UNIVERSITY OF SOUTHAMPTON

FACULTY OF SCIENCE

SOME ASPECTS OF THE EPIDEMIOLOGY OF DARK LEAF AND POD SPOT (*ALTERNARIA BRASSICAE* L.) ON WINTER OILSEED RAPE

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For Mum, Dad and my dear wife Thalia

"Even the fields of rape are turning green now, so mercifully I can stop trying to describe them."

Sher, 1985

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ABSTRACT

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SOME ASPECTS OF THE EPIDEMIOLOGY OF

DARK LEAF AND POD SPOT (ALTERNARIA BRASSICAE L.)

ON WINTER OILSEED RAPE

by Neil James Leadbitter

Dark leaf and pod spot (Alternaria brassicae) can be a serious disease of oilseed rape (Davies, 1986). In this study a detailed examination of the development of dark leaf spot on winter oilseed rape was made during three seasons (1986-1988) in Hampshire, U.K..

Disease area diagrams for dark leaf and pod spot were constructed and different measures of disease compared. Taylor 's Power Law was fitted to the data for each variable, and transformations and the sample numbers required for different levels of accuracy were derived. Relationships between different variables showed that incidence-severity relationships and selection of the plant parts assessed could be useful in reducing the time required for assessment.

Disease progress on the leaf canopy was described using simple graphical methods and contour mapping. The latter provided a succesful way of representing the vertical progression of the disease through the leaf canopy during the season. Although disease intensities differed in each epidemic the distribution pattern of disease was similar. Disease was already widely spread in the crop during the 'rosette ' stage and continued to develop slowly during the winter. During stem extension the disease was confined to the basal main stem leaves even though the leaf canopy was well spread vertically and branch leaves surrounded the mainstem leaves at the crop base. As the leaf canopy matured the disease moved up the plant so that prior to complete leaf loss the vertical distribution of the disease resembled that of the leaf canopy.

On the pod canopy disease development in each epidemic showed a similar development pattern which was adequately described by the logistic function. In each epidemic a relatively long lag phase was followed by disease development which coincided with heavy rainfall.

Plant traps were used in 1987 and 1988 to assess the levels of infection occurring during the season. In 1988 infection on the plant traps was highly correlated with spore numbers caught (R^2=78%) whereas in 1987 no clear relationship could be derived. Infection on the plant traps were almost always associated with rain induced wetness but attempts to relate infection with measures of relative humidity, rainfall and wetness using multilinear regression were unsuccessful.

In 1987 and 1988 levels of inoculum were measured using spore traps. Different patterns of spore capture occurred in each year and no clear relationships between measures of relative humidity, rainfall and wetness were developed using multilinear regression.

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PART 1: LITERATURE REVIEW AND METHODOLOGY

CHAPTER 1 LITERATURE REVIEW

1.1 Oilseed rape

Oilseed rape (*Brassica napus* L.) has recently become a major crop in the U.K., with an area in 1987 of approximately 350,000 ha, using over 6% of all agricultural land. It is used as a break crop in cereal rotations and provides oilseed for the production of vegetable oils and meal for animal feeds.

B. napus is one of a group of four *Brassica* species which are grown world wide for their oilseed. *B. carinata* L. (Ethiopian mustard) is only important in its country of origin. *B .juncea* L. (Oriental mustard) is largely confined to the Indian subcontinent and China, although it is grown in Europe for the production of condiment mustard. *B. napus* and *B. campestris* L. are the main oilseed species that are grown in Europe and Canada. There are spring sown and autumn sown varieties of both species giving the farmer a choice of four crops. Autumn varieties out-yield spring varieties of the same species by about 20% and *B. napus* out-yields *B. campestris* by about the same amount. *B. campestris* is however earlier maturing and the autumn varieties are less susceptible to winter kill. In Europe, *B. napus* is the most widely grown species, accounting for 95% of current production, whereas in Canada the production of *B. napus* and *B. campestris* is about equal (Bunting, 1986).

Introduction of rapeseed into Britain was almost certainly a consequence of land reclamation in the fenlands of eastern England during the late 16th and early 17th centuries, where it was used as a pioneer crop by the Dutch engineers (Fussell, 1955). During this period crushing mills were established and by the end of the 17th century the industry was sufficiently important to be protected by substantial tariff barriers (Bunting, 1984). During the 18th century oilseed production continued to expand, spreading away from the east coast. The 19th century saw the development of overseas sources for oilseed and indigenous production declined, so that oilseed

rape effectively disappeared from the agricultural scene. The decline in oilseed rape production in Britain was followed by a similar decline in other western countries (Bunting, 1986) and until the mid 20th century China and India became the major producers.

Although rapeseed was grown in Canada in the early 1940s to meet a demand for rapeseed oil as a lubricant for marine engines and in Germany during World War II as an edible oil, it was not until major sources of oilseeds, especially those in the Far East, suffered severe damage during World War II that the cultivation of oilseed rape was investigated in Britain as an alternative source of oil. The first agronomic trials were set up in Britain in 1948 and commercial production began in 1950. Spring varieties were grown and production was mainly confined to chalkland areas. In the late 1950's rapeseed prices plummetted and oilseed rape remained a minor crop in Britain until 1973 with an area of 4,000-7,000ha (1968-1972) (Bunting, 1984).

In 1973 Britain joined the European Economic Community (EEC) where rapeseed prices were supported via the Common Agricultural Policy (CAP). This support policy is based on a deficiency system which effectively gives the farmer a guaranteed price for his seed. Under the influence of this system U.K. production increased, even though production in other EEC countries was declining. This decline was largely due to the introduction of lower yielding low erucic acid (<5%) varieties which became mandatory in 1977. The drought of 1976 accelerated the change to autumn sown cereal varieties, and the subsequent rise in area of winter barley resolved a potential rotational problem. So from 1973 to 1979 the area of oilseed rape in Britain increased from 14,000 to 74,000 ha and production from 31,000 to 198,000 tonnes (Bunting, 1986) even though the area sown and production in the EEC as a whole showed little change. In 1979 the EEC encouraged the production of 'protein crops'. This had an immediate effect on oilseed rape production in Britain, which increased by 50% in 1980, and in 1984, was three times the 1979 level. By 1984 269,000 ha of oilseed rape was being grown in the U.K. with a yield of 914,000 tonnes (Bunting, 1986).By this time more than 95% of the crop was autumn sown (Almond *et al.* 1986).

In the 1960's and early 1970's rapeseed production was centred around the chalkland farms of Hampshire and Wiltshire (Bunting, 1984). From here the crop has moved with a north eastern trend and today there is some production in virtually all counties of England.

Until 1988 EEC policy had continued to support oilseed rape at a price which made the crop at least as profitable as cereals and the area grown continued to expand. However during 1988 support was cut and this together with the compulsory introduction of "double low" (low erucic acid and low glucocinolate content of seed) varieties for the 1991/92 season which at present yield less than "single lows" and may not consistently produce glucosinolate levels lower than the limit, is likely to reduce the hectarage of oilseed rape grown in the near future.

Autumn sown oilseed rape is grown as a break crop in cereal dominated rotations. Until recently not only did the EEC policy mean that it was highly economic but because it is harvested relatively early there is time for adequate seed bed preparation for subsequent winter wheat. Other advantages include the opportunity for effective grass weed control; reduction of levels of cereal diseases which oilseed rape does not host; the provision of a good seed bed for the following crop because of its deep and extensive root system and the lack of specialist equipment required (Almond *et al.* 1986).

Winter varieties should be sown between mid-August and mid-September (Bunting, 1956; Mendam *et al.* 1981; Scarisbrick *et al.* 1981). If conditions are moist and warm emergence can be detected in 4-5 days (Daniels *et al.* 1986). Germination is epigeal raising the two cotyledons above the soil surface. On emergence the first true leaves form a 'rosette', the form in which the crop over-winters. Leaf development is variable in the autumn depending on the date of sowing. 6-7 leaves may be produced by the end of September on early sown crops whereas only one leaf may have expanded on crops sown in early September (Daniels *et al.* 1983). Up to 30 true leaves may be produced (Daniels *et al.* 1983; Daniels *et al.* 1986) but this is influenced by sowing date, variety and season. Leaf formation is continuous between germination and the onset of reproductive development. The switch from

leaf to flower primordium initiation occurs during the latter part of the autumn. In the variety Rafal Daniels *et al.* (1986) demonstrated that a developmental switch occurred on 5 November in a crop sown on 19 August. This switch is probably triggered by a period of vernalization but little information is available on varieties grown in the U.K. (Daniels *et al.* 1986; Almond *et al.* 1986). Floret initials first become evident on the terminal raceme site of the apical meristem, the first floret producing the first pod at the base of that raceme. After a number of florets have been produced at this site floral initiation occurs on axillary buds of the upper main stem leaves. These develop to form the primary branches. Development on each raceme is acropetal and continues to occur slowly throughout the winter. The pod canopy however is not completely fixed and the plant remains indeterminate. By the beginning of winter the crop forms full ground cover and remains in its rosette form until spring.

In spring, in response to changes in temperature and day length, internodal growth causes rapid main stem elongation. During this period the flower buds become visible and a spiral arrangement of branches develops (Mendam, 1975). Secondary branches may develop but are of minor importance in determining yield (Almond *et al.* 1986). Inflorescences are initially short but during anthesis elongate and by final harvest are a major component of plant height (Askew, 1982). Flowering takes a period of 3-5 weeks. *B. napus* is inherently self-fertile and is generally 60-70% self pollinated (Williams, 1978). Not all flowers develop into pods and the number of those contributing to final seed yield is considerably less than the maximum present at early pod development. Over 4-8 weeks the long narrow pods increase to a final length of 5-10 cm (Almond *et al.* 1986). Each fruit contain up to 40 embryos and have two carpels divided by a false septum. During seed development there is a gradual decline in seed numbers until harvest (Norton & Harris, 1975). Seed ripens following a similar sequence to that of raceme development which results in uneven ripening within the plant. Mature *B. napus* seeds are dark brown or black, small round (1.75-2.00mm diam.) and 4-6 mg weight.

Having produced the mature seed there may be a problem with pod shatter before and during harvest. Pre-harvest this largely depends on weather conditions and losses can be as great as 50% if high winds and heavy showers of rain prevail (MacLeod, 1981).

1.2 The pathogen and disease

Alternaria brassicae (Berk.) Sacc. 1886 sensu Bolle 1924 is the major causal organism of dark leaf and pod spot of oilseed rape in the U.K. It is a late season disease thought to be associated with wet conditions during and after flowering. Yield loss is mainly due to the pod infection which reduces pod photosynthetic area, leading to reduced seed size, premature ripening and consequently pod shatter before and during harvest. It has also been shown that the protein content of the seed is reduced (Degenhardt *et al.* 1974; Husein & Thakur, 1963) *A. brassicicola* (Schw.) Wiltsh. causes similar symptoms and some studies have suggested that this species is more important on vegetable *Brassica* crops whereas *A. brassicae* predominates on oilseed rape (Anon, 1983; Changsri & Weber, 1963; Humpherson-Jones, 1983).

A. brassicae has been reported on many cruciferous hosts worldwide (CMI Distribution Map 353, ed. 4, 1984). Neergaard (1945) gives a list of sixty-one records of the pathogen on sixteen species on which natural infection has been recorded. Most reports were on *Brassica* species and Morton (1964) suggests that *A. brassicae* is a strong parasite of most if not all *Brassica* spp. This apparent specialisation within the crucifers may however merely reflect the interest in *Brassica* spp. as important agricultural and horticultural crops. Reports of the pathogen come from all parts of the world where *Brassica* crops are grown including Japan (Yoshii, 1933), Philippines (Fajardo & Palo, 1934), Brazil (Arruda, 1938), Canada (Conners, 1935), India (Mason, 1928) and Russia (Kikoina, 1931). New occurrences are still being found (Prasada *et al.* 1970) The pathogen is also mildly pathogenic, in inoculation experiments, on other families outside the *Cruciferae* (Groves & Skolko, 1944; Neergaard, 1945) .

As a pathogen of oilseeds *A. brassicae* was first described by Kühn (1855,1856) and has since been recorded in various parts of the world as oilseed rape has grown in popularity. In Saskatchewan, Canada, during the 1970s, *A. brassicae* and *A. raphani* were the principal causes of stem and pod spot on *B. napus* and *B. campestris* (Petrie, 1974). During the period 1970-1972 seed infection on samples taken from seed stores increased four-fold (Petrie, 1974) but then remained static over the next four years (Petrie, 1978).In Spain *A. brassicae* was first reported on *B. napus* in 1979 (Romero-Munoz & Jimenez-diaz, 1979) and caused severe damage in 1980 (Romero-Munoz & Gonzalez-Torres, 1983). In Britain surveys made during the mid 1970s as the area of oilseed rape was beginning to expand showed very low levels of *Alternaria* leaf spot to be present (Cook & Evans, 1978; Rawlinson & Muthyalu, 1979) although Rawlinson & Muthyalu (1979) did point out that their single assessment date in June may have underestimated the levels of dark leaf spot. Later work (Evans *et al.* 1984) reported an increase in levels of dark leaf spot which first became a problem in S.E England in 1979. High levels of disease were recorded in the south-east and east in 1980 and 1981 and *Alternaria* pod spot was the most serious disease on oilseed rape throughout England between 1981 and 1983 (Evans *et al.* 1984). Levels of the disease were less in 1983 than in the previous two years, results which Evans *et al.* (1984) suggested were a consequence of dry weather post-flowering and the extensive use of fungicides to control the disease. Gladders (1984) showed that in eastern England dark leaf and pod spot reached a maximum in 1981 and then declined to low levels by 1984. This reduction in disease in field surveys corresponds well with a similar drop in levels of *A. brassicae* found on commercial rapeseed over the same period (Humpherson-Jones, 1984). These declining levels reflect the adoption of fungicides against dark leaf and pod spot and unfavourable weather for the disease during pod development (Davies, 1986). In 1982 a survey in Scotland (Kothanur & Lennard, 1982) showed that *A. brassicae* was a serious disease of oilseed rape: 80% of the plants in the fields surveyed were diseased at pod filling and at harvest 80% of the seed samples were infected. In 1983 a similar survey (Anon, 1984) showed that out of forty five crops sampled for seed 75% were infected with *A. brassicae.*

The morphology of *A. brassicae* found in Denmark has been described in detail by Neergaard (1945). The hyphae grown on natural and synthetic media are septate and 3-8 μm wide. Conidiophores on natural media are 14-48 μm long by 6-13 μm wide whereas on synthetic media they are longer (30-200 μm) but narrower (4-8 μm). They are usually unbranched and erect with a single scar and on natural media often emerge through the leaf epidermis in bunches via stomata. The conidia are deep olive buff to dark olive buff; obclavate to elongated oval, gradually tapering towards a beak and constricted by septa. Both longitudinal and transverse septa are present. The spore body is 33-147 μm X 9-33 μm and divided by 3-18 transverse septa and 0-15 longitudinal septa. The beak is 9-14 μm long and 3-7.7 μm wide with 0-7 transverse septa giving a total spore length of 39-350 μm. Neergaard (1945) suggested that the species is made up of several morphological races. Prasada *et al.* (1970) described *A.brassicae* on *Eruca sativa* in N.W. India and also showed great morphological variation in the conidia. On Potato Dextrose Agar (PDA) conidial length was 22.5-65 μm and only 2-3 longitudinal septa were present. Weimer (1926) gave a conidial length of 125-225 μm for the same fungus on cauliflowers.

The symptomatology of the disease was first described by Berkeley in 1836 on *B. oleracea. A. brassicae* will attack leaves, stems and pods of *Brassica* hosts. Pod spotting may lead to seed infection which in turn may cause damping off of seedlings (Groves & Skolko, 1944; Neergaard, 1945). On leaves symptoms are variable. They start as small brown or black points (Neergaard, 1945). As lesions develop, the centre may become paler leaving a brown or black margin (Neergaard, 1945; Romero-Munoz & Gonzalez-Torres, 1983) and concentric zonation may occur giving the classic 'target ' spot symptoms (Romero-Munoz & Gonzalez-Torres, 1983; Singh, 1977). Lesions may vary in shape from circular (Neergaard, 1945; Romero-Munoz & Gonzalez-Torres, 1983; Singh, 1977) to oval (Neergaard, 1945) and on severely diseased leaves coalesce (Neergaard, 1945). Spots are often surrounded by a yellow halo (Singh, 1977) and will grow to up to 1 cm in diameter (Neergaard, 1945; Romero-Munoz & Gonzalez-Torres, 1983; Singh, 1977). Under moist conditions the spots are covered with conidia which give the lesions an olivaceous appearance although this is rarely seen in the field (Weimer, 1926).

Symptoms on pods are similar to those on leaves (Davies, 1986; Neergaard, 1945; Romero-Munoz & Gonzalez-Torres, 1983; Singh, 1977) except that they tend to be elongated (Romero-Munoz & Gonzalez-Torres, 1983; Singh, 1977). Little sporulation occurs on infected pods (Singh,1977). From an initial external infection the fungus will penetrate the pod wall to infect the seed (Davies, 1986). On *B. juncea* it was found that the pathogen survived beneath the testa and caused severe discolouration and shrivelling of the seed (Chahal, 1981). Rangel (1945) suggested that seed is the most important source of primary infection and that the infection on the seed will remain viable for at least twenty months. Chahal (1981) however, showed that infection declined during seed storage and after five months, seed that had high (84-92%) initial levels of infection was completely free of the pathogen. During seedling growth *A. brassicae* causes damping off (Groves & Skolko, 1944; Neergaard, 1945; Maude & Humpherson-Jones, 1980) the level of which has been correlated with that of seed infection (Maude & Humpherson-Jones, 1980). Damping off commences at the root and extends to the stem and leaves. It causes a yellow/brown rot or numerous brown spots and the seedling is often covered with conidia (Neergaard, 1945).

On oilseed rape the progress of the disease from its first appearance on the "rosette " leaves to its infection of the pods has rarely been described in detail. In India on *B. napus*, *A. brassicae* can cause serious damage of the leaf canopy before development on the pods occurs (Husein & Thakur, 1963). On *B. juncea* Wadhwani & Dudeja (1982) showed that during a single season the disease developed in three phases. The disease first appeared on the leaves in contact with the soil then moved up the plant during a heavy period of rain and finally progressed onto the pod canopy. In Britain infection of the leaf canopy is thought to cause no yield loss but acts as an inoculum source for infection of the pod canopy (Gladders, 1983).

The conditions necessary for disease development are warm (17-25°C), wet weather during flowering and pod development (Davies, 1986) although wet weather during stem extension may also be important (Gladders, 1983). Infection by *A. brassicae* can occur in a short time. Minimum times for infection have been variously

reported as 6h at 22°C on *B. napus* (Louvet & Billotte, 1964); 4h at 25°C on *B. campestris* (Saharan & Kadian, 1983) and 16h at 15°C on *B. oleracea* (Humpherson-Jones & Hocart, 1982). High humidity (>93% RH) (Louvet & Billotte, 1964; Mhrîda, 1983) or free water (Humpherson-Jones & Hocart, 1982) is required for infection. Increasing the length of the wetness period increases the amount of infection (Louvet & Billotte, 1964; Mhrîda, 1983) whereas alternating wet and dry (70-80% RH) periods of 16h and 8h respectively restricts pathogen development (Humpherson-Jones & Hocart, 1982). After infection lesion growth occurs quickly at temperatures greater than 18°C but is practically zero at lower temperatures (Louvet & Billotte, 1964). On cabbage seed crops Humpherson-Jones & Ainsworth (1981) showed that infection occurred on days with widely differing weather conditions and were unable to determine precisely the climatic factors necessary for infection.

Spore production is favoured by extended periods of high humidity and leaf surface wetness (Humpherson-Jones & Ainsworth, 1982). On excised leaves from field grown oilseed rape plants Humpherson-Jones & Phelps (1989) showed that abundant sporulation occurs when there is at least 12 h continuous high humidity (>91.5% RH) and a temperature of 12-24°C. Below this temperature longer periods of high humidity are required (*Ibid.*). Interrupting the period of high humidity during the first 10 h with 4 h of dryness (70 or 80% RH) completely inhibited spore production but exposing lesions to successive wet periods separated by dry periods (6 - 30 h) showed that there were no differences in the concentration of spores produced in each of the successive wet periods (*Ibid.*) Subsequent spore release from lesions is associated with a fall in relative humidity (Humpherson-Jones & Ainsworth, 1981). Louvet & Billotte (1964) suggested that there were three mechanisms of spore dispersal of *A. brassicae* in an oilseed rape crop. 1) a drop in relative humidity causing the release of spores which were subsequently dispersed by wind; 2) splash dispersal in large water droplets; 3) aerosol dispersal in small windborne water droplets during rainfall.

A. brassicae is controlled in oilseed rape using seed treatments and foliar sprays. Commercially available seed is usually treated with iprodione or fenpropimorph which controls the seed borne spread of the pathogen (Davies, 1986).

Iprodione has also been found to be one of the most effective foliar fungicide for control of dark pod spot (Davies, 1986). Evans *et al.* (1984) showed that the optimum spray timing differs from year to year. In 1982 significant yield increases were only obtained when the spray was applied when 20 pods were formed on the main raceme, whereas in 1983 sprays at 95% petal fall gave consistent yield benefits. Ogilvy (1984b) however, showed that fungicide application to pods immediately post-flowering consistently gave the most effective and economical control. A.D.A.S.now recommends that a fungicide spray should be applied if leaf spots are apparent on the upper leaves or lower pods and wet weather is forecast. A second spray may be required 3 weeks later if the weather remains conducive to disease development.

Much basic information on the progress of the dark leaf and pod spot through the oilseed rape crop both in time and space seems to be lacking, especially data relating to the leaf canopy. Such information is necessary for improving sampling and assessment; attempting to relate disease development to both biotic and abiotic factors and producing more rational control strategies. The environmental factors affecting disease development in the field also appear to be poorly understood, again restricting the development of rational control strategies. The aim of this project was to extend the information available regarding the factors controlling the development of dark leaf and pod spot on oilseed rape in the U.K., by the detailed description of several disease epidemics and the investigation of environmental conditions associated with infection and spore dispersal in the field.

CHAPTER 2 METHODOLOGY OF FIELD TRIALS

2.1 1986 Field Trial

2.1.1 Introduction

The 1986 field trial was designed to start to provide an overall picture of the way in which dark leaf and pod spot progresses through an oilseed rape crop.

Indications of plant development (e.g. leaf position, leaf height, raceme length, plant height) and disease development (e.g. lesion no., lesion area) were measured or estimated. Meteorological data were taken to compare with the biological data. Two varieties with the same levels of disease susceptibility were used to observe the effects of different crop growth patterns on the disease.

2.1.2 Experimental area

One area of oilseed rape cv. Mikado and one of cv. Rafal were sown at Bridgets Experimental Husbandry Farm, Winchester on 30 August 1985 at 9kg ha^{-1} with a row spacing of 14.5cm. Husbandry details are given in Table 2.1. Each plot was 24 x 20m and surrounded by a 6m buffer of cv. Mikado. Tramlines were made at approximately right angles to the direction of sowing, dividing each area into two plots of 6 x 20m and one plot of 12 x 20m. (Fig 2.1). Husbandry of the plots was the same as the surrounding field except that no fungicides were applied (Table 2.1).

2.1.3 Plant sampling

Plant samples for assessment were removed from the two outer plots of the Mikado and Rafal areas (Fig. 2.1). From 9 January, 15 adjacent plants were taken weekly from each sampling area. Samples from the southern plot in each cultivar area were taken systematically from west to east during the season and samples from

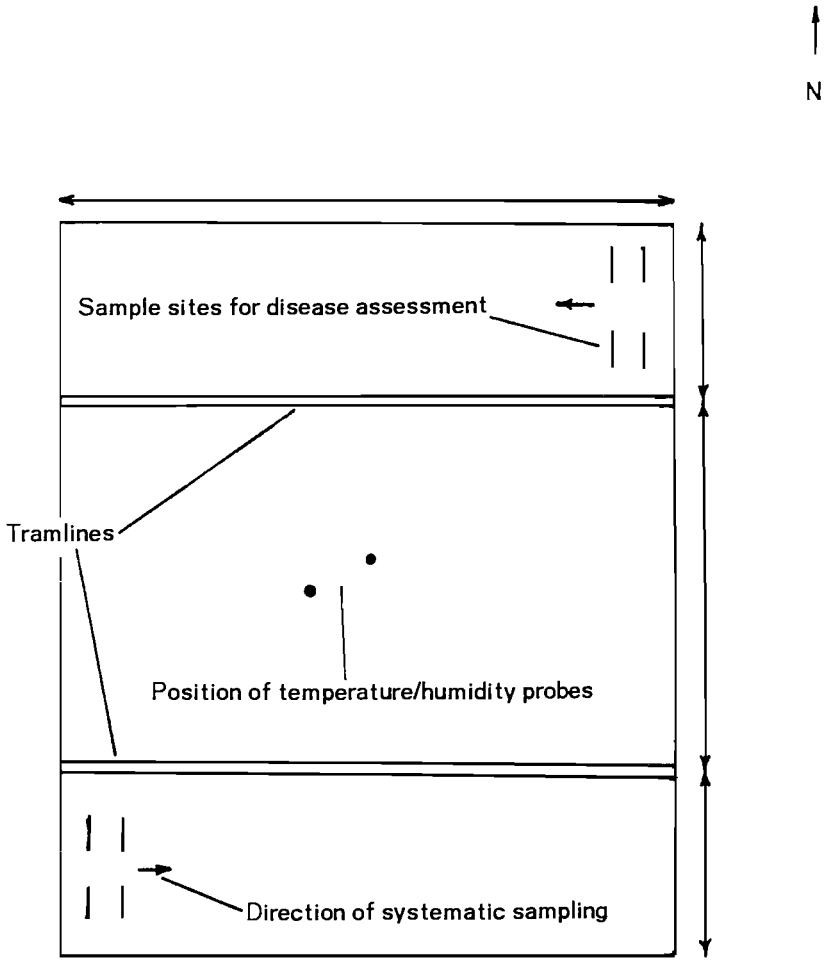


Fig 2.1: Site plan of Field trial , 1986 Mikado and Rafal identical

the northern plot from east to west. Each weekly sample was at least 1m distant in all directions from any other sample. Samples were not taken on 12, 19 and 26 February as plants were frozen in the ground.

Table 2.1: Husbandry details; Field trial,1986

Sowing Date:	30 August 1985
Seed Rate:	9.0 kg ha^{-1}
Seed bed fertilizer:	None
Top Dressing (N):	47 kg ha^{-1} 20 February 1986 123 kg ha^{-1} 2 April 1986
Herbicides:	Butisan S Pre-emergence Predone Plus Post-emergence
Fungicides:	None

2.1.4 Assessments

Sampled plants were removed carefully to the laboratory and assessed for various disease and growth parameters (Table 2.2). During assessment of leaves no distinction was made between the lower, stalked and upper, sessile leaves. Main stem leaf number was determined by counting the number of true leaf scars and the number of leaves below the leaf to be assessed. Leaves on the main stem were numbered with integers, the first leaf at the base of the stem being leaf 1. Primary branch leaves were numbered from the main stem leaf from which the branch originated together with a numerical suffix. Any secondary branches were discarded. Leaf height was defined as the height in centimetres of the leaf insertion above the first true leaf scar and plant height the distance in centimetres between the first true leaf scar and the plant apex. During

pod development, racemes were identified by their position in relation to the terminal raceme (Raceme T) and numbered consecutively down the plant, Raceme 1 being the raceme immediately below Raceme T. For the assessment of disease and the number of pods, each raceme was divided in two (see Table 2.2)

Diseases on the leaves and pods were assessed visually using a prototypes of the assessment keys for leaf and pod canopies described in Part 2, Chapter 2. Lesions of *A. brassicae* were said to be sporulating if any spores were found on the surface of the lesion using a low power binocular microscope (X50 mag.).

2.1.5 Meteorological Data

Temperature, humidity, rainfall and surface wetness were measured within or in close proximity to the crop. Temperature and humidity were measured using a negative temperature coefficient thermistor and capacitive sensors respectively (Vaisala OY, Helsinki, Finland). The humidity sensors were calibrated over the range 0 - 100% Relative Humidity (RH) using salt solutions of lithium chloride and potassium sulphate. Temperature probes are factory calibrated. Each probe contained a temperature and humidity sensor. Measurements were recorded on Rustrak chart recorders at 15 minute and 1 h intervals for humidity and temperature respectively. Throughout the season four probes were positioned in the central area of each variety. From 15 April to 12 June the probes were positioned in two pairs, one at approximately 0.5m above the crop and the other amongst the leaf canopy. After 12 June the probe positions were changed to four different heights within the crop: probe 1 approximately 0.5m above the crop; probe 2 amongst the pod canopy; probe 3 within the leaf canopy and probe 4 at ground level. Probe 3 was moved up as the leaf canopy became more concentrated at the top of the plant.

Rainfall was measured using a tilting siphon rainfall recorder, temperate pattern (Casella London Ltd., London, U.K.) set in a 3 m diameter clearing in the buffer area between the two varieties. Recordings were taken on a 7 day chart with a resolution of 0.2 ml. Surface wetness was measured using a Meteorolgical Office

Table 2.2: Parameters measured on sampled plants during 1986 trial

Mid winter until stem extension	Code
Block number	BL
Leaf number	LN
% Area of dark leaf spot	AA
Number of *A. brassicae* lesions	ALN
% Area of white leaf spot (*Pseudocecosporella capsellae*)	WLS
% Area of light leaf spot (*Pyrenopeziza brassicae*)	LLS
% Area of downy mildew (*Peronospora parasitica*)	DM
% Area of necrosis	NE

Stem extension until the end of flowering

All the parameters above plus:

Plant height	PH
Leaf height	LH
Number of sporulating lesions of *A. brassicae*	SPLN

Pod development until the end of the study

All the parameters above plus:

Raceme number	RN
Height of raceme apex (cm)	RHT
Height of lowest filled pod (cm)	PHB

Raceme divided using the formula

$$\text{Dividing height} = \frac{\text{RHT} - \text{PHB}}{2} + \text{PHB}$$

The following parameters were assessed for the portion of raceme above and below this height

Number of pods (above)	PNT
Number of pods (below)	PNB
% Area of dark pod spot (above)	APT
% Area of dark pod spot (below)	APB
Number of pods infected with *A. brassicae* (above)	ANPT
Number of pods infected with *A. brassicae* (below)	ANPB
% Area of raceme necrotic	ANT
% Area of raceme necrotic	ANB

Balance Surface Wetness Recorder with a polystyrene trapping surface placed in a 3 m diameter clearing in this buffer area. Periods of wetness were recorded as a rise in the tracing on a 7 day chart.

2.2 1987 Field Trial

2.2.1 Introduction

The field trial in 1987 was used to consolidate and improve the information obtained in 1986 about the progress of dark leaf and pod spot within the oilseed rape crop,and also to begin to define more precisely periods of infection by the pathogen.

The 1987 field trial was improved in various ways compared with 1986. Increasing the number of sampling units was intended to reduce the variability in plant and disease data; data loggers for meteorological data capture were used in an attempt to improve accuracy and consistency of measurements; leaf areas were estimated to give a clearer picture of both plant and disease development. Plant traps were used to observe infection periods of the pathogen and these will suggest conditions under which the pathogen infects in the field. Due to the abandonment of the original trial (see below), this work was carried out on cv. Bienvenu (see Section 2.2.3).

2.2.2 Rafal & Mikado

Two areas of oilseed rape (cvs. Mikado and Rafal) were sown at Bridgets Experimental Husbandry Farm on 28 August 1986. Establishment during autumn was, however, poor and there was pigeon and frost damage during the winter. This resulted in patchiness and late develpoment in the spring and the trial was abandoned.

2.2.3 Bienvenu

2.2.3.1 Experimental area

After the abandonment of the original trial a 100 x 12m area (cv. Bienvenu) was marked out in the area betwwen two M.A.F.F trials in field Kansas at Bridgets Experimental Husbandry Farm on 4 May 1987. This area was kindly provided by Bridgets at short notice. The crop was sown on 19 August 1986 at 8 kg ha^{-1}. The plot was treated the same as the surrounding field except that no application of fungicide for *A.brassicae* control was made. Husbandry details are given in Table 2.3

Table 2.3 Husbandry details; Field trial 1987

Field name:	Kansas
Soil type:	Panholes/Andover
Sowing date:	19 August 1986
Seed rate:	8.0 Kg ha^{-1}
Seed bed fertilizer:	Farmyard manure
Top dressing (N):	97 kg ha^{-1} 17 February 1987 93 kg ha^{-1} 13 April 1987
Herbicide:	Matricurb 27 September 1986
Fungicide:	Benlate + Agral 16 April 1986

2.2.3.2 Plant sampling

Plants for assessment were removed in a systematic pattern from the southern end of the plot throughout the season (Fig. 2.2). From 4 May until 13 July four samples each of eight adjacent plants were taken weekly. No two samples were within 2m of each other. The number of samples was increased in 1987 in an attempt to reduce variability in the data (see Part 2, Chapter 3).

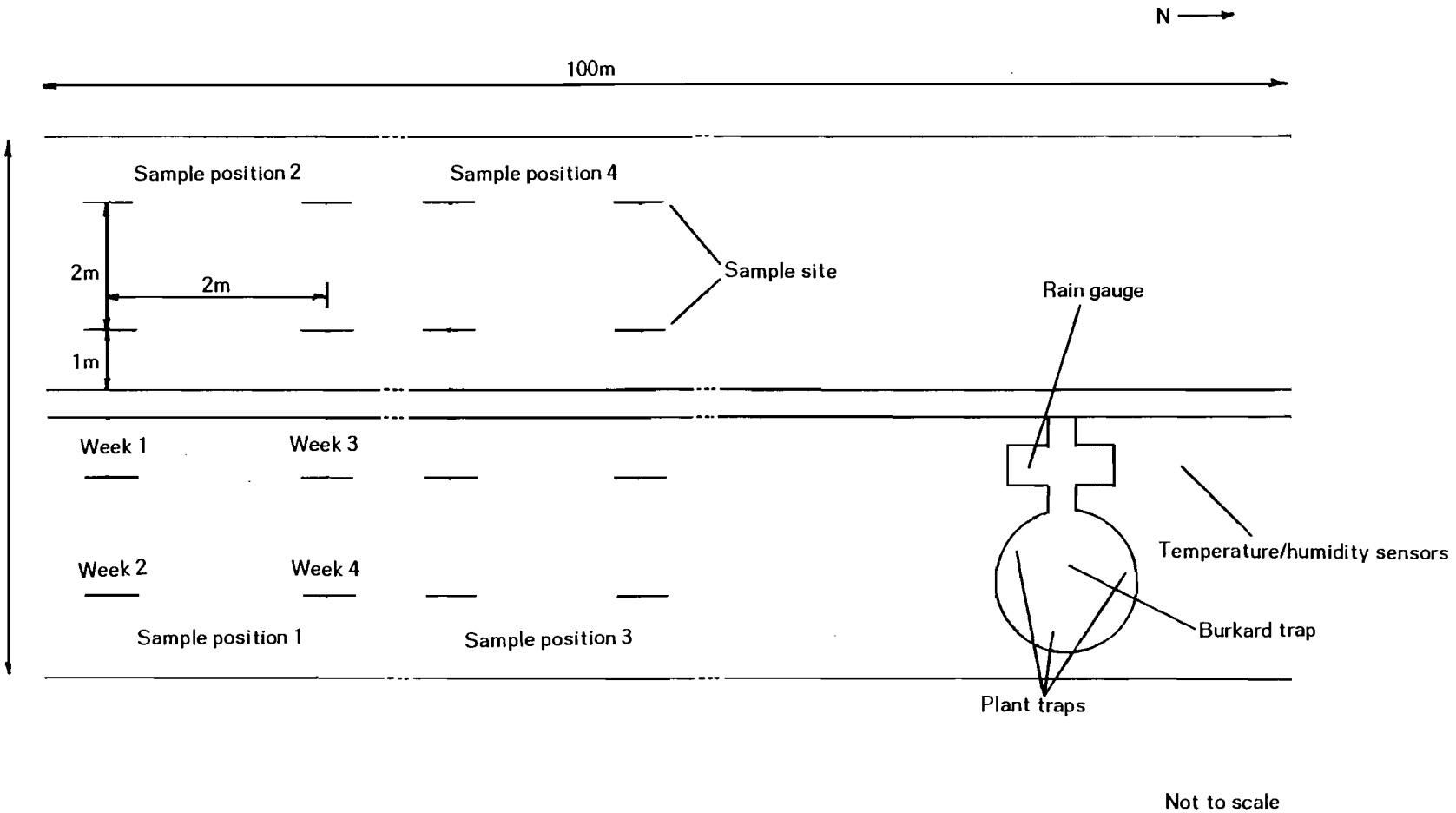


Fig 2.2: Site plan for Field trial, 1987

2.2.3.3 Assessments

Sampled plants were carefully removed to the laboratory for assessment of disease and growth parameters. The parameters measured were similar to those taken in 1986 (Table 2.4).

2.2.3.4 Spore trap

A Burkard volumetric spore trap (Burkard Manufacturing Co. Ltd.) was set in a 2m clearing within the crop (Fig 2.2). Spores are trapped when high volumes (10l/sec) of air are drawn over a sticky surface (vaseline + paraffin wax in 10% toluene) smeared onto melinex tape fixed to a rotating drum. The drum rotates at 2mm/h and has a 7 d clock. The drums were changed weekly at 16.00h on Mondays. After removal of the drum the Melinex tape was divided into 24h segments and mounted in lactophenol/cotton blue to give temporary mounts. Due to the often low numbers of spores caught the number of spores over the whole area of spore capture on each 24 h segment were counted.

2.2.3.5 Plant traps

Plant traps were set up around the Burkard trap 0.25m from the edge of the clearing (Fig 2.2). Oilseed rape, (cv. Mikado), was sown in seed trays in John Innes No. 2 potting compost and placed in a glasshouse at 13 to 30^{0}C. Seedlings were potted on after 1 wk to 8 inch (20 cm) plastic plant pots. There were three seedlings per pot. The plants then followed different temperature regimes depending on the time in the season. From the beginning of April until mid-May pots were placed in a glasshouse at 13 to 30°C for 2 wk, followed by 1 wk at 8 to 20°C and finally 2 wk in an uncovered cold frame in the University of Southampton Botanic Gardens. From mid-May to mid-June the plants were placed in the cold frame after 2 wk at 13 to 30°C and from then until the end of the season the pots were placed in the cold frame immediately after transplanting. A foliar feed (Murphy foliar feed, Murphy Chemical Ltd.) was applied to the plants 7 days before the first pots were

to be placed in the field, using a watering can fitted with a fine rose. Plants were used as traps during their sixth week after transplant when they had four to six expanded leaves. From 4 May to 13 July five pots were placed in the field daily . Each set of pots was exposed for 48 h giving a series of overlapping

Table 2.4: Parameters measured on sampled plants during 1987 trial

	Code
Block number	BL
Plant number	PN
Number of leaves lost	LL
Parameters assessed for each leaf	
Leaf height (cm)	LH
Leaf length	LLN
Number of *A. brassicae* lesions	ALN
% Area of necrosis due to *A. brassicae*	ANE
% area of dark leaf spot	AA
Number of sporulating lesions of *A. brassicae*	SPLN
% Area of white leaf spot (*Pseudocercosporella capellae*)	WLS
% Area of downy mildew (*Peronospora parasitica*)	DM
% Area of light leaf spot (*Pyrenopeziza brassicae*)	LLS
% Area of Botrytis (*Botrytis cinerea*)	BOT
% Area of yellowing	YEL
% Area of necrosis	NEC
Parameters assessed for each raceme	
Raceme number	RN
Height of raceme apex	TOPHT
Height of lowest filled pod	BOTHT
Number of pods on raceme	PODNUM
Number of flowers	FLNUM
Number of flower buds	BUDNUM
Number of pods infected with *A. brassicae*	PODINF
Number of *A. brassicae* lesions	ALRAC
% Area of pod infected with *A. brassicae*	DISRAC
Number of pods infected with Light leaf spot	LSPOD
% Area of necrosis on pods	NECPOD

exposure periods. Changeovers occurred at 1645h each evening when pots remaining in the field were also watered. After exposure pots were placed in trays in a growth cabinet (Saxcil) at 20 $\pm$1°C. Pots were placed in trays and watered from beneath to

avoid wetting the leaf surfaces. After 5 days incubation the number of lesions of *A. brassicae* on each leaf and the length of each leaf was recorded.

2.2.3.6 Meteorological data

Temperature, humidity, rainfall and surface wetness were measured within or close to the crop. Two temperature/humidity probes (Vaisala; see Section 2.1.5for details) within the crop. One was located in the leaf canopy and moved higher as the leaves at the base of the plant senesced and were lost. The other was positioned in the pod canopy. One temperature/humidity probe was placed 0.5m above the crop and a single thermistor temperature probe positioned at the top of the pod canopy.

Surface wetness was measured at the same positions as the Vaisala probes using electronic surface wetness probes (L.A.R.S, University of Bristol). Rainfall was recorded using a tipping bucket rain gauge (Rimco, Rauchfuss Inst. and Staff Pty Ltd., Victoria, Australia) calibrated at 0.33mm per tip.

Records from temperature and humidity probes, rainguage, and surface wetness sensors were collected on Grant Squirrel dataloggers (Grant Instruments). Temperature, humidity and surface wetness were recorded every 15 minutes and rainfall each time the bucket tipped. All environmental data were taken from 4 May until 13 July.

2.3 1988 Field Trial

2.3.1 Introduction

Unfortunately in 1986 and 1987 a large proportion of meteorological data was lost due to equipment failure and corruption of data on magnetic tapes respectively. It was felt that because of these problems together with the abandonment of the first trial in 1987 further fieldwork was necessary in 1988. The trial was similar to that of 1987 and was used to provide detailed meteorological data to compare with plant

trap studies and to provide a further years data on the progress of *Alternaria* dark leaf and pod spot.

2.3.2 Experimental area

An area of approximately 2500m^2 of oilseed rape (cv. Bienvenu) was marked out in field Oklahoma at Bridgets Experimental Husbandry Farm. The crop was sown at 8 kg ha^{-1} on 27 August 1987 and the trial area treated the same as the rest of the field except that no fungicides were applied. Husbandry details are given in Table 2.5.

2.3.3 Plant sampling

Plants for assessment were removed in a systematic pattern from various areas of the plot throughout the period (Fig 2.3). From 4 April until 4 July four samples each of eight adjacent plants were taken weekly. No two samples were taken within 2m of each other.

2.3.4 Assessments

Sampled plants were carefully removed to the laboratory for assessment of disease and growth parameters. The parameters measured were the same as those measured in 1987. For the pod canopy data, only Raceme T was sampled from 4 April to 4 July and Raceme 3 from 16 May until 4 July.

2.3.5 Spore traps

Spore traps were positioned at the east side of the trial area (Fig. 2.3). A Burkard volumetric spore trap was set in a 2m clearing within the crop. Trapping surfaces before and after exposure were treated as those in 1987.

Horizontal slide traps were set at various heights within the crop canopy. Microscope slides smeared with a sticky surface (vaseline + paraffin wax in 10% toluene) were heated on a hot plate until the sticky

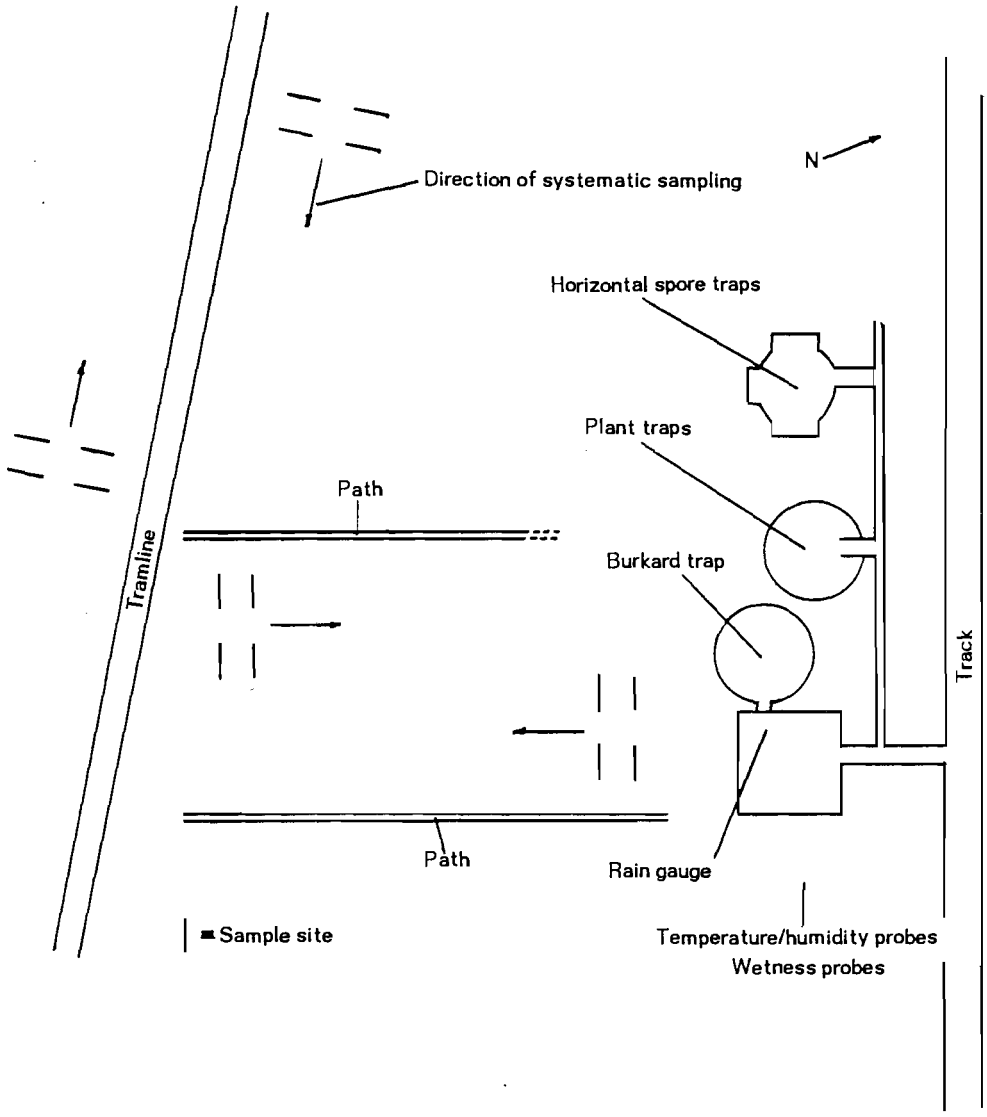


Fig 2.3: Site plan of Field trial, 1988

Table 2.5: Husbandry details; Field trial 1987

Field name:	Oklahoma
Soil type:	Panholes/Andover
Sowing date:	27 August 1987
Seed rate:	8.0 Kg ha^{-1}
Seed bed fertilizer:	None
Top dressing (N)	105 kg ha^{-1} 23 February 1988 105 kg ha^{-1} 1 April 1987
Herbicide:	Fusilade 5 25 September 1987 Kerb 27 October 1987
Fungicide:	None
Insecticide:	Fastac 20 May 1988

surface melted and then allowed to cool. This gave a smooth surface on which spores could be trapped. Groups of four slides (Fig. 2.4) were placed at four different heights within the crop canopy at a single site (Fig 2.3). A 1.5m diameter opening was cut into the crop. Three further areas, each 0.5 x 1m were cut around the edge of this opening and into each of these areas were placed groupings of slides. In site A four slides were placed at 0.25, 0.75 and 1.25m (Fig 2.5). In site B and C four slides were placed at 0.5m. Slides at site A were changed at 17.00 every 48h. At the 0.5m height two slides from site B and two from site C were changed daily, giving a series of 48h overlapping exposure periods.

After removal to the laboratory the number of *A. brassicae* spores on the whole sticky surface (25 x 55mm) were counted using a high power microscope at 100X magnification.

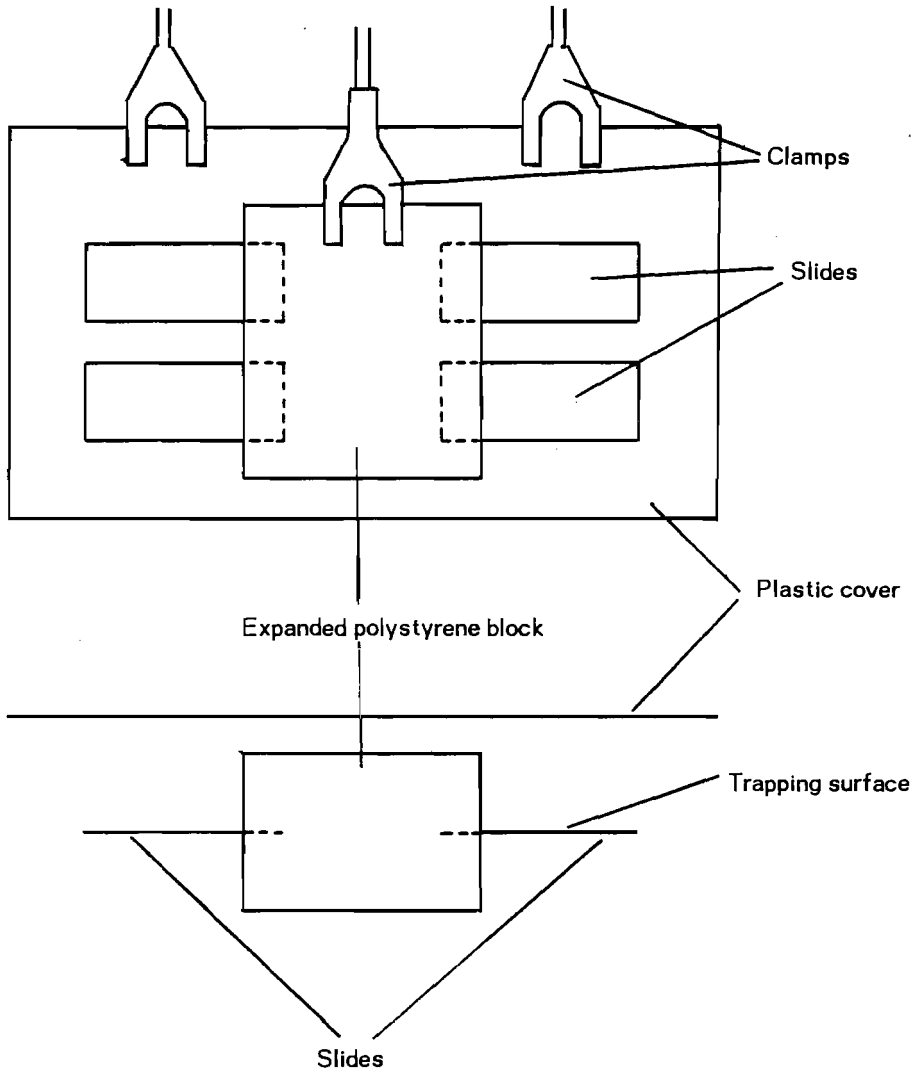


Fig 2.4: Horizontal slide trap
Single group of four

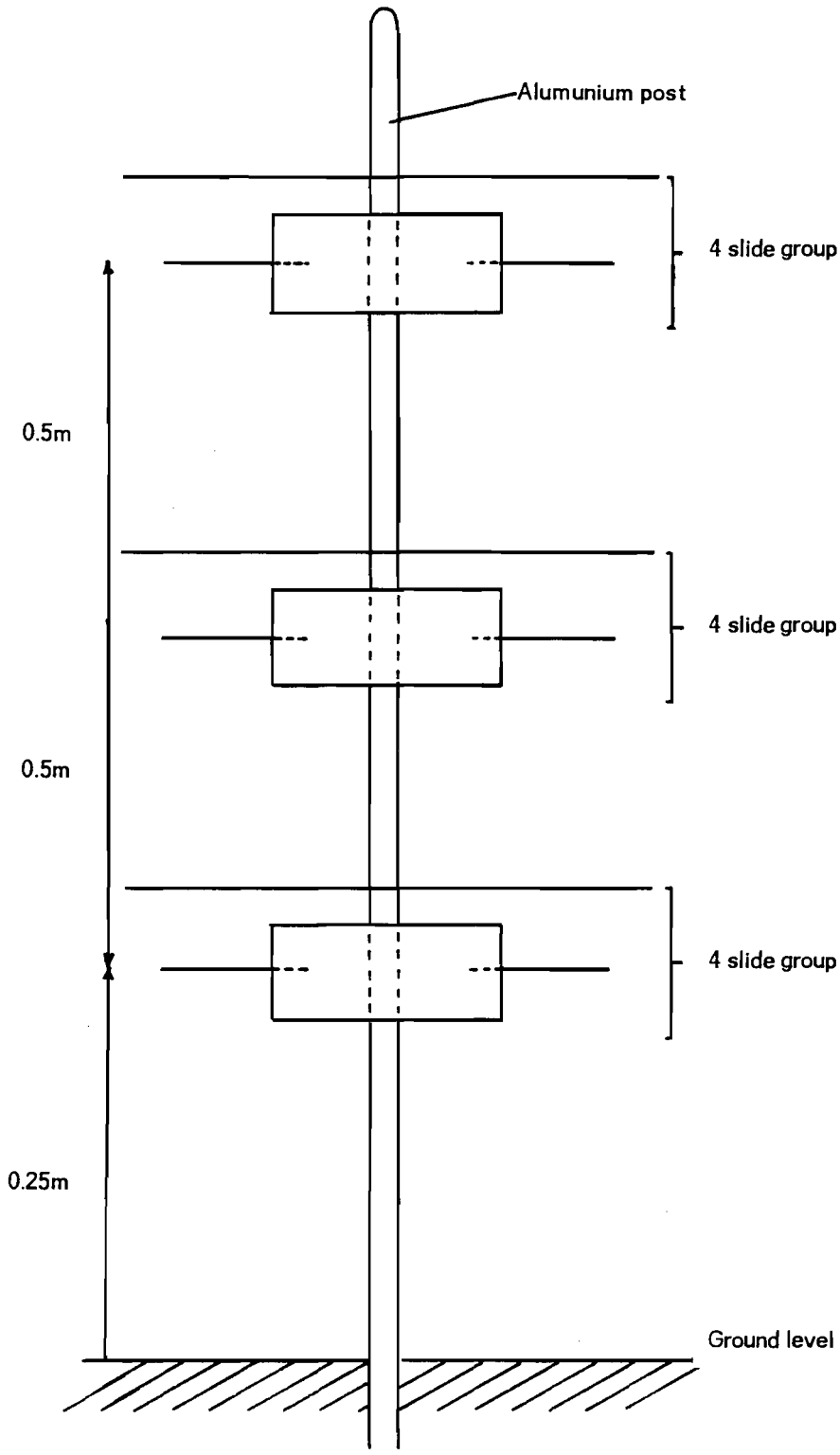


Fig 2.5: Grouping of horizontal slide traps at site A

2.3.6 Plant traps

Plant traps were set in a 1m diameter opening cut into the crop. (Fig 2.3). The plants used were produced in the same way as those in 1987. As watering had occassionally failed in 1987, the plants were placed on boards covered in capillery matting which were then placed on the top of plastic trays (350 x 210 x 37mm) containing water. The capillary matting was effective in keeping the plants watered from beneath. Two trays, each holding five pots were placed at soil level in the opening. Five plants were changed daily giving a series of overlapping exposure periods. After removal from the field the plants were treated as in 1987.

2.3.7 Meteorological data

Temperature, humidity, rainfall and surface wetness were measured close to the crop. One temperature/humidity probe was placed in the crop canopy. It was located in the leaf canopy and moved higher as the leaves at the base of the plant senesced. From 30 May it was positioned at the base of the pod canopy. A temperature/humidity probe was also positioned at 0.5m above the top of the pod canopy.

Surface wetness was measured as described in Section 2.2.3.6 at the same heights as the temperature/humidity probes. Rainfall was recorded using the same gauge as described in Section 2.2.3.6.Positions of the meteorological equipment are shown in Fig 2.3.

The data from the various probes were collected on Squirrel Data loggers. Temperature, humidity and surface wetness were recorded every 15 minutes and rainfall each time the bucket tipped. All environmental data were measured from 4 April to 7 July.

2.4 Meteorological data; Bridgets E.H.S Meteorological Site

Daily maximum and minimum temperatures, rainfall, windrun and hours of sunlight were measured on the meteorological site at Bridgets E.H.S. Information was collected at 0900h G.M.T. The site was 1 mile from the 1986 trial and adjacent to the fields containing the trials in 1987 and 1988.

2.5 Data handling

2.5.1 Data manipulation

Data from the field plants were collected and combined using an IBM3090 mainframe computer to give composite variables (Table 2.6). In 1986 when each raceme was divided for assessment, the area of disease on each complete raceme was calculated by

$$\frac{APT + APB}{2}$$

and assumes that the pod area on the two halves was the same.

Data from the meteorological probes in 1987 and 1988 were combined to give the variables

RH90 - hours of humidity >90% in any 24 or 48 period

RH80 - hours of humidity >80% in any 24 or 48 period

WET - hours in any 24 or 48 period when the probes were wet. (This gave an arbritary figure on the measured scale which differed for each probe and had been determined in the laboratory by wetting the probes with distilled water and waiting until they just dried out).

2.5.2 Plant Traps

The absolute number of lesions on each trap plant was modified to take account of the differing leaf sizes of individual plants. During the 1987 season 100 leaves of varying sizes were taken from the plant traps and their areas measured (see Part 2, Chapter 2). The areas were then related to leaf length using simple linear regression to give the equation

$$\text{Leaf area} = 1.55 \times \text{Leaf length} - 9.75$$
$$(R^2 = 89.6, p = <0.01)$$

The leaf areas of all plant traps were then estimated from the lengths of leaves on the plants, measured at the same time as the lesion counts were made. Lesion counts were then expressed as lesions/100cm ^{2} of plant trap tissue.

2.5.3 Data analysis

All data analyses and manipulations were carried out using SPSSX software on an IBM3090 mainframe computer or STATGRAPHICS on an IBMPC. 3-D and contour figures were constructed using UNIMAP (UNIRAS Ltd.) and all other figures were drawn using ALIS (Applix Co.) software on an Hewlett Packard mini-computer.

Table 2.6: Description of Composite variables

Number of *Alternaria* lesions on the complete leaf canopy of each plant	TALLF
Number of *Alternaria* lesions on the complete leaf canopy of each plant that were sporulating	TALLFSP
Total calculated leaf area per plant	TCLA*
Total calculated disease area per plant	TCDA*
Total calculated disease area per plant which was sporulating	TCDASP*
Calculated % area of the leaf tissue/plant affected by disease	TLFDIS*
Calculated % area of the leaf tissue/plant affected by disease which was sporulating	LFDISSP*
Total number of pods on each plant	PODNUM
Total number of pods on each plant infected with *Alternaria*	PODINF
Total number of *Alternaria* lesions on the pod canopy of each plant	TALPOD
Mean number of *Alternaria* lesions/pod on each plant	ALPOD

* See Part 2, Chapter 1 for explanation

PART 2: DISEASE ASSESSMENT AND SAMPLING

CHAPTER 1 INTRODUCTION

Diseases of agricultural crops are studied because they cause crop losses. To evaluate their importance, the actions needed to control them and their distribution, it is necessary to have adequate methods to measure them.

Measurement of disease is the basis for any study of plant disease, the tactics of which will differ depending on the disease being studied and the objectives of the work (Large,1966). Disease surveys require methods that are rapid and will adequately estimate levels of disease over whole fields; methods for assessing trials to evaluate crop loss relationships and control strategies often need to be more precise and related to the stage of disease development which causes damage to the plant; detailed epidemiological studies require accurate estimates of disease at low intensities (Large, 1966). Pathosystems to be studied vary widely in their requirements for methods of measurement. Consideration should be made of whether the pathogen is systemic (e.g. *Ustilago nuda*) or non-systemic (e.g. *Septoria tritici*); which plant organ is affected (e.g. *Gaumanomyces graminis*/roots) and the complexity of the host (e.g. cereals compared with coffee).

The development of adequate methods for measuring a disease involves two major components. 1) Sampling strategy; a method for defining the portion of crop to be assessed for disease whether it be which fields in a particular area, which area in a field or which plants from a particular plot together with how many samples should be taken and 2) a method for assessing the quantity of disease in the sample.

Random sampling is an assumption made when applying parametric statistics to data (Sokal & Rolfe, 1981). For practical reasons however, truly random samples are rarely taken, being replaced by systematic sampling systems, the data then being treated as if it had been collected randomly. Sampling strategies for single fields in large geographical areas are usually based on whether cooperation can be obtained

from growers, soil type, cropping rotation etc.. The choice of areas for assessment within particular fields is often defined by ease of access or where a cooperator allows work to be carried out. Individual plant samples from fields or large trials plots of arable crops are often collected using W and X type sampling systems (Gareth-Jones, 1987). For example the assessor walks in a W or X configuration across the field taking a plant every 30m. The plants collected are then assessed for disease and the mean value for disease is taken to represent disease levels in the whole field. Sampling techniques used in the German Democratic Republic for disease monitoring by Ministry of Agriculture agronomists have shown that these methods can be improved by sampling only a small area of crop in a very strict framework (Spaar and Ebert, 1985). Sampling for small plot trials (2-5m x 6-25m) is often ill defined. Instructions in assessment keys include phrases such as "sample at five sites within the plot " (Anon, 1988) and methods sections in published papers are little more explicit with comments such as "ten plants were sampled from each plot ". The interpretation of such vague terminology is generally, that samples should be taken, or were taken, at intervals throughout the whole plot. Such sampling strategies were primarily designed for cereal crops and have since been adapted to other arable crops. However, difficulties arise in crops with complex canopies, such as oilseed rape where access is difficult. Under such circumstances plant samples and/or assessments are often made along tramlines or other points of access where microclimatic differences and physical damage (e.g. tractor damage) may result in very different disease development from the bulk of the crop.

The number of samples taken is always a compromise between the statistical requirements for accuracy and the cost in terms of labour (Vickerman, 1985; Daamen, 1986). Numbers of samples taken in arable crop field trials are usually based on sample sizes required for temperate cereals which often require quite small sample numbers because of the uniformity of the crop and even distribution of disease within it (Strandberg, 1986). However, in crops where the growth habit is more variable or disease less evenly distributed much larger numbers of samples may be required.

To develop a sampling strategy for *A. brassicae* on oilseed rape the objectives of the intended study must be clear, the amount of labour available must be assessed and the statistical needs of the data gauged. Consideration must be made of the difficulty of access into an oilseed rape crop and the great variability of both individual plants within the crop and of disease development. This study has provided a limited amount of data which allowed an initial investigation into the number of samples required for a particular level of precision using a variety of measures of disease and plant development and whether the number of samples used in this study was entirely adequate (Part 2, Chapter 3).

Before sampling strategy can be developed or the number of samples estimated methods must be developed to adequately assess the levels of disease in the samples. Assessment of disease at the whole field level is obviously difficult and the use of infra-red photography to study *Phytophthora infestans* in potatoes is an isolated example (Dickinson & Lucas, 1982). Whole plot assessments used by NIAB (Anon, 1988) and some agrochemical companies give a single value for disease level (% crop area affected) for each treatment area in a trial. This is a rapid visual technique based on guidelines drawn up for each crop and is essentially based on the overall levels of disease on individual plants at several sites within a plot. The most common form of disease assesment however is carried out on individual plant samples removed from the field. Plants may be assessed whole, but more often particular plant organs are assessed to give a representation of disease on the plant and hence in the sampling area (James, 1974).

The units of disease measurement fall under two major headings as defined by Seem (1984). Incidence is "the proportion (0-1) or percentage (0-100) of diseased entities within a sample unit " e.g. proportion of infected plants or leaves in a sample; and severity is "the quantity of disease affecting entities in a sample unit " e.g. area of plant affected by disease. Disease intensity in this context is "a general characterization of disease in a particular area with incidence and severity as two of its attributes ". Incidence is the easiest assessment of disease to make, simply involving counts of entities with and without disease. The major problem of this method is disease diagnosis. The measurement of incidence is rapid, objective and can often be

carried out by untrained observers (Large, 1966). However, it is often not well related to disease severity especially at high disease levels (James & Shih, 1973; Seem & Gilpatrick, 1980). Measurement of severity is often based on the visual estimate of diseased area using pictorial keys as a guide. These were divided into two categories by James (1971b). Firstly the general descriptive key in which plants with varying amounts of disease are described (e.g. Anon,1947, *Phytophthora infestans* on potatoes) which would be used in the field to give whole plot assessments. The second category of assessment uses standard area diagrams, which are pictorial representations of various areas of disease on the particular plant organ of interest. Cobb (1892) was the first to develop standard area diagrams and since then many have been produced for a wide range of pathosystems (e.g. James, 1971a). In the case of temperate cereals, assessments are often carried out on one or two leaves (e.g. James *et al.*, 1968) as a representation of the whole plant and on crops with more complex canopies assessment of samples of leaves taken from individual plants is more usual (Plaut & Berger, 1980). In the latter case it is essential that leaves are taken from carefully selected areas so that a true picture of disease can be obtained (e.g. Butt, 1979). This allows very time consuming assessments to be reduced in number and increases the number of samples that can be taken and hence improve the accuracy of disease estimates. Disease assessment keys for dark leaf and pod spot on oilseed rape are presented in Part 2, Chapter 2.

Severity can also be represented by the numbers of pathogen-affected entities e.g. numbers of lesions/plant. If low levels of disease are present then this is probably a less error prone method of measuring disease severity than other methods e.g. the percentage area method (Daamen, 1986). The absolute area of disease is another measure of disease severity which could be used but is extremely time consuming to measure. The range of measures of disease severity leads to the question as to which should be used in a particular situation. In many cases the aim of a study is to relate levels of disease to reductions in crop yield (James, 1974). Yield loss is likely to be best related in some way to total loss of photosynthetic area on the plant as shown clearly on wheat by Samborski & Peterson (1960) and therefore a measure of the area of disease affecting the most important photosynthetic material is then likely to be most appropriate (e.g. flag leaf and leaf 1 on cereals; James *et al.* 1968). However

visual estimates of diseased area can be open to large error (Royle, Pers. comm.) and counting methods are more objective and often less time consuming, especially at low disease levels (Daamen, 1986). Relationships between counts and disease area have been studied in some pathosystems (Daamen, 1986; Seem & Gilpatrick, 1980) and their combination may lead to more accurate disease severity estimates than would otherwise be obtained. Another reason for measuring disease is to provide data for estimating further disease progress. Relative measures such as % area diseased are often suitable for this type of study however if the crop is losing photosynthetic material rapidly then the relative level of disease may be rising whilst the absolute level of disease is decreasing. Assessments of inoculum potential, for example, would then be inaccurate and under such circumstances a measurement of the absolute amount of disease would be more appropriate.

The method of disease measurement chosen for a particular study should therefore be closely linked to the objective of that study, the labour available and the type of crop canopy structure. This study has provided data with which different assessments methods for *A. brassicae* can be evaluated and methods to reduce labour can be suggested.

CHAPTER 2 DISEASE MEASUREMENT

2.1 Lesion Counts

2.1.1 Introduction

Counting lesions is a simple and objective method of disease assessment as long as the lesions are easily identifiable (Large, 1966). In the case of dark leaf and pod spot, lesions are generally clear and well defined and identifiable at an early stage of development (Plate 1).

On leaf material, where disease levels are often low, counting lesions may be a more accurate method of determining disease levels than estimates of % area affected and has been used in a number of other pathosystems (e.g. James & Shih, 1973). Similarly on the pod canopy, when disease levels are low, visual assessment of diseased area is likely to be inaccurate and lesion counts may provide a better estimate of disease.

Lesion counts provide information about the level of infection by the pathogen but this may not relate well to the damage being caused to the photosynthetic material of the plant. It is therefore essentially an epidemiological measurement unless lesion numbers can be easily related to diseased area (see Part 2, Chapter 4).

2.1.2 Methodology

Identification and the definition of discrete lesions are the main factors affecting the counting of lesions. Identification of mature dark leaf and pod spot lesions on leaves is relatively easy. Lesions are dark brown and generally circular if they are found in the centre of the leaf (Plate 2). They are often surrounded by an area of chlorosis which can be larger in area than the necrotic area of the lesion (Plate 2). When infection has occurred on the edge of the leaf lesions are often semi-circular to triangular in shape (Plate 2). The centre of a mature lesion often has a

Plate 1: *Alternaria* lesions at an early stage of development

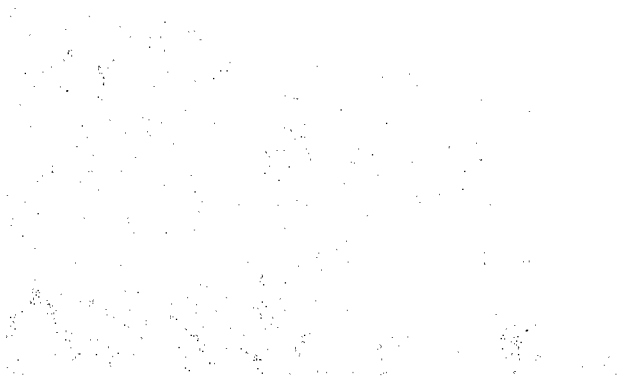


Plate 3: Mature *Alternaria* lesions with concentric rings of sporulation

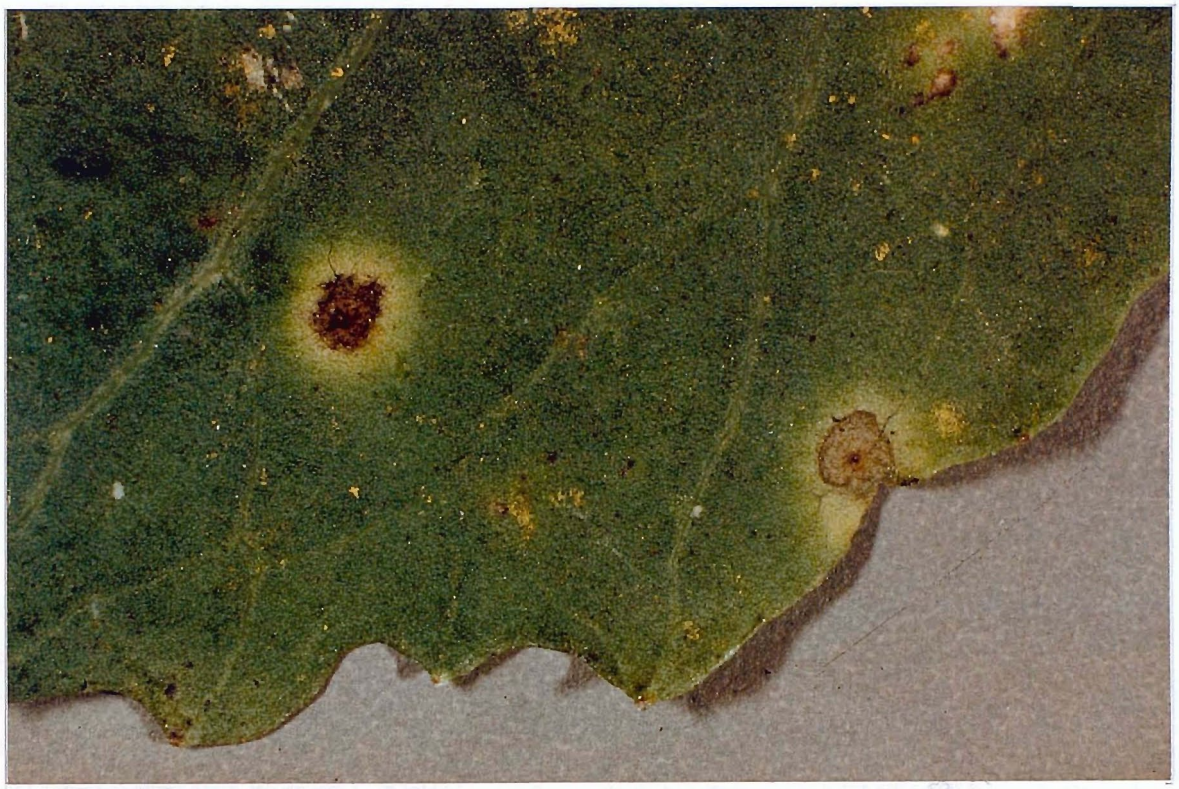


Plate 1

Plate 3

Plate 2: A variety of *Alternaria* lesions on sessile leaves

Plate 2

pattern of concentric light and dark rings where sporulation has occurred (Plate 3). These lesions are easily distinguished from the other major leaf spotting disease *Phoma* canker where the lesion centres are bleached and contain black pycnidia. Distinguishing between *A. brassicae* and *A. brassicicola* can be done quite readily with a hand lens if the lesions are mature and sporulating. *A. brassicae* spores can be seen as large, generally non-concatenated spores distributed quite sparsely over the sporulating area (Plate 4). Sporulating lesions of *A. brassicicola* however, look velvety and under a hand lens the spores can be seen to be small and in chains. If lesions are not sporulating it is impossible to tell the two species apart visually, although it is likely that if all the sporulating lesions are of one species then the majority of the non-sporulating lesions are too. This can be easily confirmed by isolating the pathogen from the lesions and identifying them when they sporulate in the laboratory. During this study only two lesions in the period 1986 - 1988 were identified visually or by isolation as *A. brassicicola* and it was therefore concluded that the majority of disease was caused by *A. brassicae.*

Immature lesions are less readily identified. The initial symptoms of both species are very dark brown pin prick dots (Plate 1). When disease is first appearing in the crop they can appear anywhere on the leaf but are especially found around the leaf margins. Even when very small an individual lesion can be seen on both sides of the leaf. These small lesions are most likely to be confused with the very early symptoms of *Phoma* canker. However, the lesions of *Phoma* tend to be more irregular in shape and soon develop bleached centres, a significant diagnostic feature.

Identification of *Alternaria* lesions on the pod canopy is also relatively simple. The symptoms on the pods are initially dark brown pin-prick lesions which gradually expand to form almost circular lesions many of which will coalesce to give large irregular lesions covering much of the pod (Plate 5). As sporulation on the pods is unusual (in this study it was only recorded in 1987) distinguishing between *A. brassicae* and *A. brassicicola* is difficult and isolation of the pathogen from a sample of lesions is the only accurate method. If sporulation by *A. brassicae* does occur on the pods, the pods look velvety and the large non-catenate spores can be easily seen

Plate 4: Spores on surface of *Alternaria* lesion.

Plate 5: *Alternaria* lesions on pod tissue

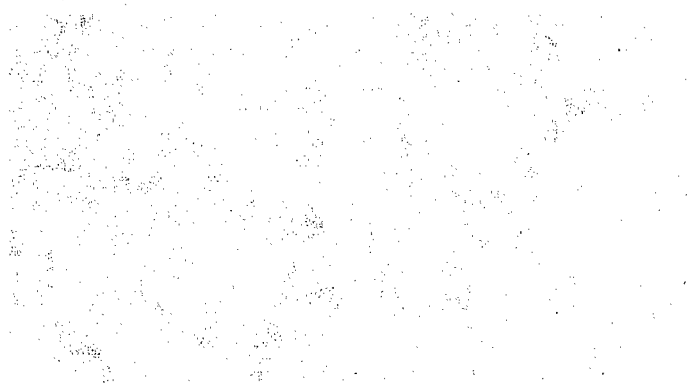


Plate 4

Plate 5

using a hand lens. Symptoms of *Alternaria* spp. may be confused with *Phoma* on the pods , but again the bleached centres soon develop with the latter.

Distinguishing between discrete individual lesions is generally easy with dark leaf and pod spot. On the pods, where little or no chlorosis surrounds the lesions, lesions remain separate until the necrotic areas coalesce. On the leaf tissue the chlorotic areas surrounding the central necrotic area may coalesce leaving the infection points represented by the necrotic areas easily identifiable. As the number of lesions is a measure of the number of infections, lesions should be counted as separate until the necrotic areas coalesce and their individual origins are no longer clear.

Counts of lesions have to be expressed in relation to some measure of the plant e.g. lesions/plant, lesions/leaf; or to an area of crop e.g. lesions/m^2, to make any statement about disease levels. The measure chosen will be related to the pathosystem, disease intensity, crop structure and statistical considerations. Whichever relation is used it is important that it is easily interpreted as a meaningful measure of disease and that it relates to the biology of the organism.

Numbers of *Alternaria* lesions on the leaf tissue of oilseed rape rarely rise to high levels in the U.K.. Expressing the number of lesions as lesions/leaf would therefore result in low numbers and would require very large numbers of samples to give accurate estimates of disease (Part2, Chapter 3). Furthermore because of the nature of the crop, its structure and rapid changes in leaf numbers, some estimate of leaf numbers/plant or /area of crop would have to accompany the estimate of lesion numbers /leaf otherwise a meaningful interpretation of disease levels could not be made. By expressing lesion numbers on the leaf canopy as lesions/plant (TALLF) the leaf number factor can be incorporated into a single measure. Expressing the lesion numbers in relation to the plant also has a more "visual" meaning in that any reader can interpret the data in relation to the crop. The relationship of this disease measure to the development of the disease will be discussed in further chapters as will the number of samples required (Part 3, Chapter 2; Part 2, Chapter 3).

On the pods, numbers of lesions would most usually be expressed as number of lesions/pod (ALPOD), number of lesions/specific raceme (ALRAC) or number of lesions on the whole pod canopy (TALPOD). Practically it is very time consuming to count all the lesions on a plant and the number of lesions on perhaps the main raceme would be an appropriate alternative and is discussed further in Part 2, Chapter 4.

2.2 Disease area measurement - Assessment Keys

2.2.1 Introduction

There are two major requirements of a disease assessment key (James, 1971b). First, observers using the key on a group of diseased plants or plant parts should be able to arrive at similar assessments consistently and second, the assessment should be achieved simply and quickly (James, 1971b). The keys presented here are based on a percentage scale because of the advantages which this offers. These advantages have been fully discussed by Large (1966) and James (1971b) and include the fixed limits and the flexibility of the scale and its universality of use for a variety of pathosystems.

Three scales are presented representing the levels of dark leaf and pod spot on individual rosette and sessile leaves and on single racemes. On the leaves the area of disease is represented by necrosis + chlorosis. Little or no specific chlorosis is associated with lesions on the pods and therefore the key for pod disease is based on area of necrotic tissue.

In disease assessment the simplest technique is the one least prone to error (James, 1971b). For example in cereals the assessment of disease on a single leaf is simple and much less prone to error than attempting to assess the whole plant. In many studies on cereals (epidemiological and crop loss) the use of assessments from only one or two leaves (often the flag leaf or the one below it) is common (e.g. James *et al.*, 1968). In oilseed rape the size and structure of the canopy and for *A. brassicae* the spatial development of the disease (Part 3, Chapter 2) precludes the use of single

leaf samples to give a good estimate of disease and therefore even if a subsample of leaves can be taken from a plant the data from each leaf assessed have to be combined to give an estimate of the proportion of area of the whole sample which is diseased. This is complicated by the differences in leaf sizes which will be present in the subsample and therefore any summation of the data has to be weighted for leaf area. This is further discussed in Part 2, Chapter 3. In the case of pod assessments, the assessment of all racemes is probably unnecessary as clear relationships are present between any raceme and total disease on the pods (Part 2, Chapter 4).

2.2.2 Preparation of the keys

Selections of leaves and racemes of oilseed rape (cvs. Rafal and Mikado) with differing levels of disease were collected from the field and photographed to keep as a record of the type, size and distribution of lesions found on the various plant parts (e.g. Plate 2). Tracings of "typical" rosette and sessile leaves and of a raceme were made from the photographs as the basic plant part outlines for the key. The outlines were then enlarged and copied several times. Using the photographs as models, necrotic and chlorotic areas were drawn onto the outlines to represent *Alternaria* lesions on the various plant parts to show different levels of disease. The drawn areas of disease were then measured by overlaying 1mm square translucent paper on to the diagrams and counting the number of squares in the complete outline and the proportion in the drawn necrotic and chlorotic (leaves only) areas. In the case of the racemes, the proportions of disease drawn on the two-dimensional diagrams were assumed to be equivalent to the proportions of disease on the whole external surface of the pods. The area designated as necrotic or chlorotic was then increased or decreased as required to give the necessary level of disease.

The levels of disease in the series of diagrams produced (Figs 2.1 - 2.3) are 0.1 - 20 or 50%. The concentration of diagrams at the lower end of the scale corresponds to the often low levels of disease found in the field.

Figure 2.1: Disease area diagrams for Rosette Leaves

Disease: Dark leaf spot

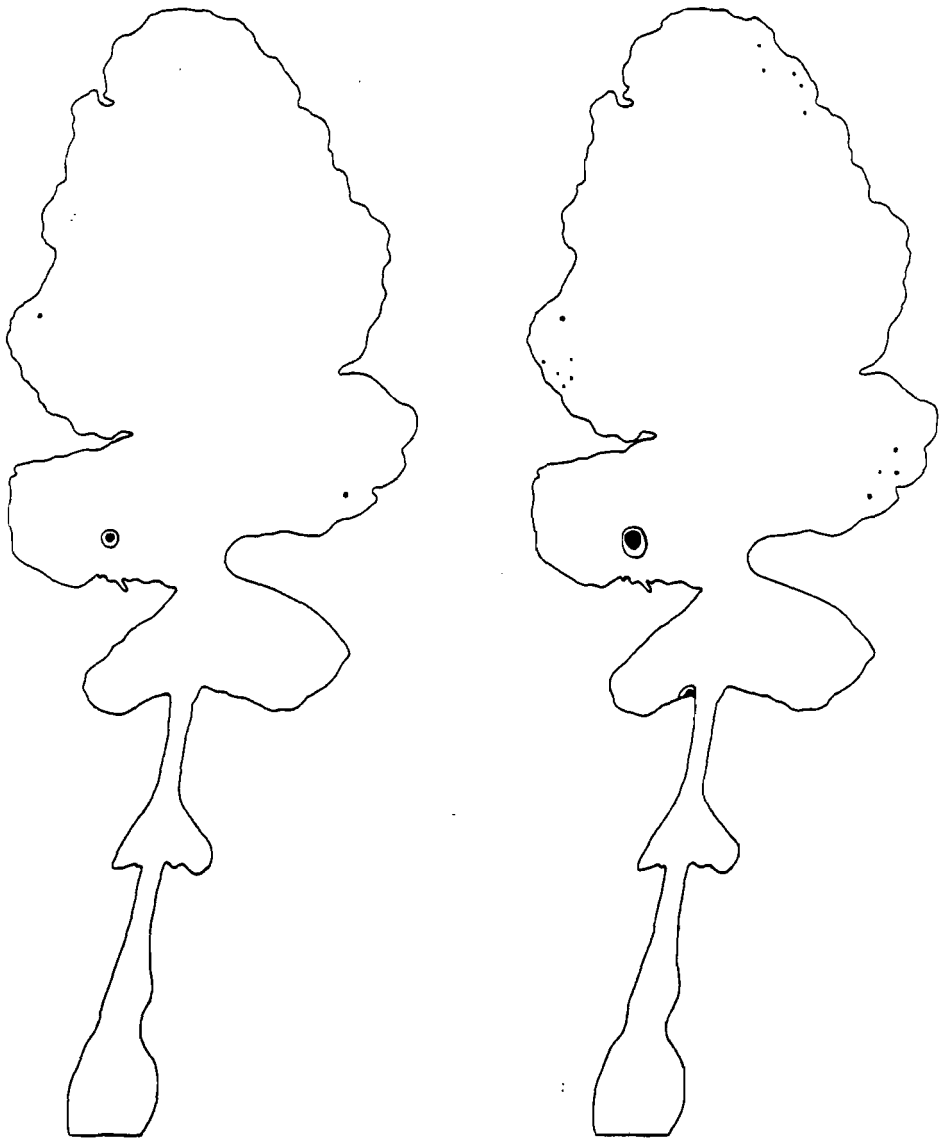
Pathogen: *Alternaria brassicae*

Crop: Oilseed rape

% Area affected

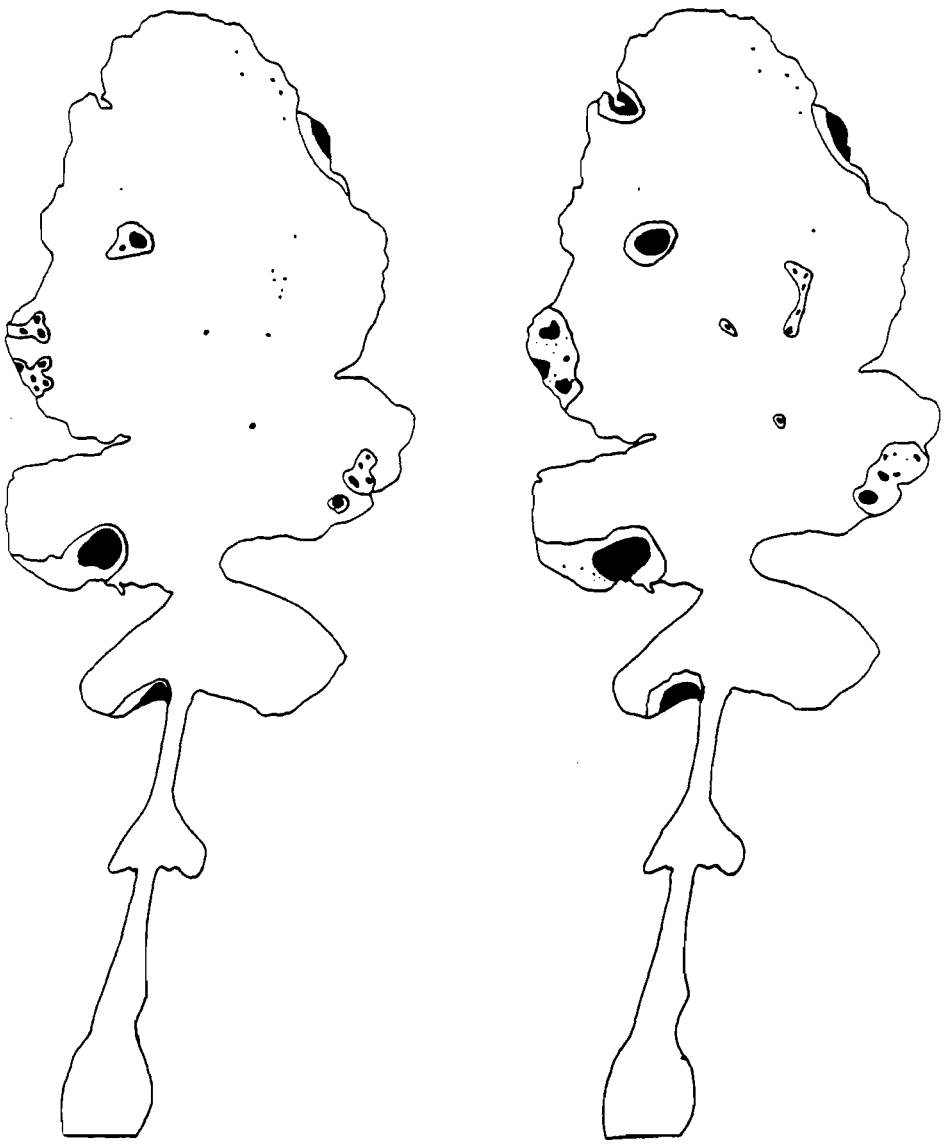
Black = necrosis

Enclosed areas = necrosis + chlorosis

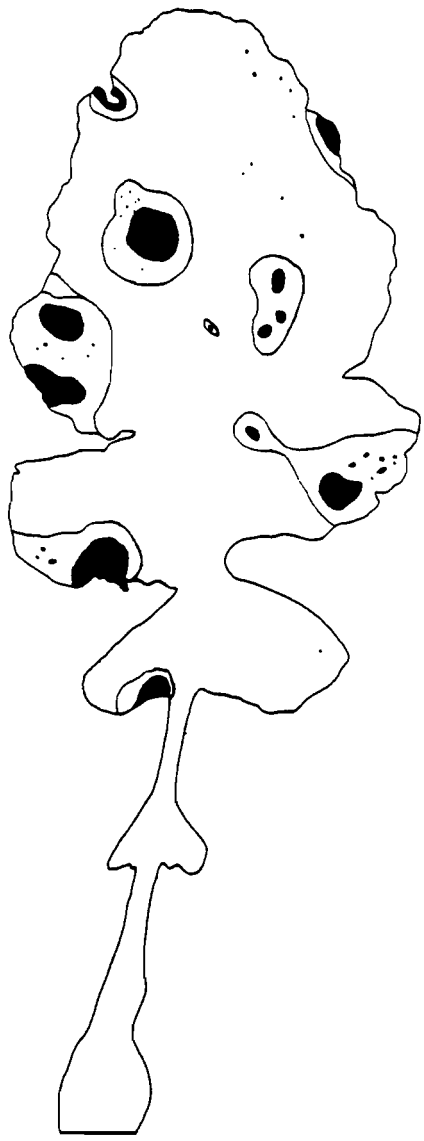


0.1 0.5

1 2



5 10



20

Figure 2.2: Disease area diagrams for Sessile Leaves

Disease: Dark leaf spot

Pathogen: *Alternaria brassicae*

Crop: Oilseed rape

% Area affected

Black = necrosis

Enclosed areas = necrosis + chlorosis

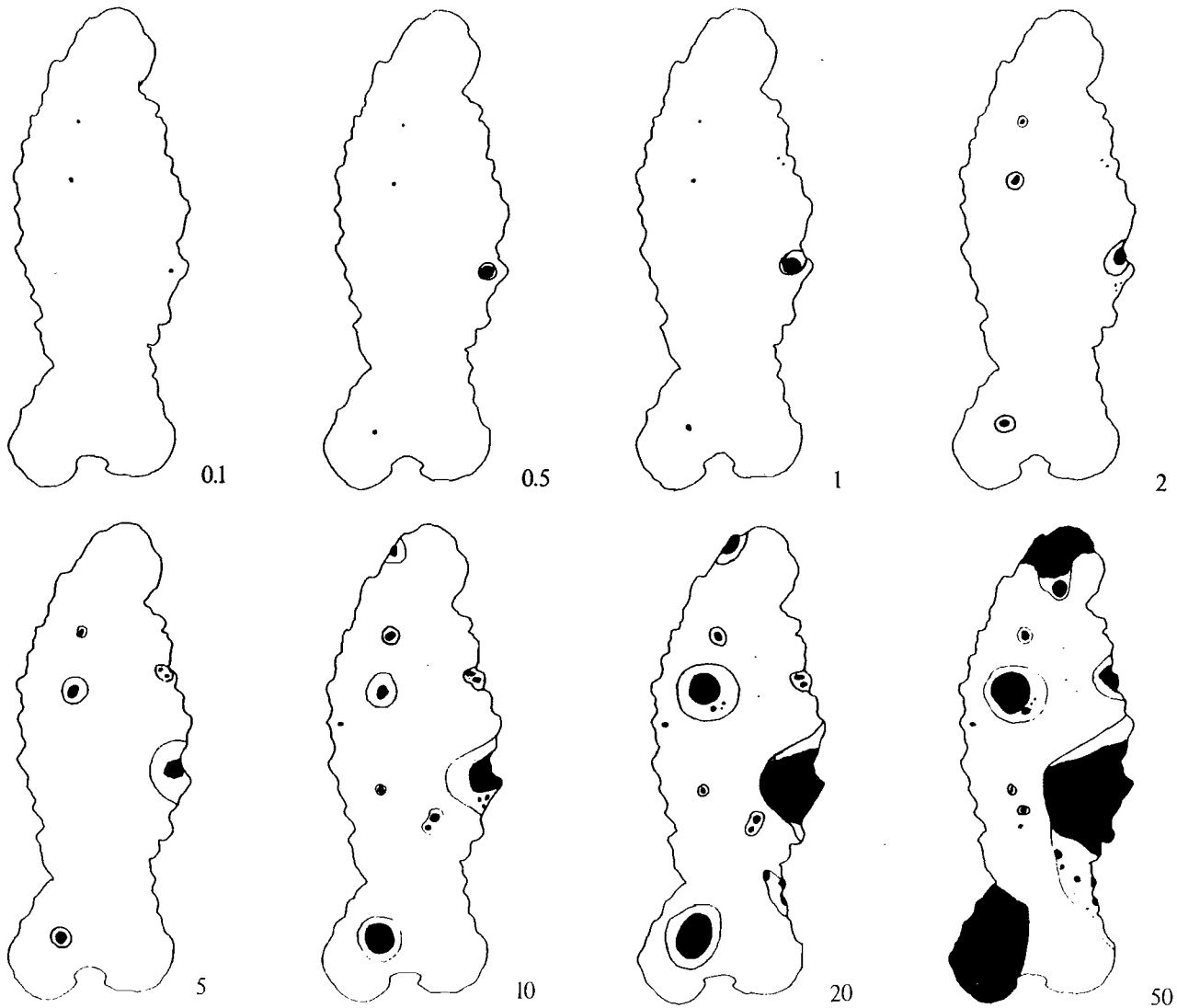

0.1
0.5
1
2
5
10
20
50

Figure 2.3: Disease area diagrams for Individual Racemes

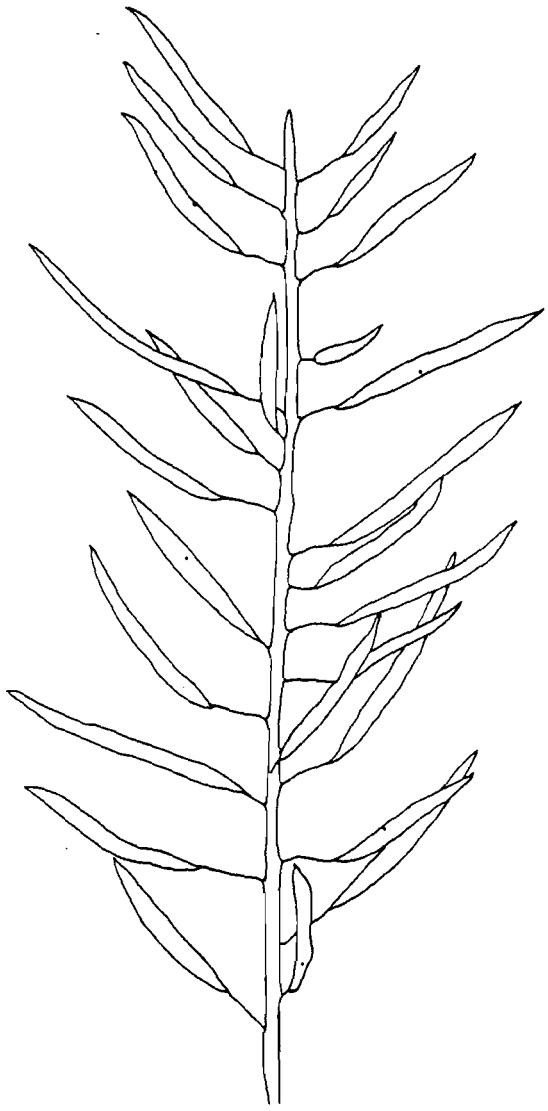
Disease: Dark pod spot

Pathogen: *Alternaria brassicae*

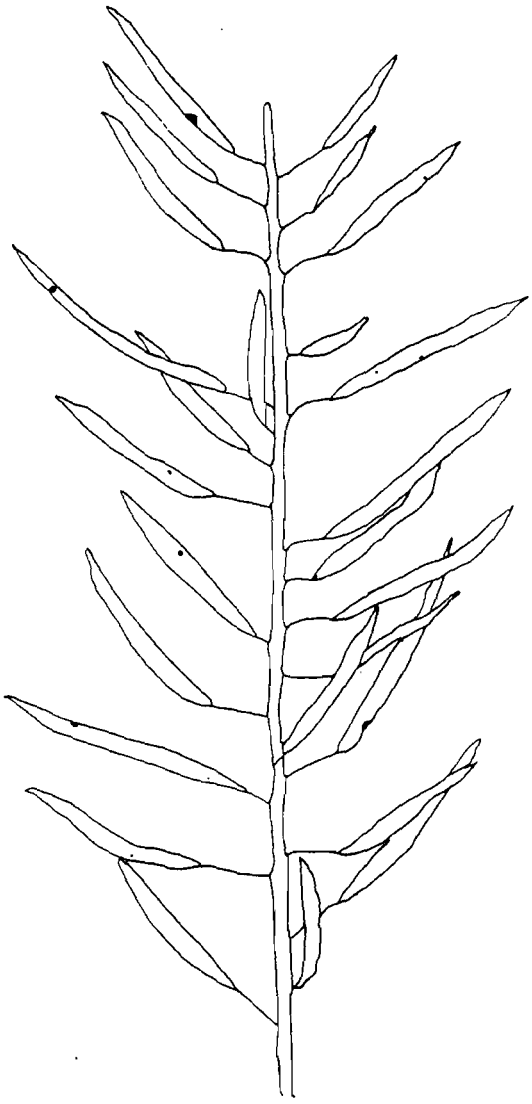
Crop: Oilseed rape

% Area affected

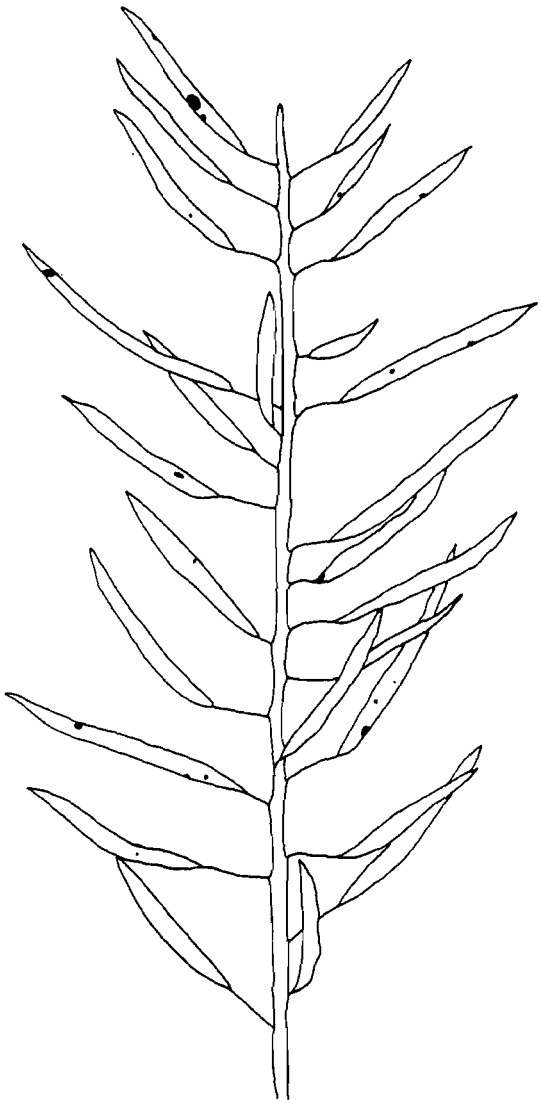
Black = necrosis



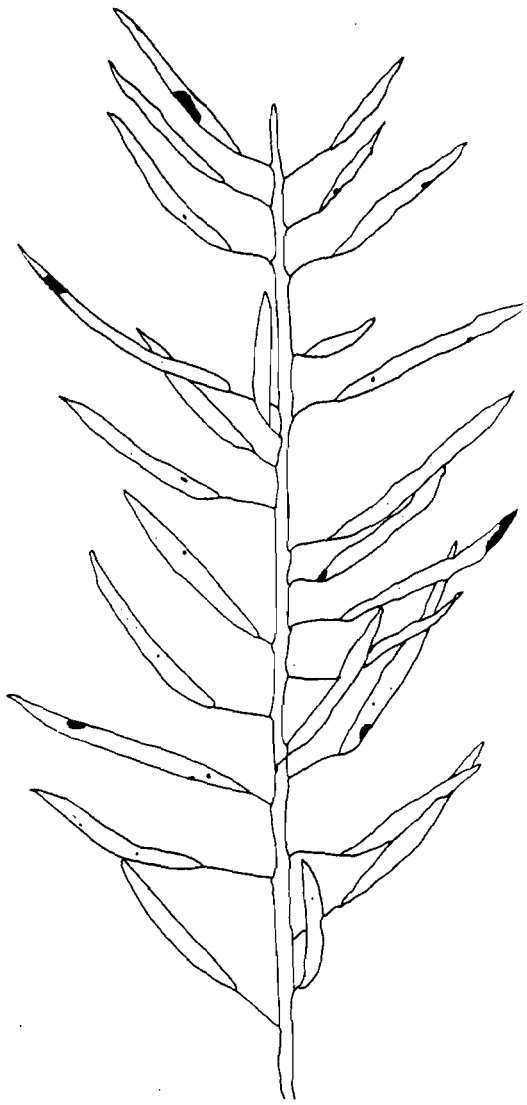
0.1



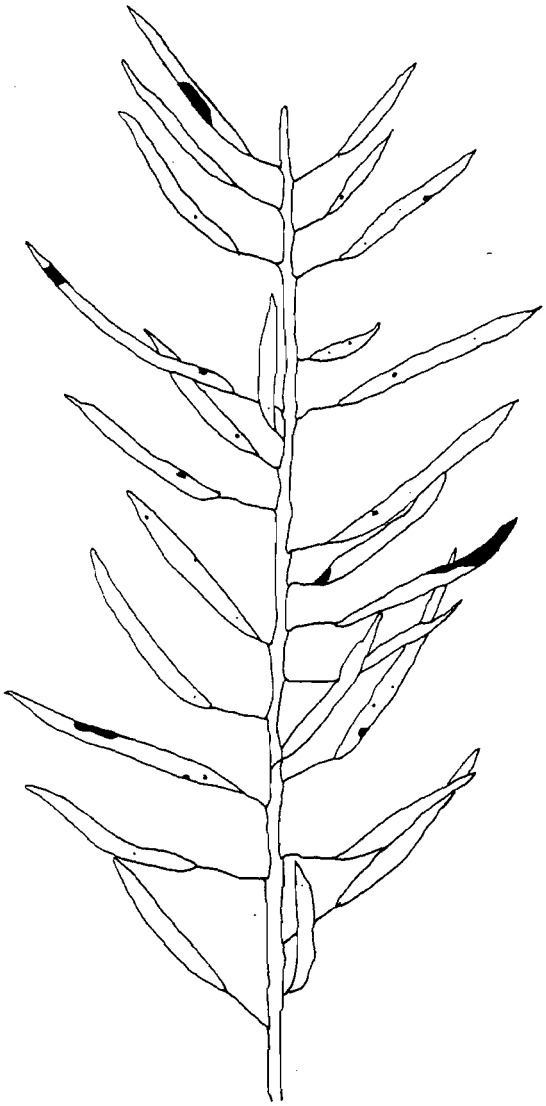
0.5



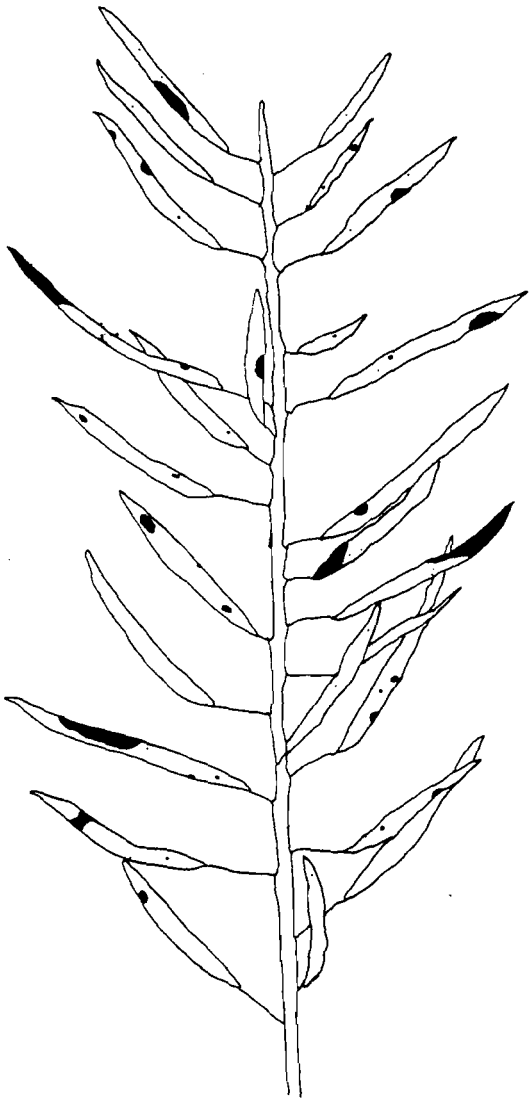
1



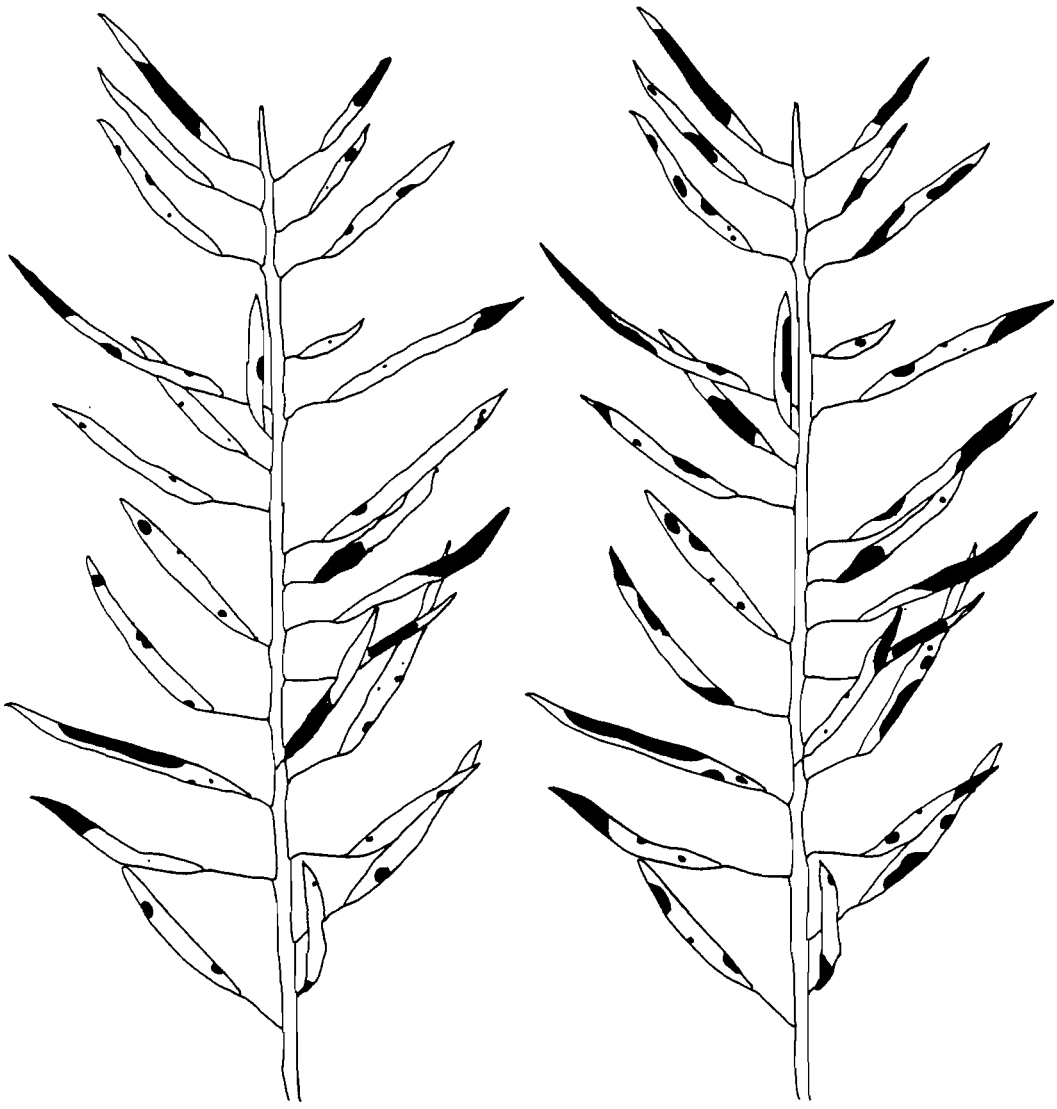
2



5



10



20

50

2.2.3 Using the Disease assessment keys.

2.2.3.1 Leaf canopy

2.2.3.1.1 Individual leaves

These keys can be used on a series of leaves sampled in some way from a field of oilseed rape. Sampling will be discussed further in Part 2, Chapter 3 but will depend on the type of study and the accuracy required. The percentage area of necrotic and total damage can then be assessed and the average area of disease can be caculated for a particular series of leaves. However, problems can arise in oilseed rape because of the highly variable canopy structure and the greatly differing sizes of leaves. Single leaf samples from an oilseed rape plant will not be representative of the disease levels on that plant as would perhaps a single leaf on a wheat plant. Whatever the number ofsamples taken data will be required from more than one leaf on each plant. These data then have to be combined to give an estimate of disease levels on the plant. As leaves are of differing sizes simple unweighted mean values have little meaning. For example if a leaf of 25 units area had 16 units of its area diseased (64 %) and another leaf of 100 units had 1 unit area of disease (1%) the simple mean value would give a proportion of disease for the two leaves of 32.5% whereas the true proportion is 13.6%. The calculated mean must therefore be weighted by a factor related to leaf area.

2.2.3.1.2 Estimation of diseased area on a plant

The measurement of absolute area of disease on a plant or plant organ is rarely considered practical due to the time required for the assessment. An alternative is to estimate the area of disease present by visually assessing the % area of a leaf or pod diseased and combining it with an estimate of leaf or pod area to provide a calculated area of diseased tissue. In oilseed rape where visual assessments of area of disease on individual leaves cannot easily be combined (see below) this can provide a method of estimating the area of disease on a whole plant. In

epidemiological terms an estimate of the absolute area of disease on a particular area of crop may provide valuable information about the level of inoculum that is available.

2.2.3.1.3 Methodology

Absolute levels of disease on a leaf can easily be calculated by multiplying the leaf area (LA) by the proportion of leaf diseased (DA). If these two values can be estimated then an estimate of the area of disease on a leaf can also be calculated. DA can be estimated visually using the area diagrams described in Section 2.2.2. LA can be estimated by the relationship between some measure of the length or width of the leaf and the area of the leaf.

To determine the relationship between leaf area and leaf length for cv. Bienvenu a combined sample of 200 leaves were taken from leaves collected throughout the season (April - July). The sample included sessile leaves from the upper parts of the plant as well as lower stalked leaves. Leaf area was determined by photocopying the leaves, carefully cutting out the copies, weighing the copies and calculating the area using the weight of a known area of paper as a reference. The length of each leaf was measured to the nearest millimetre and the relationship between length and area of leaf investigated using simple linear regression.

The data showed a distinct linear relationship between leaf area and leaf length (Fig 2.4) and was best fitted by the equation

$$\text{leaf area} = 1.88 \times \text{leaf length} - 8.53$$
$$(R^2 = 90.9\% , p = <0.001)$$

Absolute area of disease on a leaf can then be estimated by

$$\text{CDA} = \text{estimated LA} \times \text{estimated DA}$$

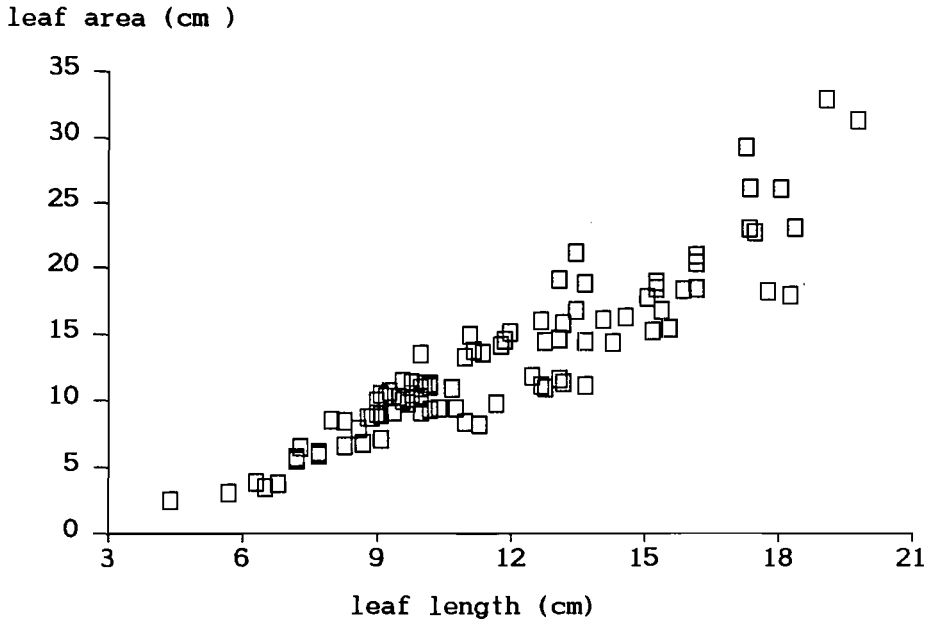


Fig 2.4: Relationship between leaf length and leaf area for oilseed rape plants from the field

and the total area of disease on a plant by

$$TCDA = \Sigma CDA_i$$

The percentage area of the whole plant which is diseased (TLFDIS) can also be calculated and is:

$$TCDA / TCLA$$

where $TCLA = \Sigma LA_i$

The results from these calculations will include considerable errors from various sources. First there is error in estimating the area of disease on the leaf where even slight and non-preventable differences between the estimate and the true area can accumulate and cause considerable differences. In the calculation of total area of disease on the plant this error is present in the estimation of disease for each leaf and then accumulated as the result for each leaf is summed. The level of the error is not easily estimated although work at Long Ashton Research Station (Royle, Pers. comm) has shown that the accuracy of disease area estimates differs considerably between workers and between different days for the same worker.

The second error is introduced by the estimation of leaf area from leaf length. This error is easily estimated from the regression statistics and is random, with there being an equal chance of under or overestimate of the leaf area. If the data are being used for comparative purposes and not as an accurate estimate of absolute diseased area then the error will be occurring equally in all treatments and will be of little consequence. It should be noted that when the percentage area of the total plant which is diseased is being estimated then this error is not only included in the estimate of TCDA but also a second time in the calculation of TCLA.

Even with the large error factor, these calculations do allow an estimate to be made of disease on the whole plant incorporating not only the levels of disease on each leaf but also the contribution which each leaf makes to the total leaf area of the plant. This is in contrast to disease assessments proposed for other broad leaf crops

(Rossi & Battilini, 1989) where the simple mean of the disease assessments for individual leaves is used to estimate total disease on the plant. These estimates have an inherent systematic error which weights the measure in favour of small leaves. In oilseed rape where leaf sizes vary considerably such a systematic error would be a considerable problem.

2.2.3.2 Pod canopy

The key presented here is designed for use on individual racemes. The raceme for assessment should be detached from the plant and held vertically in front of the assessor so that the assessor is seeing the raceme as drawn in the key. The assessment of % diseased area on the raceme (RACDIS) can then be made by comparison with the key.

Care should be taken when assessing racemes because lesions often show a particular pattern depending on the attitude of the raceme in the field. If the raceme is standing vertically in the field then the majority of the lesions especially during the early development of the disease will be on the upper surface of the pods. If the raceme is then assessed from the top or the bottom an over or under estimate of disease is likely to occur respectively. If however, the raceme has been positioned horizontally in the canopy due to lodging for example the majority of lesions again being found on the upper (towards the sky) surfaces of the pods and hence when the raceme is held verticaly the assessor should ensure that the disease levels are not being overestimated by holding the highly diseased area towards him. Alternatively two estimates of disease on the raceme can be made and then the sum of the assessments halved.

Any raceme can be used for assessment as each can be related to the total disease on the plant (Part2, Chapter 4) however Raceme T is the most appropriate as it is the first raceme to develop and remains throughout the life of the pod canopy.

The basic building blocks of disease assessment are a visual recognition of the disease together with some visual estimate of the intensity of that disease (Seem, 1984) the latter usually of the area of plant or plant part diseased or the number of lesions or diseased entities. The study of any pathosystem requires that methods of disease assessment are devised to provide a system which is appropriate for the aims of the work being carried out. A number of disease assessment methods have been described for dark leaf and pod spot of oilseed rape (e.g. Anon, 1988; Evans & Gladders, 1981; Mhrida, 1983; Petrie, 1973; Singh & Bhonmik, 1985) although no published data are available about their reliability. Only two of these methods included the leaf canopy (Mhirda, 1983; Singh & Bhonmik, 1985). These assessment techniques vary in their approach to the assessment of disease, some based on the percentage area of crop diseased (Anon, 1988; Evans & Gladders, 1981) and others on arbitary scales (Petrie, 1973; Singh and Bhonmik, 1985). None of these assessment techniques fulfilled the requirements of the present study as they did not give any information about the position of the disease within the plant canopy except whether it was on the leaf or pod canopy. The simple but time consuming assessment system of assessing each leaf and raceme visually for disease was therefore chosen.

Counting lesions as well as assessing the disease area of leaf and pod tissue allowed a wide ranging study of disease progress to be made (Part 3), although problems with diagnosis of the causal organism and lack of area diagrams for disease assessment had to be overcome. Diagnostic difficulties were associated with distinguishing between *A. brassicae* and *A. brassisicola* and were overcome by careful observation and isolation of the pathogen from a range of disease lesions in each season.

To aid disease estimation on plant tissue, area diagrams have been produced for many diseases and published in journals (e.g. James, 1971b) or as part of a manual (James, 1971a). Area diagrams for dark leaf and pod spot are conspicuously absent from these publications even though this disease is a problem on oilseed rape

in most parts of the world. Mhrida (1983) did produce an area diagram for dark leaf spot on the pod canopy but this was found to be inaccurate (Rawlinson, Pers. comm.)

The disease area diagrams described here for leaves and racemes were prepared in accordance with guidelines given by James (1971b) and are equivalent to those produced for many other crops. The rationale behind such diagrams has been fully discussed by several authors (e.g. James, 1971b; Large, 1966).

Two sets of diagrams are presented for the leaf tissue rather than the more usual single set because of the greatly differing shapes of the stalked and sessile leaves. It was considered that this difference could substantially affect the estimation of disease so the two sets of diagrams were produced. The division of disease into necrotic and chlorotic area is also unusual but was considered necessary because of the large amount of chlorotic tissue often associated with even small lesions. It gives a more representative picture of what the observer would see on the leaf material. In this study, when assessing necrotic tissue alone diagrams which were completely coloured in were used in conjunction with the sets shown here.

The two differences compared with the more standard type of area diagram with a single leaf shape and no division between necrosis and chlorosis shown was an attempt to assist the observer in making a more accurate and more rapid assessment of disease on a single leaf. However, a detailed study of the use of these standard area diagrams is required to decide whether the additions are of any assistance. Further additions to the diagrams were considered, most importantly a series of diagrams showing various sizes of the different shaped leaves but this would have made the sheets of diagrams confusing and undermine the objective of simplicity.

The area diagrams for the pod tissue conform to the more usual pattern of area diagrams with a single raceme shape and fully coloured diseased areas. James (1971b) emphasised the need to use area diagrams correctly and this is most important with the pod diagrams presented here, careful consideration of the orientation of the raceme being necessary to give the most accurate estimate of disease. One major difficulty in the preparation and use of the pod diagrams is the

narrowness of the individual pods and hence the effect of the line representing the pod outline which even if quite narrow can make up a considerable proportion of the area being viewed. In these diagrams the outline is considered as part of the area of the pod and not bounding it but a further development of the diagrams may be to have the pods completely coloured e.g. light green with the lesions remaining solid black or dark brown.

One of the major difficulties in describing dark leaf spot on oilseed rape is the estimation of disease on a whole plant basis and is largely due to the complexity of the leaf canopy and the uneven distribution of the disease. Mhrida (1983) took 10 leaves from each plant but made no consideration of the position of the leaves on the plant canopy or of leaf size. Singh & Bhonmik (1985) used an arbritary scale to record disease levels on the leaf canopy, a method which although acceptable for gross comparisons is of little use in detailed studies. Where more detailed studies of the disease are being made and data for disease is being taken from individual leaves methods of combining leaf data are required to give whole plant assessments. Similar situations arise with other pathosystems and in many cases although the strategy for sampling leaves differs a simple mean value is used for the disease estimate (Rossi & Battilini, 1989). This however takes no account of any difference in area of the leaves sampled. The method described here is an attempt to include a measure of leaf area in the combination of disease area data from the leaf canopy of oilseed rape where leaf size can vary considerably. Such a method is inevitably a compromise between accuracy and the time required for assessment.

For complete accuracy the absolute area of each leaf and disease on that leaf is required so that the calculation of diseased area on the plant can be correctly weighted for leaf size. By using estimates of each of these parameters the accuracy decreases but the number of samples that can be assessed in a given time increases. The major error in this method lies with the visual assessment of the disease on individual leaves especially at low disease levels. This error may be systematic, differ from observer to observer, or with the same observer from day to day (Royle, Pers comm.) although Shokes *et al.* (1987) showed that errors of estimatiom of peanut blight by trained observers are small. The errors associated with the estimation of

leaf area are random and therefore with large samples tend to become less significant. The accuracy of estimating disease levels on whole plants using this method has not been compared with the overall plant assessments mentioned above and further work is required to compare the various methods, as well as to explore further possibilities for better and more rapid assessments of dark leaf spot on the complete leaf canopy of oilseed rape.

One consequence of caculating the proportion of leaf area diseased on the whole plant by the method described above is the intermediate value of a calculated absolute area of disease on the leaf canopy (TCDA). This may be of little use in most circumstances but when associated with some visual estimate of sporulating tissue may be valuable in estimating the future potential of the disease. This possibility is further discussed in Part 4.

The assessment of oilseed rape as described here has used methods and rationale which have been used in many other pathosystems. With slight modifications and an understanding of the idiosyncracies of the development of dark leaf and pod spot they provide ways of estimating the disease in a wide variety of situations. The development of such assessment systems and their universal implementation would make interpretation and comparison of data from various studies less difficult (Shokes *et al.* 1987).

CHAPTER 3 SAMPLING AND DATA TRANSFORMATION

3.1 Introduction

In any experimental work it is important that the level of precision required by the experimenter can be obtained from the information gathered. The number of samples needed is always a compromise between statistical requirements and the cost in terms of labour (Vickerman, 1985) but until the sample numbers required for a particular need are known, decisions about such compromises cannot be made.

In oilseed rape Scarisbrick *et al.* (1982a) showed that large numbers of plants (>70) are needed to estimate pod dry weight from 0.54m^2 of plot to ±5g, proving that in earlier studies e.g. Ogunremi (1970), Scarisbrick *et al.* (1981) too few plants were collected. In the study of diseases of oilseed rape the numbers of plants collected varies (e.g. 25 plants, Evans *et al.* 1981; 100 plants, Regnault & Pierre, 1984) but no information is available about the validity of such sample sizes.

A number of methods are available for estimating the numbers of samples required for a given level of precision each of which is based on a particular frequency distribution. If counts from a sample fit a particular frequency distribution model then the errors of population parameters can be estimated (Elliott, 1983) and hence estimates of the numbers of samples required can be made. However, different samples of the same organism may not always have the same distribution (McGuire *et al.* 1957) and furthermore the distribution of a particular variable may change during a study period (Elliott, 1983). The benefits of a frequency distribution are therefore diminished if it does not fit samples of a variable in all situations (Taylor *et al.* 1978). Methods for estimating sample numbers based on particular frequency distributions are then limited in their value.

Estimates of the number of samples required can also be determined from the coefficient of variation (CV) (e.g. Mukerji and Harcourt, 1970). However if the standard deviation (and hence the CV) varies with the mean then the sample size

needed for a given level of accuracy will depend on the mean. Taylor (1961) put forward a functional relationship which showed that for a wide variety of organisms the variance of a population (s^2) is related to the mean population density (m) by a power law

$$s^2 = am^b$$

Taylor (1965) showed that the index b ranges widely between different organisms but remains constant for the same organism in the same environment even when sampled by different methods. This relationship between variance and mean was then utilised by Finch *et al.* (1978) to devise 'sampling plans' (i.e. number of samples required) for a range of mean values of cabbage root fly populations, the value of the power law being that a single equation can be used to estimate numbers of samples required for any particular organism at any mean population density.

As implied by the comments above, data from the field (counts or ratios) rarely follow a normal distribution. However the common parametric statistical tests e.g. t-tests, ANOVA and Regression statistics are associated with the normal distribution (Sokal and Rolfe, 1981). Furthermore parametric methods also require that the variance and the mean of the data should be independent; that components of the variance should be additive and that the effects and error should be additive (Bartlett, 1947). As well as not following a normal distribution the mean and variance of field data are often not independent. To overcome this difficulty each data point should be replaced with a suitable mathematical function to "normalise " or "transform " the data. A correct transformation should normalise the frequency distribution; eliminate the dependence of variance on the mean and ensure that the components of the variance are additive (Bartlett, 1947). Taylor (1965) has shown that using the power law the transformation needed for the further statistical analysis of the data can be calculated. The quantity (z) to be analysed is transformed from any original count (x) by the expression $z = x^{1-1/2b}$ where b is the regression coefficient as described above. This is a popular method of estimating the required transformation as it can be used in most situations even though the biological interpretation of the method is not fully understood (1) (Taylor, 1972).

In the present study, various parameters were measured for oilseed rape and dark leaf and pod spot on oilseed rape (see Part 1, Chapter2) in four different crops and epidemics. Estimation of the number of samples required and transformations needed for further analysis are calculated using Taylor 's Power Law.

3.2 Methodology

The data were taken from the four epidemics; Rafal (1986), Mikado (1986), Bienvenu (1987) and Bienvenu (1988) as described in Part 1, Chapter 2. The variables which were analysed in this section are given in Table 3.1.

Table 3.1: Variables analysed to estimate variance v mean relationships, sample numbers and transformations required

Leaf Canopy

Number of leaves/plant (LNN)

Calculated leaf area/plant (TCLA)

Number of *A. brassicae* lesions/plant (TALLF)

Calculated disease area/plant (TCDA)

% area diseased on each plant (TLFDIS)

Number of sporulating lesions/plant (TALLFSP)

Calculated area of sporulating tissue/plant (TCDASP)

Pod Canopy

Number of pods/plant (PODNUM)

Number of pods/plant infected with *A. brassicae* (PODINF)

Number of *A. brassicae* lesions/raceme (ALRAC)

% area diseased on each raceme (RACDIS)

% area diseased on each plant (PODDIS)

3.3 Statistical Analysis

According to Taylor (1961) the variance (s^2) of a sample varies with the mean (m) according to the following Power Law

$$s^2 = am^b$$

The relationship is expressed logarithmically as

$$\ln (s^2) = \ln (a) + b \times \ln (m) \qquad \text{I}$$

Equation I is used to test whether the regression accounts for a significant proportion of the variance and whether it is linear.

The sample size (N) needed for a given level of precision (p) can be caculated (Finch *et al.*, 1978) from:

$$\ln (N) = (\ln (a) - 2\ln (p)) - (2 - b)\ln (m) \qquad \text{II}$$

if equations I can be justified. From the 'sampling plan' it is then possible to estimate the number of samples required based on a preliminary investigation of the mean density of the parameter being studied.

If b in equation I is common for a particular parameter then the transformation required for further statistical analysis of the data can be calculated from

$$z = x^{1-1/2b} \qquad \text{(Taylor, 1965)} \qquad \text{III}$$

Means and variances were calculated for the different variables at each sampling date and the extent to which the Power Law applied to each variable was investigated. Where they were required, comparisons between regression coefficients and intercepts were carried out using a method described by Sokal and Rolfe (1981).

Where appropriate, the numbers of samples required for particular estimates of precision and the transformations needed for further statistical analysis were estimated.

3.4 Results

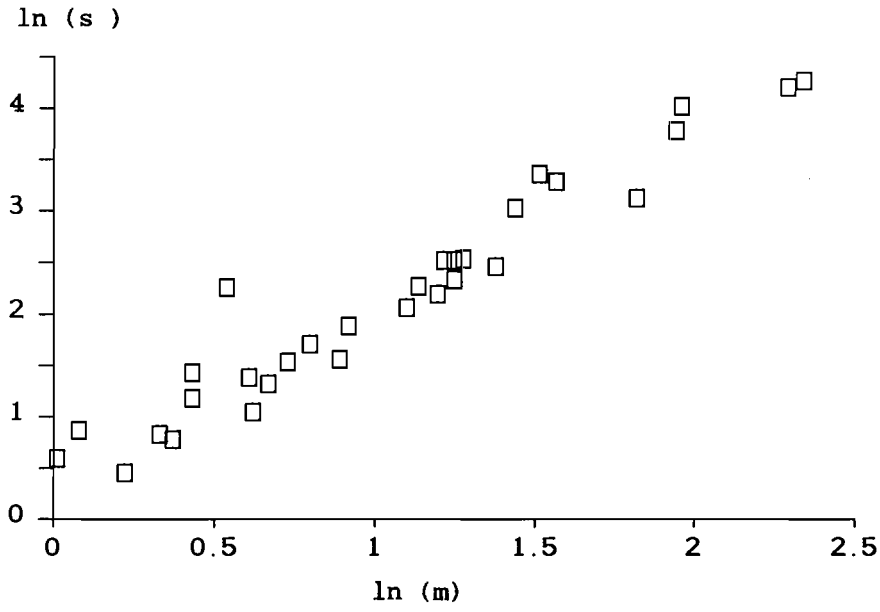
3.4.1 Relationship between variance and mean.

The results of regression analysis for the various parameters are given in Tables 3.2 - 3.4. and graphical examples of the relationships between ln (s^2) and ln (x) are given in Fig 3.1. In only two cases was there no significant relationship between the sample means and variance (Table 3.2). In one of these situations (TCDA, 1987) the range of the means was small and any linear relationship was probably masked by the error. For the other parameters from the leaf canopy the regressions accounted for 70.8 to 98.2% of the variance and in all cases except one were significant at the 1.0% level. The variance accounted for by the regression when using the pod canopy data was greater than for the leaves ranging from 90.7 to 99.9% except for ALRAC with variety Mikado (R^2=79.4%).

For several variables comparisons were possible between ln (a) and b values obtained for different years and/or different cultivars. For LNN, TCLA, and TALLF no significant differences were found between the values of b for 1987 and 1988 when cv. Bienvenu was used. Similarly for LNN and TCLA there was little difference between the values of ln (a). Although there appeared to be a considerable difference between the values of ln (a) for TALLF between 1987 and 1988 they were found not to be significantly different. Where cv. Mikado was also compared with cv. Bienvenu there was no significant difference between the b values for TALLF or LNN.

On the pod canopy comparisons for a and b were made between between different racemes within the same year and between years for cv. Bienvenu. No significant differences were found for PODINF, ALRAC or RACDIS between racemes or year. The only significant difference (p=0.01) was between ln (a) for 1987 total and the single racemes with respect to the number of *Alternaria* lesions

a)



b)

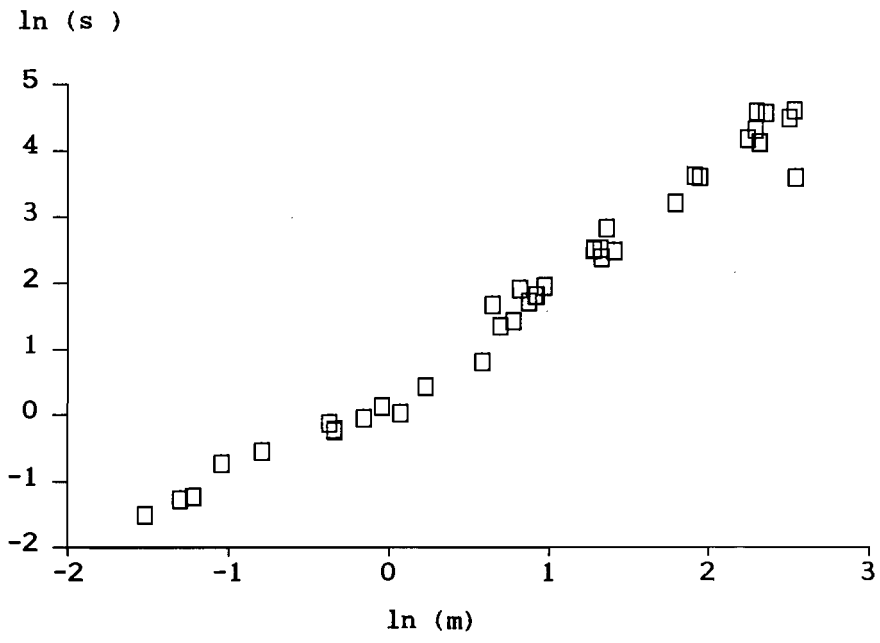


Fig 3.1: Regression of ln (s^2) on ln (m) for
a) TALLF*
b) ALPOD*
for the combined data of 1986 - 1988

* see text for explanation

Table 3.2: Statistics for the regression of ln (s^2) on ln(m) for various parameters of the leaf canopy of oilseed rape.

Variable	ln (a)	b	% s^2 accounted for	sig*	range of m
LNN					
1986	0.99	0.95	80.5	++	2.5-33.7
1987	0.87	1.15	90.8	+++	5.3-27.3
1988	0.83	1.78	91.0	+++	6.5-27.0
TCLA					
1987	1.49	1.66	96.4	+++	114-1238
1988	1.25	1.69	89.1	+++	85-1031
TALLF					
1986	0.77	1.69	93.5	+++	1.0-27.8
1987	0.48	1.77	86.1	++	24.4-220.2
1988	1.35	1.60	78.5	+++	1.2-33.3
TCDA					
1987	6.57	0.08	0.05	NS	21.3-44
1988	0.74	2.0	80.8	+++	0.5-8.2
TLFDIS					
1987	0.74	1.14	98.2	+++	1.7-16.4
1988	0.71	1.67	89.7	+++	0.06-8.2
TALLFSP					
1987	1.67	1.12	70.8	+	2.5-11.7
1988	0.98	1.40	96.3	+++	0.06-2.69
TCDASP					
1987	2.05	0.99	44.9	NS	1.4-23.7
1988	1.21	1.30	76.7	+++	0.13-1.94

* NS = not significant, + = $p < 0.05$, ++ = $p < 0.01$, +++ = $p < 0.001$

Table 3.3: Statistics for the regressions of ln (s^2) on ln (m) for various parameters on the pod canopy of oilseed rape

Variable	ln (a)	b	% s^2 accounted for	sig*	range of m
PODINF					
1987					
Raceme T	0.80	1.10	93.1	+++	1.4-32.9
Raceme 1	0.57	1.16	99.8	+++	0.25-20.0
Raceme 2	0.70	1.15	97.1	+++	0.3-25.0
Raceme 3	0.71	1.11	98.2	+++	0.09-21.9
Raceme 4	0.57	1.21	97.9	+++	0.05-23.2
Raceme 5	0.60	1.17	97.6	+++	0.06-23.1
1988					
Raceme T	0.44	1.19	96.6	+++	0.03-27.6
Raceme 3	0.35	1.09	91.5	+++	0.36-18.3
1987 + 1988	0.24	1.17	97.1	+++	
ALRAC					
1987					
Raceme T	0.76	1.64	99.1	+++	1.4-269.4
Raceme 1	1.06	1.63	99.7	+++	0.25-165
Raceme 2	0.93	1.64	99.7	+++	0.3-192
Raceme 3	1.29	1.42	96.1	+++	0.09-173
Raceme 4	1.15	1.6	98.9	+++	0.05-194
Raceme 5	1.21	1.56	99.6	+++	0.06-206
1988					
Raceme T	0.96	1.54	95.5	+++	0.03-198
Raceme 3	0.66	1.75	96.8	+++	0.13-225
1987 + 1988	0.49	1.54	97.7	+++	0.03-269

* see Table 3.2

Table 3.4: Statistics for the regressions of ln (s^2) on ln (m) for various on the pod canopy of oilseed rape

Variable	ln (a)	b	% s^2 accounted for	sig*	range of m
RACDIS					
1986					
Mikado	-0.14	1.39	79.4	+++	0.04-29.2
Rafal	-0.16	1.61	91.3	+++	0.03-5.8
1987					
Raceme T	-0.35	1.95	99.3	+++	0.06-40.3
Raceme 1	0.02	1.80	99.5	+++	0.03-40.9
Raceme 2	0.35	1.68	96.1	+++	0.04-40.1
Raceme 3	-0.35	1.89	97.3	+++	0.04-33.5
Raceme 4	-0.14	1.91	99.3	+++	0.04-34.3
Raceme 5	0.18	1.78	99.9	+++	0.03-30.2
1988					
Raceme T	-0.06	1.65	98.3	+++	0.01-25.4
Raceme 3	-0.17	1.73	93.7	+++	0.03-27.5
1987 + 1988	-0.10	1.65	90.7	+++	0.03-40.9
TPODINF					
1987	0.48	1.14	99.7	+++	2.1-158
TALPOD					
1987	0.39	1.83	99.9	+++	2.1-1271
PODDIS					
1987	-0.39	1.86	96.9	+++	0.03-30.2

* see Table 3.2

(TALPOD and ALRAC respectively). Furthermore the values of b for *A. brassicae* lesions for the pod canopy and the leaf canopy did not differ significantly. For data combined over the whole pod canopy in 1987 (TPODINF, TALPOD, PODDIS) the relationship between s^2 and m was highly significant with the variance accounted for rising to 96.9 - 99.9%.

Table: 3.5: Statistics for the regressions of ln (s^2) on ln (m) for various parameters of the leaf canopy of oilseed rape Combined data 1986 - 1988

Variable	ln (a)	b	% variance accounted for	sig*	Range of (m)
LNN	0.08	1.34	78.9	+++	2.5-33.7
TCLA	0.56	1.68	91.7	+++	84.7-1238
TALLF	0.40	1.67	93.3	+++	1-220
TCDA	0.40	1.78	92.7	+++	0.5-44
TLFDIS	0.46	1.45	91.8	+++	0.06-16.4
TALLFSP	1.02	1.42	97.5	+++	0.06-11.7
TCDASP	1.29	1.39	91.7	+++	0.13-23.7

* see Table 3.2

For each variable on the leaf canopy and pod canopy the data were combined and new regression statistics calculated (Table 3.5). For TCLA, TALLF and TCDA these combined regressions were highly significant ($p > 0.001$) and accounted for 91.7 - 93.3 of the variance. For number of leaves although the regression was highly significant the variance accounted for was only 78.9%. For the pod canopy, regression calculations were only made for the combination of all data from 1987 (Table 3.3 &

3.4). For all variables the regressions were highly significant and accounted for between 90.6 and 97.7% of the variance.

3.4.2 Sampling precision

Taylor 's power law adequately described the relationship between x and s^2 for a variety of parameters on the leaf and pod canopy so equation II was used to derive sampling plans (Finch *et al.* 1978) using TALLF, TCDA and TLFDIS on the leaf tissue and PODINF, ALRAC and RACDIS. Sampling plans were devised using the relationships developed for the combined data sets from the four trials (see Table 3.3-3.5) to give the number of plants required to give a 5, 10 and 20% coefficient of variation. The numbers of samples presented in Table 3.6 represent the numbers required for mean values of the parameter at the extremes and the middle of the range of data used to prepare the sampling plans. The results show that for all the parameters the number of samples required changes non-linearly with the mean value of the parameter. This relationship was adequately linearised using Taylor 's Power law allowing the number of samples required for a given mean value to be easily calculated.

The different parameters require greatly varying numbers of samples to give the same level of precision at a given mean value (Table 3.6). On the leaf tissue TLFDIS requires almost four times the number of samples as TCDA and in turn this requires approximately twice the number of samples needed to estimate TALLF at a sampling precision of 10%. On the pods the differences in numbers of samples required for the different parameters are not so great. Estimates of PODINF require the most samples, approximately 1.5 times the number required for RACDIS and almost twice the number for ALRAC.

Where the numbers of samples required for the same parameter on both pods and leaf tissue (Number of *A. brassicae* lesions and % diseased area) are compared fewer samples are generally required for the pod tissue.

Table 3.6: Number of sample units required to obtain population estimates with a coefficient of variation (CV) of 5, 10 and 20% for different parameters of dark leaf and pod spot of oilseed rape

Variable	mean	% CV		
		5	10	20
Leaf Tissue				
TALLF	10	279	70	18
	80	140	35	9
	200	104	26	7
TCDA	1	596	149	37
	10	360	90	22
	30	282	71	18
TLFDIS	0.1	2248	562	141
	1	634	158	40
	10	179	45	19
Pod tissue				
PODINF	1	508	127	32
	10	75	19	5
	30	30	8	2
ALRAC	10	229	57	14
	80	89	22	6
	200	59	15	4
RACDIS	1	362	90	24
	10	161	40	10
	25	116	29	7

Table 3.7: Appropriate Transformations for various parameters of the leaf and pod canopy of oilseed rape

	Absolute transformation	Approximate transformation
Leaf tissue		
LNN	$x^{0.33}$	log (x+1)
TCLA	$x^{0.16}$	log (x)
TALLF	$x^{0.16}$	log (x)
TCDA	$x^{0.11}$	log (x)
TLFDIS	$x^{0.28}$	log (x+1)
TALLFSP	$x^{0.29}$	log (x+1)
TCDASP	$x^{0.31}$	log (x+1)
Pod tissue		
PODINF	$x^{0.41}$	$\sqrt{x}$
ALRAC	$x^{0.23}$	log (x)
ALPOD	$x^{0.15}$	log (x)
RACDIS	$x^{0.18}$	log (x)

3.4.3 Data transformation

The good fit of Taylor 's power law to the data also allowed the calculation of appropriate transformations for the different variables (Table 3.7).

3.5 Discussion

Taylor 's Power Law adequately fitted much of the data collected in this study for a variety of variables used to measure dark leaf and pod spot on oilseed rape. On one of the two occasions where there was no significant fit of the data the range of data was limited and when combined with the data from other years significant relationships between x and s^2 were achieved. The reason for the poor relationship in 1987 for TCDASP is difficult to explain although again when the data from the two years are combined the relationship between x and s^2 is good. The similarity of parameter b for the same measurement variable between different years and cultivars when the power law fitted is consistent with findings of Taylor *et al.* (1978), Taylor & Woiwood (1980) and Taylor (1962) who showed that the power law adequately described data for a number of species from a wide variety of taxa. Although no microfungi were included in their studies Taylor (1972) considered that data from other taxa would be likely to be well fitted.

Taylor (1970) has shown that where the power law fitted the parameter b tends to remain constant for counts of the same organism even when sampled by different methods and has suggested that parameter b can be thought of as a species characteristic. The practical importance of this is that transformations which are then derived from b, are also species characteristics and can therefore be applied to all future samples of the organism. In plant pathology Taylor 's power law has been used by a number of authors to develop transformations for a variety of measures of incidence and severity (Analytis, 1973; Analytis, 1979; Butt & Barlow; 1980; Seem & Gilpatrick; 1980).

The data transformations caculated from the results of this study show that a range of transformations is required for the different parameters. Many of the transformations can be approximated by those more usually used in statistical analysis (Perry, Pers. comm.; Taylor,1970) although some of the transformations which are approximated are not those normally associated with that type of parameter. On both leaf and pod tissue the transformation for % diseased area can be approximated by log (x) (b=2) an unexpected transformation for a percentage disease scale. A more

usual transformation would be arcsin $\sqrt{(x)}$ (the angular transformation) which takes account of the upper boundary of the percentage scale (Sokal & Rolfe, 1981). This transformation stretches out both tails of a distribution of percentages or proportions and compresses the middle. The reason why a log (x) transformation has been found appropriate for % disease on oilseed rape is a consequence of the generally low levels of disease which were noted so that the mean values did not range over the whole of the 0 - 100 scale. The data therefore showed no apparent top limit and hence acted as if its increase could be infinite. This transformation then is only applicable to data which lie in the ranges found within this study i.e. 0 - 40%. Because of the likelyhood of zeros being found in the data set the transformation chosen for use in this study was log(x+1).

When data consist of integers (i.e. counts) an often used transformation is $\sqrt{x}$ (Bartlett, 1947). Since such count data e.g. bacterial colonies, plant numbers will often follow a Poisson distribution Bartlett (1947) showed that to stabilize the variance a square root scale should be used. With the Poisson distribution the variance equals the mean (Sokal and Rolfe, 1981) but even where this is not the case the square-root transformation may stabilize the data (Bartlett, 1947). In this study however the transformations suggested for the number of lesions on the leaves and pods were approximated by log (x) (b=0.2) with only the transformation for the number of pods infected lying close to a square root function (b=0.5). With lesion count data although the variance and the mean were equal at very low mean densities, in most cases the variance was greater than the mean. Bartlett (1947) showed that when the variance is greater than the mean, a more usual transformation would be log (x) and if zeroes were likely to be found in the raw data a log (1 + x) transformation should be used. Furthermore a log (1 + x) transformation tends to approximate a square root transformation at low mean values and a log (x) transformation at high ones (Perry, Pers. comm) which is consistent with the raw data from this study. In this study then a log (x+1) transformation was used for any data where lesion counts were involved.

The value of transforming data, as discussed previously, is not only to stabilise the variance but also to normalise the data and make real effects linear and additive.

Fortunately the transformation of a scale to meet the first condition often improves the others (Bartlett. 1947). However, a power transformation as proposed by the power law although fulfilling the first and second requirements is less likely to fulfil the third (Perry, Pers. comm.). Logarithmic and square root scales are more likely to fulfil all three requirements (Perry, Pers. comm) and have therefore been used in this study.

Taylor 's power law has led to the estimation of the transformations required for the data collected in this study. The functions suggested here can probably be applied universally as the parameter b, as discussed earlier, appears to remain reasonably constant over years and varieties. However, further work is required to confirm this view. The difference between the transformations chosen for this work and those which would have been suggested by the data type show how important an investigation of the data prior to analysis can be and is the major value of using Taylor's Power Law to evaluate transformations.

Taylor (1970) suggested that where the power law fitted, although the parameter b tended to remain constant for counts of a particular species the value ln (a) would probably differ between different investigations as a consequence of different sampling techniques, and demonstrated this with *Aphis fabae* on beans (Taylor, 1970). Vickerman (1985) on the other hand showed that for a range of beetles ln (a) as well as b remained constant with a range of sampling techniques. In this study where a significant relationship occurred between variance and mean no significant differences were shown within each parameter for the leaf tissue between ln (a) although some differences were close to significance. Where the differences were visually quite large but not significant it appeared to be a consequence of a poorer relationship between variance and mean making the errors greater and hence differences less significant. On the pod canopy the reason for the significant difference between ln (a) for the 1987 Total and the racemes for the number of lesions on the racemes is unknown but in any case slight. Similarly with % disease on the raceme the differences between the values for ln (a) amongst the different racemes cannot be explained and is undoubtedly the reason for the poorer fit of the power law to the total data for 1987 and 1988.

Although these results show some slight differences between the parameter ln (a) on the pod canopy when estimated from different data sets the very adequate fit of the power law to the combined data sets does suggest that sampling plans developed from the combined data from leaf and pod canopy for different years will give a good estimate of the sample numbers required for different mean densities of each disease assessment variable.

The use of Taylor 's Power Law combined with the equation defined by Finch *et al.* (1978) with data from this study has shown that for many of the disease parameters measured, an estimate of sample numbers required for a particular level of precision and mean value can be made. The numbers of samples required, as was expected, are high especially at low mean values of the parameters. This is undoubtedly a consequence of the uneven distribution of disease and the irregular nature of the crop. The results clearly demonstrate the usefulness of lesion numbers as a measure of disease on the leaf canopy where sample numbers required for a particular level of precision are substantially lower than TCDA or TLFDIS, although at high levels of precision (5%) sample numbers are too high for most field studies. At the 10% level of precision, one which is generally considered adequate for field work, numbers of plants required in a sample are more managable although for low levels of disease are still probably too high for studies other than those of the most detailed kind.

With the pod data the less irregular pattern of disease distribution and the more consistent nature of the raceme structure compared with the leaf canopy probably explain the need for fewer samples for disease on the pods than on the leaf material and the reduced difference between the numbers required for the different parameters. Although ALRAC is the parameter requiring the least number of samples RACDIS does not require substantially more and as well as being a more rapid assessment would probably be of use in more studies especially those related to yield loss. However as with the leaf material when disease is at low levels large and probably unmanagble numbers of plants are required even at the 10% level of precision.

These results together with those of Scarisbrick *et al.* (1982a) and Henderson (Pers. comm.) show that for a range of measurements on oilseed rape both of the plant and of disease, large numbers of samples are required often outside the scope of normal field studies. The results show that even in this study, too few plants were assessed to adequately measure low levels of disease on either the leaf or pod canopy. Of published studies of dark leaf and pod spot of oilseed rape only Regnault & Pierre (1984) took adequate samples. It is therefore essential that prior to any study of dark leaf and pod spot on oilseed rape an evaluation is made of the sample requirements for the level of precision and the mean value of the parameter being measured, from this it may be concluded that assessments should not begin until the disease reaches a certain level as work prior to this will require too much time or will be wasted if too few samples are taken. This would be particularly true where different control measures of the disease are being compared, these data indicating that if 40 plants per sample can be assessed then work should wait until until disease levels rise to 10%. This however, is complicated by yield loss figures (Ogilvey, 1984b) which suggest that very low levels of disease can cause significant yield loss (i.e. <5% of pod area infected).

CHAPTER 4 RELATIONSHIPS BETWEEN DIFFERENT MEASURES OF DISEASE

4.1 Introduction

As described in Part 2, Chapter 1 the method of assessment used for a particular pathosystem should be tailored to the needs of the experimenter. However in some cases even the most limited data can be time consuming to obtain. In oilseed rape this can clearly be the case especially when disease is at low levels on the leaf canopy (Part2, Chapter3). There is therefore a need to explore ways of reducing the work required to collect a particular data set. Several methods could be utilised. Relationships have frequently been found between different measures of disease one being easier and quicker to collect than the other. These relationships can be described as either incidence-severity or severity-severity relationships (Seem, 1984). In the former a measure of incidence, for example the number of leaves of a plant infected is related to the number of lesions on the plant or perhaps the area of the plant diseased. Incidence is not only a much quicker assessment to make but is generally also easier and more objective (e.g. James & Shih, 1973, Seem & Gilpatrick, 1980). Severity-severity relationships relate to measures of severity one of which is simpler and less time consuming to make than the other (Daamen, 1986). Another method of reducing the work required to obtain the relevant data, especially where the crop is complex is to select a particular portion of the plant, the disease on which can be related to the level of disease on the whole plant.

In this study it was found that detailed assessments of both the leaf and pod canopy of oilseed rape for dark leaf and pod spot were very time consuming taking up to one hour per plant. Although the detailed measurement of disease carried out in this study is clearly unnecessary in most work, there is potential for finding simpler and less time consuming ways of measuring the disease. In this chapter possible methods for reducing labour input for disease assessment are explored using incidence-severity, severity-severity relationships and the relationships between disease/on different parts of the plant.

4.2 Severity-Severity relationships

4.2.1 Introduction

Severity of disease can be measured in a variety of ways as discussed in Part 2 Chapter 1. In this study three measures of severity were assessed or calculated from leaf material (lesion numbers (TALLF), calculated diseased area (TCDA), and calculated % area diseased (TLFDIS)) and two measures on the pod canopy (lesion numbers/raceme (ALRAC) and % area diseased on each raceme (RACDIS)). On the leaf material both the calculation of TCDA and TLPOD require not only the visual assessment of the area of disease on each leaf but also the measurement of each leaf length. The latter two measures of disease are therefore much more time consuming to obtain than simply counting the number of lesions. Estimating some measure of diseased area from the number of lesions would therfore be an advantage.

On the pods the opposite is true in that the visual assessment of diseased area is much quicker than counting the total number of lesions on the raceme. However in studies of infection numbers of lesions can be important and so a method of estimating lesion numbers from area diseased could be useful.

4.2.2 Relationship between Lesion numbers, diseased area and TCDA

4.2.2.1 Methodology

Disease data from 1987 (leaves and pods) and 1988 (leaves) were used to obtain the variables:

Leaf Canopy

Number of lesions/plant (TALLF)

Number of lesions/leaf (ALLF)

Caculated diseased area/plant (TCDA)

% diseased area on each plant (TLFDIS)

Pod Canopy

Number of lesions/raceme (ALRAC)

% diseased areaon each raceme (RACDIS)

Variables for leaf material were also divided into data from the mainstem (MS) leaves and the branch (BR) leaves.

Variables from leaf tissue were transformed using log (x+1) and simple linear regression was used to investigate the relationship between;

a) TCDA v TALLF

b) TLFDIS v TALLF

MS and BR data were investigated separately as well as combined.

Pod data from 1987 were taken from Raceme T and the cumulative percentage frequency of lesion numbers for each percentage area class was plotted (Fig. 4.1).

4.2.2.2 Results

4.2.2.2.1 Leaf canopy

Results of the various regression analyses for the leaf data are given in Table 4.1. When the all the data were combined from MS and BR leaves and for all weeks in each year then the regression of TALLF on to TCDA gave an R^2 value of 80.8 and 55.3% for 1987 and 1988 respectively. Splitting the leaves into BR and MS showed that the relationship was better for BR leaves than MS leaves in both years. The slopes of the regression line (b) were not significantly different within or between years but the value of the intercept log (a+1) did differ significantly ($p=0.01$) between 1987 and 1988.

In the case of the relationship between TALLF and TLFDIS the relationship was poorer when data from all weeks were combined than TALLF v TCDA except

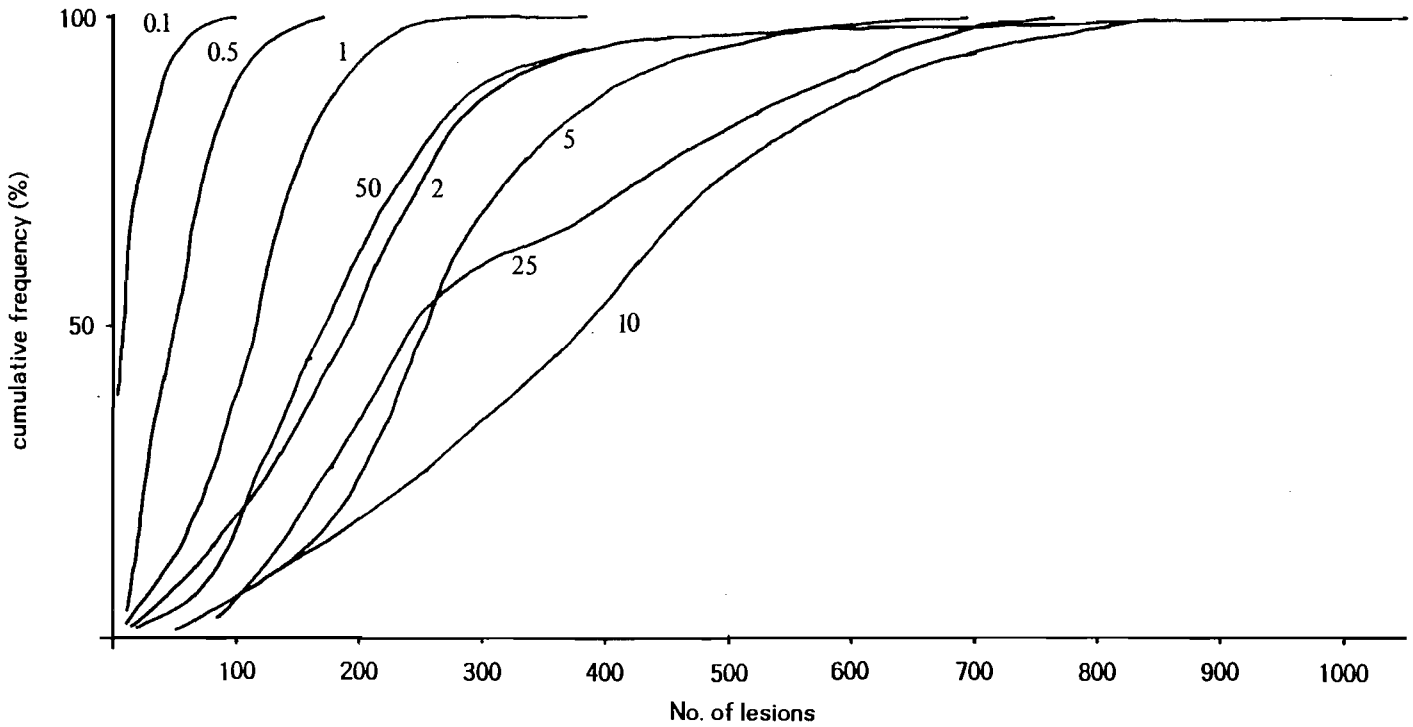


Fig 4.1: Cumulative percentage frequency of the number of lesions affected relating to specific percentage area

for BR leaves in 1988. In both years the fit of the regression was better for the BR leaves than the MS leaves.

4.2.2.2.2 Pod canopy.

Fig. 4.1 shows the relationship between ALRAC and RACDIS for individual racemes. For each estimate of diseased area (classes 0.1,0.5,1,2,5,10,25,and 50%) the percentage cumulative frequency curve is drawn against the number of lesions associated with the different areas. The graph clearly shows that there was a considerable range of lesion numbers for each area estimate and that generally upto 10% diseased area the numbers of lesions increased for area class. However the overlap of lesion numbers for different disease areas precludes the use of disease area as a prediction for lesion numbers.

As the diseased area increased above 10% the associated number of lesions declined such that the frequency distribution for 25 and 50% were similar to the those of 5 and 2% respectively, probably as a result of the merging of lesions on the pods.

4.3 Incidence- Severity relationships

4.3.1 Introduction

Perhaps an even more rapid and time saving method of disease assessment is the measurement of disease incidence, simply recording the proportion of leaves or pods affected by the disease. If this could be related to a measure of disease severity that is required then disease assessment would be considerably simplified.

As discussed in Part 3 Chapter 2 measurements of disease on the leaf tissue may be used as a prediction for further disease development rather than for predicting yield loss and it is suggested that the most appropriate measure would be

Table 4.1: Statistics for the regression of
log (x+1) TCDA on log (x+1) TALLF
log (x+1) TLFDIS on log (x+1) TALLF
Total of all plants in all weeks

	log (a+1)	b	R^2	n*
TCDA v TALLF				
Bienvenu 1987				
MS	-0.31	0.94	71.7	198
BR	-0.39	0.77	92.4	162
MS+BR	-0.33	0.82	80.8	261
Bienvenu 1988				
MS	0.00	0.65	53.7	203
BR	-0.16	0.70	67.7	130
MS+BR	-0.01	0.62	55.3	272
TLFDIS v TALLF				
Bienvenu 1987				
MS	-0.10	0.40	48.8	198
BR	-0.15	0.31	78.3	162
MS+BR	-0.15	0.34	58.9	261
Bienvenu 1988				
MS	0.03	0.23	37.1	203
BR	-0.15	0.35	68.0	130
MS+BR	0.00	0.26	50.1	272

* number of data points

the number of *A. brassicae* lesions on the leaf canopy. Counting the number of lesions on the leaf canopy can be time consuming especially later in the season when numbers increase. An estimation of lesion numbers from a count of the number of leaves infected could therefore be useful.

On the pod canopy diseased area is relatively easy to assess visually. However, counting the number of lesions present can be very time consuming and therefore a

Table 4.2: Relationship between number of leaves infected (incidence) and number of lesions/leaf (severity)

	log (a+1)	b	R^2	n*
Mikado 1986	0.68	0.73	59.6	192
Rafal 1986	0.60	0.72	28.3	115
Bienvenu 1987	0.46	1.05	81.1	215
Bienvenu 1988	0.69	1.16	82.7	171
Bienvenu 1987+1988	0.49	1.32	84.9	387

* n = no. of data points

method of estimating lesion numbers from the proportion of pods infected could also be very useful. In the following sections both these options will be explored.

4.3.2 Leaf canopy

4.3.2.1 Methodology

Simple regressiom analyses were used to assess the relationship between the proportion of leaves infected/plant (LFINF) and TALLF. As the number of lesions on a plant is related to the number of leaves on that plant the parameter for the number of lesions was taken as the mean number of lesions per leaf i.e. number of lesions/number of leaves (ALLF). After fitting Taylor 's Power Law to this variable the estimated transformation for the data was $x^{0.15}$. As with other variables which include lesion counts an approximate transformation is log (x+1) which also provides other benefits as discussed in Part 2, Chapter 3. The percentage of leaves infected ranged from <0.001 to 100 and therefore the transformation most likely to give an

adequate result was arcsin √(proportion of leaves infected) (Sokal and Rolfe, 1981). The regression analyses were therefore carried out on log (TALLF + 1) v arcsin √(LFINF). Relationships were investigated for Mikado (1986), Rafal (1986) and Bienvenu (1987 & 1988).

4.3.2.2 Results

Results of the regression analyses (Table 4.2) showed that in 1986 the relationship between proportion of leaves infected and lesion numbers was poor for both varieties. In 1987 and 1988 however approximately 80% of the variation was accounted for in the relationship by linear regression. When data from the two years were combined almost 85% of the variance was accounted for. There was no significant difference between b or log (a+1) for the 1987 and 1988 data. Comparisons with the 1986 data were not carried out because of the poor incidence - severity relationship.

4.3.3 Pod canopy

4.3.3.1 Methodology

Simple regression analysis was used to investigate the relationship between PODINF and ALRAC. Both sets of data were transformed before the regressions were carried out, the proportion of pods infected using arcsin √PODINF (Sokal & Rolfe, 1981) and log (ALRAC+1). Data from Racemes T and 3 from 1987 and 1988 were used in the analysis.

4.3.3.2 Results

The results of the regression analysis are given in Table 4.3. The variance accounted for by the regessions was between 80 and 90% except in the case of Raceme T in 1988 where the relationship was poorer (R^2=67.4%). Values of log

Table 4.3: Relationship between number of pods infected (incidence) and number of lesions/raceme (severity on the pod canopy

Year	Raceme	log (a+1)	b	R^2
1987	T	0.41	0.99	89.5
	3	0.45	0.97	84.3
1988	T	0.48	0.90	67.4
	3	0.45	0.97	80.2
1987+1988	T	0.46	0.95	81.9
	3	0.45	0.97	84.0

(a+1) and b were very similar in all the analyses differing by a maximum of only 0.07 and 0.09 respectively.

4.4 Methods for selective assessment

4.4.1 Introduction

Assessment of selective parts of the plant for disease is widely used in plant pathology. In oilseed rape a good example is the assessment key for dark pod spot published by NIAB (Anon, 1988) which suggests that only the terminal raceme need be assessed to give an adequate representation of disease on the whole plant. If selected plant parts are used then obviously there is a concommitant reduction in the time required for assessment. It is however essential that the relationship between the disease on that part and the whole plant is explored and in this section the relationship between different racemes and disease on the whole pod canopy is described.

4.4.2 Methodology

Data from the pod canopy of Bienvenu (1987) were combined to give values for the number of pods (TPODNUM), the number of pods infected (TPODINF), the number of *A. brassicae* lesions (TALPOD) and the % diseased area (PODDIS) on the complete pod canopy. For the first three variables numbers were summed over all the racemes on the plant and meaned over the sample to give a mean total value for the parameters. For % diseased area the simplest method of combining the data from all the racemes from each plant would be:

$$\text{PODDIS} = \frac{\Sigma\, \text{RACDIS}_i}{n}$$

where n is the number of racemes. However this assumes that each raceme contributes equally to the total area of that raceme. To take account of the different contributions to the total area by each raceme the number of pods on the raceme was used to weight each value for % disease. So that

$$\text{PODDIS} = \frac{\Sigma\, (\text{RACDIS} \times \text{PODNUM})_i}{\text{TPODNUM}}$$

The data from each plant in every week were then used to explore the relationships between the variables on individual racemes and those on the whole plant using simple linear regression. All variables were transformed using log (x+1) before analysis.

4.4.3 Results

Table 4.4 gives the results of the regression analyses for the relationships between data from individual racemes and the whole plant. For the number of pods the relationship between individual racemes and the whole plant was poor, Raceme 1 showing the best predictive ability still only having an R^2 value of 60%.

Table 4.4: Relationships between various parameters for different individual racemes and the total of all racemes

Variable	Raceme	log (a+1)	b	R^2	maximum value for variable
PODNUM v	T	0.41	1.12	34.3	33
TPODNUM	1	0.92	0.95	60.1	20
	2	1.01	0.85	57.1	25
	3	1.15	0.74	49.5	22
	4	1.01	0.87	57.1	23
	5	1.38	0.63	45.4	23
	6	1.77	0.39	33.0	23
	7	1.79	0.41	45.8	29
	8	1.78	0.44	57.6	24
PODINF v	T	0.11	1.29	83.2	33
TPODINF	1	0.65	1.15	91.1	20
	2	0.54	1.20	90.3	25
	3	0.74	1.05	88.1	22
	4	0.94	0.96	89.3	23
	5	0.93	0.97	89.5	23
	6	1.04	0.90	90.2	23
	7	1.39	0.68	85.8	29
	8	1.08	0.96	94.9	24
ALRAC v	T	0.22	1.13	90.9	270
TALPOD	1	0.82	1.03	90.6	165
	2	0.70	1.07	87.2	192
	3	0.78	1.03	90.5	173
	4	0.94	0.96	89.7	195
	5	1.03	0.95	89.1	206
	6	1.14	0.91	90.7	230
	7	1.42	0.83	85.9	226
	8	1.13	0.98	91.0	220
RACDIS v	T	-0.05	0.89	99.5	40.3
PODDIS	1	-0.02	0.88	99.7	40.9
	2	-0.02	0.85	99.1	40.1
	3	-0.03	0.91	99.4	33.5
	4	-0.02	0.91	99.6	34.3
	5	0.00	0.95	100	30.2
	6	-0.04	1.07	98.1	18.1
	7	0.00	1.02	98.7	19.6
	8	0.03	0.98	99.6	27.2

For the three disease parameters the relationships were much stronger. The % variance accounted for being close to 90% for the PODINF and ALRAC and 98 - 100% for RACDIS.

The linear nature of the relationships developed here showed that the absolute relationships between these parameters at different mean values were non-linear. The changing values of b, and a in the case of number of pods infected and number of

A. brassicae lesions describe these changing relationships between the individual racemes and disease on the whole plant.

4.5 Discussion.

The data presented here show that simple relationships between different measures of severity give little opportunity for predicting TCDA or TLFDIS from TALLF. For the predictive equations to be of use they would have to account for >90% of the variation of the dependent variable (Mann, Pers. comm.) and not vary from year to year or between weeks. Only data from BR leaves gave reasonably high R^2 values (67.4 -92.4) but is still not adequate for use as a predictive equation. Severity-severity relationships have proved particularly successful where individual lesions or pustules remain small and of a similar size (e.g. Daamen,1986; *Erysiphe graminis* on wheat) the relationships breaking down only when the pustules begin to coalesce. With dark leaf spot where the areas of lesions can differ considerably relationships between total area of lesions and number of lesions would be expected to be poor.

On the pods where lesions tend to be similar in size and remain isolated for much of the early development of the disease on the pod canopy a better relationship between diseased area and lesion number might be expected. The data described in Fig 4.1 showed clearly however that no such relationship exists. This is undoubtedly because of the visual assessment system used to estimate diseased area. A visual assessment system relies on the observer being able to distinguish between different levels of disease using the disease key as a guide. By interpolating the key, the observer can assess levels of disease between those on the key but because of the difficulties of this type of assessment only one or two extra levels are usually added. Each assessment of area is therefore already covering a range of true diseased areas. This has a significant effect on the relationship between estimated disease area and the number of lesions. Furthermore lesions are very small in relation to the total area of the canopy and an estimate inaccurate by only 1% may include or exclude a large number of lesions. The relationship between diseased area and lesion numbers could therefore probably be improved if a better estimate or an absolute measure of

diseased area could be used. Although this relationship would have little practical use for estimating the number of lesions on a raceme from the diseased area as it would probably take longer to measure the diseased area than to count the lesions, in detailed work on the pod canopy such a relationship could allow a better estimate of diseased area from counting lesions.

The incidence-severity relationships described here show that measures of incidence are in some cases good predictors of severity although generalisations are difficult to make because of the small data set. On leaf tissue the two years' data from cv. Bienvenu suggest that a consistent relationship between LNINF and TALLF may be obtainable and that year has little effect on the relationship. This is in contrast to Seem & Gilpatrick (1980) who showed that for apple powdery mildew the I-S relationship differed between years. The results from 1986 are difficult to interpret because it cannot be determined whether year or cultivar was responsible for the poor relationship but it does show that the relationship found for cv. Bienvenu in 1987 and 1988 is not universally applicable and that further detailed study is required. With the pod canopy the I-S relationships again show potential for use in the field situation although the poorer relationship with the data from Raceme T, 1988 shows that further study is required to confirm these findings.

The relationships between disease data from individual racemes and the total measures of disease on the whole plant suggest that any raceme can be used to estimate disease on the whole plant. However the relationships are not always linear as has been assumed in assessment techniques in the past (Anon, 1988). With lesion numbers and number of pods infected the increasing values of a from Raceme T to Raceme 8 is a consequence of the earlier development of lesions on the older racemes and this is compensated for by a reducing value of b from Raceme T to Raceme 8. This indicates that when there are few lesions on the plant (e.g. early in the season) counting the number of lesions on Raceme T will tend to overestimate the amount of disease present whereas when there are higher levels of disease on the plant, counting the number of lesions on Raceme T will underestimate the total number of lesions on the pod canopy. In contrast with % diseased area the assessment of the terminal raceme will always over estimate the amount of disease

on the whole plant. Clearly these data are very limited and a considerable amount of further work is required before any generalisations can be made about the results described here.

PART 3 DESCRIPTION OF PLANT AND DISEASE DEVELOPMENT

CHAPTER 1 INTRODUCTION

Any pathosystem is a dynamic relationship between the host plant and the pathogen causing the disease. During a disease epidemic both increases and decreases in the level of disease may occur but the system is never static.

In the study of pathosystems one of the important initial areas of investigation is disease progress, a description of the way in which the level of disease changes with time. Kranz (1974) describes a progress curve as "the graph of an epidemic " and it is in their most simple graphical form that disease progress curves (DPCs) allow a visualisation of disease progress: they describe the overall pattern of disease development. Many authors (e.g. Shenoi & Ramalingam,1983; Plaut & Berger, 1980) have used this method and frequently, comparisons of disease epidemics on different host varietes, under different fungicide treatments etc. have been made by simple visual comparisons of DPCs. Recently for example, Royle *et al.* (1986) described two patterns of leaf blotch epidemics in wheat caused by *Septoria nodorum.*

As shown by Royle *et al.* (1986) perhaps the primary reason for examining DPCs is to formulate questions about disease development. In the case of their data, "why two different patterns of disease progress? " Waggoner (1986) puts it concisely when he states "since it is evident that cause precedes effect, we make a series of observations over a period of time and ask not only what level has disease attained but what level it came from. Only when we have "before " and "after " can we reason about causes ". So before starting to compute we should examine disease progress using simple diagrams (Bliss,1970).

Simple diagrams provide the basic information necessary for the comparison of disease epidemics. They show whether disease increases or decreases over a particular time period; they show whether disease increases gradually over the season or whether there is explosive development. They give the investigator a "feel " for

how the disease progresses. Simple diagrams, however can provide only somewhat limited information on disease progress. For example they are general, in that they are often used to describe the progress of global measures of disease e.g. proportion of plant diseased or % of plants infected.

Developments of these simple diagrams have been made by a number of authors. For example Plaut & Berger (1980) divided peanut foliage into different levels by height and estimated the intensity of *Cercosporidium* leaf spot disease at each canopy level. Diagrams of disease v time were then used to describe disease progress in those layers. Frinking & Linders (1986) used 3-D diagrams for the description of powdery mildew on radishes, extending the information content of DPCs still further.

Another refinement of the simple graphical representation of DPCs is their mathematical analysis. Although mathematical models describing DPCs had been proposed previously (Large,1952), Van der Plank is generally considered to be the "father " of the analysis of DPCs. Taking several examples from the literature, Van der Plank (1963) used the logistic model (and variations of it) to describe a variety of DPCs from different pathosystems. By introducing factors such as latent period and removal of sporulating tissue he explored theoretically and empirically various rate parameters which were predicted by the use of the logistic equation. Linearising epidemics using transformations such as the logistic equation allows the formal comparison of DPCs using methods of regression and consequent comparisons of the regression coefficients. Such comparisons will be discussed in more detail in Chapter 3 of this Part.

Since 1963 many authors have described epidemics using the logistic model and the apparent infection rate, r, (see Part 3, Chapter 3) has become a universal measure of epidemics even in situations where it may not be truly valid (see Part 3, Chapter 2). Other models have also been used for example the Gompertz model (e.g. Waggoner,1986) and the Richards model (Richards,1959) which fit DPCs with skewed curves where either disease is at very low levels for a long period and then increases

rapidly (Richards model) or increases rapidly and then remains at high levels (Gompertz and Richards models).

All these additional methods add to the value of DPCs by refining the "why" questions that can be asked after analysis. In the case of sub-dividing the disease progress into various levels of crop canopy, questions can be asked of the difference in progress between those levels. The mathematical modelling on the other hand allows formalised comparisons of the curves to be made and to ask why, for a particular pathosystem, the curves differ or do not differ from year to year, or why curves change their skewness or even fit one model in one year and another in another year.

Any discussion of the development of disease on a plant must of necessity include descriptions of plant development. Many authors appear to disregard the plant (e.g. Shenoi & Ramalingam, 1983), especially where a simple mathematical analysis of DPCs is made (see Part 3,Chapter 3). Disease progress can be substantially affected by plant development. Adult plant resistance in cereals may slow disease progress (Kranz & Hau, 1980); leaf drop may quickly reduce disease inoculum levels and hence slow disease progress (Large, 1966) and increased susceptibility of ageing plant material to the pathogen may cause an explosive increase in disease at the end of the season. The effective interpretation of DPCs should therefore include some consideration of plant development.

In the following two chapters of Part 3 the progress of dark leaf and pod spot on the leaf canopy (Chapter 2) and pod canopy (Chapter 3) are described. Disease progress on the leaf canopy is shown to follow patterns that cannot easily be analysed mathematically and distribution within the canopy is described using 3-D graphics. On the pod canopy four epidemics are described using the logistic model and disease progress on different racemes is compared. The Gompertz and Richards models are compared with the logistic model as descriptors of the DPCs. The DPCs are then related to plant growth and development and simple hypotheses are proposed to explain the patterns of disease development.

Details of field trials and data manipulation are given in Part 1, Chapter 2. For graphical representation data are presented without transformation as this allows easier interpretation of the visual image. Where data are transformed for further numerical analysis this is discussed in the relevant section.

CHAPTER 2 LEAF CANOPY

2.1 Plant development

2.1.1 Rafal 16 January-26 June 1986

Plant development during the winter and early spring was slow. The mean number of leaves/plant (LNN) remained at approximately five (Fig 2.1) from 16 January to 7 February. Heavy snow and hard frosts during the period 14 February to 28 February made sampling impossible and severely damaged the plants. Leaves suffered frost damage which led to wilting and loss from the plant. After the cold weather, pigeon damage was severe leaving only the apex intact on many plants. Reliable assessments were not possible again until 3 April.

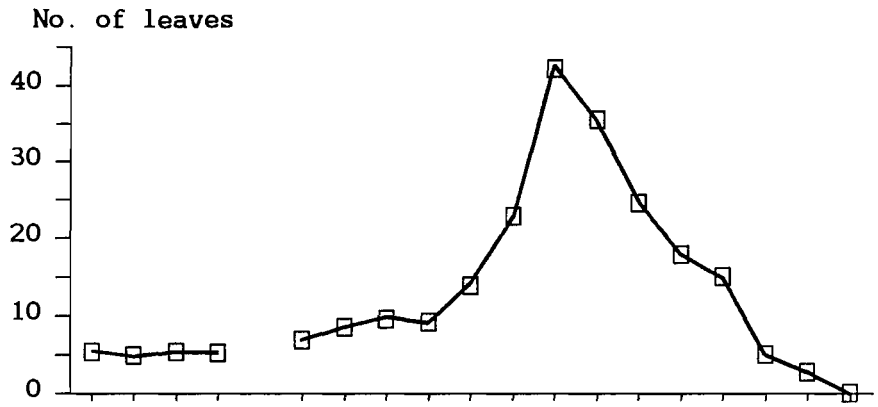
On 3 April the number of mainstem leaves lost had risen from 5 to 8 (Fig 2.2), showing the effect of the cold weather. The mainstem leaves/plant had also increased to 7 (Fig 2.3). From 3 April until 24 April leaf number increased only slightly (c.1 leaf/plant). Between 1 May and 15 May leaf numbers increased substantially, a rise due entirely to the development of leaves on primary branches. During this period stem extension occurred and there was a corresponding increase in plant height and almost no leaves were lost.

Between 15 May and 22 May there was a loss of main stem (MS) leaves from the base of the plant together with the loss of some branch (BR) leaves (Fig 2.3). From 22 May until 26 June leaf loss followed an approximately linear pattern. The rates of loss were 1.05 and 3.84 leaves/plant/week from the MS and BR leaves respectively. By 3 July no leaves remained on the crop.

2.1.2 Mikado 16 January-26 June 1986

As in Rafal, plant development during the winter and early spring was slow. LNN remained at approximately 5 until the severe weather in the middle of February (Fig 2.1).

a)



b)

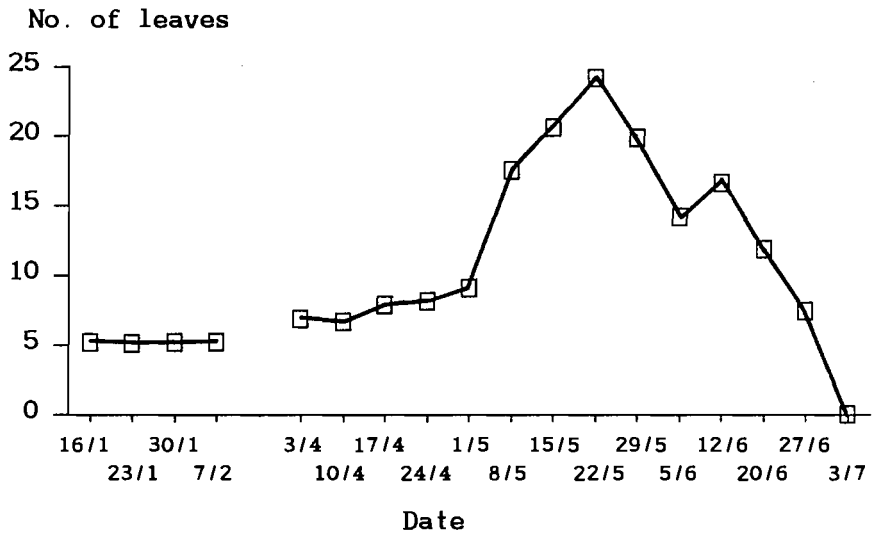


Fig 2.1: Number of leaves on Rafal and Mikado, 1986

a) Rafal
b) Mikado

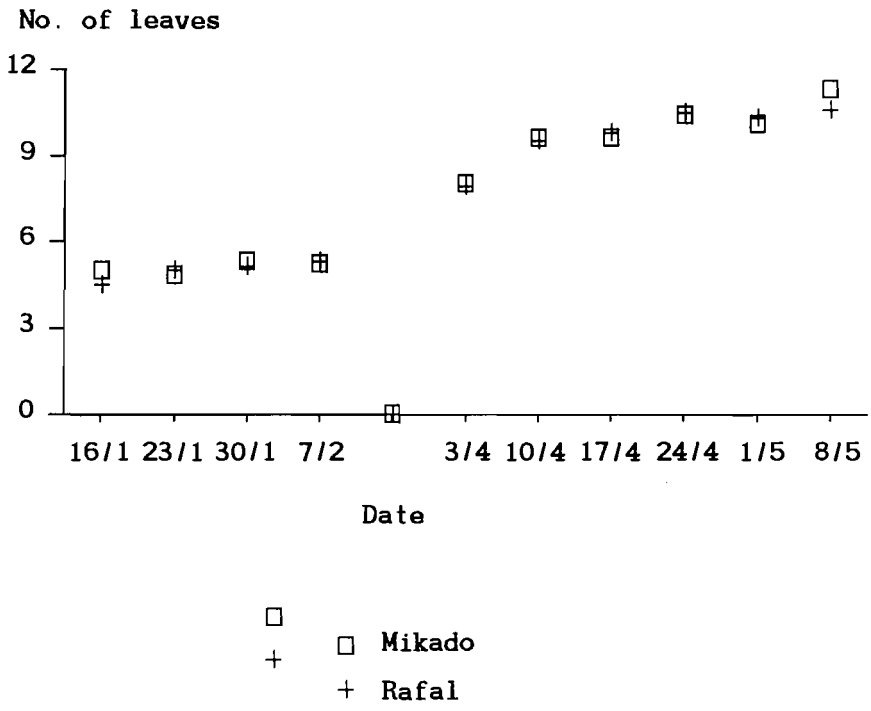
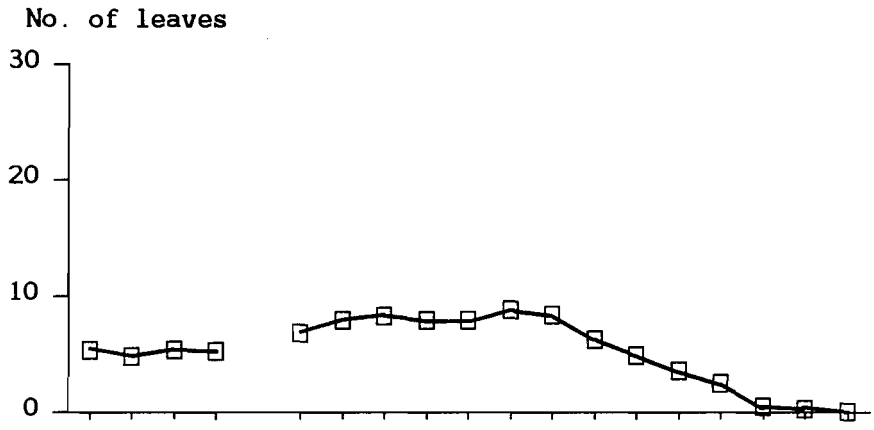


Fig 2.2: Number of leaves lost from the mainstem Rafal and Mikado, 1986

a)



b)

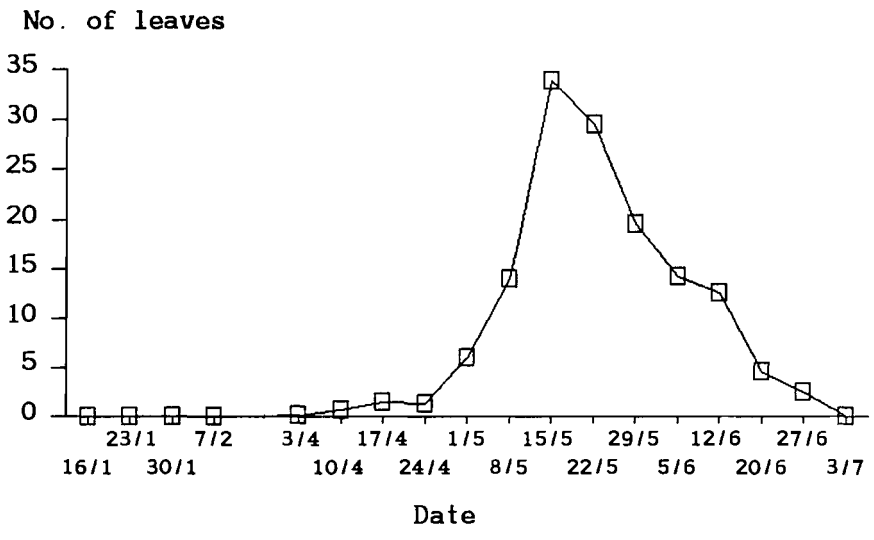


Fig 2.3: Number of leaves on the main stem and branches cv. Rafal, 1986

a) Main stem
b) Branch

Similarly after the cold weather the number of mainstem leaves lost and the mean number of mainstem leaves/plant remained approximately constant on Mikado at 5 and 7 respectively (Fig 2.1 & 2.2). From 3 April until 1 May, leaf numbers increased only slightly (c.1 leaf/plant). Between 1 May and 22 May there was a substantial increase in the mean number of leaves/plant which reached a maximum of 25, less than on cv. Rafal. As in Rafal, virtually no leaves were lost during this period.

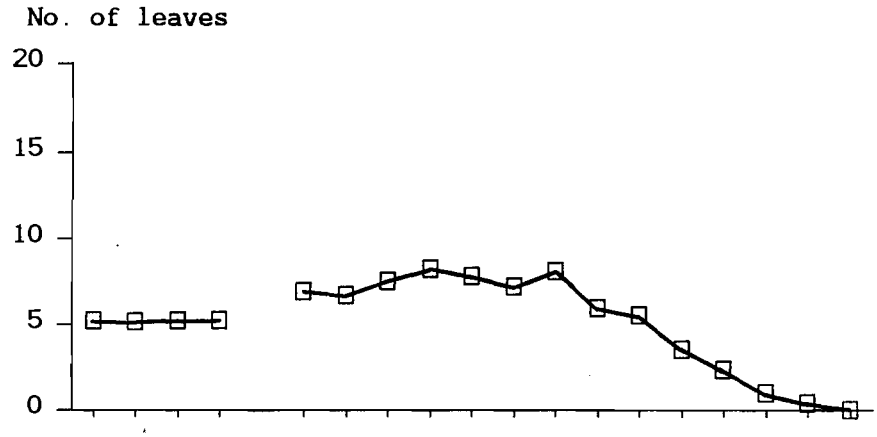
On Mikado leaf loss from the main stems began between 15 May and 22 May and during the following week on the branches (Fig. 2.4). The initial decline in leaves from the main stem was not as great as that in Rafal and a steady leaf loss occurred (0.98 leaves/plant/week) until 26 June. Leaf numbers on the branches began to decline between 22 May and 29 May and this continued until 3 July with a mean weekly leaf loss of 2.26 leaves/plant/week. Leaf loss from Mikado was not as rapid as that from Rafal. No leaves remained on Mikado by 3 July.

2.1.3 Bienvenu 4 May-15 June 1987

Compared with 1986, data collection from the trial in 1987 started late in the development of the crop. Leaf numbers on the main stem were already begining to decline on 4 May and continued to fall until the 22 June when no leaves were found on the plants (Fig. 2.5). The loss of leaves from the mainstem showed a linear pattern with a rate of loss of 1.3 leaves/plant/week. From 4 may until 25 May the numbers of branch leaves increased from 16.9 to 21.3 leaves/plant and then began declining until no leaves were present on 22 June (Fig. 2.5). The rate of loss was again approximately linear with a rate of 5.3 leaves/plant/week.

LNN varied only slightly between 8 May and 25 May (range: 25.6-27.3 leaves/plant) after which date a decline in leaf numbers began. This then continued until 22 June (Fig. 2.6). Total calculated leaf area/plant (TCLA) (see Part 2, Chapter 1) showed a very similar pattern (Fig. 2.6) of development with a decline in TCLA being approximately linearly between 25 May and 22 June.

a)



b)

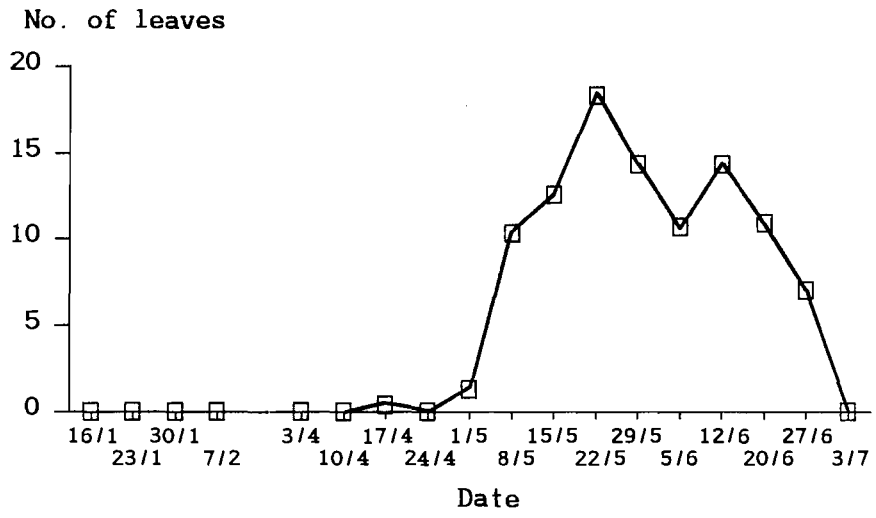
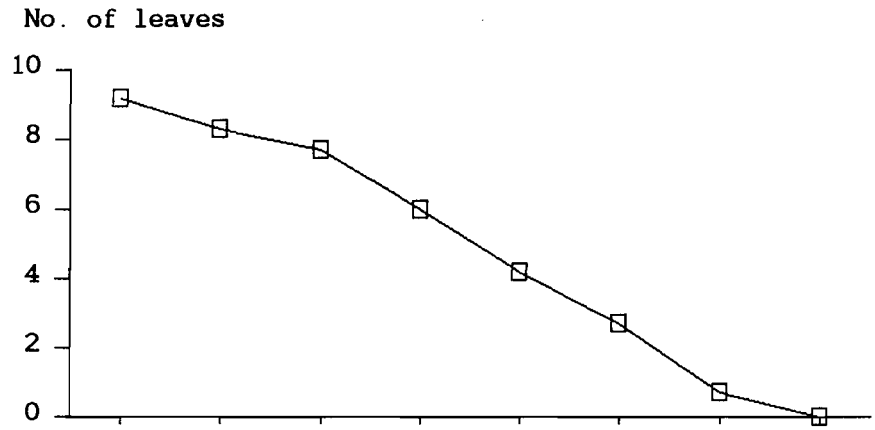


Fig 2.4: Number of leaves on the main stem and branches cv. Mikado, 1986

a) Main stem
b) Branch

a)



b)

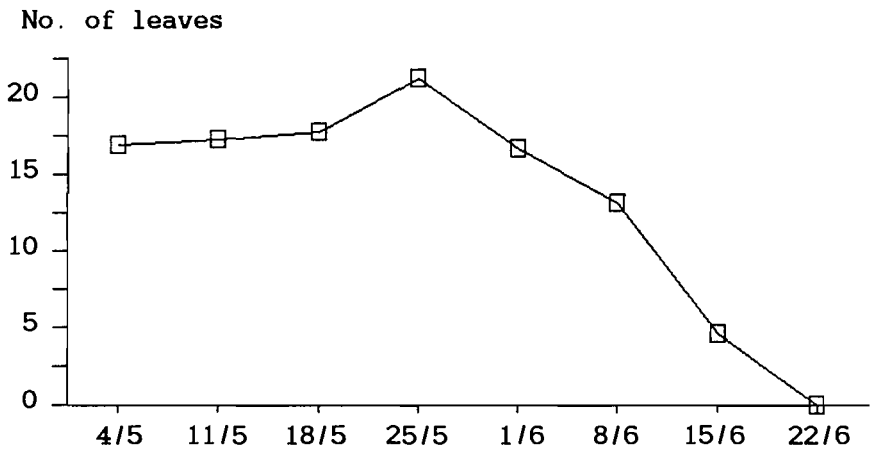
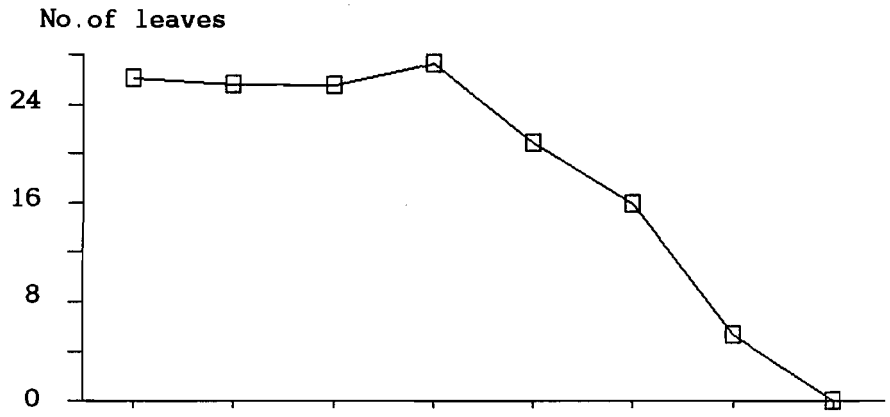


Fig 2.5: Number of leaves on the main stem and branches cv. Bienvenu, 1987

a)Main stem
b) Branch

a)



b)

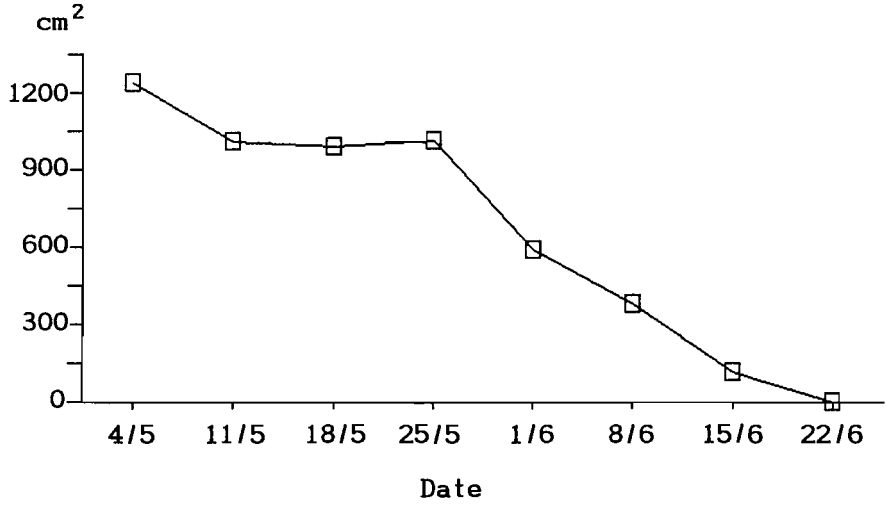


Fig 2.6: Leaf development; cv. Bienvenu, 1987

a) LNN*
b) TCLA*

*see text for explanation

The pattern of change of TCLA on MS and BR was also similar to that described for leaf numbers (Fig. 2.7). As with leaf numbers, the area of MS leaves declined approximately linearly over the period 4 May to 22 June (rate: 94.2cm^2/plant/week) and BR leaves from 25 May to 22 June (rate: 163.6cm^2/plant/week). A divergence from this pattern occurred between 4 and 11 May when the number of branch leaves appear to increase slightly (0.4 leaves/plant) whereas the area of branch leaves decreased substantially (172.5cm^2/plant). Between 11 May and 25 May branch leaf areas and leaf numbers both appeared to increase.

The proportion of the TCLA provided by the MS leaves changed during the period of the study (Table 2.1). At 53.3-59.9% over the first three weeks of the study, this declined to 10.8% by 15 June. These proportions are higher than those of the number of leaves except on 15 June (Table 2.1) when the proportion of leaves on the MS was 13.0% and the proportion of leaf area on the MS was 10.8%.

Table 2.1 Percentage of total number of leaves/plant and TCLA occurring on the mainstem

Bienvenu 1987			Bienvenu 1988		
Date	TCLA	LNN	Date	TCLA	LNN
			4 Apr	87.4	46.4
			11 Apr	77.6	40.1
			18 Apr	68.9	35.9
			25 Apr	65.5	34.0
4 May	53.3	35.3	2 May	54.8	28.9
11 May	59.9	32.4	9 May	45.3	25.4
18 May	55.8	30.2	16 May	40.9	25.8
25 May	35.7	22.1	23 May	26.7	20.8
1 Jun	27.1	20.0	30 May	11.3	13.8
8 Jun	21.4	16.9	6 Jun	9.1	8.2
15 Jun	10.8	13.0	13 Jun	8.2	7.6

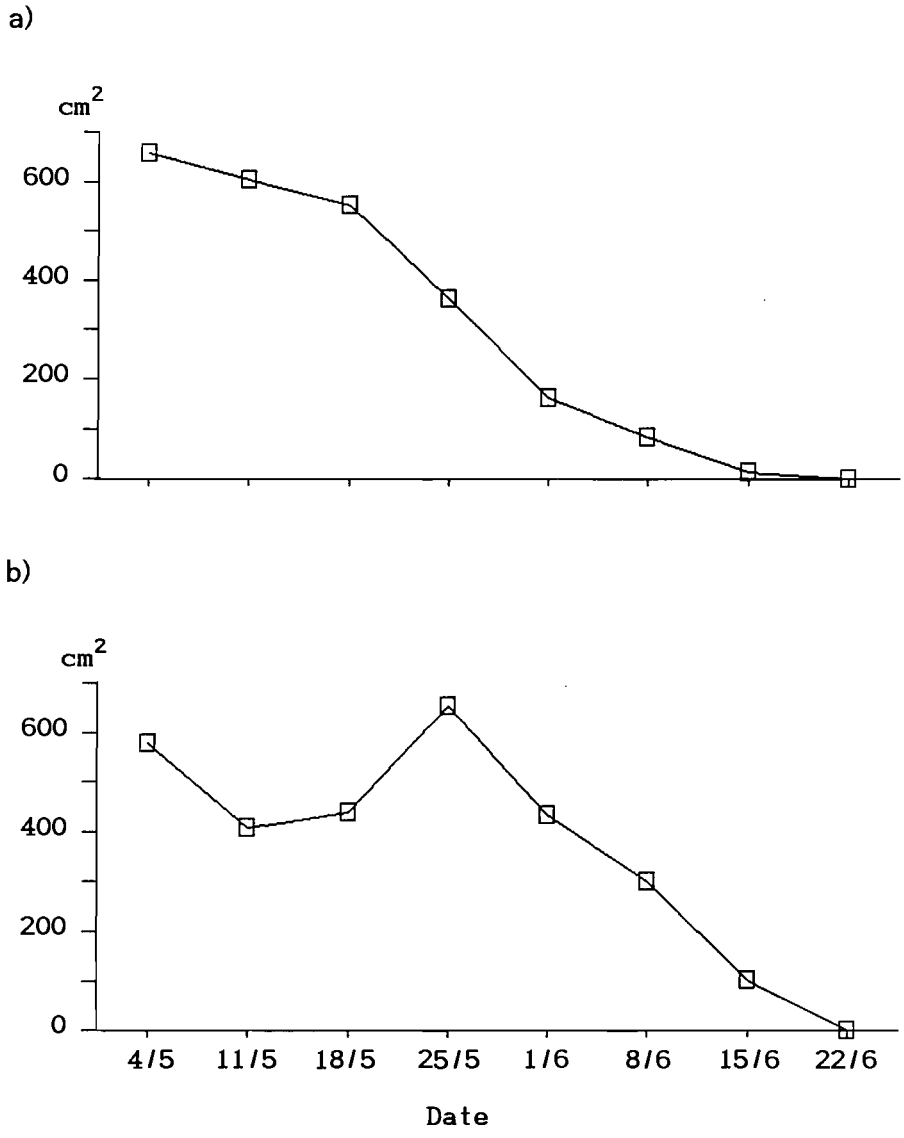


Fig 2.7: TCLA* on the main stem and branches
cv. Bienvenu, 1987

a) Main stem
b) Branch

* see text for explanation

2.1.4 Bienvenu 4 April-13 June 1988

In 1988 data capture started earlier in the season than in 1987 and LNN remained approximately constant for six weeks until the 9 May (Fig. 2.8). After this date leaf numbers declined steadily so that by 27 June no leaves were left on the plant. TCLA showed a different pattern from that observed in 1987 with the decline in TCLA beginning between the 2 May and 9 May and then continuing almost linearly until 27 June (Fig. 2.8).

Loss of main stem leaves commenced as early as 4 April and loss continued approximately linearly at a rate of 1.18 leaves/plant/week until 20 June by which date all MS leaves had been lost (Fig. 2.9). The numbers of BR leaves increased between the first and second recording and then remained relatively constant until 23 May. From this date until 27 June an approximately linear decline of the number of leaves occurred (rate: 3.24 leaves/plant/week). On the main stem TCLA showed a similar progress pattern to leaf numbers, TCLA declining steadily between 4 April and 27 June at a rate of 90.2cm^2/plant/week (Fig 2.10). On the branches however leaf area did not reach a peak until 2 May and began declining again after 9 May. The decline was approximately linear with a rate of loss of 70.8cm^2/plant/week. The maximum TCLA was measured on 4 April at 1031.9cm^2/plant. The proportion of the total number of leaves found on the MS declined during the period of the study from 46.4% to 8.2% and similarly the proportion of total leaf area found on the MS declined from 87.4% to 7.7% (Table 2.1).

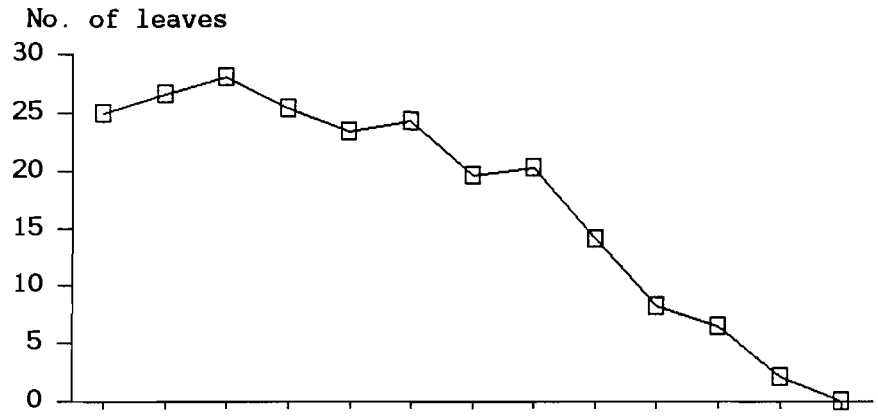
2.2 Disease development

2.2.1 General description of the development of dark leaf spot

2.2.1.1 Rafal 16 January-26 June 1986

Levels of dark leaf spot caused by *A. brassicae* remained low throughout the season. The maximum occured on 15 May when the mean number of lesions/plant

a)



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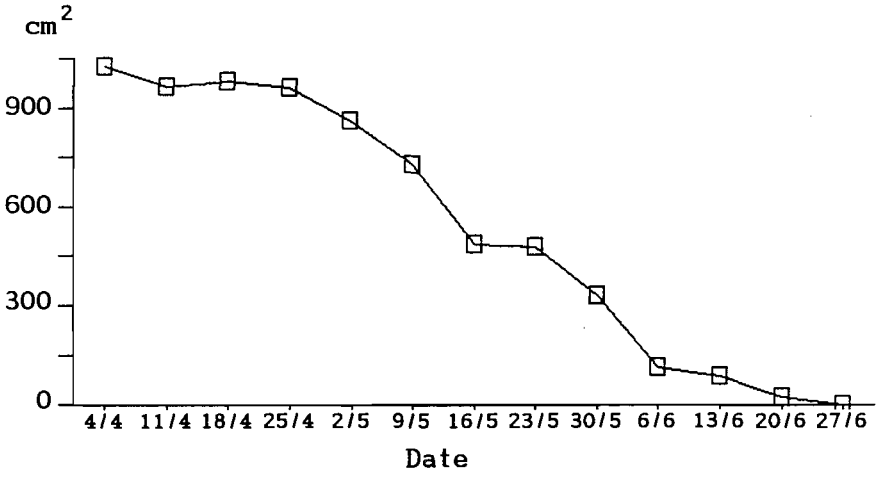
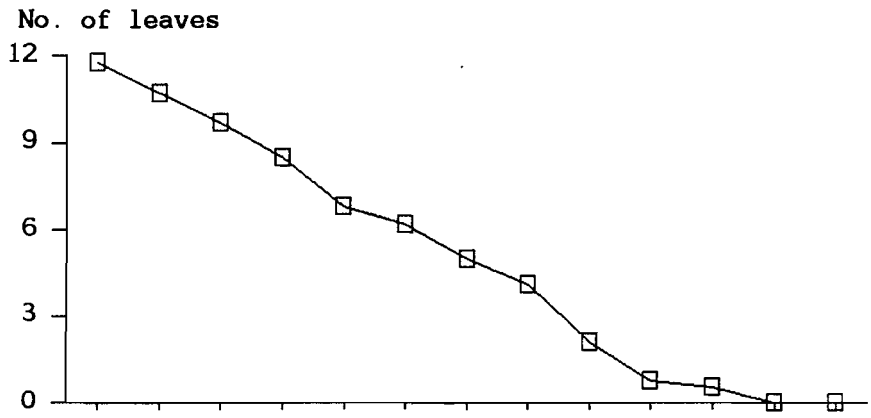


Fig 2.8: Leaf development; cv. Bienvenu, 1988

a) LNN*
b) TCLA*

* see text for explanation

a)



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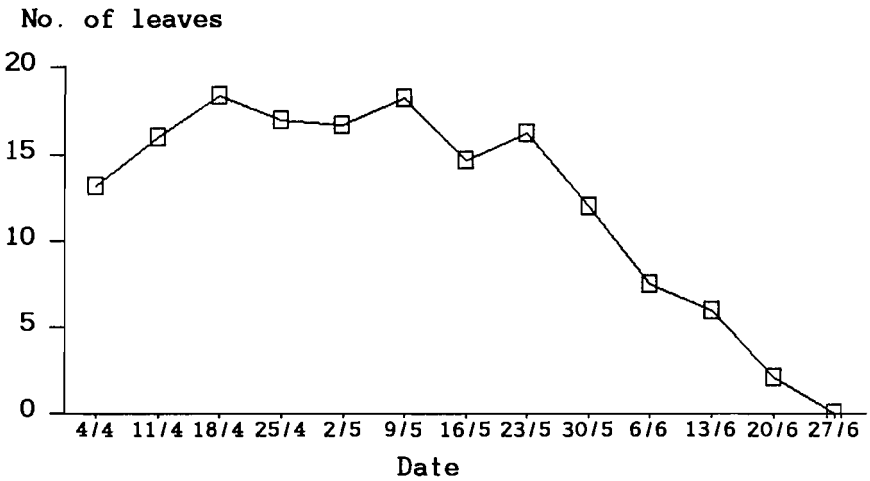
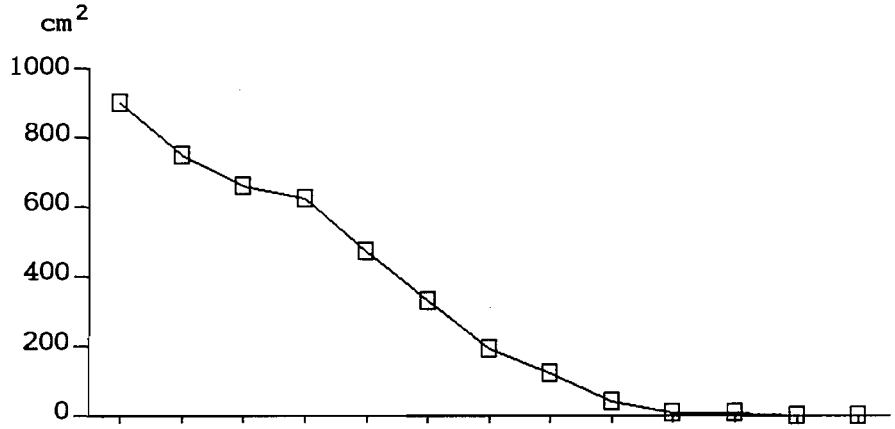


Fig 2.9: Number of leaves on the main stem and branches cv. Bienvenu,1988

a) Main stem
b) Branch

a)



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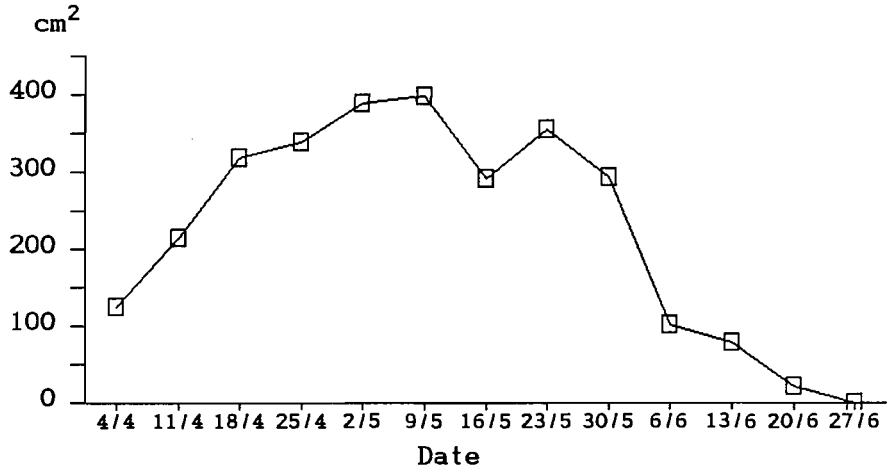


Fig 2.10: TCLA* on the main stem and branches
cv. Bienvenu, 1988

a)Main stem
b) Branch

*see text for explanation

(TALLF) reached 28 (Fig 2.11) and the mean number of lesions/leaf was 0.82.

During the winter and early spring levels of leaf spot remained almost constant at 2-3 lesions/plant until the middle of April. An increase in disease began after 17 April and TALLF reached a peak on 15 May. This peak was immediately followed by a sharp decline in lesion numbers so that on 22 May the mean number of lesions/plant had dropped to 7. There was then a more gentle decrease in the level of disease until 26 June.

2.2.1.2 Mikado 19 January - 26 June 1986

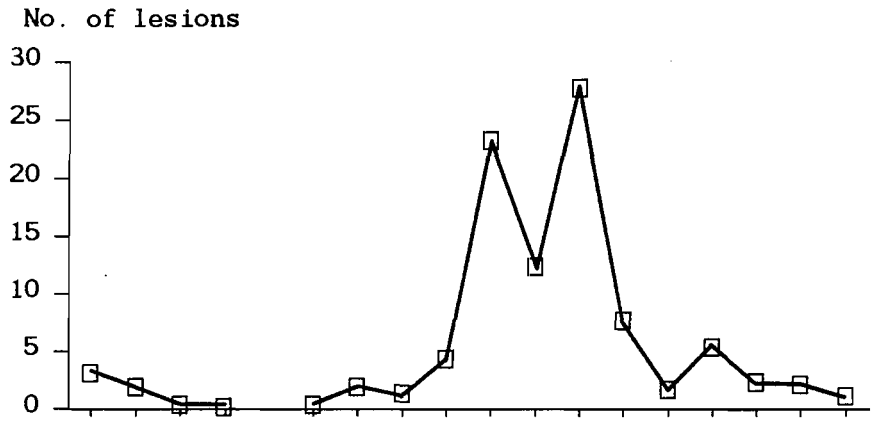
As in Rafal, leaf spot disease in Mikado reached only low levels (Fig 2.11). At the maximum on 20 June there was a mean of 24.8 lesions/plant (2.35 lesions/leaf). During the winter the mean number of lesions/plant remained at 2-3 until the cold weather in February. By 3 April only 10% of plants were infected. The number of lesions then gradually increased until 17 April (2 lesions/plant) when a more rapid increase of disease began. Mean numbers of lesions/plant rose to a peak of 17.9 on 22 May. An apparent decline of the disease then occurred but was followed by another rise to give a maximum 30.4 lesions/plant on 20 June. This second peak of dark leaf spot was in contrast to Rafal where disease levels declined from the peak on 15 May until 26 June.

2.2.1.3 Bienvenu 4 May-15 June 1987

Much higher levels of dark leaf spot were found in 1987 than in either 1986 or 1988. TALLF reached a maximum of 220.2 lesions/plant on 1 June corresponding to a calculated diseased area (TCDA) of 44cm^2/plant (Fig.2.12).

Early season data were not collected in 1987 and on the first sampling date TALLF had already reached 24.4 (25.1cm^2 TCDA/plant). There was a steady increase in disease until 25 May when TALLF was 88.1. Between 25 May and 1 June TALLF increased by a factor of 2.5 to 220.2. The comparable increase in TCDA was from 30.5-44cm^2/plant. There was a slight decline in TALLF in the next week

a)



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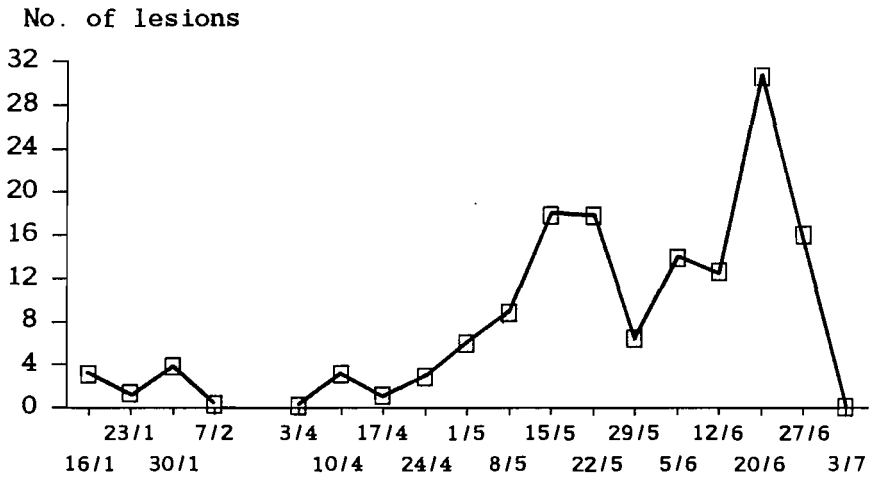
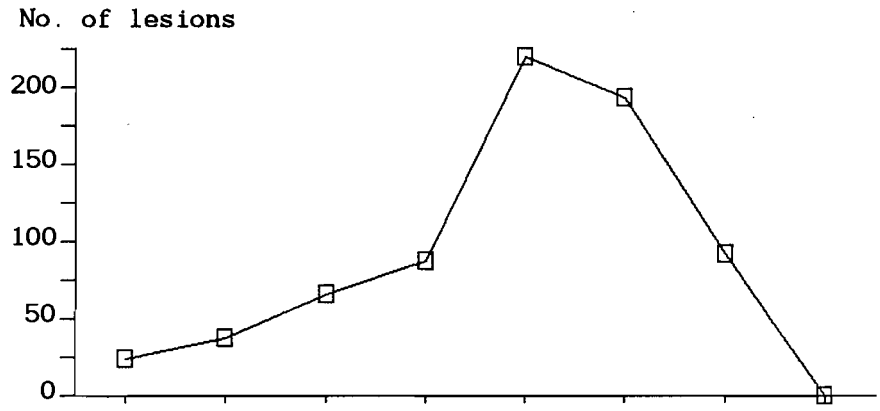


Fig 2.11: Disease development (TALLF*) on Rafal and Mikado, 1986

a) Rafal
b) Mikado

*see text for explanation

a)



b)

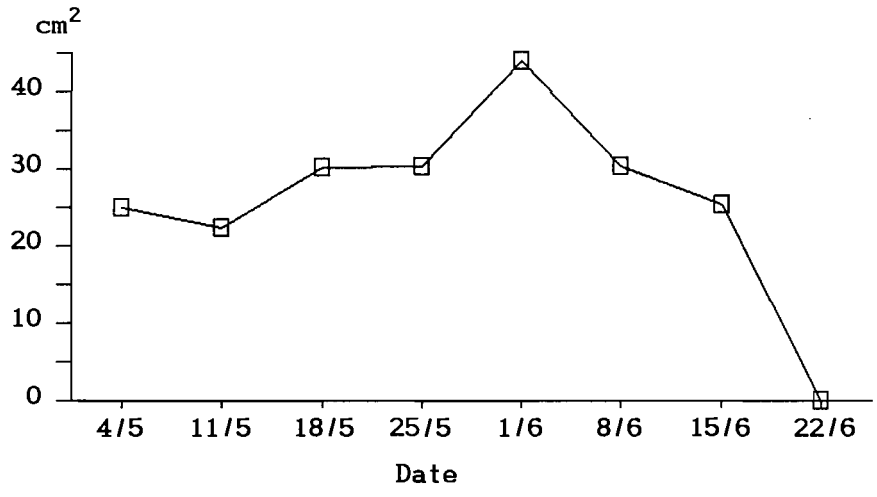


Fig 2.12: Disease development; cv. Bienvenu, 1987

a) TALLF*
b) TCDA*

* see text for explanation

followed by a steeper decline between 8 June and 22 June by which time there were no leaves left on the plants.

The pattern of the change of TCDA was similar to, but less extreme than the number of lesions/plant (Fig 2.12). The TCDA was 25.1cm^2/plant on 25 May and reached a maximum of only 44cm^2/plant on 1 June. Although there was an increase between 25 May and 1 June it was not as substantial as that of TALLF. The range of TCDA for the seven assessment dates was 21.3 - 44.0 cm^2/plant.

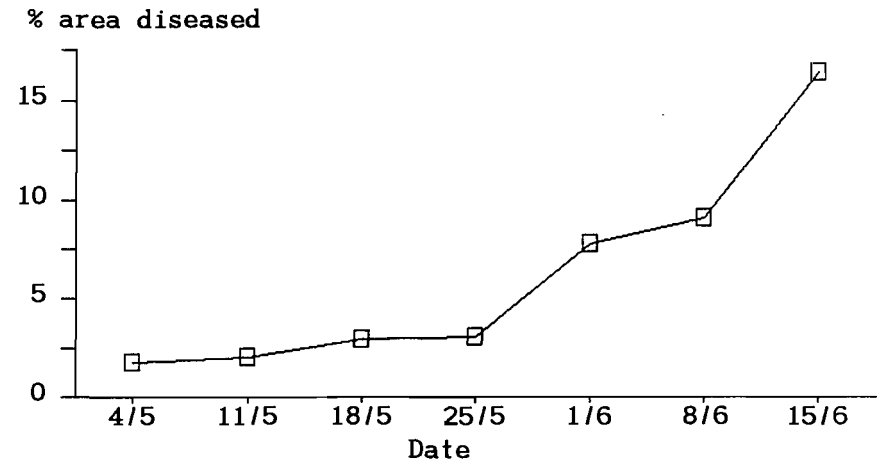
The percentage area of the leaf canopy diseased (TLFDIS) showed a very different pattern from that of the more absolute measurements of disease described above. This parameter increased during the period of study from 1.73% on 4 May to 16.4% on 15 June (Fig 2.13). The relationship between the different measures of disease is discussed in Section 2.3 of this Chapter.

2.2.1.4 Bienvenu 4 April-13 June 1988

As in 1986, only low levels of *Alternaria* leaf spot were found during 1988 with a maximum of 33.3 lesions/plant on 4 April (Fig. 2.14).The development of disease in 1988 showed a different pattern to that in 1986 and 1987. The peak of disease was on the first sample date whilst the crop was still in stem extension (GS 2,00-2,20; Sylvester-Bradley & Makepeace, 1984). There was then a decline in TALLF so that by 11 April there were only 2.7 lesions/plant. TALLF then remained very low for six weeks (3.5-4.1 lesion/plant) after which there was a rise to 19 and 16.5 lesions/plant on 6 and 13 June respectively.

The progress of TCDA, as in 1987, showed a similar but not identical pattern to lesion number (Fig. 2.14). The maximum TCDA was found on 4 April (8.8cm^2/plant) after which there was a drop to 1.2cm^2/plant on 11 April. Following this the TCDA remained between 2.1 and 3.1cm^2/plant until 6 June except on 2 May when it dropped to 0.5cm^2/plant. On 13 June the TCDA had risen to 5.7cm^2/plant whereas TALLF remained approximately constant.

a)



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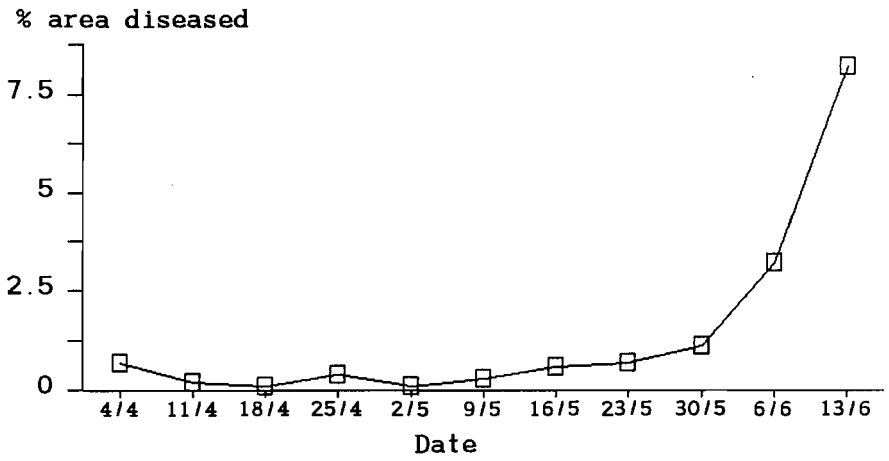
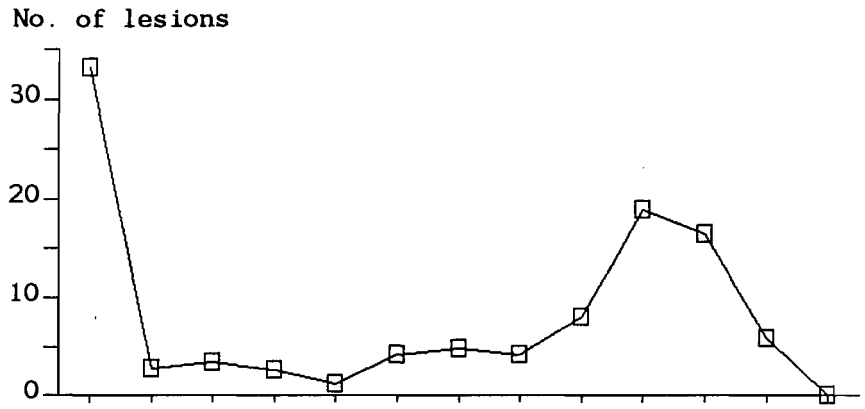


Fig. 2.13: % area of leaf tissue afectedd by *A. brassicae*

a) Bienvenu, 1987
b) Bienvenu, 1988

a)



b)

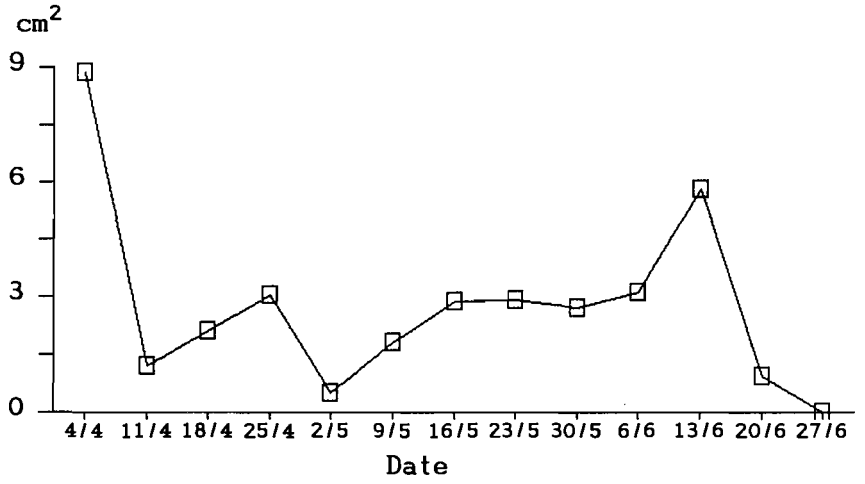


Fig 2.14: Disease development; cv. Bienvenu, 1988

a) TALLF*
b) TCDA*

*see text for explanation

In the 1988 trial TLFDIS did not rise above 8.2% (Fig. 2.13). Until 6 June LFDIS remained at or below 1% and then in the following two weeks it rose to 3.2 and 8.2%.

2.2.2 The relationship between mainstem and branch leaves in the development of dark leaf spot

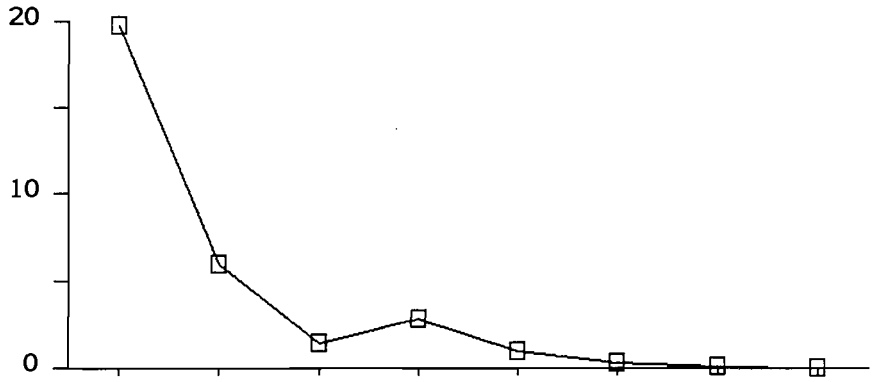
2.2.2.1 Rafal 15 May-26 June 1986

The development of disease (lesions/plant) on MS and BR leaves over the period 15 May-26 June is shown in Fig 2.15. After a peak on 15 May the mean number of lesions/plant on the MS leaves decreased rapidly until 29 May and then remained almost constant until 26 June. On the branch leaves however, the disease level was relatively constant except for a distinct drop on 29 May. The peak of disease that occurred on Rafal on 15 May was therefore largely associated with lesions on the mainstem leaves.

2.2.2.2 Mikado 15 May - 26 June 1986

The development of disease (lesions/plant) on MS and BR leaves is shown in Fig 2.16. In Mikado the decline in TALLF on MS leaves began between 22 May and 29 May. The decrease then continued steadily until 26 June except for an apparent slight increase between 29 May and 5 June. The decrease in disease on MS leaf tissue was later and less steep than on Rafal. In contrast to Rafal, disease on the BR leaves although remaining at low levels until 9 May then showed an increase so that by 20 June it reached a peak of 27.9 lesions/plant. Following this there was a sharp decline in lesion numbers on BR leaves probably associated with the corresponding leaf loss. During the first peak of disease on Mikado in May most lesions were associated with MS leaves whereas in the second peak they were found on the BR leaves.

a)



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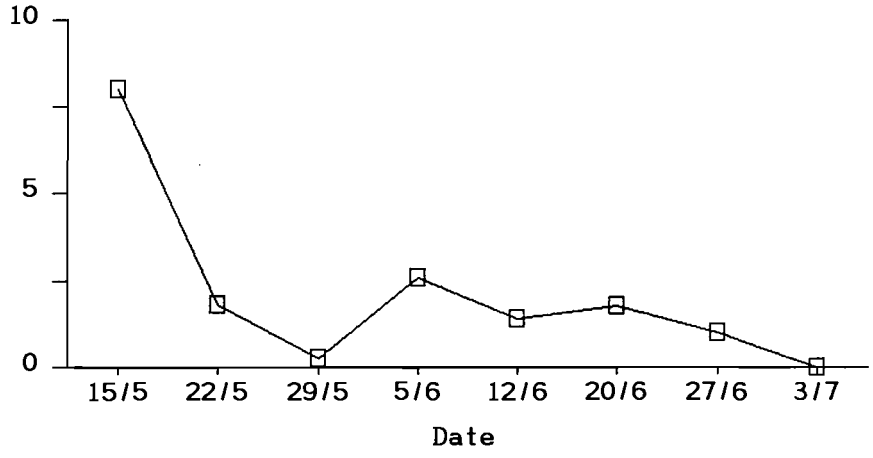
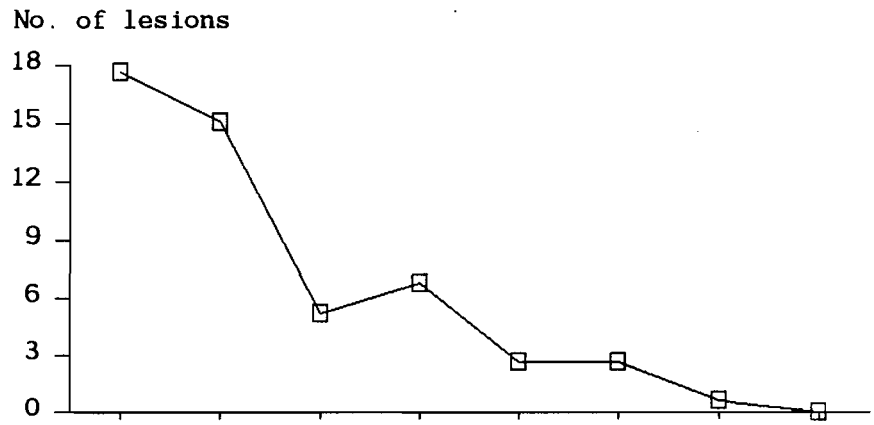


Fig 2.15: Distribution of TALLF* between mainstem and branch leaves cv. Rafal, 1986

a) MS leaves
b) BR leaves

*see text for explanation

a)



b)

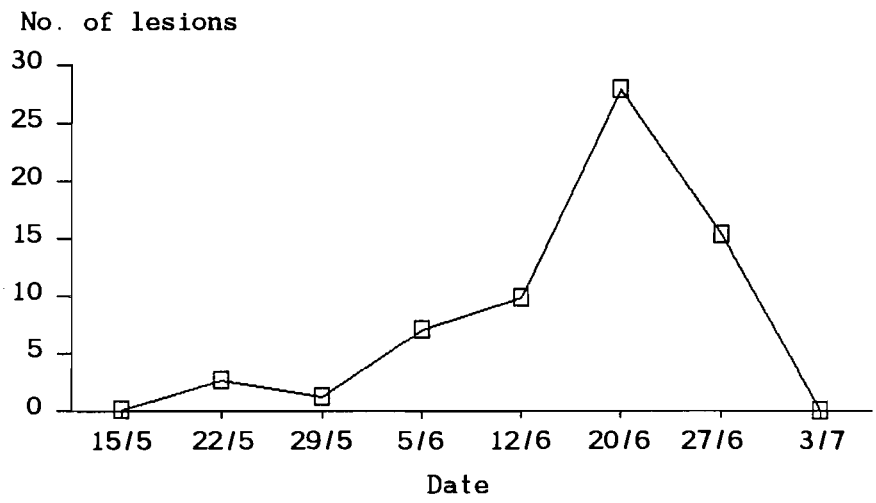


Fig 2.16: Distribution of TALLF* between mainstem and branch leaves cv. Mikado, 1986

a) MS leaves
b) BR leaves

* see text for explanation

2.2.2.3 Bienvenu 4 May - 15 June 1987

TALLF on the MS leaves showed a rise to a peak on 18 May and then some inconsistent variation between 18 May and 8 June (range:38.8 - 67 lesions/plant) and a decline to 0 lesions/plant by 22 June (Fig. 2.17). On the BR leaves the number of lesions increased between 4 May and 1 June, remained approximately constant for one week and then declined rapidly so that by 22 June there were no *Alternaria* lesions on the leaf tissue of the plants. The peak in lesion numbers/plant on 1 June was therefore due almost entirely to an increase in disease lesions on the BR leaves, with only a slight increase being seen on the MS leaves. In consequence the data (Table 2.2) shows that until 11 May more than 80% of all lesions were found on the main stem, after which this proportion gradually declined through the season.

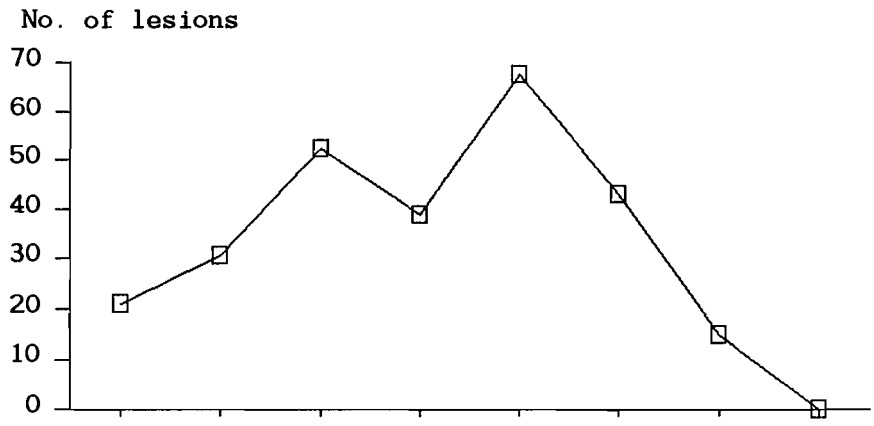
The distribution of TCDA between MS and BR leaves (Fig 2.18) showed a similar pattern to TALLF in that the increase in the disease that occurred around 1 June was mainly associated with disease development on the BR leaves. However the proportion of TCDA found on the MS leaves was always greater than the proportion of TALLF found on the same tissue, so that even on 1 June when TCDA and TALLF were at a maximum, 55.7% of TCDA was on the MS leaves whereas only 30.7% of TALLF was on that tissue.

2.2.2.4 Bienvenu 4 April-13 June 1988

The low levels of disease during 1988 make detailed analysis of distribution between MS and BR leaves difficult. However, the general patterns are similar to those of the other epidemics already discussed.

Until 2 May almost all *Alternaria* lesions (93.3-100%) and an even higher percentage of TCDA (98.6-100%) were found on the MS leaves (Table 2.2). During this period TALLF and TCDA (Figs 2.19 & 2.20) showed a peak on 4 April and then declined to low levels for the following three weeks. From 2 May until 28 May, lesion numbers remained approximately constant on the MS leaves and any increase in lesion numbers was associated with the BR leaves. TCDA seemed to show a different

a)



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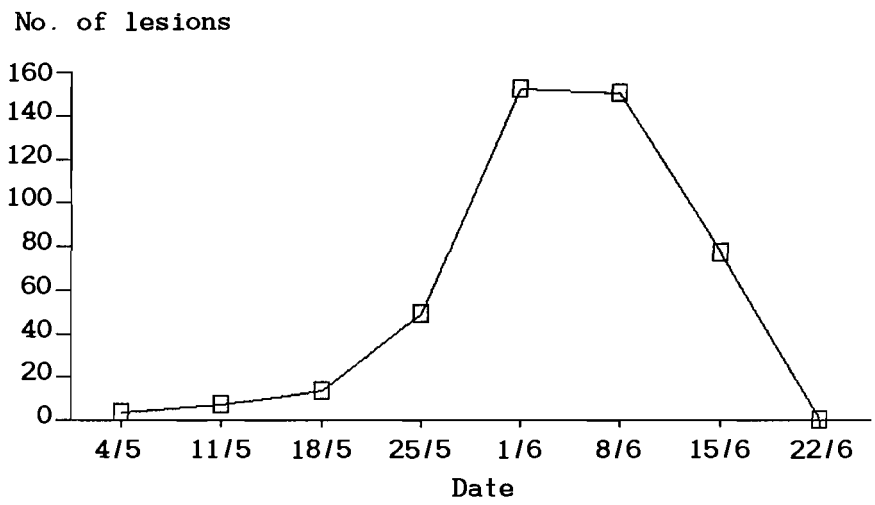
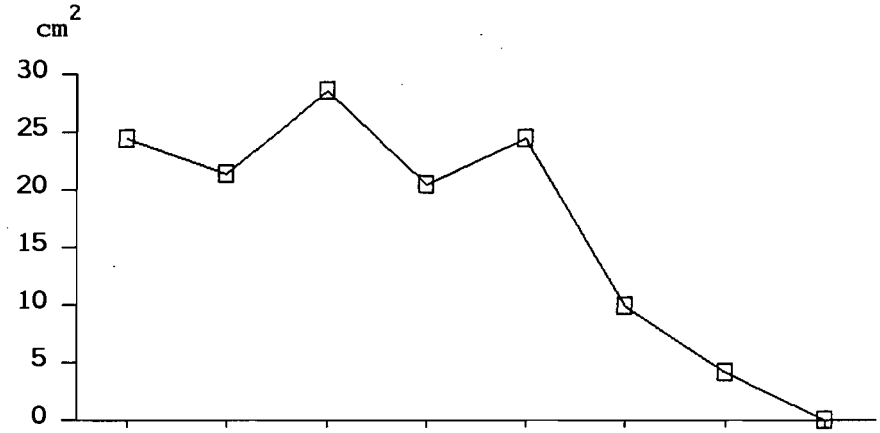


Fig 2.17: Distribution of TALLF* between main stem and branch leaves cv. Bienvenu, 1987

a) MS leaves
b) BR leaves

* see text for explanation

a)



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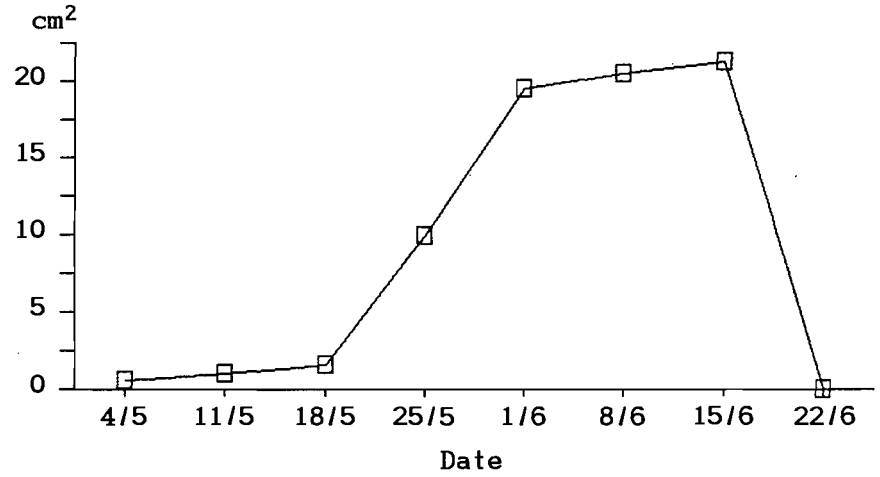
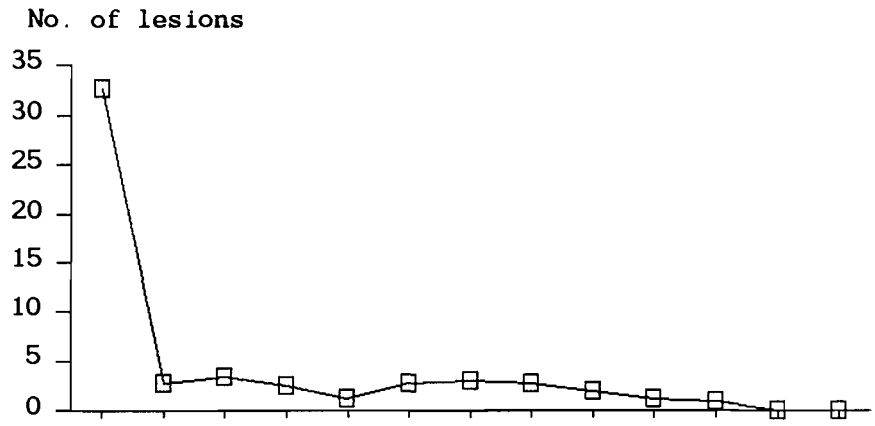


Fig 2.18: Distribution of TCLA* between main stem and branch leaves cv. Bienvenu, 1987

a) MS leaves
b) BR leaves

* see text for explanation

a)



b)

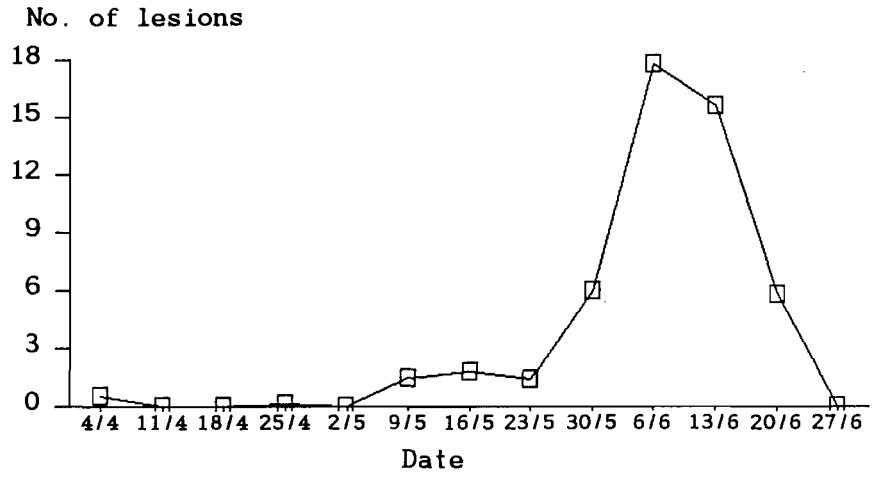
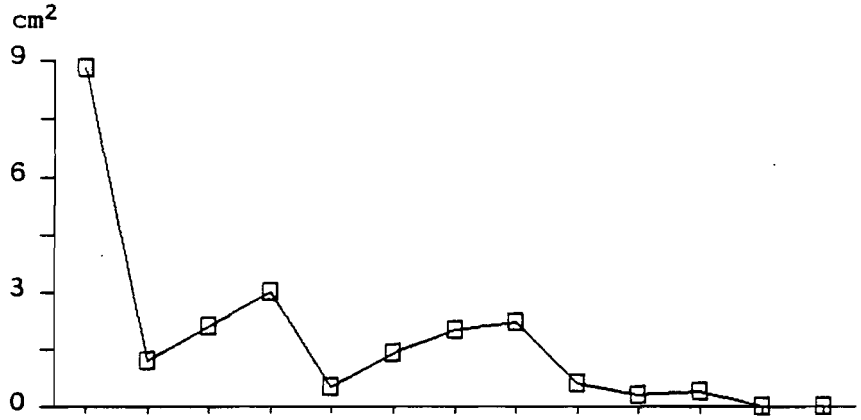


Fig 2.19: Distribution of TALLF* between main stem and branch leaves cv. Bienvenu, 1988

a) MS leaves
b) BR leaves

* see text for explanation

a)



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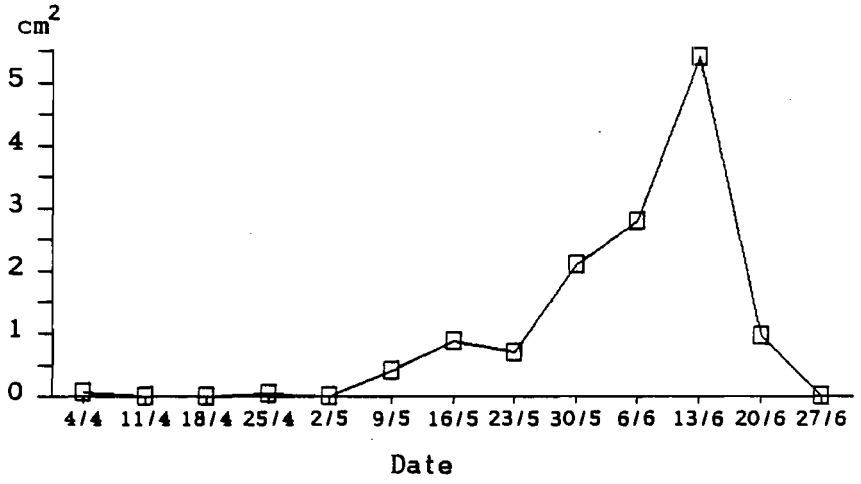


Fig 2.20: Distribution of TCDA* between main stem and branch leaves cv. Bienvenu, 1988

a) MS leaves
b) BR leaves

* see text for explanation

pattern to lesion numbers during this period when disease increased on both MS and BR leaves although the low disease levels make interpretation difficult. In the weeks following this period (30 May - 13 June) disease development was associated with an increase in TALLF and TCDA on the BR leaves and an apparent decrease on the MS leaves.

Table 2.2: Percentage of total number of *Alternaria* lesions and CDA occurring on MS leaves

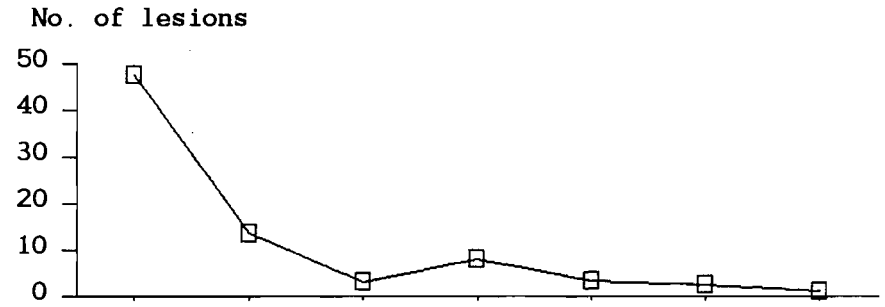
Bienvenu 1987			Bienvenu 1988		
Date	TCDA	TALLF	Date	TCDA	TALLF
			4 Apr	99.7	98.3
			11 Apr	100	100
			18 Apr	98.6	98.6
			25 Apr	99.3	93.3
4 May	97.6	85.6	2 May	99.9	99.2
11 May	94.8	81.6	9 May	77.3	64.9
18 May	94.6	79.5	16 May	69.2	64.5
25 May	67.3	44.1	23 May	78.6	66.0
1 Jun	55.7	30.7	30 May	21.1	23.9
8 Jun	32.4	22.2	6 Jun	9.8	6.2
15 Jun	19.3	16.1	13 Jun	6.8	5.5

2.2.3 The development of sporulating tissue on the leaf canopy

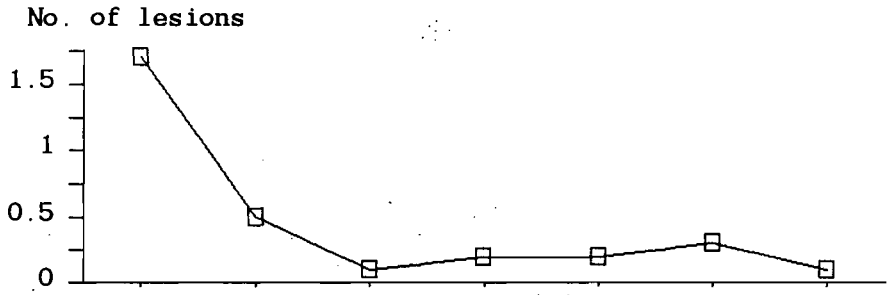
2.2.3.1 Rafal 15 May-26 June 1986

The proportion of *Alternaria* lesions found to be sporulating was small (3.1 - 11.7). and varied throughout the season. The pattern of development of the numbers of sporulating lesions/plant (TALLFSP) matched closely that of TALLF (Fig 2.21) with a peak on 15 May. The proportion of leaves which were sporulating was small and was never greater than 12%. The apparent increase in the proportion of the lesions sporulating on 19 June and 26 June is difficult to interpret because of the very small numbers of lesions counted.

a)



b)



c)

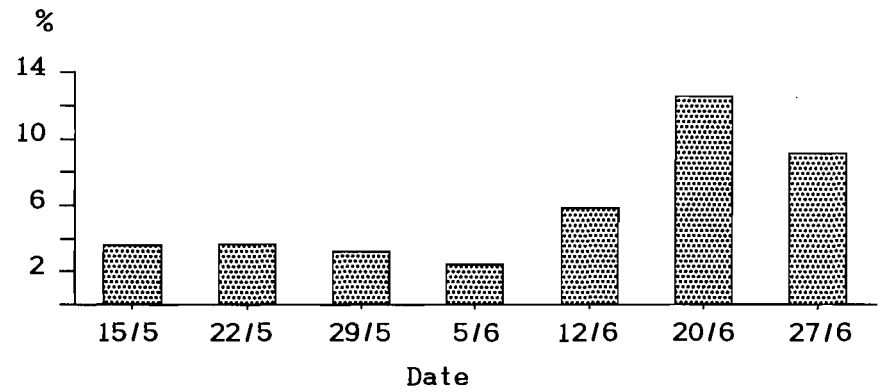


Fig. 2.21: Sporulating tissue on Rafal, 1986

a) TALLF*
b) TALLFSP*
c) Proportion of lesions sporulating

* see text for explanation

2.2.3.2 Mikado 15 May-26 June 1986

On Mikado the proportion of *Alternaria* lesions that were sporulating varied considerably throughout the season (Fig 2.22), but was never greater than 32%. Between 15 May and 19 June the proportion of lesions sporulating was small, in the range 1.8-6.3%. There was then a sharp increase by 26 June when 24.4% of the lesions were sporulating.

The pattern development of the numbers of sporulating lesions matches closely that of the total numbers of lesions on the plant until 19 June (Fig 2.22). Between 19 June and 26 June although there was an increase in TALLFSP, TALLF decreased.

The distribution of sporulating lesions between MS and BR leaves showed a similar pattern to that of the total lesion numbers. During the peak of disease on 22 May the majority of the sporulating lesions were found on the MS leaves (94.7%) whereas during the peak of disease later in the season an increase in the sporulating lesions was associated with the BR leaves.

2.2.3.3 Bienvenu 4 May-15 June 1987

During most of the season the development of sporulating lesions (TALLFSP) and their area (TCDASP) followed a similar pattern to the development of TCDA and TALLF with the proportion of lesions and diseased area sporulating, remaining relatively constant (Figs 2.23 & 2.24). The exception was on 11 May when the proportion of sporulating tissue (TALLFSP & TCDASP) was greater than in other weeks. Distribution of sporulating tissue (TCDASP & TALLFSP) between MS and BR leaves showed a similar pattern to that of TCDA and TALLF so that generally there was a greater proportion of TCDASP than TALLFSP on the MS leaves in each week and the proportion of sporulating tissue on the MS leaves declined through the season.

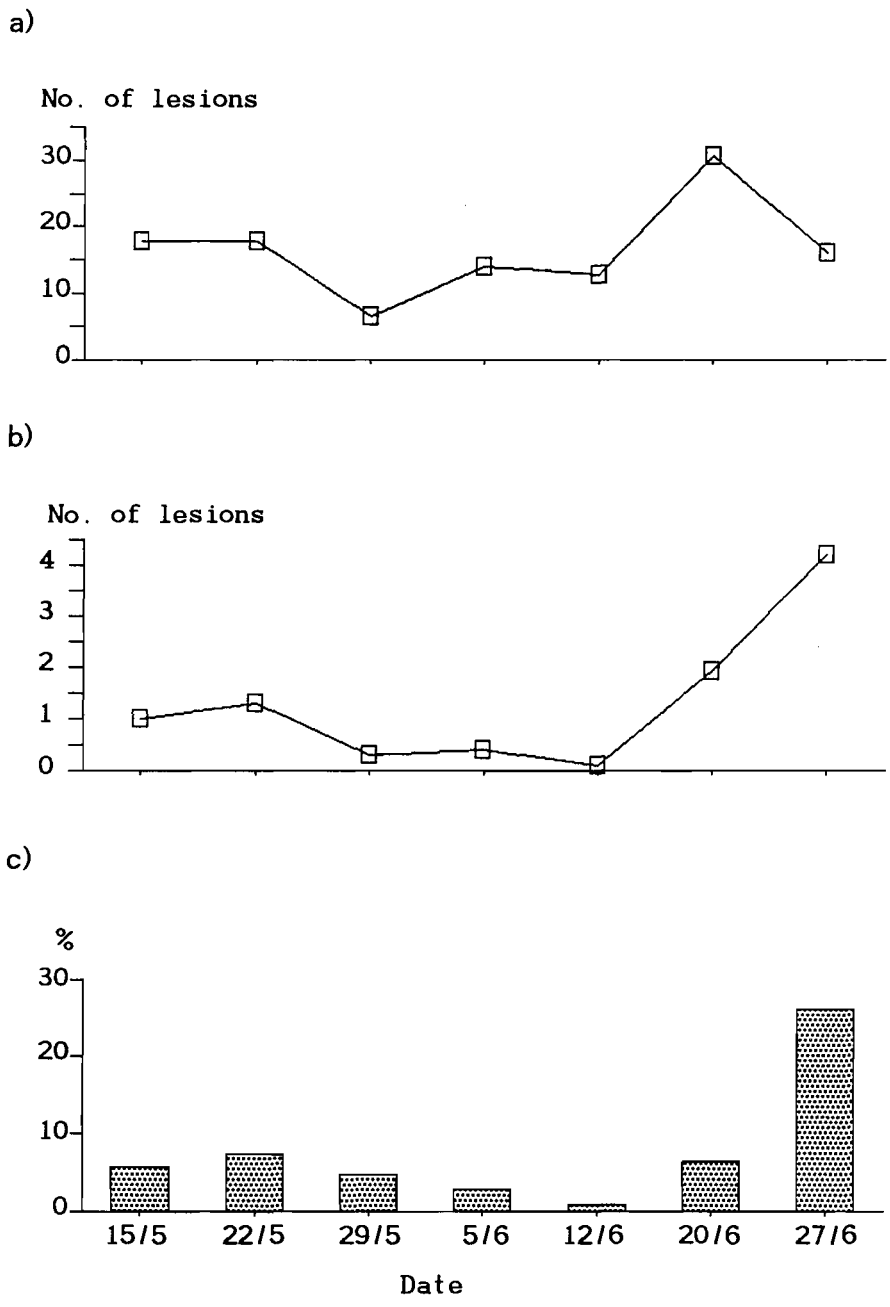
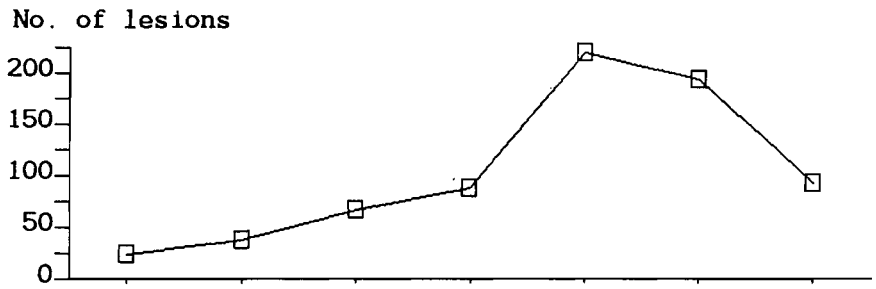


Fig. 2.22: Sporulating tissue on Mikado, 1986

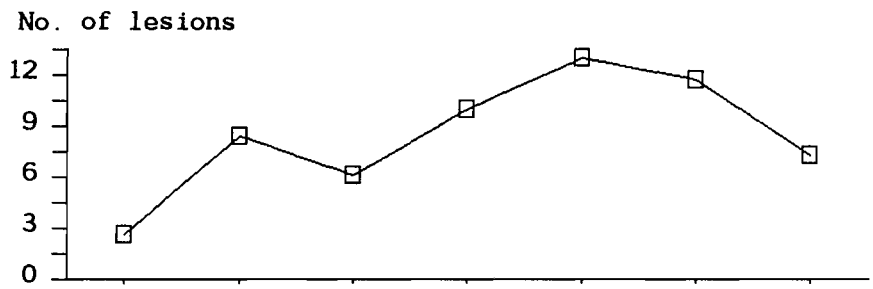
a) TALLF*
b) TALLFSP*
c) Proportion of lesions sporulating

* see text for explanation

a)



b)



c)

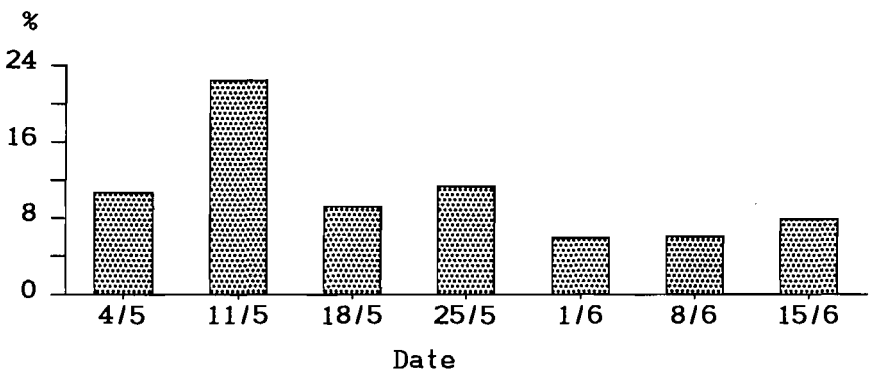
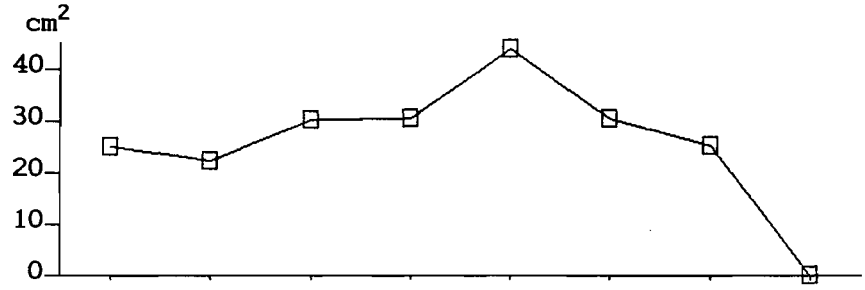


Fig. 2.23: TALLF*, TALLFSP* and proportion of lesions sporulating on the leaf canopy; cv. Bienvenu, 1987

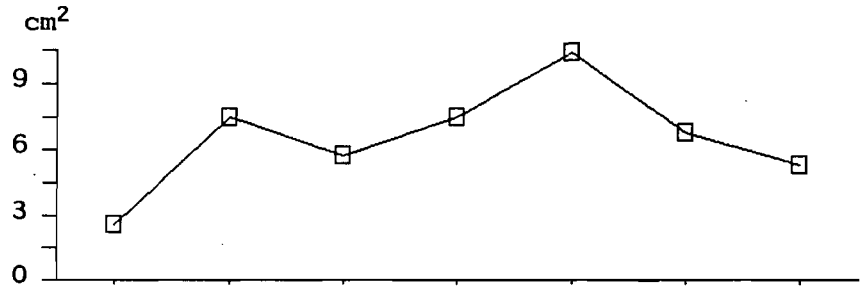
a) TALLF*
b) TALLFSP*
c) Proportion of lesions sporulating

* see text for explanation

a)



b)



c)

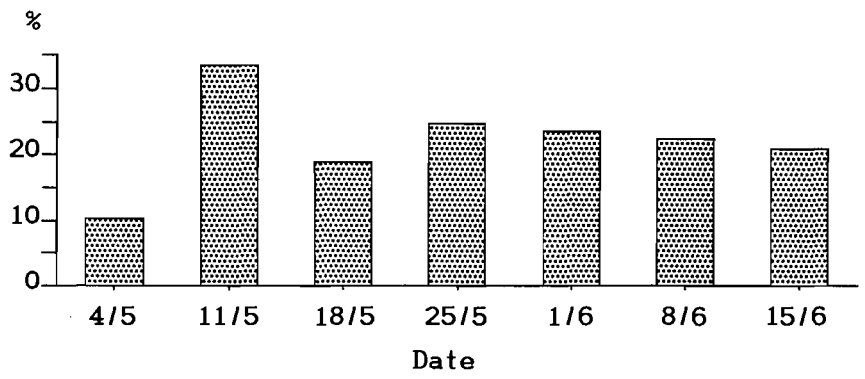


Fig 2.24: TCLA*, TCLASP* and proportion of diseased area sporulating cv. Bienvenu, 1987

a) TDLA*
b) TDLASP*
c) proprtion of diseased area sporulating

* see text for explanation

Table 2.3: Percentage of total number of *Alternaria* lesions sporulating and TCDASP occurring on MS leaves

Bienvenu 1987			Bienvenu 1988		
Date	TCDASP	TALLFS	Date	TCDASP	TALLFS
			4 Apr	100	100
			11 Apr	100	100
			18 Apr	100	100
			25 Apr	94.3	90.1
4 May	97.6	97.6	2 May	100	100
11 May	96.0	89.4	9 May	97.0	96.8
18 May	96.5	91.8	16 May	93.4	88.5
25 May	69.3	52.0	23 May	69.8	66.0
1 Jun	53.8	43.0	30 May	28.6	30.8
8 Jun	47.0	27.3	6 Jun	11.8	13.9
15 Jun	26.4	19.7	13 Jun	4.6	2.2

2.2.3.4 Bienvenu 4 April - 20 June 1988

In 1988 disease levels were low (Section 2.2.1.4)and consequently the amount of sporulating tissue was low (max: 2.7 lesions/plant, 1.9 cm^2/plant). With such very low levels of sporulating tissue interpretation of the data is difficult but does suggest that as in other years the pattern of development of sporulating tissue (TCDASP & TALLFSP) is similar to that of TCDA and TALLF (Figs 2.25 & 2.26). An apparent exception to this was on 4 April when the maximum number of lesions/plant (33.3 lesions/plant) were found but only a very small proportion (1.6%) were sporulating. The distribution of sporulating tissue between MS and BR leaves (Table 2.3) again showed that like TCDA and TALLF early in the season sporulation was almost entirely associated with MS leaves moving to BR leaves during the season. However, in contrast to the 1987 trial the proportion of TCDASP was similar to that of TALLFSP in each week.

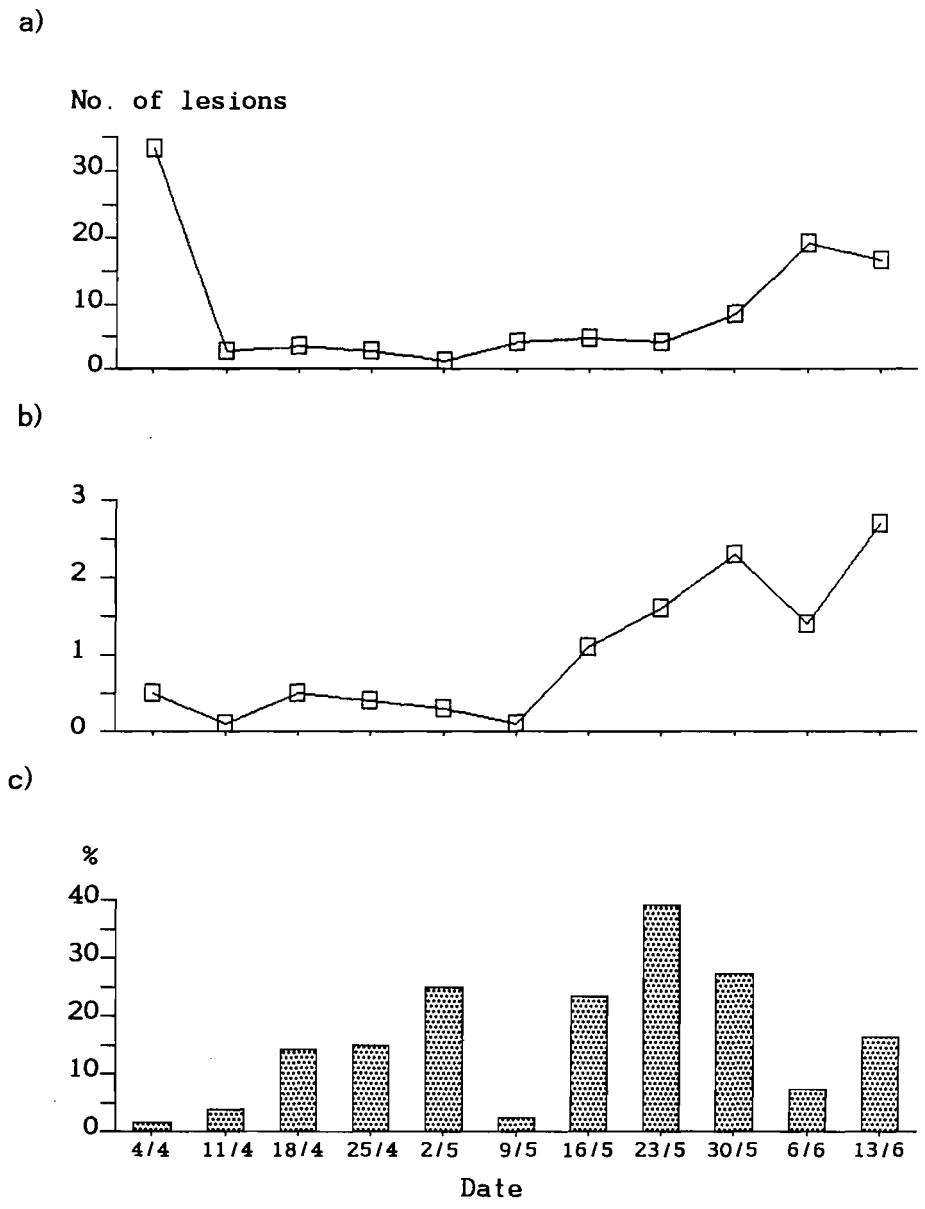


Fig. 2.25: TALLF*, TALLFSP*, and proportion of lesions sporulating on the leaf canopy; cv. Bienvenu, 1988

a) TALLF*
b) TALLFSP*
c) Proportion of lesions sporulating

* see text for explanation

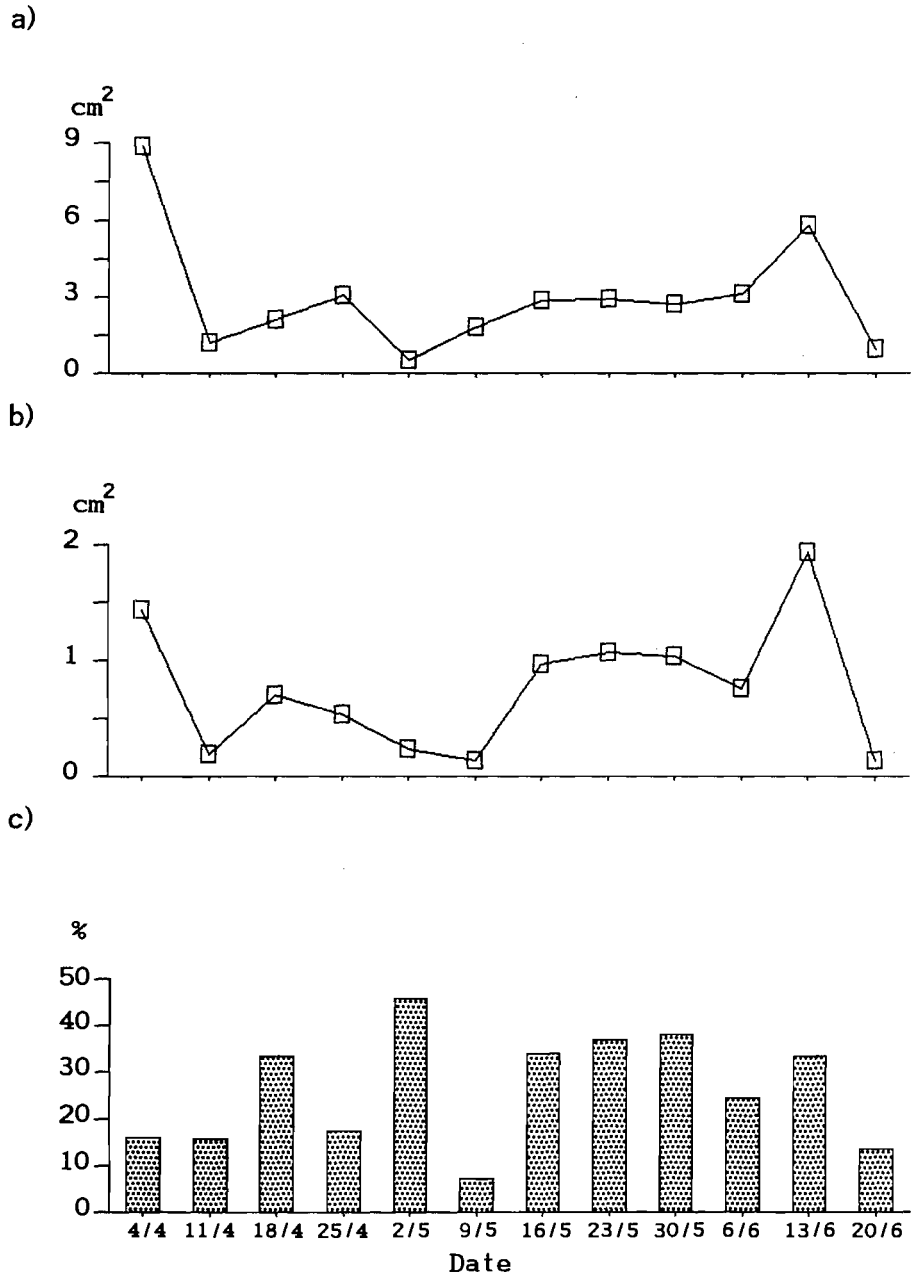
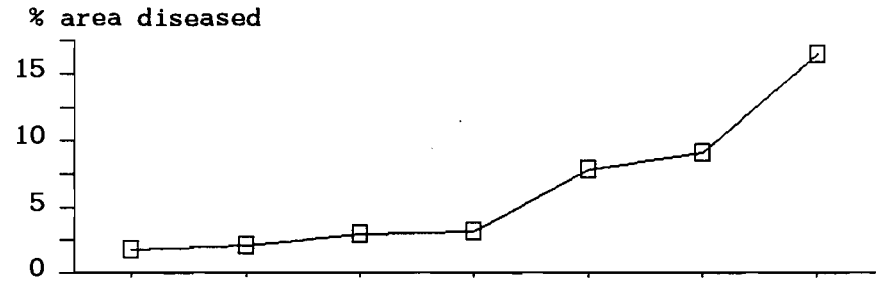


Fig 2.26: TCLA*,TCLASP*, and proportion of diseased area sporulating cv. Bienvenu,1987

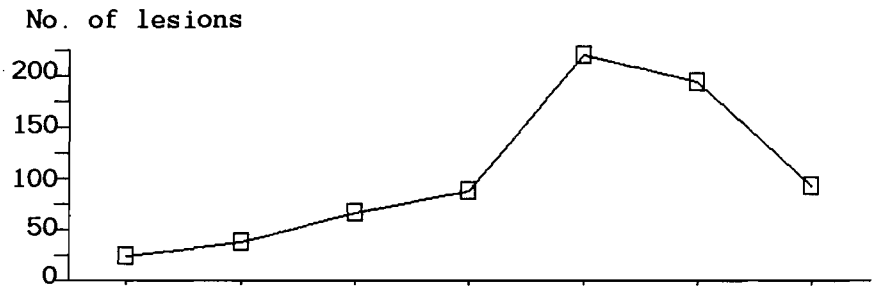
a) TDLA*
b) TDLASP*
c) proportion of diseased area sporulating

* see text for explanation

a)



b)



c)

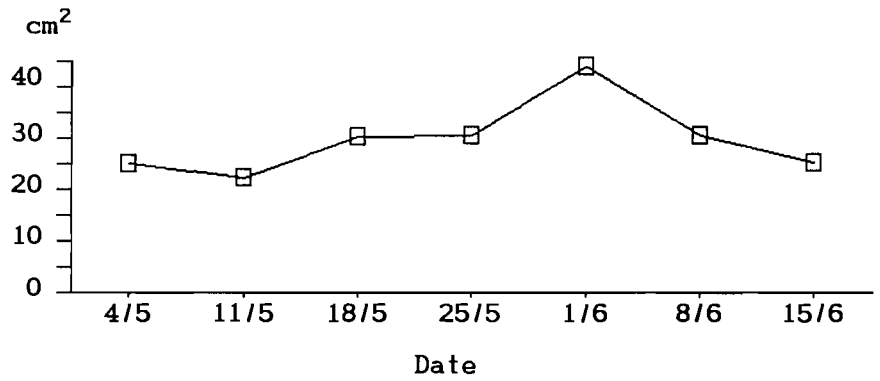
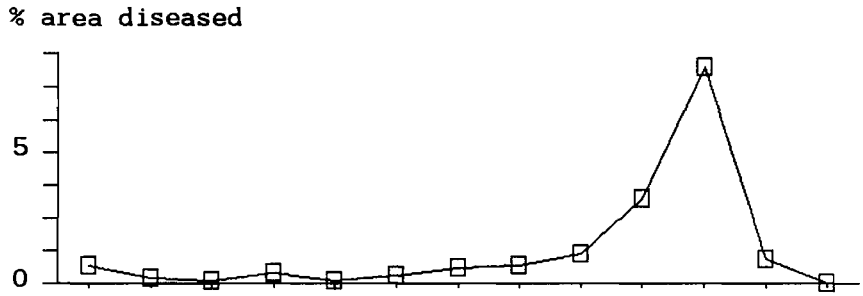


Fig. 2.27: Comparison of different measures of disease on the leaf canopy cv. Bienvenu, 1987

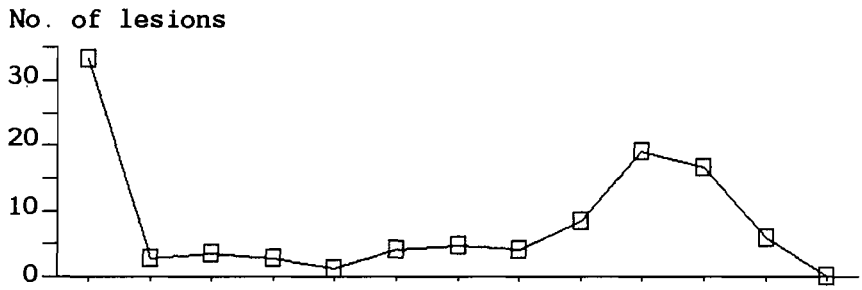
a) TLFDIS*
b) TALLF*
c) TCDA*

* see text for explanation

a)



b)



c)

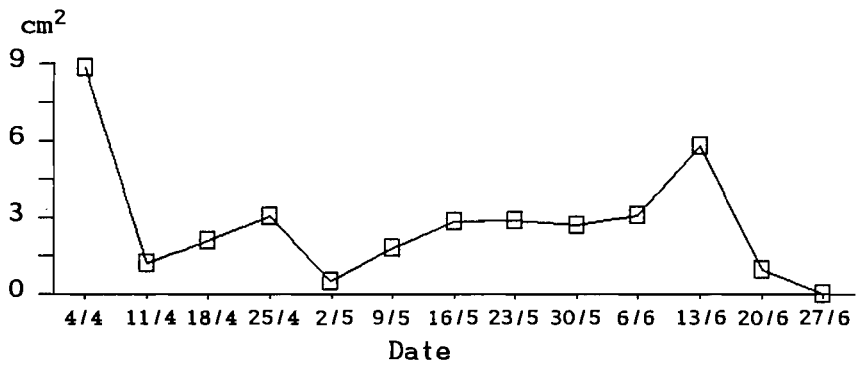
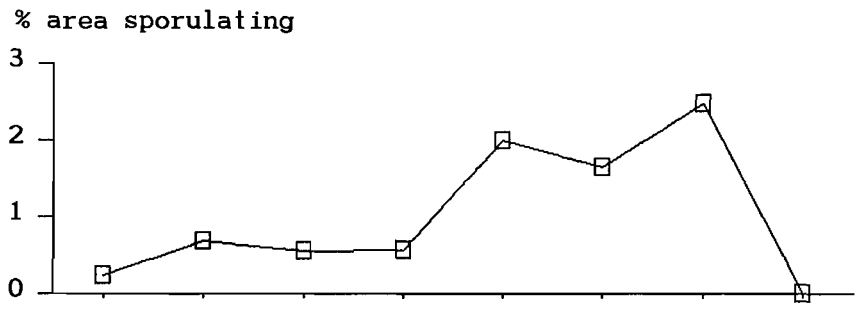


Fig. 2.28: Comparison of different measures of disease
cv. Bienvenu, 1988

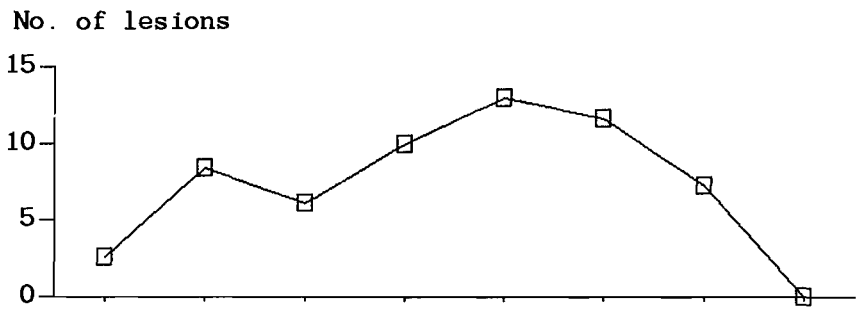
a) TLFDIS*
b) TALLF*
c) TCDA*

* see text for explaination

a)



b)



c)

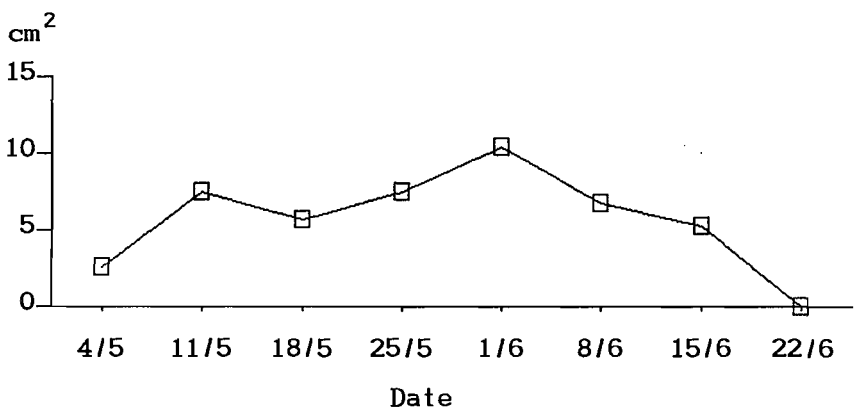
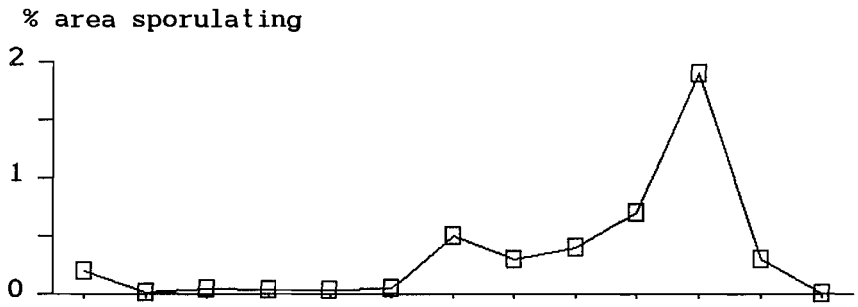


Fig 2.29: Comparison of different measures of sporulating tissue Bienvenu, 1987

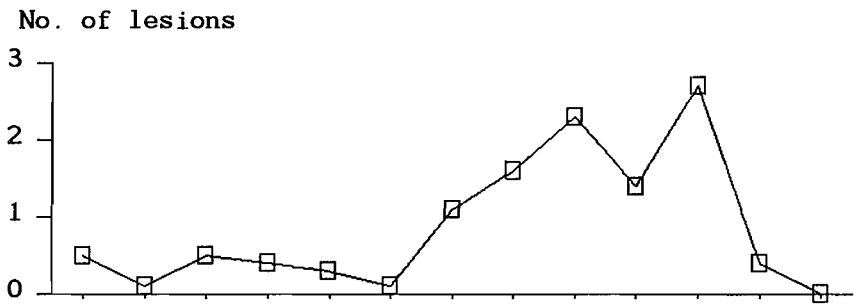
a) TLFDISSP*
b) TALLFSP*
c) TCDASP*

* see text for explanation

a)



b)



c)

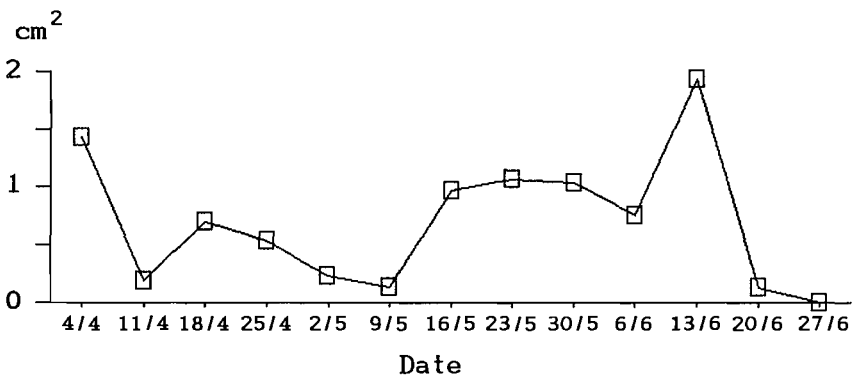


Fig 2.30: Comparison of different measures of sporulation on the leaf canopy cv. Bienvenu, 1988

a) TLFDISSP
b) TALLFSP*
c) TCDASP*

* see text for explanation

2.3 Comparison of various measures of the severity of dark leaf spot on oilseed rape

2.3.1 Results

Figs 2.27 - 2.30 show the progress of dark leaf spot on oilseed rape for the 1988 and 1987 seasons using six different variables. In both years the % area of leaf tissue affected (LFDIS) showed a similar pattern of development remaining at low levels through most of the season and then rising during the three to four weeks prior to complete leaf loss. In contrast the development patterns of both TCDA and TALLF differed markedly between seasons. In 1987 both variables described an inverted 'V'- shaped curve and in 1988 a 'U'-shaped curve.

Within each season although the general shape of the curves for TCDA and TALLF were similar, differences were clear. In both 1987 and 1988 the curve for TCDA is much less extreme than that for TALLF and in 1988 TCDA appeared to be more variable than TALLF when disease levels were low. When variables for sporulating tissue are considered (TALLFSP, TCDASP & LFDISSP) the patterns of development were generally similar to those for the total diseased tissue, the major difference being the much lower levels of sporulating tissue compared with those of total levels of disease. Consequently small differences in the patterns are difficult to interpret. Differences on 11 May 1987 between the patterns of TCDA and TALLF and TCDASP and TALLFSP respectively should however, be noted as well as the low numbers of sporulating lesions on 4 April 1988 compared with the total number of lesions counted.

2.4 Three-Dimensional description of the development of dark leaf spot on the leaf canopy of oilseed rape

2.4.1 Introduction

The development of disease in a crop canopy is most often described in terms of a measure of disease representing the whole plant and studying the change in this

variable with time. Such descriptions for dark leaf spot are described in sections 2.2.2 and 2.2.3 and they give some idea of how the disease develops. However, these descriptions give no indication of the position of the disease within the leaf canopy, an important factor in determining the future potential development of the disease. For example if during early-pod development the disease is found at the base of the plant then infection of the pods during this period is perhaps likely to be less than if the disease is near the top of the canopy assuming that leaf tissue provides an inoculum source for infection of the pod canopy. A pictorial representation of the vertical development of the disease within the crop canopy would therefore be useful. Few examples of pictorial representations of vertical movement of disease in crop canopies can be found in the literature. Royle *et al.* (1986) described the DPCs of leaves at different positions on the plant to represent development of *Septoria tritici* on wheat and Plaut & Berger (1980) divided the canopy of peanuts into layers of 15cm and presented DPCs for disease caused by *Cercosporidium personatum* on each layer. Frinking & Linders (1986) went further and used 3-D graphics to describe similar data from downy mildew on radishes.

In oilseed rape apparently no literature is available on the development of *A.brassicae* at different heights in the leaf canopy. This section shows various graphical methods for describing the vertical development of *A.brassicae* in oilseed rape and the use of contour mapping for the comparison of four different epidemics of dark leaf spot.

2.4.2 Methodology

Data for each variable at each assessment were extracted from the total data set for a series of height ranges (Table 2.4) the height for each data point representing the height of insertion of the relevant leaf. The mean value for each height range was classified as being the value of that variable at the mid-range height (Table 2.3). These means were then plotted in various ways as described below.

2.4.3 Different graphical techniques and their "pros and cons"

The simplest method of picturing the progress of disease through different heights in a leaf canopy with time is a series of x-y graphs for each date (Fig 2.31, x = height) or different heights (Fig 2.32, x = date). Although such diagrams give a good visualisation of the development of disease within each height category, it is difficult to visualise the overall pattern of disease distribution within the leaf canopy and hence comparisons between epidemics are difficult.

Table 2.3: Height ranges and mid-height points for 3-D figures to describe plant and disease development

Height Class	Height Range (cm)	Mid-Height (cm)
1	0 - 20.0	10
2	20.1 - 40.0	30
3	40.1 - 60.0	50
4	60.1 - 80.0	70
5	80.1 - 100.0	90
6	100.1 - 120.0	110
7	120.1 - 140.0	130
8	140.1 - 160.0	150

Frinking & Linders (1986) went further and used 3-D figures to represent progress of downy mildew on radishes. They used simple diagrams, the 3-D lattice being constructed of a matrix of date v leaf position and a measure of disease drawn at each intersection on the matrix. The diagrams so drawn provided a good visual picture of disease development. Data for the number of lesions/plant for the 1987 field trial were constructed into a similar 3-D figure (Fig 2.33). In this case a more complex plotting procedure was used than by Frinking & Linders (1986). From the basic matrix of x points, other points were interpolated within the matrix to give a much finer matrix from which the figures were drawn. The data were analysed using UNIMAP (UNIRAS Ltd) on an IBM3090 mainframe computer. The interpolations

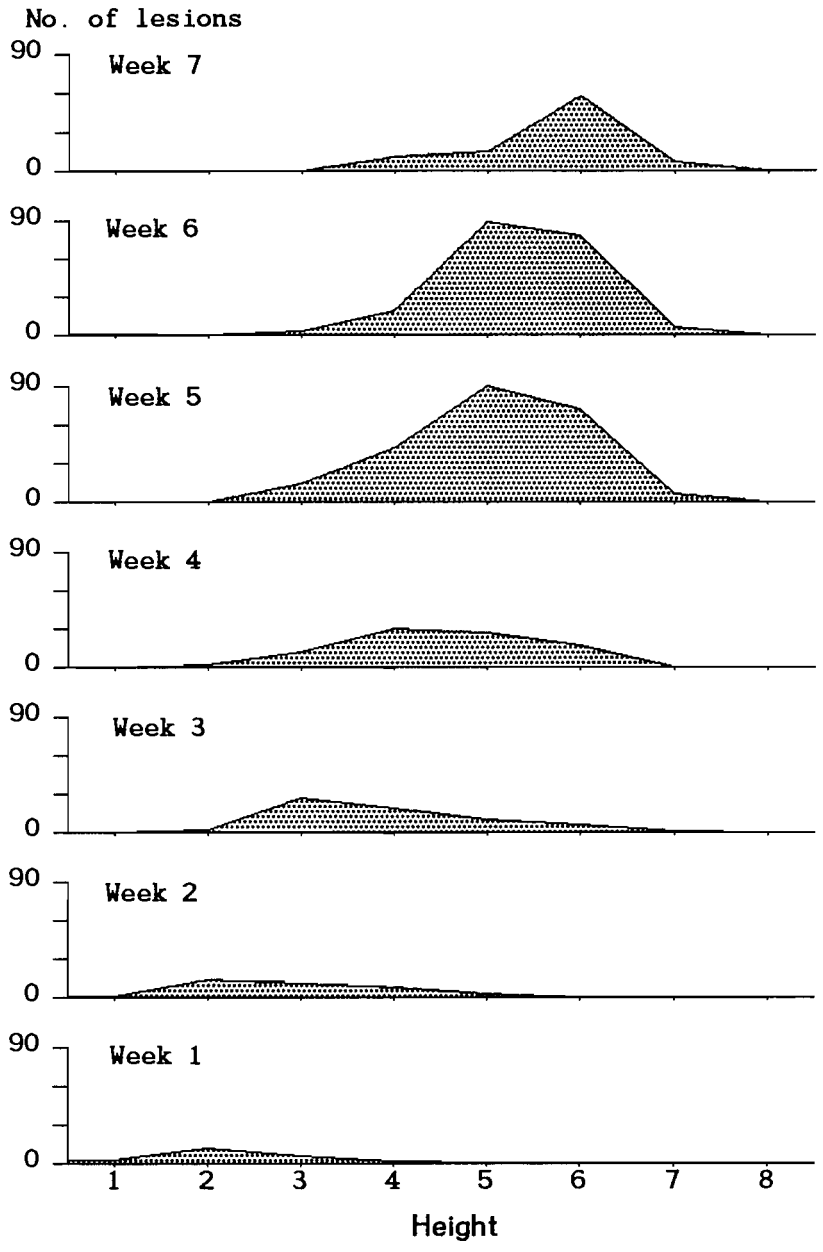


Fig 2.31: Number of lesions in each height class on different sampling dates cv. Bienvenu, 1987

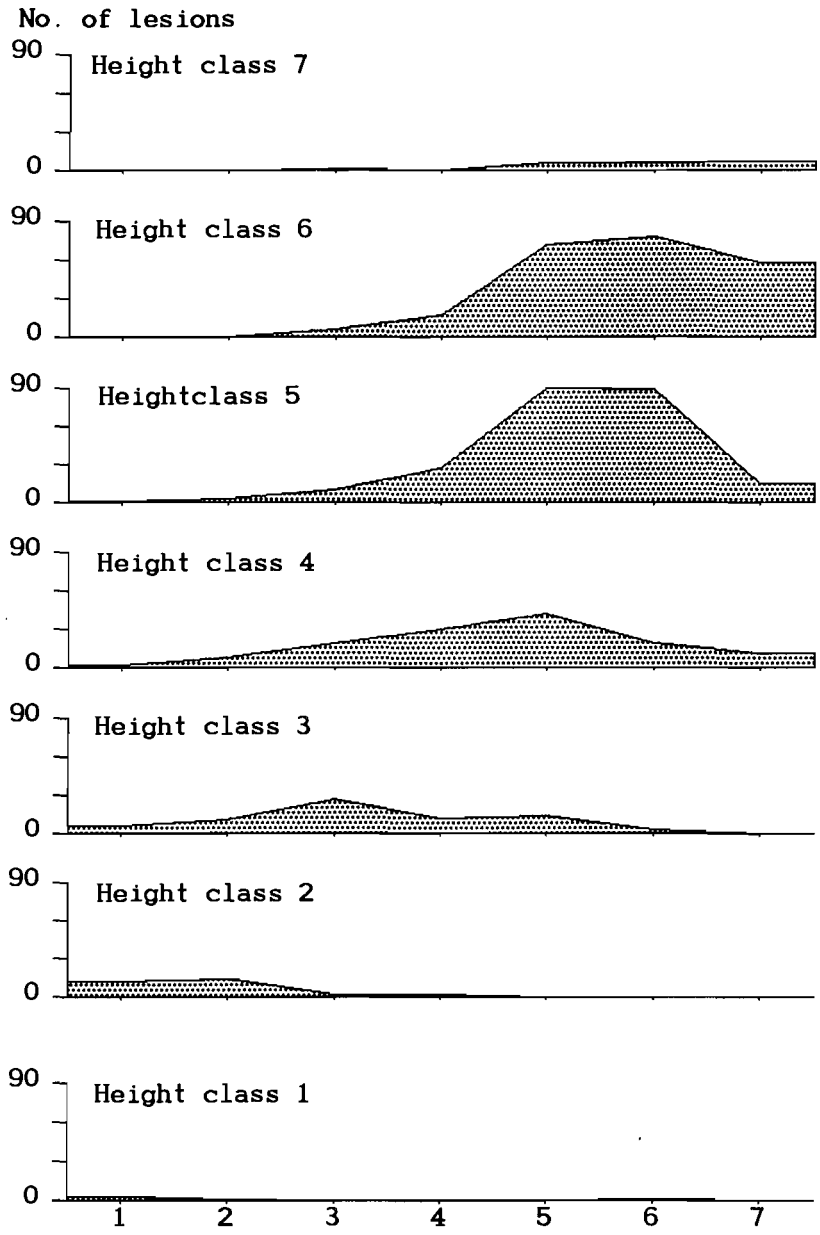


Fig 2.32: Number of *A. brassicae* lesions in each height class on different sampling dates

used were based on polynomial expressions (Anon, 1985). The data so formulated into 3-D figures are presented colour filled (Fig 2.33). It gives a powerful visual picture of where the disease is present within the canopy at any particular time during the season. The use of interpolation has allowed the smoothing of curves to give a better image.

Although such 3-D images give a good visualisation of disease (or crop) development, they have distinct drawbacks. To obtain a complete view of the image, it often needs to be viewed from more than one angle (Fig. 3.34) so that the greatest amount of information can be shown. This was clearly a difficulty in the data presented by Frinking & Linders (1986) where two views of each plot were presented. Furthermore as with 2-D graphs, comparison of data from different epidemics is difficult.

Three dimensions can however be presented in a much simpler 2-D format. Contour mapping has been used by geographers for many years to map the topographical details of the landscape. Each contour line joins points of similar height giving a pattern of concentric lines describing the changing height of a landscape. In areas between the lines, no detail is given about the changes in height which occur, and there may be small peaks and troughs between the contour lines. In many cases colour is used to fill the areas between the lines to help improve the interpretation of the map. Contours can similarly be used to map the different levels on a surface of 3-D graphs. Figs 2.35-2.41,show this type of mapping used for the data of the four epidemics previously described in Section 2.2. The mappings were produced using UNIMAP and again the data were interpolated. Filled colour was used to accentuate the contours. Grids were drawn to assist comparisons between years, but more especially between the maps of crop canopy development and those of disease development within a single year.

Such mappings give an immediate visual impression of disease distribution in the leaf canopy which is easy to interpret. Using the colour fill maps it is easy to see at which heights the concentrations of disease were on any particular date. The

mappings are easy to present without colour fill and could be overlayed for comparison. For presenting data of this sort they are concise and informative.

Improvements of the method can however be suggested. Problems were found in using UNIMAP for these particular data sets. A contour of zero could not be set, so that zero i.e. no disease was always incorporated into a range containing some disease. This gives no clearly defined borders between no plant and some plant or no disease and some disease. This limits the interpretation of the data at points where disease or leaves are at low levels and even shows plant or disease material to be present where there is none. Another limit of the UNIMAP software is the inability to define the same colour for the same level of variable in each data set. This means that the category 18.0 - 21.0 lesions for the 1988 data set is given the same colour as 51.0 - 59.5 lesions in the 1987 data. Although this enables the patterns of disease development to be compared easily (the original aim of this mapping) the colour ranges could lead to misinterpretation of the data. A further and more fundamental difficulty arises from the use of interpolation of the data to present the mappings in this form. Interpolation within data sets is often used, consciously or unconsciously when using graphical representation of data. In section 2.2.2 for example where lines are drawn between data points on different dates, the implication is that disease development or plant growth is linear between these points. The line however describes only what happens "on-average " between those two points. It is clear that similar implications are made in the contour mapping procedures described here, although in this case as polynomial interpolation has been used linear progress is not assumed. The contour lines have been produced by calculating data points between those actually taken from the field. In this case those data supplied from the field are a mean over a 20cm height range and the height for graphical analysis is given as the mid-point of the range. So not only are points within the matrix estimated, but the original data are mean values. This clearly differed from geographical mapping where the original data are absolute.

Over-interpretation of the mappings presented here is therefore unwise and only comparisons of the general patterns of disease development in relation to leaf canopy data should be attempted. To obtain better data sets for this type of mapping,

data could be taken from smaller sections of the canopy e.g. 2-3cm lengths at 6-7 heights. These would then give a series of points between which interpolation would be more appropriate. Data collection could be further refined by taking samples at more heights. Collecting such data would however be extremely time consuming and a detailed study of various sampling techniques is required if this type of data analysis is to be pursued.

In other pathosystems data could be collected more easily. The ideal pathosystem would be where the host leaf canopy is tall, dense and complex and the disease is severe, so reducing the number of samples required. Examples would be oilseed rape/ *A.brassicae* in other parts of the world, peanuts/ leaf spot diseases and bush fruits/ leaf spot diseases. On cereals and other crops of simple morphology such contour mapping would be inappropriate. Disease data need to be measured on individual leaves and in a crop where each tiller may only have five leaves and each leaf is long compared with the height of the plant data could not easily be assigned to a particular height.

2.4.4 Comparison of leaf canopy data from each of the years 1986-1988 using contour mapping

2.4.4.1 Rafal 15 May-26 June 1986

On Rafal the numbers of leaves reached a maximum on 15 May when they were spread throughout the height of the plant the densest area being 10-40cm (Fig.2.29). During the following weeks leaves were lost from the base of the crop so that by 12 June the densest area was c.50cm.

The low levels of disease in Rafal gave poor data for this type of mapping (Fig. 2.35). However, the data do show that at its maximum on 15 May disease was confined to the very base of the crop canopy (0-10cm). After this date the disease, at low levels, moved slowly up the crop canopy.

2.4.4.2 Mikado 15 May-26 June 1986

The leaf canopy in Mikado was well developed by 15 May with the greatest density of leaves (5.6-8.0leaves/plant) between 10 and c.60cm above the soil surface (Fig. 2.36) Between 15 and 22 May the leaf canopy became denser at c.50cm and then in the following weeks leaves were lost from the base of the canopy. Disease devolopment showed a different pattern. On 15 May disease was confined to the base of the crop, mostly 10-30cm above the soil surface. The majority of disease remained low in the crop until 5 June when it began to spread up through the canopy. From 12-17 June the distribution of disease was similar to that of the leaves. The initial peak of disease 15-22 May was therefore associated with disease at the base of the leaf canopy and the second (19 June) with a wider distribution similar to that of the leaves.

2.4.4.3 Bienvenu 4 May-15 June 1987

By 4 May the leaf canopy was already well developed. The mapping of leaf numbers suggested that the densest part of the leaf canopy lay between 70 and 100cm from 4-11 May and between 70 and 120cm for the rest of the season (Fig. 2.37). The vertical range of the canopy decreased during the season as leaves were lost from the base of the canopy. The distribution of TCLA shows a different pattern (Fig 2.38). Using this variable the leaf canopy appears to be denser over a wider vertical spread. On 4 May there were 210-330cm^2 TCLA/plant over the range c.20-95cm. As time progressed leaves were lost from the base of the canopy so that by 1 June the same range of TCLA was only found between c.80 and 100cm above soil level. Between 1 June and 15 June TCLA decreased throughout the canopy.

As in Mikado, 1986 disease did not show the same distribution pattern as the leaf canopy. A distinct difference is also seen between the distribution pattern of lesion numbers and TCDA. At the beginning of sampling, lesions were associated with the base of the leaf canopy and during the season the disease was seen to progress up the plant (Fig. 2.37). By 5 June lesion numbers had a similar vertical distribution to leaf numbers. TCDA showed a different pattern of development. As

with the number of lesions the map of TCDA shows that disease was close to the ground on the first assessment (Fig 2.37). There was then a gradual, almost linear development of disease up through the leaf canopy. The densest area of disease was generally found at the bottom edge of the leaf canopy where leaf material was being lost. TCDA did not take on a similar vertical distribution to TCLA until 8 June. The numbers of sporulating lesions had a distribution pattern very similar to TCLA rather than the total number of lesions (Fig 2.39).

2.4.4.4 Bienvenu 4 April-13 June 1988

The pattern of crop canopy development described by the number of leaves/plant was similar to that in other years. The earlier sampling showed that during April the densest part of the canopy was moving upwards and then from 2 June until the end of the season remained at c.70cm above soil level (Fig 2.40). Using TCLA to describe the canopy the pattern of development was different (Fig 2.41). On 4 April the densest part of the canopy was found at c.50cm above ground level. During the season the location of the densest part moved vertically following a linear pattern so that by 30 May it was at c.70cm. The TCLA representing the densest portion of the canopy drops from c.550cm^2/plant on 4 April to c.250cm^2/plant on 30 May.

As on Rafal in 1986 the very low disease intensities in 1988 reduced the value of such mapping procedures. However, the pattern of vertical development of such low intensities was still obvious. The disease on 4 April was associated with the base of the crop and then throughout the season the position of the disease moved vertically higher so that by 30 May any lesions which were present were situated close to the pod canopy.

3-D Descriptions of plant and dark leaf spot development on oilseed rape, 1986 - 1988

Figures 2.33 - 2.41

Keys for x and y axes

x - axis: variable = week

Week	1986	1987	1988
1	15 May	4 May	4 Apr
2	22 May	11 May	11 Apr
3	29 May	18 May	18 Apr
4	5 Jun	25 May	25 Apr
5	12 Jun	1 Jun	2 May
6	19 Jun	8 Jun	9 May
7	26 Jun	15 Jun	16 May
8			28 May
9			30 May
10			6 Jun
11			13 Jun
12			20 Jun

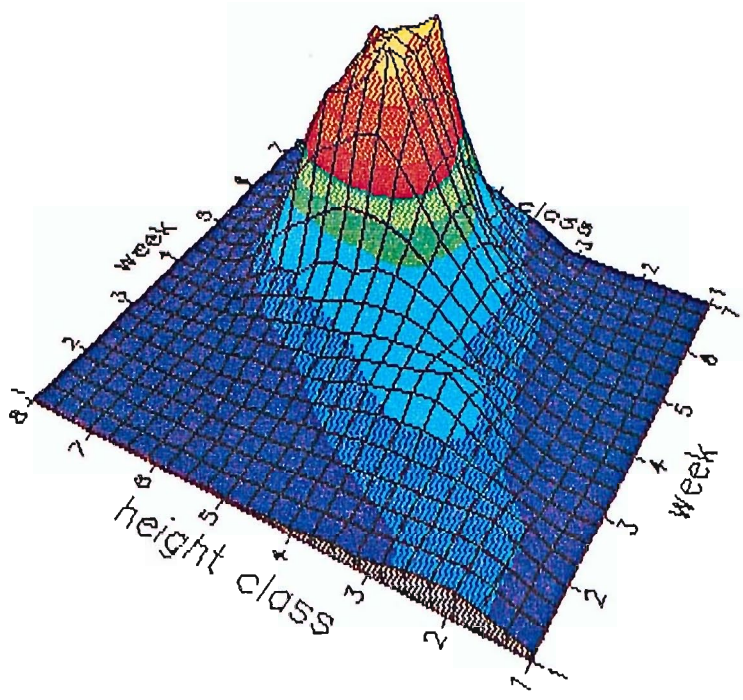
y - axis: variable = mid-range point of height class
(see Section 2.4.2)

Height	Mid-range point (cm)
1	10
2	30
3	50
4	70
5	90
6	110
7	130
8	150

Fig 2.33: 3-D figure of the number of *A. brassicae* lesions in each height class/plant

Contours = No. of lesions

cv. Bienvenu, 1987



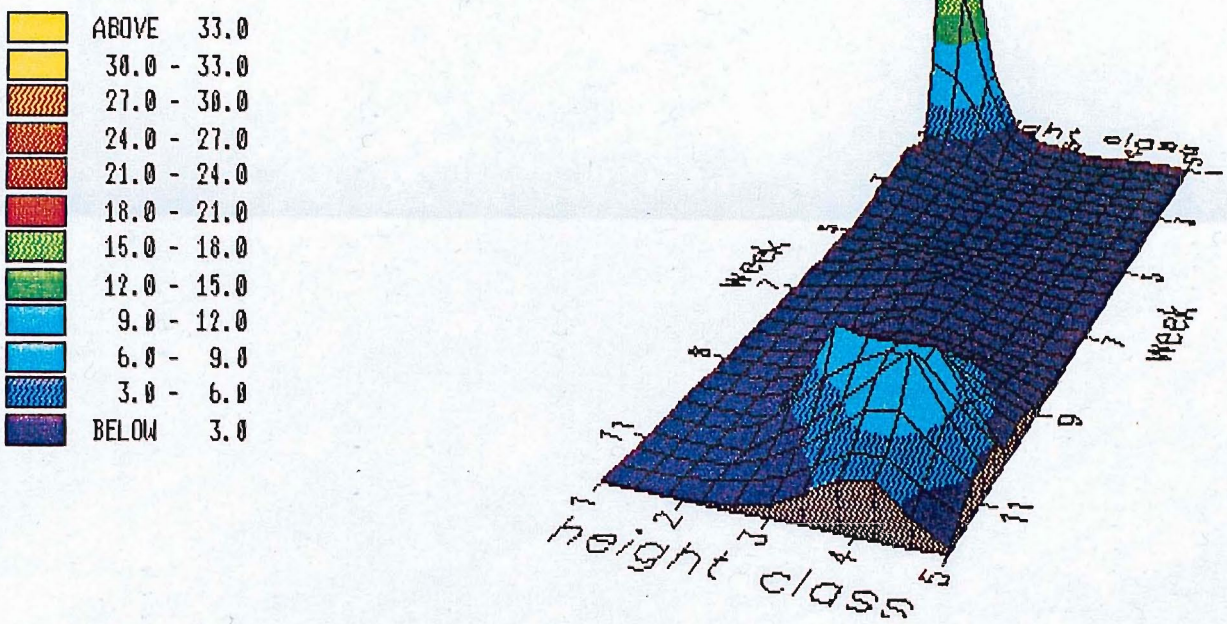
ABOVE	93.5
85.0 -	93.5
76.5 -	85.0
68.0 -	76.5
59.5 -	68.0
51.0 -	59.5
42.5 -	51.0
34.0 -	42.5
25.5 -	34.0
17.0 -	25.5
8.5 -	17.0
BELOW	8.5

Fig 2.34: Two views of the number of lesions in each height class/plant

Contours = No. of lesions

cv. Bienvenu, 1988

A


ABOVE 33.0
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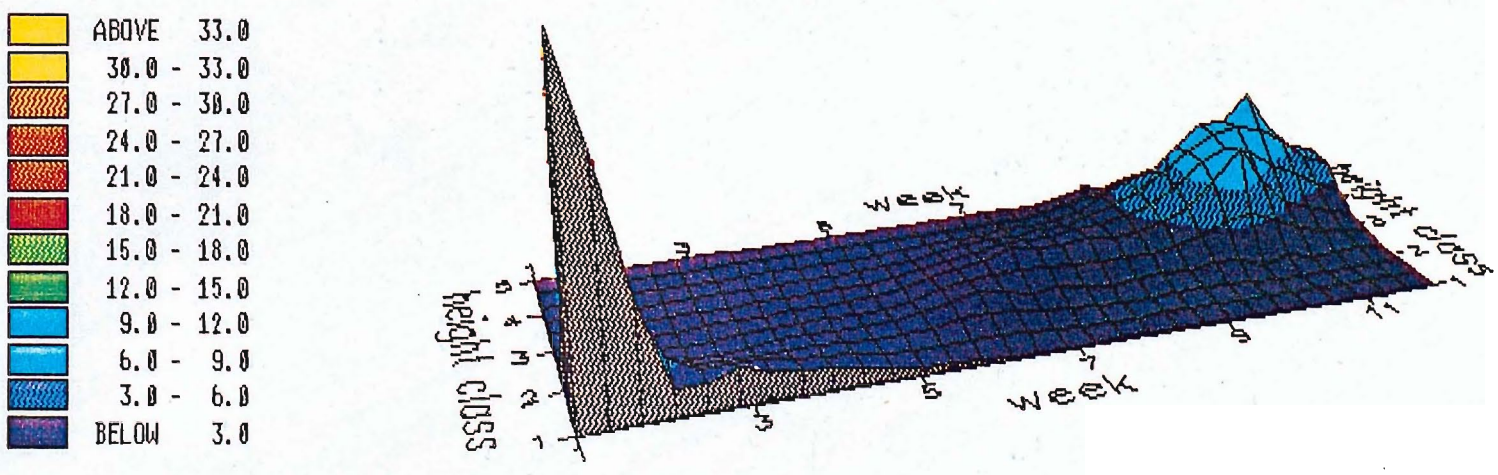
27.0 - 30.0
24.0 - 27.0
21.0 - 24.0
18.0 - 21.0
15.0 - 18.0
12.0 - 15.0
9.0 - 12.0
6.0 - 9.0
3.0 - 6.0
BELOW 3.0
week
height class

B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27.0 - 30.0
24.0 - 27.0
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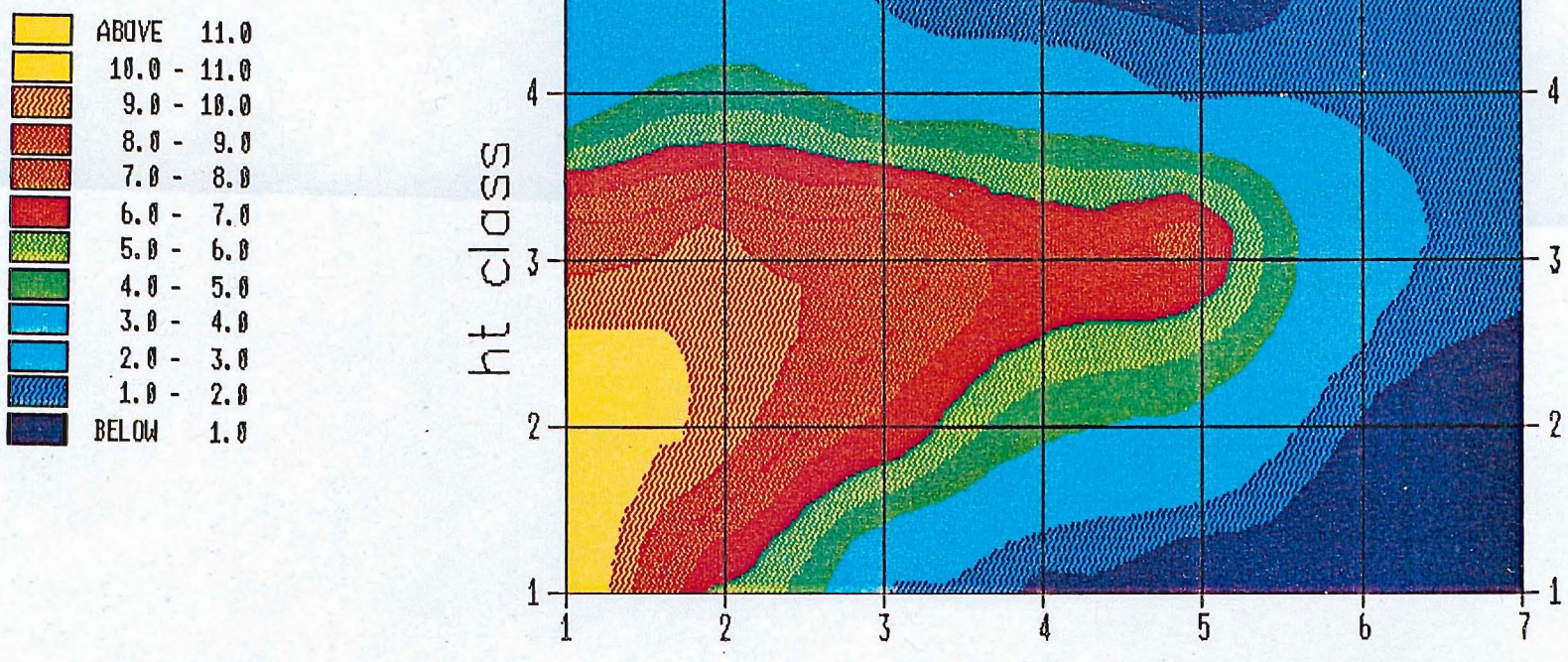
18.0 - 21.0
15.0 - 18.0
12.0 - 15.0
9.0 - 12.0
6.0 - 9.0
3.0 - 6.0
BELOW 3.0
height class
week

Fig 2.35: Rafal, 1986

A: Number of leaves in each height class/plant
Contours = No. of leaves

B: Number of *A. brassicae* lesions in each height class/plant
Contours = No. of lesions

A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10.0 - 11.0
9.0 - 10.0
8.0 - 9.0
7.0 - 8.0
6.0 - 7.0
5.0 - 6.0
4.0 - 5.0
3.0 - 4.0
2.0 - 3.0
1.0 - 2.0
BELOW 1.0
week
ht class
1 2 3 4 5 6 7
1 2 3 4 5

B

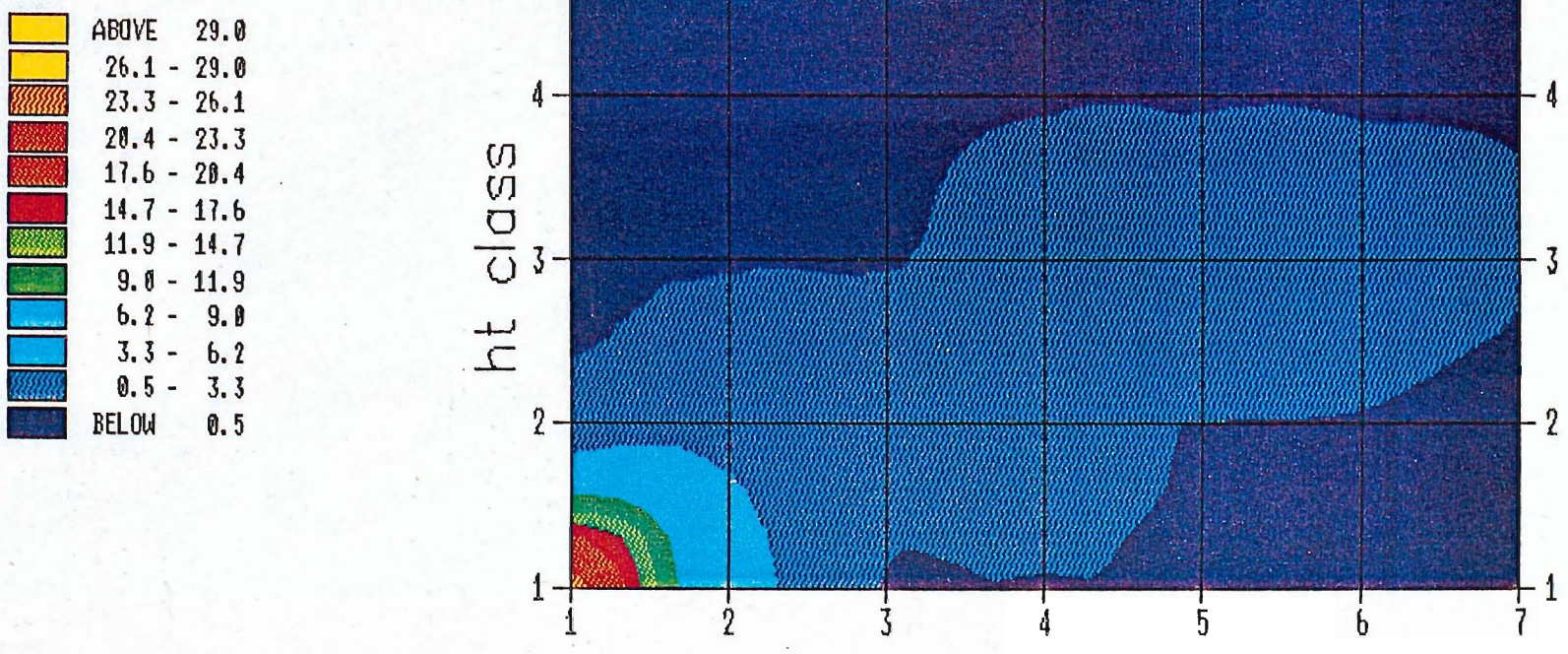
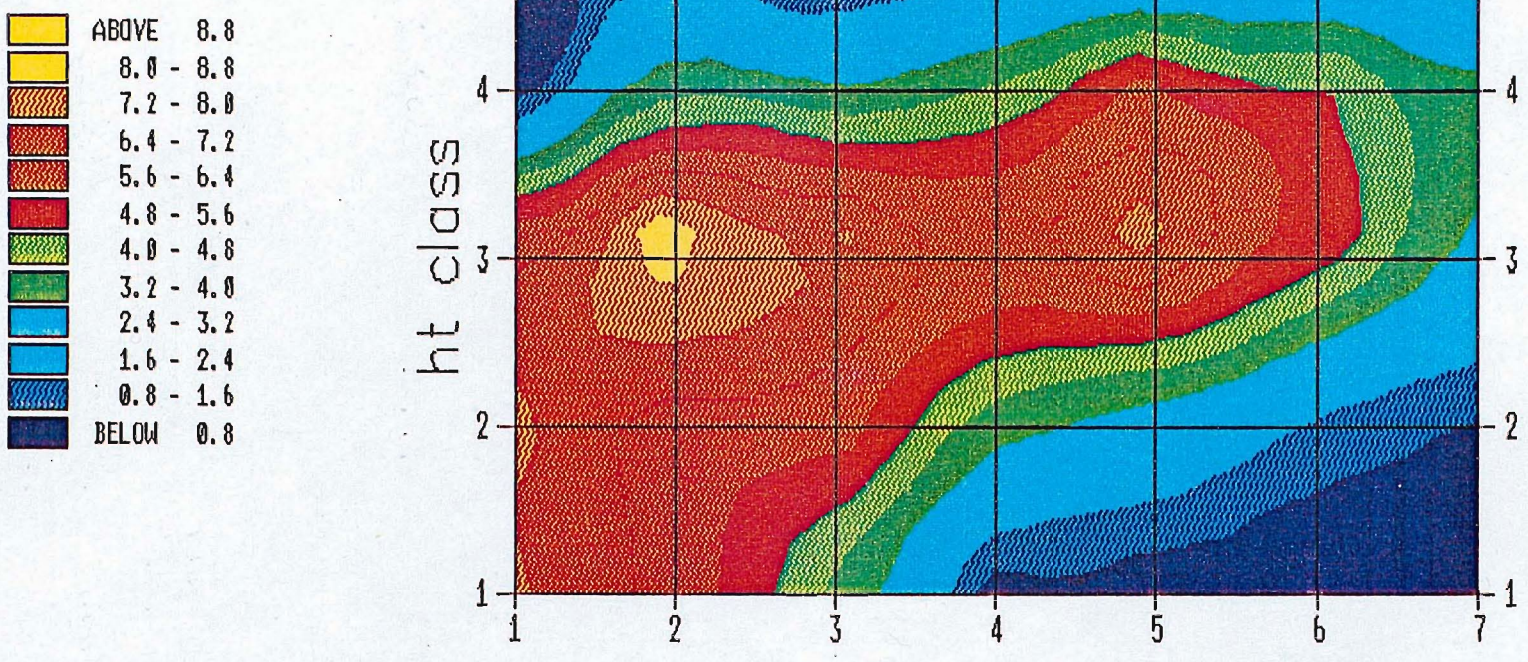

ABOVE 29.0
26.1 - 29.0
23.3 - 26.1
20.4 - 23.3
17.6 - 20.4
14.7 - 17.6
11.9 - 14.7
9.0 - 11.9
6.2 - 9.0
3.3 - 6.2
0.5 - 3.3
BELOW 0.5
week
ht class
1 2 3 4 5 6 7
1 2 3 4 5

Fig 2.36: Mikado, 1986

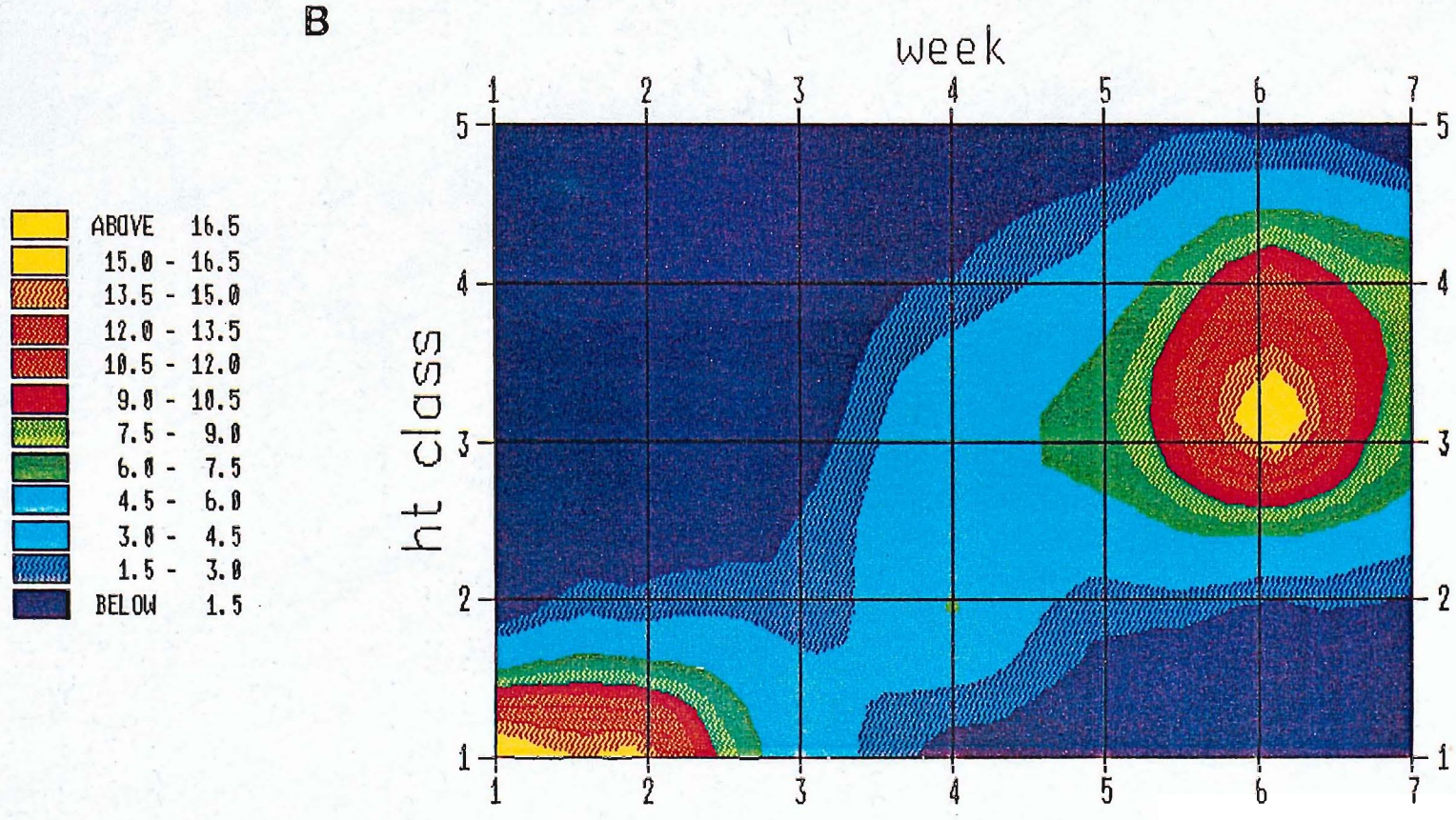
A: Number of leaves in each height class/plant
Contours = No. of leaves

B: Number of *A. brassicae* lesions in each height class/plant
Contours = No. of lesions

A


ABOVE 8.8
8.0 - 8.8
7.2 - 8.0
6.4 - 7.2
5.6 - 6.4
4.8 - 5.6
4.0 - 4.8
3.2 - 4.0
2.4 - 3.2
1.6 - 2.4
0.8 - 1.6
BELOW 0.8
week
ht class
1
2
3
4
5
6
7

B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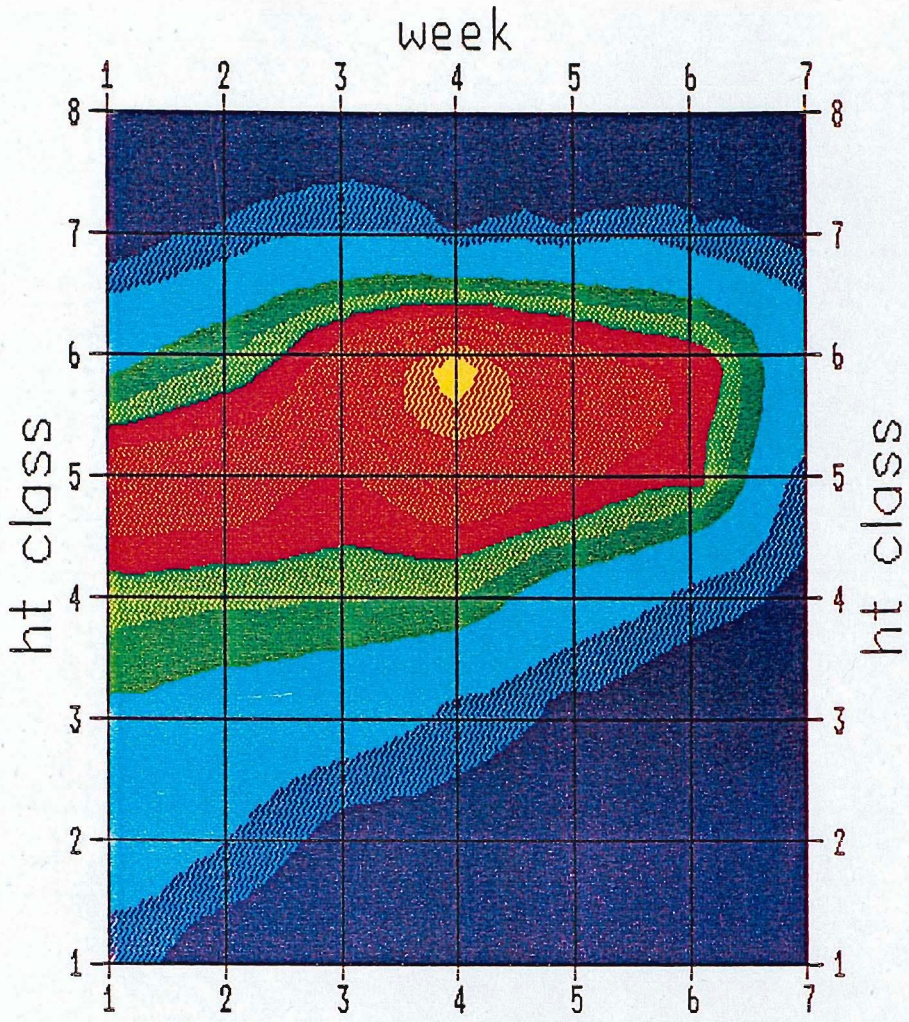
15.0 - 16.5
13.5 - 15.0
12.0 - 13.5
10.5 - 12.0
9.0 - 10.5
7.5 - 9.0
6.0 - 7.5
4.5 - 6.0
3.0 - 4.5
1.5 - 3.0
BELOW 1.5
week
ht class
1
2
3
4
5
6
7

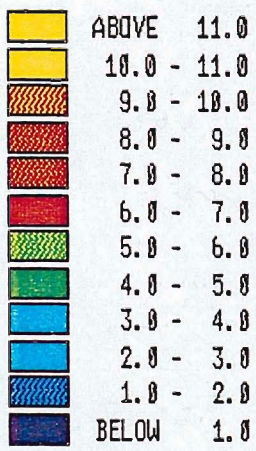
Fig 2.37: Bienvenu, 1987

A: Number of leaves in each height class/plant
Contours = No. of leaves

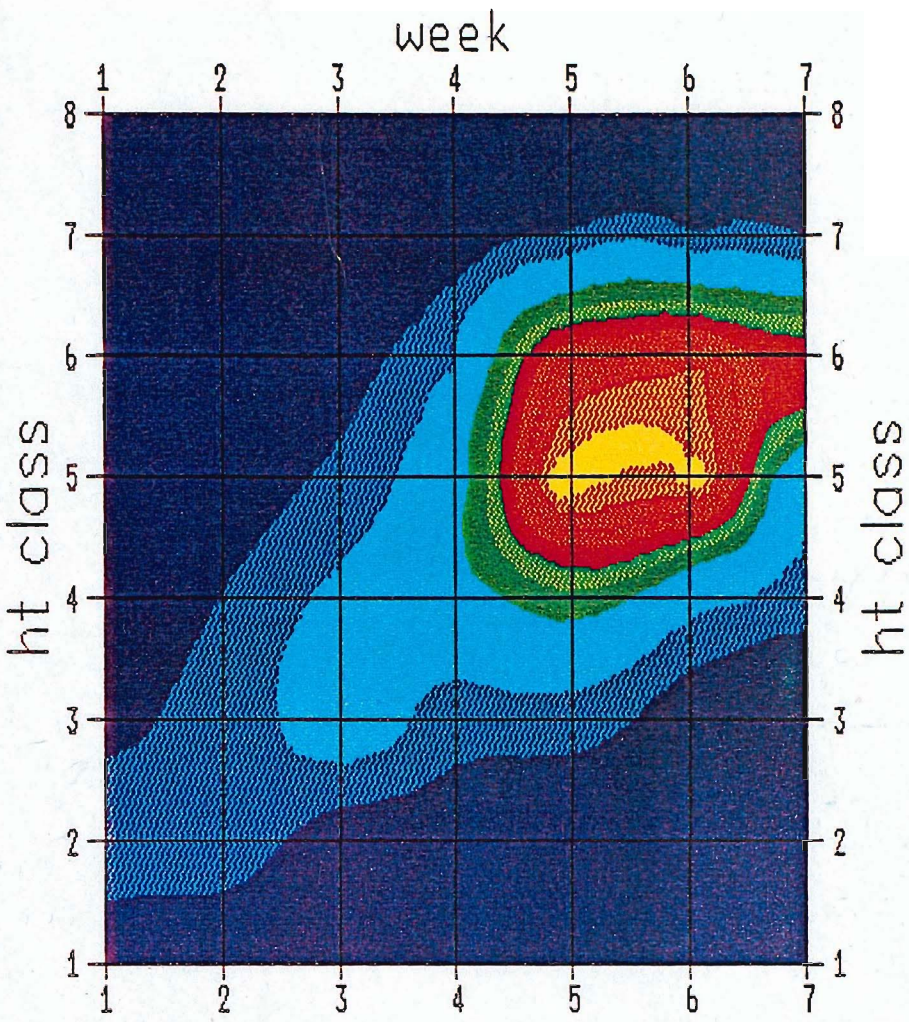
B: Number of *A. brassicae* lesions in each height class/plant
Contours = No. of lesions

A


week
1
2
3
4
5
6
7
8
ht class
ht class


ABOVE 11.0
10.0 - 11.0
9.0 - 10.0
8.0 - 9.0
7.0 - 8.0
6.0 - 7.0
5.0 - 6.0
4.0 - 5.0
3.0 - 4.0
2.0 - 3.0
1.0 - 2.0
BELOW 1.0

B


week
1
2
3
4
5
6
7
8
ht class
ht class

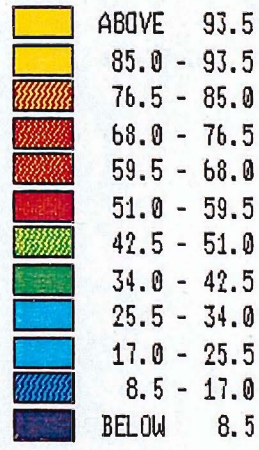
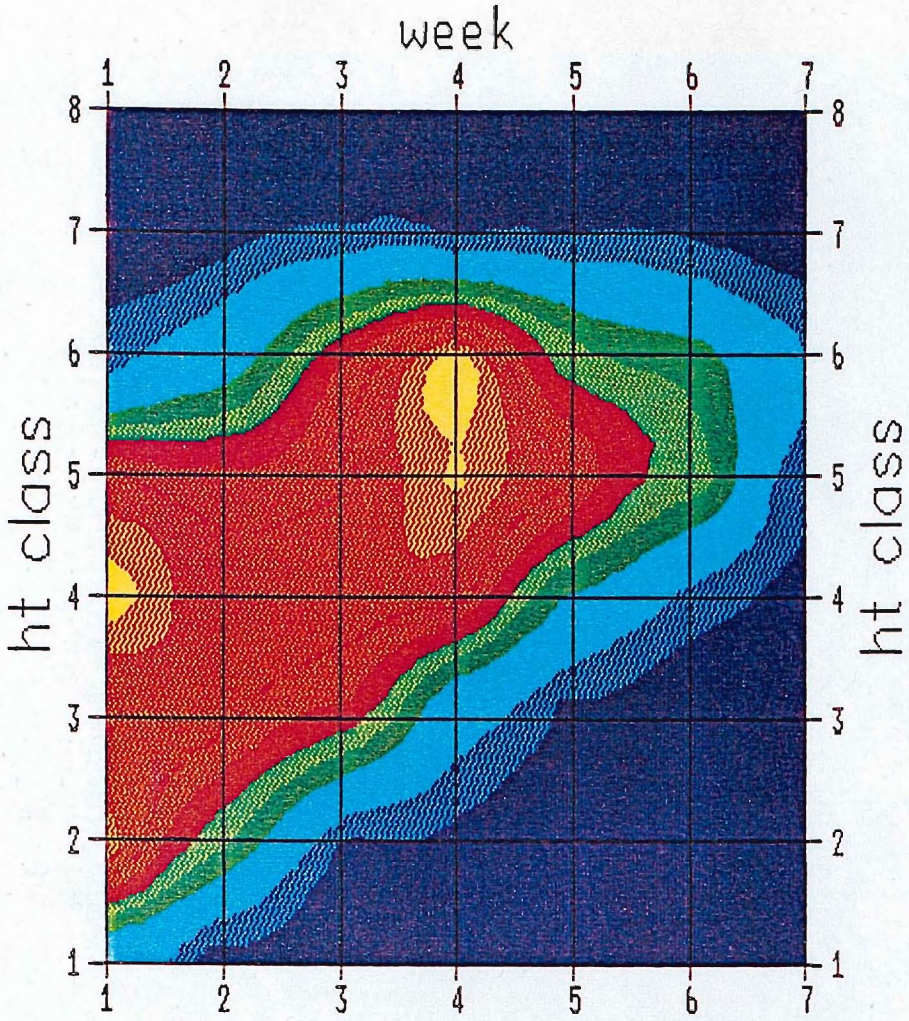

ABOVE 93.5
85.0 - 93.5
76.5 - 85.0
68.0 - 76.5
59.5 - 68.0
51.0 - 59.5
42.5 - 51.0
34.0 - 42.5
25.5 - 34.0
17.0 - 25.5
8.5 - 17.0
BELOW 8.5

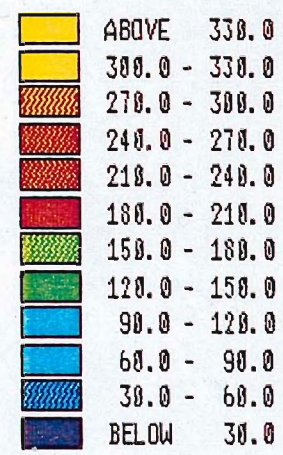
Fig 2.38: Bienvenu, 1987

A: TCLA in each height class/plant
Contours = cm^2

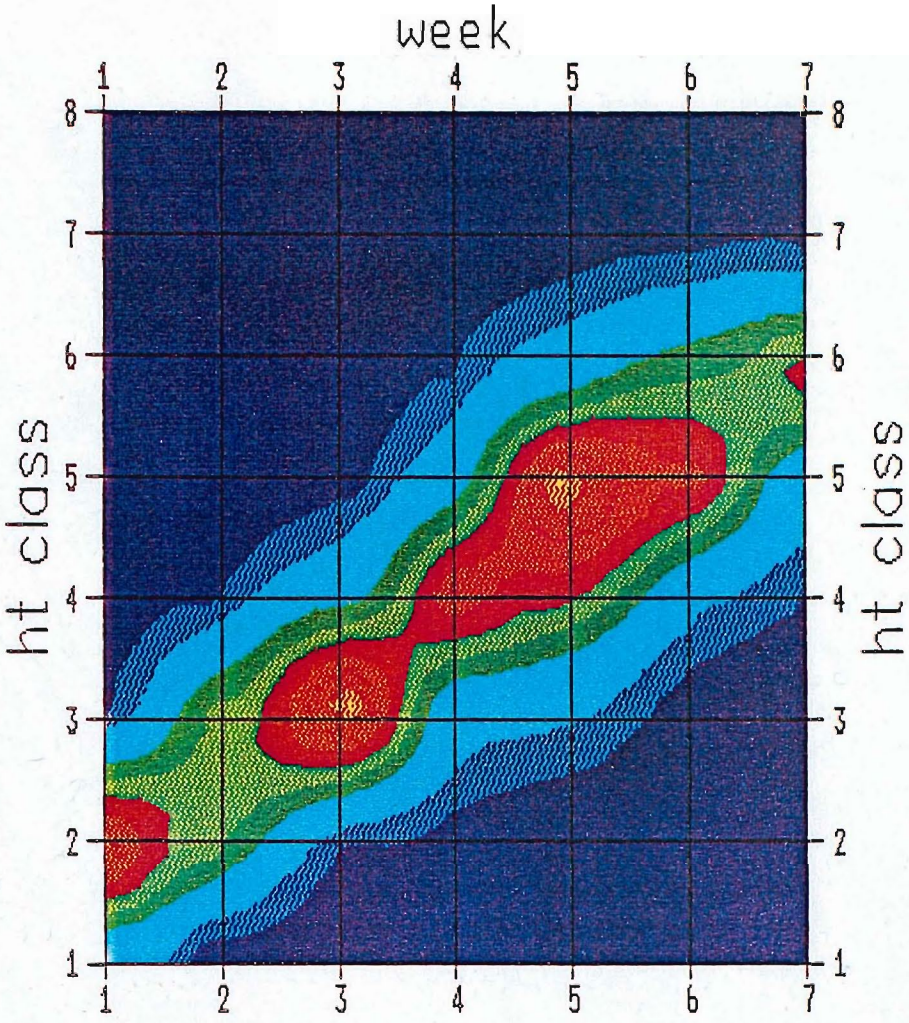
B: TCDA in each height class/plant
Contours = cm^2

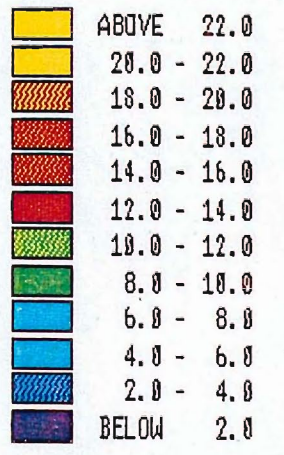
A


week
1
2
3
4
5
6
7
8
ht class
ht class


ABOVE 330.0
300.0 - 330.0
270.0 - 300.0
240.0 - 270.0
210.0 - 240.0
180.0 - 210.0
150.0 - 180.0
120.0 - 150.0
90.0 - 120.0
60.0 - 90.0
30.0 - 60.0
BELOW 30.0

B


week
1
2
3
4
5
6
7
8
ht class
ht class


ABOVE 22.0
20.0 - 22.0
18.0 - 20.0
16.0 - 18.0
14.0 - 16.0
12.0 - 14.0
10.0 - 12.0
8.0 - 10.0
6.0 - 8.0
4.0 - 6.0
2.0 - 4.0
BELOW 2.0

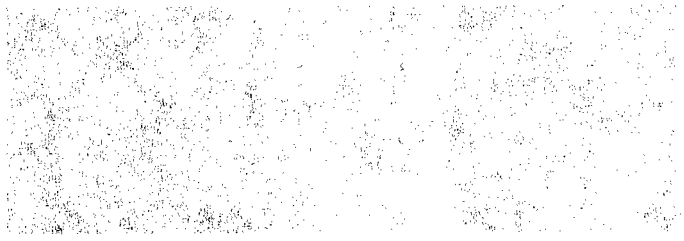
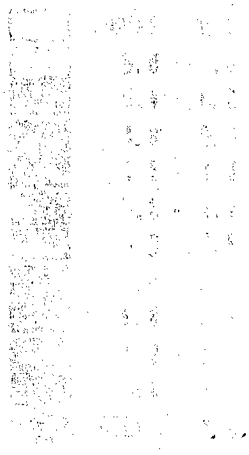


Fig 2.39: Number of sporulating lesions in each height class/plant

Contours = No. of lesions

Bienvenu, 1987



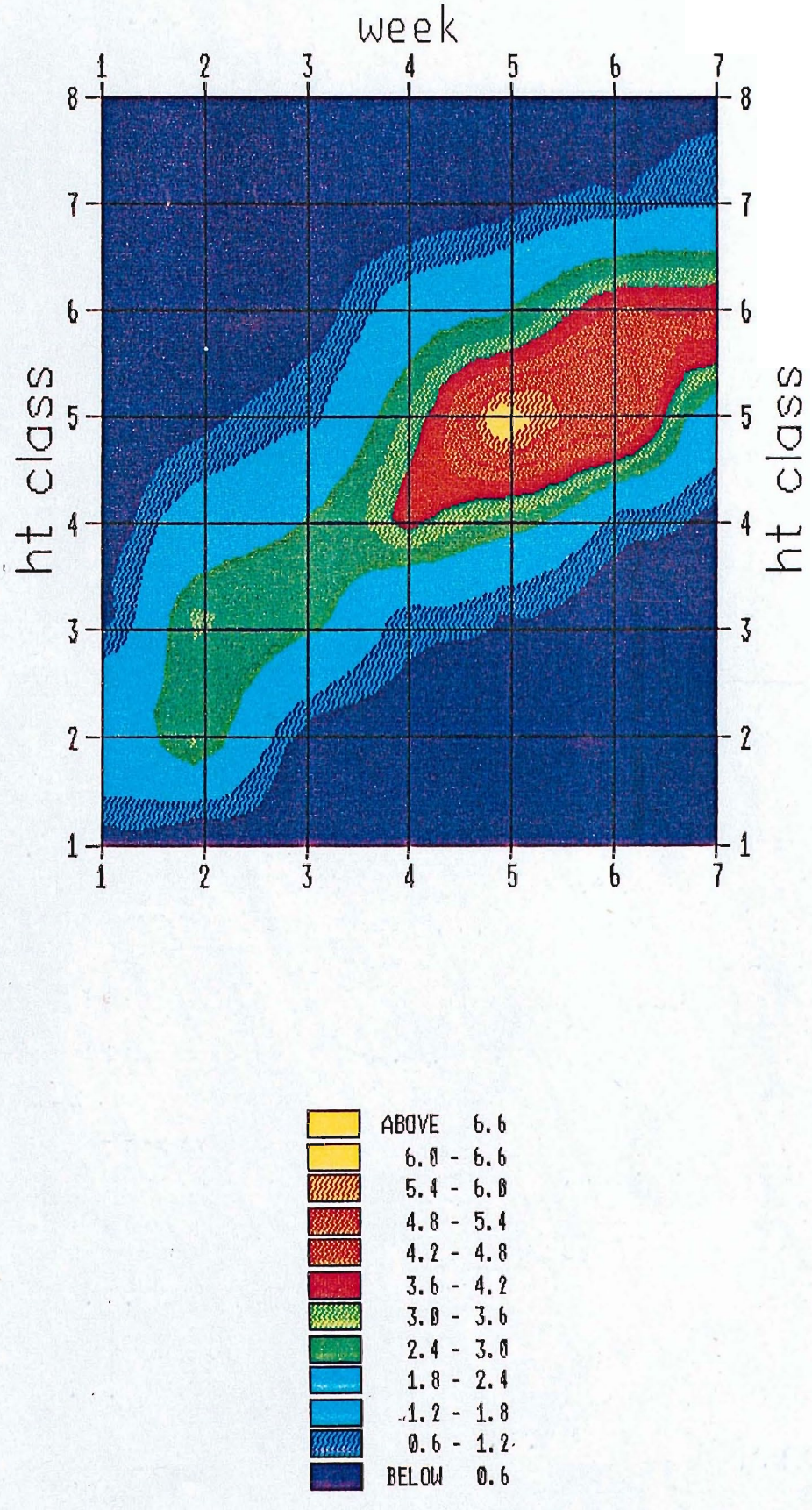
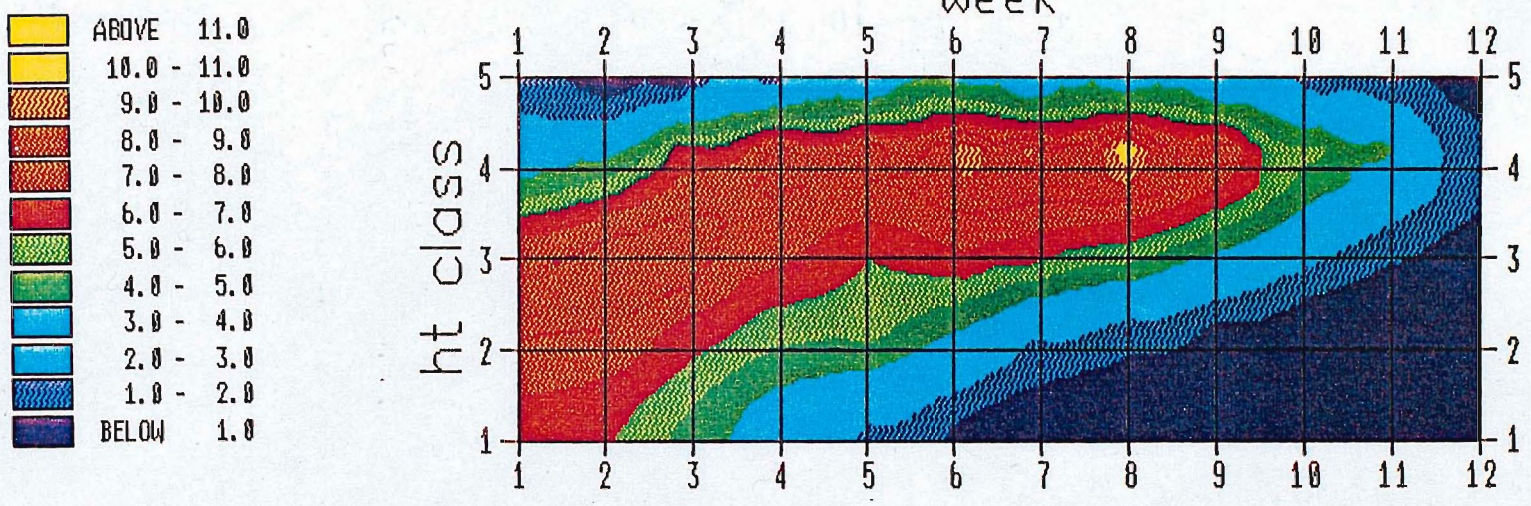

week
1
2
3
4
5
6
7
8
ht class
ht class
ABOVE 6.6
6.0 - 6.6
5.4 - 6.0
4.8 - 5.4
4.2 - 4.8
3.6 - 4.2
3.0 - 3.6
2.4 - 3.0
1.8 - 2.4
1.2 - 1.8
0.6 - 1.2
BELOW 0.6

Fig 2.40: Bienvenu, 1988

A: Number of leaves in each height class/plant
Contours = No. of leaves

B: Number of *A. brassicae* lesions in each height class/plant
Contours = No. of lesions

A


ABOVE 11.0
10.0 - 11.0
9.0 - 10.0
8.0 - 9.0
7.0 - 8.0
6.0 - 7.0
5.0 - 6.0
4.0 - 5.0
3.0 - 4.0
2.0 - 3.0
1.0 - 2.0
BELOW 1.0
week
ht class
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5

B

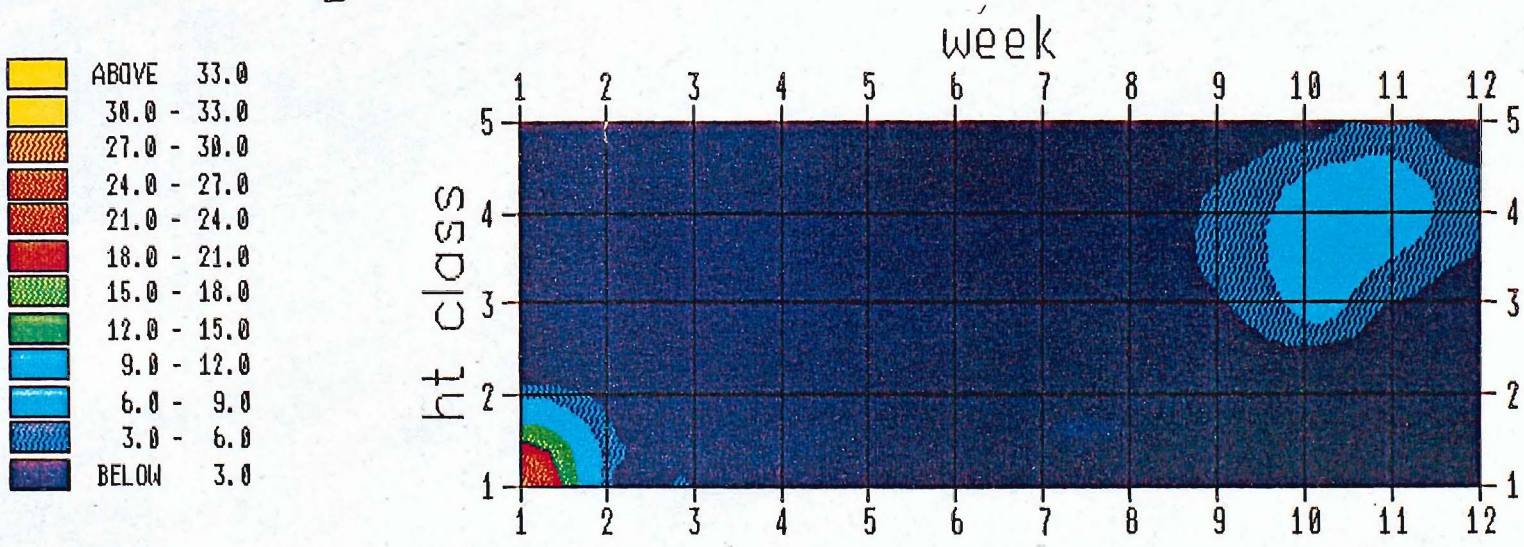
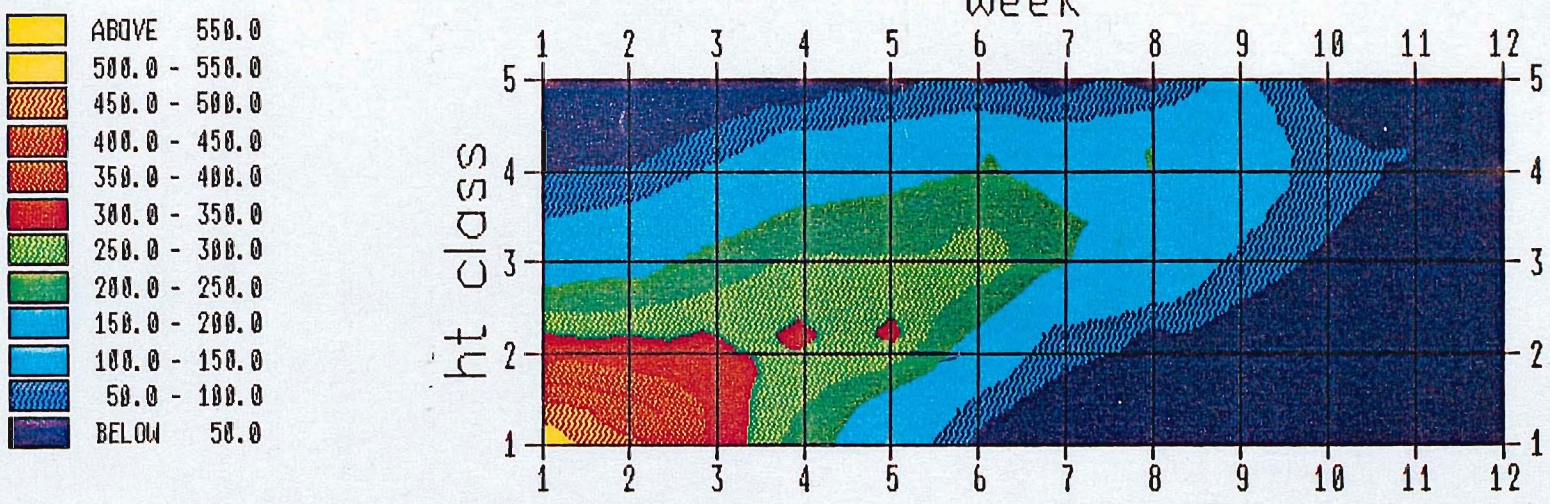

ABOVE 33.0
30.0 - 33.0
27.0 - 30.0
24.0 - 27.0
21.0 - 24.0
18.0 - 21.0
15.0 - 18.0
12.0 - 15.0
9.0 - 12.0
6.0 - 9.0
3.0 - 6.0
BELOW 3.0
week
ht class
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5

Fig 2.41: Bienvenu, 1988

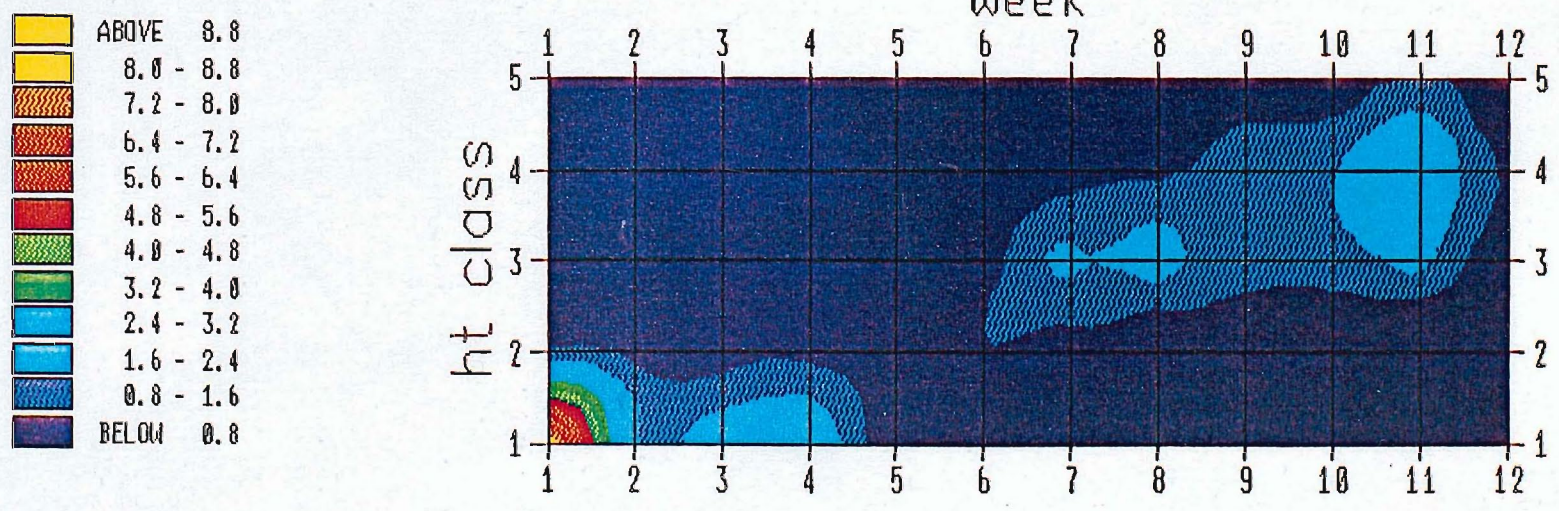
A: TCLA in each height class/plant
Contours = cm^2

B: TCDA in each height class/plant
Contours = cm^2

A


ABOVE 550.0
500.0 - 550.0
450.0 - 500.0
400.0 - 450.0
350.0 - 400.0
300.0 - 350.0
250.0 - 300.0
200.0 - 250.0
150.0 - 200.0
100.0 - 150.0
50.0 - 100.0
BELOW 50.0
week
ht class
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5

B


ABOVE 8.8
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7.2 - 8.0
6.4 - 7.2
5.6 - 6.4
4.8 - 5.6
4.0 - 4.8
3.2 - 4.0
2.4 - 3.2
1.6 - 2.4
0.8 - 1.6
BELOW 0.8
week
ht class
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5

2.5 Discussion

2.5.1 Plant development

The morphological development of the leaf canopy of oilseed rape is incompletely described in the literature. Daniels *et al.* (1986) in their review present a single graph of the development of cv. Bienvenu. Others (e.g. Allen & Morgan, 1972; Allen & Morgan, 1975) give data representing single dates but do not describe a general picture of development. The development of the leaf canopy of oilseed rape can be divided into three phases: the "rosette " phase in which the plant overwinters; the stem extension phase during which the apex of the plant is raised well above the ground and new branches and leaves develop; the leaf loss phase when leaf loss is greater than leaf development and leads to complete loss of leaf tissue from the plant. In this study, the four crops show very similar patterns of development although differing in their rapidity of development and their size, even when the same cultivar was grown (i.e. 1987 and 1988). Only 1986 data are available for the "rosette " phase of plant development. During this period cvs. Rafal and Mikado behaved similarly with a slow development of new leaves following the the very cold weather conditions in February. All development occurred on the mainstem.

Data from 1986 and 1988 described plant development during the stem extension phase. Rafal, began stem extension one week before Mikado (Fig 2.1), a time lag which remained until harvest. This agrees with the NIAB ratings for earliness of these varietes (Anon,1986). Total leaf numbers on Rafal were greater than on Mikado and as the vertical distribution of leaf material was similar in each variety (Fig 2.35 & 2.36) this led to a denser crop canopy in Rafal. In 1988 (cv. Bienvenu) stem extension started earlier than with cv. Rafal and Mikado in 1986. This was probably due to the mild, wet spring which encouraged plant growth. Final leaf numbers were similar to those on cv. Mikado in 1986. The vertical distribution of leaves on cv. Bienvenu in 1988 changed considerably during this stem extension period so that by the end of stem extension the largest number of leaves occurred approximately two thirds up the plant. A different pattern of vertical development

with TCLA showed that the maximum area of leaf material changed its vertical position throughout the life of the crop, gradually moving upwards towards the pod canopy. This is in contrast to some other crops (e.g. cereals, Butler, Pers. comm.) which have their greatest leaf area density approximately two thirds from the base of the crop by the time that leaf numbers reach their maximum. The increase in leaf material during stem extension was due almost entirely to development on the primary branches. Although new leaves were developing on the MS an overall loss of leaf material on the MS was occurring because of the senescence of large stalked leaves at the crop base. The beginning of the leaf loss phase of development commenced when leaf loss from the plant was greater than the number of new leaves appearing. At this time a very similar pattern of leaf development was seen on each crop. The number of leaves on the primary branches was substantially greater than the numbers on the main stem. With TCLA the difference was not so great due to the generally smaller leaf size on the BR leaves. The size of leaves in different positions on the canopy also explains the difference between the pattern of vertical development of leaf numbers/plant and TCLA. Whereas the vertical distribution of leaf numbers takes no account of leaf size, TCLA responds greatly to leaf area. The contribution of the large stalked leaves at the canopy base to TCLA is therefore greater than that of the small sessile leaves higher in the crop. The vertical position of maximum leaf area therefore changed only as the larger leaves were lost from the crop base. Loss of leaf material from the plants was almost linear in all crops, although the rates of loss were different (Section 2.1). In all cases apparent leaf loss began earlier from the main stem than from the branches. Leaf loss occured from the base of the plant upwards (Figs 2.35-2.41) so that when only a few leaves were left they were found just below the pod canopy.

The pattern of plant development in the four crops was therefore similar and results from the MS leaves corresponded to the data presented by Daniels *et al.* (1986). However, the timing of development and the size of the plants differed considerably between crops which may have had a substantial effect on the microclimate within the crop and the distance that disease has to progress from the lower part of the leaf canopy to the pods.

2.5.2 Disease development

Little published work is available on the development of *Alternaria* leaf spotting on the leaf canopy of oilseed rape. Wadhwani & Dudeja (1982) suggested three phases for the development of *Alternaria* disease on *Brassica juncea*. In the first phase the disease appeared on leaves in contact with the soil; it then moved up the plant during heavy rain (second phase) and finally developed on to the pods. In India Husein & Thakur (1963) showed that high levels of leaf spotting could occur on the leaf canopy before pod canopy development, but gave no details about disease progress. Apparently no detailed descriptions of leaf spot development have been published using U.K. data although Gladders (1983) suggested that leaf spotting should only be considered as an inoculum source for disease on the pods.

This study has confirmed the view (Gladders, 1983) that *Alternaria brassicae* is only found at low intensities on the leaf canopy of oilseed rape in the U.K.. Even in 1987, when disease on the pods eventually reached >40% pod area diseased on most racemes the percentage area of leaf tissue diseased was never greater than 16.5%.

The patterns of disease development (TALLF and TCDA) can be divided into three phases corresponding to the phases of plant growth described in Section 2.5.1. i.e. the "rosette "phase, the stem extension phase and the leaf loss phase. The 1986 data indicate that *Alternaria* leaf spot was present at low levels throughout the late winter and early spring during the rosette period of plant development (Fig 2.8). During this time the disease became widespread spatially (>90% of plants infected). Although some infected lower leaves were lost during this period, levels of *Alternaria* remained constant indicating that some infection was taking place. Infection at low temperatures which would have been encountered during this time has been demonstrated in the laboratory (Mhrida, 1983).

Data describing disease development during stem extension were also mainly available from 1986. An increase in disease on cv Mikado and cv. Rafal appeared to coincide with BR leaf production. However, lesions were mainly confined to MS

leaves at the base of the plant (Figs 2.35 & 2.36). The level of disease on cv. Bienvenu, 1988 on 4 April also suggests that disease had developed on the basal MS leaves during this plant development stage. In this phase disease levels still remained low in comparison with potential levels (e.g. late 1987).

Although data are limited for disease development during the "rosette " and stem extension phases of oilseed rape growth (GS 1,00-2,10) a typical pattern of development can be suggested. During the "rosette " phase the disease becomes well dispersed (possibly early after emergence) but remains at low intensities. During stem extension, any disease which does develop will be confined to the basal MS leaves. Therefore at the end of stem extension disease can be found on most plants confined to the base of the crop and generally on the MS leaves. The number of lesions/plant may differ considerably but are still low compared with the levels which might occur later in the season.

As discussed previously (Section 2.5.1) the third period of leaf canopy development corresponds to a gradual loss of leaves from the plant. Interpretations of disease development must therefore take into account this dramatic change in plant structure. Many similarities can be seen between the development of disease during the leaf loss phase in three of the epidemics studied (Mikado, 1986; Bienvenu, 1987 & 1988) despite the differences in disease severity. The general pattern which emerges is the progress of disease from basal MS leaves to upper BR leaves.

The confinement of disease to the base of the crop at the beginning of leaf loss is attributable to the rapid growth in height of the plant during stem extension. From its "rosette " form with the top leaves 5-10cm above ground level stem extension causes the top of the crop canopy to rise quickly so that by the end of stem extension the leaf canopy has a substantial vertical range. The older leaves infected with *A. brassicae* remain close to the ground with expanding BR leaves forming the bulk of the new canopy. The disease then progresses slowly up through the leaf canopy towards the pod canopy. This step by step progression is similar to that noted in other pathosystems (e.g. Royle *et al.* 1986).

Another clear pattern which arises from these data is the changing distribution of disease between MS and BR leaves during the season. This is expected as the proportion of BR leaves increases and the disease moves toward the upper part of the plant. However, in each crop few *Alternaria* lesions occurred on BR leaves until some weeks after BR leaf production was completed even though BR leaves were spatially close to infected MS leaves and during a period when lesion numbers were still increasing on the MS leaves. The spatial proximity of BR leaves with infected MS leaves seems to preclude the possibility that such a difference in infection was caused by differences in inoculum level, although dataareneeded to support this view. A difference in susceptibility to infection by *Alternaria* between the MS and BR leaves would, however, clearly lead to the effect described above. Kohle & Hoffmann (1989) and Mhrida (1983) have both shown that susceptibility of oilseed rape leaves to infection by *A. brassicae* increases with maturity although Mhrida (*Ibid.*) also showed that susceptibility decreases again during senescence. In the field such differences in susceptibility between young and older leaves would, during leaf canopy development lead to leaves lying in close spatial proximity which have very different susceptibilities to the pathogen. (e.g. BR and MS leaves). Therefore a different intensity of infection may occur on closely associated leaves as described in this study.

Although other factors are likely to be important (e.g. meteorological parameters and inoculum levels) a difference in susceptibility to the pathogen of different age leaves could also be a contributory factor in the gradual development of disease up the plant. Stem extension not only causes a spatial division between the leaves at the top and the bottom of the canopy but also an age gradient, so that leaves in the upper part of the canopy are younger than those at the base. Vertical spread of the pathogen may therefore be initially limited by the less susceptible leaf tissue in the upper and middle parts of the leaf canopy. Circumstantial evidence for this is provided by the contour mapping for 1987 which showed initial disease development to be associated with the bottom edge of the leaf canopy. Futhermore with TCDA in 1987 the maximum areas of diseased tissue remained at the bottom edge of the leaf canopy throughout the season suggesting that the larger disease lesions were associated with maturing tissue. This may be a consequence of the time taken for lesion growth or the effect of leaf age on lesion development. The pattern

of vertical distribution of the number of sporulating lesions in 1987 is also related to this hypothesis, because sporulation generally occurs on the older, larger lesions (Humpherson-Jones & Phelps, 1989) and hence the vertical distribution of TALLSP resembles that of TCDA rather than TALLF.

By the time leaf loss was virtually complete, disease was spread throughout the small amount of leaf material remaining. It was only during this period that the proportion of tissue attacked by *A. brassicae* increased rapidly in three of the four crops. and even then did not reach high levels of severity. These increases were in part the result of new infections but especially in 1987, where numbers of lesions and TCDA were dropping in the final two weeks of assessment the increase in proportion of disease was mainly attributable to the loss of uninfected leaf tissue.

The low severity levels of leaf spotting noted in this study are unlikely to have caused a direct effect on yield at this stage of crop development when most of the photosynthetic tissue is contained in the stems and pods. However, it is important to note that whatever levels of disease are present they are positioned very close to the pod canopy and could act as a source of inoculum for infection on the pods.

Levels of disease were very different in 1987 compared with the other seasons. Even on the first assessment levels were high compared with similar periods in other years. Unfortunately no early disease data are available and the reason for such disease intensities is unknown. Although high levels continued to be found throughout the season, there was no substantial difference in weather conditions compared with the other years. (see Part 4). However these data do suggest that the levels of disease early in the season may have a substantial effect on disease severity later in the season.

In summary, the levels of dark leaf spot caused by *A.brassicae* did not reach high levels in any of the four disease epidemics studied. The pattern of disease development during the epidemic was similar in all cases except in the latter stages of leaf fall in Rafal 1988. During the "rosette " stage of plant development disease remained at low levels but was spatially widespread in the crop. During stem

extension the disease continued to develop slowly but was confined to the basal MS leaves. After this period disease gradually progressed vertically up the canopy until it was distributed in a pattern similar to that on the leaves. Vertical movement of the disease and distribution of disease between MS and BR leaves may be influenced by leaf age but the evidence is circumstantial and a detailed study is required to investigate this hypothesis. The proportion of leaf tissue attacked by dark leaf spot was never high and it is unlikely that in the four epidemics examined that this phase of the disease had a direct effect on yield.

2.5.3 Sampling methods, disease levels and disease prediction

Different measures of disease describe different aspects of disease development (Part 2, Chapter 2) and therefore the assessment technique chosen for a particular study must depend on the objectives of that study. The % area of leaf material diseased gives a measure of the proportion of photosynthetic area which has been destroyed by disease and is often related to crop yield losses (James, 1974). During this study, even in 1987 when the numbers of *Alternaria* lesions on the leaf canopy were high, the % area of disease did not reach substantial levels (Section 2.5.2). It is therefore likely that disease assessment on the leaf tissue may need to fulfil objectives other than an estimate of leaf material destroyed. The most obvious uses for assessment of *Alternaria* on the leaf tissue are therefore in detailed studies of disease development or measurements of disease potential for systems used to predict disease development on the pods.

As a measure of disease potential, % leaf area diseased may be of little use. Being a measure of disease, relative to the plant material it only gives an estimate of the actual amount of disease present if the size of the leaf canopy remains constant throughout the development of the disease epidemic. In oilseed rape the size of the leaf canopy is changing throughout the season and hence the % area of leaf material diseased represents the combined factors of the change in disease and leaf area. It is this factor that also makes mathematical analysis of the progress curve difficult. The analysis of DPCs using the simple mathematical techniques developed by Van der Plank (1963) (see Part 3, Chapter 3) and others (e.g. Richards, 1959),

assume that the area of crop canopy remains constant throughout the epidemic. Complex modifications to incorporate crop development have been made (Van der Plank, 1963; Waggoner, 1986) but in this situation where disease levels are low they are of limited value. However, % area assessments can be made on a whole plant or whole plot basis, and many studies will continue to use this method. In such cases careful interpretation of the data is required, as erroneous conclusions could be drawn because of the obvious similarity with disease development curves of other pathosystems.

TCDA and TALLF are measures of disease which although affected by leaf loss (and hence removal of disease tissue) are not relative measures of disease; they both estimate absolute disease levels. However, they do measure different attributes of the disease. The number of lesions records the number of infections which have taken place and developed to form lesions. It integrates spore dispersal, infection and leaf loss in a single measure. It estimates the past potential of the pathogen to infect and produce lesions and will give some indication of future potential assuming that some lesions will develop and produce spores.

TCDA gives no direct information of infection except that at some time infection must have occurred. For example TCDA would continue to increase even if all the infection occurred on a single day as long as the lesions produced continued to grow. TCDA provides an estimate of the total amount of disease area present. (Part 2, Chapter 1). It gives little information about the pathogen 's former potential for infection, but assuming that TCDA is related to TCDASP it could give a good estimate of future potential. In this study TCDA gave the best estimate of disease on the leaf canopy even though the errors incorporated into its measurement were large (see Part 2, Chapter 3). This is because it gives some estimation of the true area of disease which neither TLFDIS or TALLF can give. The pattern of disease development described by TCDA differed substantially between 1987 and 1988 (Figs 2.12 & 2.41) and both differed considerably from the development pattern described by % leaf area infected. Of particular interest is the difference observed between 1 June and 15 June 1987 where the proportion of leaf area infected was increasing rapidly (7.8-16.4%) and the TCDA was decreasing, showing the inappropriateness of

% leaf area infected as a true measure of disease in the oilseed rape canopy. The major difficulty with measurement of TCDA is the time required for assessment, due to the need to assess and measure each leaf individually and the large number of samples required, compared with TALLF (see Part 2, Chapter 2). An assessment method which is simpler, quicker and less prone to error is to count the number of lesions. However, it is less likely to represent the true area of disease present. A visual comparison between the patterns of development of TCDA and number of lesions in both years showed a similar basic pattern, an inverted "V" in 1987 and "U" in 1988. However the changes in the amount of disease appeared greater when the number of lesions was used as is shown by the ranges in TCDA (21.3 - 44.0) and number of lesions (24 - 220) for 1987. These differences between lesion numbers and TCDA are further reinforced with the failure of attempts to relate the different measures of disease using linear regression (Part 2, Chapter 3).

The calculated area of sporulating tissue (TCDASP) and number of sporulating lesions (TALLFSP) also give different information about disease development. Counting the number of sporulating lesions improves the information content compared with counting the number of lesions, for estimating the potential of disease development, by ennumerating the sites from which dispersion can occur. TCDA of sporulating tissue improves this still further by giving an estimate of the total amount of that sporulating tissue. Information, is however lost as TCDASP does not estimate the total levels of disease at the time of assessment.

If assessment is required for a predictive system, the evaluation of the area of disease which is sporulating would probably give a better indication of the level of inoculum potential in the crop. In 1987 and 1988 assessment of sporulating tissue using TCDASP and TALLFSP shows that the general pattern of development was similar to that of the TCDA or TALLF (i.e. inverted "V" shape, 1987; "U" shaped, 1988). There are however substantial problems in using sporulating tissue for assessment. Firstly, this type of assessment would be very time consuming, each lesion having to be looked at with a hand lens or binocular microscope. Secondly the areas and numbers of lesions that sporulate remain small, which would require a very large

increase in the numbers of plants sampled compared with TCDA or TALLF and probably preclude the use of such data in any practical situation.

In summary, if the main aim of assessment of *A. brassicae* on the leaves of oilseed rape in the U.K. is to predict further disease development, % leaf area diseased may be of little use. Of the "absolute " estimates of disease TCDA gave the best estimate of disease in this study, but number of lesions was a quicker and simpler measure, less prone to error and giving at least similar patterns of development to TCDA. The measurement of sporulating tissue although, perhaps giving a more relevent measure of inoculum potential would be very time consuming and unless large samples were taken very error prone.

CHAPTER 3: POD CANOPY

3.1 Plant development.

3.1.1 Rafal 26 June-17 July, 1986.

Flowering had begun by 8 May and continued for approximately four weeks, being completed by 5 June. Pods began to appear on Rafal during flowering in the middle of May. Over the period 26 June-17 July, pod numbers per plant remained relatively constant (Fig. 3.1).

3.1.2 Mikado 26 June-17 July, 1986.

Flowering in Mikado had just started on 8 May but did not finish until ten days after Rafal on 15 June. Pods began to appear on Mikado during flowering in the middle of May. As with Rafal pod numbers in the samples remained relatively constant between 26 June and 17 July (Fig. 3.1).

3.1.3 Bienvenu 11 May - 6 July, 1987.

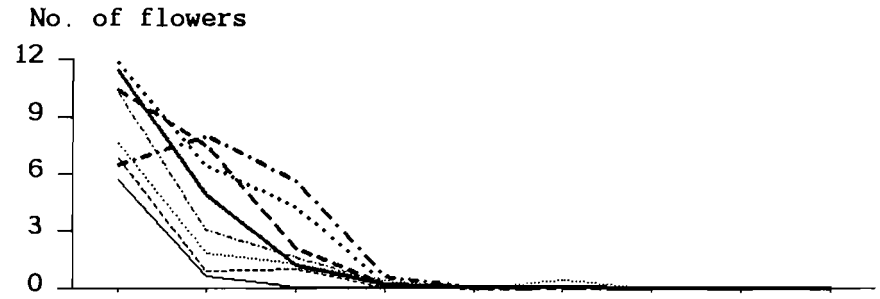
In 1987 sampling started during flowering. On 4 May the numbers of flowers and buds on each raceme had either reached a peak or were already declining (Fig 3.2), except on raceme 7 where a slight increase in the numbers of flowers was observed between 4 and 11 May. The pattern of flower loss was similar in all racemes but took place in order of raceme position i.e. Raceme T, Raceme 1,...Raceme 7.

The decrease in numbers of flowers was mirrored by an increase in pod numbers on the racemes (Fig. 3.2). Maximum pod numbers were reached by the different racemes by different dates; Raceme T by 11 May, Raceme 1 to 3 by 16 May, Raceme 4 and 5 by 25 May, Raceme 6 by 1 June and Raceme 7 by 8 June.

Maximum pod numbers were greater on Raceme T than on the other racemes and Raceme 1 had the lowest number of pods. A comparison using one way ANOVA

a)

b)



c)

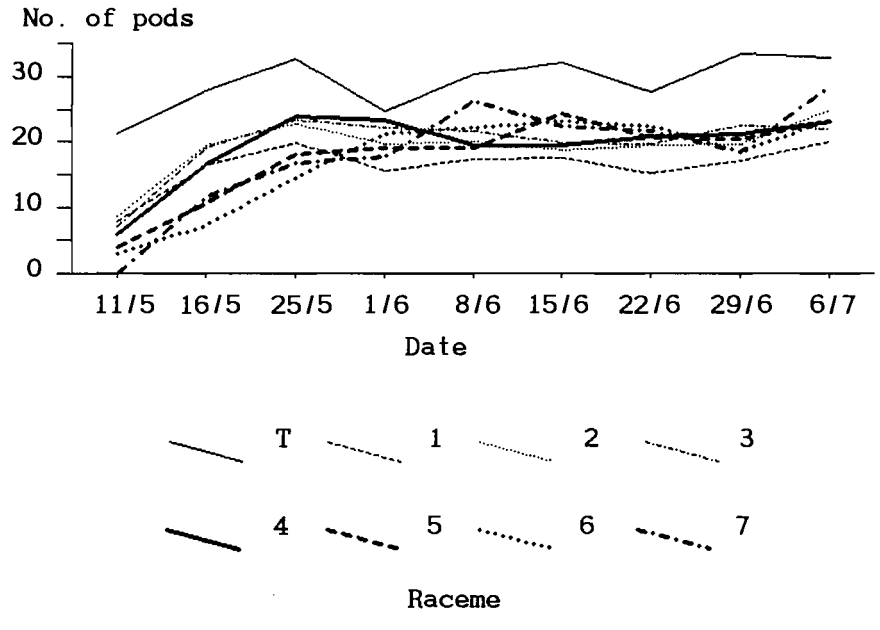


Fig. 3.2: Numbers of buds, flowers and pods on different racemes; Bienvenu, 1987

a) buds
b) flowers
c) pods

shows the differences for the seven weeks 25 May - 6 July, Racemes T to 4 to be inconsistent at the 5% level of significance (Tukey test) (Table 3.1). The number of pods on Raceme T was significantly different from Raceme 1 on only five of the seven weeks analysed, although pod numbers were less on Raceme 1 by between 9.3 and 14.6 during this time. The number of pods on Raceme T differed significantly from those on Racemes 2 to 4 on only three occassions but during the seven weeks analysed numbers of pods were less on Racemes 2 to 4 by 1.5 to 13.7. The number of pods on Raceme 2 did not differ significantly from numbers on the lower racemes but generally numbers were slightly lower (1-5 pods). Although enough data for statistical comparison using Racemes 5 - 7 were not available because of the small numbers of lower branches on the plants the pod numbers appeared to very similar to those on the mid canopy racemes (Racemes 2 - 4).

3.1.4 Bienvenu 4 April - 4 July, 1988.

In 1988 Raceme T was assessed throughout the period of study. Raceme 3 was assessed only after *Alternaria* lesions were first seen on Raceme T (16 May). On Raceme T flowers were already present on 4 April (Fig. 3.3) and rose to a maximum between 18 April and 2 May. Pods were first noted on 18 April rose to a maximum between 9 and 16 May and then remained constant (Fig. 3.3) throughout the rest of the sampling period. Differences between the numbers of pods on Racemes T and 3 were inconsistent (Table 3.2) although the general trend indicated that there were more pods on Raceme T than Raceme 3.

3.2 Disease development - General Description.

3.2.1 Rafal 26 June-17 July, 1986.

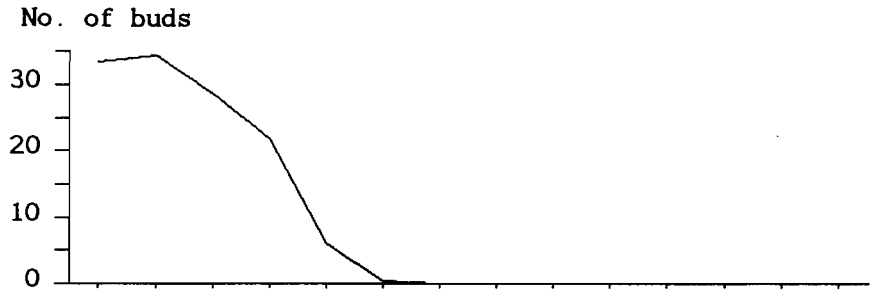
Disease was first seen on the pods on 26 June and during the next four weeks progressed so that on 17 July 2.5-5.2% of the pod area was attacked on Racemes T-4 (Fig. 3.4). The percentage of pods infected rose to 78-85%.

Raceme	Date								
	11/5	18/5	25/5	1/6	8/6	15/6	22/6	29/6	6/7
T	21.3	27.8	32.6a	24.8a	30.4a	32.2a	27.6a	33.4a	32.9a
1	7.8	16.3	19.8b	15.5a	17.3b	17.6b	15.1a	17.2b	20.0b
2	8.6	19.5	22.7ab	19.7a	20.0b	18.6b	19.4a	19.7b	25.0b
3	7.1	19.0	23.4ab	22.1a	21.6ab	19.9b	19.6a	22.5b	21.9b
4	5.9	16.5	23.9ab	23.3a	19.5ab	19.5b	20.8a	21.2b	23.2b
5	4.0	10.5	18.2	19.1	19.1	24.4	20.6	20.4	23.1
6	3.0	7.3	14.4	21.3	22.2	23.2	22.4	18.5	23.4
7	0.0	11.5	16.8	17.8	26.3	22.4	21.7	18.4	28.6

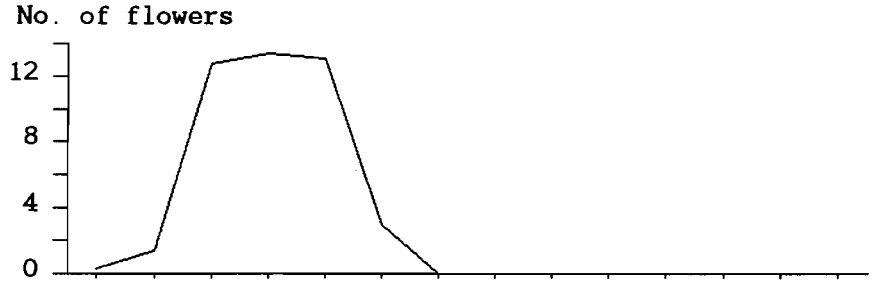
Table 3.1: Mean number of pods on different racemes
Bienvenu, 1987

Within each column numbers not followed by a letter in common are significantly different at $p<0.05$

a)



b)



c)

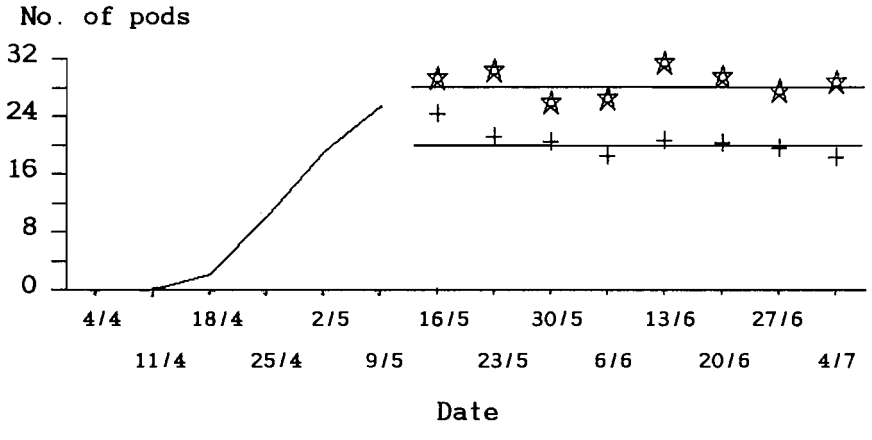


Fig. 3.3: Number of buds, flowers and pods on Raceme T and Raceme 3 Bienvenu, 1988

a) Buds, Raceme T
b) Flowers, Raceme 3
c) ☆ Pods, Raceme T
\+ Pods, Raceme 3

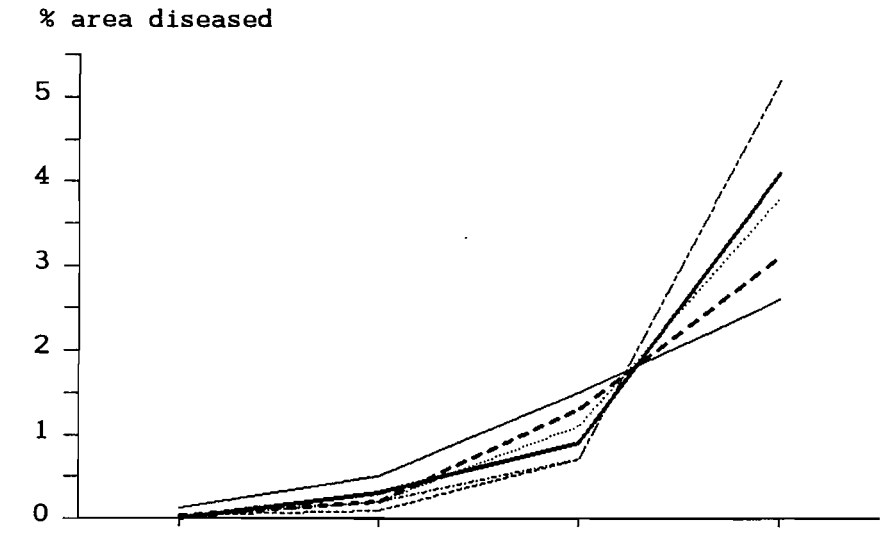
Raceme	Date						
	11/4	18/4	25/4	2/5	9/5	16/5	23/5
T	0.0	2.2	10.1	19.0	25.4	29.3a	30.4a
3	-	-	-	-	-	24.2a	21.2b

Raceme	Date					
	30/5	6/6	13/6	20/6	27/6	4/7
T	25.9a	26.5a	31.5a	29.6a	27.5a	28.8a
3	20.4a	18.5a	20.7a	20.2b	19.5a	18.3b

Table 3.2 Mean number of pods on Raceme T and 3
Bienevenu, 1988

Wthin each column numbers not followed by a letter in common are significantly different at $p<0.05$

a)



b)

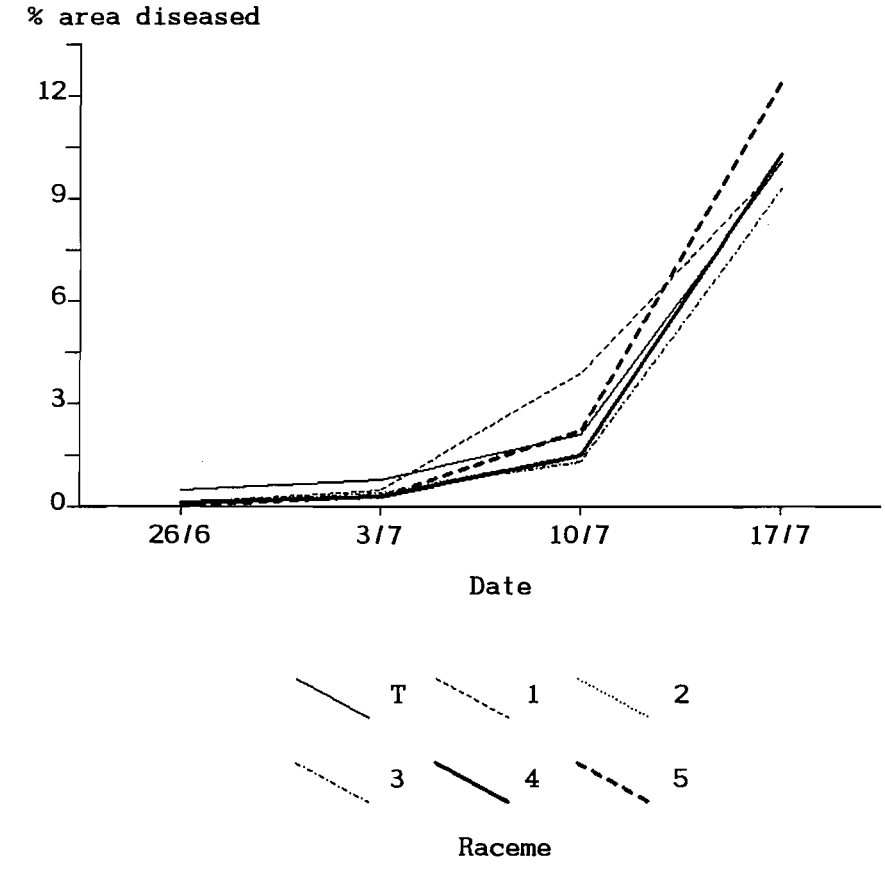


Fig. 3.4: Disease development (RACDIS*) on different racemes, 1986

a) Mikado
b) Rafal

* see text for explanation

3.2.2 Mikado 26 June-17 July, 1986.

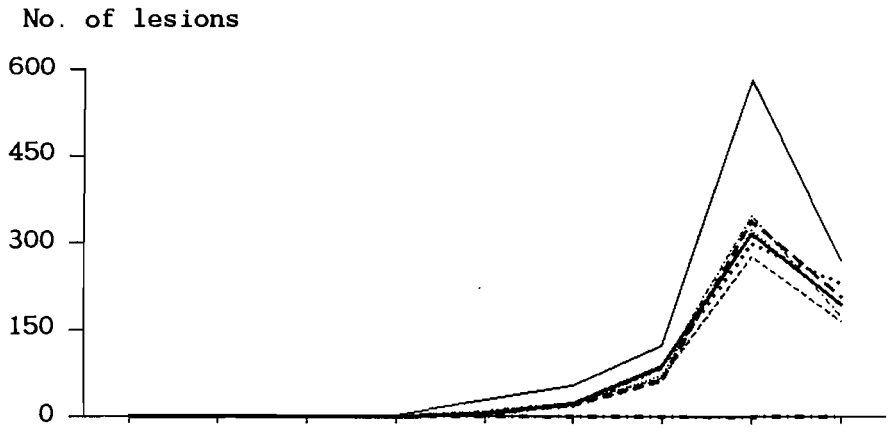
As on Rafal disease was first noticed on the pods on 26 June when 5-14.2% of pods were infected on Racemes T-4 (Fig. 3.4). By 17th July 79-99% of pods were infected on the different racemes and the percentage pod area attacked by the disease had risen to 9.5-12.2%.

3.2.3 Bienvenu 4 May - 6 July, 1987.

In 1987 assessment started prior to the onset of pod infection and the assessment of the number of lesions gave a more objective measure of low disease levels than assessment of % pod area infected. Pod disease was first noted on 25 May on Racemes T-5. Disease did not appear on the lower racemes until later weeks (Raceme 6, 1 June; Raceme 7, 8 June) (Fig 3.5). The number of lesions then increased so that by 29 June there was a mean of 1271 lesions/plant and percentage area of pods attacked was 5.8%. By the following week (6 July) the numbers of lesions had dropped due to coalescence but the mean pod area attacked had risen to 25.4%. By this date all pods on the plant were infected.

The pattern of disease development was similar on all racemes. The number of lesions was generally greater on Raceme T than on the other racemes although the differences were only significant on 1,8 and 15 June (Table 3.3). The numbers of lesions on the other racemes did not differ significantly from one another. Only on 1 and 8 June when disease levels were very low (0.03 - 0.28%) were significant differences found between % disease attack on the different racemes. On all other dates no significant differences were noted. These comparisons were only made between Racemes T to 4 because there were often low numbers of Racemes 6-8 in the sample but with the small amount of data available levels of disease on Racemes 6 and 7 did appear lower than on the other racemes.

a)



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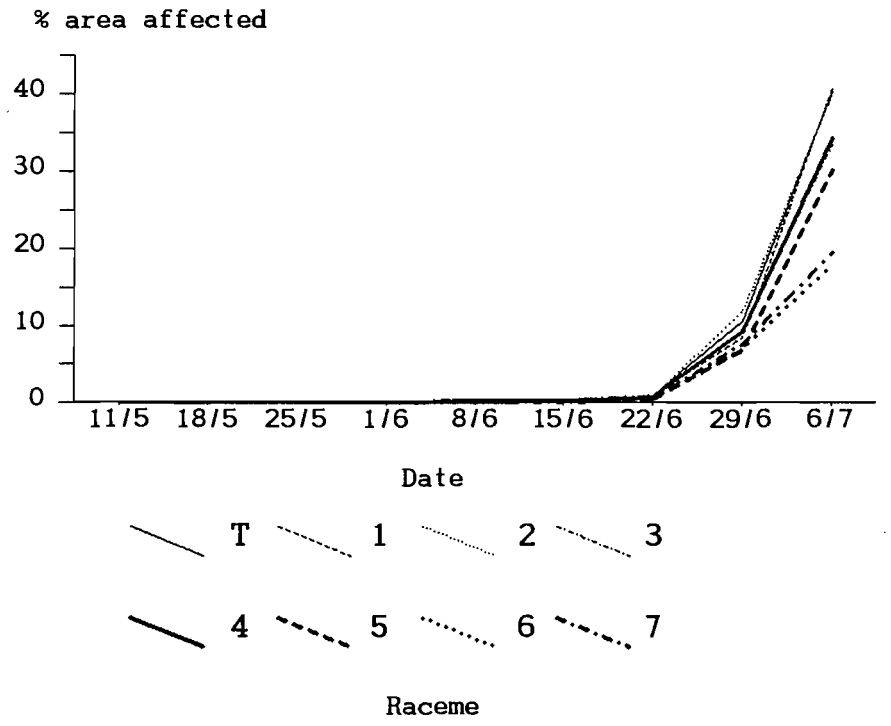


Fig 3.5: Disease development on the pod canopy; Bienvenu. 1987

a) ALRAC* on different racemes
b) RACDIS* on different racemes

* see text for explanation

a) Raceme

	Date								
	11/5	18/5	25/5	1/6	8/6	15/6	22/6	29/6	6/7
T	0.0	0.0	1.4a	2.4a	29.6a	53.7a	122.4a	582.8a	269.4a
1	0.0	0.0	0.3b	0.4b	8.1b	25.3b	65.9a	275.5b	165.3a
2	0.0	0.0	0.3b	0.9b	9.3b	21.9b	71.6a	322.5b	192.3a
3	0.0	0.0	0.1b	0.9b	8.3b	20.8b	81.8a	347.2ab	173.0a
4	0.0	0.0	0.1b	0.7b	5.0b	22.9b	87.0a	315.7ab	194.6a
5	0.0	0.0	0.1	0.5	4.5	19.2	62.0	337.2	206.5
6	0.0	0.0	0.0	0.4	6.2	19.0	84.1	297.1	230.0
7	0.0	0.0	0.0	0.0	3.9	17.4	57.7	258.1	225.9

b) Raceme

	Date								
	11/5	18/5	25/5	1/6	8/6	15/6	22/6	29/6	6/7
T	0.0	0.0	T	0.06a	0.28a	0.31a	0.78a	10.7a	40.3a
1	0.0	0.0	T	0.03b	0.13b	0.18a	0.78a	8.5a	40.9a
2	0.0	0.0	T	0.04b	0.15b	0.16a	0.64a	12.1a	40.1a
3	0.0	0.0	T	0.04ab	0.13b	0.19a	0.71a	9.5a	33.5a
4	0.0	0.0	T	0.04ab	0.11b	0.18a	0.64a	9.3a	34.3a
5	0.0	0.0	T	0.03	0.11	0.15	0.40	6.9	30.2
6	0.0	0.0	0.0	0.02	0.08	0.13	0.82	7.1	18.1
7	0.0	0.0	0.0	0.0	0.12	0.10	0.34	7.7	19.6

Table 3.3 Disease develoment on the pod canopy; Bienvenu, 1987
a) ALRAC* on different racemes
b) DISRAC* on different racemes

T = trace of disease
* = see text for explanation

Within each column numbers not followed by a letter
in common are significantly different at $p<0.05$

3.2.4 Bienvenu 16 May - 4 July, 1988.

Disease was first observed on Raceme T on 16 May and Raceme 3 one week later (Fig. 3.6). It then developed very slowly until 13 June after which a more rapid increase in disease levels began so that by 27 June 5% of pod area was attacked (Raceme T) and the following week 29% of the area was affected. Levels on Raceme 3 showed a very similar pattern.

Differences in the levels of disease (number of lesions and % area attached) between Racemes T and 3 were only significant on 16 and 23 May when disease levels were very low (Table 3.4).

3.3 Disease development-Mathematical analysis.

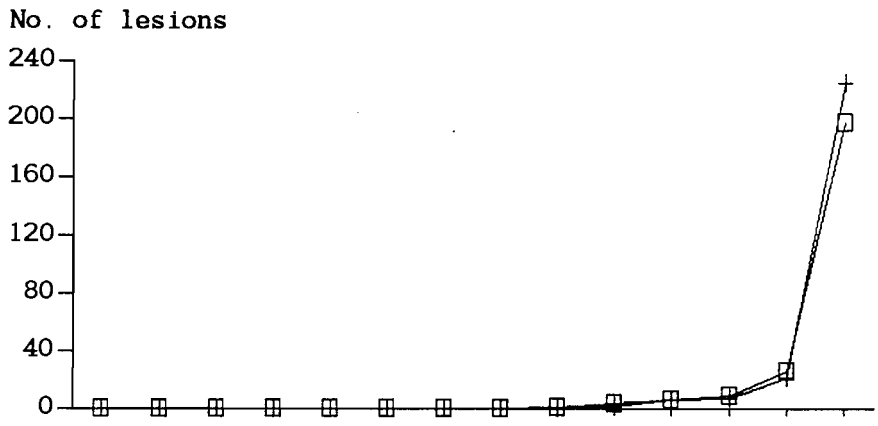
3.3.1 Mathematical Models.

As discussed earlier (Part 3, Chapter 1) the description of DPCs using mathematical models allows the formal comparison of different epidemics and the measurement of skewness in disease progress. Unlike the DPCs from the leaf spot data (Chapter 2) DPCs from the pod data were suitable for simple mathematical modelling.

3.3.1.1 Logistic Model

Although mathematical models had been used previously (e.g. Large, 1952) to describe DPCs, Van der Plank (1963) was the first to describe their use in detail. Van der Plank (1963) discussed, using empirical and theoretical examples, a series of equations based on a logarithmic increase of disease (and modifications thereof), models which have since been used extensively in plant pathology (e.g. Berger, 1977; Zadoks & Schein, 1979; Merrill, 1967; Plaut & Berger, 1980) In particular he describes the use of the equations

a)



b)

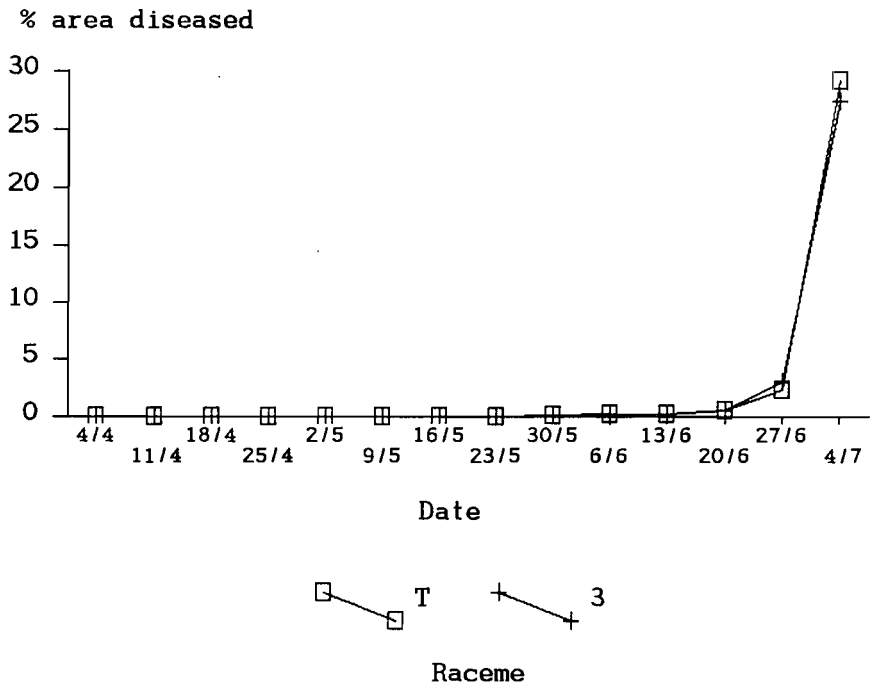


Fig 3.6: Disease development on Raceme T and Raceme 3
Bienvenu, 1988

a) ALRAC*
b) RACDIS*

*see text for explanation

a) Raceme

	Date							
	16/5	23/5	30/5	6/6	13/6	20/6	27/6	4/7
T	0.1a	0.2a	1.2a	3.8a	6.1a	9.4a	25.9a	197.9a
3	0.0b	0.0b	0.4b	1.7b	6.6a	7.4a	21.4a	224.7a

b) Raceme

	Date							
	16/5	23/5	30/5	6/6	13/6	20/6	27/6	4/7
T	T	T	0.1	0.2a	0.3a	0.6a	2.3a	29.2a
3	0.0	0.0	T	0.1b	0.3a	0.6a	3.0a	27.5a

Table 3.4 Disease development on the pod canopy; Bienvenu, 1988

a) ALRAC* on different racemes
b) DISRAC* on different racemes

T = trace of disease
* = see text for explanation

Within each column numbers followed by a letter in common are sugnificantly different at $p<0.05$

$$r_l = \frac{1}{t_2 - t_1} \ln \frac{x_2}{x_1} \qquad (a)$$

$$r = \frac{1}{t_2 - t_1} \ln \frac{x_2 (1 - x_1)}{x_1 (1 - x_2)} \qquad (b)$$

where

r_l and r are rate parameters

x_1 is proportion of disease at time t_1

x_2 is proportion of disease at time t_2

Equation (a) gives r_l the apparent rate of infection during the initial phase of the epidemic ($x < 0.05$) when the area of healthy tissue available for infection is not considered to be limiting to disease progress and hence increase in disease is not proportional to x. This is described as the logarithmic phase of the epidemic and r_l as the logarithmic infection rate. Equation (b) gives r, the apparent infection rate, and includes a correction factor (1-x) for the proportion of host material still available for infection. Equation (b) traces a progress curve for x which is sigmoid and symmetrical around the inflection $x=0.5$.

Van der Plank (1963) used these equations to describe disease progress both on a weekly basis and over the complete period of an epidemic. He showed that for many epidemics of polycyclic pathogens r remained constant over a range of disease levels throughout an epidemic as shown by regressing time against log x/1-x and it is the value r which is quoted by many authors (e.g. Merrill, 1967; Plaut & Berger, 1980) as a descriptor of epidemic progress. However, although being a very convenient figure to compute and apparently fitting a wide range of situations, the

linearity of r should be interpreted with care. Van der Plank (1963) arbritarily splits the disease progress curve into three stages.

1) A logarithmic stage when r and r_1 are almost identical.

2) A stage when x does not exceed 0.35 when the correction factor (1-x) is important to allow for overlapping lesions and multiple infections. During this stage the latent period, overlapping lesions or multiple infections combine to increase r above r_1 whereas because the correction factor (1-x) is inadequate (except in perfectly uniform conditions for infection) it leads to under-correction. The two errors of using r as a measure of r_1 are in opposite directions and in practice balance one another to the same extent.

3) A stage when x exceeds 0.35. If interaction of host and environment stays constant r would increase and the correction factor (1-x) becomes more inadequate. If r stays nearly constant throughout this part of the epidemic then conditions have varied not stayed constant. If the objective then, is to refine the "why" questions about disease development and compare different curves r is not a good measure to use as not only is it affected by the disease levels already present but it can be affected by factors unknown to the investigator. A better measure would be r_1, as unlike r it is not proportional to x and is therefore a simple and direct measure of the balance of factors affecting the epidemic. However, it is often difficult to estimate accurately because of the problems of assessing low levels of disease. Here r becomes important because although the linearity above $x = 0.35$ is difficult to interpret it is a good estimator of r_1 up to this level.

These infection rates seem to be very sensitive to external changes (Van der Plank, 1963) and are therefore suitable for immediate comparisons of effects on the epidemic behaviour of particular diseases (Kranz, 1974). The most frequent method used for comparing such transformed DPCs is simple regression analysis. Similarities in DPCs are expressed by b, the regression coefficient (representing the infection rate), and a, the y intercept. The regression coefficients can be tested for statistical significance within and between b values, essential tests which avoid conclusions from differences in DPCs which are due to chance variation. Regression equations may also lead to the classification of DPCs (Kranz, 1974). For example James *et al.*

(1972) using regression analysis, showed that 96 epidemics of potato late blight did not differ significantly, suggesting that they may be considered to form one class. Models can then be derived which may be of general application (e.g. James *et al.* 1972; Eversmyer & Burleigh, 1970).

Although seemingly general in application and reasonably easy to interpret biologically the simple logistic models have their drawbacks. They assume that the total area of host tissue remains constant throughout the epidemic. Modifications to the basic logistic model have been made but are not easy to apply (Waggoner, 1986) and this is why such equations are not applicable to the disease data from the leaf canopy of oilseed rape in this study. The pod canopy also changes its area during the early period of disease development as pods grow after fertilisation of the flowers. However, it was noted that in general pods were well developed before disease was seen on them and therefore the assumption that pod area remains constant during disease development was made for the data in this study.

Another limitation of these models is that they assume that the DPC has an inflection at $x = 0.5$. Waggoner (1986) clearly showed that this is not always true. Using epidemics of a variety of pathogens he showed that some DPCs are negatively skewed (inflection > 0.5) and some positively skewed (inflection < 0.5). Berger (1981) pointed out that logistically transformed disease progress curves are frequently curvilinear rather than linear indicating an asymmetric growth curve. Of the 113 DPCs from 9 pathosystems that he evaluated all were positively skewed. To overcome the difficulty with skewness different mathematical models can be used to fit the data. Waggoner (1986) and Berger (1981) both used the Gompertz model to fit DPCs skewed to the left as have others (Analytis, 1973). To fit DPCs skewed to the right Waggoner (1986) described the Richards model which was also used by Analytis (1973). Other models have also been used but are not described here, e.g. the Bertalanffy and Mitscherlich models (Berger and Mishoe, 1976) and the Weibull model (Pennypacker *et al.* 1980).

3.3.1.2 Gompertz Model

This model was developed by B. Gompertz for acturial tables (Berger, 1981) and has since been used frequently by ecologists to explain biological phenomena. The linear form of the model is described by

$$r_g t = -\ln (\ln 1/x) + \ln (\ln 1/x_o)$$

r_g, the rate specific to the Gompertz model, can be estimated by the regression of t against -ln(ln 1/x). This model gives a good fit when the inflection occurs at about x = 0.37.

3.3.1.3 Richards Model

The Richards Model (Richards, 1959) is a general equation for growth and includes a parameter m which changes depending on the skewness of the progress curve. The linear forms of the equations are

$$r_h t = -\ln (1-x^{1-m}) - \ln (1-x_o^{1-m}) \quad m < 1$$

$$r_h t = -\ln (1-x^{1-m}) + \ln (1-x_o^{1-m}) \quad m > 1$$

If m = 1 the model approximates the Gompertz model and for m = 2 it approximates the logistic model. As m increases it describes curves which are negatively skewed.

3.3.1.4 Biological Interpretation

Waggoner (1986) points out that when looking for equations to describe DPCs, not only should there be a quantitative resemblance between the collected data and the model but that the concept underlying the model should be examined for resemblances to nature. The rationale behind the logistic model is clear, it conceives that pathogens simply increase in proportion to their population and to the remaining

opportunity for infection (Van der Plank, 1963) although as described above (Section 3.3.1.1) the meaning of constant r is far from clear. The rationale proposed by Gompertz for the Gompertz model is that in equal small intervals of time the organism loses equal proportions of its power to increase (Waggoner, 1986), a rationale somewhat less clear than that of the logistic model. For the Richards model which moves from one form to another the rationale must change likewise. (Waggoner, 1986).

The logistic model is therefore the easiest to interpret but will not fit all situations with the accuracy of the other models. If biological interpretation of the data is most important then the logistic model should be considered. However, statistical comparisons between DPCs will be less error prone if the curves are fitted using the most accurate equation.

In the following sections the DPCs of disease on the pod canopy from this study will be fitted using the models described above and detailed comparisons between years and racemes will be carried out using the logistic model.

3.3.2. Methodology

Four models were fitted to the DPCs of pod disease for 1986 - 1988 data. The models fitted were:

Logistic	$\ln (x/1-x)$ v t	
Gompertz	$-\ln (\ln 1/x)$ v t	
Richards(1)	$-\ln (1 + x^{1-m})$ v t	m=3
Richards(2)	$-\ln (1 + x^{1-m})$ v t	m=4

where: x = proportion of pod tissue diseased at time t

m = constant.

Each model was fitted to the data using simple regression utilising least squares fitting. No methods are available to compare coefficients of variation, so comparisons

were made by non statistical comparisons of the coefficients of variation and visual comparisons of graphical representations of the transformed data.

For the logistic model, regression coefficients (b) and intercepts log (a) were calculated, together with their standard errors and confidence limits. Regression coefficients were compared using a method described by Bailey (1981) based on the t - statistic.

3.3.3 Results

Values for the regression coefficents for the four models, using all racemes from 1986 and 1987 and the terminal raceme and raceme 3 in 1988 are given in Tables 3.5 and 3.6. All regressions were highly significant ($p = <0.001$).

No single model gave the best fit in all cases, although the logistic and Richards (1 + 2) models generally appeared to fit better than the Gompertz model. The exceptions are Mikado, 1986 Raceme 1 and Rafal, 1986 Racemes T, 2 and 5 where the Gompertz model fitted better. Overall in 1986 for both varieties the fit for the Gompertz model was better (86.8 - 99.3% of variation accounted for than its fit on the 1987 and 1988 data (79.4 - 91.9% of variation accounted for). Graphical representation of the data (Fig 3.7) shows that where the Gompertz model fitted less well, the graph of the transformed data was curvilinear.

Difference between the fit of the logistic model and the two Richards models were slight, never greater than 1% between the R^2 values. The two Richards models were even closer in their fit, the values of R^2 not differing by more than 0.15%. In all cases the logistic and Richards models accounted for >92% of the variation.

The logistic model was used to compare the development of disease on different racemes and in different years because in all cases it accounted for >92% of the variation and is the easiest model to interpret biologically. None of the regression coefficients differed significantly from one another at the 5% probability level. The regression coefficients (Table 3.7) represent r (section 3.3.2) during the

Mikado, 1986

	Raceme					
Model	T	1	2	3	4	5
Logistic	93.79	98.45	98.93	98.55	98.34	99.96
Gompertz	88.76	98.84	93.82	93.38	93.12	97.05
Richards (1)	94.29	98.21	99.17	98.80	98.59	99.93
Richards (2)	94.26	98.23	99.16	98.79	98.58	99.93

Rafal. 1986

	Raceme					
Model	T	1	2	3	4	5
Logistic	96.99	98.72	99.23	93.85	94.16	97.65
Gompertz	98.88	94.65	99.81	97.92	86.80	99.28
Richards (1)	96.89	98.80	99.16	93.66	93.84	97.55
Richards (2)	96.89	98.80	99.16	93.66	93.84	97.56

Table 3.5 Comparison of Logistic, Gompertz and Richards models for describing DPCs on different racemes

Coefficients of Determination

Mikado and Rafal, 1986

Bienvenu, 1987

	Raceme							
Model	T	1	2	3	4	5	6	total
Logistic	92.38	95.10	92.18	94.40	93.99	92.61	97.68	93.13
Gompertz	82.37	83.51	83.03	85.17	84.44	82.88	91.96	84.01
Richards (1)	93.36	96.08	92.88	95.05	94.70	93.30	97.46	93.71
Richards (2)	93.29	96.00	92.84	95.01	94.65	93.25	97.46	93.62

Bienvenu, 1988

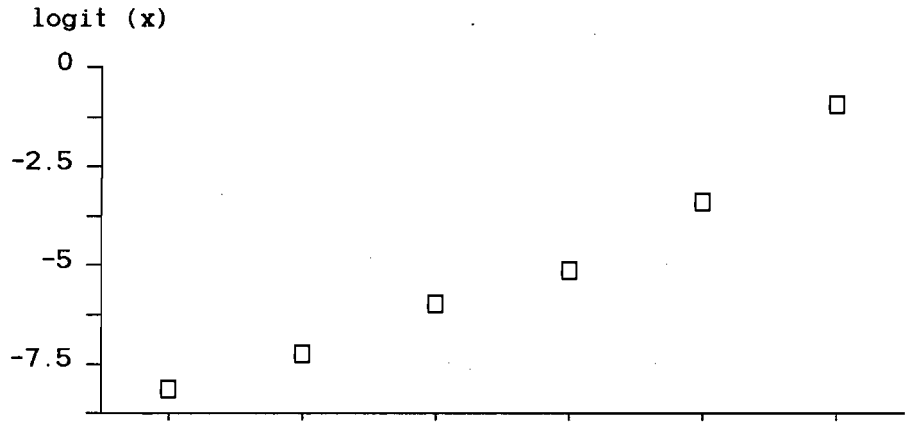
	Raceme	
Model	T	3
Logistic	94.54	95.94
Gompertz	79.40	82.75
Richards (1)	95.27	97.01
Richards (2)	95.21	96.69

Table 3.5 Comparison of Logistic, Gompertz and Richards models for describing DPCs on different racemes

Coefficients of Determination

Bienvenu, 1987 & 1988

a)



b)

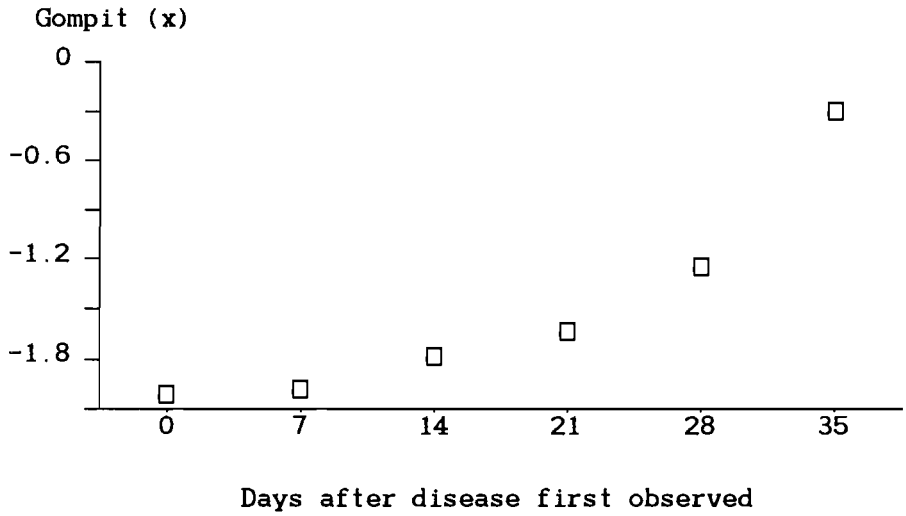


Fig 3.7: Comparison of logistic and Gompertz model for describing DPCs Raceme 3; Bienvenu, 1988

a) Logistic model
b) Gompertz model

Raceme	1986, Mikado	1986, Mikado	1987, Bienvenu	1987, bienvenu
T	0.15±0.074	0.15±0.050	0.19±0.071	0.17±0.039
1	0.23±0.057	0.25±0.061	0.22±0.063	
2	0.22±0.046	0.23±0.040	0.21±0.079	
3	0.22±0.051	0.34±0.171	0.20±0.064	0.20±0.048
4	0.23±0.052	0.19±0.051	0.21±0.067	
5	0.27±0.011	0.22±0.069	0.20±0.074	
6			0.21±0.044	
7			0.20±0.070	

Table 3.7 Regression coefficients representing r for all years and racemes from the logistic model

± 95% confidence limits

period of the epidemic. They ranged from 0.147 - 0.340 with the majority lying in the range 0.193 - 0.232. The actual and predicted values for diseased area for raceme T in 1987 and 1988 are given in Fig 3.8. In both years although the values predicted from the logistic curve were similar to those from the actual data, disease area tended to be overestimated early in the epidemic and underestimated at the end.

3.4 Relationship between disease development on the leaf canopy and on the pod canopy.

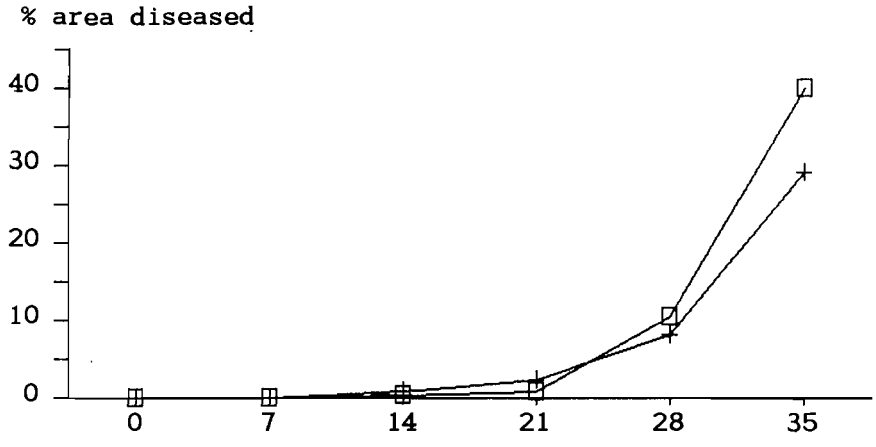
Figs 3.9 - 3.11 show the development of disease on both the leaf and raceme T during the 1986 - 1988 trials (disease = TALLF). The 1988 data show that disease development on raceme T began seven weeks after pods first appeared on raceme T and two to three weeks after maximum pod numbers had been reached. Disease development in each epidemic began during a period of increase of disease on the leaf tissue (except Rafal, 1986) and at a time when infection of the leaf was positioned just below the pod canopy (Part 3, Chapter 2,). The rapid increases in disease development on the pods at the end of the season occurred when no leaves were left on the plant.

The relationship between numbers of lesions on the leaf canopy one, two and three weeks prior to complete leaf fall and final area of disease on the pod canopy are shown in Fig 3.12. No statistical analysis of the relationship was attempted because of the small number of data points. These results suggest a positive linear relationship between leaf disease and pod disease for Bienvenu in 1987 and the 1986 data but the relationship is different for Bienvenu 1988. A better relationship seems to be achieved with data for the leaf canopy just prior to final leaf fall.

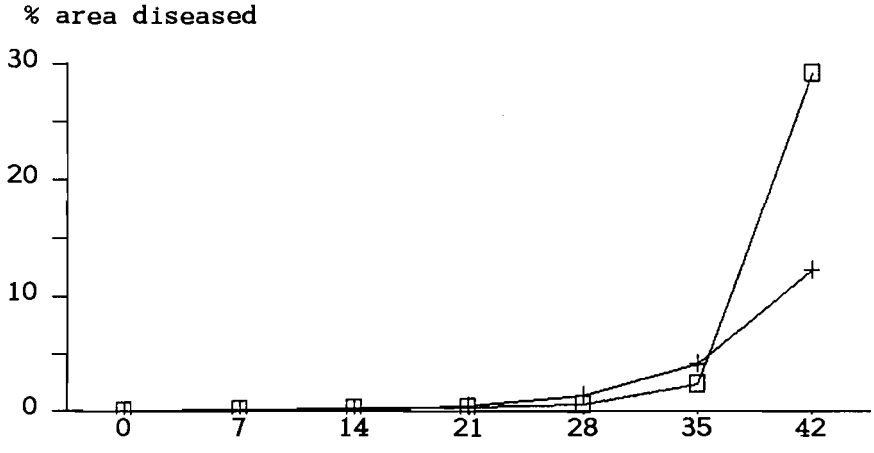
3.5 Discussion

Pod canopy development in the four crops was similar to and followed the pattern described for other studies (Norton & Harris, 1975, Scarisbrick *et al.*, 1982b; Allen & Morgan, 1972, Allen & Morgan, 1975). Pods began to appear during flowering and increased in number until flowering was complete, after which pod

a)



b)



Days after first disease observed

observed

predicted

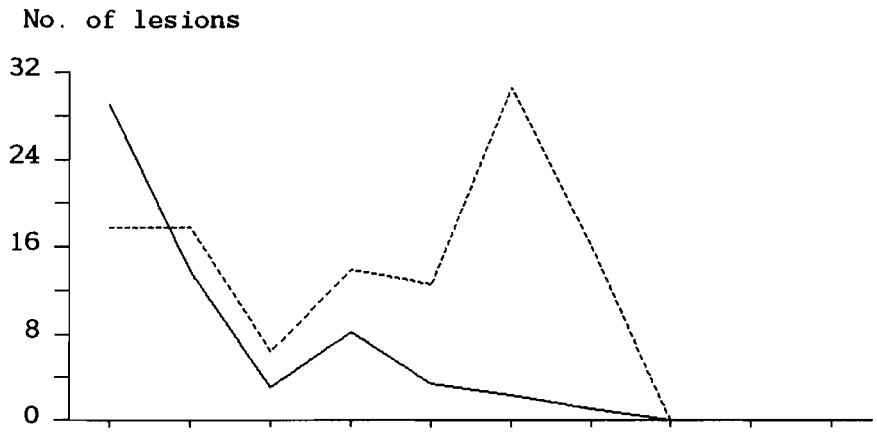
Fig 3.8: Observed and predicted values for RACDIS* on Raceme T

a) Bienvenu, 1987
b) Bienvenu, 1988

Predicted values calculated from the logistic model

* see text for explanation

a)



b)

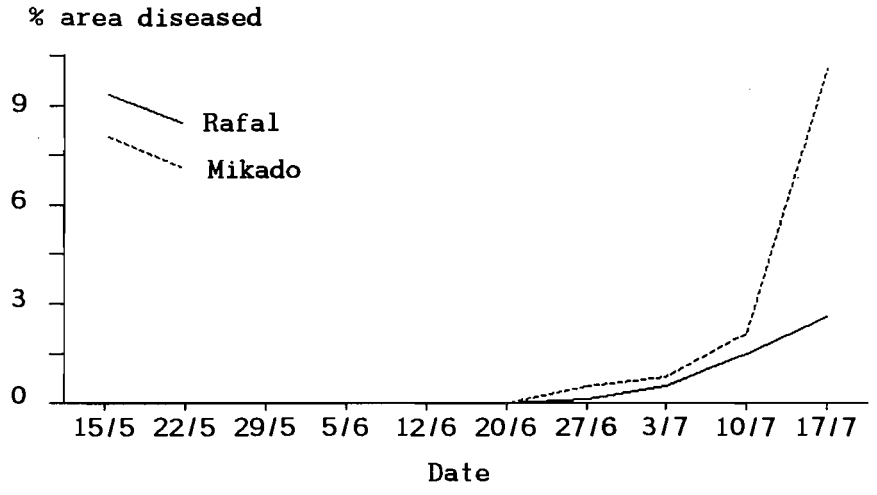


Fig 3.9: Disease development on the leaf and pod canopy
Rafal and Mikado, 1986

a) TALLF*
b) RACDIS* on Raceme T

* see text for explanation

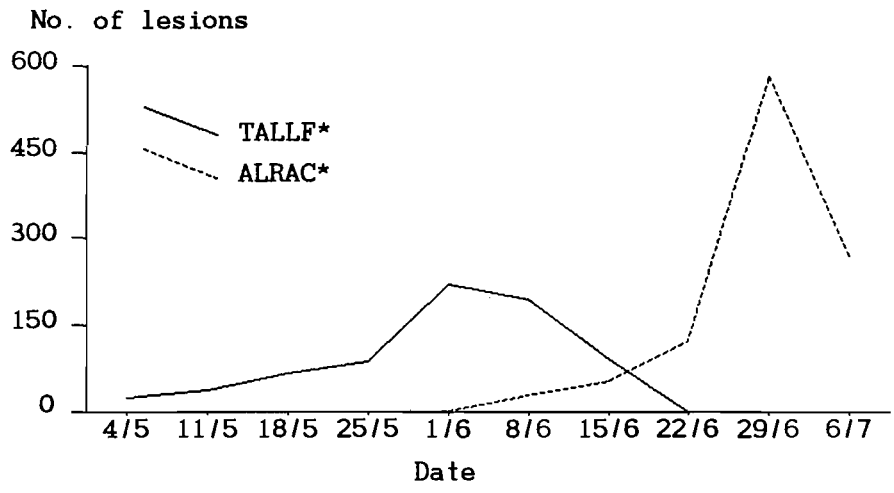
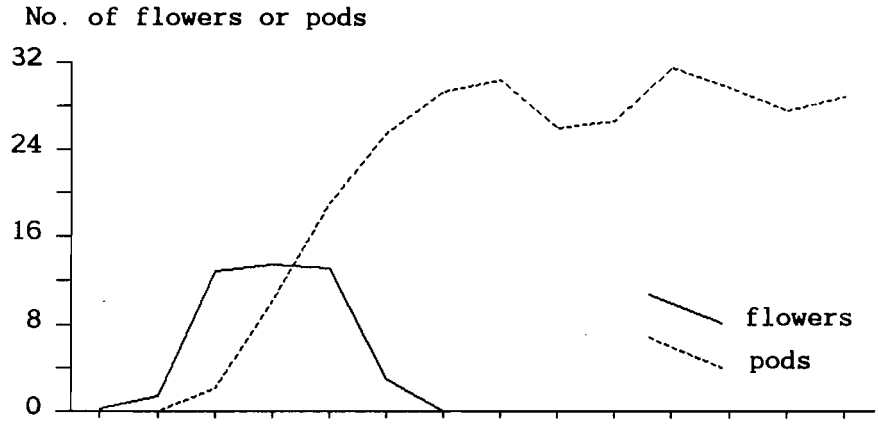


Fig 3.10: Disease development on the leaf and pod canopy; Bienvenu. 1987

a)



b)

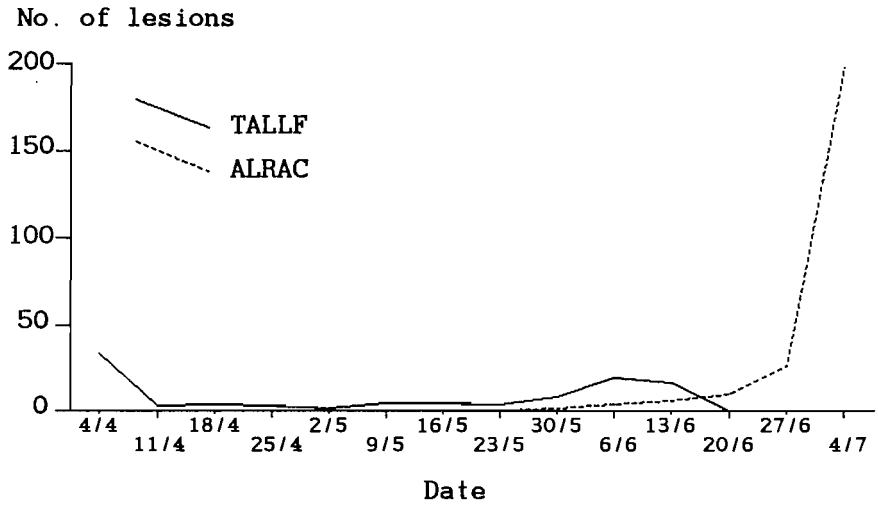


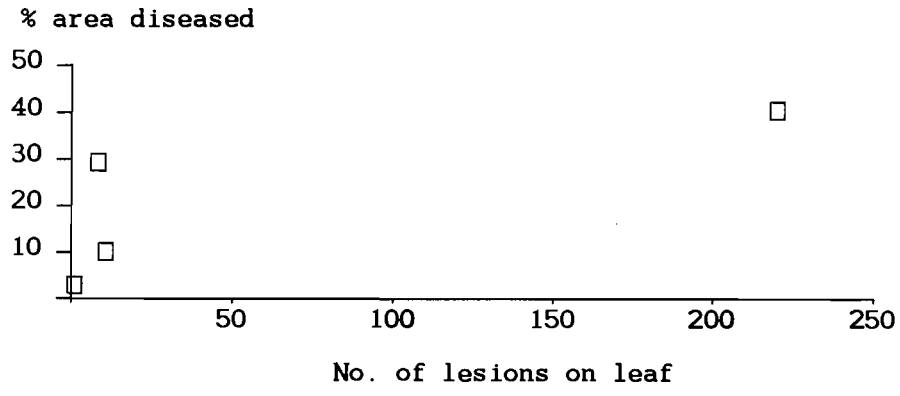
Fig 3.11: Disease and plant development; Bienvenu, 1988

a) No. of flowers
No. of pods on Raceme T

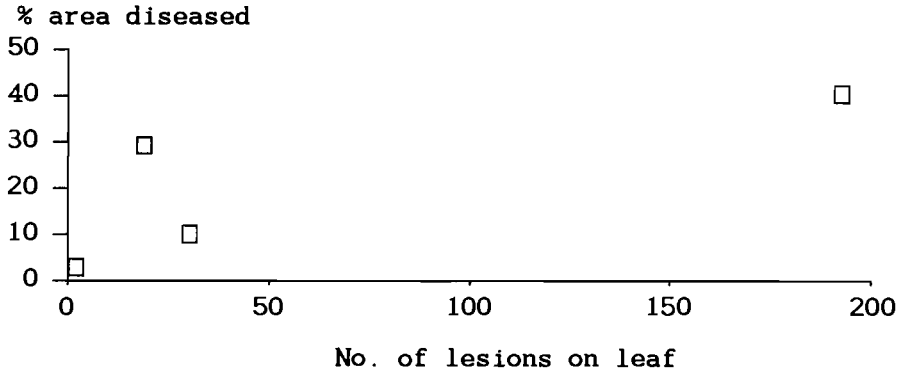
b) TALLF*
ALRAC* for Raceme T

* see text for explanation

a)



b)



c)

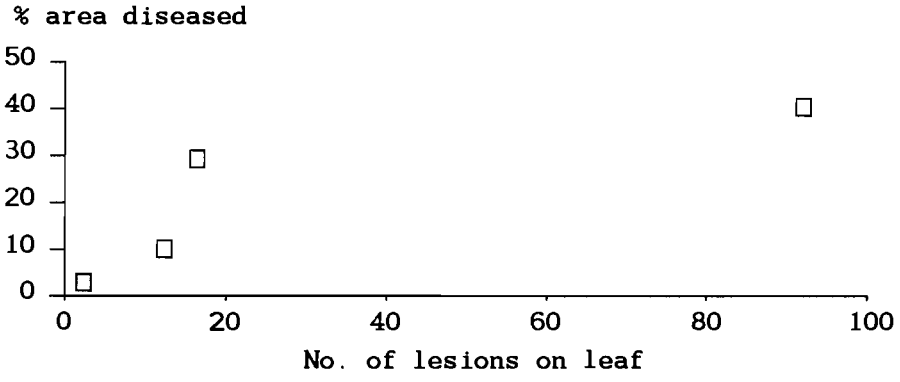


Fig 3.12: Relationship between TALLF* on different times before final leaf fall and final area of disease on Raceme T, 1986-1988

a) 3 weeks before final leaf fall
b) 2 weeks before final leaf fall
c) 1 week before final leaf fall

* see text for explanation

numbers on each raceme remained constant. In 1987 and 1988 pod development began first on the terminal raceme with the lower racemes following. In 1987 pod numbers were generaly greater on raceme T than on the others although the differences were not always statistically significant. Similarly in 1988 pod numbers tended to be greater on Raceme T than on Raceme 3. Allen and Morgan (1975) described a similar situation in spring oilseed rape, although in that case the terminal raceme accounted for 45% of the pods. Daniels *et al.* (1986) describing cv. Bienvenu also showed that the terminal raceme carried more pods than the other racemes and that Raceme 1 had fewer pods than the other lower racemes. In Bienvenu in 1987 numbers of pods were generally slightly lower on Raceme 1 than on the other lower racemes but never significantly so. From the middle of flowering, new pods were becoming available for infection by spores of *A. brassicae.* Pods on raceme T were available first followed by those on the lower racemes. Many of these pods were situated close to the upper leaves on the plant.

Development of dark pod spot caused by *A. brassicae,* on the pod canopy of oilseed rape, is considered to be associated with warm, wet weather during flowering and pod fill (Davies, 1986; Louvet and Billotte, 1964) and that inoculum for this stage of disease development is provided by lesions on the leaf tissue (Gladders, 1983). In this study disease development on the pods did not occur until well after pods had appeared and began to develop. In 1988 when pod development was followed from first pod appearance, disease was not apparent until seven weeks after the first pods appeared on the Raceme T and four weeks after the number of pods became constant on that raceme.

The initial development of disease on the pods occurred at the same time as increasing numbers of lesions on the leaf canopy in all epidemics except on Rafal in 1986, and at a time when lesions on the leaf canopy were at the top of the crop close to the pod canopy. However, the major increase in lesion numbers on the pod canopy occurred after all leaf material had dropped from the plant. With incubation periods of 2-3 days at this time of year (Mhrida, 1983) the inoculum for this expansion could not have been leaf material still attached to the plant. One possible source of inoculum was that lesions already present on the pods had begun to sporulate.

Although no detailed information of sporulation was taken for the pod canopy, it was noted that only in 1987 did substantial numbers of pod lesions sporulate and then only in the final weeks of assessment. In other years virtually no sporulation was noted on the pod tissue; an observation also made by Louvet & Billotte (1964). Another possibility is that senescing leaf material, at soil level provided inoculum for the pod canopy. It has been shown that *A. brassicae* lesions will continue to sporulate on senescing tissue (Mhrida, 1983; Humpherson-Jones, 1989) and therefore decaying leaf tissue on the soil surface would have been active source of spores. However, the distance between soil and pod canopy would have made distribution difficult (see Part 4, Chapter 2 for further discussion).

Although it is difficult to pinpoint the actual source of sporulating material that provides the inoculum for the pod canopy there is evidence for a relationship between numbers of *A. brassicae* lesions on the leaf tissue just prior to complete leaf fall and final levels of disease on the terminal raceme. However further investigation with much larger numbers of epidemics would be required to validate the trend shown here. The possible relationship here does not imply cause and effect in terms of inoculum source for the pod canopy. It may simply mean that the higher the general level of disease at that time in the season the higher the final level of disease. It does not preclude either of the suggestions proposed previously for the actual inoculum producing material. However, if fallen leaf material is the main inoculum source, then an improving relationship between lesion numbers on the leaf and final disease with time would be expected.

Even if a useful relationship, like the one above, was defined it might be of little practical value since even low levels of pod spotting can cause economically important yield losses (Ogilvey, 1984b) and therefore the accurate prediction of disease levels >5% may be of little use. However, to predict whether disease levels would reach a particular threshold level may be a valuable consequence of the further development of such a relationship, especially where number of lesions is the independent variable, as it is an easily measured, objective assessment method.

The general development of dark pod spot on the pod canopy in the four epidemics was similar. The first observations of disease were on the terminal raceme and subsequently infections were seen on the lower racemes 1-3 weeks later. The differences in disease development between the terminal raceme and other racemes caused by this delay was not sustained and after 3-4 weeks no differences in the area of pods diseased was seen between the different racemes. The difference in timing of the early development of the disease on Raceme T compared with the other racemes may have several explainations. Raceme T is the first raceme to develop and therefore is exposed to inoculum longer than the other racemes. However, infection of the pod canopy by the pathogen does not occur until well after pod development has started on all racemes. It might therefore be expected that disease would first appear on the lower racemes which are closer to the inoculum source (leaves or senescent tissue). This suggests that the tissue on Raceme T during this period is more susceptible to infection than the lower racemes which again is circumstantial evidence for the view (Kohle and Hoffman, 1989) that younger tissue is more resistant to infection than older tissue. Differences in numbers of lesions on the differet racemes was probably a consequence of differences in pod numbers on those racemes.

This similarity between racemes and years was seemingly supported by the results of analysing the epidemics using various simple mathematical models. Comparisons of the four models used, showed that in most cases the Gompertz model gave the least good fit to the data and that the logistic model and Richards models (1+2) gave a better and almost equivalent fit. Where the Gompertz model fitted better it is probably a consequence of lack of data for the early part of the epidemic. The generally better fit of the logistic model is in contrast to the findings of Berger (1981) who showed that the Gompertz model gave a better fit than the logistic model for the 113 epidemics of the nine pathosystems tested. However Carsen (1985) showed that for *Alternaria* blight of sunflowers (*A. helianthi*) the logistic model gave a better fit than the Gompertz model.

As already discussed (Section 3.3.1)the four models describe different shapes of curve. The poor fit of the Gompertz model, especially in 1987 and 1988 is because

disease does not develop quickly and reach an early maximum. Disease progress showed a distinct lag phase, followed by a rapid increase in disease, which fitted the logistic and Richards models. The partial curves represented by these data probably explain the similarities between the fit of the logistic and Richards models. These models differ most substantially in the latter part of disease development when the proportion of diseased material exceeds 50%. Partial curves of low disease levels are therefore likely to fit these models equally well. Although the logistic model fits the data well (R^2 = 92-95%) the small amount of data available does suggest that when disease progress is recorded throughout pod development this model does not accurately predict the length of the lag phase or the explosive disease development later in the season. A large number of data sets are required to confirm this view and allow the testing of other models which may give a more adequate prediction of disease development.

Comparison of the development of disease on each raceme and in each year using the logistic models, showed that the regression coefficients did not differ significantly within or between years. This is similar to the findings of James *et al.* (1972) who showed that for 96 epidemics of potato late blight their DPCs did not differ significantly from one another. This implies that whatever the environmental factors affecting disease development e.g. climate, variety, once disease reaches the pods the level of disease in any future week can be determined and hence if the date of harvest can be estimated, final disease levels and therefore yield losses estimated. However, a more detailed examination of the data shows that care should be taken in making such conclusions. Firstly, the data used to determine these relationships is incomplete in two of the four epidemics. (Rafal and Mikado, 1986). Data for the very early part of the epidemic are missing and this may have served to change the shape of the DPC. Although comparison with the DPCs of 1987 and 1988, suggests that the early data may fit the logistic model just as well. Secondly, the data set is very small and it could be by chance that the DPCs were found to be similar. A large data set, including descriptions of epidemics from different parts of the country, under different environmental conditions, and on a range of varieties is required before any generalisations can be made , but these data do suggest that such an approach would be worthwhile. Thirdly, sample numbers were too small at the beginning of the

epidemic to accurately estimate low disease levels (Part 2, Chapter 2). Although this is unlikely to have a substantial effect on the overall pattern of the data taken i.e. long lag phase followed by explosive increase, difference between very low levels of disease should be analysed with care and further studies should use adequate sampling methods.

The pattern of disease development with a long lag phase followed by an explosive development of disease also implies that early in disease development on the pods there is some restriction to infection by or growth of the pathogen. Factors affecting such restriction could include meteorological parameters and levels of inoculum which are discussed in Part 4. However, as on the leaf canopy another possibility is an effect of the age of the plant material. If young pod tissue in the field is less susceptible to infection by *A. brassicae* than older tissue then the development of disease on the pod canopy may show a long lag phase as seen here. Any further work carried out to investigate development of disease on the pod canopy should include the effect of pod age as part of the investigation.

In summary, the pattern of disease progress was similar in all the epidemics studied, generally being first associated with the terminal raceme and subsequently with lower racemes. Differences in disease levels are lost within three four weeks. Each epidemic was adequately described by the logistic model and the value of r was similar in all years and for all racemes, which suggests a classification for this pathogen using r.

PART 4 DISEASE DEVELOPMENT; METEOROLOGICAL AND BIOLOGICAL FACTORS

CHAPTER 1 INTRODUCTION

In Part 3 the development of dark leaf and pod spot on and through the canopy of oilseed rape was described and discussed, providing details of the general patterns of epidemic progress. Superimposed on these general patterns are the relationships between disease development and meteorological factors which in turn incorporate the effects of the weather on the polycyclic processes of the pathogen, for example sporulation and infection. In Part 4 an initial investigation of these relationships is described.

One approach to investigating the effects of enviromental factors on the development of a pathogen is to carry out laboratory investigations under controlled conditions. Published studies on the meteorological factors affecting the development of *A.brassicae* have been centred on these methods and have included germination in axenic culture (Mukadam & Deshpande, 1979), infection (e.g. Louvet & Billotte, 1964), post-infection processes (e.g. Louvet & Billotte, 1964) and sporulation (e.g. Humpherson-Jones & Phelps, 1989). The studies in axenic culture provide a guide to the likely temperature optima for pathogen development and in the case of germination possibly for infection. The relationship between this type of study and the development of the pathogen in the plant is however unclear. Studies which utilise the host/pathogen relationship provide more appropriate information although studies under controlled enviroments may also be difficult to relate to the field situation. With this type of study the effect of temperature and wetness on infection was investigated by Mhrida (1983). This author (*Ibid.*) also presented results on the effect of temperature on post infection processes. Sporulation has been studied by Humpherson-Jones & Phelps (1989) and Mhirda (1983) both of whom used lesions from the field in an attempt to identify meteorological factors which affect spore production.

Another approach to the study of the effects of meteorological factors on pathogen development is to study them in the field under natural enviromental conditions. Initially this may simply entail relating meteorological data collected in the field to disease progress curves. This simple visual method can give vital leads about the environmental conditions which affect disease development especially if single weather events are important. For example Royle *et al.* (1986) showed that in some years development of *Septoria tritici* is related to a single rainfall event.

Distinct parts of the pathogen's life cycle can also be studied in the field, of which the events surrounding sporulation and infection are those most readily investigated. Spore traps of various kinds have been widely used to study the air spora in diseased crops (Fitt & McCartney, 1986). Horizontal slide traps (Hirst, 1953) are one example of static traps which are often used to collect spores on a daily basis, data from which over a period of time can be related to daily measurements of various enviromental factors. Hirst (1953) developed a spore trap in which the spore trapping surface moved continuously and air was impacted on to the surface through a narrow orifice so that a detailed picture of spore release during the day could be investigated. Modern equivalents of this trap are frequently used to investigate the air spora in crops (i.e. Burkard trap). From these traps both hourly and daily spore counts can be made and subsequently related to meteorological factors (e.g. Royle & Thomas, 1972) The first approach to comparing spore trap data with meteorological information is a visual comparison of the various data sets (Butt & Royle, 1974). This will allow the identification of any obvious relationships between single weather events and spore release and may lead to conclusions about the effect of general weather patterns on spore capture. To investigate the effect of meteorological factors more closely simple regression techniques can be used which will indicate whether spore release is correlated with any of the meteorological variables measured (Butt & Royle, 1974). This method was used by Royle & Thomas (1972) when investigating spore capture data for *Pseudoperonospora humuli* and they showed that a variety of meteorological variables were correlated with the capture of the spores of this pathogen. Further investigations of the relationships between meteorological variables and spore trap data can be carried out using multilinear regression where the effects of several variables combined can be assessed (Butt &

Royle, 1974). Spore trapping was used by Louvet & Billotte (1964) in a field study of *A. brassicae* in oilseed rape. Their data were presented for visual comparison but no further analysis was attempted to study the relationships between meteorological variables and spore capture.

Infection can be investigated in the field with the use of plant traps, plants of the particular crop grown apart from any source of inoculum and placed in the field to capture spores and allow infection to occur. These have been used in a variety of studies (Butt & Royle, 1974) but often just to give a qualitative measure of whether infection did or did not occur during the exposure period. They can however be used in a much more detailed study of the relationship between enviromental factors and infection e.g. Royle (1973). By exposing traps over short periods (e.g.48h) and then removing them to conditions suitable for lesion development but not for infection, count of the resulting lesions gives a measure of infection that occured during the exposure period. These data can then be compared and correlated with meteorological data in a similar fashion to the spore trap counts as described above. One added factor can also be used in the analysis, that of the spore trap counts for the same period. Plant traps were used by Humpherson-Jones & Ainsworth (1981 & 1982) in the study of *A. brassicae* on cabbages but the exposure period was relatively long (3-4 days) and no detailed information has been published. Other studies of infection by the pathogen have concentrated on controlled enviroment experimentation (e.g. Louvet & Billotte, 1964).

These two approachs of laboratory and field work in the study of meteorological factors on the life cycle of a pathogen are complementary and should be carried out simultaneosly. However, studies carried out in the field for this purpose are time consuming both in collecting and analysing the data and are often considered secondary to laboratory work. They can however, provide invaluable information to direct laboratory studies as well as giving data to test hypotheses and models constructed from laboratory work. Due to the apparent paucity of detailed field information to direct or test laboratory work, such field work was chosen for this study.

The objective of this work was to provide sufficient data so that various meteorological factors could, by simple visual comparisons and regression methods, be related to data collected from spore traps and plant traps.

CHAPTER 2 DISEASE DEVELOPMENT; FIELD PLANTS

2.1 Visual comparisons

2.1.1 Rainfall and temperature

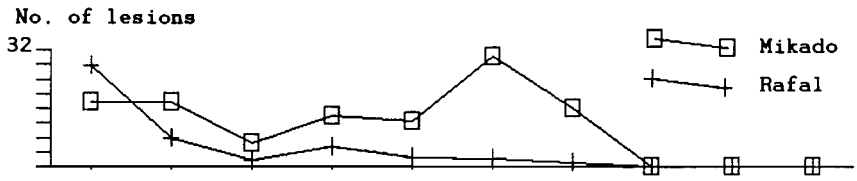
Data for the development of dark leaf and pod spot on the leaf and pod canopies of Rafal and Mikado, 1986 and cv. Bienvenu 1987 and 1988 are presented in Figs 2.1 - 2.3 together with rainfall and maximum temperatures taken from the meteorological site at Bridgets E.H.F. The number of lesions on the plants is given for 1987 and 1988 because as already discussed (Part 3, Chapter 2) this gives a better representation of the number of new infections that are occuring than the area of diseased tissue. These data are not available for the pod canopy of cvs. Rafal and Mikado and so disease area is used. Data from the pod canopy in all years are from Raceme T.

Neither rainfall or maximum temperature were obviously associated with disease development in every year. In 1986 the rapid development of disease on the pod canopy late in the season followed heavy rainfall on 4 and 5 July. Earlier in the season there was no obvious relationship between rainfall and disease development.

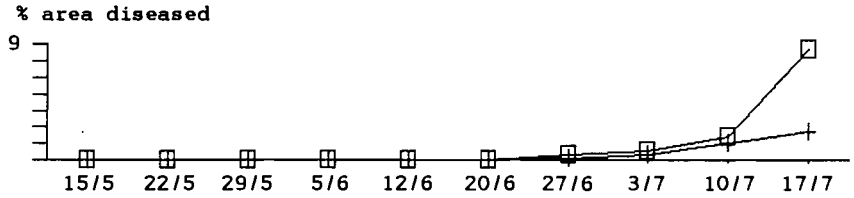
In 1987 (Fig 2.2) a sharp increase in lesion numbers between the 25 May and 1 June coincides with heavy rainfall on 30 May together with a general rise in maximum temperatures, however in the following week when there was rainfall on most days the number of lesions was declining on the leaf tissue and increasing only slowly on the pod canopy. Between 22 and 29 June a similar sharp increase in lesion numbers on the pod canopy was preceded by another period of heavy rain.

In 1988 (Fig 2.3) although periods of heavy rainfall occurred throughout April and May numbers of lesions remain at very low levels on the leaf tissue. However, the slight increase in lesion numbers on the leaf canopy and the beginings of disease devevlopment between 28 May and 6 June on the pod tissue coincided with heavy

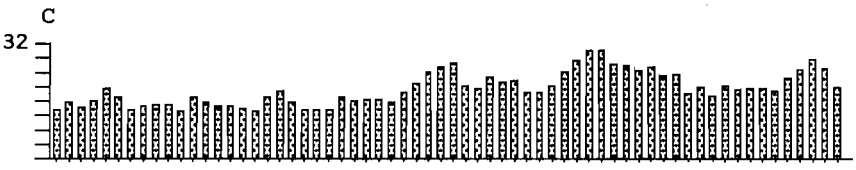
a)



b)



c)



d)

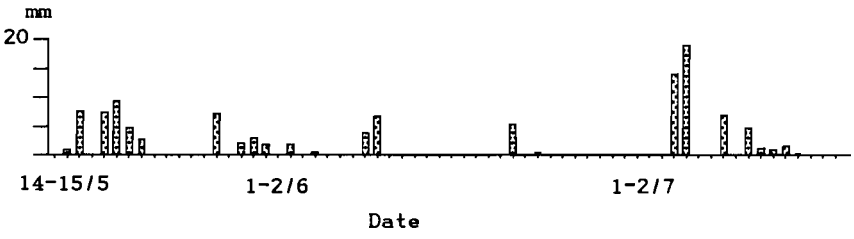


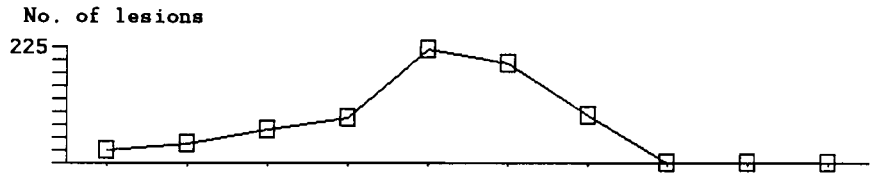
Fig 2.1: Visual comparisons of

a) TALLF*
b) RACDIS* for Raceme T
c) Maximum temperature (Bridgets met. station)
d) Rainfall (Bridgets met. station)

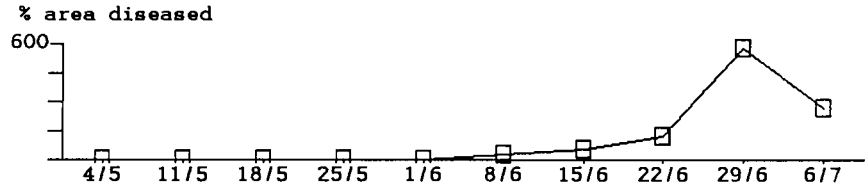
for Mikado and Rafal, 1986

* see text for explanation

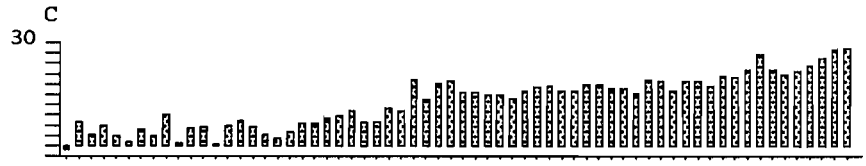
a)



b)



c)



d)

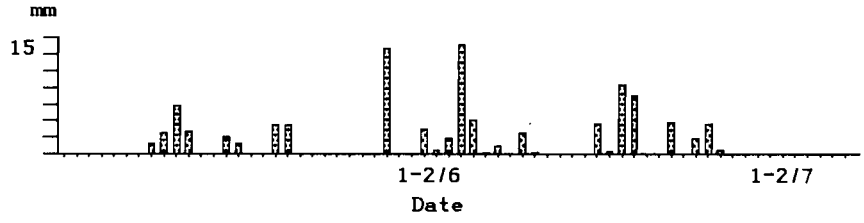


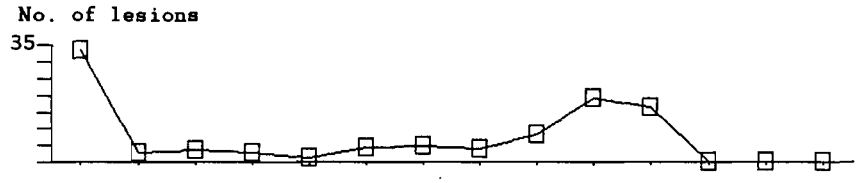
Fig 2.2: Visual comparisons of

a) TALLF*
b) ALRAC* for Raceme T
c) Maximum temperature (Bridgets met. station)
d) Rainfall (Bridgets met. station)

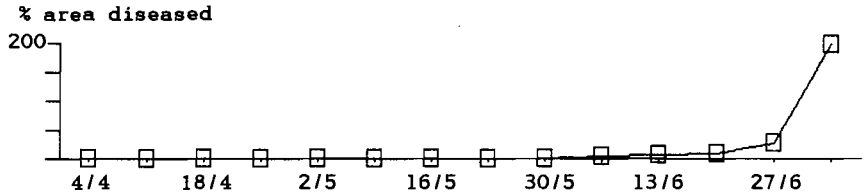
for cv. Bienvenu, 1987

* see text for explanation

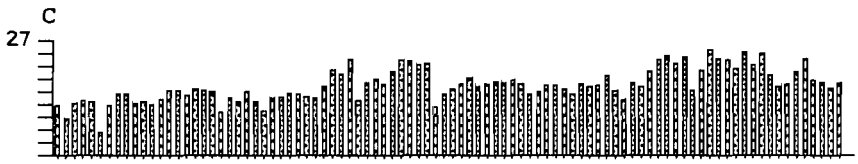
a)



b)



c)



d)

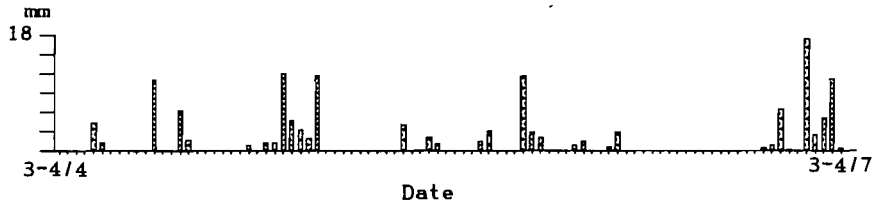


Fig 2.3: Visual comparisons of

a) TALLF*
b) ALRAC* for Raceme T
c) Maximum temperature (Bridgets met. station)
d) Rainfall (Bridgets met. station)

for cv. Bienvenu, 1988

* see text for explanation

rainfall on 28 - 30 May. The increase of lesion numbers on the pod canopy between 20 and 27 June occured despite a preceding period of very dry weather, however it is clear from the plant trap data that infection did occur on 25 and 26 May and lesions from these infection periods may already have been obvious at the assessment on the 27 May. Between 27 June and 4 July heavy rainfall occurred together with a large increase in the number of lesions on the pod canopy. In 1988 maximum temperatures bore no obvious relationship to disease development.

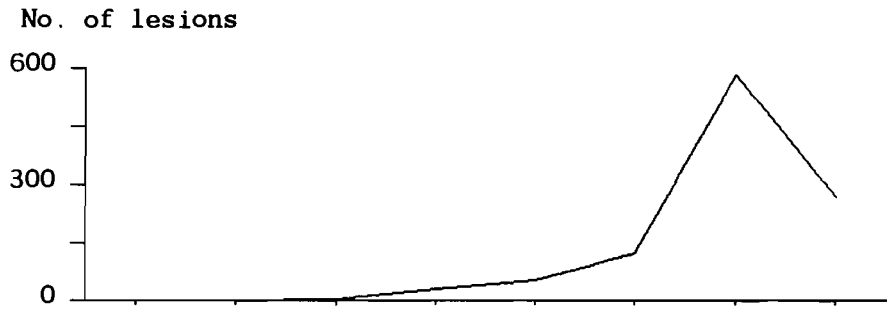
2.1.2 Sporulation and Infection periods

In 1987 and 1988 data from spore traps and plant traps were available to compare development of disease on the crop canopy with spore release and infection periods. In Figs 2.4 & 2.5 cumulative numbers of spores collected in the Burkard spore traps and the cumulative number of lesions on the plant traps were compared with the mean number of lesions on the pod canopy of the field plants during the period of disease development on the pods. Data for leaf disease were not included because of the difficulty of interpreting disease data on a continuosly changing crop canopy (Part 3, Chapter 2). The plant trap data were accumulated over six and eight day intervals as the plant traps were placed in the field for 48-hour periods. In 1988 only the final three weeks ' data are presented for the plant traps because of missing data in the preceding weeks (for details of methodology see Part 1, Chapter 2).

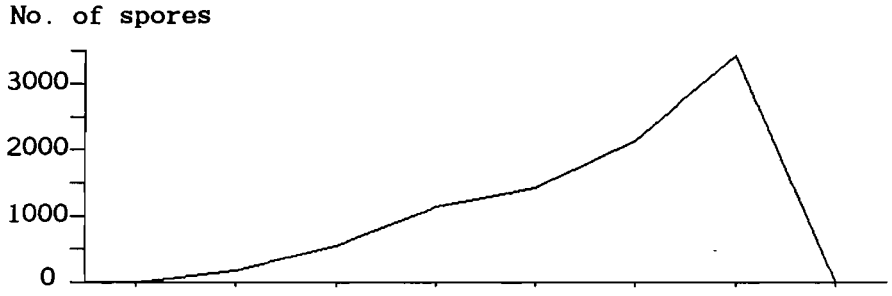
In 1987 (Fig. 2.4) the patterns of cumulative spore numbers (Burkard trap) and cumulative lesion numbers from the plant traps showed a different pattern to the development of lesions on the pod canopy in the field plants. The cumulative number of spores showed a curvilinear progress which did not exhibit the logarithmic shaped curve of lesion numbers on the pod canopy. The pattern exhibited by the number of lesions on the plant traps was also different to that on the pod canopy of the field crop, showing an almost linear increase between 15 June and 29 June but with little increase in the preceding week (9 - 15 June).

In 1988 the picture was different (Fig 2.5), with both the number of spores caught (Burkard) and the cumulative numbers of lesions on the plant traps showing

a)



b)



c)

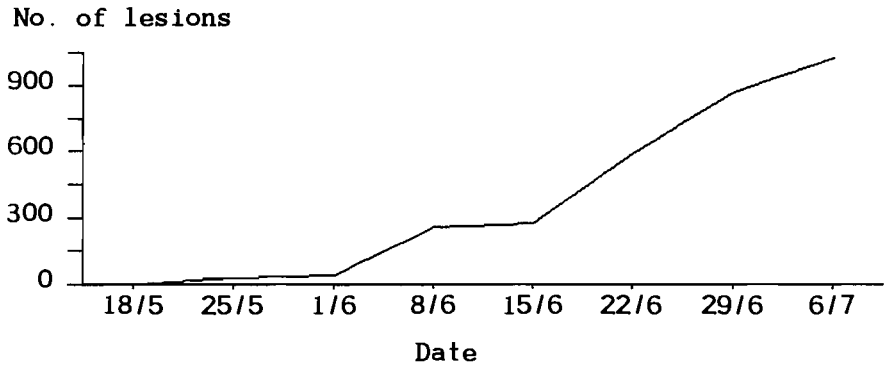


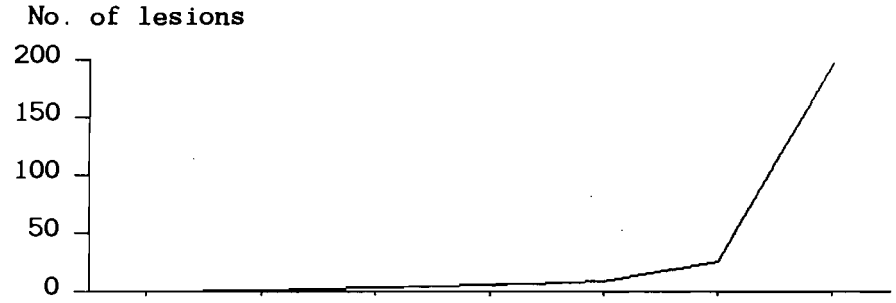
Fig 2.4: Visual comparison of

a) ALRAC* on Raceme T
b) Cumulative spore counts from Burkard trap
c) Cumulative lesion numbers from plant traps

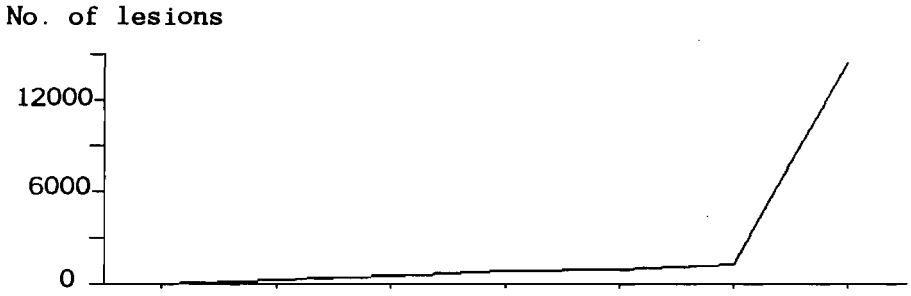
for Bienvenu,1987

* see text for explanation

a)



b)



c)

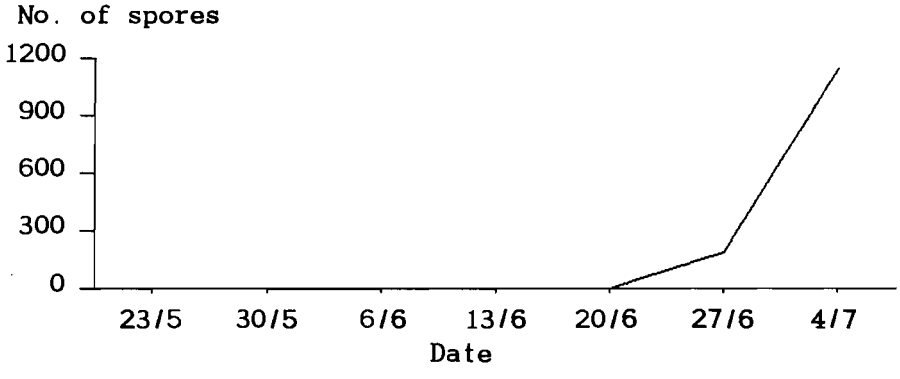


Fig 2.5: Visual comparison of

a) ALRAC* on Raceme T
b) Cumulative spore counts from Burkard trap
c) Cumulative lesion numbers from plant traps

for Bienvenu,1988

* see text for explanation

similar patterns to the number of lesions on the field plants with little activity until the final two weeks of the season.

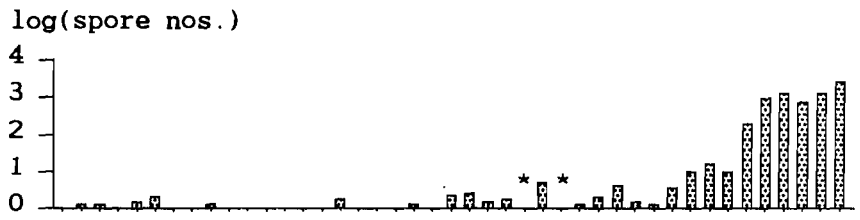
2.1.3 Spore capture at different canopy heights

As described in Part 1, Chapter 2, horizontal slide traps were placed at four heights within the crop canopy. Spore traps were changed at 48-hour intervals and therefore data are presented as 48-hour adjacent periods (Fig 2.6). The date at the base of the bar correspond to the day on which the trap was placed in the field.

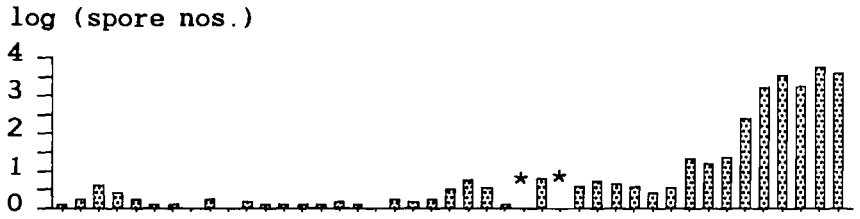
These data showed that although only low numbers of spores were collected before the middle of June there were differences between the numbers collected at the different positions within the crop canopy even in the early part of the season. Spores were being caught consistently at the base of the crop throughout the early part of the study whereas the number and consistency of spore capture declined at higher levels within the canopy. At the highest level (1.25m), which coincided with the centre of the pod canopy at its full development, few spores were captured prior to the beginning of June and after that only at very low levels until 27 June when a considerable increase in the number of spores caught was noted at all heights. However, even at the end of the season more spores were being caught on the traps at 0.25m, 0.5m and 0.75m than on the highest trap. This increase of trapped spores at the end of the season coincided with the increase of new lesions on the pod canopy.

As already discussed in Chapter 2 the position of the disease gradually increased in height as the leaf canopy developed and by 27 June there were no leaves remaining on the plants and therefore no disease lesions below the pod canopy. This suggests that during the latter part of the season no sporulating tissue was present at the same levels as the bottom (0.25m) and middle (0.5-0.75m) slide traps even though the numbers of spores caught by these traps increased substantially in the two weeks prior to the end of the study.

a)



b)



c)

d)

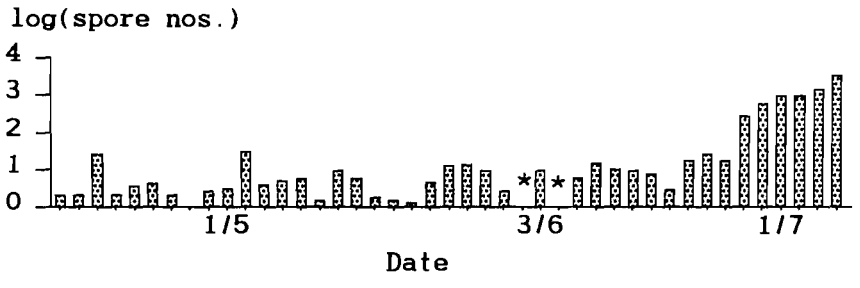


Fig 2.6: Spore trap counts (48h periods) from horizontal slide traps at different heights; Bienvenu, 1988

a) 1.25m
b) 0.75m
c) 0.5m
d) 0.25m

* missing data

2.2 Discussion

Visual comparisons of raw data will often give indications about the relationships between disease progress and meteoroloical factors (Butt & Royle, 1974). From the data presented here no consistent picture of the relationship between rainfall, temperature and disease development is shown. The lack of evidence that temperature has a marked effect on disease development is to be expected as temperatures were high enough for infection and disease development to occur (Louvet & Billotte, 1964; Humpherson-Jones & Hocart, 1982).

Rainfall also showed no consistent relationship with disease development. On two occassions in 1988 rainfall following a period of dry weather can clearly be associated with substantial increases in lesion numbers which may have been a consequence of an effect on inoculum levels (Part 4, Chapter 4). Royle *et al.* (1986) showed a similar pattern with *Septoria nodorum* and in this case it was an effect on spore dispersal. On other occassions when rainfall occurred, little or no disease development appeared to occur (April 1988). It seems therefore that care should be taken in concluding from these gross data that dark leaf and pod spot is exclusively a "wet" disease as suggested by Gladders (1983) although in each year rainfall did coincide with some period of disease development.

The two different patterns of relationship between the spore trap counts, plant trap data and disease development in 1987 and 1988 show that although patterns of disease development as measured on the pods may be similar (Part 3, Chapter 3), the underlying background for such development may differ from year to year. The 1988 data gave a more expected result, with the level of infection on the plant traps mirroring the increase in number of infections on the field plants suggesting that the suceptibility of the pod canopy to infection, and the level of inoculum reaching the pod canopy, bore a direct relationship to the susceptibility of the plant traps and the inoculum reaching them, and that the environmental conditions were similar for both. In 1987 these relationships were different. However, from these data it is impossible to assess whether the relative inoculum loads reaching the pod canopy of the field plants, compared with the plant traps or their relative susceptibilities to infection

were different or, whether the environmental conditions differed between the crop canopy and trap plants. Circumstantial evidence from spore trapping at different heights in 1988 suggests that although absolute numbers of spores may have been lower at the top of the crop canopy the pattern of inoculum available daily was likely to be similar at the pod canopy and at plant trap levels. Similarly from 1988 where plant trap and field plant infections were well matched it suggested that environmental conditons which affect infection were similar at the two levels. With susceptibility to infection the plant traps and field plants may have differed considerably. Mhirda (1983) and Kohle & Hoffmann (1989) have both shown that as tissue of oilseed rape ages it becomes more susceptible to infection by *A. brassicae* although in both cases work was carried out on plants grown under controlled environment conditions. If the susceptibility of the pods was increasing during the season then this may explain the discrepancy between the plant trap and field plant data during early pod development in 1987.

Similar differences and similarities between 1987 and 1988 occurred with the number of lesions on the field plants and the number of spores caught by the Burkard trap. Spores of *A. brassicae* are usually produced on lesions >3.5mm diameter and take 24 - 48h to develop following the correct environmental conditions (Humpherson-Jones, 1989). This taken together with the observation that very few sporulating lesions were found on the pod canopy in 1988 (Part 3, Chapter 2) suggests that the relationship between spore numbers and lesion numbers on the field plants in 1988 was caused by an increase in spore numbers giving increased numbers of infections rather than the increased infections giving rise to more spores. The source of these spores is unknown. Clearly it was not leaf tissue on the plants as very little infected leaf tissue was left at this time and the pod canopy was producing few spores. The major resevoir of sporulating tissue in the crop was likely to have been necrotic tissue at the crop base. Humpherson-Jones (1989) showed that necrotic tissue can sporulate for long periods if the weather conditions are conducive. This author suggested that the necrotic tissue would provide a resevoir of inoculum following periods when disease development was inhibited. Further evidence for this is provided by the data from spore traps set at different heights which showed that the greatest density of spores was found at or close to the bottom of the crop.

Deadman & Cooke (1989) showed that this was also the case for *Pyrenophora teres* in barley but concluded that this was mainly due to the leaching of the spores from the air by rain. Spore capture by horizontal slide traps with their rain covers is likely to be little affected by rain leaching although some spores may be deposited through splash depositon. Gradients of spore capture are always noted from an area of spore release (Fitt & MacCartney, 1986) and if rain leaching had little effect on the spore trap counts then the data suggests a source for the spores at the crop base.

Although there wass no clear relationship between spore numbers and infections on the pod canopy in 1987, the spore source may have been similar at least at the end of the season when lesions in the pod canopy increase rapidly. Again at this time little or no sporulating tissue was left on the plant so the inoculum source was likely to have been necrotic tissue at the plant base. The reason for the poor relationship between the spore trap and disease development on the pods is unclear although similar arguments can be put forward as were suggested for the plant trap data. A further consideration of the spore trap data is that conditons for spore production are likely to be differnt from infection conditions.

Although the spore traps at different heights within the crop canopy showed that there is a gradient up the plant, considerable numbers of spores were still caught at pod canopy height. If during the period of disease development on the pod canopy most of the sporulating tissue was at the base of the crop, spore movement through the canopy must have been considerable. No data are available from this study to elucidate the mechanism of movement and this type of investigation should be considered a high priority in the further study of *A. brassicae* on oilseed rape.

These general visual observations have shown that there are no obvious consistent relationships between rainfall, temperature, a measure of infection and a measure of inoculum levels and the development of dark leaf and pod spot. Furthermore between years considerable differences can be seen.

CHAPTER 3 INFECTION

3.1 Introduction

Studies of the infection processes of *A. brassicae* in the field are limited. Humpherson-Jones & Ainsworth (1981 & 1982) showed that infection on plant traps in a cabbage crop occured on many occasions during a season but suggested that data did not clearly relate to meteorological factors.

In his study of *Pseudoperonospora humuli* Royle (1973) described a method of using plant traps to investigate infection of this pathogen in the field. By placing plant traps (in this case hops) in the field for 48h periods and then removing them to a controlled enviroment he was able to quantify the relationship between the level of infection on the trap plants and the weather conditions during the exposure period. The use of 48h periods was dictated by the need to give enough time for infection to occur but to have short enough time periods so that the details of meteorological events during the period were not lost in the combined data from many days. A similar method of using plant traps was considered appropriate for this study and published data from laboratory studies (e.g. Louvet & Billotte, 1964; Humpherson-Jones & Hocart, 1982) showed that 48h exposure periods would allow enough time for infection by *A. brassicae* to occur.

Laboratory studies (Louvet & Billotte, 1964; Humpherson-Jones & Hocart, 1982) have also shown that periods of wetness or high humidity are required for infection and so in this initial analysis of the available data, only measures of wetness and humidity are considered in detail although data for wind and temperature are available for later analyses. A detailed description of the methods used for the capture are given in Part 1 Chapter 2.

3.2 Visual comparisons

Data from 1987 and 1988 are presented (Figs 3.1 & 3.2) to compare the levels of infection on the plant traps with meteorological and biological factors (Table 3.1) in a simple visual format. Wetness and humidity data were collected from probes at the base of the leaf canopy and spore trap data for 1988 are from the trap set at 0.5m above the ground. The limited nature of the meteorological data from 1987 is explained in the methodology section (Part 1, Chapter 2). The date on the x-axis of each graph represents the date on which the plant traps were placed in the field. So adjacent dates show data from overlapping periods of time for each variable. All data are therefore presented in bar chart format and cannot be directly compared with the graphs in Section 2.1 Meteorological and spore trap variables have been summed over this 48h period.

Table 3.1 Meteorological & Biological factors.

Meteorological *

WET	Hours of wetness as recorded at base of crop
RAIN	Amount of rainfall (mm)
RH80	Hours of relative humidity > 80%
RH90	Hours of relative humidity > 90%

Biological *1

BURKSP	Absolute numbers of spores on Burkard spore trap
SLIDSP	Spore numbers on horizontal slide traps

* for methodology of data capture see part 1, chapter 2.

1 all data for 48h periods

3.2.1 Comparison between years

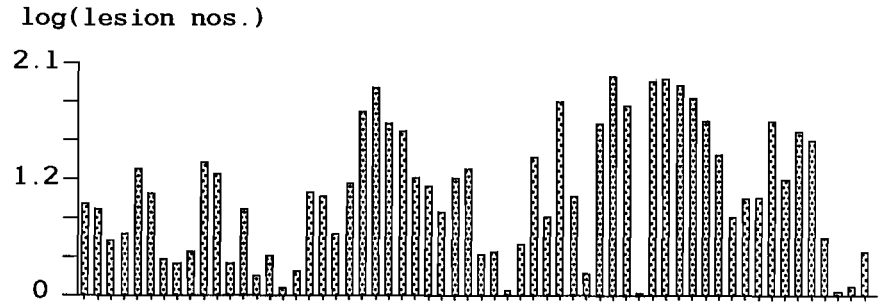
The pattern and numbers of lesions which developed on the plant traps differed considerably between 1987 and 1988 (Figs 3.1 & 3.2) In 1987 the maximum number of lesions which developed in any 48h exposure period was 186/100cm^2 of leaf tissue whereas in 1988 it was 480/100cm^2 of leaf tissue. This occured even though the final levels of disease in the field plants were much higher in 1987.

In 1987 the pattern of numbers of infections on the plant traps showed a series of peaks and troughs during the study period. During May the peaks rose to a maximum of 23.9 lesions/100cm^2 of leaf tissue and then during June and July peaks of infection reached 20.4 to 186 lesions/100cm^2. Over the whole period there was no general trend of increase in the number of infections which occurred. In contrast in 1988 the numbers of infections occurred at peaks of infection increased throughout the season and although in 1987 there were no days when infections did not occur in 1988 there was no infection on 27 days.

3.2.2 Comparison with spore trap catches

Figs 3.1 & 3.3 show that the relationship between spore numbers and lesion numbers on the plant traps differed considerably between the two years. In 1987 there was no clear relationship between the spore numbers as recorded in the Burkard trap and numbers of lesions on the plant traps. The spore trap catch gradually increased throughout the season whereas lesion numbers on the plant traps varied considerably and showed no overall trend of increase. In 1988 there was a closer relationship between infection and spore numbers. With spore numbers from the Burkard trap three distinct levels of spore capture occurred (see Chapter 4 for further discussion). These changes in spore numbers coincided with a similar overall changing pattern of lesion numbers on the trap plants except between 9 and 23 June when very low levels of infection occured during a period when considerable numbers of spores were caught. When the spore numbers caught on the horizontal slide traps were considered the general relationship between lesion numbers and spore numbers appeared more consistent, although again between 9 and 23 June low levels of

a)



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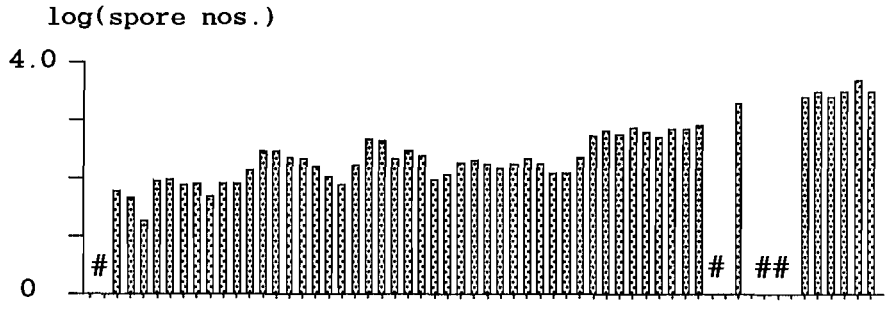


c)

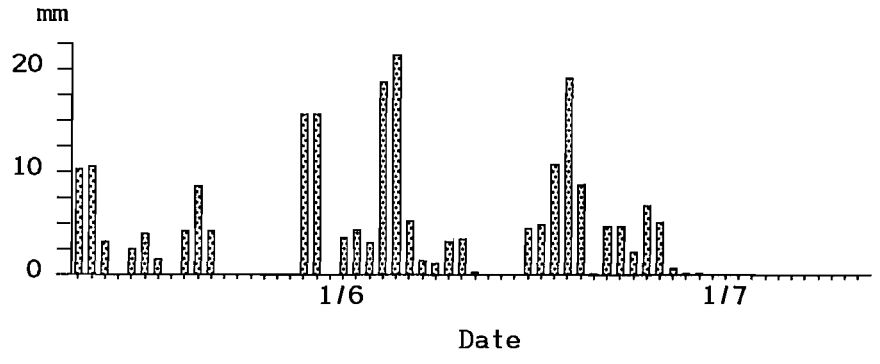


Fig 3.1: Visual comparison of

a) Lesions on plant traps (/100cm^2 leaf tissue)
b) log(BURKSP)*
c) Rainfall at Bridgets met. station

for Bienvenu, 1987

*see text for explanation
missing data

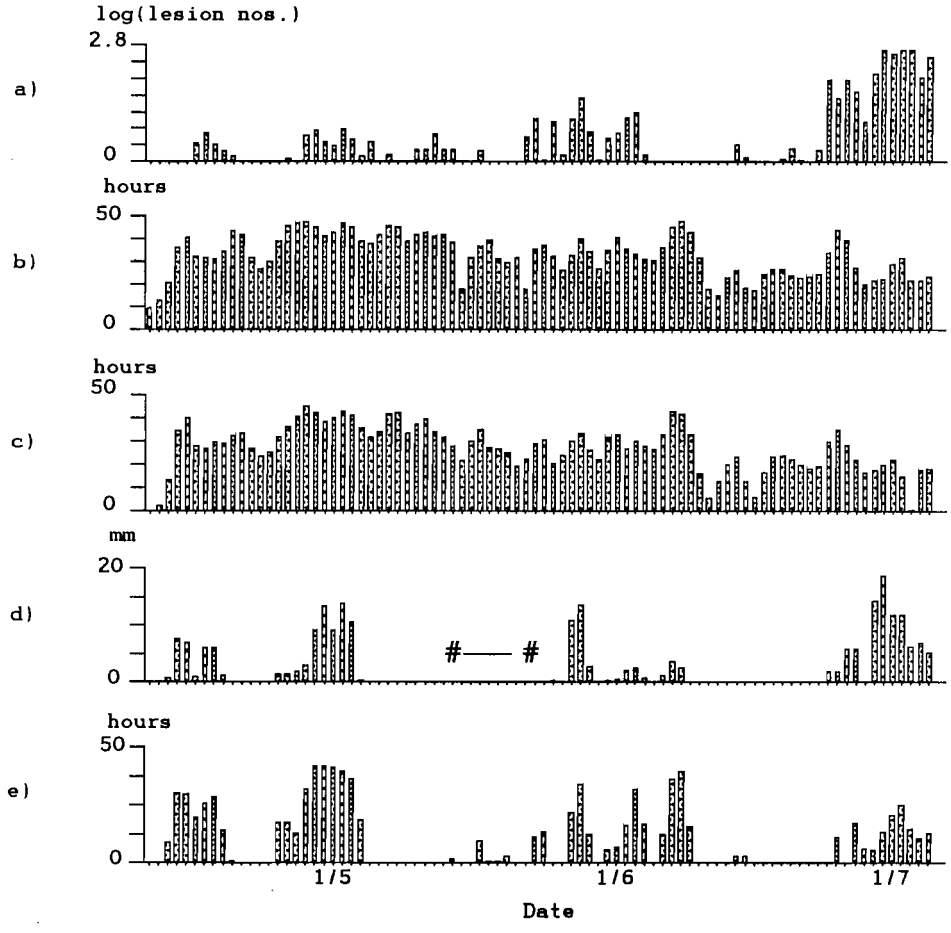


Fig 3.2: Visual comparison of

a) Lesions on plant traps (/100cm^2 leaf tissue)
b) RH80*
c) RH90*
d) RAIN*
e) WET *

*see text for explanation
missing data

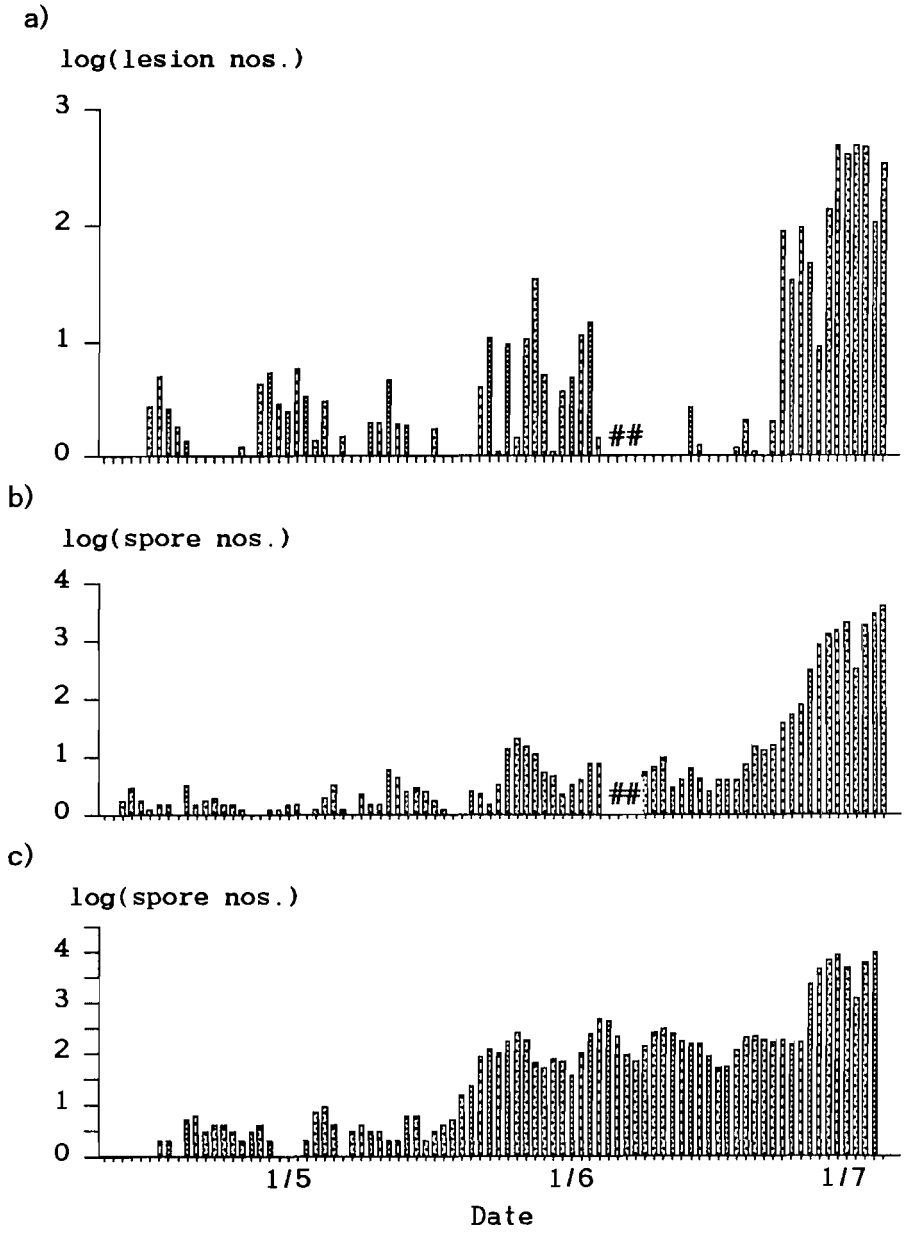


Fig 3.3: Visual comparison of

a) Lesions on plant traps (/100cm^2 of plant tissue)
b) SLIDSP*
c) BURKSP*

* see text for explanation
# missing data

infection coincided with high levels of spore capture. Underlying these general trends however were the daily variations in both plant trap and spore trap catches. A comparison of days where both spores were caught and lesions developed with those where spores were

Table 3.2 Occurrence of single and combined events with plant trap numbers, spore counts and wetness.

EVENTS	NO OF DAYS
+ LESIONS	55
- LESIONS	27
+ SPORES	
- LESIONS	
BURKARD	22
SLIDE	19
- SPORES	
+ LESIONS	
BURKARD	5
SLIDE	3
+ LESIONS	
+ SPORE	
- WET	10
- LESIONS	
+ SPORES	
+ WET	
BURKARD	3
SLIDE	2
+ WET	
- LESIONS	4

trapped but no lesions noted showed that on 19 days (horizontal slide trap) and 22 days (Burkard trap) when spores were caught no lesions developed. Conversely on only three (horizontal slide trap) and five (Burkard) occasions did infection occur when no spores were caught.

3.2.3 Comparison with meteorological variables

In 1987 where only limited data were available to compare lesion numbers on the trap plants with meteorological data (Fig 3.4) no strong relationships were clear. A peak of infection on 21 and 22 May coincided with rainfall but on 5 June when lesion numbers were high no wetness was recorded. A similar relationship was obvious with RH80 and RH90.

In 1988 simple general relationships were clearer (Fig. 3.2) and were characterized using a comparison of whether certain events did or did not occur during the same 48h period. Comparisons were made using $\pm$ infection on the plant traps, $\pm$ wetness, $\pm$ spores trapped on either horizontal slide traps or Burkard traps (Table 3.2). On only 9 out of 55 48h periods when infection occurred was there no period of wetness and during five of these (30/5-1/6, 26/5-28/5, 21/6-23/6, 19/6-21/6, 7/5-9/5) only very low numbers of infections were noted (0.08-1.03 lesions/100cm ^{2} leaf tissue). During only two 48h periods were there wetness periods but no infection on the trap plants. On 13-15 April only a very low spore count was noted (0.75 spores/slide trap) and on the other occasion (18 - 20 May) the wetness period only lasted 0.5h.

The majority of the wetness measured by the probe at the base of the canopy during this period was rain related (Fig 3.2). No relationship was obvious between infection and either RH80 or RH90.

3.3 Simple linear regression

3.3.1 Methodology

Data from 1987 (limited) and 1988 were used to study the quantitative relationship between the number of infections on the trap plants (dependent variable) and a number of biological and meteorological factors (Table 3.1) using simple linear regression. The dependent variable, lesions/100cm ^{2} leaf tissue, was transformed using a log (x+1) transformation before analysis. This transformation was considered

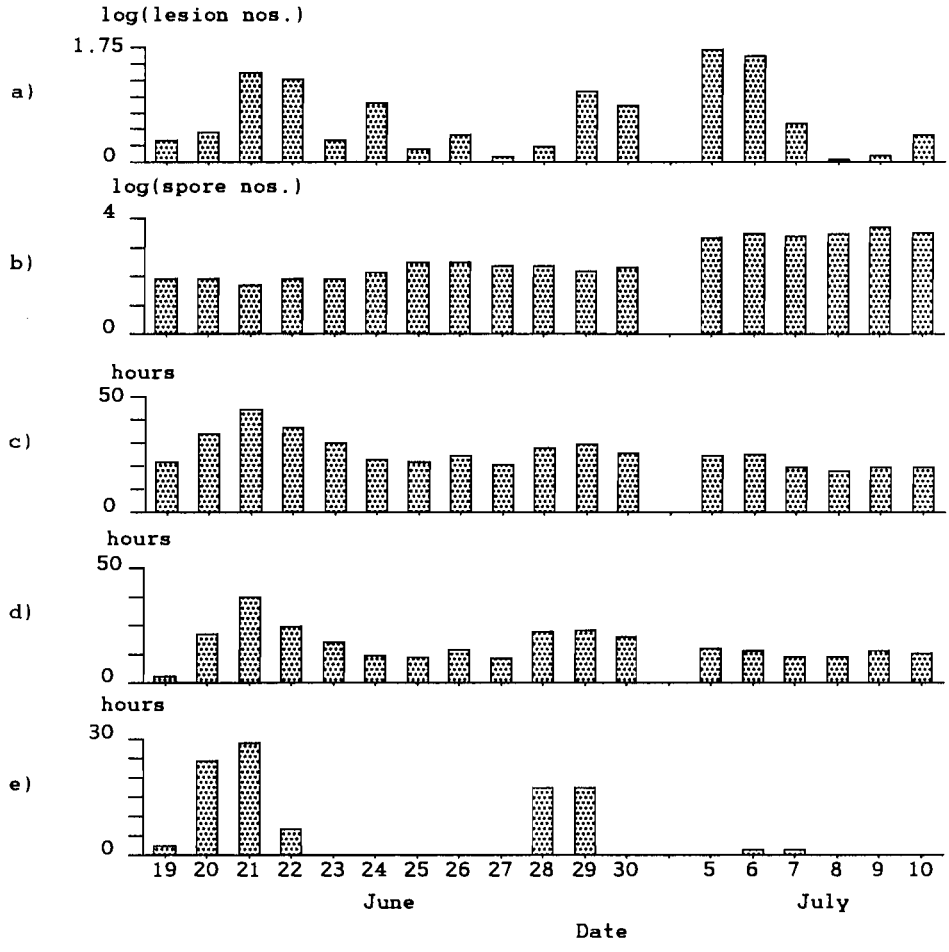


Fig 3.4: Visual comparison of

a) Lesions on plant traps (/100cm^2 of plant tissue)
b) BURKSP*
c) RH80*
d) RH90*
e) WET*

Bienvenu, 1987; Limited data

*see text for explanation

necessary following the results of analyses of lesion number data as described in Part 2, Chapter 3.

A major assumption of regression analysis is that each data point of the dependent variable is independent of every other (Sokal and Rolfe, 1981). In a series of data collected daily over a period of weeks this is often untrue (Butt & Royle, 1974). For example if a plant was exposed to infection over a period of weeks and initial infections made the plant resistent to later infections then new infections appearing on day 10 would be dependent on those which occurred earlier. However, these data from the plant traps are independent because infection on one set of plants cannot influence the amount of infection on any other set. The data can therefore be analysed directly following transformation.

Spore counts were also transformed using a $\log(x+1)$ transformation as this was likely to linearise the data (Royle & Thomas, 1972). No transformations were carried out on the meteorological data.

3.3.2 Results

In 1987 (Table 3.3) with the complete data set there was no significant relationship between BURKSP and LES whereas in 1988 over 34% ($p < 0.001$) of the variance was accounted for by BURKSP. Furthemore in 1988 SLIDSP gave a better relationship accounting for 64.5% ($p < 0.001$) of the variation.

With the meteorological variables differences between the two years were also clear. In 1987, with the limited data set RH80 showed the best relationship ($R^2 = 24.07\%, p < 0.01$) with RH90 being weaker ($R^2 = 19.57\%, p < 0.05$) and wetness showing no significant relationship. In 1988 however, wetness was the only meteorological variable to account for a significant amount of the variance ($R^2 = 10.71, p < 0.01$).

Table 3.3: Coefficients of determination for the regression of the number of *Alternaria* lesions on plant traps on a variety of biological and meteorological variables

Variable	R^2	sig	R^2	sig
WET	10.7	++[c]	5.0[a]	NS
RH80	1.0	NS	24.1[a]	++
RH90	2.6	NS	19.6[a]	+
BURKSP	34.7	+++	1.4[b]	NS
SLIDSP	64.6	+++	-	-

a limited data only
b full data
c NS, non-significant; +, $p < 0.05$; ++, $p < 0.01$; +++, $p < 0.001$

3.4 Multilinear regression

3.4.1 Methodology

In Section 3.3 several independent variables were investigated, by simple regression analysis, for their individual contributions to lesion numbers on the trap plants during the 48h exposure period. Simple linear regression evaluates the linear model to see if it adequately represents the observed data using the method of least squares to give an estimated regression line. This is a predictive equation and defines the linear relationship between the dependent and independent variables. These simple linear regressions can be expanded into Multiple (partial) regression when the lesion numbers on the plant traps is considered to be a linear function of two or more independent variables. This fuction is described by the equation

$$y = a + b_1x_1 + b_2x_2 \ldots\ldots b_nx_n$$

where constants b_1 to b_n are partial regression coefficients and are estimates of the net linear effect of the independent variables x_1 to x_n on the total response of the dependent variable (Butt & Royle, 1974).

The analysis of variance which accompanies multiple regression analysis is important in allowing the model to be tested and compared with others. The basis for the analysis of variance is the total variation in the observed values of the dependent variable from their mean (the total sum of squares). This is made up of the sum of squares due to regression i.e. the proportion of error accounted for by the independent variables and the residual error. The latter which describes the difference between observed and predicted values is a measure of the inadequacy of the independent variable to account for the total variation in the dependent variable. The mean square of the residuals is therefore an estimate of the variance of the error term in the model and is used in variance ratio tests of the statistical significance of the combined and individual effects of the independent variables.

The coefficient of multiple determination R^2 is the proportion of total variation in the dependent variable which is accounted for by multiple regression on the independent variables. It is a convenient assessment of the fit of the regression equation since when R^2 is near unity almost all the variation has been explained. An alternative to R^2 which Butt & Royle (1974) suggest is more useful is the ratio mean square due to regression/total mean square. This is the proportion of total variance accounted for by multiple regression. Whereas the addition of each new independent variable to the equation increases R^2 towards unity, the proportion of total variance accounted for can reach a maximum of less than unity and subsequently decline as each new predictor is added. As R^2 continues to rise, the precision of estimation by the equation progressively declines as the optimum number of independent variables is exceeded (Butt and Royle, 1974).

Data from 1987 (limited) and 1988 were analysed using multilinear regression in an attempt to relate a range of variables to infection on the plant traps. The independent and dependent variables were treated as for simple linear regression as the same assumptions apply. Multilinear regression was initially carried out using a forward enter procedure. Following this automatic procedure variables were added and removed manually so that all possible combinations of variables were tested.

3.4.2 Results

As anticipated from the general visual comparisons and simple linear regression, results (Table 3.4) showed that in 1987 no combination

Table 3.4: Coefficients of determination (modified) of multilinear regressions for 1987

Variables included	R^2 (modified)*
RH80	0.19
RH80, BURKSP	0.32
RH80, BURKSP, RH90	0.40
RH80, BURKSP, WET	0.35

* see text for explanation

of the variables explained a high proportion of the variation in the dependent variable. The combination of RH80 and BURKSP only accounted for 32.9% of the total variation of the dependent variable and the addition of RH90 or WET improved the relationship slightly but not significantly.

In 1988 (Table 3.5) the combination of SLIDSP, WET and RH80 accounted for 78.5% of the variation of the dependent variable whereas replacing SLIDSP with BURKSP reduced this to 59.4%. The replacement of RH80 with RH90 gave very similar results (78 and 57.5% respectively). The removal of RH80 from these analyses showed that even with only two variables, SLIDSP and WET or BURKSP and WET, 76.3 and 54.2% of the variation was accounted for respectively. If the biological factor of spore numbers was removed then no significant relationships could be developed although WET and RH90 in combination accounted for 19.2% of the variation in the dependent variable.

3.5 Prediction

Using the formula constructed from the results of the multilinear regression using BURKSP, WET and RH80 for 1988 lesion numbers for the periods 19 - 29

Table 3.5: Coefficients of determination (modified) of multilinear regressions for 1987

Base variable	Variables included	R^2 (modified)*
SLIDSP	SLIDSP	0.65
	SLIDSP, WET	0.76
	SLIDSP, WET, RH80	0.79
	SLIDSP, WET, RH90	0.78
BURKSP	BURKSP	0.35
	BURKSP, WET	0.54
	BURKSP, WET, RH80	0.59
	BURKSP, WET, RH90	0.58
	BURKSP, WET, RH80, RH90	0.59
Met variables only	WET	0.10
	WET, RH90	0.19

* see text for explaination

May and 30 Jume - 10 July 1987 were predicted (Fig 3.5). The prediction, as expected from the poor relationships of lesion numbers with meteorological data in 1987, was weak and could not be considered useful.

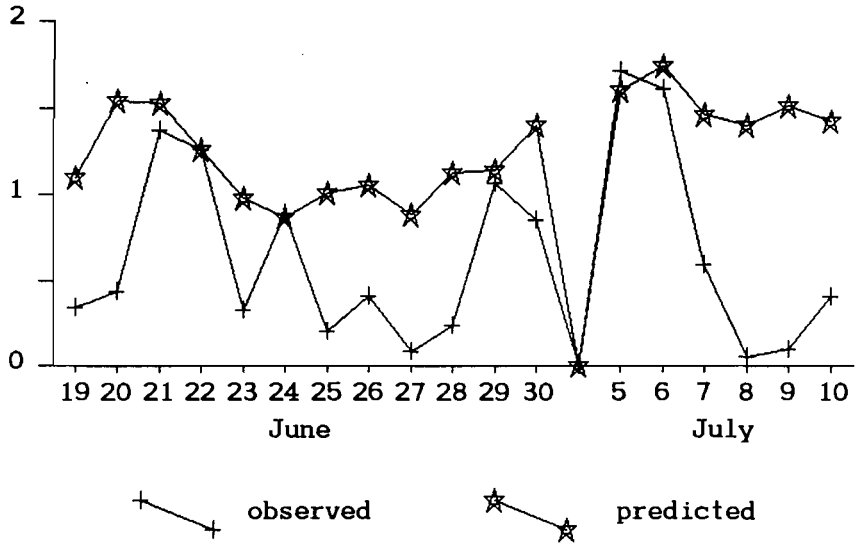


Fig 3.5: Prediction of lesions on plant traps in 1987 using equation developed from 1988 data

3.6 Discussion

Although plant traps have been used to study *A. brassicae* infection in the field (Humpherson-Jones & Ainsworth, 1980 & 1981) only relatively long periods of exposure were used, limiting the usefulness of the data for interpretation of the effect of meteorological factors on infection. With the 48h exposure periods it was hoped to overcome this problem and develop relationships between biological (spore counts) and meteorological variables and lesion numbers on the plant traps.

The clear difference in the pattern of infections on the plant traps between the two seasons appeared to be closely related to the levels of inoculum present. The close linear relationship between inoculum levels (as measured by the spore traps) and plant trap counts in 1988 suggested that at least during the first two phases the level of inoculum was limiting maximum infection levels on the plant traps. In 1987 when spore counts remained relatively constant throughout the experimental period the maximum levels of infection also remained constant. In 1987 there was a suggestion that infection levels were slightly lower at the beginning of the study when spore capture was lower and later in the season when spore capture increased, environmental conditions (see later) were probably not optimal for infection and so maximum infection levels may not have been reached. This probably explains the discrepency between the maximum levels of infection at the end of the season in the two years compared with the levels of spore capture by the Burkard traps.

The closer relationship between the horizontal spore traps than the Burkard trap with infection on the plant traps is probably a consequence of the closer relationship between spore capture on a leaf surface and that on the horizontal slide trap than with the Burkard trap. Although ineffecient, spores are impacted on the slide trap in the same way as they are on a leaf, by wind eddies around the trapping surface whereas with a Burkard trap the spores are forced to impact on the trapping surface by pumped air which is in effect scrubbed.

The substantial linear relationship between spore counts and lesion numbers on the plant traps in 1988 was most likely a consequence of factors affecting spore

production and release and reflected the large changes in inoculum levels which occurred during that year. Where maximum inoculum levels remained relatively constant over a period (1987, and during plateaus in 1988) the relationship between spore release and lesion numbers was poor. This is similar to the findings of Royle (1973) who showed that airspora variables for *P. humuli* did not relate closely to infection on trap plants. He concluded that this was because of the different climatic conditions required for spore release and infection. With *A. brassicae* a similar situation may arise as climatic conditions for spore release and infection are likely to be different.

In 1988 the qualitative relationship between climatic factors which are related to wetness fit well with laboratory studies on infection which show that some period of wetness is required for infection (Humpherson-Jones & Hocart, 1982). The high percentage of occasions when rain was related to the wetness events which were allied to infection is probably a consequence of the position of the trap plants in the crop. By being placed in a clearing at the crop base they were often protected from dew, wetness caused by dew therefore having little chance to affect infection on the plant traps at this level. Data from plant traps placed at pod canopy level are yet to be analysed. Futhermore because for this analysis data from the probes placed within the leaf canopy were used wetness caused by dew was recorded on only few occasions. The poor quantitative relationship between wetness and lesion numbers may have been related to the overiding effect of spore numbers on infection which affects all the simple linear relationships with meteorological variables.

The equations developed from multilinear regression were also dominated by spore count variables but showed that wetness significantly improve the prediction of lesion numbers on the plant traps. However, the improvement is not as great as might have been expected from a variable which was so closely related qualitatively to infection. This may have been a consequence of the fact that infection may only require short periods of wetness at high temperatures (e.g. Louvet & Billotte, 1964) and that in some cases during the season the length of the wetness period was super-optimal for maximum infection. This suggests that a combined variable of

temperature and wetness which takes account of an upper cut off point would be superior to simple wetness and temperature variables.

The significant but small increases in R^2 (mod) which occurred by the addition of RH80 and RH90 were undoubtedly a result of their close relationship to wetness. However, it does suggest that, if as noted by Humpherson-Jones & Hocart (1982) *A. brassicae* has an absolute requirement for wetness during infection that the relationship between wetness on the probes and on the plant trap surfaces was not adequate.

Although providing some leads about the likely requirements for infection by *A.brassicae* the overriding effect of spore numbers in 1988 masked any clear relationship which might have been developed. To overcome this, two approaches could be taken with the data. First data from a period in the season when spore release was consistent could be used. This however substantially reduces the data available for analysis. Second, spore numbers could be assimilated into the dependent variable by dividing the lesion numbers on the plant traps by spore numbers (Alderman *et al.*, 1987).

CHAPTER 4 SPORULATION

4.1 Introduction

Although recently published data (Humpherson-Jones & Phelps, 1989) have provided detailed information about the requirements for sporulation by *A.brassicae* under controlled enviromental conditions little information is available of the relationship between spore production and release in the field. Louvet & Billotte (1964) used a volumetric spore trap to capture spores in a field of oilseed rape heavily infected with *A. brassicae.* They showed by visually comparing graphical representations of various meteorological data and spore trap counts that spore capture was related to wind intensity, changes in RH and rainfall. Furthermore, periods of high spore capture often followed rain which was preceded by several days of dry weather. These relationships described by Louvet and Billotte (1964) suggest that a quantitative approach to the comparison of various meteorological parameters with data from spore traps may be of value.

Spore trapping can be carried out using a variety of techniques from simple static traps (Hirst, 1953) which collect spores by impaction due to a air movement onto a sticky surface to volumetric traps which by pulling air through a narrow orifice at high volume impact spores onto a moving sticky surface. Of the former, two types of trap are commonly used, the vertical cylinder and horizontal slide (Fitt & MacCartney,1986). They both have the benefit of being simple to use and requiring no maintenance although the vertical rod traps need a greater preparation time both before and following the trapping period. Hirst (1953) showed that for a wide variety of airspora the vertical cylinder was a more efficient trap than the horizontal slide, but that both were adequate for the capture of large spores such as *A. brassicae.* One benefit of these types of trap over a volumetric type trap with its higher efficiency is that they model the capture of spores on a leaf surface better than the Burkard trap (Putter, Pers. comm.). However, the volumetric type trap (e.g. Burkard) generally have higher trapping efficiencies than the static traps (Hirst, 1953) especially at low wind speeds which means that they will catch spores at lower airspora densities than

the static type trap. They also benefit from being able to catch spores on a moving surface which allows the analysis of diurnal patterns of spore release from the data. They are designed to catch wind dispersed spores and will underestimate any spore release which occurs during rainfall.

In this study spore traps were used in 1987 and 1988. In 1987 only a Burkard volumetric trap was used because a low time input system was required as the main objective was to compare spore catches with data from plant traps. It was considered that although some secondary dispersal was likely to be rain initiated, the major periods of spore dispersal would be during dry conditions when spores are wind dispersed due to the large surface area of the *A. brassicae* spores (McCartney, Pers comm.). In 1988 when a more comprehensive study of spore capture was carried out static traps were also included. The choice of horizontal spore traps was made because these were less time consuming to prepare and count. Furthermore although they are not designed to trap rain dispersed spores, droplets landing on the traps during rain will deposit their spore load. The inclusion of the horizontal slide traps not only allowed a comparison between different traps but also a comparison of spore capture at different heights within the crop canopy. Other traps were included in 1988 but are not discussed here.

As with the plant trap data simple and multiple linear regression analyses were used to analyse the data following initial visual interpretation. The analyses presented here are preliminary and only include the same meteorological variables considered for the plant trap data.

4.2 Visual comparisons

Data are presented in a simple visual format to compare the spore trap between years, different traps and with meteorological data. Except where Burkard trap counts are compared with horizontal slide trap counts periods of 24h are considered, the date below the bars corresponding to the day on which the count was started.

Where horizontal slide traps are included the trap height used for comparison is 0.5m and the time period extended to 48h as the horizontal slide traps were placed in the field for 48h periods. In this case the date on the x-axis represents the date on which the horizontal slide traps were placed in the field and so adjacent dates show data from overlaping exposure periods.

Data are presented for the visual comparison of Burkard trap counts for 1987 with rainfall (Bridgets Met Station) and 1988 with rainfall (Tipping bucket, 1988), WET, RH90 and RH80.

4.2.1 Comparison between years

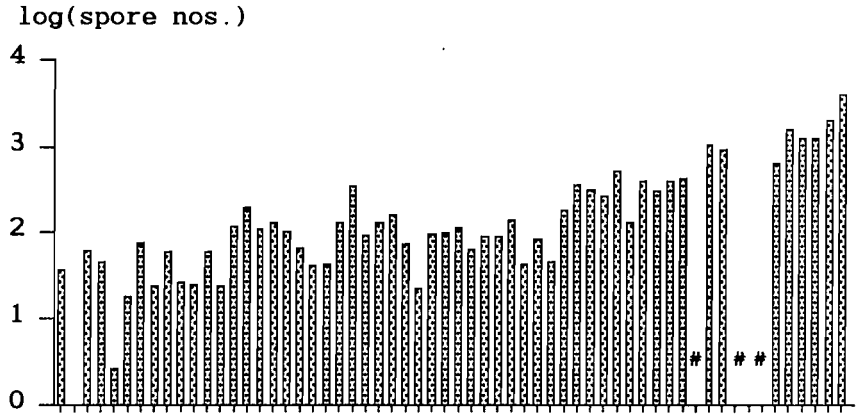
The numbers of spores caught and the pattern of their release throughout the study period differed considerably between 1987 and 1988 (Fig 4.1 & 4.2). In 1987 spore numbers showed a gradual increase throughout the season with peaks and troughs superimposed onto this increase. These peaks and troughs did not follow an obvious pattern throughout the season although between 26 May and 28 June there was a suggestion of a cylic pattern with a periodicity of eight to nine days. The maximum number of spores caught on any day was 5021.

In 1988 the pattern of spore release was different. Data from the Burkard trap showed three distinct phases which are discussed in more detail in Section 4.2.2. During each phase the range of spore catches was distinct from the range in the other phases. Peaks and troughs were apparent in each phase and between 22 May and 27 June have a periodicity of 5 to 6 days. The maximum number of spores caught in any 48h period was 9437, substantially higher than in 1987.

4.2.2 Meteorological variables

In 1988 the gradual increase in spore numbers (Burkard trap) throughout the season found in 1987 was replaced by three distinct phases (Fig 4.2). During the first, 11 April to 20 May very low levels of spores were trapped (0 - 5) in any 24h period. At the end of this phase, over a period of two days the numbers of spores increased

a)



b)

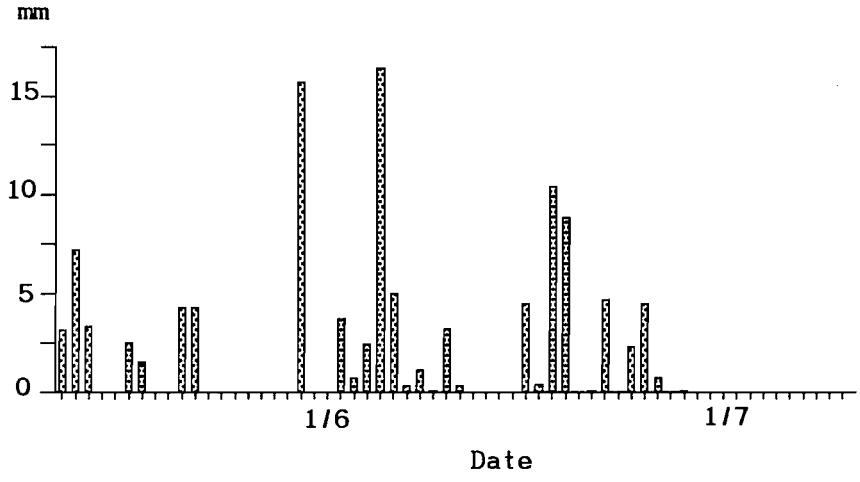


Fig 4.1: Visual comparison of

a) log(BURKSP)*
b) Rainfall at Bridgets met. station

Bienvenu, 1987; Variables accumulated over 24h

*see text for explanation
missing data

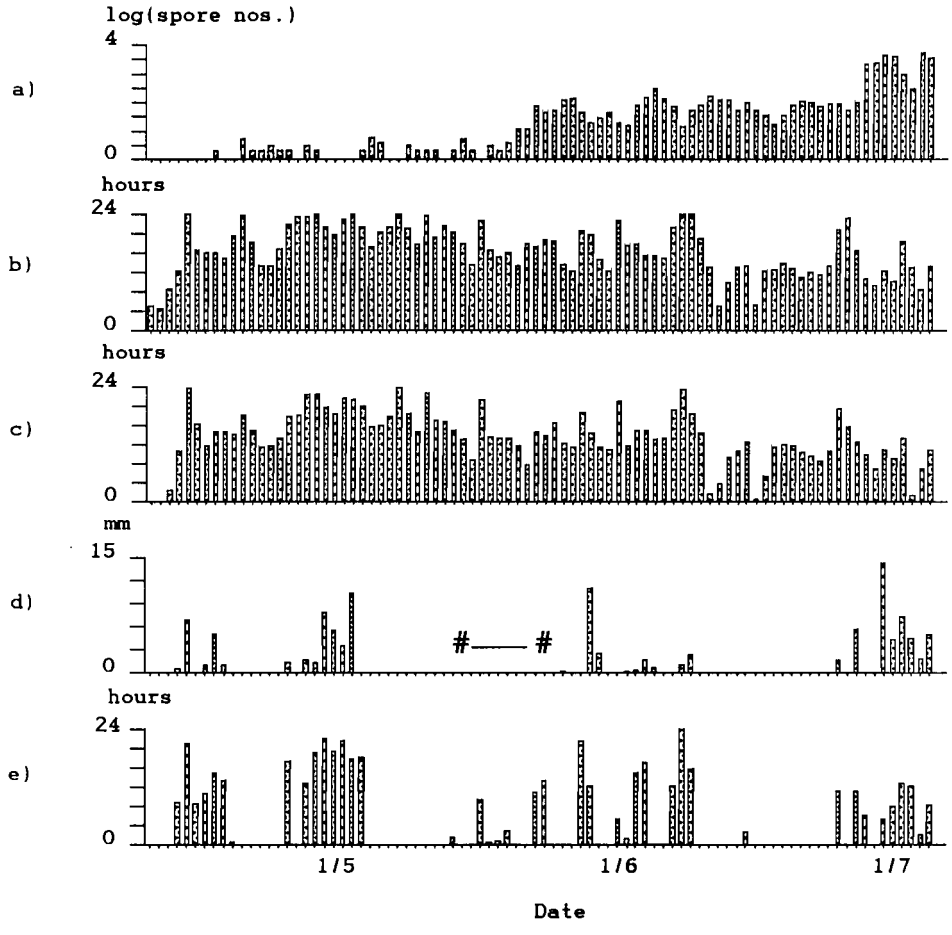


Fig 4.2: Visual comparison of

a) BURKSP*
b) RH80*
c) RH90*
d) RAIN*
e) WET*

Bienvenu, 1988

* see text for explanation
# missing data

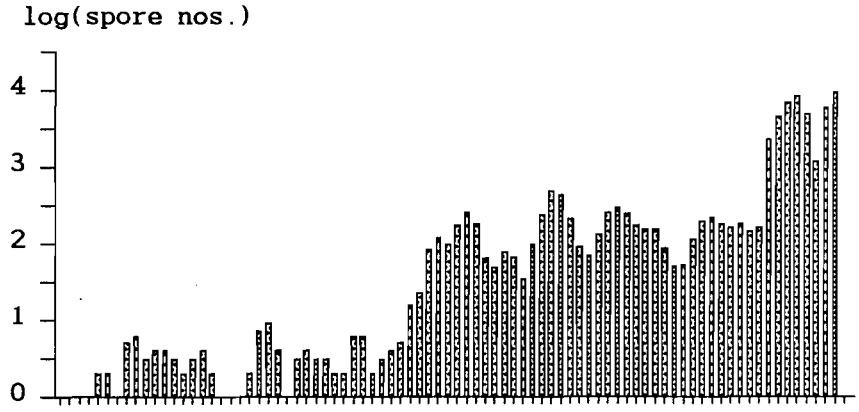
greatly so that between 23 May and 27 June the range of spores trapped was 34 - 486 in any 24h period. During this period distinct peaks and troughs of spore release were noted. On three occassions, the peaks of spore release occuring on 27 May, 5 June, 12 June, followed four days after a distinct wet period, caused by rainfall, which itself was preceded by a period of 2 - 4 days when no wetness was recorded. The other major distinct peak on 22 June followed a period of wetness 6 days previously. In each case the peak of spore capture occurred on a day when no wetness was recorded. On 28 June the spore numbers again increased dramatically so that between 27 June and 5 July the range caught was 1188 to 9437 in any 24h period. Similarly in this period a peak of spore release noted on 1 July was preceded by wetness on 26 June following a long period (9 days) when wetness had not been recorded. These peaks of spore release as in 1987 were again superimposed onto general and in some cases high (28 June - 4 July) level of daily spore release.

The increase of spore release representing the change between phases on 21 - 23 May and on 28 June could both be related to periods of wetness caused by rainfall occurring in the preceding 4-5 days which followed a period of days when wetness was not recorded or only for very short periods. Between 5 May and 16 May only 1.75 hours of wetness was recorded followed by heavy rain and a long period of wetness (nine hours) on 17 May which was then followed by increased spore release on 21 and 22 May. Similarly a period of heavy rain and long wetness (11 hours) on 25 June following 15 days when only 0.75 hours of wetness had been recorded, preceded the large increase in spore release on the 28 June. In 1987 the pattern of spore release bore no obvious relationship to rainfall as recorded at the Bridgets Met Station. In 1988 RH80 and RH90 although following closely the pattern portrayed by wetness showed no obvious relationship to spore release except those given by rainfall and wetness.

4.2.3 Comparison between spore traps

Data from the Burkard and horizontal slide traps in 1988 (Fig 4.3) showed that there were different patterns of spore capture by the two traps. Each type of trap showed three distinct phases during the season. The first phase from 11 April to

a)



b)

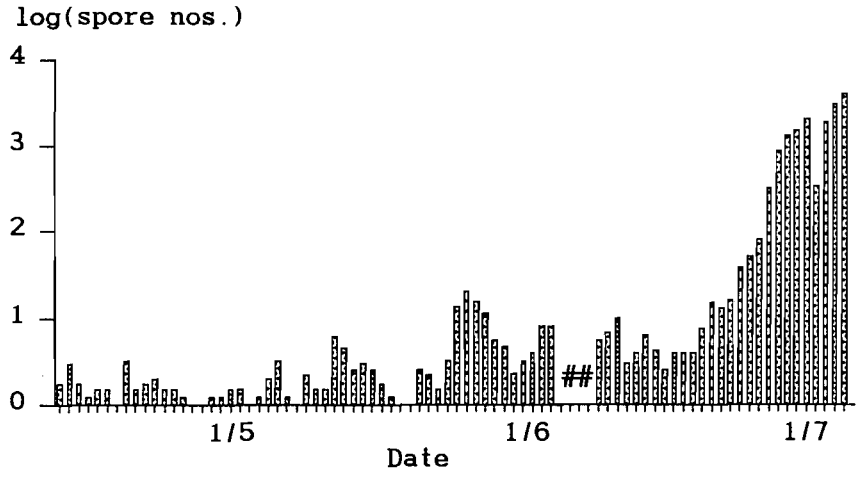


Fig 4.3: Visual comparison of

a) BURKSP*
b) SLIDSP*

Bienvenu, 1988

* see text for explanation
# missing data

approximately 20 May showed a very low level of spore capture by both types of trap. During some two day periods the Burkard trap caught more spores and during other periods the horizontal slide trap did. During the second phase (21 May - 26 June) the Burkard trap consistently captured more spores than the slide trap however the relative levels of spore capture varied considerably during this period. Fig 4.4 shows the ratio BURKSP/SLIDSP for the whole of the 1988 season. In the second phase of spore capture this ratio ranged from 9.1 to 81.3, the peaks corresponding to the peaks of spore capture by the Burkard trap. At the end of phase two however spore capture by the horizontal slide trap increased compared with the Burkard trap (Fig 4.3) and the ratio BURKSP/SLIDSP increased. During phase three when both types of trap were trapping large numbers of spores the ratio of BURKSP/SLIDSP was much lower (2.3 to 7.2) than during phase 2.

Disregarding the differences in numbers of spores captured, the pattern of capture during the study period was similar especially from 22 May onwards when peaks and troughs on the two trap coincided exactly. Early in the season when the numbers of spores caught by each trap were small the patterns of capture did differ (Fig 4.3) but with no clear pattern.

4.3 Regression analysis

4.3.1 Methodology

Suitable data for regression analysis to study possible relationships between spore trap data and meteorological variables were only available from 1988 where the full range of micrometeorological data were available. Only data from the Burkard trap were used because the counts could be reduced to 24h periods. Furthermore because of the three phases of spore release described above only a selected portion of these data could be used. Spore numbers trapped between 11 April and 21 May were too small to be used in these analyses as the error associated with them was likely to be too great. Between 28 June and 4 July although spore numbers were adequate the number of sample points was too small. The period chosen for analysis was therefore the 23 May to 27 June.

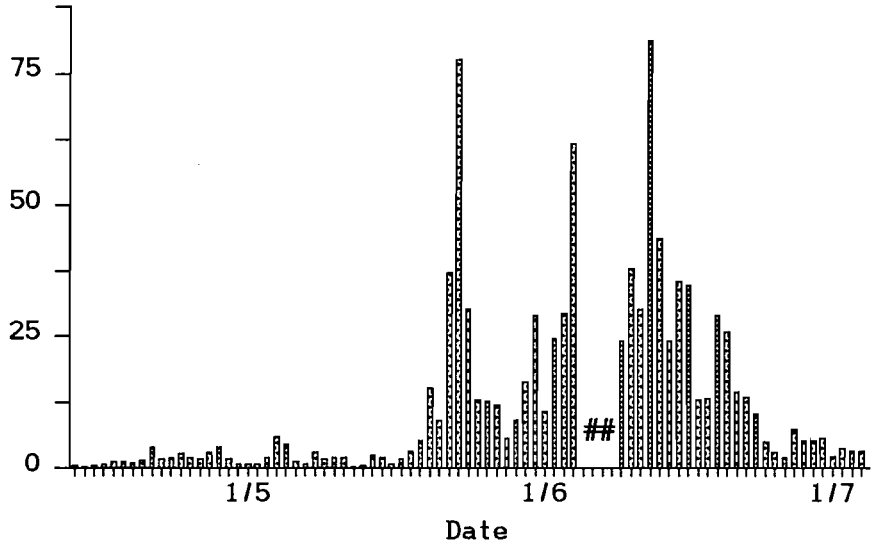


Fig 4.4: Ratio of BURKSP*/SLIDSP*; Bienvenu, 1988

* see text for explanation
# missing data

As discussed previously it is an assumption of regression analysis that each value of the dependent variable should be independent from any other. It is clear from the data presented above showing peaks and troughs for spore release that daily spore catches were unlikely to be independent of those adjacent to them. It was therefore important to modify the data so that values of the dependent variable are independent.

Royle & Thomas (1972) used a method based on days of maximum spore release in a given period of days. The maximum spore catch for periods of 1,2,3...7 days were regressed against the adjacent maximum spore catch. The period of 4 days was chosen which gave the weakest relationship between adjacent maxima. Regressions for 1, 2, 3 and 4 days for the spore trap data of this study are presented in Table 4.1. Clearly spore catches from adjacent days were highly correlated but when maxima were extracted from the varying periods i.e. 2,3 and 4 days there was no significant correlation (Table 4.1) although the 4 day maximum did show the least correlation. For this study the 2 day maximum was chosen because this provided the maximum number of data points for analysis.

Table 4.1: Correlation coefficient for regressions of spore maxima for 1,2,3 & 4 day periods

Period	r
Adjacent days	0.87
2 day maximum	0.11
3 day maximum	-0.33
4 day maximum	-0.01

Simple and multiple regression analyses were then carried out using the maximum spore count (log(x+1) transformed) from each pair of days and the

independent variables WET, RAIN, RH80 and RH80[1]. The methods used for multiple regression analysis are given in Part 4, Chapter 3, Section 3.4.

4.3.2 Results

The results of the simple regression analyses (Table 4.2) showed that there is no significant relationship between spore release and WET, RAIN, RH90 or RH80 on the same day and similarly a combination of all the variables in multiple regression analysis did not give a significant relationship.

Table 4.2: Coefficients of determination for the regression of the number of *A. brassicae* spores on Burkard trap against a variety meteorological variables

Variable	R^2	sig*
RH80	0.05	NS
RH90	0.03	NS
WET	0.15	NS
RAIN	0.03	NS

* NS = non significant

4.4 Discussion

Spore traps give an integrated view of the level of sporulation in the crop and the amount of spore release. Distinguishing between the effects of independent variables on spore release and spore production is difficult from spore trap data although circumstantial evidence will often give clues about which is being affected.

The data show clearly that the pattern of spore capture in different years may vary considerably. The three phase pattern which occurred during 1988 were linked

[1] variables accumulated over 24h periods

to specific wetness events closely allied to rainfall. This pattern of spore capture following rainfall which itself follows a dry period is similar to that described by Louvet and Billotte (1964) who suggested that high levels of sporulation will occur when heavy rainfal follows a period of dry weather. Humpherson-Jones & Phelps (1989) showed that wetness or very high humidity is necessary for sporulation and this suggests that the spore traps were recording an increase in sporulation rather than in spore release. The time delay between the onset of a wetness period and the increase was short (24-48h) although maximum spore capture following the wetness period occurred some time later. This supports the work of Humpherson-Jones (1989) who showed that spore production occurred within 24h of wetting a non sporulating lesion.

High levels of spore capture especially during phase 2 suggests that either spore production was continuing during this time or that spores already produced were being released. If the latter was the case then a drop in spore numbers caught between 10 and 22 June might have been expected when a long period of dry weather occurred, however this was not the case, possibly indicating that further spore production had occurred. Although during this period no wetness was recorded the relative humidity at the crop base did rise above 90% for more than 10h on most days during the dry period which may have induced spore production (Humpherson-Jones, 1989) on the senescent tissue. This ability for spore production to occur without free water is an indication as to why spore capture can vary considerably from infection on the plant traps.

As already discussed (Part 4, Chapter 2) little is known of the inoculum source for *A. brassicae* in an oilseed rape crop. The 1988 spore capture data beg the same question, especially in relation to the dramatic increases in spore numbers which occurred on 20 - 21 May and 27 June. The rapidity of increase suggests that tissue with potential for sporulation was already present in the crop. However, on 27 June very little tissue with the potential for sporulation was present on the canopy of the plant, leaf or pod, again leading to the conclusion that necrotic tissue at the base of the canopy provided the inoculum source. Humpherson-Jones (1989) has shown that the lesions of *A. brasicae* can develop on the senesced tissue and retain the potential

for sporulation for some time. These results suggest that at least in 1988 the primary source of inoculum was the necrotic tissue at the base of the crop.

In 1987 although the pattern looked considerably different similar conclusions may be drawn. During the majority of the season no long periods of dry weather occurred and therefore conditions were probably conducive for spore production throughout this time. However, during the first part of July no rainfall was recorded although the numbers of spores caught continued to rise. Unfortunately no data are available for relative humidity during this time and it can only be suggested that high RH or wetness due to dew was recorded even though rainfall was not. At this time although some sporulation was occurring on the pod tissue only a small number of lesions were involved. The level of spore capture suggests another source which as in 1988 could not have been the leaf tissue as there were none left on the crop and therefore was probably senescent tissue at the bottom of the crop.

The failure to be able to demonstrate a relationship between daily meteorological variables and the spore counts was not unexpected. In other studies (Royle & Thomas, 1972) spore trap data have been more closely related to meteorological variables from limited periods of the day e.g. prior to the period of maximum hourly spore release or to events occurring in previous days. These data will be further analysed by modifying the meteorological data so that not only daily meteorological data from previous days can be regressed on the spore trap counts but so that meteorological data from six hourly periods on the day of the spore count and in previous days can be analysed.

PART 5 GENERAL DISCUSSION

Dark leaf and pod spot (*A. brassicae*) can be a serious disease of oilseed rape in the U.K. (Davies, 1986). However, the incidence of the disease is sporadic so that in some years it is of no economic significance (Davies, 1986) and chemical control is inappropriate and wasteful. Under such circumstances the ability to forecast disease development would prove a useful tool in rationalising control measures. Such a forecasting system can only be developed after a detailed study of the develpment of the pathogen and disease using a combination of field and controlled environment experimentation (Kranz & Hau, 1980). Although several studies have been conducted to investigate pathogen development under controlled environmental conditions (see Part 1, Chapter 1) field studies appear to have been limited to surveys (e.g. Cook & Evans, 1978) and evaluation of chemical control measures (e.g. Ogilvy, 1984a) or varietal susceptibility. Apparently little has been published as to the temporal or spatial development of the disease on the crop canopy, especially on leaf tissue, or the environmental factors that affect that development. The objective of this study was therefore to make a detailed examination of the development of dark leaf spot on oilseed rape in the field and to attempt to relate disease progress to environmental factors measured in the field.

In any detailed study of disease development in the field one of the most important tools of the plant pathologist is relevant methods of disease assessment (Kranz & Hau, 1980). Disease assessment methods used in other studies (e.g. Anon, 1988; Evans & Gladders, 1981) were not appropriate for this investigation as they gave little or no information as to the position of disease in in the leaf canopy. As the objective of this investigation was to develop a detailed picture of disease progress on the leaf and pod canopy of oilseed rape it was considered appropriate to use a number of different variables (Part 2, Chapter 2) to describe the disease, each of which measured a slightly different aspect of the disease (Part 2, Chapter 2). The comparison of these different variables as measures of plant disease (Part 2, Chapters 3 & 4; Part 3, Chapter 3) showed that on the leaf canopy, where low levels of disease are usually encountered, lesion numbers/plant gives the most rapid and objective measure of disease for general studies and that on the pod canopy both

lesion numbers/raceme and % area of raceme affected are both easy and relatively rapid to assess. The numbers of samples required to accurately estimate these variables is however high when disease is at low levels (Part2, Chapter 3). The data showed that during this study too few samples were taken in many weeks when disease levels were low even to give a coefficient of variation <20%. These low sample numbers were however unavoidable as data capture was very time consuming taking >1 h/plant and furthermore the removal of large numbers of plants would have required a much larger trial area. The major consequence of the need for large sample numbers is that the data requirements for further studies should be considered with care and other sampling methods should be investigated. For example with sporulating tissue washing the leaf tissue and counting the number of spores recovered may be a more accurate and convenient method. Relationships between one measure of disease and another were investigated in an attempt to reduce the amount of work required for assessment (Part 2, Chapter 4). Only the incidence-severity relationship for lesion numbers on the leaf tissue and the relationship between disease on individual racemes and that on the whole pod canopy showed potential and should be investigated further.

Description of disease epidemics is important for understanding the causes of disease development (Kranz, 1974). The pattern of DPCs will undoubtedly indicate areas of further work as well as being a potential aid to rational disease control. In this study the canopy of oilseed rape was divided into leaf and pod canopy and disease progress described on each. This division, although somewhat arbritary, was used because of the different developmental patterns of the different portions of the crop. The rapid development of the leaf canopy in the spring followed by its complete loss from the plant is in contrast to the longer development and more permanent nature of the pod canopy. The continuous changes of the leaf canopy structure made the interpretation of disease progress difficult and emphasised the inadequacy of the standard % area assessment method often used by plant pathologists (James, 1971b). Distinctive patterns of disease development did however become clear. From the small amount of data available it showed that the disease is already widely spread in the crop during the 'rosette ' stage and may continue to develop slowly during the winter. Then, as seen in each of the four epidemics, during stem extension the disease

is confined to the basal MS leaves even though the leaf canopy is well spread vertically and BR leaves surround the MS leaves at the crop base. As the leaf canopy matures and leaves are lost from the base of the plant the disease moves up the plant so that prior to complete leaf loss the vertical distribution of the disease resembles that of the leaf canopy. In terms of disease severity, in three of the four epidemics an increase in disease was noted on the leaf tissue 2-3 weeks prior to complete leaf loss. Circumstantial evidence suggests that this pattern of disease development on the leaf tissue may be mediated by the changing susceptibility of the leaf canopy to infection by the pathogen (Part3, Chapter 2; Kohl & Hoffmann, 1989). No evident relationships were noted between rainfall, temperature and disease development on the leaf canopy (Part 4, Chapter 2) even though wetness or very high humidity was associated with infection on plant traps (Part 4, Chapter 3).

On the pod canopy disease development in the four epidemics showed a similar developmet pattern which was adequately described by the logistic function. In each epidemic a relatively long lag phase was apparent, followed by explosive disease development which in each epidemic coincided with heavy rainfall (Part 3, Chapter 3). As with disease development, the circumstantial evidence suggests that this pattern of development may be associated with the changing of host susceptibility to the pathogen.

In addition to describing in detail the progress of the various epidemics, spore traps and plant traps were used in an attempt to relate development of the pathogen under field conditions with various meteorological variables. This initial investigation, the analysis of which was limited by time, showed no quantitative relationship between measures of wetness, rainfall and humidity and the spore trap or plant trap data, although a qualitative relationship between wetness and infection on the plant traps was noted as was an apparent relationship between rainfall and spore capture in 1988. This difficulty in relating simple meteorological measures with spore trap and plant trap data is similar to that described by Humpherson-Jones & Ainsworth (1980, 1981) with *A. brassicae* on cabbages.

These data therefore need to be reanalysed using a wide range of modified meteorological variables e.g. power functions, complex functions (Royle, 1973; Royle & Thomas, 1972), variables extracted from narrower time periods within the exposure period and inclusion of other available data (i.e. wind speed and temperature). This would hopefully lead to better multilinear models than those described here and a better understanding of basic pathogen processes in a fied situation.

Although only poor relationships were developed with meteoroloical data, the plant trap and spore trap data provided other useful information. Spore trapping showed that the pattern of spore capture can differ considerably from year to year (e.g. 1987 and 1988; Part4, Chapter 4). These differences are important because they would probably not be predicted from laboratory studies and show that complementary field studies are vital. These data also demonstrated that levels of new infection may or may not be restricted by the level of inoculum available suggesting that laboratory studies of inoculum thresholds are required.

The pattern of spore capture during the season and vertically within the crop together with time of development of disease on the pod tissue has also identified senescent leaf tissue at the base of the crop canopy as a possible source of inoculum for disease development later in the season. Although circumstantial, the evidence is supported by Humpherson-Jones (1989) who demonstrated the potential of such leaf tissue for sporulation. Again it is clear that much further work is required in this area.

The data from this study have provided detailed descriptions of the development of dark leaf and pod spot on the canopy of oilseed rape in the U.K. and useful data as to patterns of sporulation and infection during several epidemics. These data together with laboratory studies (see Part 1, Chapter 1) especially those published recently (Humpherson-Jones, 1989; Humpherson-Jones & Phelps, 1989; Kohl & Hoffmann, 1989) provide a significant database from which further work can be developed. Clearly, areas of importance are the relationship between pathogen development and plant age under field conditions; the source and levels of inoculum during the season; the dispersal of that inoculum, especially from senescent tissue at

the crop base; the relationship between pathogenic processes in the field and meteorological factors and post infection processes in the laboratory and field.

Due to the complex nature of this pathosystem and the numerous areas of investigation required, a framework for further work should be developed. Such a framework could be provided by a systems approach (Kranz & Hau, 1980) which regards the plant/pathogen relationship as a system of "interlocking processes characterized by many reciprical cause-effect pathways " (Kranz & Hau, 1980). This type of approach develops a conceptual framework of the system within which important relationships between processes can be identified. It should allow experimental work to be organized so that time and effort is utilised effeciently.

A detailed quantitative description of any pathosystem is time consuming and laborious but a necessary complement to other experimental approaches e.g.controlled environmental studies. This study has provided much detailed information for the development of dark leaf spot on oilseed rape in the U.K. together with some additional information on pathogenic processes in the field. These data have complemented the laboratory studies already published and together they provide an adequate database to develop an effecient and rational approach to further study of this disease and its control.

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