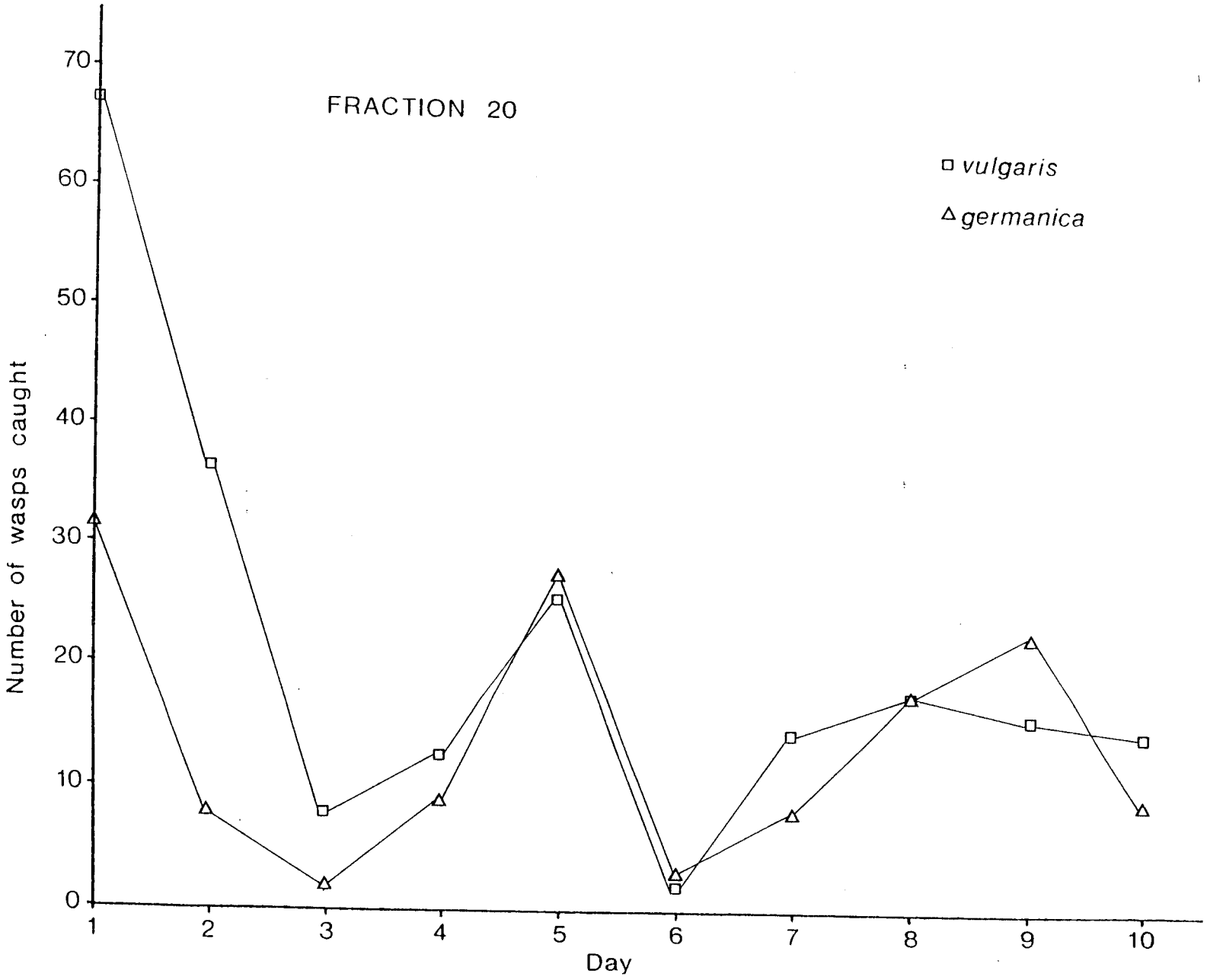


Fig. 60. Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

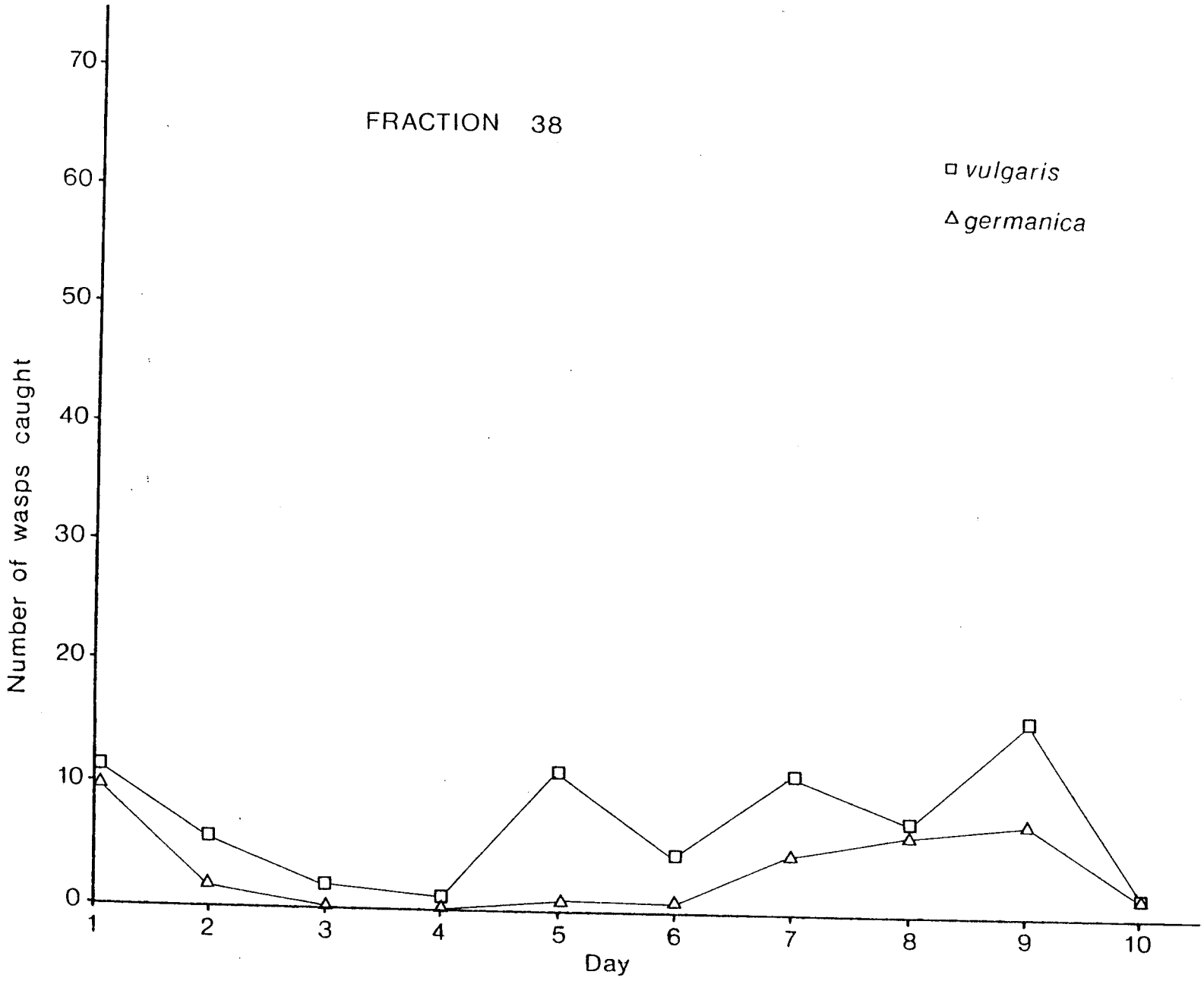


Fig. 61. Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

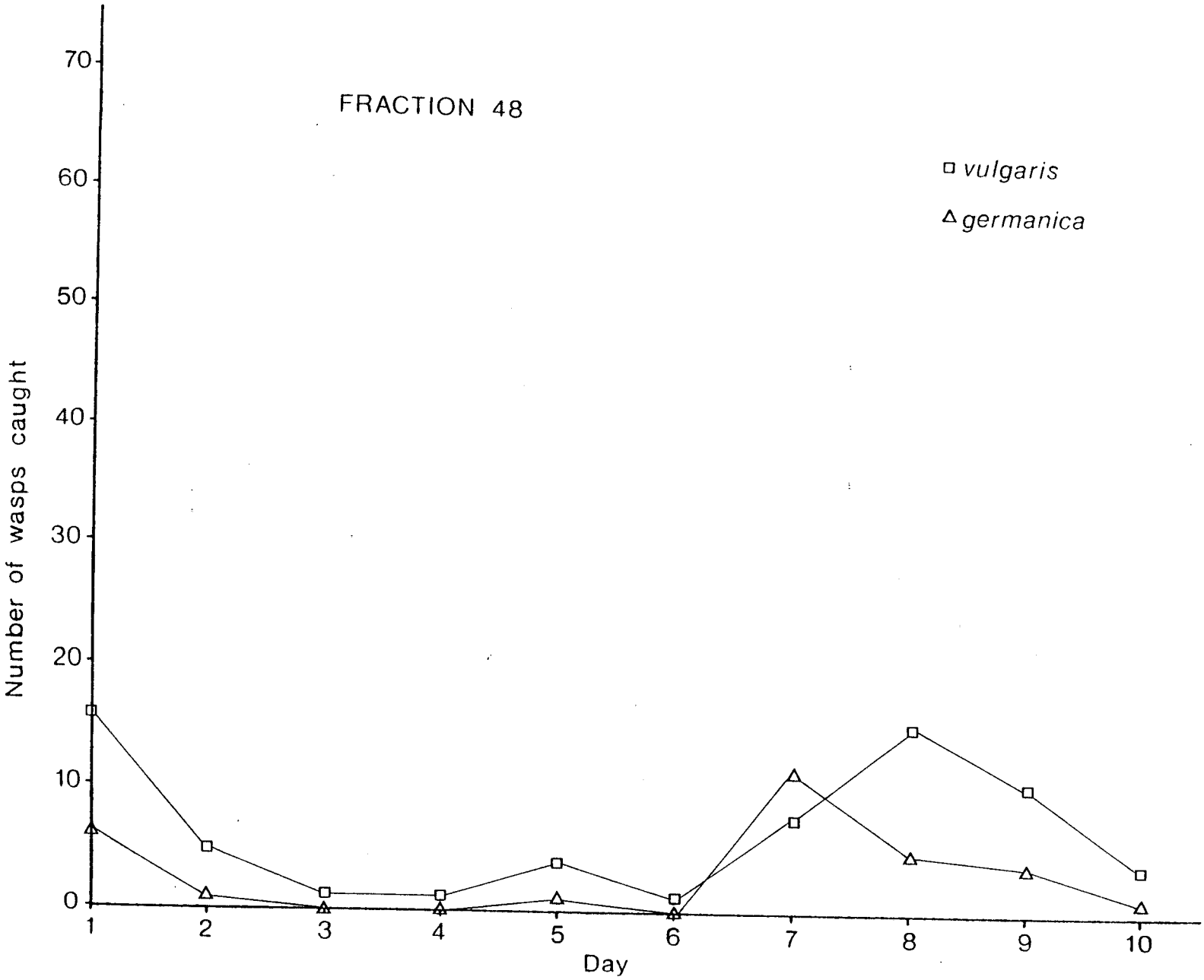


Fig. 62. Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

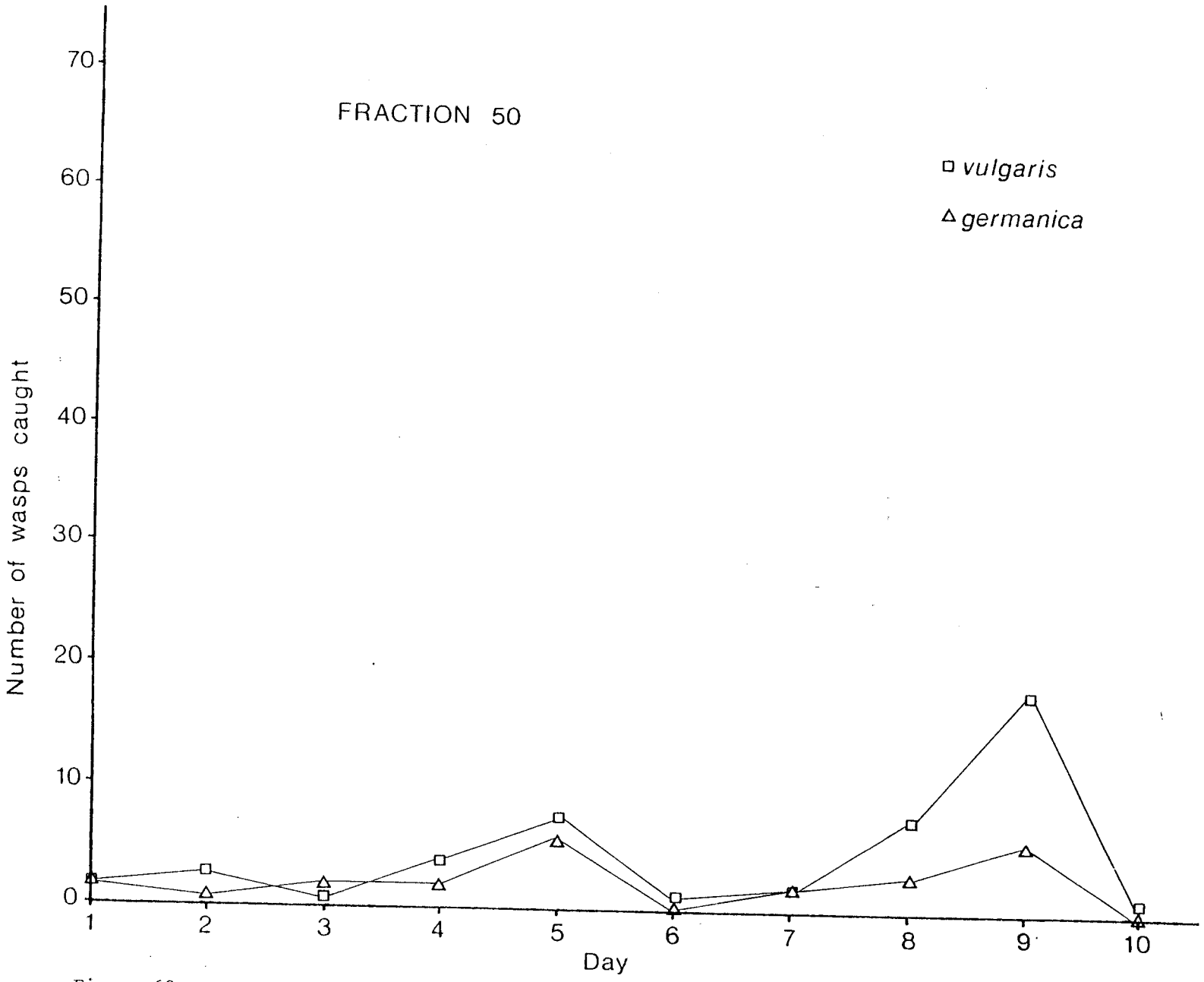


Fig. 63. Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

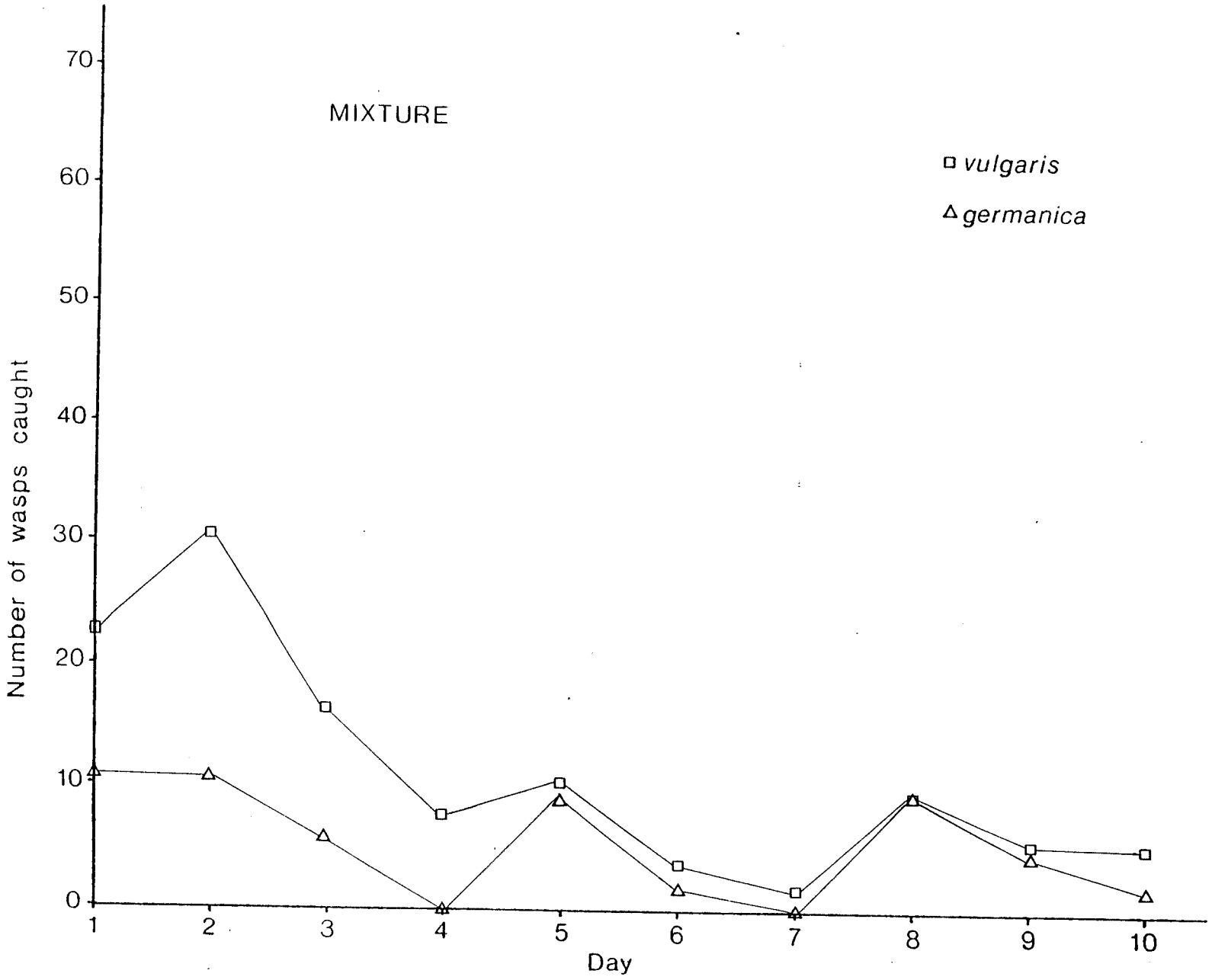


Fig. 64. Comparative attractiveness of ginger fractions (III). Daily catches of wasps.

The numbers of *germanica* attracted to most of the fractions, including the control, did not differ significantly from the expected catch of 33.3%, but fractions 3 and 5 caught fewer than expected and fraction 14 more.

Although the graphs in figure 52 (p. 185) indicate that rather more wasps were caught in the first 5 days than in the last, this downward trend was not reflected in all the fractions. Expressing the numbers of wasps caught in the last 5 days as percentages of the whole catch in each category, the values are 40% for all wasps, 45% for *germanica* and 37% for *vulgaris*. The percentages of wasps caught in each trap in the latter half of the experiment are tabulated in table 54 below.

Taking 37% to be the expected percentage catch of *vulgaris* in the latter half of the 10 days it can be seen that fraction 5 and the mixture attracted significantly fewer wasps, but fractions 38, 48 and 50 attracted significantly more. Figures 57, 61, 62, 63 and 64 illustrate this.

The figures from *germanica* differ from these. Taking 45% to be the expected catch for the last 5 days, fraction 12 attracted fewer wasps than expected and fractions 38 and 48 considerably more. Figures 61, 62 and 62 illustrate t

Trap (Fraction) No.	Percentage of wasps caught in last five days			
	vulgaris	Significance	*germanica*	Significance
14	44	NS	51	NS
50	63	***	46	NS
5	24	*	45	NS
38	57	**	62	*
48	60	***	73	***
12	29	NS	26	**
20	30	NS	44	NS
3	34	NS	40	NS
Control	31	NS	37	NS
Mixture	24	*	33	NS

Table 54. The catch of wasps in the latter half of the experiment expressed as a percentage of the whole. (NS = not significant; * = $p < 0.05$; ** = $p < 0.01$ *** = $p < 0.001$ compared to expected value of 37% for *vulgaris* and 45% for *germanica*; χ^2 test).

(c) Honeybees

Honeybees were comparatively scarce during the time of the experiment and comprised only 9% of the total insects caught. The graph in figure 52 (p. 185) shows that, as for wasps and flies, the catch was initially high but progressively decreased thereafter, and it had become very small by day 3. As with the other insects, the number of honeybees reached minima on days 3, 6 and 10 and peaked on day 5.

The cathc for each trap over the 10 days is shown graphically in figure 65 p. 202, the fractions being arranged in ascending order of attractiveness. The four fractions 12, 14, 20 and the mixture were the most attractive to honeybees and statistical analysis shows that the differences between them were not significant at $p = 0.05$ (χ^2 test). However, all the other fractions were significantly less attractive than these ($p < 0.05$, χ^2 two sample test on fraction 30 and the mixture) and did not differ significantly from the control (at $p = 0.05$, χ^2 test). The order of attractiveness of the fractions is tabulated in table 52 p. 185.

(d) Flies

Various families of Diptera were represented and no attempt was made to separate the different species. Included in the daily catches were various nematocerans, muscids and drosophilids, some of which had entered the traps at night.

Figure 66 (p.204) is a bar graph showing the trap totals for flies, arranged in ascending order. Fraction 20 was the most attractive, the difference between this and the total catch from fraction 12 being highly significant ($p < 0.001$, χ^2 two sample test). With the exception of fraction 3, the others (including 12) were not significantly different from the control in their attractiveness to flies. Fraction 3 was significantly less attractive ($p < 0.05$, χ^2 two sample test). The order of attractiveness of the fractions is shown in table 52 p. 187.

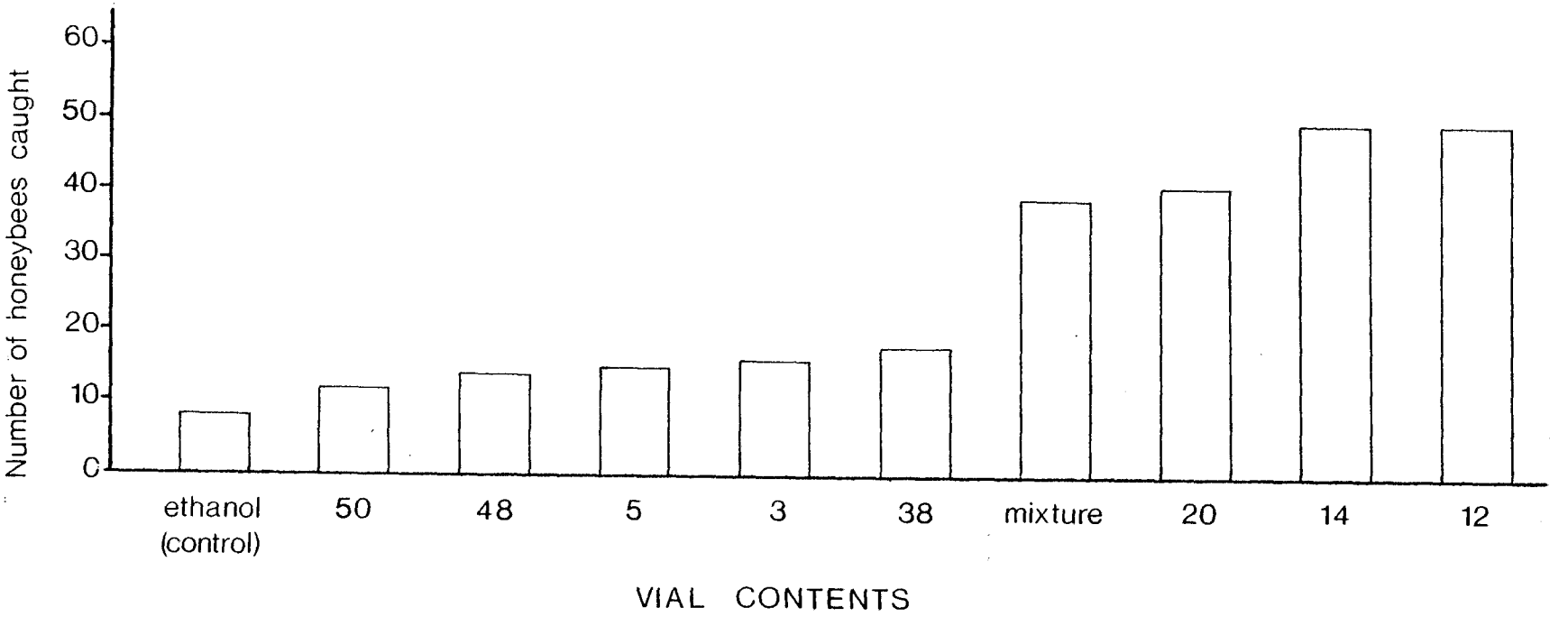


Fig. 65. Comparative attractiveness of ginger fractions (III). Total catches of honeybees.

Discussion and conclusions

1. All insects

Wasps, being highly attracted to waste ginger at the foraging site, were trapped in the largest numbers, and, as the weather was rather warm and dry, many flies also came to the traps. Honeybees, however, were only present in small numbers, presumably due to the onset of frost.

The results show that all the insects were affected by the weather, in that fewer were trapped on days which were overcast; also, that the provision of more attractive food at the foraging site resulted in an increased catch in all categories (see figure 52).

The general decline in the numbers of insects caught throughout the period was probably due to the occasional days of poor weather, coupled with the advancing season.

Fractions 12, 14, 38 and the mixture were attractive but fraction 20 surpassed them all. The other fractions were no more effective than ethanol (table 52 p. 187).

2. Wasps

Large numbers of wasps were present in the vicinity of the traps throughout the period and many were attracted to them. This was because, having come to associate the smell of ginger with an abundant source of food, the wasps' attention was diverted by the more concentrated odours emanating from the vials.

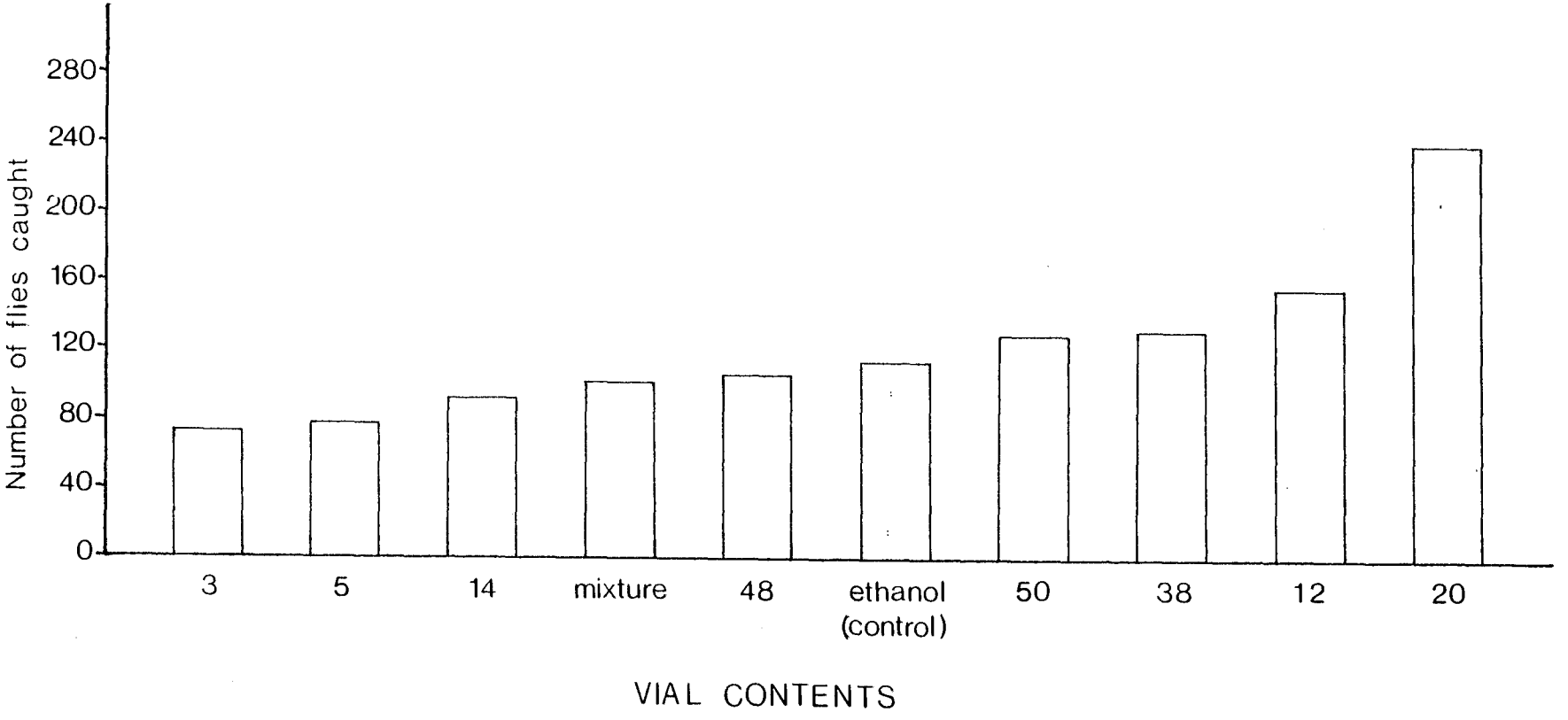


Fig. 66. Comparative attractiveness of ginger fractions (III). Total catches of flies.

Some of the fractions were extremely attractive, but the majority were no more effective than the ethanol used as the control.

Overall the traps caught twice as many *vulgaris* as *germanica* but the proportions attracted to each fraction were not the same. This would seem to indicate that the two species have different 'thresholds of attraction' for the component odours of ginger, i.e. for any given fraction a greater concentration would be required to attract one species than the other, from the same distance. The fact that greater numbers of wasps were caught when more ginger syrup was made available (on day 5) seems to add weight to the argument that some form of rudimentary recruitment takes place (see p.215). Thus, worker wasps in the nest, when fed with syrup rather than the inferior whole ginger they had become accustomed to, might be stimulated to collect the syrup themselves, and spend less time on other duties. Such opportunism would ensure the efficient exploitation of rich (but possibly transient) food sources, to the advantage of the whole colony.

Although *germanica* and *vulgaris* workers appeared to react differently to some of the ginger fractions, their reaction to number 20, the most attractive fraction, was apparently the same. Being a considerably better attractant than any of the other fractions and ginger syrup, fraction 20 and some of its component compounds (α-curcumene, γ-and δ-elemene, α-cubebene etc) are potentially useful as baits. On the contrary, compounds such as borneol and other low boiling compounds together with some high - boiling sesquiterpenes appear to be repellent.

The decrease in numbers of wasps caught throughout the trapping period can be ascribed to the destructive nature of the experiment coupled with a gradual decline in the wasp populations caused by bad weather and the onset of autumn. The foraging population of *vulgaris* decreased more drastically than that of *germanica* and this can be correlated with the slightly earlier colony-breakdown characteristic of the former (Edwards 1980).

More difficult to explain, however, are the large increases and decreases in catches by individual fractions (see table 54 p.200). Fraction 5 and the mixture caught fewer *vulgaris* than expected during the last half of the experiment and fractions 38, 48 and 50 caught more. Fraction 12 caught fewer *germanica* than expected during this time and, as with *vulgaris*, fractions 38 and 48 caught more. Inflated totals caused by proximity of attractive fractions to less attractive ones can be discounted because the traps were placed in different positions relative to one another every day.

One possible reason to explain these phenomena concerns the relative volatilities of the different fractions and the fact that, apart from on day 5, no new ginger waste was put in the bins. The longer the ginger waste was exposed to the air, the more volatile constituents would be lost, especially on dry sunny days. Once the concentration of low-boiling point volatiles reached a certain low value, wasps would no longer be attracted by them and other, less volatile components would become relatively more attractive as a result. The wasps would then begin to associate their source of food with these less volatile components, until the ginger was replenished. According to this theory, the components of fractions 3 and 5, occurring naturally in the ginger waste, would volatilize first, leaving larger concentrations of the higher-boiling point components characteristic of fractions 38, 48 and 50. Therefore, although wasps would still be attracted to the traps containing fractions 3 and 5, there would, nevertheless, be an increase in the numbers lured by fractions 38, 48 and 50.

3. Honeybees

Honeybees were comparatively scarce towards the end of the experiment and, even at the beginning, were far less numerous than at the same time the year before. This was probably due to a reduction of the number of hives in the area. Notwithstanding the small catches in the traps, it is possible from the results to see

that bees were less specific in their attraction to the fractions than wasps - there was no significant difference between the four most attractive. These fractions (12, 14, 20 and the mixture) were, however, significantly more attractive than the others, and this could cause problems if a wasp attractant which does not lure bees is to be developed.

4. Flies

Like the hymenopterans, flies were affected by the prevailing weather, and the catch was depressed on the dull days. It seems likely that at least some of the flies were attracted to the traps, not by virtue of the ginger fractions but because of odours released by dead wasps and bees in the initial stages of decay, although decomposition would be somewhat retarded by the detergent. If, however, the smells of decay were the major attraction, then fractions 14 and the mixture should have caught larger numbers of flies (unless certain components were particularly repellent to all Diptera, which seems unlikely); so it would appear that the Diptera caught had been attracted by ginger fractions. Fraction 20, containing α-curcumene and other sesquiterpenes, was again the most attractive, but none of the others was any better than ethanol, and fraction 3 was worse.

(e) Efficiency of various traps: Bendicks, 1979

(i) Comparison of the trapping efficiencies of modified and unmodified 'Wasp-wizard' traps (I)

Introduction

Although the traps used in the previous experiment were quite efficient, especially when used in conjunction with a wetting agent, some wasps could, and did, escape, by climbing over the dead insects and up the sides of the trap from where, once they had reached the top, they could fall out of the entrance hole. It was in order to reduce this chance of escape that a modified design was tried. The trial was also used as a way of testing the attractiveness of ethanol, which had been the control in the previous experiment.

Method

A 'Wasp-wizard' trap was modified as shown in figure 67 (p.209), two horizontal rings of perspex, 0.5 cm wide, being glued to the central pillar and outer edge of the trap above the water line and a vertical cylinder of perspex being glued to the roof of the trap to prevent climbing wasps from falling through the entrance hole.

The modified trap was baited with a 10% solution of fraction 20 in ethanol and half-filled with water to which had been added 0.4 ml of 'Savona' liquid detergent. An unmodified trap was baited likewise. Two further, unmodifed traps were half-filled with water plus 0.4 ml of the wetting agent. One was baited with pure ethanol, but the other was merely stood over an empty vial. The four traps were set out in the factory yard at Bendicks (Mayfair) Ltd., on the tops of contiguous 40-gallon drums, as in previous experiments. The two traps baited with the ginger fractions were placed between the other two and their positions were swapped every 24 hours throughout the 4 days of the experiment. The two outer traps were swapped round after 2 days. This arrangement ensured that the traps were equally exposed to factors which might influence the numbers of wasps caught, e.g. wind direction. The wasps caught each day were removed and counted every morning and the traps were washed out, recharged with water and wetting agent, and set out above fresh vials.

Results

1. Weather

DAY 1 (1 OCTOBER 1979)

Warm and mostly sunny. Wind very light SSE

DAY 2

Warm and sunny. Wind light SSW

DAY 3

Starting warm but hazy. Overcast with some rain in afternoon.

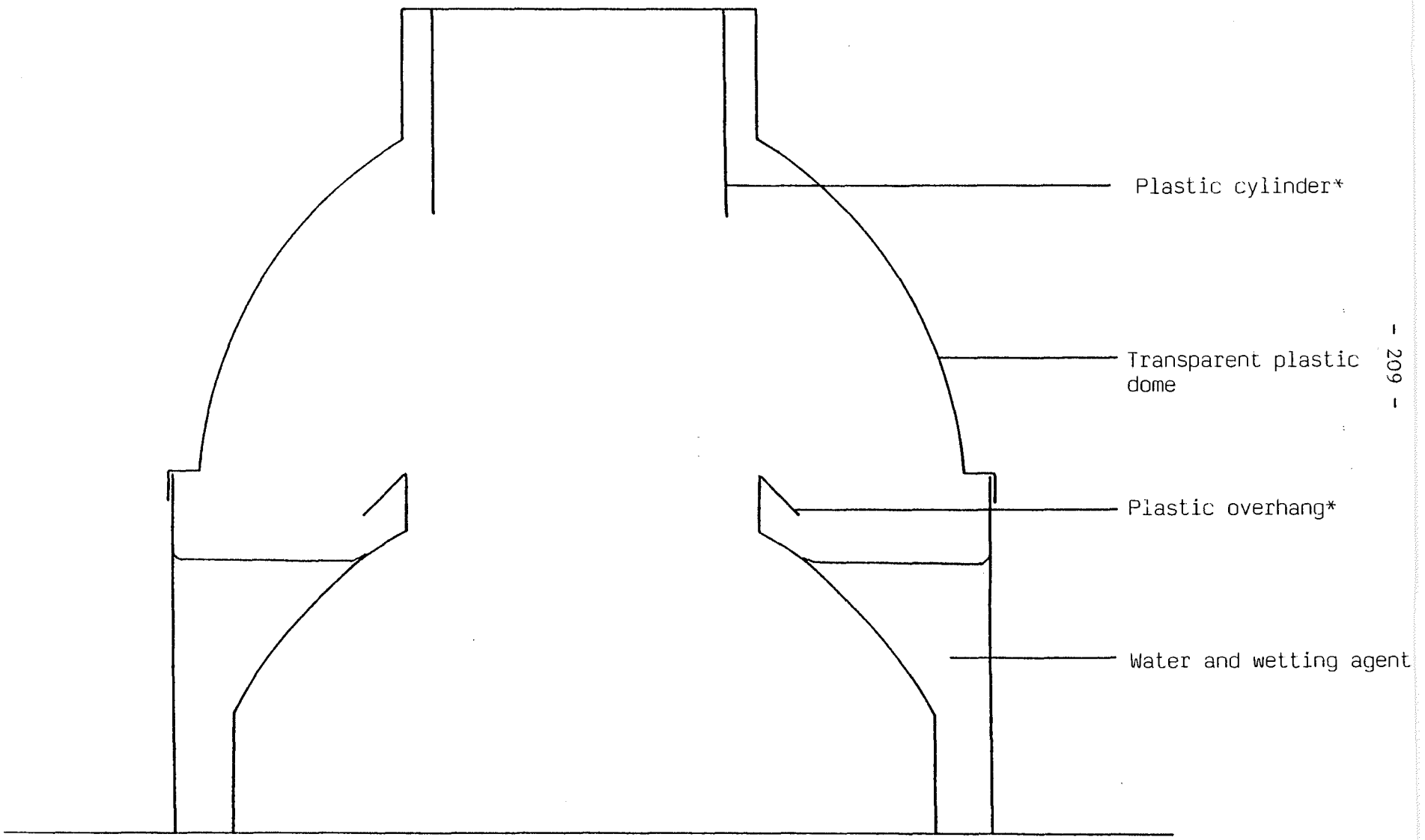


Fig. 67. Cross-section of modified 'Wasp-wizard' trap (Actual size. Parts added to original trap are marked *)

DAY 4

Calm and very cloudy with occasional showers at first, becoming sunny but cooler with more showers.

2. Trapping data

Fraction 20			ethanol	blank	
modified	unmodified	significance	unmodified	unmodified	Significance
46	150	***	10	27	NS

Table 55. Comparison of catches of modified and unmodified 'Wasp-wizard' traps, using fraction 20 or ethanol as bait. (NS = not significant, *** = $p < 0.001$, χ^2)

The difference between the numbers of *vulgaris* and *germanica* entering the traps was not significant at $p = 0.05$, so the data were combined.

Observations indicated that when wasps entered the modified trap the ledges provided by the perspex rings allowed the insects to 'test' the water without falling in, as a result of which many escaped. Also the rings reduced the effective surface area of the water.

There was no apparent reason why wasps entered the trap placed above the blank vial, although there did seem to be a slight attraction once other wasps were inside.

Conclusions

The modified trap was worse at catching wasps than the ordinary one. Ethanol was no better than a blank vial for attracting wasps into the traps. The fact that wasps were caught in these is possibly due to a slight attraction either to the detergent, or to volatile components of the plastic trap, coupled with inquisitiveness. In addition, it is likely that wasps were attracted by the sight of others in the trap. This would agree with observations made by other workers (Free 1970, Pflumm 1975, Parrish and Fowler, 1983).

(ii) Comparison of the trapping efficiencies of modified and unmodified 'Wasp-wizard' traps (II)

Method

Following the previous experiment, which highlighted a number of design faults in the modified trap, the experiment was repeated for another 5 consecutive days, using a trap with certain refinements i.e. the outer ring of perspex was removed and the inner one was angled downwards, so as to increase the effective surface area of the water without allowing wasps to climb up the central pillar. The cylinder of perspex fixed to the roof of the trap was unchanged (Fig. 67).

As before, two unmodified traps were placed outside the ones baited with fraction 20 and one was baited with ethanol, whilst the other was left blank. The details of the experiment were the same as before.

Results

1. Weather

DAY 1 (5 OCTOBER 1979)

Starting cold, but no frost. Becoming very warm and sunny. Calm.

DAY 2

Windy but warm and sunny following overnight rain. Wind fresh to strong ESE.

DAY 3

Warm and windy with some cloud and rain. Wind strong S.

DAY 4

Warm and windy. Heavy rain by late afternoon. Wind strong S.

Day 5

Warm with some sun. Wind moderate, S.

2. Trapping data

Trap	Number of wasps caught	Significance
Fraction 20		
modified	25	NS
unmodified	28	
Ethanol unmodified	12	NS
Blank unmodified	8	

Table 56. Comparison of catches of modified and unmodified 'Wasp-wizard' traps, using fraction 20 or ethanol as bait.
(NS = not significant, χ^2 test).

Discussion and conclusions

The weather and advancing season were responsible for the low numbers of wasps caught during this experiment. However, enough were trapped to indicate that the second modification of the 'Wasp-wizard' trap was no worse at catching wasps than the standard. It is likely that, had large numbers of wasps been attracted to the traps, the modified one would have retained more, because it is at the stage when the entire water surface is covered in bodies that wasps can sometimes crawl out. Had this happened in the modified trap wasps would have been unable to negotiate the overhang on the central pillar or, if they had managed to climb the wall of the trap they would have fallen back into the water on reaching the central cylinder attached to the roof.

In accordance with the previous experiment the ethanol and blank vials were not significantly different in their effects on the catches of wasps.

IV.D. Experiments to determine whether wasp saliva contains a short-range attractant

IV.D.1 Introduction

Observations at the Havant nest during the alarm-behaviour experiments of 1978 indicated that workers of *vulgaris* co-operated in removing obstructions such as twigs from the nest entrance. When a twig was placed in front of the nest it would be ignored for half an hour, but many outgoing wasps, and some incoming ones, would collide with it, only to re-orientate and continue on their way. Eventually one of the outgoing workers would land on the twig and begin to chew through it. Thereafter, more interest would be shown by other wasps, some hovering in front of the stick before flying off. After about 10 minutes of chewing, the worker wasp would fly away but thereafter, numerous others would gather at the point where it had been biting and some would begin to chew the stick themselves. However, if work on the twig ceased for any length of time, such as overnight, then outgoing wasps would again tend to ignore it until another had chewed it once more.

These observations suggest that *vulgaris* workers are attracted in some way to objects chewed by others. Although visual cues undoubtedly play a part in the onset of this 'obstruction-removing' behaviour, this does not explain why wasps seem to be attracted to the point where another has chewed, *after* it has flown off, especially as the object in question is usually ignored if a wasp has not been working on it. The most likely explanation for this behaviour is that the saliva of *vulgaris* contains a short-range attractant and that it acts as a releaser for chewing behaviour. Such an attractant would account for the increase in numbers of wasps hovering at the part of the twig which had been bitten and it would ensure that objects blocking the nest entrance would be speedily removed by co-operative biting at one point. An attractant in the saliva might also be invoked to explain how nest-building is synchronised in the darkness of the nest cavity: wasps returning with a load of wood-pulp would be able to find the point of last application by means of such a pheromone.

In order to test this hypothesis more rigorously the following methods were used.

IV.D.2 Methods

In the first experiment an untreated, wooden boiling stick was placed upright in the ground directly in front of the entrance to the *Vespula vulgaris* nest in Bassett, the intention being to record the time taken for the first bite to occur, followed by the time interval between this and subsequent bites by different workers.

The second experiment involved the placing of two boiling sticks in the ground 2 cm apart and directly in front of the same nest. One stick was impregnated with a drop of an extract made from the heads of *vulgaris* workers in dichloromethane whilst the other, the control, was treated with a drop of the solvent only. Here again the intention was to take the time for the first bite and compare it with the intervals between subsequent bites.

IV.D.3 Observations

The mean rate of exit of wasps was 18 per 30 seconds. In the first experiment, though the stick was observed for an hour, no wasps landed on it and none began to chew it, even though there were numerous collisions.

In the second experiment, lasting 30 minutes, though there was no chewing, 3 wasps landed on and investigated the control, compared to 6 on the test stick. This difference was not significant (binomial test).

IV.D.4 Discussion and conclusion

Though these results are inconclusive, the observations in experiment 2 indicate that some wasps were interested in the sticks. If the sticks had been larger and more of an obstruction, perhaps the wasps would have attempted to remove them.

IV.E. Location of wasp nests in the vicinity of Bendicks (Mayfair) Limited 1979

By following the lines of returning foragers it was possible to trace the nests of those wasps which were using the factory waste as their source of carbohydrates. As the map in figure 68 (p.216) shows, one *germanica* and 4 *vulgaris* colonies were located, all to the west of the factory on Winnall Moor. Another *germanica* nest was situated beyond the river, but was inaccessible.

Observations at the sites of the five nests indicated that the majority of foragers flew in the direction of the factory on leaving the colony. The prevailing wind in the area is generally south westerly, which indicates that some form of 'communication' of foraging sites exists, as odours from the factory would seldom be wafted towards the more distant nests.

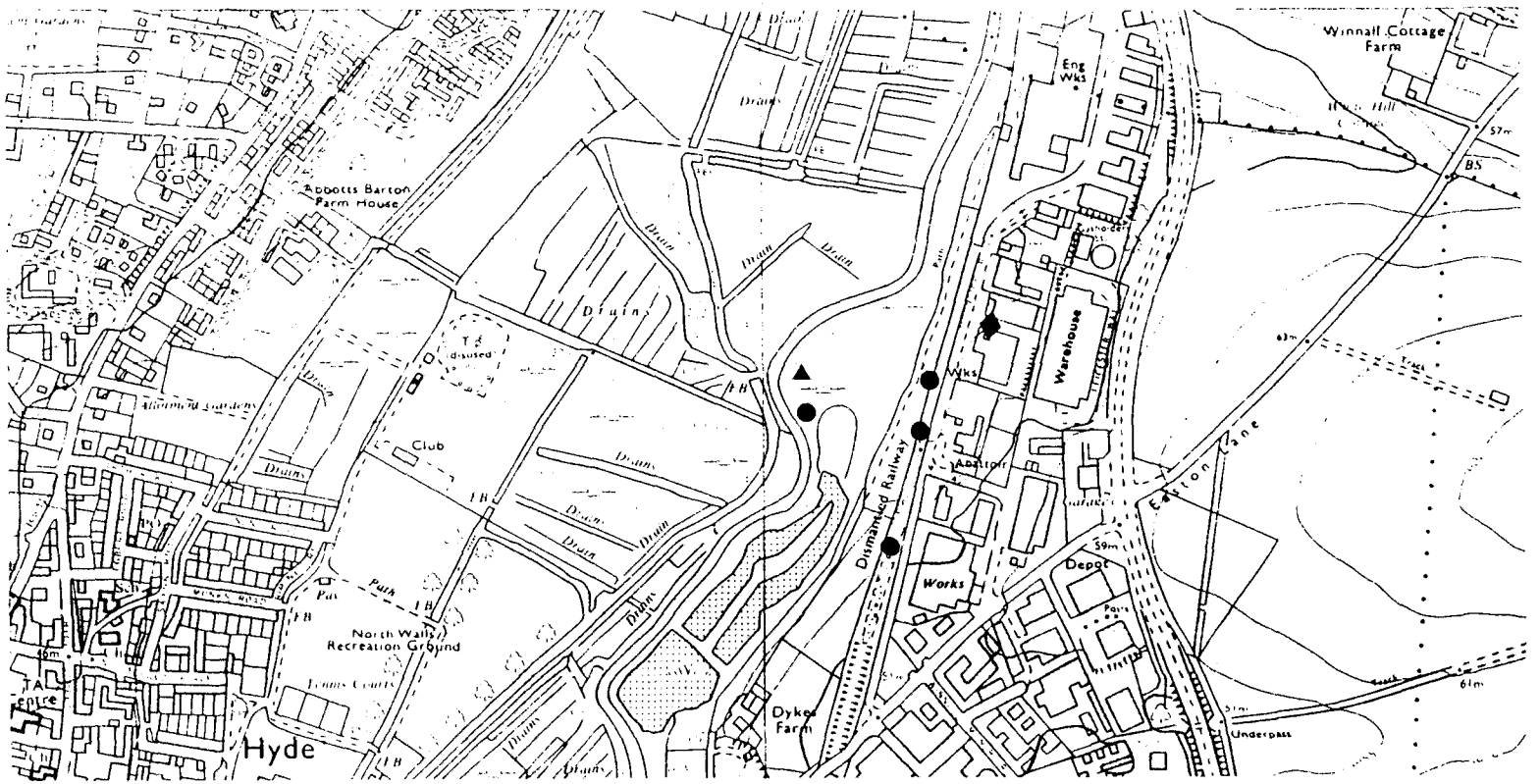


Fig. 68. Winnall Moor and Trading Estate, Winchester, showing colonies of wasps whose workers were exploiting the ginger waste in the factory yard at Bendicks (Courtesy: Ordnance Survey)

Scale = 6 inches to 1 mile

◆ = factory yard

● = *vulgaris* nest

▲ = *germanica* nest

IV.F. Chemical analysis of attractants

IV.F.1 Introduction

Ginger contains a large number of components, many of which are not particularly attractive to wasps. To discover which of the constituents were effective attractants it was necessary to fractionate large volumes of essential oil by the methods described elsewhere (p.166) and test them in the field. Various components, both of the oil as a whole and of its most attractive fraction, were then analysed in an attempt to identify them.

IV.F.2 Method

Samples of essential oil of Chinese ginger and fraction 20 dissolved in dichloromethane were injected onto a 5% OV101 column coupled to the mass spectrometer (for specifications and conditions see p. 38). Major peaks were analysed by the computer for later identification.

IV.F.3 Results

(a) Essential oil

A chromatogram of the essential oil is shown in figure 19 (p.118). In the analysis below the numbers refer to the peaks marked on the chromatogram.

Peak	Identity
3*	camphene
4*	myrcene
5	α-phellandrene (α-terpinene?)
6	monoterpene alcohol
8*	borneol
10	neral?
11	geranial?
12	bornyl (iso-bornyl?) acetate
14*	δ-elemene
16*	α-cubebene

Peak	Identity
20*	zingiberene + α-curcumene
21*	β-farnesene
24*	β-eudesmol

The mass-spectra for compounds marked with an asterisk are given in appendix C (p. 233).

(b) Fraction 20

A chromatogram of this fraction is shown in figure 47 (p.171) included among the components of this fraction are the following:

(i) * geranial
(ii) citronellal?
(iii) α-citronellol?
(iv) * terpineol
(v) * borneol
(vi) * α-curcumene
(vii) * γ-elemene
(viii) * δ-elemene
(ix) * α-cubebene

The mass spectra of compounds marked with an asterisk are given in appendix C (p. 233).

IV.G. Discussion

From these experiments a number of conclusions can be drawn regarding the role of attractants in the foraging behaviour of *Vespula vulgaris* and *V. germanica*.

Ginger syrup is a good attractant, but only at sites where wasps are not already exploiting a different source of carbohydrates, in which case the syrup is largely ignored, until the alternative food is no longer available (expt. (b)(ii) p.135). This explains why certain control methods depending on specific attractants do not always achieve satisfactory results. As far as the wasp colony is concerned such behaviour (as suggested on page 143) would ensure efficient utilisation of carbohydrate resources by reducing the time spent by foragers searching for other, possibly more transient, sources of food in response to new, apparently more attractive odours.

An analogy can be drawn here between the wasp and the source of carbohydrate it is exploiting, and a lock and key. Thus, once a wasp (the key) has become conditioned to a certain attractive source of carbohydrate (the lock), such as ginger syrup or rotting pears, the sudden appearance of another attractant will have no effect, because the wasp has learned to associate a definite combination of odours with food i.e. the key will not fit the new lock. However, just as it is occasionally necessary to change a lock and buy a new key to go with it, so, when its food supply is exhausted, does the wasp begin to respond to new combinations of odours and to search for fresh supplies of carbohydrate.

The attractive principle of ginger syrup is, in the first instance, the essential oil it contains. This was proved by many of the above experiments in which vials of sugarless distillates were disguised and covered to prevent imbibition, yet attracted numerous wasps, some of which gnawed at the protective netting in their attempts to reach the contents. Similar results have been obtained in the U.S.A. with *Vespula pensylvanica* and 2,4-hexadienyl

butyrate, a powerful attractant with no apparent food-value (MacDonald *et al* 1976).

The experiments involving sucrose solution (Pilot surveys p.116) showed the importance of taste as a stimulus in foraging wasps. When presented in an open watch-glass adjacent to others containing aromatic solutions, sucrose was ignored, presumably because the wasps could not smell it. After the positions of the watch-glasses had been changed, however, wasps returning to what had been ginger syrup discovered the sucrose solution. As the solutions were moved on subsequent occasions, wasps returned to the last-known position of the sucrose, using visual cues, and then resorted to tasting each solution until they rediscovered it. These observations suggest that aromatic sources of carbohydrate are initially located by smell, but thereafter by sight and taste.

The concentation of the attractive odour is an important aspect of foraging behaviour in wasps. It has been shown in the experiments above that concentrated distillates are considerably more attractive than their parent syrup when both are presented in equal amounts, (eg. expt (a)(i) p.125). However, the surface area of the attractant is vitally important: thus a small vial of highly concentrated ginger distillate is little more attractive than a waste-bin full of rubbish coated with ginger syrup, except at close range, even though the syrup contains, weight for weight, 1,600 times less essential oil (see p. 107).

Wasps at the chocolate factory were more attracted to certain fractions of ginger oil than to others, including the whole oil (expt. (d)(iv) p.180). Experiments showed that fractions consisting mainly of sesquiterpenes, especially the major ones characterisitic of ginger viz. α- and β-zingiberene, α-curcumene β-farnesene and zingiberol, were attractive to wasps, whereas fractions containing larger proportions of substances with higher or lower boiling points than the aforementioned were considerably less so.

The relative proportions of these sesquiterpenes in the various fractions differ considerably and this might explain why fractions 20, 14 and the steam distillate were so attractive compared to the rest.

During the experiment in which wasps were trapped over 10 days, certain fractions caught more in the latter half of the time even though the total number of wasps was gradually dropping (expt. (d)(iv) p.180). The fractions in question were those containing the less volatile constituents such as sesquiterpene alcohols (e.g. β-eudesmol) and were in general, the least attractive. This shift in emphasis ties in with the gradual depletion of the source of ginger syrup which, on continued exposure to the air, was losing its volatiles at an unequal rate, the more attractive sesquiterpenes evaporating fastest. In conjunction with this gradual change the wasps, though having become conditioned to return, would gradually associate the syrup with a subtly different combination of odours; to extend the metaphor employed above, small alterations to the tumblers of a lock require compensatory adjustments to the key. Hence the wasps became more attracted to fractions 38, 48 and 50 as time progressed.

It is useful here to discuss the possible reason for the relative unattractiveness of the high-boiling sesquiterpenes. Although, as explained above, the less effective fractions tended to attract more wasps in the latter half of the experiment, they were still poor in comparison to fraction 20: also the total number of wasps coming to the traps, though fluctuating on a daily basis due to changes in weather etc., showed a tendency to dwindle as time progressed. This can be explained by considering the quality of the syrup and the differential evaporation of its constituent volatiles. Thus at the start of the experiment, and on day 5, when fresh ginger syrup was available to the foragers, low-boiling volatiles were at their most concentrated and the syrup was of high quality. However, as more of the syrup was collected, and the surface of the stem ginger was reached, wasps had to resort

to careful searching between the chunks, or to biting off pieces of ginger - a less satisfactory source of sugar. As a result the foraging wasps would begin to associate the relatively higher proportions of the less volatile sesquiterpenes with poor rewards.

Interspecific differences in foraging behaviour were evident from some of the latter experiments (eg. expt. (d)(iv) p.180). This is not surprising, since it is rare among animals indigenous to a region that two species occupy an identical niche. Thus the data show that foragers of *Vespula vulgaris* found certain fractions more attractive than did *V. germanica*. Fewer of the latter than expected were attracted to fractions 3 and 5 (containing low-boiling volatiles) and conversely, fraction 14 (containing both mono- and sesquiterpenes) attracted more. This suggests that the two species are attracted to the syrup by virtue of different combinations of odours: to further the analogy, different locks require different keys. The control of wasps in the USA by means of attractants such as heptyl butyrate confirms this (Macdonald *et al* 1973). This work showed that when traps baited with heptyl butyrate were placed in separate cages containing colonies of *Vespula atropilosa* or *V. pensylvanica*, though workers of both species were attracted, 40 times more *atropilosa* were caught. Results of field trials, in which workers from wild colonies were trapped, complicated the picture because more *pensylvanica* than *atropilosa* were caught in the month of September. However, this was simply due to the fact that colonies of the former were at their peak, whereas those of the latter were declining, and it serves to illustrate the importance of thoroughly understanding the biology of a species if control measures are to be taken.

Evidence for social facilitation among wasps was forthcoming in some of the attractant work (e.g. expt. (e)(i) p.207). Free (1970) describes how workers of *Vespula vulgaris* and *V. germanica* were more attracted to dishes of sugar syrup already attended by others than to those where no wasps were foraging. His findings are

supported by observations made by MacDonald *et al* (1974) of captive *Vespula pensylvanica* workers co-operating in the preparation of large prey prior to returning the pieces to the nest. Pflumm (1975), in his experiments on the social facilitation of *V. germanica* workers, showed that the visits to a source of carbohydrate depended upon the concentration of the solution (see discussion on sucrose above), the orientation patterns with regard to the foraging site (i.e. visual stimuli) and the group effect at the food source. Recently Parrish and Fowler (1983) have demonstrated that social facilitation is mediated visually and that *Vespula germanica* prefers to feed in the company of others. Results from the present research agree with the last observation in so far as apparently unattractive traps (such as the control, which contained no attractant) occasionally caught a number of wasps. In earlier bioassays in which vials covered by netting cones were used, honeybees were especially attracted to those already being visited by others. On one occasion, during a preliminary trial, erroneous results were obtained when two honeybees alighting near a relatively unattractive vial to rest, were joined by 8 others.

Much of the behaviour described above also applied to honeybees, though they were less liable to ignore ginger syrup in places where other sources of carbohydrate were available. Thus the honeybees were attracted in the first instance by smell and the more concentrated the attractant, the more bees were attracted. Preference for different fractions was shown by honeybees, but not to such a great extent. Whereas fraction 20, of all the ginger components, was the most attractive to wasps, bees showed no apparent discrimination between fractions 12, 14, 20 and the mixture. This is perhaps an indication that honeybees, which rely heavily on visual stimuli when visiting flowers (Von Frisch and others in Wilson, 1971), do not require such a delicate perception of odour combinations as is evident in wasps, and it lends indirect support to the idea that chemotaxis is the primary means by which wasps locate carbohydrates.

Intergeneric differences in the numbers of

insects caught by the various fractions is encouraging from the point of view of those interested in controlling wasps, as it indicates that attractants may be found which will not affect honeybees: such is the case with 2,4-hexadienyl butyrate and similar attractants in the U.S.A. (MacDonald *et al*, 1973).

The weather, as might be expected, affected the numbers of wasps foraging for carbohydrates (expt. (d)(iv), p.180). However, whereas overcast conditions and heavy rain drastically reduced the foraging population of honeybees, wasps continued to forage in bad weather, albeit at a reduced rate. It would appear from some observations that the major factor affecting the foraging population of wasps is the prevailing light intensity. Blackith (1958) showed the importance of this with respect to the initiation and completion of foraging at dawn and dusk respectively, and suggested that activity was independent of temperature above 2°C. Measurements of worker exit rates from subterranean nests (this research) agree with these findings, as does the fact that the foraging population at the chocolate factory was lower on overcast days. That fewer wasps should forage for carbohydrates on dull days than on sunny ones, regardless of temperature, can be explained if prey capture is taken into account. Broekhuizen and Hordijk (1968) showed that the favourite prey of *Vespula vulgaris* was flies, especially *Musca domestica*, and observations during this research are in agreement: hover-flies are also an important food of both *vulgaris* and *germanica* (personal observation). It is a well known fact that many insects, particularly diurnal flies of the types just mentioned, are more active on sunny days and that dull weather can drastically reduce this activity. On such days foraging wasps, which depend on the visual stimuli afforded by dark, moving objects in bright light for efficient prey capture (personal observation), would be at a disadvantage and would have to hunt for longer. Therefore, proportionately fewer wasps would be foraging for carbohydrates, and activity within the nest would be generally suppressed owing to the reduced input of food, both protein and carbohydrate.

Although wasps visit certain flowers for carbohydrates it is possible that visual cues are not of immediate importance. Without exception the British plants adapted for pollination by wasps (e.g. Figwort *Scrophularia nodosa*) have small, dull or faintly-coloured flowers which are unlikely to be noticed except at close range: this would accord with Scudder's observations on the visual acuity of wasps (1889). Furthermore, wasp-pollinated plants often have flowers which are dull red, a colour which wasps tend to confuse with dark grey or black (Schremmer 1941, Beier and Menzel 1972). Hence attraction to 'wasp-flowers', such as those of Figwort *Scrophularia nodosa* or Snowberry *Symphoricarpos rivularis*, is possibly chemical at first and then augmented at very close range by visual cues, when small, reddish flowers would become obvious as dark objects against the paler foliage. This raises the interesing possibility that wasps are ultimately attracted because of a similarity in size and apparent colour of some wasp-flowers to the small flies, especially *Musca domestica*, which constitute a large part of the diet of *Vespula* species.

Some flowers, though not specifically pollinated by wasps are nevertheless, visited by them: among these are Hogweed *Heracleum sphondylium* and Wild Carrot *Daucus carota* (Personal observation). Both have white flowers arrayed in showy umbels which are attractive to many species of flies and beetles as well as wasps, but here again, even though the flowers are very conspicuous, the initial attraction may be triggered chemically, as, in common with most other umbellifers, these two plants are highly aromatic.

At the height of the wasp season in the British Isles fallen fruits and aphid secretions form the major sources of carbohydrates, and attraction to these is probably a chemically-mediated one, as visual cues are generally inconspicuous, except at close quarters.

CHAPTER V

GENERAL DISCUSSION

The experiments described in this work illustrate the complexity of foraging and alarm behaviour in British social wasps and suggest that adequate control measures are still far from being realized - at least until foraging behaviour is more fully understood.

Workers of *Vespula vulgaris* and *V. germanica* spend a large proportion of their adult life foraging and, unless they are within a few metres of the nest entrance, only use their stings in self defence. If, however, the colony is threatened and a wasp leaving the nest to forage encounters an unfamiliar object near the entrance, alarm behaviour to a varying degree is precipitated, depending upon the 'severity' of the threat. This type of alarm behaviour appears to be entirely altruistic and is rendered all the more effective because the venom injected when the wasp stings contains an alarm pheromone which can evoke an attack response in any sister worker nearby: most predators are quickly deterred by this cumulative action.

The various categories of alarm response shown by *Vespula vulgaris* are summarized in figure 69 (p.227) which is a simplified scheme based on the findings of this research. The flow chart also indicates the possible sequence of events taking place during the course of a foraging expedition and relates this to colony defence.

As the flow chart shows, alarm behaviour varies in intensity, depending on the combination of provoking stimuli. Motionless, white objects which have not been stung only evoke weak investigatory responses, whereas rapidly moving, black objects impregnated with venom incite the wasps to display the most intense form of aggression i.e. strong attack responses. This variation is due to the large number of stimuli which can evoke alarm and it ensures that the form of colony defence displayed is tailored to requirements. Apart from the visual and chemical stimuli described in figure 69(p.227), other factors known to affect the alarm reaction include prevailing weather conditions, unfamiliar odours and chemicals released from damaged wasps, which seem to have an attractive function.

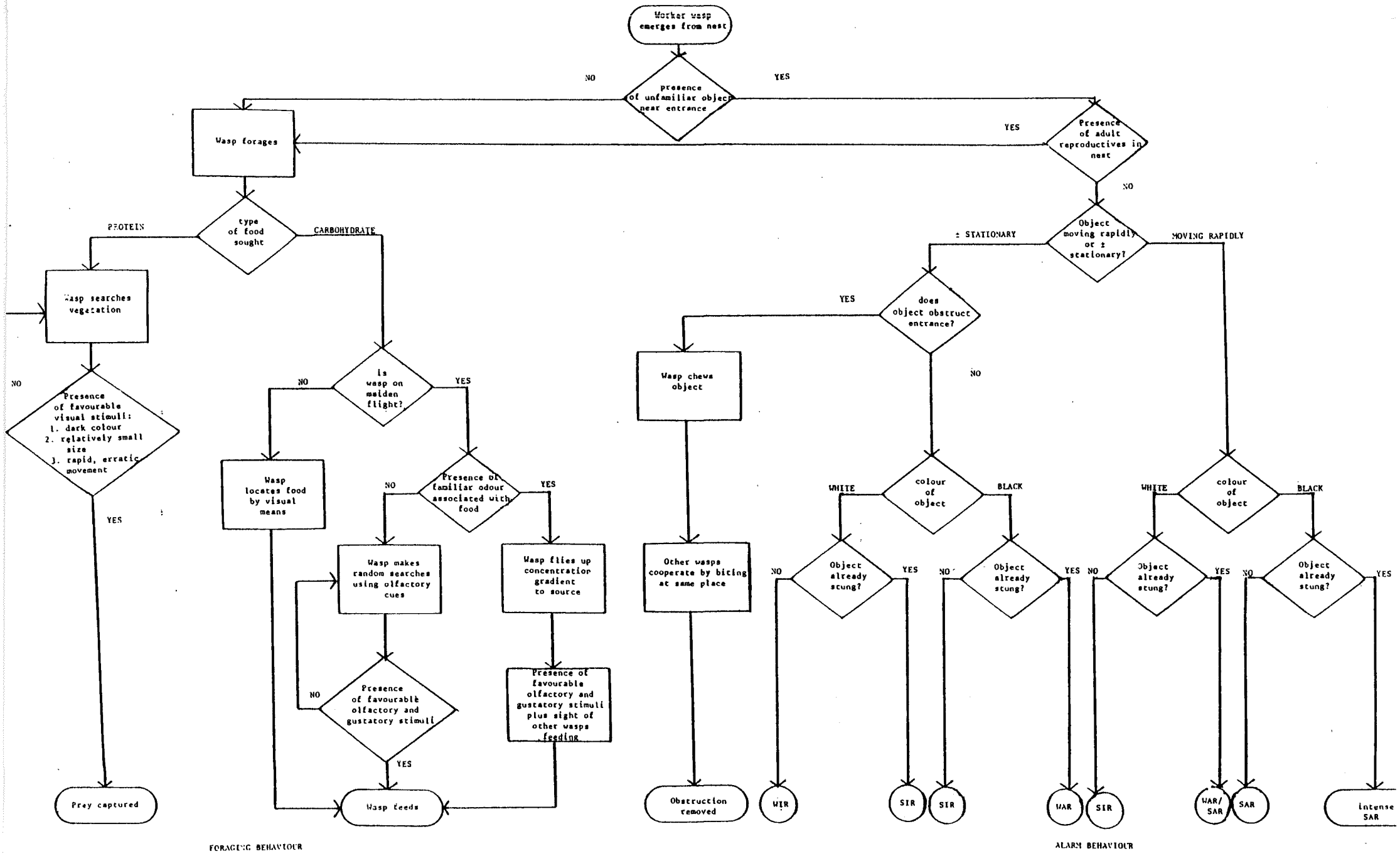


Fig. 69. Flow chart summarizing foraging and alarm behaviour of a *Vespula vulgaris* worker. (± = more or less, WIR = investigatory response, SIR = strong investigatory response, WAR = weak attack response, SAR = strong attack response).

Foraging behaviour among social wasps also appears to be influenced by sequences of stimuli of various kinds, but whereas the factors which kindle the alarm reaction are quite clear-cut it is not known what stimulates a wasp to search for carbohydrates or proteins.

If the wasps is foraging for flesh, visual stimuli coordinate the process, but if carbohydrates are the goal it seems that chemical stimuli may be involved at first. Thus, as the flow chart indicates, a wasp on its maiden flight may recognize the smell of the carbohydrate which has recently been brought to the nest, and will search for food with this odour. Flying up the concentration gradient, it will eventually reach the source and will be stimulated to feed by the sight of other wasps (social facilitation) and by the smell and taste of the food. Navigation to the food source on future occasions depends, not so much on olfactory stimuli, but on visual ones (landmarks) and feeding often begins even if the attractive food has been replaced by an unpalatable one whilst the wasp has been away.

The presence of other species members at a food source may be an important attraction at close range but olfactory cues appear to dominate initially. The fact that wasps can detect extremely small concentrations of attractive molecules offers some hope to researchers involved in wasp control, but this hope is offset by the many other parameters involved: accidental capture of honey-bees, foodstuffs competing with attractants, weather conditions, fluctuations in wasp populations and difficulty in locating nests, are just some of these.

Although ginger fractions containing certain sesquiterpenes (α-curcumene, α-cubebene etc) are highly attractive to wasps familiar with ginger, no such attraction can be demonstrated at sites where wasps are taking different foodstuffs. Moreover, the two species of wasps react differently to each fraction. Problems such as these preclude the possibility of effective wasp-control unless, by chance, a simple substance can be found which is as attractive to British wasps as heptyl butyrate is to certain American ones.

The results of this research serve to illustrate the complexity of alarm behaviour in British social wasps and indicate the importance of chemical stimuli in this and foraging behaviour. Neither avenue offers any immediate hope for those involved in wasp control, but they emphasize the need for a multifaceted approach to the problem.

CHAPTER VI

APPENDIX

VI.A. Wasp-separating antechamber

Figure 70 (p. 231) shows the antechamber designed to fit over the entrance hole of an artificial nest box. Made of galvanised iron, the box was constructed to serve as a means of separating outgoing wasps from ingoing, and it had a central compartment into which chemical or biological material could be put for the purposes of behavioural bioassays.

From the nest box wasps entered the antechamber through the large hole (diameter 2.54 cm) rather than the smaller glass tube (outside diameter 9.5 mm) because the former was more obvious and was flush with the entrance hole; also the glass tube could only take wasps in single file and was usually obstructed by returnin foragers. Once inside the antechamber, outgoing wasps were segregated from incoming ones by the partition wall, and could only reach the outside by leaving through the glass tube: returning foragers entered the adjoining hole which was larger, darker and more obvious.

The floor of the antechamber was of zinc gauze, below which was a sliding pane of red glass. To allow for access the roof consisted only of a sliding pane of glass which could be either colourless or red. The antechamber was designed to function as part of the wasp colony, insofar as it formed a confined and darkened extension of the entrance tunnel. Observation was afforded by means of the red glass which, however, created the impression of darkness to the wasps within, these insects being insensitive to certain red lights.

The central cage of the antechamber was made from zinc gauze and contained a removable gauze canister in which could be placed venom extracts, parts of wasps etc. The apparatus could enable the observer to compare the differences in behaviour between ingoing and outgoing wasps and to contrast their alarm behaviour within the entrance tunnel to that outside.

The antechamber was attached to a colony of vulgaris in the summer of 1978 but could not be used for behavioural work owing to the moribund state of the nest.

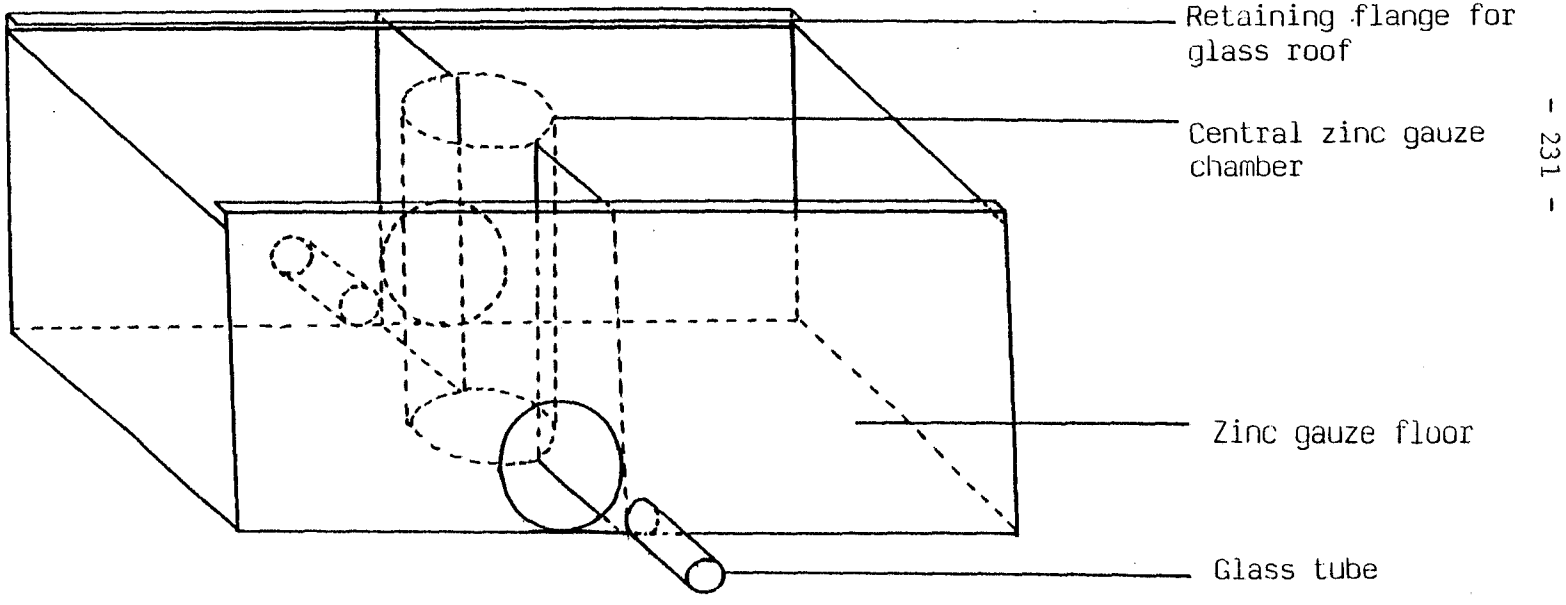


Fig. 70 Wasp separating antechamber (half actual size).

VI.B. Experiments with trap design

VI.B.1 Introduction

The preliminary tests on essential oil of ginger at the chocolate factory using vials and netting cones emphasised the need for an efficient method of trapping wasps. The traps available, though reasonably good, had their disadvantages and modifications were made to try to alleviate these problems.

VI.B.2 Modified 'drain-pipe trap'

A 'drain-pipe trap' of the type designed by Edwards (1980) was modified by removing its flat, nylon gauze base and replacing it with a truncated cone of wire mesh. To test the effect of the aerodynamics of the modified and unmodified traps on the flow of vapour from them, both kinds were placed in a wind-tunnel with beakers of dry ice and hot water inside them. The traps were then inverted and any change in the vapour flow was noted. Whereas the vapour from the unmodified trap under the influence of moving air, came through both ends, whichever way up it was, vapour only flowed out of the concave end of the modified trap, even when it was upside-down. These results indicated that the modified design would be more useful in the field, as wasps would only be attracted to the end containing the entrance hole. Comparisons were made, using baits of ginger syrup and essential oil in both traps, at the chocolate factory, an orchard and a bakery but neither trap was very effective and there was no significant difference between the small numbers of insects caught by either. It was noted however, that, when the modified trap was stood with its entrance facing downwards fewer honeybees attempted to enter than when the entrance faced up, whereas wasps crawled in upside-down more readily. This difference could be an important aspect to consider in future trap designs if the trapping of honeybees is to be avoided.

VI.B.3 Funnel and jar trap

This consisted of a stemless glass funnel fixed into the neck of a wide-mouthed glass storage jar containing a vial of attractant and 70% ethanol as a killing agent.

Although the trap was tried in an orchard, a bakery, a chocolate factory and a baker's shop, no wasps or bees were caught. This was probably because the ethanol was repellent at close range and the sides of the funnel were too smooth.

VI.B.4 'Wasp-wizard' trap and modifications

These were used successfully to catch wasps and honeybees at the chocolate factory. Comparisons between the modified and unmodified designs are discussed on p.207.

VI.C. Mass spectra

The mass spectra of N-3-methylbutylacetamide and 11 positively identified ginger components are given on the following pages.

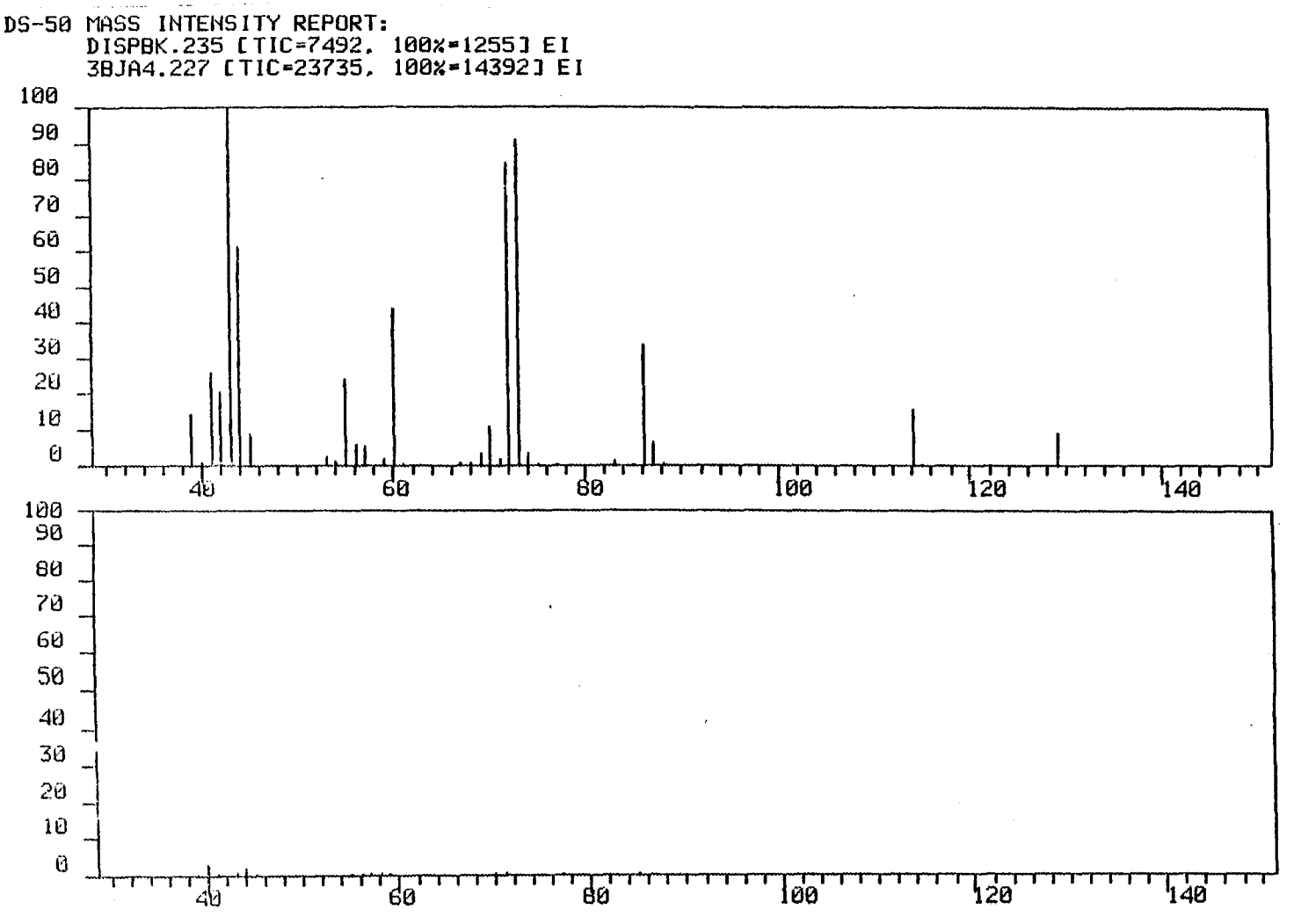


Fig. 71. N-3-methybutylacetamide.

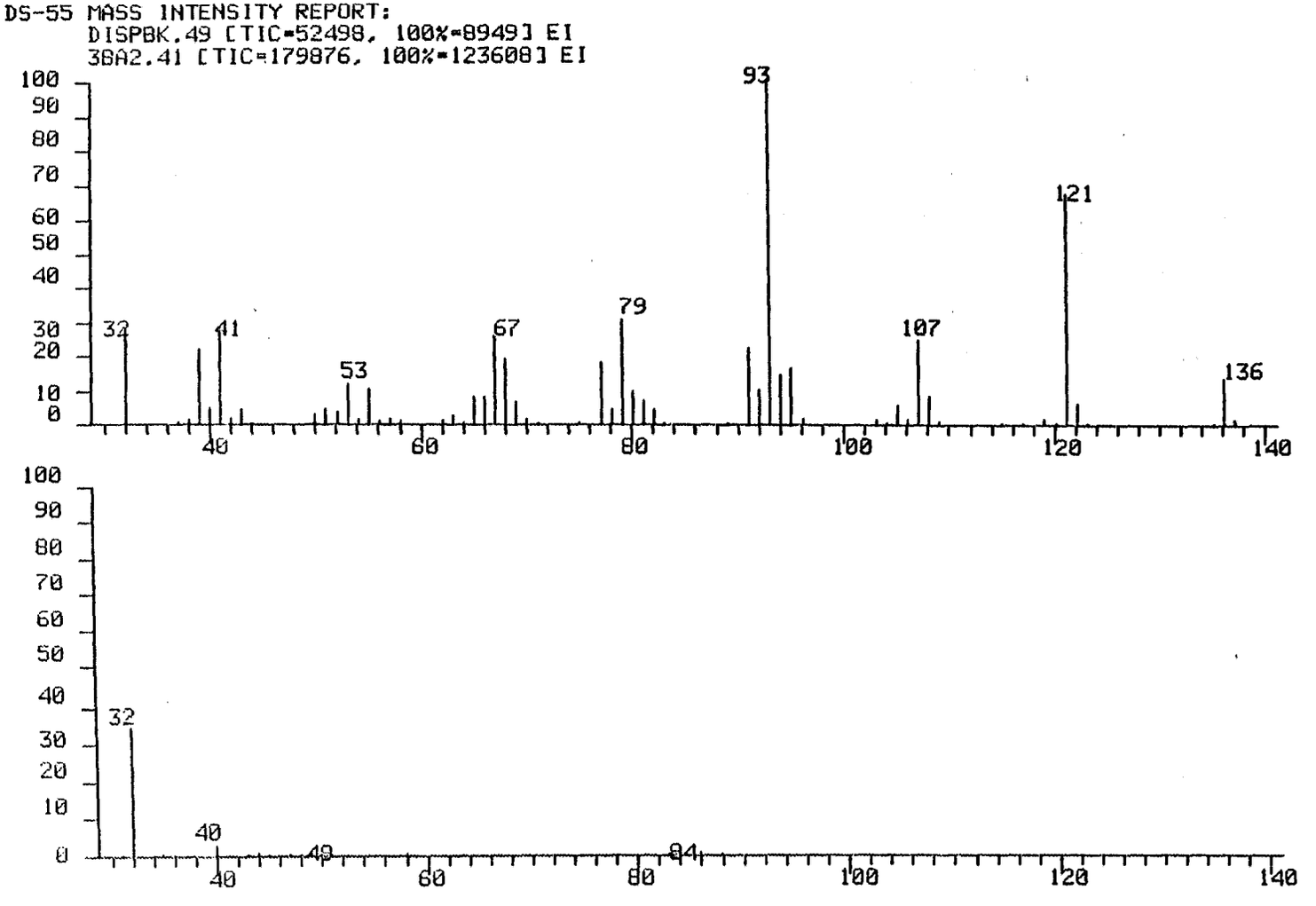


Fig. 72. Camphene

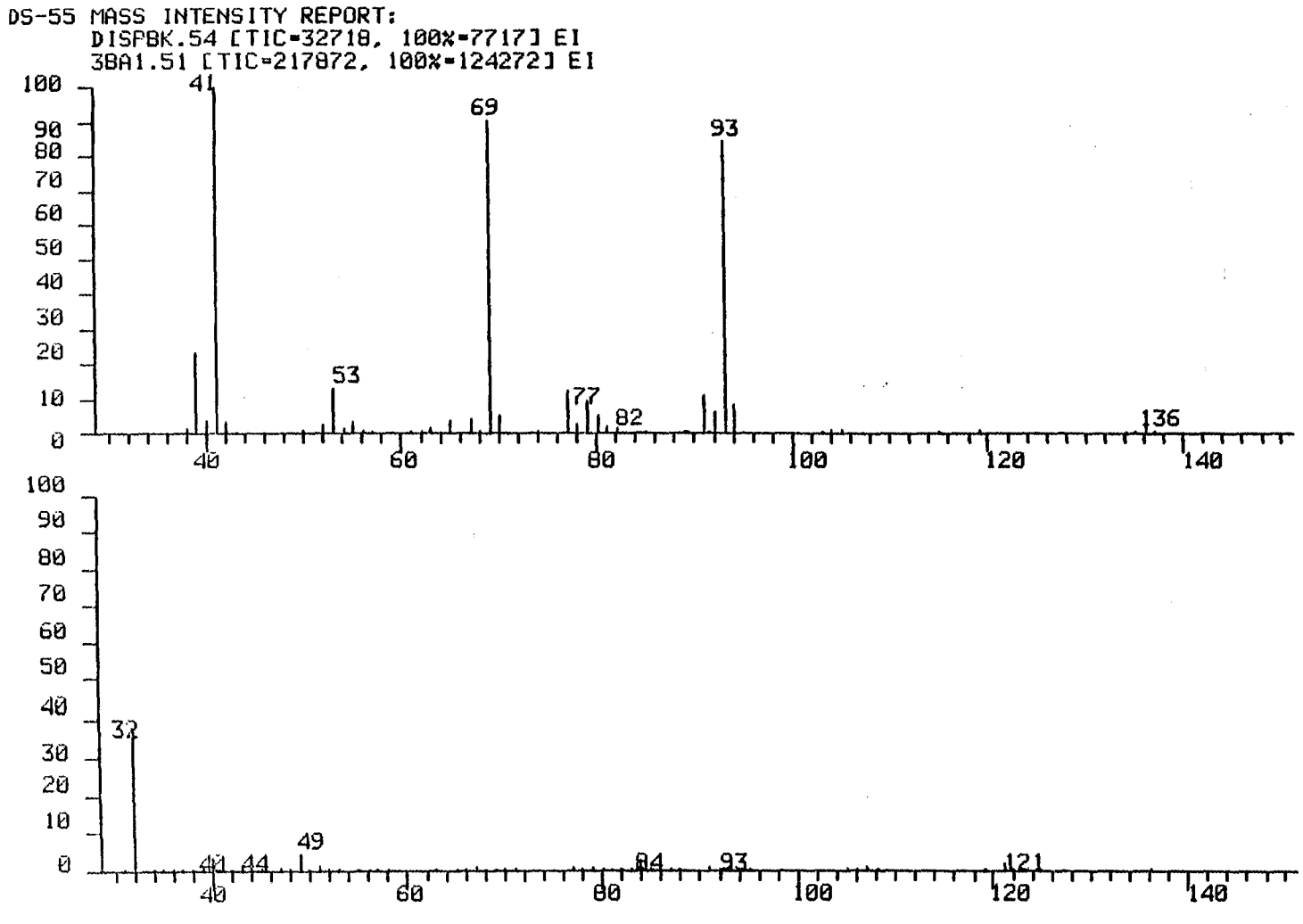


Fig. 73. Myrcene.

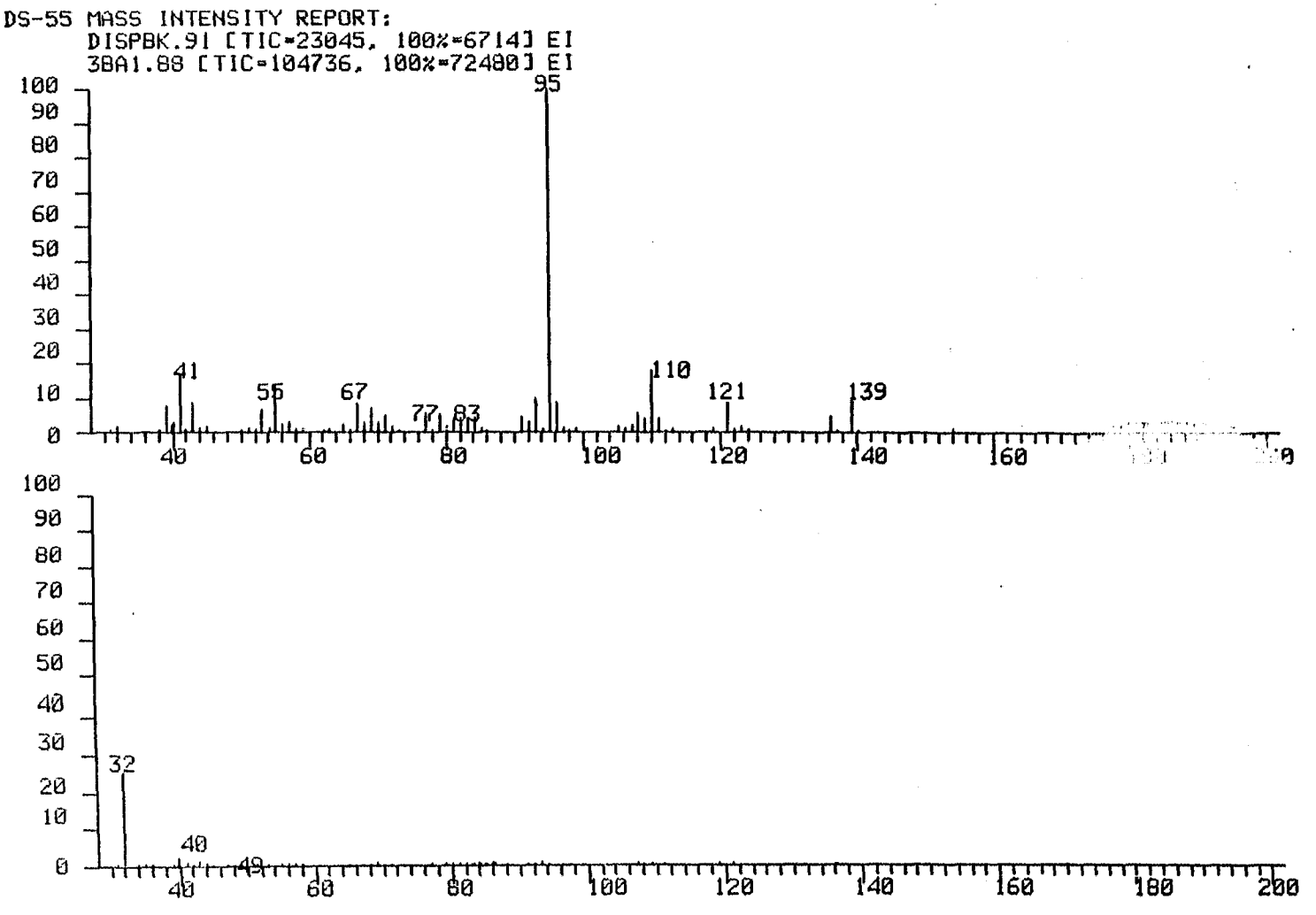
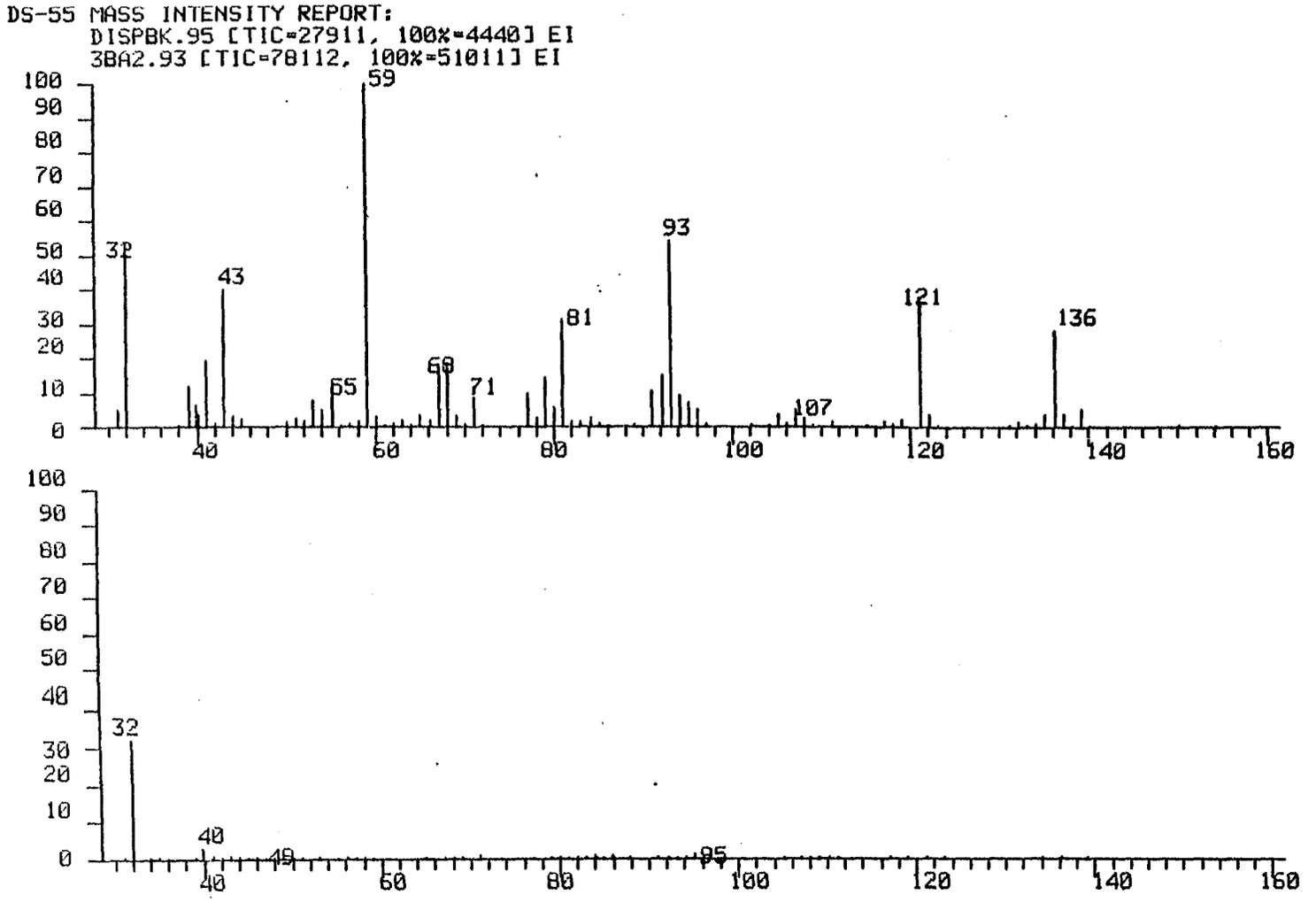


Fig. 74. Borneol.



α-terpineol

Fig. 75.

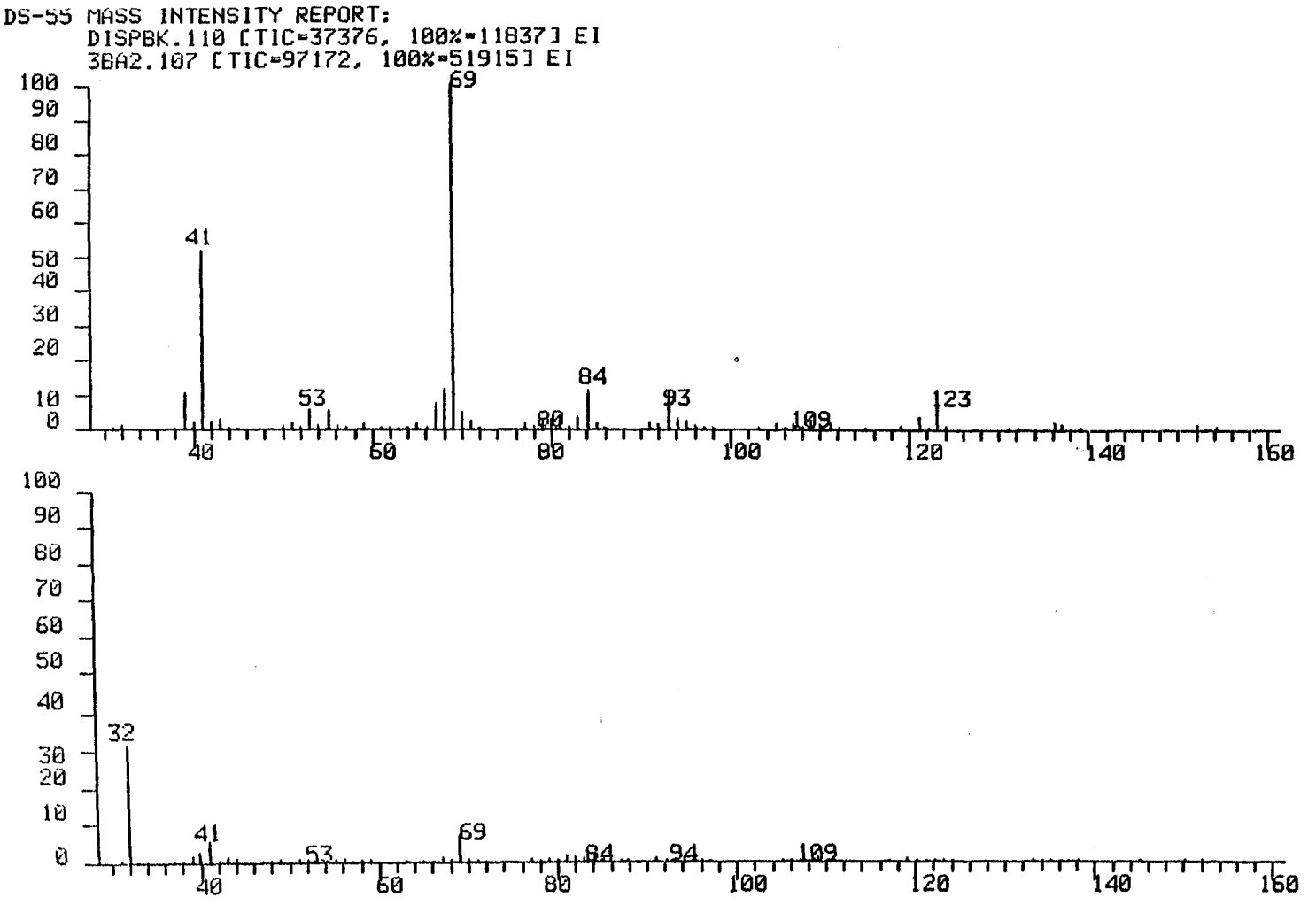


Fig. 76. Geranial

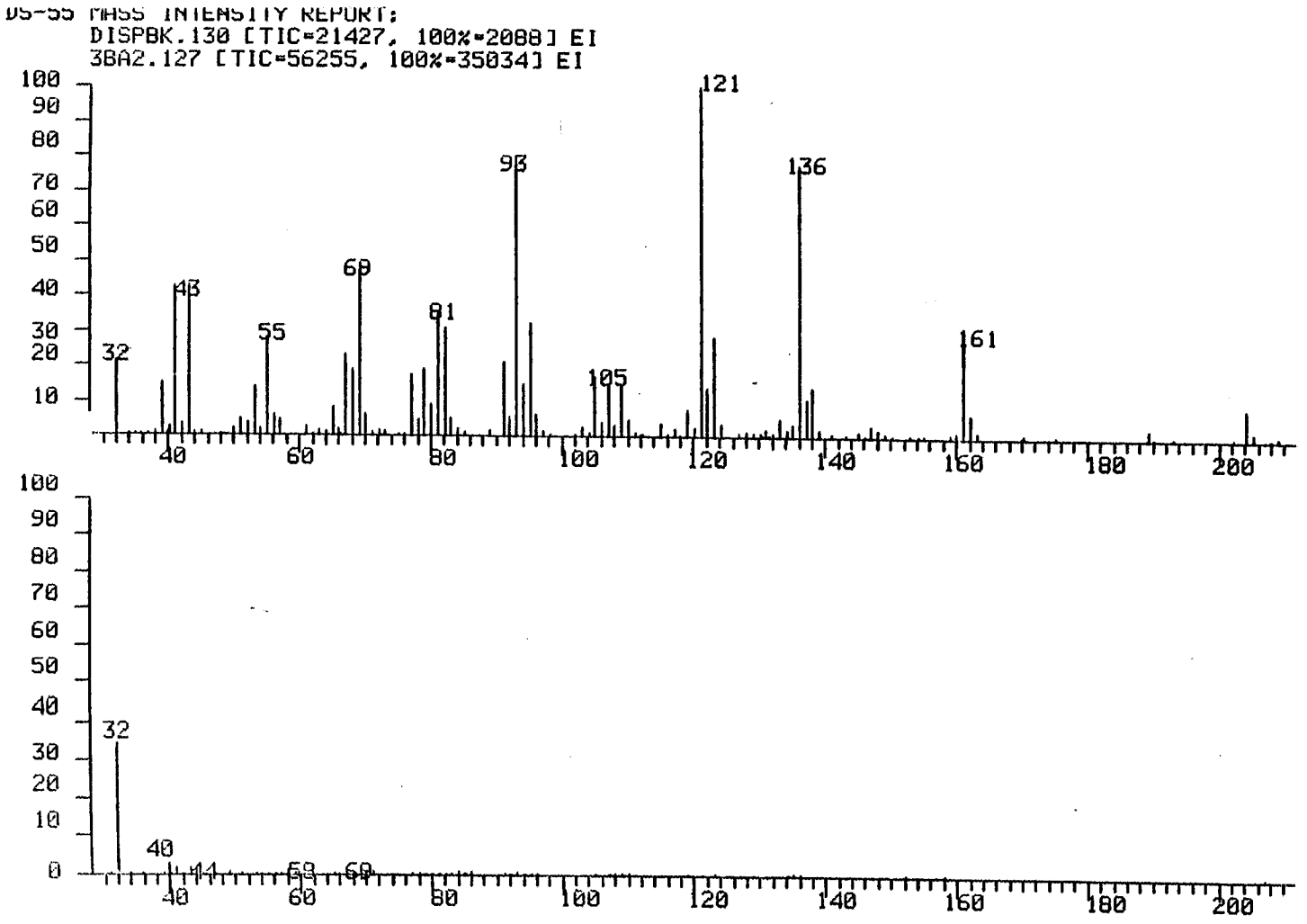


Fig. 77.

δ-elemene

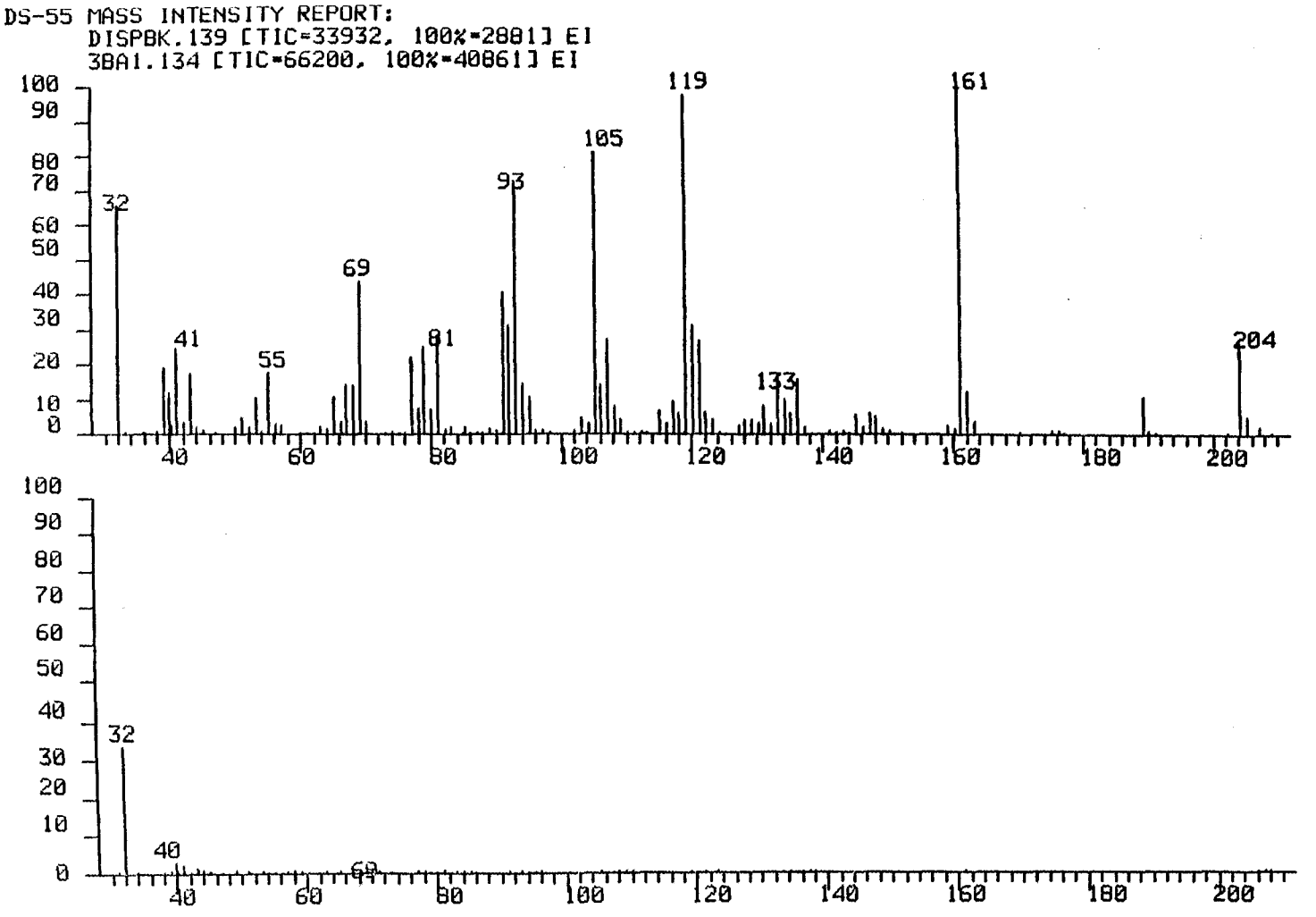


Fig. 78. α-cubebene

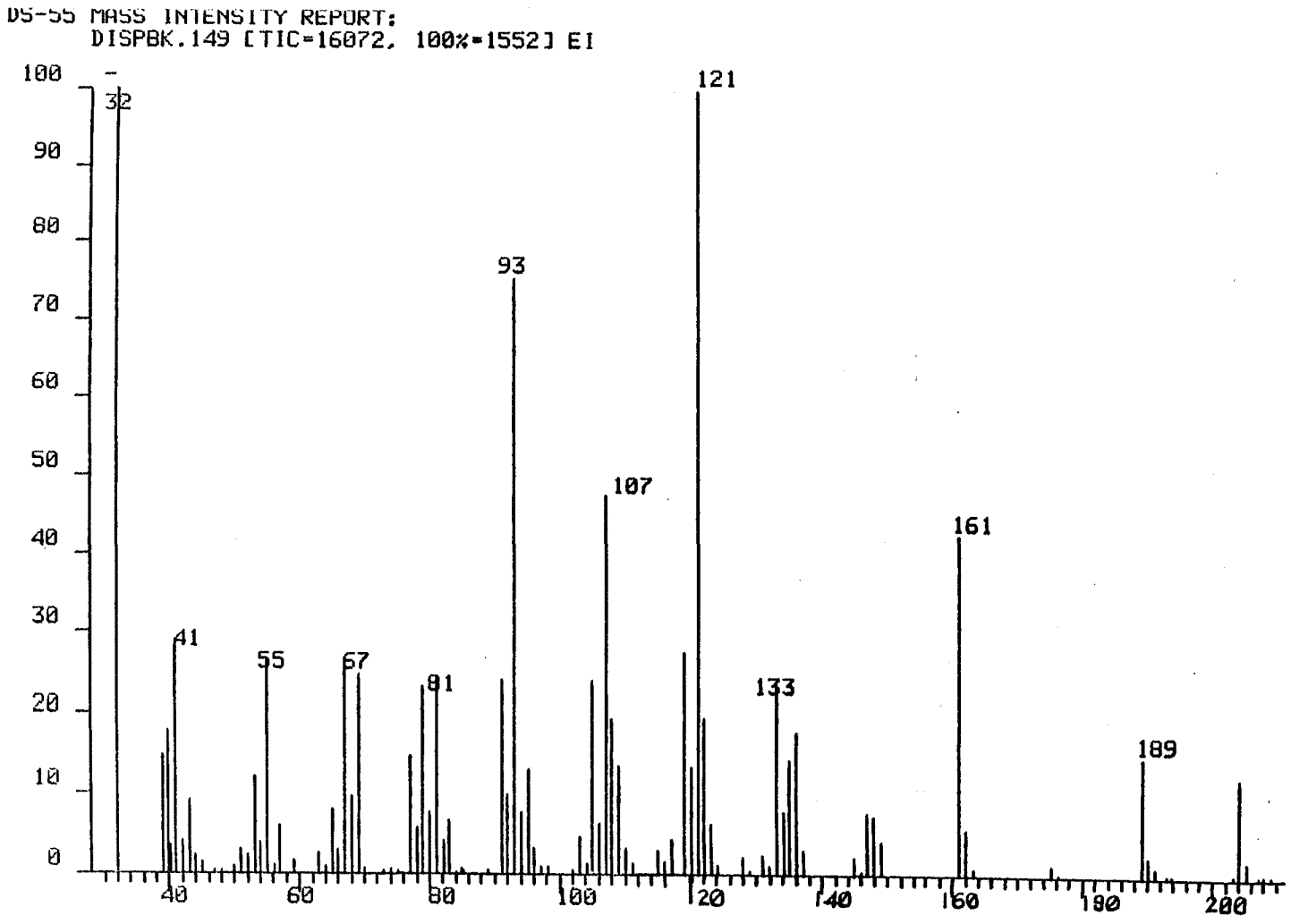


Fig. 79. γ-elemene

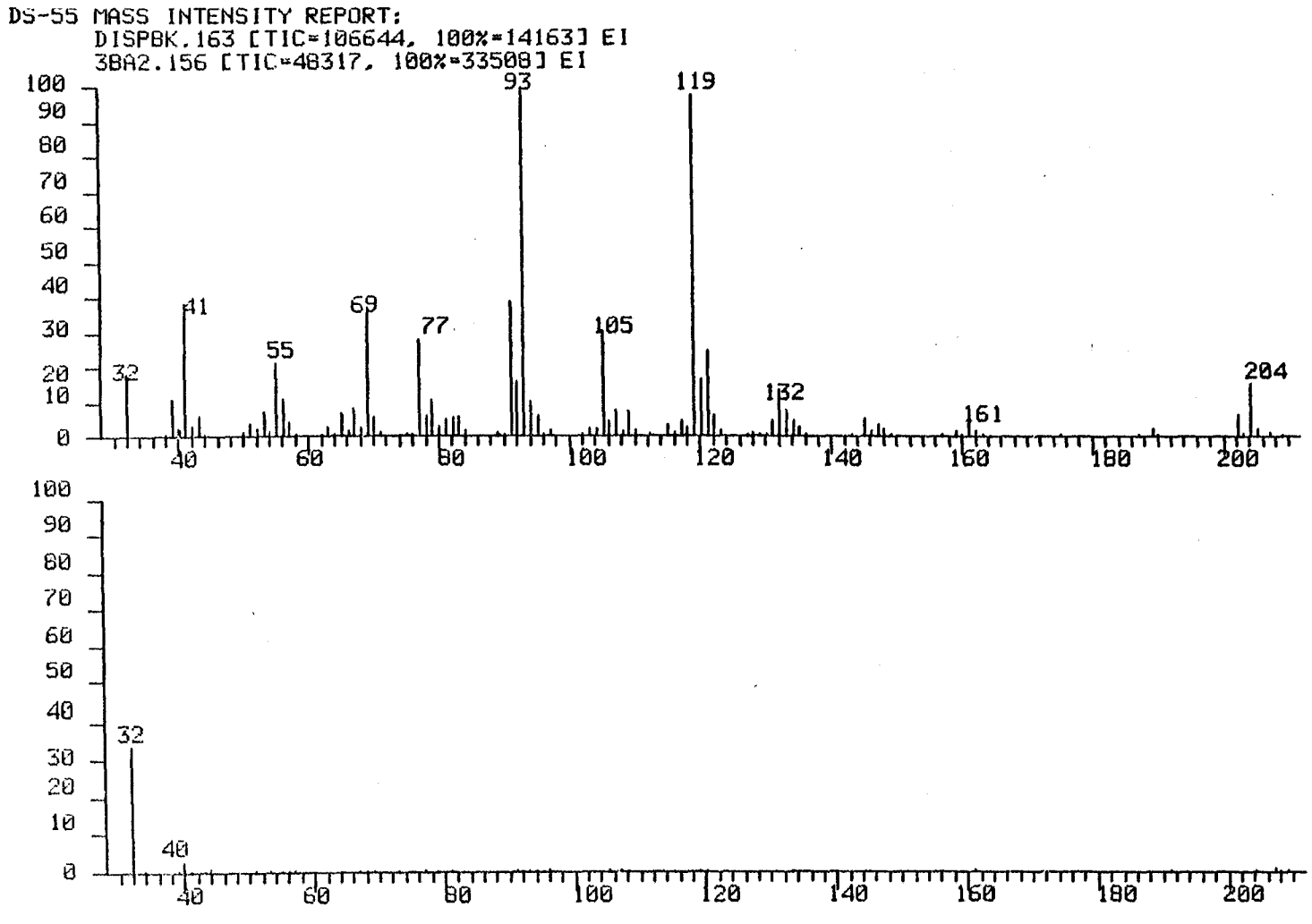


Fig. 80. Zingiberene + α-curcumene

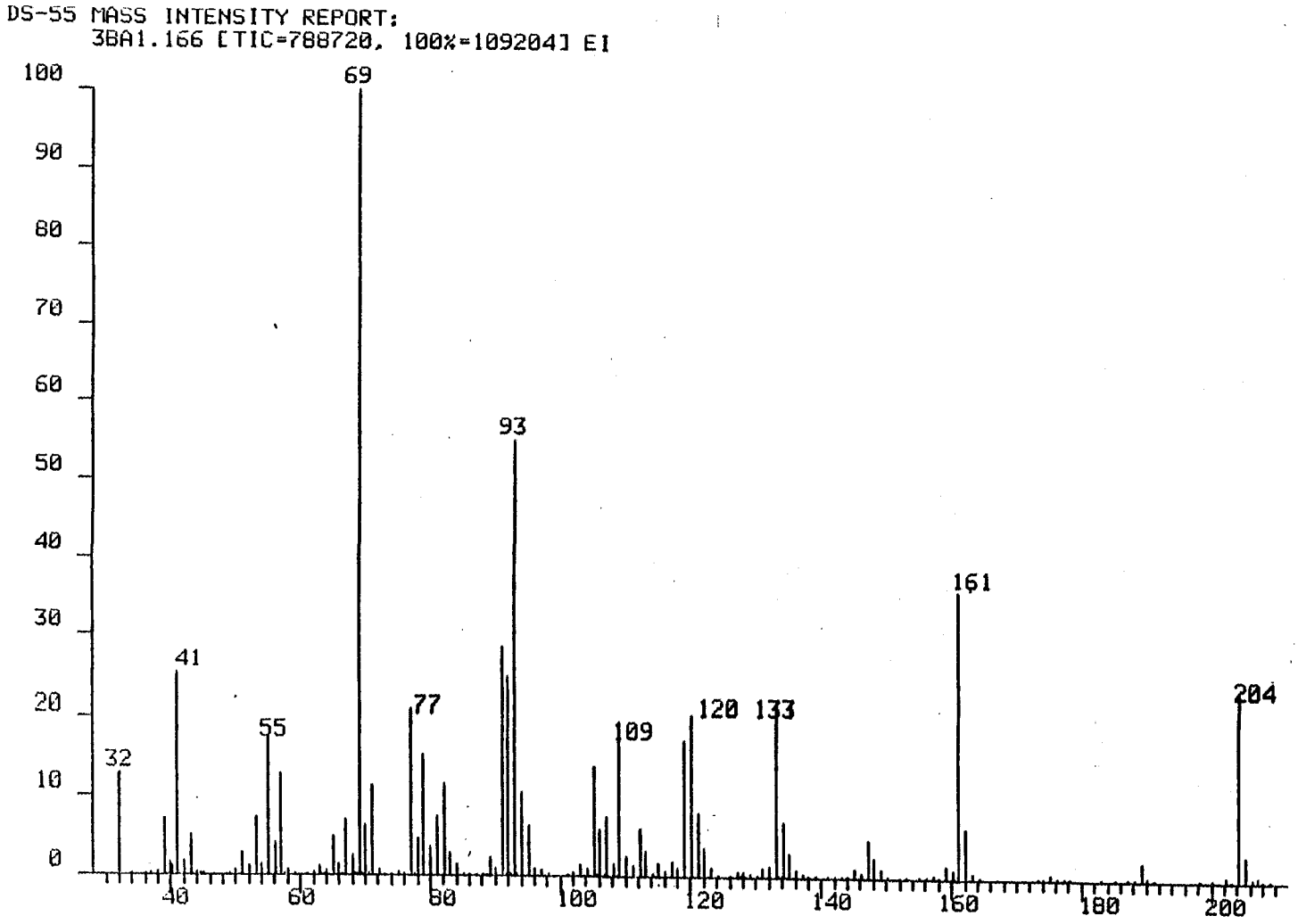


Fig. 81. β-farnesene

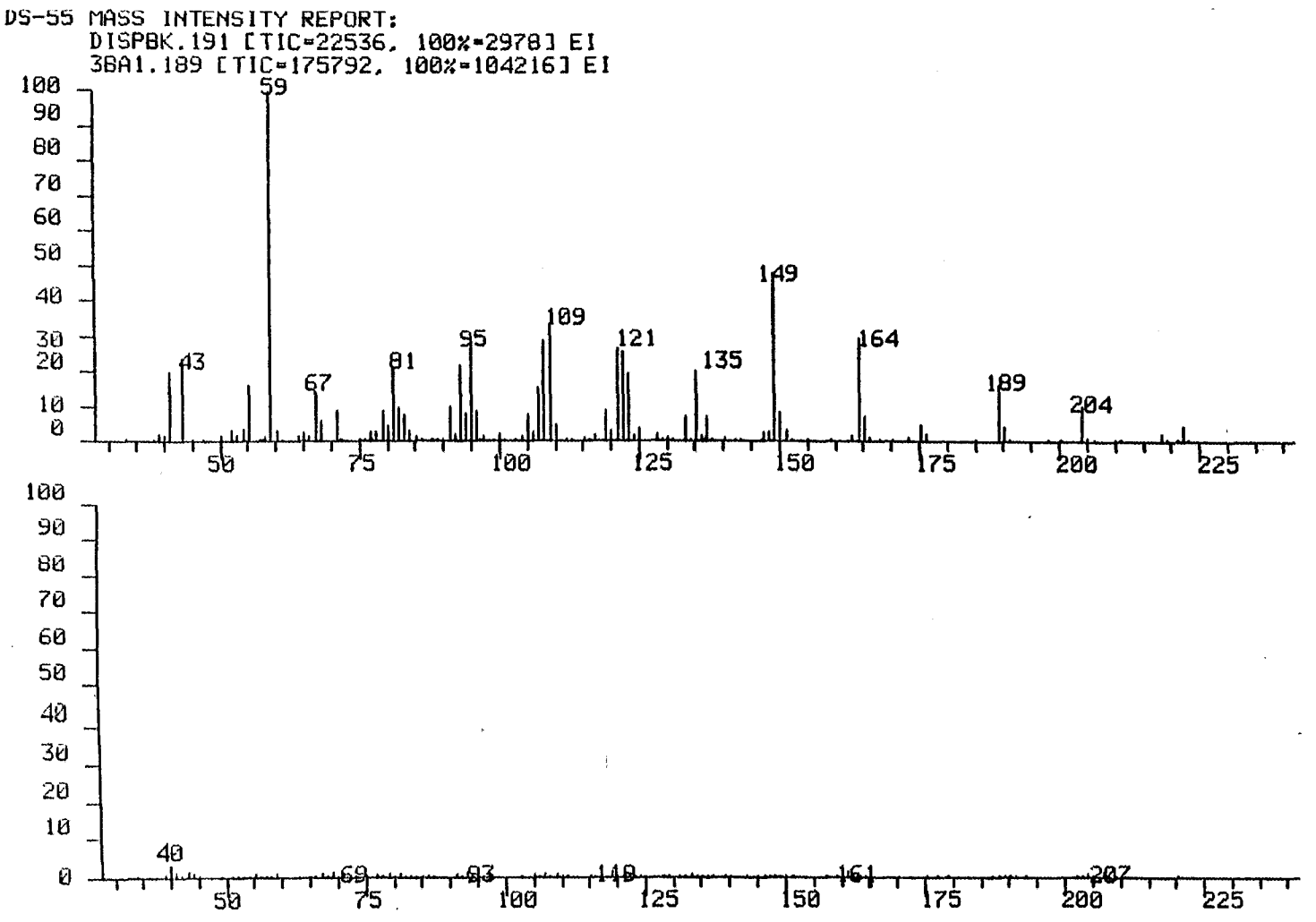


Fig. 82. β-eudesmol

REFERENCES

AKRE, R.D. (1977). *Personal communication.*

AKRE, R.D., HILL, W.B. and MACDONALD, J.F. (1973). Artificial housing of yellowjacket colonies. *J. Econ. Ent.*, 66: 803-805.

AL SAAD. (1979). *Personal communication.*

ANON (1909). Preserving ginger. *Agricultural News.*, 8: 56.

ARCHER, M.E. in EDWARDS, R. (1980). *Social wasps: their biology and control.* Rentokil Limited, Felcourt, East Grinstead. 398 pp.

ARNOLD, T.S. (1966). Biology of social wasps. Comparative ecology of the British species of social wasps belonging to the family Vespidae. *Unpublished M.Sc. thesis, University of London.*

BEIER, W. and MENZEL, R. (1972). Untersuchungen über den Farbensinn der deutschen Wespe (*Paravespula germanica* F., Hymenoptera, Vespidae): Verhaltensphysiologischer Nachweis des Farbensehens. *Zool. Jb. Allg. Zool. Physiol. Tiere*, 76: 441-454.

BETHE, A. (1932). Vernachlässigte Hormone. *Naturwissenschaften*, 20: 177-181.

BLACKITH, R.E. (1958). Visual sensitivity and foraging in social wasps. *Insectes Soc.*, 5: 159-169.

BOCH, R., SHEARER, D.A. and STONE, B.C. (1962). Identification of isoamylacetate as an active component in the sting pheromone of the honeybee. *Nature*, 195: 1018-1020.

BOSSERT, W.H. and WILSON, E.O. (1963). The analysis of olfactory communication among animals. *J. Theoret. Biol.*, 5: 443-469.

BRADSHAW, J.W.S., BAKER, R. and HOWSE, P.E. (1975). Multicomponent alarm pheromones in the weaver ant. *Nature*, 258: 230-231.

BRIAN, M.V. and BRIAN, A.D. (1952). The wasp, *Vespula sylvestris* Scopoli: feeding, foraging and colony development. *Trans. R. Ent. Soc. Lond.*, 103: 1-26.

BROEKHUIZEN, S. and HORDIJK, C. (1968). Untersuchungen über die Beute von *Paravespula vulgaris* L. (Hym., Vespidae) und ihre Abhängigkeit von der Beutetierdichte. *Z. angew. Zool.*, 62: 68-77.

BROWN, W.L. (1968). A hypothesis concerning the function of the metapleural glands in ants. *Amer. Natur.*, 102: 188-191.

BROWN, W.L., EISNER, T. and WHITTAKER, R.H. (1970). Allomones and kairomones: transspecific chemical messengers. *Bioscience*, 20: 21-22.

BUTLER, C.G., FLETCHER, D.J.C., and WATLER, D. (1969). Nest-entrance marking with pheromones by the honeybee, *Apis mellifera* L., and by a wasp, *Vespula vulgaris* L. *Anim. Behav.*, 17: 142-147.

DAVIS, H.G., EDDY, G.W., McGOVERN, T.P. and BEROZA, M. (1967). 2-4 Hexadienyl butyrate and related compounds highly attractive to yellowjackets (*Vespula spp.*). *J. Med. Ent.*, 4: 275-280.

DAVIS, H.G., McGOVERN, T.P., EDDY, G.W., NELSON, T.E., BERTUN, K.M.R., BEROZA, M., and INGANGI, J.C. (1968). New chemical attractants for yellowjackets. (*Vespula spp.*). *J. Econ. Ent.*, 61: 459-462.

DAVIS, H.G., PETERSON, R.J., ROGOFF, W.M., McGOVERN, T.P. and BEROZA, M. (1972). Octyl butyrate, an effective attractant for the yellowjacket. *Environ. Ent.*, 1: 673-674.

EBERHARD, M.J.W. (1969). *The social biology of Polistine wasps.* Misc. Publ. Mus. Zool. Univ. Michigan No. 140. 101 pp.

EDWARDS, R. (1980). *Social wasps: their biology and control.* Rentokil Limited, Felcourt, East Grinstead. 398 pp.

EDWARDS, R. and SMITH, C.P. (1980). *Personal communication.*

ENNIK, F. (1973). Abatement of yellowjackets using encapsulated formulations of diazinon and rabon. *J. Econ. Ent.*, 66: 1097-1098.

FRANCKE, W., HINDORF, G. and REITH, W. (1978). Methyl-1, 6-dioxaspiro- (4-5) -decanes as odors of *Paravespula vulgaris* (L). *Angew. Chem. Int. Ed. Engl.*, 17: 862.

FREE, J.B. (1961). The stimuli releasing the stinging response of honeybees. *Anim. Behav.* 9: 193-196.

FREE, J.B. (1970). The behaviour of wasps (*Vespula germanica*. L. and *V. vulgaris* L.) when foraging, *Insectes Soc.*, 17: 11-19.

FREE, J.B., and SIMPSON, J. (1968). The alerting pheromones of the honeybee. *Z. verg. Physiologie.*, 61: 361-365.

GAUL, A.T. (1952). Additions to vespine biology X. Foraging and chemotaxis. *Bull. Brooklyn ent. Soc.*, 47, 138-140.

GUENTHER, E. (1952). *The essential oils*, 5. p. 106. New York, Van Nostrand Co. Inc.

HEIM, F. (1893). Un procédé de déstruction des Frelons. *Bull. Soc. ent. Fr.*, 72: 106-9.

HERBERT, R. (1982). *Personal communication.*

HIGGS, M.D. (1976). Microscale structure elucidation of the monoterpenes in the frass of *Hylotrupes bajulus* (L) (Coleoptera: Ceromycidae) and their role in oviposition attraction. *Ph.D Thesis, University of Southampton.*

HÖLLDOBLER, B. (1978). Ethological aspects of chemical communication in ants. *Adv. Stud. Behav.* 8: 75-115.

HOWSE, P.E. (1964). The significance of the sound produced by the termite *Zootermopsis angusticollis* (Hagen). *Anim. Behav.*, 12 (2-3): 284-300.

IKAN, R., GOTTLIEB, R., BERGMANN, E.D. and ISHAY, J. (1969). The pheromone of the queen of the oriental hornet, *Vespa orientalis*. *J. Insect Physiol.*, 15: 1709- 1712.

JEANNE, R.L. (1977). Behaviour of the obligate social parasite *Vespula arctica* (Hymenoptera: Vespidae). *J. Kans. ent. Soc.*, 50: 541-557.

JEFKINS, F. (1961). Control of Wasps in Food Factories. New delayed action bait capable of destroying whole colonies. *Food Trade Rev.*, 31: 47-8, 54.

KARLSON, P. and BUTENANDT, A. (1959). Pheromones (ectohormones) in insects. *Ann. Rev. ent.*, 4: 39-58.

KEMPER, H. (1962). Nahrung und Nahrungserweb der heimischen sozialen Vespiden. *Z. angew. Ent.*, 50: 52-5.

KEMPER, H. and DÖHRING, E. (1961). Soziale Wespen als Schädlinge des Obstbaues und des Obsthandels. *Anz. Schädlingsk.*, 34: 17-19.

MACDONALD, J.F., AKRE, R.D. and HILL, W.B. (1973). Attraction of yellow jackets (*Vespula* spp) to heptyl butyrate in Washington state (Hymenoptera: Vespidae) *Environ. Ent.*, 2: 375-379.

MACDONALD, J.F., AKRE, R.D. and HILL, W.B. (1974). Comparative biology and behaviour of *Vespula atropilosa* and *V. pensylvanica* (Hymenoptera: Vespidae). *Melanderia*, 18: 1-66.

MACDONALD, J.F., AKRE, R.D. and MATTHEWS, R.W. (1976). Evaluation of yellowjacket abatement in the United States. *Bull. Entomol. Soc. Am.* 22: 397-401.

MARKL, H. (1965). Stridulation in leaf-cutting ants. *Science*, 149: 1392-1393.

MARKL, H. (1967). Die Verständigung durch Stridulationssignale bei Blattschneiderameisen. I-Die biologische Bedeutung der Stridulation. *Z. vergl. Physiol.*, 57 (3): 299-330.

MARKL, H. (1968). Die Verständigung durch Stridulationssignale bei Blattschneiderameisen. II - Erzeugung und Eigenschaften der Signale. *Z. Vergl. Physiol.*, 60(2): 103-150.

MASCHWITZ, U. (1964). Gefahrenalarmstoffe und Gefahrenalarmierung bei sozialen Hymenopteren. *Z. vergl. Physiol.*, 47 (6): 596-655.

MASCHWITZ, U. (1978). *Personal communication.*

MATHEW, A.G., KRISHNAMURTHY, N., NAMBUDIRI, E.S. and LEWIS, Y.S. (1973). Oil of Ginger. *The Flavour Industry*. 226-228.

MORGAN, E.D. and WADHAMS, L.J. (1972). Gas chromatography of volatile compounds in small samples of biological material. *J. Chromatogr. Sci.*, 10: 528-529.

NEEDHAM, P.H. and STEVENSON, J.H. (1966). Insecticides and beekeeping in England and Wales. *Bee World*, 47: 65-70.

NESTEROVODSKY, V.A. (1947). Wasp Control (in Russian) *Pchedlovdstvo*, 24: 49-51.

PALMER-JONES, T., WHITE, E.P., DEVINE, B.W. and PATTERSON, C.R. (1949). Developments in control of wasps. *N.Z. Jl. Agric.*, 229-32.

PARRISH, M.D. and FOWLER, H.G. (1983). Contrasting foraging related behaviours in two sympatric wasps (*Vespula maculifrons* and *V. germanica*). *Ecological Entomolgy.*, 8: 185-190.

PFLUMM, W. (1975). The influence of different sucrose syrup concentrations and presence of species members on the foraging behaviour of wasps. *Entomol ger.*, 2(1):7-21.

POTTER, N.B. (1964). A study of the Biology of the Common Wasp *Vespula vulgaris* L., with special reference to the foraging behaviour. *Ph.D Thesis, Bristol Univ.*

PRAVATOROFF, N. (1967). Ginger: the properti es and chemistry of some natural spicy compounds. *Manufacturing Chemist and Aerosol News*, 40-41.

RENTOKIL TECHNICAL REPORT, (1963). Screening tests for wasp attractants. *Technical Committee Report No. 63/122*

RICHARDS, O.W. (1962). *A revisional study of the masarid wasps. (Hymenoptera: Vespoidea).* British Museum (Natural History).

RICHARDS, O.W. (1971). The biology of the social wasps (Hymenoptera Vespidae). *Biol. Rev.*, 46: 483-528.

RIVNAY, E. and BYTINSKY-SALZ, H. (1949). The Oriental Hornet *Vespa orientalis* L.; Its biology in Israel (in Hebrew). *Bull. agric. Res. Stn. Rehovet, Israel*, 52: 1-32.

ROLAND, C. (1969). Rôle de l'involucre et du nourrissement en sucre dans la régulation thermique à l'intérieur d'un nid de Vespides. *C.r. hebd. Séanc. Acad. Sci., Paris*, 269D: 914-916.

SASLAVASKY, H., ISHAY, J. and IKAN, R. (1973). Alarm substances as toxicants of the oriental hornet, *Vespa orientalis*. *Life Sciences*, 12(2): 135-144.

SCHREMMER, F. (1941). Versuche zum Nachweis der Rotblindheit von *V. rufa*. *L.Z. Vgl. Physiol.*, 28: 457-466.

SCUDDER, S.H. (1889). Power of vision in Vespidae. *Psyche, Camb.*, 5: 279-280.

SHEARER, D.A. and BOCH, R. (1965). 2-Heptanone in the mandibular gland secretion of the honeybee. *Nature*, London. 206: 530.

SMITH, C.P. (1982). *Personal communication.*

SPRADBERY, J.P. (1973). *Wasps: an account of the biology and natural history of social and solitary wasps.* Univ. Wash. Press. Seattle. 408 pp.

THOMAS, C.R. (1960). The European Wasp (*Vespula germanica* Fab.) in New Zealand. *Inf. Ser. Dept. Sci. Ind. Res. New Zealand*, 27: 1-74.

TROUGHT, T.E.T. (1982). A proposal for research into wasp (*Vespula germanica* F.) control in New Zealand. *Ministry of Agriculture and Fisheries, Agricultural Research Division, Lincoln, New Zealand.*

WEYRAUCH, W. (1935). *Dolichovespula* und *Vespa*. Vergleichende Übersicht über zwei wesentliche Lebenstypen bei sozialen Wespen. *Biol. Zbl.*, 55: 484-524.

WHEELER, W.M. (1928). *The social insects: their origin and evolution.* Kegan Paul, Trench, Trubner and Co. Ltd., London. xviii + 378 pp.

WILSON, E.O. (1971). *The insect societies.* Belknap Press, Harvard, Mass. x + 548 pp.

WRATTEN, S.D. (1982). *Personal communication.*