Ascospores as primary inoculum for epidemics of white leaf spot (*Mycosphaerella capsellae*) in winter oilseed rape in the UK

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In the UK, conidia of *Pseudocercosporella capsellae*, the anamorph of *Mycosphaerella capsellae*, were observed on white leaf spot lesions on leaves throughout the growing season. Ascomata were not observed on lesions on either green or senescent leaves, although stromatic knots and spermogonia were occasionally seen in summer. However, spermogonia and protoascomata were produced in white leaf spot pod and stem lesions in early summer. Protoascomata continued to mature after harvest in these lesions on the debris. Mature ascomata subsequently developed by early autumn, but were exhausted by early January and did not overwinter. A diurnal periodicity in numbers of air-borne *M. capsellae* ascospores discharged from infected debris was observed with a Burkard spore sampler, with greatest numbers of ascospores collected near the middle of the day; the records also suggested that ascospores were released in response to wetting by dew or rain. Studies of natural white leaf spot epidemics in winter oilseed rape provided evidence that air-borne ascospores are the primary inoculum for initiating epidemics in the autumn in the UK. White leaf spot disease gradients over 100 m across a winter oilseed rape crop at Rothamsted were fitted by both negative exponential and inverse power-law models, with gradient slopes suggesting the deposition of air-borne spores dispersed from a single local source of inoculum. In comparison, no obvious white leaf spot gradients were observed over 250 m in a severely diseased crop near North Petherton, Somerset, suggesting that the air-borne spores were dispersed from a number of more distant sources in the area. Both patterns of disease were unlikely to have been initiated by *P. capsellae* conidia, which are dispersed only very short distances by rain-splash. However, once epidemics have been initiated by air-borne ascospores in the autumn, subsequent disease spread within an infected crop is dependent only on splash-dispersed conidia. A revised disease cycle of the pathogen is proposed.

Keywords: ascospores, disease cycle, *Mycosphaerella capsellae*, oilseed rape, white leaf spot

Introduction

In the UK, white leaf spot has historically been an uncommon, autumn disease of fodder brassicas such as turnip and swede in the west, south-west and north. This apparently reflects the general distribution of rainfall in the UK and the concentration of such fodder crops in these areas (Gladders *et al.*, 1984; Inman, 1993). On winter oilseed rape (*Brassica napus* ssp. *oleifera*), which has become the most important arable crop in the UK after cereals in the last few decades, the disease is restricted mostly to the south-west and south-east of England. However, it was reported more recently in the main oilseed rape-growing areas of central and eastern England, e.g. in Hertfordshire and Suffolk (Anonymous, 1988) and in Lincolnshire (Anonymous, 1992). Although outbreaks have sometimes been severe in individual winter oilseed rape crops, no general epidemics have occurred (Hardwick *et al.*, 1989). In France, however, the disease appears to have become widespread on winter oilseed rape and has caused significant yield losses when it has spread onto the pods (Penaud, 1987). *Mycosphaerella capsellae* was described as the sexual state of *Pseudocercosporella capsellae*, the cause of white leaf spot of oilseed rape and other brassica crops (Inman *et al.*, 1991). It is distinguished from the closely related species *Mycosphaerella brassicicola*, the cause of ringspot of brassicas, by ascospore shape, colony morphology, life history, host preference and symptoms (Inman *et al.*, 1991).

Ascospores of *M. capsellae*, discharged from ascomata in old white leaf spot lesions on pod debris onto oilseed rape leaves in the glasshouse, produced typical white leaf spot lesions from which conidia of *P. capsellae*

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were produced. Similarly, ascospores discharged onto V-8 juice agar produced typical *P. capsellae* colonies with conidia that produced white leaf spot lesions when inoculated onto oilseed rape leaves. In the UK, the development of the sexual stage on crop debris was first observed in the summer of 1990 and mature ascomata were found from early autumn to early winter. The pattern in concentrations of air-borne ascospores over a 10-day period in November 1990 suggested that they were discharged in response to wetting by rain or dew (Inman *et al.*, 1991). However, the role of the sexual stage in the epidemiology of the disease has not been demonstrated.

Until the discovery of the sexual state, conidia were the only infective spore stage known in the life-cycle of *P. capsellae* (Paul, 1988). Survival between crops was thought to be by means of stromatic knots (microsclerotia), which were believed to produce conidia in the autumn (Crossan, 1954; Penaud, 1987; Paul, 1988). However, such stromatic knots are unlikely to have an asexual role in survival since they appear not to be sclerotial but to be the primordia for spermogonia and ascomata (Inman *et al.*, 1991). Although there are reports of conidiophores originating from these stromatic structures (Crossan, 1954), the stromata are unlikely to be involved in the production of conidia; indeed, such positional associations between sexual stromata and conidiophores are common in many species of *Mycosphaerella* (Jenkins, 1939; Wolf, 1943). Furthermore, since the conidia of *P. capsellae* are dispersed over only short distances by rain-splash (Fitt *et al.*, 1989, 1992), they are unlikely to be the primary inoculum for infecting new crops of winter oilseed rape, which usually follow several crops of cereals. Although seed transmission of *M. capsellae* was demonstrated in North America, it appeared to occur at only a very low frequency and was not considered important in initiating white leaf spot epidemics (Crossan, 1954; Petrie & Vanterpool, 1978). Since air-borne ascospores produced in the autumn have the potential to be dispersed over large distances, unlike splash-dispersed conidia, ascospores therefore appear most likely to be the primary inoculum for infecting autumn-sown crops of oilseed rape in the UK (Inman *et al.*, 1991). This paper presents evidence for the role of ascospores in initiating epidemics of white leaf spot and reviews the disease cycle of the causal fungus.

Materials and methods

Ascospore development

The development of stromatic structures in white leaf spot lesions on naturally infected leaves of winter oilseed rape crops at Rothamsted, UK, was studied from December 1989 to May 1990, March to July 1991 and January to July 1992. To investigate the development of stromata on pods during the summer, pods of 73 plants in a crop of winter oilseed rape (cv. Cobra) at Rothamsted were inoculated with a conidial spore suspension (5×10^4 conidia mL^{-1}) on 17 June 1991 at growth stage (GS) 6,2 (Sylvester-Bradley & Makepeace, 1985). The inoculated racemes were covered for 4 days with sealed, transparent polyethylene bags sprayed inside with water. Lesions appeared within 11–12 days and were regularly monitored thereafter for the development of stromatic structures (spermogonia and ascomata). The diseased plants were removed from the crop on 5 August, immediately prior to harvest. Pods with white leaf spot lesions (total dry weight 705 g) were placed outside on soil in several trays (36×22 cm) covered with plastic netting (mesh diameter 3 mm). Freezing microtome sections were cut through the old white leaf spot lesions on these pods on 21 August and 4 September. On 20 September, the pod debris was taken from the trays and placed under a circular piece of plastic netting 2 m in diameter in the centre of a field at Rothamsted sown 4 days earlier with winter oilseed rape (cv. Cobra). Samples of pod debris were taken at weekly intervals until December 1991 and 100 ascomata were excised with a sharp needle each time. The excised ascomata were placed in lactophenol/cotton blue on glass microscope slides, gently squashed under a cover slip and examined under a light microscope for the presence of mature asci.

In 1992, the development of ascomata was studied in old white leaf spot lesions on naturally infected stems of winter oilseed rape (cv. Libravo) collected from a crop on Fordgate Farm, near North Petherton, Somerset, UK. Stems were brought back to Rothamsted on 25 July and placed outside on soil in a box frame (90×120 cm) covered with plastic netting. Samples of debris were taken weekly from 20 August to 8 December and each time 100 ascomata were excised and examined for the presence of mature asci.

Ascospore dispersal

Ascospore dispersal was studied at Rothamsted from 10 to 19 November 1990 and from 28 September to 18 October 1991, using a Burkard volumetric spore sampler (Burkard Manufacturing Co., Rickmansworth, UK). In 1990, a sample of infected pod debris was placed in a tray (36×22 cm) of soil covered with a piece of plastic netting. The tray was then placed below the inlet of the spore sampler, which sampled 10 L of air per minute. Methods for the use of the Burkard spore sampler were described by McCartney & Lacey (1990). In 1991, ascospore dispersal from the diseased pod and raceme debris, placed in the centre of the crop of winter oilseed rape (cv. Cobra) sown on 16 September 1991, was studied using a Burkard spore sampler placed in the centre of the debris. Air-borne ascospores collected were identified on the basis of their morphology (see Fig. 2f; Inman *et al.*, 1991; Inman, 1993). In both 1990 and 1991, hourly rainfall data were obtained from an automatic weather station situated within 500 m of the Burkard spore sampler. In 1990, the duration of surface wetness was also monitored with an automatic

meteorological station placed in a nearby winter oilseed rape crop using a leaf wetness resistive sensor at crop height (McCartney & Lacey, 1990); data on surface wetness gave an indication of the presence of dew.

White leaf spot disease gradients

Two epidemics of white leaf spot were studied in crops of winter oilseed rape: at Rothamsted in the 1990–91 season and on King's Farm, near North Petherton, Somerset in 1991–92. In both crops, the horizontal distribution of disease in the crop was studied to determine whether splash-dispersed conidia or air-borne ascospores were most likely to be the primary inoculum.

Rothamsted

Well-developed symptoms of white leaf spot were first noted on winter oilseed rape leaves in a field (Black Horse) at Rothamsted in March 1991. The field had been sown with cv. Cobra on 4 September 1990 at a seed rate of 6 kg ha^{-1}, following two winter wheat crops and a winter barley crop in previous seasons and was directly adjacent to a field (Bylands) that had grown oilseed rape in the 1989–90 season. The two fields were separated only by a ploughed strip 0·5 m wide. After harvest, the oilseed rape stubble in Bylands had been topped, and the soil worked with a shakerator and rolled between 25 July and 2 August 1990. Volunteer oilseed rape seedlings were sprayed with a herbicide (diquat as Gramoxone) on 26 September. The field was then cultivated with a flexitine on 4 October and sown with winter wheat on 7 October 1990. Bylands and Black Horse were part of a block of four fields that all grew oilseed rape in rotation with one another every fourth year.

The incidence of white leaf spot appeared to be highest at the edge of the crop adjacent to the field that had grown oilseed rape in the previous season, and a transect was sampled away from this edge in a south–north direction up to a distance of 100 m. Plants were sampled on 26 March (GS 3,1), 23 April (GS 4,0) and 15 May (GS 5,4) 1991, after which disease progress was halted by a period of prolonged dry weather in May and the loss of infected leaves. The number of plants and the distances at which they were sampled differed slightly between sampling dates. On 26 March and 23 April, 10 plants were sampled at distances of 1, 2, 3, 5, 9, 13 and 17 m from the edge of the crop; on 23 April, 10 plants were also sampled at 21 and 25 m, and 25 plants were sampled at 50, 75 and 100 m. On 15 May, 10 plants were sampled at all previous distances from 1 to 100 m, except at 2 m. For each sampling date, the number of plants with white leaf spot lesions, the number of leaves with lesions and the number of lesions per plant were assessed. Lesions were identified by their appearance (Fig. 1; Brun, 1986; Inman, 1993) and, when necessary, were checked microscopically for conidia of *P. capsellae*.

North Petherton

An epidemic of white leaf spot was studied in a crop of winter oilseed rape (cv. Libravo) near North Petherton,

Somerset. The crop was sown on 6 September 1991 at a rate of 5 kg ha^{-1}. The field had not grown oilseed rape previously and had been under permanent pasture until 1987. It was then ploughed and sown with a crop of swedes, followed in subsequent years by two crops of wheat, one of barley and then oilseed rape in the 1991–92 season. No immediately adjacent fields had grown winter oilseed rape in the 1990–91 season; however, white leaf spot epidemics were consistently observed on winter oilseed rape crops in the area of North Petherton each season, usually from autumn and early winter onwards (P. Gladders, ADAS Boxworth, personal communication). Two transects across the winter oilseed rape crop, perpendicular to each other, were sampled to determine if there were any gradients of white leaf spot within the crop, the first on 17 January 1992 in an east–west direction over a distance of approximately 230 m, and the second on 28 February in a south–north direction over approximately 270 m. On each occasion, 25 plants were taken at approximately 15 m intervals and the number of plants with white leaf spot lesions, the number of leaves with lesions and the number of lesions per plant were assessed.

Statistical analyses

For the Rothamsted epidemic, the two empirical models used to describe the decrease in disease (y) with distance (x) were the inverse power-law model and the negative exponential model (Fitt *et al.*, 1987). So that gradients could be compared more easily, the equations for both models were made linear by taking natural logarithms of both sides.

The inverse power law model becomes:

$$\ln(y) = \ln(a) - b.\ln(x)$$

The negative exponential model becomes:

$$\ln(y) = \ln(c) - dx$$

The exponents b and d are then the slopes of the respective linear equations. Values for b and d were estimated by linear regression. This was done for the disease gradients in March, April and May, using as y-values the parameters % plants with white leaf spot, % leaves with white leaf spot and mean number of white leaf spot lesions per plant. The percentage of leaves with white leaf spot was calculated using assessments on only the lowest two leaves per plant in March and on only the lowest three leaves per plant in April and May. Data for percentage of plants with white leaf spot and percentage of leaves with white leaf spot were transformed using the multiple infection transformation (Gregory, 1973). The goodness of fit of each model was estimated by calculating the percentage variance accounted for (R^2) for each regression. The exponential model has the property that the amount of disease decreases by half as the distance from the source increases by a constant increment, the half-distance (α). The half-distance is related to the exponent d (Fitt *et al.*, 1987) and was
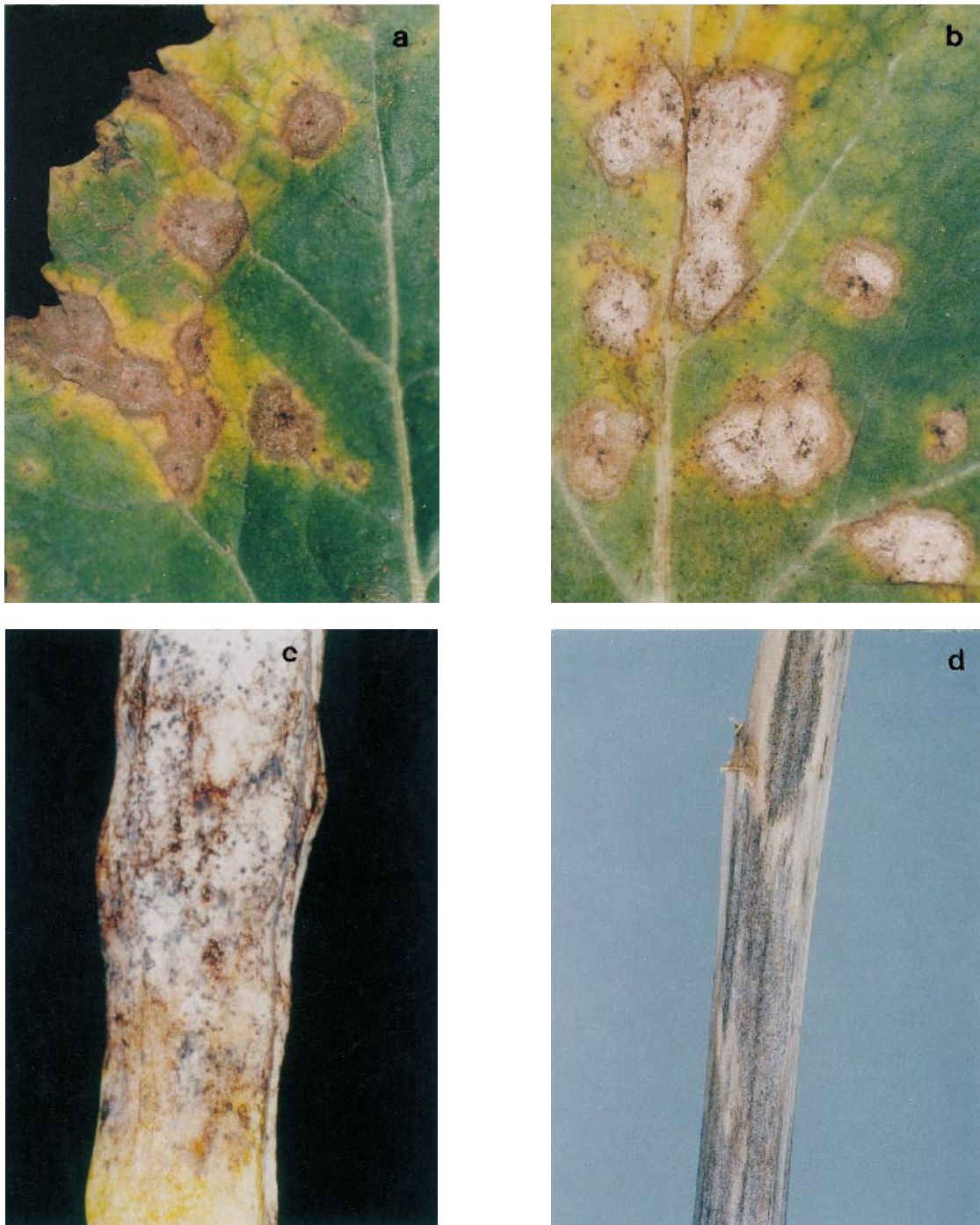
Figure 1 White leaf spot lesions on winter oilseed rape: (a) tan-brown lesions becoming white as conidia are produced on a leaf; (b) mature lesions with abundant white conidial sporulation on a leaf; (c) mature, bleached lesion with developing black stromata (spermogonia and protoascomata) on a pod; (d) lesions with developing black stromata (protoascomata) on a stem.

calculated for each gradient:

$$\alpha = 0 \cdot 693/d$$

Analyses of position and parallelism were done to assess whether, for each disease assessment, the gradients on the three dates were best fitted by a single regression line or by three parallel lines or by three non-parallel lines.

Seed transmission studies

Plants with pods with white leaf spot lesions were removed on 5 August 1991 from the crop of winter

oilseed rape (cv. Cobra) at Rothamsted and left outside until seeds were harvested on 13–15 August. Two samples of seeds were collected: (1) from pods without white leaf spot lesions; (2) from pods with white leaf spot lesions (where possible, seeds were taken from directly beneath lesions). From each sample, 1000 seeds were sown in trays (30 × 58 cm) of soil-less compost containing a slow-release fertilizer (Croxden compost produced by Nursery Trades (Lea Valley) Ltd, Cheshunt, Herts, UK) and the seedlings were grown in a controlled environment room operating an 18-h light cycle (fluorescent lighting emitting 150–270 μE m^{-2} s^{-1} at plant height) at 15°C and a 6-h dark cycle at 10°C. Each tray was covered with a clear plastic propagator hood. Seedlings were sampled 20 days after sowing and the cotyledons were assessed for the presence of white leaf spot lesions.

Results

Ascospore development

Conidia of *P. capsellae* were produced abundantly on white leaf spot lesions on green attached leaves (Fig. 1a,b) in winter oilseed rape crops, but no stromata were seen in these lesions or in dead detached leaves during autumn, winter or spring. However, stromata measuring 45–60 μm diameter were observed in four out of 14 lesions on senescent attached leaves on 12 July 1991 (Julian day 193). Stromata measuring 30–60 μm in diameter were also observed in seven out of 12 lesions on attached living leaves, 18 out of 20 lesions on attached dead leaves, and 13 out of 14 lesions on dead fallen leaves collected on 25 June 1992 (day 176); most stromata appeared to be loose knots of large, brown cells 9–12 μm (occasionally up to 15 μm) diameter, but five stromata, 30–60 μm in diameter, contained spermatia and were therefore considered to be spermogonia (Fig. 2a).

On inoculated pods, black stromatic bodies were observed in white leaf spot lesions on 15 July 1991 (day 196). Most lesions were dark brown and stromata were seen only in those lesions that had developed white, bleached areas (Fig. 1c). Most (90%) of these stromata were spermogonia containing spermatia (Fig. 2b). In those stromata without spermatia, no trichogynes were seen. However, sections taken from diseased pod debris on 21 August 1991 (day 233) showed both spermogonia and protoascomata to be present in all lesions, with protoascomata being slightly more numerous. Most spermogonia still contained large numbers of spermatia, although some were exhausted; most protoascomata contained only nutritive cells (Fig. 2c) but a few had well-developed ascogenous tissue, occasionally with ascus initials. By 4 September (day 247), spermogonia were exhausted while most protoascomata now contained immature asci (Fig. 2d), although some still contained only nutritive cells, and a few had produced mature ascospores. By 18 September (day 261), all

ascomata contained asci with mature ascospores (Fig. 2e,f). The proportion of ascomata with asci then gradually declined during October and November (Fig. 3) and subsequently was over-estimated, as only whole ascomata were sampled. Those ascomata that had collapsed or disintegrated after becoming exhausted were neither sampled nor accounted for, despite being the most abundant. By 16 December (day 350), asci were no longer observed in any of the ascomata.

In 1992, the pattern of ascospore development on naturally infected stems was similar to that in 1991 on inoculated pods (Fig. 3). Black, stromatic structures were present in white leaf spot lesions on stems collected on 16 July (day 197; Fig. 1d). Freezing microtome sections taken on 30 July (day 211) showed that only protoascomata were present. Ascospores were not observed until 27 August, when 5% of ascomata contained ascospores. This proportion steadily increased until 99% contained mature asci on 24 September. The proportion then steadily declined during October and November until all ascomata observed were exhausted on 8 December (day 342).

Ascospore dispersal

In experiments on the diurnal periodicity of dispersal of *M. capsellae* ascospores, the maximum numbers of ascospores were collected near the middle of the day with nearly all of the ascospores being collected between 0500 and 1700 h (GMT) in both 1990 (93%; Fig. 4a) and 1991 (98%; Fig. 4b). The diurnal periodicities in ascospore dispersal were not smooth curves because they were affected by the timing of rainfall, which occurred on eight out of nine sampling days in 1990 and 12 out of 21 sampling days in 1991. Few spores were collected during the hours of darkness, even when dew or rainfall occurred. The data on hourly periodicity in ascospore dispersal in relation to occurrence of rainfall in September–October 1991 (data not presented; see Inman, 1993) were consistent with those of November 1990 (Inman *et al.*, 1991); ascospores of *M. capsellae* were collected by the Burkard spore sampler at times when wetting by dew or rain occurred (sometimes as little as 0·2 mm of rain). Spores were collected almost immediately after wetting, with numbers typically reaching a maximum within 2–3 h. Few spores were collected when rainfall was prolonged and heavy.

White leaf spot disease gradients

Rothamsted

The incidence and severity of white leaf spot in the winter oilseed rape crop at Rothamsted in the spring of 1991 decreased with increasing distance from the edge adjacent to the field that had grown oilseed rape in the previous season. Gradients of the decrease in disease with distance up to 100 m from the edge of the field fitted both the inverse power-law model (Fig. 5) and the negative exponential model. The percentages of the
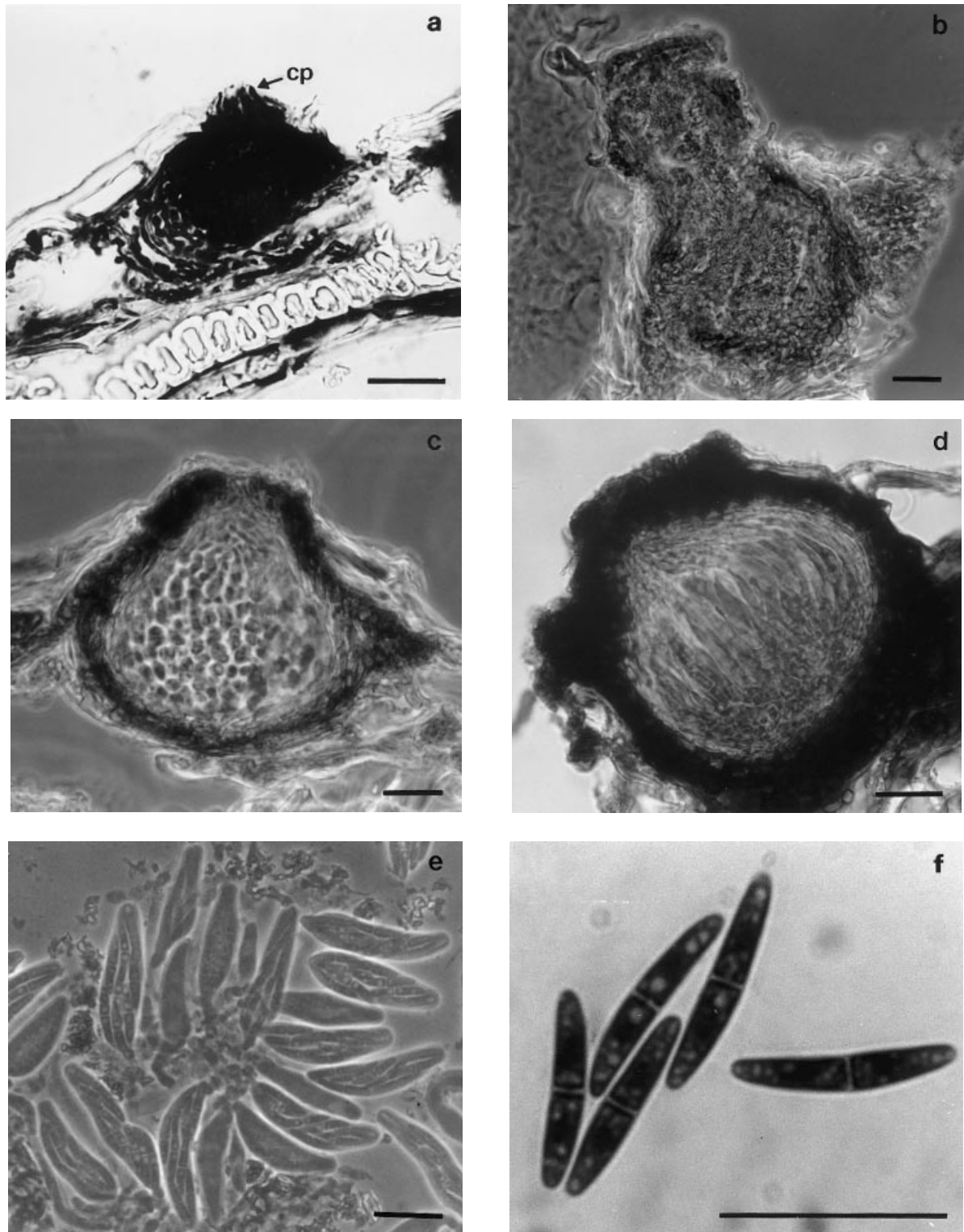
Figure 2 Spermogonia and ascomata of *Mycosphaerella capsellae*: (a) spermogonium with spermatia developing in a white leaf spot leaf lesion during early summer (note conidiophores (cp) above); (b) spermogonium with spermatia in a pod lesion; (c) developing ascoma with nutritive cells in a pod lesion; (d) maturing ascoma with asci; (e) asci with ascospores; (f) ascospores. Bar = 20 μm (a, c, d and f, specimens stained with cotton blue in lactophenol; b and e, observed by phase-contrast microscopy).
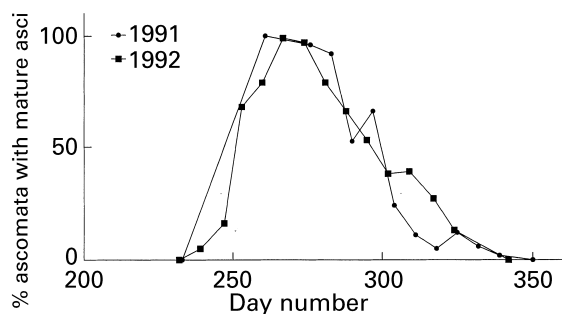
Figure 3 Changes in the proportion of ascomata in old white leaf spot lesions on oilseed rape debris that contained mature asci: on pod debris in 1991 from 21 August (day 233) to 16 December (day 350); and on stem debris in 1992 from 20 August (day 232) to 18 December (day 342).

variance accounted for by each model were generally very similar (Table 1) and differed, on average, by only 11·6% over all dates and disease assessments. Although both models adequately described the gradients, those in March and April fitted slightly better to an inverse power-law model and those in May to a negative exponential model. The analyses of position and parallelism suggested that the gradients for the three dates were best fitted by three non-parallel lines. The slopes of the regression lines and the half-distances calculated from the exponential models also indicated that the primary gradient became less steep with
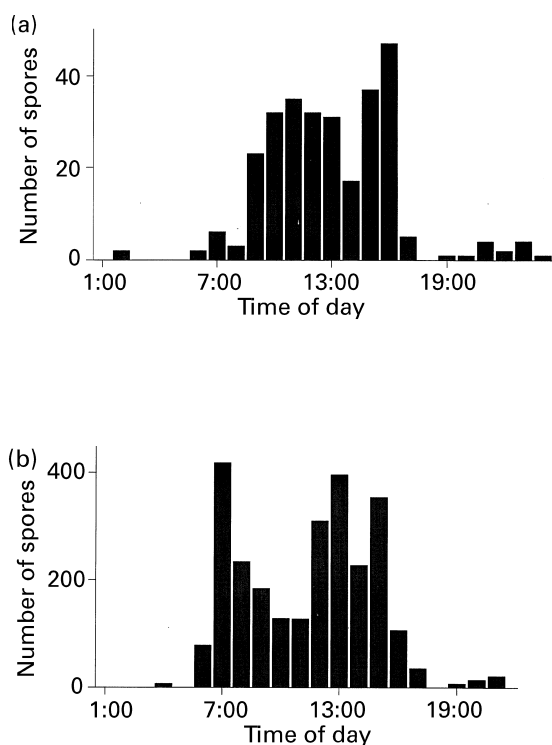

Figure 4 The total numbers of *Mycosphaerella capsellae* ascospores collected by a Burkard spore sampler positioned near debris of oilseed rape with old white leaf spot lesions in each hour of the day (i.e. from 0000 h to 0100 h, etc.): (a) between 10 and 19 November 1990; (b) between 28 September and 18 October 1991.
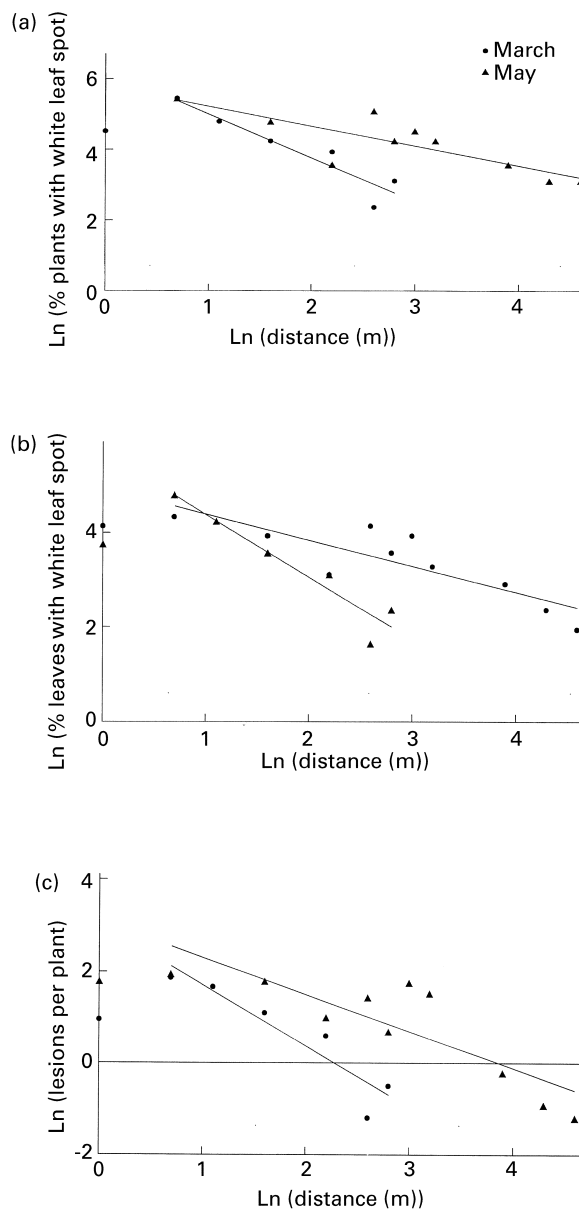

Figure 5 Gradients of white leaf spot (y) decreasing with distance (x) across a crop of winter oilseed rape at Rothamsted on 26 March and 15 May 1991, fitted by the inverse power-law model: (a) percentage of plants with white leaf spot (transformed by the multiple infection transformation); (b) percentage of leaves with white leaf spot (multiple infection transformation); (c) number of white leaf spot lesions per plant. Regression equations of the form $y = a - bx$; values of regression parameters are given in Table 1; regressions were calculated omitting values for distance 1 m.

time (Table 1). White leaf spot lesions were also observed on plants up to a distance of 200 m at the furthest edge of the field.

North Petherton

The incidence and severity of white leaf spot along both the south–north and east–west transects suggested that there was no obvious disease gradient in the crop (Fig. 6).

Table 1 Values of parameters for white leaf spot (WLS) disease gradients observed in a winter oilseed rape crop at Rothamsted on 26 March, 23 April and 15 May 1991, with disease (y) assessed as percentage of plants with WLS, percentage of leaves with WLS and number of WLS lesions per plant with distance (x) from the edge of the field. For each date and disease assessment, linear regressions were fitted for the inverse power-law model (Power, $\ln(y) = \ln(a) - b.\ln(x)$) and the negative exponential model (Exponential, $\ln(y) = \ln(c) - dx$); values are given for the percentage variance accounted for by each model (R^2), the intercepts on the y-axis ($\ln(a)$ and $\ln(c)$) and the slopes of the regression lines (b and d); half-distances were calculated for the negative exponential model, where the half-distance $= 0.693/d$

Disease assessment/model	% variance accounted for			Intercept			Slope			Half-distance (m)		
	March	April	May	March	April	May	March	April	May	March	April	May
Percentage of plants + WLS												
Power	83·5	75·5	65·0	6·24	6·66	5·78	1·236	1·055	0·556			
Exponential	74·1	72·8	60·0	5·35	5·10	4·80	0·168	0·051	0·020	4·13	13·59	34·65
Percentage of leaves + WLS												
Power	87·1	88·5	67·6	5·70	5·21	4·94	1·317	0·850	0·548			
Exponential	76·7	80·4	79·9	4·74	3·94	4·04	0·178	0·041	0·022	3·89	16·90	31·50
Lesions per plant												
Power	82·2	85·6	66·9	3·05	2·78	3·12	1·344	0·892	0·807			
Exponential	80·7	48·7	84·9	2·13	1·29	1·83	0·189	0·035	0·033	3·67	19·80	21·00

Incidence in the crop was high (67% and 98% of plants with white leaf spot on 17 January and 28 February 1992, respectively) and the horizontal distribution of disease across the crop was fairly even. There was a very slight trend for disease incidence and severity to decrease in an easterly direction, but the rate of decrease was low and very gradual over a distance of approximately 230 m.
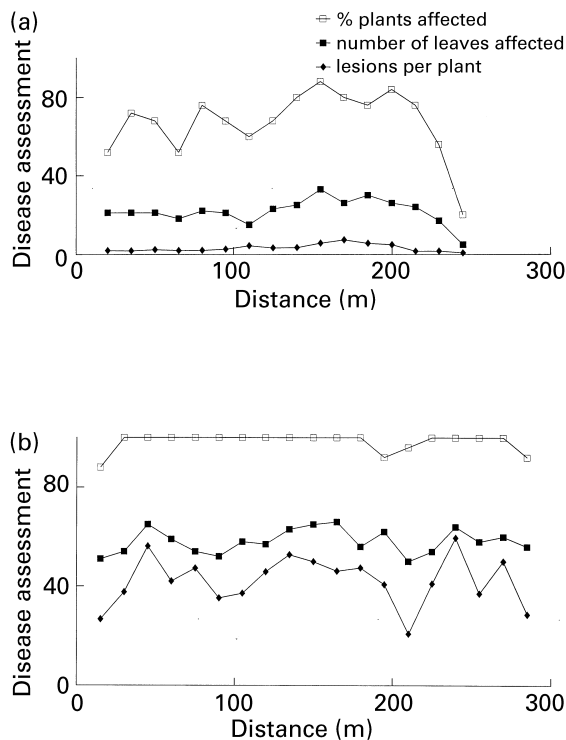
Seed transmission studies

Two out of 1000 seeds (0·2%) collected from pods with white leaf spot lesions produced seedlings with white leaf spot lesions on their cotyledons. The identity of the lesions was confirmed microscopically by the presence of *P. capsellae* conidia. No white leaf spot lesions were recorded on the cotyledons of seedlings grown from seeds taken from pods without white leaf spot.

Discussion

Ascospores as primary inoculum for white leaf spot epidemics

The patterns of disease in both the Rothamsted and North Petherton crops provide good evidence that air-borne ascospores of *M. capsellae* (Inman *et al.*, 1991) are the primary inoculum for initiating epidemics of white leaf spot in winter oilseed rape crops in the autumn in the UK. The gradient at Rothamsted extended over a distance of 200 m from the edge of the field and the even distribution of disease in the crop at North Petherton occurred over distances of 230–270 m. These patterns of white leaf spot distribution in first oilseed rape crops after cereals could not have been produced by *P. capsellae* conidia dispersed in splash droplets, because they are dispersed over only short distances by rain-splash (Fitt *et al.*, 1989, 1992).

    The white leaf spot gradient at Rothamsted, which fitted both negative exponential and inverse power-law models, was typical of a horizontal gradient produced by air-borne spores dispersed from a single local source of inoculum (Gregory, 1973). In this case, the most likely
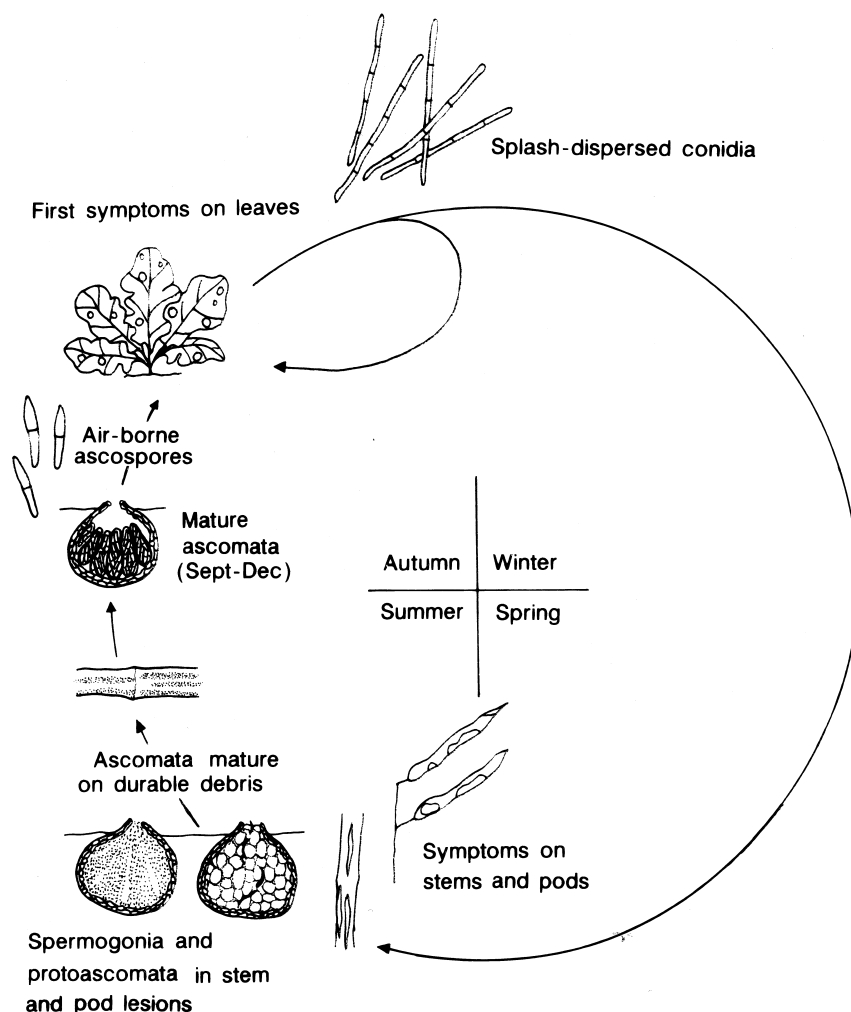

Figure 6 The distribution of white leaf spot in a crop of winter oilseed rape near North Petherton, Somerset: (a) along an east–west transect sampled on 17 January 1992; (b) along a south-north transect sampled on 28 February 1992; (□),% plants with white leaf spot; (■), total number of leaves with white leaf spot (in a sample of 25 plants); (◆), mean number of lesions per plant.

Splash-dispersed conidia

First symptoms on leaves

Air-borne
ascospores

Mature
ascomata
(Sept-Dec)

| Autumn | Winter |
| Summer | Spring |

Ascomata mature
on durable debris

Symptoms on
stems and pods

Spermogonia and
protoascomata in stem
and pod lesions

Figure 7 A proposed disease cycle for *Mycosphaerella capsellae* (anamorph *Pseudocercosporella capsellae*) on winter oilseed rape in the UK.

source was the adjacent field, which had grown winter oilseed rape in the previous season. The high incidence and even distribution of disease at North Petherton suggested a number of more distant sources capable of producing a high background concentration of air-borne spores in the area (Gregory, 1973). This is consistent with the regular appearance of white leaf spot from year to year on winter oilseed rape in the area surrounding North Petherton.

The changes with time in the disease gradients at Rothamsted supported the conclusion that conidia of *P. capsellae* are not dispersed over large distances by wind and rain. It is possible that conidia dispersed in small splash droplets could become air-borne as a result of aerosol formation (Fitt *et al.*, 1989), although the mucilage surrounding the conidia probably prevents dispersal by wind in the absence of rainfall. However, since most *P. capsellae* conidia are dispersed in droplets greater than $200\,\mu$m diameter (Fitt *et al.*, 1992), it is unlikely that large numbers of conidia are aerosol-dispersed. Although the white leaf spot gradients

at Rothamsted did gradually become less steep with time, they were not greatly changed by secondary spread (Gregory, 1973). This indicated that the white leaf spot foci were expanding only slowly, an observation consistent with conidia being dispersed over short distances by rain-splash (Fitt *et al.*, 1992). These experiments also confirmed the suggestion that seed transmission is not an important source of primary inoculum for epidemics of white leaf spot. The rate of seed transmission was very low (0·2%), even though the sample was highly biased because the seeds were collected from directly below white leaf spot lesions on infected pods. However, seed transmission might be a mechanism for introducing white leaf spot disease to new areas.

The observations on the development of *M. capsellae* ascospores in infected tissues and their dispersal under natural conditions provide further evidence that ascospores initiate white leaf spot epidemics in oilseed rape crops in the autumn. The production of ascomata at the end of the growing season enables the pathogen to survive between crops, since ascospore release then
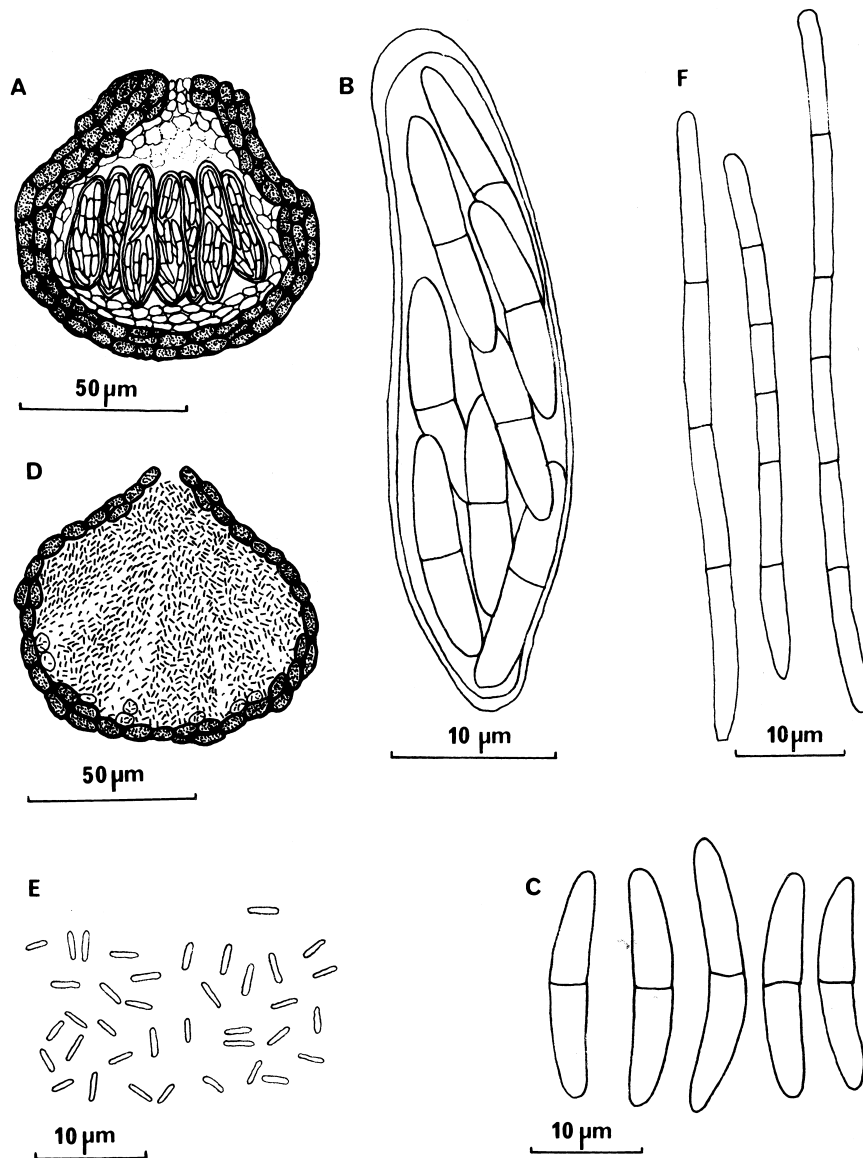
Figure 8 *Mycosphaerella capsellae*: (a) ascoma, 70–116 μm; (b) ascus, 32–53×8–14 μm; (c) ascospores, 15–23×3–3·5 μm; (d) spermogonium, 58–116 μm; (e) spermatia, 3–4×1 μm; (f) conidia, 30–60(−90)×2–3(−4) μm.

coincides with the emergence of young oilseed rape plants in crops sown in late August or early September. Although the factors affecting the production of spermogonia and protoascomata of *M. capsellae* are not clearly understood, stromatic knots can be produced throughout the year in senescent leaf material if it is incubated in moist chambers (Inman *et al.*, 1991; Inman, 1993). However, stromata were observed under natural conditions only in the summer months. Daylength, wavelength and intensity of light, temperature and nutritive status of host tissue are therefore factors likely to be important in the development of these structures. The factors affecting ascospore discharge are clearer; discharge was initiated as a result of wetting by dew or rain, which ensures that moisture is available to

favour subsequent germination and infection. Wetting by dew or by short periods of rain is most likely to favour dispersal, since ascospores discharged during prolonged rainfall are likely to be washed out of the air. However, wetting may not be the only factor involved in initiating dispersal, as ascospores were rarely collected at night even if dew or rain occurred. An almost identical diurnal pattern of ascospore release has been reported for *Mycosphaerella populorum* (Luley & McNabb, 1989).

Proposed revision of the disease cycle of *M. capsellae*

The disease cycle of *M. capsellae* on oilseed rape in the UK can now be revised to incorporate a *Pseudocercosporella* conidial state, a spermatial state and a *Mycosphaerella*

ascospore state (Figs 7, 8; Inman *et al.*, 1991; Inman, 1993). The sexual cycle appears to be initiated in summer with the production of spermogonia and protoascomata in white leaf spot lesions on pods, racemes or stems in June or July. Leaf lesions are not considered to be important in the production of sexual structures or, therefore, in survival of the pathogen between crops, since few leaves are left on the plant when the sexual cycle is initiated in the summer; leaf debris also decays quickly and is unlikely to support the development of ascomata into the autumn. The sexual cycle is thought to involve both spermogonia and protoascomata. Spermatia produced by spermogonia are thought to act as male sexual elements because they are small and unable either to germinate in culture or to infect host plants (Higgins, 1936; Dring, 1961; Inman *et al.*, 1991). Protoascomata are produced concurrently with spermogonia and contain one or more coiled basal ascogonia from which a slender trichogyne extends out of the protoascoma (Inman, 1993). The trichogyne is thought to be the receptive structure for spermatia to fertilize the ascogonia (Jenkins, 1939). Whereas spermogonia rapidly become exhausted, protoascomata of *M. capsellae* develop further and asci containing mature ascospores first appear by late August or mid-September; this time can vary slightly between years and it was 1 month later in 1990 (Inman *et al.*, 1991) than in 1991 and 1992. Ascospores continue to be produced throughout the autumn, the main period of production occurring between September and November. By mid-December to early January ascomata are exhausted and they do not appear to overwinter in the UK. Subsequent disease spread within an infected crop through winter, spring and summer is then dependent on splash-dispersed conidia.

Although the proposed disease cycle for *M. capsellae* in the UK is probably applicable to other regions of Europe and the world where winter oilseed rape is grown, the situation is likely to be different in regions, such as parts of Canada, where only spring cultivars of *Brassica campestris* and *B. napus* are grown. In these regions, stromatic mycelium of the type described by Petrie & Vanterpool (1978) may have a role in survival of the pathogen between crops. Alternatively, it is possible that protoascomata may overwinter in Canada, because the growing season is shorter and the onset of winter more rapid than in Europe. Structures identical to the protoascomata described in this study were observed in white leaf spot lesions on stems of Canadian oilseed rape provided by G. A. Petrie in July 1991. However, mature ascomata have not yet been reported in Canada and such overwintering of protoascomata has not been demonstrated.

Acknowledgements

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