ASPECTS OF THE BIOLOGY AND EPIDEMIOLOGY OF PSEUDOCERCOSPORELLA CAPSELLAE, THE CAUSE OF WHITE LEAF SPOT ON OILSEED RAPE.

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INTRODUCTION

White leaf spot on oilseed rape is caused by the fungus Pseudocercosporella capsellae (Ell. & Ev.) Deighton. In the UK the disease is mostly restricted to crops in the south west and south east of England. Outbreaks here have sometimes been severe in individual fields, but no general epidemics have occurred. If warmer, wetter winters occur in the UK as a result of predicted climate change, it is possible that white leaf spot may become more important. In France, the disease decreased yields significantly when it spread on to the pods in 1985 (Penaud 1986) and it has also been reported in Germany (Amelung and Daebler 1988).

There is little information on the epidemiology of the pathogen. Conidia of the fungus are splash-dispersed (Crossan 1954; Fitt et al 1989) and disease spread is therefore dependant on rainfall. Infection takes place through stomata and disease symptoms can be seen on crops throughout the season. A spermatial state has been reported in culture and on Capsella bursa-pastoris (L.) Med. The function of spermatia is uncertain, although they may act as male sexual gametes for other fungi which produce spermogonia (Higgins 1920). Since no teleomorph has been reported for P.capsellae, survival has been attributed to the production of dark, stromatic bodies in older necrotic lesions, considered sclerotial in character (Penaud 1986; McKay 1956; Davis 1927). These stromatic bodies are commonly associated with conidiophores and have been thought to be responsible for the production of primary inoculum for infecting autumn-sown crops.

The disease is also found on other cruciferous and brassica species and these may be a source of inoculum for spread on to oilseed rape crops. Brassica napus and Brassica campestris are apparently better hosts than Brassica oleracea (Petrie and Vanterpool 1978). Swedes (B.napus var rapifera) and turnips (B.campestris var rapifera) are known to be hosts in the UK, whilst Chinese mustard (B.campestris var chinensis) and Chinese cabbage (B.campestris var pekinensis) are the main crops affected in continental Europe and North America.

This paper reports for the first time the occurrence of the spermatial state and a Mycosphaerella teleomorph for P.capsellae on oilseed rape, and provides data on other aspects of the epidemiology of the pathogen.

METHODS

Field Studies

Diseased leaves sampled from a crop of autumn-sown oilseed rape (cv. Cobra) between December 1989 and May 1990 were examined for the presence of stromatic structures, prior

to or after senescence. Pods were artificially inoculated on 14 May 1990 and resulting lesions were sectioned with a freezing microtome 4, 6 and 8 weeks after the first appearance of lesions. Diseased pods were collected from the crop on 12 July and placed outside on soil in plastic trays covered with a plastic mesh. Freezing microtome sections were made from samples taken every 2-4 weeks. All sections were stained in either lactophenol/cotton blue or Heidenhain's iron haemotoxylin.

For a ten day period between 10-19 November 1990, a Burkard spore sampler was used to collect airborne ascospores of the Mycosphaerella state of P.capsellae. The diseased pods collected from the crop were placed below the inlet of the spore sampler which sampled 10 l of air per minute. Rainfall, leaf wetness (dew) and other environmental variables were measured concurrently at an automatic weather station 500m from the sampler.

Teleomorph-Anamorph Connections

Ascospores were obtained by placing wetted pod material containing mature pseudothecia over an agar plate. Individual ascospores or ascospore groups discharged on to the agar were isolated on V8 juice agar and incubated at 20°C. Two-week-old cultures were placed under near ultraviolet light (nuv) to induce conidial production. The conidia obtained were compared morphologically with those of P.capsellae and their pathogenicity was tested by inoculating them on to leaves of oilseed rape. In addition ascospores were discharged on to oilseed rape leaves in the glasshouse and plants were incubated in moist chambers for 3 days to provide conditions favourable for infection.

Laboratory Studies

The development of stromatic structures in leaf and petiole lesions produced in the glasshouse was studied under controlled conditions. Lesions were placed on moist filter paper in sealed Petri dishes and incubated at either 7°C or 20°C, in the dark or under nuv light, or outside. Plant tissue varied from green to senescent and yellow. Material was studied directly under the light microscope or after sectioning with a freezing microtome.

For cultural studies mycelial plugs from cultures on V8 juice agar were transferred to distilled water agar plates. These were incubated for 2-4 weeks at 20°C in the dark and then at 7°C under continuous nuv light. Spermogonia could readily be produced using this method and attempts were made to produce cultures from spermatia by transferring exuding spermatial droplets with a sterile needle on to plates of PDA, V8, malt agar, corn meal agar, or rape leaf decoction agar. Plates were incubated at either 7°C or 15°C in the dark, and assessed after two weeks for the presence of colonies or ungerminated spermatia. Attempts were also made to infect rape plants with spermatia.

In an additional experiment 10 single conidial isolates were paired in all possible combinations and incubated as above. When mature spermogonia were produced drops of sterile distilled water were applied to each pair of isolates and spermatia carefully exchanged between them using a sterile loop.

Latent Period Studies

A series of six experiments was done to determine the effect of temperature on the latent period of P.capsellae. Plants were grown in a 10°C (night)/15°C (day) controlled environment room operating a 16 hour light regime for four weeks. The 3rd and 4th leaves of each plant were inoculated with a conidial spore suspension containing 10,000 spores/ml. Plants were covered with polythene bags for three days and then transferred to controlled environment cabinets at 5, 10, 15, or 20°C. Each temperature was replicated in time with the others to give a series of six experiments. Each cabinet was maintained at a constant vapour pressure deficit of 350 Pascals, and 21,000 lux fluorescent lighting with a 16 hour daylength. There were 24 plants per cabinet. The number of lesions which appeared was recorded daily at each temperature.

RESULTS

Field Studies

Lesions on attached or fallen leaves sampled between December 1989 and May 1990 contained no spermogonia or dark stromatic mycelium, although conidia were almost always present. However, small black bodies were seen in the bleached centres of pod lesions on 19 July 1990. Minimum and maximum temperatures (°C) in the four preceding weeks were 0.2-11.4 (mean 6.7) and 12.2-22.5 (mean 17.3) respectively. Sectioning showed these black bodies to be of two types:

1) Spermogonia (44)-82-(116) μm in diameter, filled with spermatia (Fig. 1a).

2) Protoascomata (58)-87-(128)μm in diameter, containing nutritive cells and structures corresponding to ascogonia and trichogynes (Fig. 1b).

Spermogonia were exhausted by 30 August and showed no further development, whilst protoascomata steadily matured. Ascogenous tissue and ascus initials could be seen within the ascomata on 30 August and by 21 September asci were well developed. Ascospores had formed by 25 October (Fig. 1 c-e).

Ascospores collected by the spore sampler were discharged in response to wetting by rain or dew, but ascospore numbers also appeared to follow a diurnal pattern. Most ascospores were released between 0500 and 1700 hours, with a maximum at around 1100 hours (Fig. 2). Ascomata were almost all exhausted by January 1991, and would therefore appear not to overwinter. In this study ascospore release occurred for three months between mid October and early January.

Teleomorph-Anamorph Connections

Ascospore cultures on V8 agar were identical to those derived from conidia of P.capsellae, and readily yielded typical conidia under nuv light (Fig. 1f). When these conidia were inoculated onto rape leaves in the glasshouse, typical white leaf spot lesions were produced. These in turn yielded conidia of P.capsellae when incubated in a moist chamber. Ascospores discharged on to leaf surfaces infected through stomata and produced white leaf spot lesions from which conidia of P.capsellae were obtained. The teleomorph of P.capsellae was identified as belonging to the genus Mycosphaerella, and was differentiated from Mycosphaerella brassicicola (Duby) Lindau, which also occurs on rape.

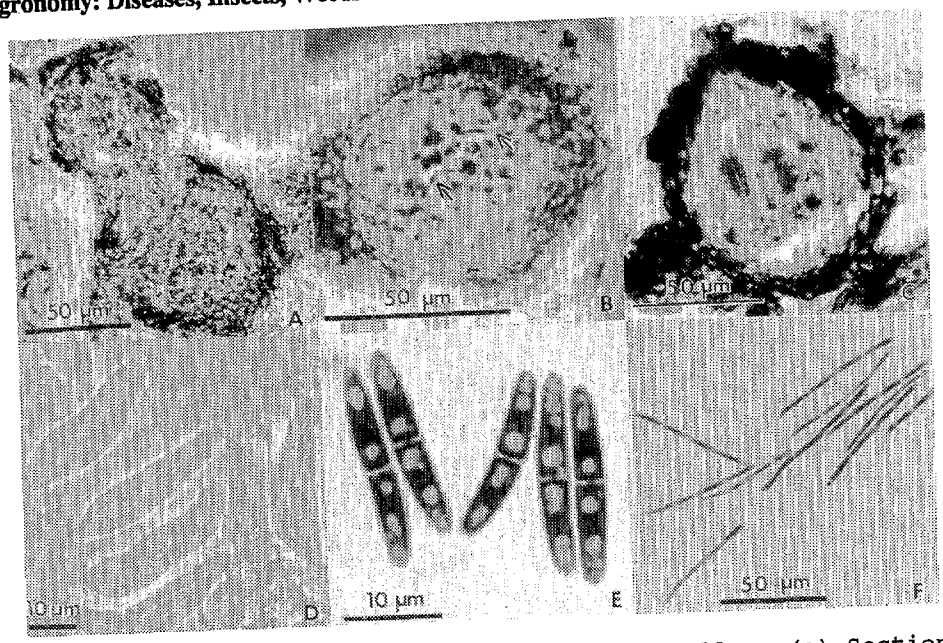

Fig. 1. The Mycosphaerella state of P.capsellae. (a) Sectioned spermogonium with spermatia. (b) Sectioned protopseudothecium with trichogyne (arrowed). (c) Pseudothecium with asci. (d) Asci. (e) Ascospores. (f) Conidia from ascospore cultures.

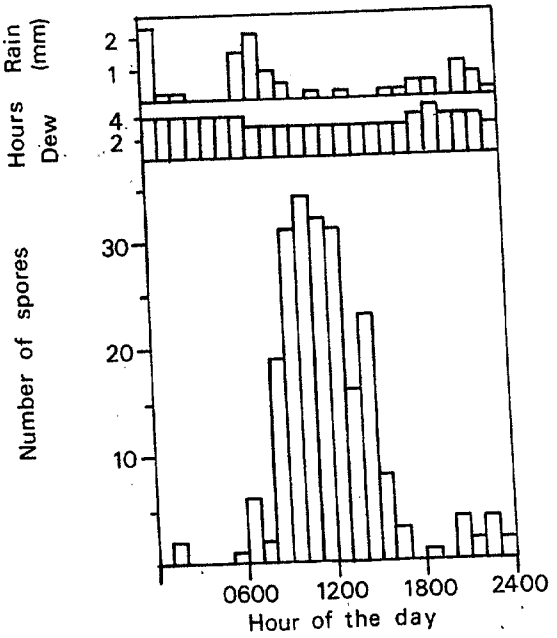

Fig. 2. Numbers of ascospores of the Mycospharella state of P.capsellae collected in relation to leaf wetness (dew) and rainfall in each hourly period from 10-15 November 1990. Data are totals for the six-day period.

Laboratory Studies

Mature spermogonia were produced in less than a week in leaf and petiole lesions on senescent yellow material incubated at 7°C under nuv light in a moist chamber. They were not produced in lesions on living green material, or in the dark, under nuv light at 20°C, or outside. In such cases dark stromatic knots 30-60μm in diameter with no spermatia were produced. Many stromatic knots in lesions incubated outside in early July had 1-2 long slender hyphae extending from their apices. These were assumed to be trichogynes. All attempts to infect plants with spermatia, or to germinate spermatia in culture, or to produce the teleomorph in spermatized cultures were unsuccessful.

Latent Period

The latent period is defined here as the time from infection to the appearance of lesions, and is described as a range and as the time when 50% of lesions (Lp50) had appeared. As temperature increased both the range and the Lp50 decreased (Table 1). The distribution of lesions with time fitted a slightly skewed normal distribution. There were no differences in the numbers of lesions produced between temperatures of 10, 15 and 20°C, but 50% fewer lesions were produced at 5°C.

Table 1. The effect of temperature on the latent period (range and Lp50) of P.capsellae on 3rd and 4th leaves.

Temperature(°C)	Range(days)	Lp50(days)
5	18-36	25.0
10	11.5-19	14.5
15	8.5-13	9.0
20	6.5-11	7.5

DISCUSSION

P.capsellae has been demonstrated to have both a sexual and an asexual life cycle. The sexual cycle begins with the production of spermogonia and protoascomata in late summer. Ascospores are produced in late autumn and are aerially dispersed as the source of primary inoculum. Subsequent disease spread and development is dependant on splash-dispersed conidia. The natural occurrence of a teleomorph of P.capsellae has important implications for our understanding of the epidemiology of white leaf spot. The production of pseudothecia at the end of the growing season enables the pathogen to survive between host generations. Air-borne ascospores ensure dispersal and their release coincides with vegetative growth of the crop in autumn.

It is unlikely that microsclerotia have an asexual role in survival as suggested by Crossan (1954), McKay (1956) and Penaud (1986), as they appear not to be sclerotial in character. Rather they represent a switch from asexual to sexual reproduction and are the primordia for spermogonia and pseudothecia. These primordia typically form below existing conidiophores but are not involved in the production of

conidia. Primordia can apparently be formed throughout the year in senescent leaf material if moisture is not limiting. However, their subsequent development into spermogonia or protoascomata seems to require light and relatively cool temperatures. Light intensity, quality, and day length are all likely to be important, although their contributions have not been determined.

The results of the latent period study show that it is possible for the disease to become established quickly during mild autumns. This correlates with reports of outbreaks at this time. Although further development is likely to be much reduced during the winter, temperatures in spring and early summer would permit quite rapid cycling. This is potentially damaging if the disease becomes established on the pods.

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