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THE DEVELOPMENT OF LEPTOSPHAERIA MACULANS IN WINTER OILSEED RAPE AND ITS IMPLICATIONS FOR DISEASE CONTROL

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Summary A high incidence (c 74% plants infected) of stem canker (Leptosphaeria maculans) was found in winter oilseed rape crops in Eastern England in 1977 and 1978. Particularly dry weather conditions in October and November 1978 resulted in a lower incidence of stem canker at harvest in 1979. Studies in commercial crops indicated that rape stubbles were the main source of infection. Leaf spots first appeared in October but maximum infection occurred in the spring during early flowering. Stem cankers appeared in the spring during extension growth and continued to develop up to and after harvest. As the early stages of plant development were most susceptible to infection by L. maculans, a number of control strategies are suggested.

Résumé Une incidence importante (ca. 74% de plantes infectées) de taches brunes (Leptosphaeria maculans) a été constatée dans les cultures de colza d'hiver dans l'est de l'Angleterre en 1977 et 1978. Des conditions météorologiques particulièrement sèches en octobre et novembre de 1978 aboutissaient à une incidence réduite de taches brunes lors de la récolte en 1979. Des études effectuées sur des cultures commerciales ont donné l'indication que les éteules due colza étaient la source principale d'infection. Des taches apparaissaient sur les feuilles pour la première fois en octobre mais l'infection a atteint son maximum au printemps pendant la floraison prématurée. Des taches apparaissaient sur les tiges au printemps pendant leur croissance longitudinale et rapide et continuaient à se développer jusqu'à la récolte et après. Les premières étapes du développement de lat plante étant celles où l'infection par L. maculans risquait de se produire le plus facilement, un certain nombre de stratégies de contrôle sont proposées.

INTRODUCTION

Diseases in winter oilseed rape crops (Brassica napus L. ssp oleifera) have been monitored by ADAS in Southern and Eastern England for the last three years. Particular attention has been paid to stem canker (Leptosphaeria maculans, asexual stage Phoma lingam) which has caused serious yield losses in rapeseed crops in France (Alabouvette and Brunin, 1970) and in Australia (Bokor et al, 1975; McGee and Emmett, 1977).

Observations have been made throughout the year in commercial crops in Eastern England to identify periods in the development of both disease and pathogen at which control measures can be aimed.

METHODS AND MATERIALS

Disease monitoring. Studies were carried out on two farms in South Cambridgeshire in conventionally grown crops on shallow chalky boulder clay (Hanslope Series). In October 1977 a crop (cv. Primor) was selected on each farm adjacent to a ploughed stubble of an oilseed rape crop harvested in July 1977. Samples of 100 plants were collected at intervals throughout the growing season from areas (approx. 100 m x 20 m) close to the ploughed stubble and as far as possible from the stubble. This was 400 m south of the stubble at one farm and 800 m north of the stubble at the second farm. Growth stages were identified by the key suggested by Harper and Berkenkamp (1975).

The severity of stem canker was assessed on a 0–6 scale as follows :-

0	Healthy
1	10% or less stem circumference infected
2	11–25% stem circumference infected
3	26–50% " " "
4	51–75% " " "
5	76–100% " " "
6	Plant severely infected and prematurely ripened or dead

A mean disease index was calculated as the mean severity score for all plants in the sample.

In 1977 one crop was drilled in early September and treated in mid-November with a tank mix of propyzamide at 0.7 kg/ha and dalapon at 0.94 kg/ha. The second crop was drilled on 19 September and received TCA pre-emergence at 4.4 kg/ha and propyzamide at 0.42 kg/ha post-emergence in late December.

In September 1978 two crops of cv.Jet Neuf and two crops of cv. Primor were selected on the same farms, one farm providing three crops and the other a crop of cv. Primor. Samples of 50 plants were collected for disease assessment from each crop at each visit. The area of crop sampled was similar to that used in 1977/78 and the crops were approximately 800 m south of rape stubbles ploughed after harvest in 1978.

In 1978 the crops were drilled in late August/early September following pre-emergence treatment with TCA at 8.4-11.0 kg/ha. Propyzamide was applied at 0.35 kg/ha in late October. All crops received a compound fertiliser in the seedbed and c 200 kg/ha of nitrogen in early March.

Disease survey In 1977 and 1978, 50 stem bases were collected from winter oilseed rape crops throughout Eastern England. Most sampling was carried out before swathing (GS 5.3-5.4) in 1977 but shortly after swathing in 1978. In 1979 a survey was carried out by Bedford ADAS within that county. Samples of 25 stems were collected shortly before or shortly after swathing. The incidence and severity of canker infection were assessed as described.

Spore trapping Ascospores of *L.maculans* were trapped using a sticky cylinder spore trap (Jenkyn, 1974). In August 1977 stubble infected with *L. maculans* was collected from crops of cv. Primor in S. Cambs and placed on bare soil plots near Cambridge. A cylinder spore trap was positioned 1 m above the centre of a circular area of stubble, 2 m in diameter. The trap was changed twice weekly and counted using the method described by Jenkyn (1974).

The 1979 survey indicated that the lower incidence of stem canker this year may, in part, be attributed to the introduction of the resistant cultivar Jet Neuf (range of infection 4-52%). A lower incidence of canker was recorded in susceptible cultivars (range 16-68%) than in the previous two years suggesting a seasonal effect.

In 1977-78 a strong correlation (r = 0.96) was found between the maximum incidence of leaf spot infection in the autumn and the incidence of severe stem canker at harvest (severe is >50% stem circumference infected). This was based on only 9 sites, but results obtained in 1978-79 (only 6% severe canker in crops in Fig. 2) support the hypothesis that plants with leaf spot infection in the autumn are likely to develop severe canker infection at harvest. Total canker infection is more closely correlated with the maximum incidence of leaf spot infection in the spring as can be seen in Fig. 1 and Fig. 2. Thus plants which become infected in the spring can develop stem cankers by harvest but they are unlikely to be severe.

Closer analysis of meteorological records for autumn 1977 and autumn 1978 suggested that rainfall distribution rather than temperature or wind direction effects was a major factor (Table 2).

Table 2

Rainfall distribution in Autumn 1977 and Autumn 1978

Month	1977 Rainfall (mm)	1977 No. Days $\geq$1.0 mm	1977 No. Days $\geq$0.1 mm	1978 Rainfall (mm)	1978 No. Days $\geq$1.0 mm	1978 No. Days $\geq$0.1 mm
Sept	14.2	4	9	28.9	5	11
Oct	31.2	7	20	5.8	3	10
Nov	58.5	9	17	20.7	4	10
Dec	57.2	8	21	113.0	19	23
Total	161.1	28	67	168.4	31	54

The weekly ascospore catches in Figs. 1 and 2 are from mid-September to the end of June. There was little difference between the two years over the whole period September to December, but rainfall was exceptionally low during October and November 1978. Although ascospore catches in 1977-78 and 1978-79 are not comparable because different amounts of stubble were used, fewer ascospores were released in October and November 1978 (Fig. 2) than in the previous year (Fig. 1). Dry conditions may also have reduced host infection by ascospores, spread of pycnospores within the crop and even the maturation rate of perithecia of *L. maculans*.

Ascospores are released throughout the life of the growing rape crop, but monthly catches increased from October to January in both years. Low spore catches in some weeks in January 1979, February 1978 and March 1978 were the result of snow cover. Although ascospores and pycnospores are present throughout the year the relative importance of these propagules has not been determined. In the spring, however, leaf spots increased more rapidly on leaves which already had a pycnidial lesion than on healthy leaves suggesting pycnospores are important at this stage.

Fig. 1

Development of *L. maculans* in cv. Primor 1977–78.

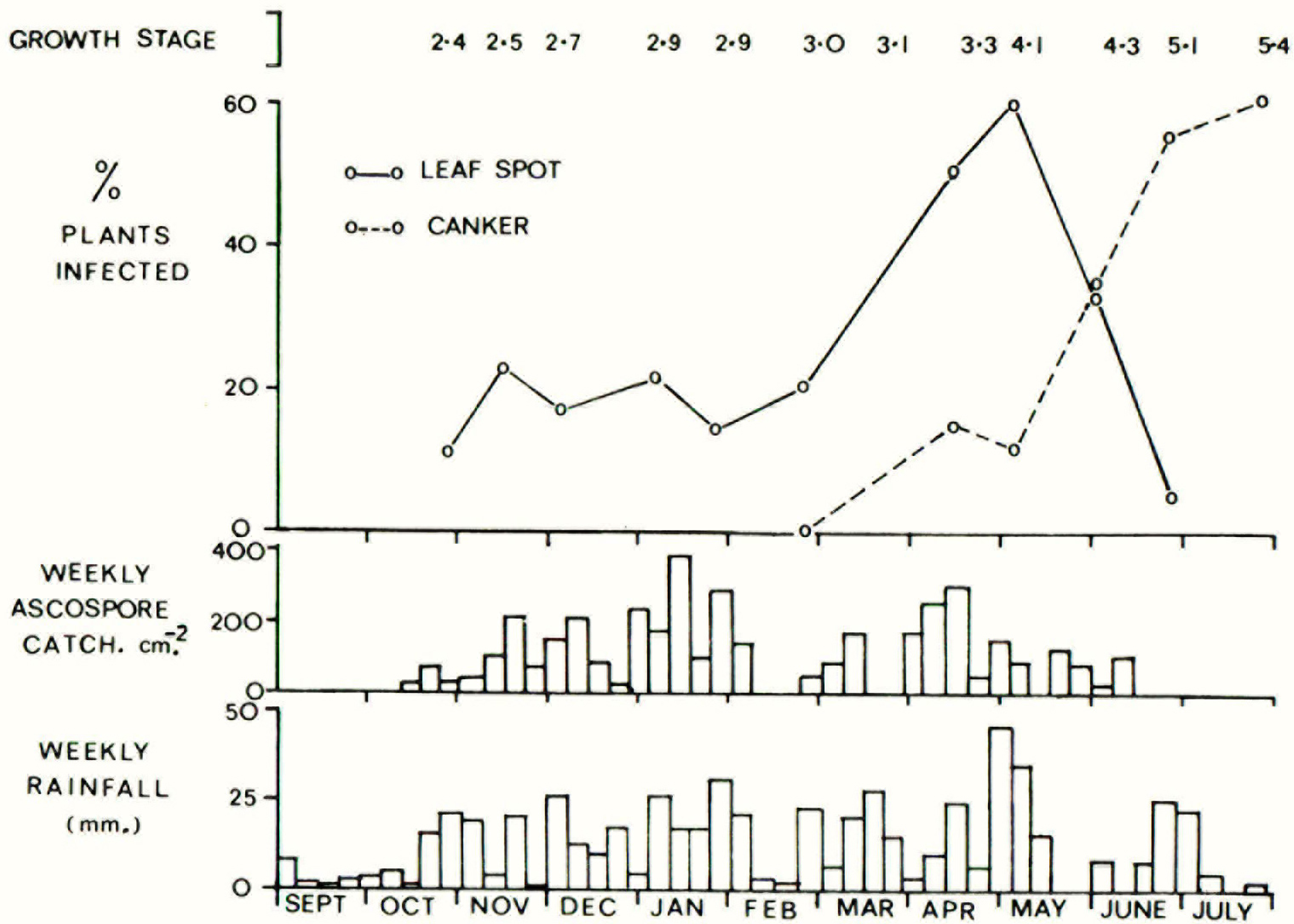
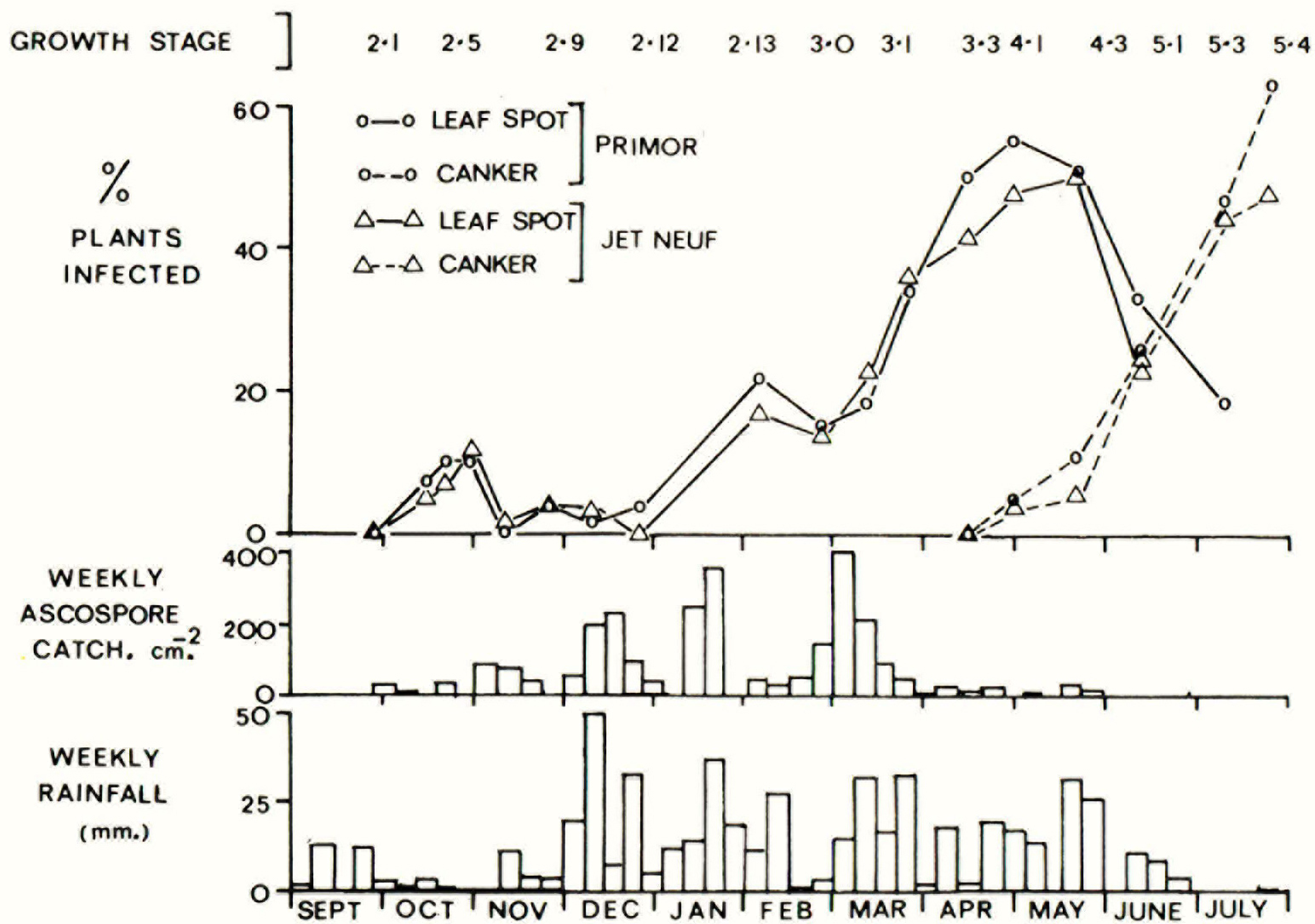


Fig. 2

Development of *L. maculans* in cvs. Jet Neuf and Primor 1978-79.



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In August 1978 infected stubble was again collected from crops of cv. Primor in S. Cambs and arranged as a single layer of stubble 0.3 m wide around the circumference of a circle 10 m in diameter. The spore trap was set up at the centre of the circle, 1 m above the soil surface.

Daily meteorological records were obtained from Stansted Airport which is situated about 28 km south of the study area.

RESULTS

The mean incidence of leaf spot and stem canker from four areas is given in Fig. 1. The results in Fig. 2 are the means of two crops of each cultivar. Leaf spot infection first appeared in October and then either increased in incidence (Fig. 1) or decreased (Fig. 2). In the spring the incidence of leaf spot increased during the phase of extension growth (GS 3.1-3.3), reached maximum incidence during early flowering (GS 4.1) and then decreased. In most crops more infection was present in the spring than in the autumn. Stem cankers were first seen in the spring and new infections continued to appear up to and after swathing. There was little difference in disease development on cvs. Primor Jet Neuf in 1978-79 (Fig. 2).

The main difference between crops monitored in 1977-78 and 1978-79 was the low incidence of leaf spot in November and December 1978 (Fig. 2). The importance of leaf spot infection at this stage is indicated by disease survey results (Table 1). The mean incidence and severity for each year are given in Table 1. The results for 1979 are separated into those from susceptible cultivars and those from the canker 'resistant' cultivar Jet Neuf.

Table 1

Incidence and severity of stem canker at harvest

Year	No. of crops surveyed	Main cultivars	Mean % plants with canker	Main disease index
1977	16	Primor/Rapora	76	2.36
1978	16	Primor/Rapora	73	3.29
1979	20	Quinta/Primor	41	0.60
1979	6	Jet Neuf	29	0.32

A high incidence of stem canker was first recorded in the UK in 1977 when the range of infection was 20-100%. The most severely infected crops were adjacent to unploughed stubbles from the previous year's rape crop. In one or two crops yields were estimated to have been reduced by 50%. In 1978 a similar incidence of stem canker was recorded (range 18-100% plants infected). The higher disease index in 1978 reflected the later sampling date rather than more severe canker infection in the growing crop. In one crop next to unploughed stubble, 96% seedlings were infected in mid-November when the plants had 2-3 true leaves. Complete crop loss occurred up to 100 m from the stubble as a result of canker infection and pigeon grazing of weakened plants.

DISCUSSION

Stem canker is now widespread in Eastern and Southern (Cook and Evans, 1978) England and has occasionally caused serious yield losses. Current control measures emphasize ploughing to bury infected stubble, crop rotation and treatment of seed with fungicide (Cook and Evans, 1978). Observations on disease developments in the UK support more detailed experiments in France (Brunin and Lacoste, 1970) and Canada (McGee and Petrie, 1979) which showed that rape seedlings infected before the 6 true leaf stage are likely to develop severe canker. Control measures should therefore be aimed at this early stage of plant development.

To date poor control of both leaf spot and canker has been achieved with single sprays of benzimidazole fungicides (Brown et al, 1976). Multiple sprays of fungicide which include autumn and spring treatments show more promise but results have been inconsistent in ADAS trials. An alternative treatment may be seed pelleted with fungicide so that seedlings are protected post-emergence, preferably until the 6 true leaf stage. Preliminary trials have been initiated by ADAS Bristol.

The most neglected phase of the canker life cycle is the development of *L. maculans* on stubbles after harvest. Within days of cutting the crop, there is extensive spread of mycelium from both cankers and small stem lesions on all cultivars. Most perithecia are formed on tissues colonised at this stage. Stubble treatments should therefore be seriously considered. They could act in one or more of the following ways :-

a) by reducing saprophytic colonisation of stubble
b) by delaying perithecial initiation/ascospore formation
c) by killing ascospores and perithecia

Such treatments should effectively control canker infections if ascospores are prevented from reaching crops before the 6 true leaf stage. A stubble treatment would be attractive to farmers in direct drilling sequences and growers on heavy land who are unable to produce a satisfactory seedbed for cereals after ploughing rape stubbles.

In contrast to the dry autumn conditions of 1978, mild wet autumn conditions are likely to favour the development of severe stem canker infections. This has been demonstrated by Pierre et al (1978) who have artificially produced severe canker in trials to compare the susceptibility of oilseed rape cultivars to *L. maculans*.

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LIGHT LEAF SPOT OF OILSEED RAPE: AN APPRAISAL WITH COMMENTS ON STRATEGIES FOR CONTROL

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Summary Light leaf spot (*Pyrenopeziza brassicae*) is now a common disease of winter oilseed rape in England; it has occurred on commercial crops each year since 1975. Severe infection can kill plants and in commercial crops of susceptible cultivars is estimated to decrease yield by up to 20%. Location, cultivar, seasonal conditions and herbicides are important factors in disease development. Frequent or large early applications of systemic fungicide are often necessary for satisfactory disease control, but predicting the need for such control measures sufficiently early in the growing season remains a problem. New resistant cultivars would be desirable in order to obviate less certain forms of control. Aspects of occurrence, spread, effects and control of the disease and likely profitable areas for further research are discussed.

INTRODUCTION

Light leaf spot (*Pyrenopeziza brassicae* (*Cylindrosporium concentricum*)), formerly a minor disease of vegetable brassicas, first caused severe damage in some autumn-sown rape crops in 1974-5. The results of subsequent surveys and studies on the biology, epidemiology and control of the pathogen have been reported (Rawlinson *et al*. 1978a, 1978b; Rawlinson & Muthyalu, 1979).

Conidia splashed from infected plants and from rape stubble appear to be the main source of inoculum, but spores can be trash-borne with seed and Rawlinson and K. Fovargue (unpublished) have recently confirmed seed infection. Infected seedlings arising from such seed could provide initial foci from which further conidia are spread. The cycle from spore deposition to production of secondary conidia on leaves can take up to 30 days in a susceptible cultivar at temperatures above 5°C. Symptoms are first seen in late autumn and winter as blanched papery lesions on older leaves. Leaves and leaf and bract primordia can carry latent infection until the spring when sporing lesions appear, often causing severe leaf scorch, gross distortion and stunting. By early May infection can extend to the stem causing splitting of the epidermis and also to all parts of the inflorescence, killing buds and preventing flowers from opening. By mid July pods may be extensively infected, prematurely ripened and split.

Surveys of incidence and severity of light leaf spot were made in 1975-9 and experiments on control in 1976-8.

MATERIALS AND METHODS

Surveys

In each year 1975-9 on ten farms in Hertfordshire and Bedfordshire 50 plant samples were taken along a diagonal across fields in late May to early June.

Percentage plants infected and number of infected leaves and bracts on the main stem and central rachis of infected plants were recorded from an average of ten scored per plant.

Chemical control

Plots of cv. Eurora were grown from either untreated seed or seed dressed with modified Benlate T (5 g benomyl and 1.5 g thiram kg^{-1}) sown on 6 September 1977. Plots were given a foliar spray of benomyl (1.12 kg a.i. ha^{-1}) on 10 February 1978 and some plots received a second spray on 28 April. Disease assessments on 20 plant samples from each plot were made on 6-14 June when the percentage of plants and leaves infected were recorded and a disease index was derived from eight visual scores per plot. Scores on a four point scale 0-3 corresponded to none, <10 (slight), 10-50 (moderate) and $>50\%$ (severe) foliage affected by light leaf spot. Plots were combine harvested after haulm desiccant on 13 August when mean grain dry matter was 83%.

Spore trapping

P. brassicae spores were caught on rotorod samplers, a pre-impinger and a Burkard trap, all operated automatically in fields during periods of rain by a battery-powered acoustic device sensitive to the impact of rain drops (modified after Faulkner & Colhoun, 1977). Other traps designed to catch and retain rain splash and wind-blown rain, and pots of rape seedlings as trap plants, were also exposed in growing crops. In laboratory experiments on splash dispersal, droplets were caught on strips of 35 mm film exposed beneath a rain tower at Rothamsted; 5 mm water droplets fell on 0.5 mm deep spore suspensions (M. Fatemi, B. D. L. Fitt & Rawlinson, unpublished).

RESULTS

Surveys

A five year survey of crops in May/June shows that light leaf spot is now a very common disease of winter oilseed rape (Table 1).

Table 1

Mean incidence and severity of light leaf spot in surveys of winter oilseed rape crops 1975-9

Year (No. of crops)	Incidence % crops infected	Incidence % plants infected	Severity No. leaves infected (0-10) per infected plant
1975 (21)	100	42	3.5
1976 (11)	91	16	2.3
1977 (15)	100	38	3.6
1978 (17)	94	28	2.6
1979 (13)	92	22	1.7

Almost every crop on the ten farms surveyed was infected each year and approximately one third of all plants examined during the period were infected. The disease was most prevalent in 1975 and 1977 - both following a wet autumn or winter, whereas autumn periods preceding other survey years were unusually dry. However, the extent of damage to crops was characteristically less predictable. Individual plants and occasionally whole crops could be severely damaged, with the majority of the foliage distorted or withered, whereas nearby plants or crops of the same cultivar might remain comparatively healthy. Crops most at risk appeared to be those close to a previous infected rape crop and those sprayed with the herbicide dalapon. Of the cultivars surveyed (Victor, Rapol, Lesira, Primor, Rapora, Expander, Erra, Quinta and Jet Neuf) occasional crops of Rapol, Lesira, Rapora and Primor suffered severe damage at some time during the survey period. Interestingly, the new canker-resistant cv. Jet Neuf, grown commercially for the first time in 1979 when disease levels were generally low, appears to be relatively susceptible to light leafspot since one crop of this cultivar had 64% of plants infected with inflorescences and pods attacked.

Chemical control

In field experiments large amounts of systemic fungicide were necessary to decrease incidence and severity of disease and increase yield significantly. In one experiment (Table 2) light leaf spot was the most important disease present and increases in yield following benomyl were found to be closely related to control of this disease.

Table 2

Effect of benomyl on light leaf spot and yield of susceptible oilseed rape

Benomyl treatment	% plants infected	% leaves infected	Disease index ($\pm$ 0.09)	Yield (t ha^{-1} $\pm$ 0.080)
Untreated	92	69	2.5	1.74
Seed	95	55	2.3	1.89
1 spray	24	8*	0.6*	2.06
2 sprays	5*	2*	0.3*	2.32
Seed + 1 spray	5*	2*	0.8*	2.24
Seed + 2 sprays	1*	1*	0.3*	2.23

* Values significantly different from untreated ($P < 0.05$) after log transformation of % values.

Correlations between disease and yield allowed an estimate of yield loss to be calculated - yield was decreased by 8.3% (0.19 t ha^{-1}) per unit increase in severity of light leaf spot measured on a scale 0-3. These calculations were confirmed by inspection of unanalysed data where plots had yields close to the calculated values (Table 3).

Table 3

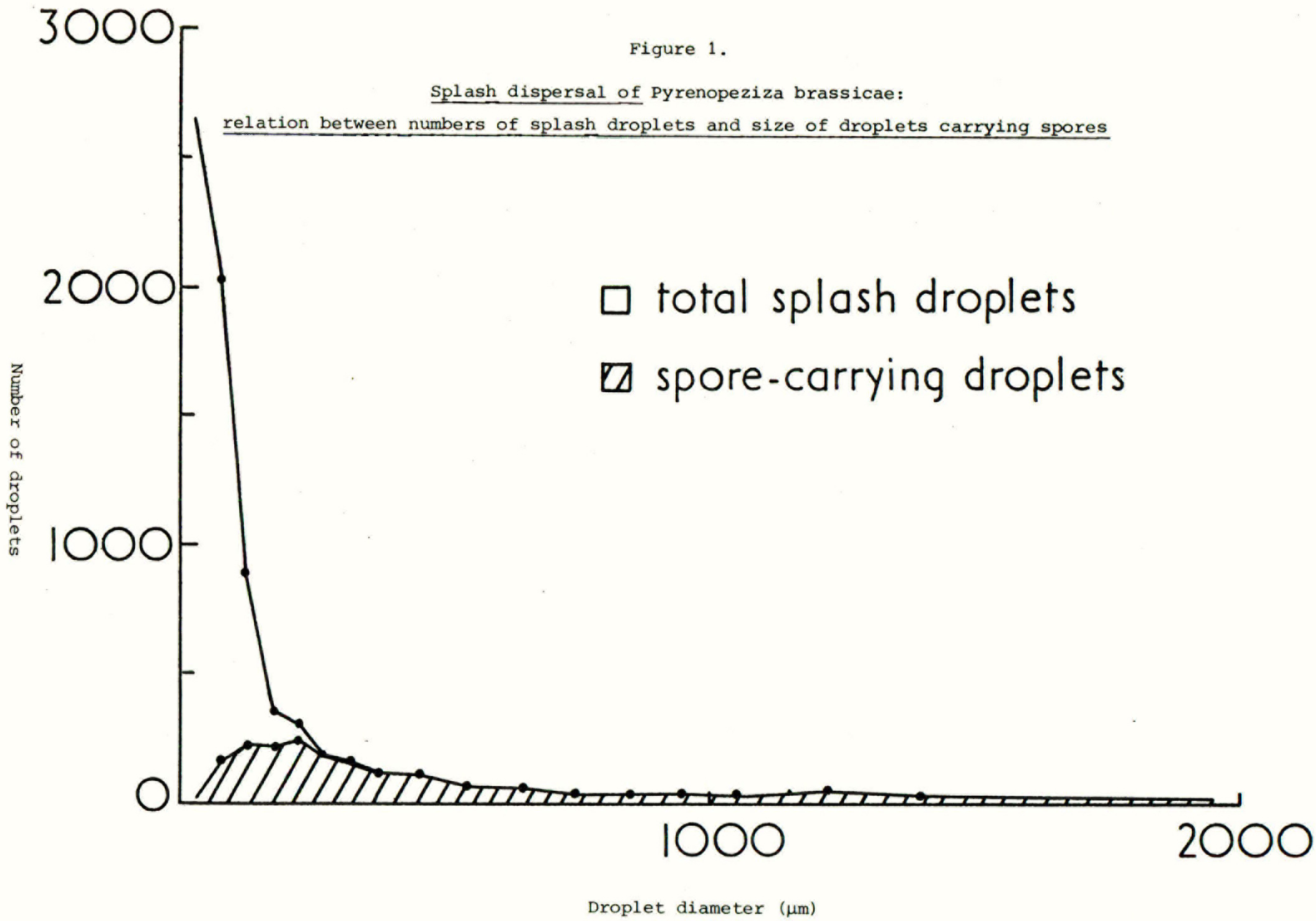
Effect of light leaf spot on actual and calculated yield in a susceptible cultivar

Disease index (scale 0-3)	Calculated yield (t ha^{-1})	Actual yield
0-1	2.11	2.24 $\pm$ 0.183
1-2	1.92	2.03 $\pm$ 0.340
2-3	1.73	1.83 $\pm$ 0.205

Figure 1.

Splash dispersal of Pyrenopeziza brassicae:

relation between numbers of splash droplets and size of droplets carrying spores



Spore trapping

Rain-splash is an important mechanism for both infection and spread of light leafspot. Data from different types of spore trap showed that spore concentrations in and around rape crops follow closely the pattern of rainfall during the growing season. Spores from infected crops were detected from late autumn up to harvest. Peak catches on spore traps coincided with periods of heavy rainfall from February to June, except that few spores were caught when the temperature was below $0^{o}C$ or when snow had fallen. Most spores were dispersed locally by run-off water from leaves and by large splash droplets distributed over fairly steep gradients near soil level. However, because spores can be caught on traps sampling air rather than splash around infected crops, it is likely that smaller droplets can carry spores over greater distances. Laboratory experiments have shown that a single incident drop can generate $>$7000 splash droplets of which 20% carry spores and splash can disperse spores up to a distance of 95 cm in still air. Moreover, 37% of splash droplets were $<$50 µm diam. (Fig. 1) and the smallest droplet carrying spores was 27 µm. Under field conditions with turbulent air these small droplets could be responsible for initiating spore dispersal over much greater distances. Measurement of the surface tension of water containing a concentrated suspension of P. brassicae spores showed decreases of $>$ 20% which is likely to increase the efficiency of droplet spread on waxy rape leaf surfaces (Rawlinson, unpublished).

DISCUSSION

Until recently light leaf spot was not recorded as a problem in other rape growing areas of the world. However, infected plants were seen in May 1979 (Rawlinson, oilseed rape study tour) in France, Germany and Sweden. Severe damage was recorded on some plots (including Jet Neuf) in trial sites in Finistère, France (H. Brun & M. Renard, pers. comm. 1979) where climatic conditions seem favourable for disease development. The increasing area of winter rape and the prevalence of other overwintering brassica hosts in certain areas seem likely to ensure that the disease can continue to spread. Because the new cv. Jet Neuf can be attacked, and is expected to occupy a substantial proportion of the future acreage, the disease is likely to remain a problem for rape growers.

The association of dalapon and wet seasons with increased damage by light leaf spot is likely to be due to effects on leaf surface wax (Rawlinson et al 1978b). Dalapon decreases the amount of wax and changes its physical form and distribution on rape leaves, increasing their wettability. Heavy rain can have a similar effect, increasing the spread and retention of spores leading to increased infection. It has also been noted that leaves of some susceptible cultivars are inherently more wettable than resistant types. Since 1975 dalapon has gradually been replaced by herbicides or mixtures less damaging to leaf waxes, but despite changes in herbicides and the range of cultivars grown and variations in seasonal conditions the disease remains common and occasionally severely damaging. If correlations between disease and yield in the susceptible cv. Eurora are validly extended to other equally severely infected cultivars, then yield loss in some crops of Rapol, Lesira, Rapora and Primor encountered in the 1975-9 surveys could have been as much as 20%. The disease may affect yield in several ways, but the rape plant is capable of making much compensatory growth and yield loss in crops is more difficult to prove than in individual plants, making the economics of chemical control difficult to assess. Mycelial growth of the fungus in vitro is sensitive to small amounts of some systemic fungicides (0.1 ppm benomyl or thiabendazole can inhibit growth on agar), and topical applications are directly toxic to spores. Despite this sensitivity either frequent or large early applications of systemic fungicide to crops are apparently necessary to achieve yield increases. From the limited information available at present it seems that sprays applied between October and April are most effective. Sprays applied after the disease has become obvious in spring have little effect on yield even though they afford some control of the disease. However, decisions on the need for chemical control are difficult to make sufficiently early for sprays to be economic in terms of yield response. The often long latent period before the re-appearance of symptoms, making difficult the definite visual diagnosis of light leaf spot during winter, contributes to the problem.

The increasing practice of seed treatment may largely prevent seed transmission but will not prevent infection from other sources. Minimum cultivation techniques which leave surface trash are likely to help spread the disease so the recent recommendation of deep ploughing rape stubble after harvest may prove valuable, but nothing is known about the capacity for survival of the fungus on stems in soil. The isolation of new-sown rape crops as far as possible from previous rape stubble should also help reduce the risk of infection, but the likelihood of long distance spore dispersal after splash liberation makes this measure uncertain and its practicability is doubtful on many farms and in many areas.

New resistant cultivars would be desirable in order to obviate less certain forms of control. Sources of resistance to the disease exist in oilseed rape (Thompson & Capitain, 1979) and some plant breeders are making selections for resistant types. Many aspects of the behaviour of the pathogen on rape are imperfectly understood and much more information will be needed before pathologists can assist plant breeders by identifying likely resistance mechanisms. Leaf surface wax may be involved in resistance to light leaf spot, as it is in resistance to *Alternaria* blackspot on rape (Skoropad & Tewari, 1977) and is worthy of closer attention. Another promising line of investigation involves the sulphur-containing compounds common in rape and other brassica tissues: currently oilseed rape breeding programmes aim, among other objectives, at a low concentration of glucosinolates, some derivatives of which have long been known to be fungitoxic (Hooker, Walker & Smith, 1943) so the consequences of this trend need examining in the general context of rape diseases. Preliminary investigations suggest that some volatile sulphur compounds are more abundant at certain times in leaves of light leaf spot-resistant rape cultivars than in susceptible types (Rawlinson & K. A. Lord, unpublished). At least one of these compounds, allyl isothiocyanate, is toxic at low concentrations to *P. brassicae*; solutions containing $<$0.5 ppm decrease mycelial growth in vitro and in the vapour phase inhibit spore germination (Rawlinson & J. Emmenegger, unpublished). Allyl isothiocyanate is thought to be involved in resistance to other brassica pathogens (Greenhalgh & Mitchell, 1976; Harthill, 1978) and production of such volatile constituents of brassicas can be affected by growing conditions and certain agricultural practices (Macleod & Nussbaum, 1977; Macleod & Pikk, 1978).

Light leaf spot is clearly no longer a minor disease of vegetable brassicas. Greater understanding of the physiology and biochemistry of the host/pathogen interaction and the elucidation of the epidemiology of the disease are essential for the development of effective control measures.

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