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Tijs Gilles

**Epidemiology of light leaf spot  
(*Pyrenopeziza brassicae*) on winter  
oilseed rape (*Brassica napus*)**

**De epidemiologie van 'light leaf spot'  
(*Pyrenopeziza brassicae*) in  
winterkoolzaad (*Brassica napus*)**

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## Chapter 2

### Mini review

#### **Epidemiology in relation to methods for forecasting light leaf spot (*Pyrenopeziza brassicae*) severity on winter oilseed rape (*Brassica napus*) in the UK**

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#### **Abstract**

*Pyrenopeziza brassicae*, cause of light leaf spot of oilseed rape, has a complex polycyclic life cycle. It can be difficult to control light leaf spot in winter oilseed rape in the UK since it is not easy to optimise fungicide application timing. Early autumn infections are usually symptomless and recognisable lesions do not develop until the epidemic has progressed further by the spring. Light leaf spot often has a patchy distribution in winter oilseed rape crops and estimation of disease incidence can be difficult. There is evidence that epidemics are initiated primarily by ascospores produced from apothecia that survive the summer inter-crop period on infected debris. Subsequent development of the epidemic during the winter and spring is maintained by rain-splashed conidia that spread light leaf spot from initial foci. Understanding the relative roles of ascospores and conidia in the light leaf spot life cycle is crucial for forecasting epidemic severity and developing control strategies. The current web-based regional forecast system provides an autumn forecast of the incidence of light leaf spot that can be expected the following spring. This is based on survey data which assesses the occurrence of disease the previous July, and weather factors, such as deviations from summer mean temperature and winter rainfall. The forecast can be updated throughout the autumn and winter and includes crop-specific elements so that growers can adjust risks by inputting information about cultivar, sowing date and fungicide use. Crop-specific forecasts can be confirmed by assessing the incidence of light leaf spot. Such assessments will become easier when immunodiagnostic methods for detection of the disease become available. Incorporation of information on spore biology (e.g. apothecial maturation, ascospore release and infection conditions) is considered as a component of the interactive, continuously updated, crop-specific, web-based forecasts which are needed in the future.

**Keywords:** Apothecial development, ascospores, conidia, disease assessment, primary inoculum, regional forecasts.

## Introduction: the need to forecast light leaf spot severity

An accurate understanding of the epidemiology of light leaf spot (*Pyrenopeziza brassicae*; anamorph *Cylindrosporium concentricum*) on winter oilseed rape (*Brassica napus*) is essential for forecasting epidemic severity to achieve effective control of the disease. In recent seasons, yield losses of up to 22% of the total potential seed yield were estimated to have been caused by light leaf spot in the UK (Fitt *et al.*, 1997). Light leaf spot epidemics can also be severe on winter oilseed rape crops in other northern European countries (Paul and Rawlinson, 1992). None of the currently grown winter oilseed rape cultivars are completely resistant to light leaf spot (Anonymous, 1998), and fungicides remain the only effective means of light leaf spot control. However, because of great variation in disease incidence between seasons (Figure 1), regions and individual crops within a region (Fitt *et al.*, 1996), different numbers of fungicide applications and dosages are needed for each season, region and individual crop. In practice, fungicide applications in certain regions and seasons were not related well to disease incidence, as fungicides were often applied unnecessarily, for example when the risk of a severe epidemic was low or at times during the season when they were less effective at controlling the disease (Fitt *et al.*, 1996).

In the UK, winter oilseed rape crops are sown in the autumn, during late August and throughout September, although planting date varies with latitude. Planting occurs earlier in more northern latitudes, as cooler temperatures dictate the need for a longer growing season in order to maximise yield potential. Harvest occurs during the summer, from mid-July through to late August, with more northerly crops being harvested later. In Scotland and northern England this creates the potential for overlap of growing seasons, particularly in wet years when growers have to delay harvest and consider an early sowing date (Sutherland *et al.*, 1995). Light leaf spot can infect crops soon after emergence and can cause yield loss at two periods in the season. An early, widespread epidemic can cause plant stunting and sometimes death at the rosette stage of growth during the winter (December to February). Later in the season, following stem extension (late February to April), infection of developing floral structures and seed pods (April to July) can result in malformation of pods/ seeds, premature senescence of pods and pod shatter before harvest.

Research has shown that sprays in the autumn are often needed to control light leaf spot effectively in the UK (Rawlinson and Cayley, 1984; Jeffery *et al.*, 1989; Gladders, 1990; Rawlinson *et al.*, 1984; Sutherland *et al.*, 1995; Fitt *et al.*, 1997). However, the visual diagnosis of light leaf spot can be difficult in the autumn because of the long incubation period between infection and appearance of recognisable necrotic lesions (Figuroa *et al.*, 1995b; 1993; 1992; Fitt *et al.*, 1998a), and fungicide sprays are often applied after the time that would give optimal control. In general, fungicides need to be applied before light leaf spot symptoms are visible in a crop (Fitt *et al.*, 1997). There is thus a need to accurately forecast, at the time when spray decisions need to be taken, the risk that severe light leaf spot epidemics will develop. The forecasts currently being developed are designed to assist farmers in making decisions about when to apply fungicides, particularly during the early part of the season when symptoms of light leaf spot may not be visible.

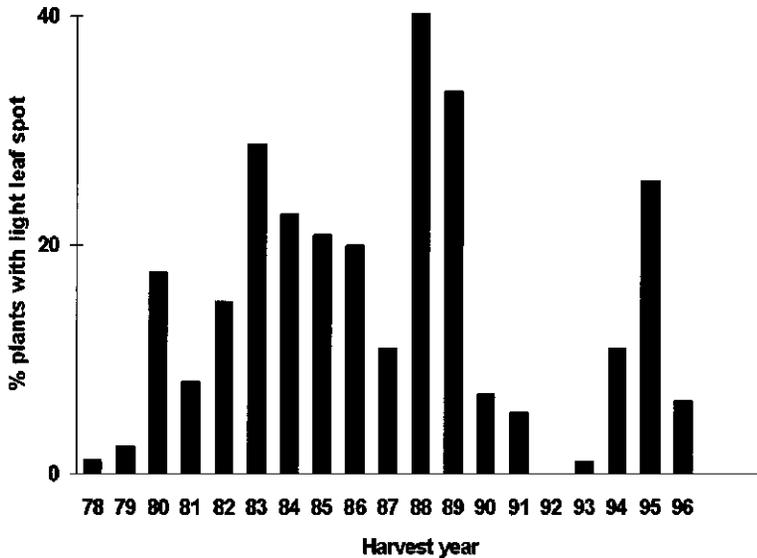


Figure 1 Incidence (% plants with leaves affected) of light leaf spot (*Pyrenopeziza brassicae*) in eastern England in March, assessed in a survey of diseases on commercial crops of winter oilseed rape in harvest years 1978 to 1996. Amended from Fitt *et al.*, (1994).

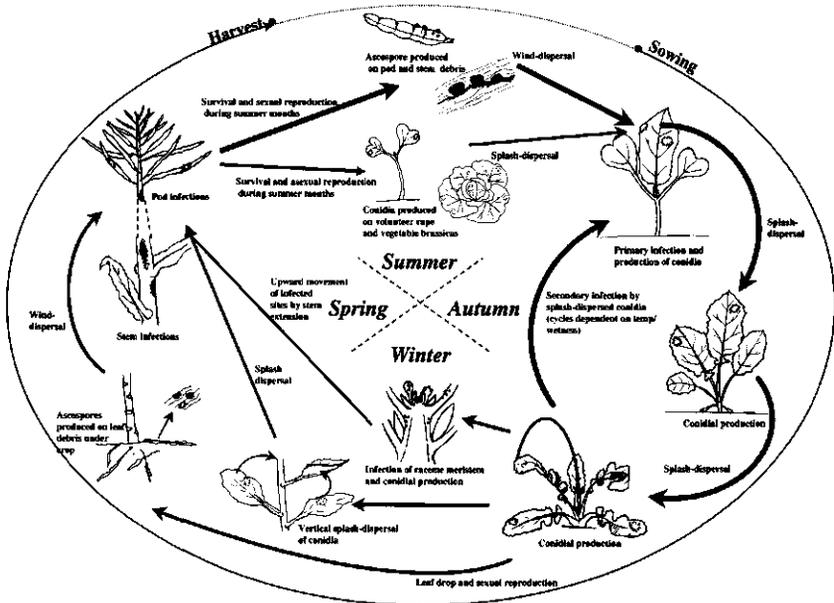
A thorough understanding of the processes that contribute to light leaf spot epidemics can be used to develop forecast systems. These processes, such as the survival of *P. brassicae* during the inter-crop period in the summer, maturation and release of ascospores, infection by ascospores, the latent period and cycles of conidial production and infection, are influenced by weather factors. For example, leaf wetness duration and temperature influence the infection of winter oilseed rape leaves by conidia (Figueroa *et al.*, 1995a; 1995b; Gilles *et al.*, 2000b). The influence of weather factors on some of these components of light leaf spot epidemics can be described as mathematical functions, which can be incorporated into models to forecast the risk of severe epidemics. This review discusses current knowledge of the critical stages in light leaf spot epidemics and the factors that influence them in relation to development of methods for forecasting light leaf spot severity in the UK.

#### **Epidemiology of light leaf spot: the role of ascospores and conidia**

To accurately forecast the onset and severity of light leaf spot epidemics, it is necessary to understand the role that ascospores and conidia play in the disease cycle. Sexual ascospores of *P. brassicae* develop in apothecia during saprophytic survival on oilseed rape debris under favourable moist conditions and are dispersed by wind (Lacey *et al.*, 1987). However, asexual conidia are produced in acervuli during parasitic growth of *P. brassicae* in living plant tissue and are dispersed by rain-splash (Maddock and Ingram, 1981).

### Summer survival strategy – sexual or asexual?

The mode of survival of *P. brassicae* over the summer, between successive winter oilseed rape crops in the UK, influences the type of spores that can act as the primary inoculum, although the relative importance of each spore type in eventual epidemic development is unclear. Two main survival strategies have been identified; saprophytic survival on stem, pod and leaf debris after harvest (Lacey *et al.*, 1987; McCartney and Lacey, 1989; McCartney and Lacey, 1990) and pathogenic survival on green tissues of late harvested oilseed rape crops, volunteer oilseed rape (McCartney and Lacey, 1990; Maddock and Ingram, 1981), vegetable brassicas (Maddock *et al.*, 1981; Cheah and Hartill, 1985; Staunton, 1967) and cruciferous weed species (Maddock and Ingram, 1981) (Figure 2).



**Figure 2** Epidemic cycle of light leaf spot (*Pyrenopeziza brassicae*) in winter oilseed rape in the UK, indicating the potential roles of wind-dispersed ascospores produced in apothecia and splash-dispersed conidia produced in acervuli.

In southern England, the pathogen has to survive an inter-crop period of about two months between harvest in mid-July and emergence of the new crop in September. The most likely mode of survival for the initiation of epidemics in newly sown winter oilseed rape crops, as with diseases of many winter sown arable crops in Europe, is on the stubble and crop debris left after harvest of the previous crop in the summer. *P. brassicae* forms its sexual state on infected debris; the apothecia develop and ascospores are produced within them (Lacey *et al.*, 1987). Senescent infected leaves of volunteer oilseed rape in previously cropped fields and along headlands and pathways and vegetable brassicas have been reported to act as sources of ascospores in several countries (McCartney and Lacey, 1990; Staunton, 1967; Staunton and Kavanagh, 1966; Cheah *et al.*, 1980). However, the frequency of sexual reproduction on

senesced leaves of volunteer oilseed rape or vegetable brassicas in the UK is unknown. Recent evidence from population studies of field isolates, collected in spring (Majer *et al.*, 1998) and summer (Ball *et al.*, 1990), indicates that both mating types of *P. brassicae* are present throughout the UK and that the sexual stage occurs frequently. The sexual phase of the life cycle is therefore important in the survival of the pathogen in the UK.

However, in northern England and Scotland, late harvested crops have been known to form a "green bridge" and act as a primary inoculum source for newly sown crops (Sutherland *et al.*, 1995). There is thus the potential for asexual survival of *P. brassicae* in these areas. Conidia, produced during biotrophic growth on these late harvested winter oilseed rape crops, may therefore play a role in the transmission of light leaf spot (Rawlinson *et al.*, 1978), although they are only dispersed over short distances by splash (Fatemi and Fitt, 1983; Rawlinson *et al.*, 1978). It has been suggested that conidia produced on volunteer oilseed rape, vegetable brassicas or other host plants (Rawlinson *et al.*, 1978) could play a role in the transmission of *P. brassicae* to newly sown winter oilseed rape crops on a localised scale (Maddock and Ingram, 1981). Weeds probably play a lesser role in the transmission of *P. brassicae* to winter oilseed rape from one cropping season to the next. Of 19 weed and closely related cruciferae species tested, only a few individuals of *Brassica rapa* (6 out of 21 plants tested), *B. nigra* (4 out of 21) and *B. juncea* (1 out of 17) developed a small number of light leaf spot lesions (Maddock and Ingram, 1981).

#### *Initiation of epidemics in autumn*

Considering current rotational practices in the UK, with the winter oilseed rape crop as an important break from cereal crops, and the limited dispersal range of splash-dispersed conidia, light leaf spot epidemics are most likely to be initiated by ascospores in the autumn. As ascospores are wind-dispersed (McCartney and Lacey, 1989), they travel further than splash-dispersed conidia and have a greater opportunity to land on uninfected winter oilseed rape crops. Recently, Evans *et al.* (1999b) suggested that dispersal patterns of light leaf spot provide further evidence that wind-dispersed ascospores form the primary inoculum source. Early in the season, at low levels of light leaf spot incidence (<10%), affected plants were randomly distributed. It was suggested that this pattern was caused by wind-borne ascospores arriving from distant inoculum sources (Evans *et al.*, 1999b).

Controlled environment studies have indicated that ascospores may be much more infective than conidia. This provides further evidence that they may play the major role in causing primary infections and initiating epidemics (Gilles and Fitt, 1999). However, the infection conditions for ascospores have not been investigated fully and may differ from infection conditions for conidia (Figueroa *et al.*, 1995a; 1995b; Gilles *et al.*, 2000b). If ascospores are more infective than conidia and infect winter oilseed rape under a broader range of infection conditions, ascospores are more likely to cause the primary infections in newly sown winter oilseed rape crops each autumn. Gilles and Fitt (1999) suggested that this may be the case, as infection conditions would generally be less limiting for ascospore infection and fewer spores would be required for successful infection.

Further evidence for the importance of ascospores was provided by Lacey *et al.* (1987), who observed apothecia on crop debris from the previous season in the autumn, at the start of the following season. Large numbers of air-borne ascospores have been collected near oilseed rape crops at Rothamsted in the autumn of several seasons (McCartney, unpublished data). Observations from field experiments suggested that there was a requirement for the debris to be moist for apothecial development and ascospore release to proceed (McCartney and Lacey, 1990). Thus, the production of ascospores depends on both the presence of infected debris (colonised by both mating types of the pathogen) on the soil surface and the occurrence of weather conditions favourable for development of apothecia. Indirect evidence for this was

provided by Cheah and Hartill (1985) who demonstrated that, in New Zealand, ploughing after harvest greatly decreased both the amount of infected debris on the soil surface and the production of ascospores. Weather conditions favourable for ascospore production and release occur frequently during the autumn in the UK.

#### *Light leaf spot progress in winter*

In northern England and Scotland, plant death during winter has been reported to be an important cause of yield loss from light leaf spot in winter oilseed rape (Sutherland *et al.*, 1995). The increase in the incidence of light leaf spot resulting from primary infection in the autumn to the levels observed in March may be determined by the number of cycles of asexual reproduction that have occurred during the winter (Figure 2). Dispersal of conidia, success of infection and the length of the latent period are influenced by occurrence of rain events (Fatemi and Fitt, 1983), temperature and leaf wetness duration (Figueroa *et al.*, 1995b), and temperature (Figueroa *et al.*, 1995a; Gilles *et al.*, 2000b), respectively (Figure 2). Evidence for a major role for rain-splashed conidial secondary infection in the increase in severity of epidemics has been suggested by Evans *et al.* (1999b). Following the observation that light leaf spot has a random distribution early in the season, Evans *et al.* (1999b) observed that, as the season progressed and incidence increased to more than 10% plants affected, the spatial pattern of light leaf spot showed aggregation. It seemed likely that the observed aggregation was a direct consequence of rain-splashed secondary spread and that this could explain the "patchiness" of light leaf spot reported by Fitt *et al.* (1996).

#### *Infection of floral structures/developing pods in spring*

Little information is currently available about the most important mechanism for infection of the developing floral and pod structures in the spring and the effect of this on subsequent yield loss. Three possible mechanisms that have been identified for the infection of the floral structures (Fig. 2). From fungicide experiments, Rawlinson and Cayley (1984) suggested that infection may take place relatively early in the season, probably through the development of latent infections on leaf and flower primordia, which have been initiated by conidia splashed to the central meristematic tissues of prostrate plants at the rosette stage. Dissection of such meristematic tissues in January showed that they may be infected by *P. brassicae* (Figueroa, pers. comm.). Early infection of these tissues prior to stem extension could explain how the disease progresses from leaf infections at the rosette stage to infections on flowers and pods.

It is possible that the developing floral and pod structures are infected by the ascospores observed during April, May and June, which have been produced on infected leaf debris lying on the ground under crops (McCartney and Lacey, 1990). Alternatively, the presence of abundant conidia on leaves during spring/early summer suggests that infection of the upper stem, flowers and developing pods can take place through the upwards movement of rain-splashed conidia (Pielat *et al.*, unpublished). Further work is required to characterise the relationship between early infections and the occurrence of light leaf spot on the upper sections of the plant after stem extension. Apart from reducing yield through pod shatter, it appears that light leaf spot on stems and pods in the upper part of the crop canopy provides the majority of the inoculum source for the infection of the crop the following season (Gladders *et al.*, 1995).

## Methods for forecasting severity of light leaf spot epidemics

The seasonal, regional and inter-crop variation in severity of light leaf spot is the main reason why a robust forecasting system is necessary if winter oilseed rape is to remain economically sustainable as a break crop in the UK (Figure 1). Current and potential future methods for forecasting severity of light leaf spot on winter oilseed rape in the UK are based on pathogen inoculum, disease assessment or *P. brassicae* spore biology (Table 1).

### *Inoculum based risk assessment forecasts*

Currently the only forecasting system widely available to growers in the UK is an inoculum-based system for forecasting regional risk, with an option to modify the regional risk to produce a crop risk (Fitt *et al.*, 1996). The regional forecasting system (Table 1, Figure 3) has been produced using a number of disease and weather factors; national disease survey data for incidence of light leaf spot on pods in July and deviations from the 30 year mean for summer temperature and winter rainfall (Fitt *et al.*, 1996; Welham *et al.*, 1999). A preliminary light leaf spot risk assessment forecast, based on incidence of light leaf spot on pods and deviations in summer temperature, is issued in the early autumn (in October). The forecast is then updated the following March after the inclusion of winter rainfall data (Welham *et al.*, 1999). The observed relationship between current and past disease incidence may occur because the amount of infected debris on which the pathogen can survive during the summer, and thus the numbers of ascospores produced, is related to incidence of light leaf spot on pods in July. Variation in temperature over the summer period affects the development and maturation of apothecia on the debris (Gilles and Fitt, 1999). The inclusion of winter rainfall allows the updated model to take account of the effects of rainfall on dispersal of conidia and infection of leaves at this time and thus the number of disease cycles that can occur early in the season.

Analysis of the ADAS/CSL winter oilseed rape disease survey data suggests that England and Wales can be divided into specific "light leaf spot regions" that consist of areas which have similar levels of light leaf spot from season to season (Welham *et al.*, 1999). Weather parameters that clearly predicted the incidence of light leaf spot differed between some of these regions and as a result, the forecast model was regionally specific. Currently, the forecasting scheme has only been used in England and Wales and it needs to be extended to Scotland. The regional forecast has been adapted for delivery via the Internet to growers since 1998. Recently the forecast has been improved by the introduction of active server pages (ASP's) (Fig. 4), which can be found at the URL: <http://www.iacr.bbsrc.ac.uk/lightleafspot> (Evans *et al.*, 1999a). These allow the grower to input three crop-specific parameters which influence the risk that a severe epidemic may develop; cultivar (to take account of resistance rating), sowing date and autumn fungicide application information (Welham *et al.*, 1999).

Table 1 Methods for forecasting in autumn/winter, severity of light leaf spot (*Pyrenopeziza brassicae*) epidemics on winter oilseed rape in the UK

Current and potential forecast methods	Advantages	Disadvantages	Potential for improvement
<p>Inoculum based Regional risk Crop risk</p>	<p>Advance warning for growers (based on previous disease). Updated in autumn/winter (inclusion of weather data). Not complex, easily delivered. Interactive, allowing grower input.</p>	<p>Problems in defining 'regions'. Likely inaccuracies at crop level. Empirical models, not continuous. Perturbation over time.</p>	<p>Improve crop forecasts. Include pathogen/host/environment information. Include yield loss and economics.</p>
<p>Disease assessment based. Sampling and diagnosis</p>	<p>Crop-specific. Provides disease data. Improves spray timing.</p>	<p>Time-consuming, may need repeating (monthly). Patchy spatial distribution. Diagnosis difficult. Yield loss thresholds needed.</p>	<p>Improve sampling strategy. Develop rapid PCR and immunodiagnosics. Calculate thresholds.</p>
<p>Spore biology based. Apothecial development/ Ascospore release Infection criteria</p>	<p>More reliable advance warning. Improves spray timing. Allows farm/crop specific forecast. Potential continuous interactive system.</p>	<p>Requires scientific knowledge. Difficult to collect ascospore data. Time consuming interpretation. Requires up-to-date weather data.</p>	<p>Regional depots for regional forecasts. Diagnostic automation for crop-specific forecasts. Incorporate into dynamic models. Continuous updates with automatic weather data.</p>

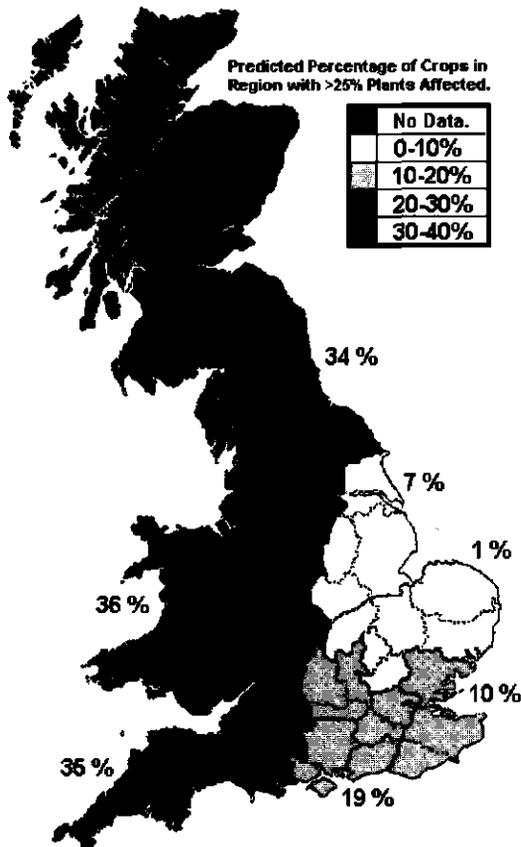


Figure 3 Map of the UK, illustrating, for different forecast regions, the % crops predicted in October 1999 to have >25% of plants with light leaf spot (*Pyrenopeziza brassicae*) in March 2000.

The advantages of the inoculum-based forecasting system are that it provides the grower with an advance warning of the risk of severe light leaf spot, which allows a period of several weeks in which to make a decision about fungicide applications, and the risk can be updated during the autumn/winter period. Furthermore, it is not complex and can be easily delivered to the user via the Internet, press releases and other methods. Although the model is based on large light leaf spot regions, the inclusion of the interactive choices allows the forecast to be more crop-specific. This interaction also allows the grower to carry out role-play scenarios, for example to calculate the decrease in light leaf spot risk for the following spring if an autumn spray application is applied. The interactive system can also be used before the start of the season to assess the decrease in light leaf spot risk if a more resistant cultivar is grown or the crop is sown later in the season.

a)

## Forecast for North of England

Please enter your details to refine the forecast for your farm:

Cultivar:

Apex

Sowing date:

- Early: before 1st September  
 Late: after 31st August

Autumn Fungicide Spray:

- No  
 Yes

Submit Query    Reset



b)

## Light Leaf Spot Forecast

Customized forecast for a farm in North of England

Cultivar	Apex
Resistance Rating	6
Sown	early
Autumn spray	unsprayed

The model predicts

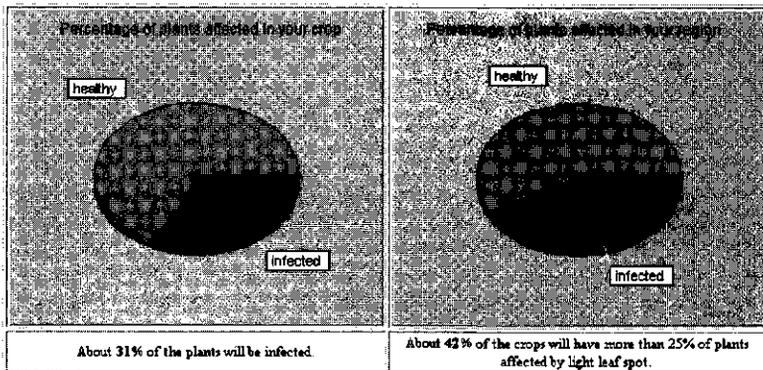
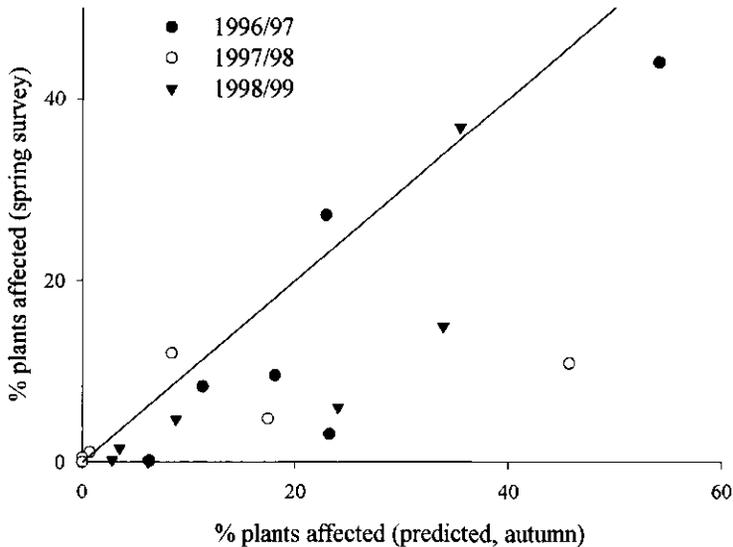


Figure 4 Internet-based, interactive light leaf spot (*Pyrenopeziza brassicae*) forecast. a) Grower input page with cultivar choice, sowing date and fungicide application information. b) Output page with regional (right pie chart) and crop-specific (left pie chart) light leaf spot risk forecast. (Evans *et al.*, 2000)

A disadvantage of the inoculum-based forecasting system is that it is difficult to define the light leaf spot regions or to assess risks for crops near to the borders of regions with different risks. Furthermore, the system is open to inaccuracy, especially at the crop-specific level, because it is based on a few simple empirical relationships which do not fully describe the dynamics of the biological processes influencing light leaf spot epidemics. Although the forecasts can be updated by incorporation of weather information, the risk assessment system is still susceptible to perturbation over time. However, a comparison of predicted regional risks against the actual incidence of light leaf spot the following March over three seasons (1996/97, 1997/98 and 1998/99) indicated that the model provides growers with reasonable information with which to make spray decisions (Fig. 5). The light leaf spot risk assessment model appears to have slightly overestimated the percentage of plants that will be affected with light leaf spot the following March. However, the predictions were made before fungicide sprays were applied in autumn; the fact that observed incidences were less than predicted incidences may have been the result of treatments applied in response to the predictions.



**Figure 5** Validation of the regional light leaf spot (*Pyrenopeziza brassicae*) risk forecasting system. Figure indicates a comparison of the regional risk (% plants with light leaf spot predicted) in October 1996 to 1998 against incidence of light leaf spot observed in March for 1997 to 1999 in winter oilseed rape disease surveys.

Improvements could be made to the inoculum-based forecasting system by incorporating more survey information on agronomic, environmental and crop factors affecting the epidemics. For example, the lengths of the periods between harvest, ploughing, sowing and crop emergence and the weather conditions during these periods influence maturation of apothecia, release of ascospores and potential for disease escape. The value of these factors for prediction of light leaf spot incidence the following March needs to be investigated on a regional basis. The disease data collected in the autumn winter oilseed rape survey could be used to improve the accuracy of the updated risk for each region. Analysis of survey data for

light leaf spot severity (as opposed to incidence) might also help to improve the accuracy of the crop-specific component of the forecasting system. To overcome one factor influencing the observed variation between forecasts for regions and for individual crops within a region (Fitt *et al.*, 1996), it should be possible for growers to input their own local weather information to improve the accuracy of predictions for their own crops, using the ASPs on the forecasting web-site. This would move the responsibility for updating the forecast from the scientist to the individual grower and decrease the cost of maintaining it. Since Su *et al.* (1998) observed that the incidence of light leaf spot at early flowering in March gave a good prediction of yield losses caused by light leaf spot, incorporation of yield loss models into the forecasting system would allow growers to assess more accurately whether or not a fungicide application is justified economically.

#### *Disease assessment based forecasts*

Disease assessments are already being used to improve the accuracy of inoculum-based forecasts of the risk that severe epidemics will develop since the light leaf spot forecasting web-site includes a recommended protocol for sampling crops so that growers can confirm the occurrence of the disease (Fitt *et al.*, 1998a). Growers are recommended to inspect crops at monthly intervals from October to April and to collect samples to assess the incidence of the disease, if they consider the crop to be at high risk or symptoms are beginning to appear. During the winter, plants need to be sampled in clusters because light leaf spot has a patchy distribution (Fitt *et al.*, 1996) and disease symptoms are not randomly distributed (Evans *et al.*, 1999b). Diagnosis of light leaf spot can be improved by incubating sampled plants in polyethylene bags under humid conditions for 3-4 days (Fitt *et al.*, 1996) because infected leaves are frequently symptomless early in the season. Incubation creates conditions conducive to asexual sporulation of *P. brassicae* and characteristic white spore pustules become visible on infected leaves (Fitt *et al.*, 1998a). The assessment of light leaf spot incidence at various times during a season can be used to provide crop-specific information to update and verify risk forecasts and improve the accuracy of spray timing decisions. The relationship between light leaf spot incidence at early flowering in March and yield loss (Su *et al.*, 1998) can be used as a basis for calculating economic thresholds. However, further work is needed to establish detailed relationships between light leaf spot incidence in the autumn/winter, when spray decisions need to be made, and incidence/severity in spring and subsequent yield loss before disease assessment based forecasts can be developed effectively.

However, such disease assessments are time-consuming, particularly if they need to be repeated on several occasions during the autumn/winter period. Accurate diagnosis of light leaf spot, when infections are symptomless or show ambiguous symptoms which can be confused with frost damage or other diseases, is difficult, even if plants are incubated to induce pustule development. Forrer (1992) cited the requirement for accurate disease diagnosis as the main reason why decision support systems have had little impact on European agriculture. He suggested that the future integration of immuno-diagnostic tests with decision support systems may simplify the process of disease identification and improve the accuracy of assessments and that this would make the decision making process more robust. The effectiveness of the disease assessment based light leaf spot forecasting can be improved by developing novel molecular diagnostic techniques to detect light leaf spot in winter oilseed rape crops before symptoms appear (Foster *et al.*, 1999; Hollomon, 1998). A PCR-based technique that has been developed to detect *P. brassicae* in symptomless leaves is expensive, and requires specialised equipment and knowledge. Whilst it can be used for research purposes, it cannot easily be used by growers. There is a need to develop a simple immuno-diagnostic test specific for *P. brassicae*, which can be used in crops

However, a light leaf spot forecasting system based on sampling and disease assessment alone could be unreliable because the spatial pattern of the disease is not fully understood. There is a need for a robust sampling protocol to effectively assess disease incidence. Procedures that detect nanogram quantities of target DNA or antigen cannot provide meaningful results unless the plant sample tested is representative of all susceptible host tissue from the larger population being sampled (Putnam, 1995). Further research is required to clarify the spatial distribution of the disease and to design specific sampling strategies (Evans *et al.*, 1999b).

#### *Spore biology based risk forecasts*

Recent improvements in the understanding and modelling of the epidemiology of light leaf spot, together with improvements in information technology, provide the potential for delivery via the Internet of two-way interactive, continuous, crop-specific forecasts. As light leaf spot epidemics appear to be initiated by ascospore infection (Fig. 2), the development of a forecasting scheme based on aspects of apothecial development, ascospore release and/or ascospore infection conditions should provide more specific advance warnings for growers than regional or crop risk forecasts. Subsequently, spore biology based forecasts could be improved by using information about the latent period of *P. brassicae*, production of conidia and infection conditions for conidia. They could also include forecasts of disease spread from leaves to pods, based on apothecial maturation and ascospore release in the spring (McCartney and Lacey, 1990) or vertical splash dispersal of conidia (Pielaat *et al.*, unpublished).

It is likely that such spore biology based forecasts would initially need to be developed on a regional scale. A network of "depots" of infected oilseed rape debris could be developed around the UK. The debris could be monitored on a weekly basis, at ADAS/SAC regional centres, for example, to provide regional warnings that weather conditions have been favourable for apothecial maturation. A "depot" system has been used to monitor sclerotinia apothecial development in Sweden (Nordin *et al.*, 1992) for a number of years and has been evaluated as part of a risk forecasting scheme in the UK (Sansford, 1995). Monitoring of apothecial maturation, through the use of depots, could provide growers with a regionally based forecast of the likely onset of the epidemic to allow the first control spray to be targeted more efficiently. Furthermore, ascospore release could be monitored at these depots with a Burkard spore sampler and used to improve the accuracy of warnings. It would not be practical to monitor apothecial maturation and ascospore release in this way on individual farms. Identification of apothecia and ascospores is currently both difficult and time-consuming. Ascospores of *P. brassicae* are very small in size, hard to identify and easily confused with ascospores of the saprophyte *Ungularia* cfr. *raripila* (Inman, Fitt and Evans, 1992).

When models have been developed to describe relationships between weather factors (e.g. temperature, wetness duration) and ascospore maturation (Gilles and Fitt, 1999), ascospore release and ascospore infection conditions, to complement those for conidial infection conditions, the latent period and sporulation (Figueroa *et al.*, 1995a; 1995b; Gilles *et al.*, 2000b), it may be possible to use regional weather as a basis for regional warnings. This would be considerably less time-consuming than monitoring apothecial maturation or ascospore release in depots. It seems unlikely that forecasts based on infection criteria alone, such as the Beaumont period (Beaumont, 1947) and Smith period (Smith, 1956) utilised to guide potato blight control, would be successful with light leaf spot since prior knowledge of the presence of ascospores or conidia would be essential. Forecasts based on ascospore occurrence and infection criteria would allow fungicides to be applied at the onset of epidemics. These forecasts could be updated in response to occurrence of conidial infection

criteria, the latent period, and sporulation and dispersal criteria (which would allow estimates of the number of asexual reproductive cycles that could be expected). From this, the severity of the epidemic could be predicted much more effectively. The work of Gilles and Fitt (1999) and Figueroa *et al.* (1995a; 1995b) indicates that the timing of the forecast need not be that precise, as the light leaf spot pathogen has a long latent period (c. 250 degree-days), which creates a large spray "window" for effective control. If the relationships between weather factors and these phases in the epidemiology of light leaf spot can be accurately modelled, then it should be possible to produce farm-specific forecasts for farms with local meteorological data available.

In the future, forecasts based on ascospore release may become more practicable as automated systems for sampling and identifying spores become available. Foster *et al.* (1999) recently developed a PCR-based diagnostic for symptomless *P. brassicae* in infected leaves and an immuno-diagnostic test specific for *P. brassicae* could be developed (S. Foster, pers. comm.). Although current PCR-based diagnostics are not of direct use to growers because of their cost and requirement for specialised equipment, it may be possible to develop a PCR-based diagnostic test to identify *P. brassicae* ascospores in air samples. A similar system has been successfully used for *Penicillium roqueforti* (H.A. McCartney, pers. comm.). If samples could be quickly and efficiently assessed for the presence of *P. brassicae*, it would save time and remove the possibility of misidentification of ascospores. Furthermore, development of immuno-diagnostic techniques for use with simple spore samplers, which could be used on farms, may allow automatic incorporation of spore data with meteorological data to produce more accurate crop-specific forecasts.

Developments in information technology now provide the possibility that epidemiologically based forecasts could be delivered to growers and advisers and updated continuously via the Internet. Unlike a forecast based only on past survey data, such a system would be based on "real time" epidemiological events and would be less susceptible to perturbation. Monitoring throughout the season could improve the accuracy of forecasts and maximise efficiency of fungicide spray timing. Such a forecasting system will be effective only if it is based on models which accurately describe the progress of light leaf spot epidemics in time and space. Internet delivery could allow forecasts to be updated continuously in response to daily weather data. Two-way interaction would be possible. Warnings could be issued automatically to growers by e-mail. Growers could input their own data, for example assessments of disease in specific crops, from mobile phones used in the field to get instantaneous information with which to make decisions. Furthermore, the cost of maintaining and updating such an Internet-based forecasting system would be considerably less than for systems delivered by traditional methods.

## Discussion

This review indicates how improvements in understanding the epidemiology of light leaf spot can be exploited to improve current inoculum-based risk forecasts and disease assessment based forecasts or to develop new forecasting systems based on *P. brassicae* spore biology (Table 1). In combination with improvements in diagnostic and information technology, these improvements offer the prospect of developing more accurate, interactive, crop-specific forecasts of the risk of severe light leaf spot epidemics. Recent advances in understanding the effects weather factors have on progress of light leaf spot epidemics will allow improvements to be made to inoculum-based regional risk forecasts (Fitt *et al.*, 1996). It should be possible to use information about relationships between temperature/wetness and apothecial development, ascospore release (McCartney and Lacey, 1990), ascospore infection conditions (Gilles and Fitt, 1999), the latent period and conidial infection conditions (Gilles *et al.*,

2000b) to provide weather-based updates to regional risk forecasts more frequently than at present. Furthermore, this epidemiological information can also be used to improve updating of the interactive, crop-specific component of these inoculum-based forecasts delivered via the Internet (Welham *et al.*, 1999). Nevertheless, the accuracy of these regional and crop risk forecasts will always be limited by the simplicity of the empirical models on which they are based. There is also an urgent need for regional and crop risk forecasts for stem canker to be developed and combined with those for light leaf spot in southern England, where both diseases cause serious problems on winter oilseed rape.

An understanding of the epidemiology of light leaf spot, especially the mechanisms of disease spread, can also be used to improve the disease assessment based forecasts. Since spatial analyses of light leaf spot epidemics indicate that initial infections in the autumn fit a random distribution (Evans *et al.*, 1999b), suggesting that primary infections are caused by wind-blown ascospores, in the autumn samples can be taken as individual plants collected at regular intervals along a transect across a crop. However, later in the season aggregation of light leaf spot is observed, providing evidence for the importance of localised spread of the disease from initial foci by rain splashed conidia (Evans *et al.*, 1999b). This "patchy" pattern of disease observed in crops (Fitt *et al.*, 1996) suggests that samples then need to be taken as clusters of plants to get an accurate estimate of light leaf spot incidence. Whatever the sampling strategy, current disease assessment based forecasting methods are time-consuming; the prospect of using immuno-diagnostic methods to obtain rapid, accurate diagnoses (Foster *et al.*, 1999) is to be welcomed.

There are prospects for using assessments of *P. brassicae* apothecial development and ascospore release to develop new, more accurate systems for providing growers with advance warning of onset of light leaf spot epidemics, but the expertise and time required to make the assessments will mean that they can only be used on a regional basis. However, recent improvements in diagnostic (Foster *et al.*, 1999) and information technology (Evans *et al.*, 1999a) offer real potential for development of continuous, interactive, crop-specific forecasts based on the spore biology of *P. brassicae*. Understanding and modelling of factors such as local primary inoculum sources, dispersal processes (primary and secondary) and how weather factors influence disease progress through the season will allow new forecasts to be much more crop-specific and "real time" in nature. Furthermore, the delivery of such forecasts via the Internet will allow them to be interactive and updated continuously. Thus, a detailed understanding of the epidemiology of light leaf spot can be used to develop forecasts that are robust, accurate and easily delivered to growers to guide decisions about strategies for control of light leaf spot on winter oilseed rape.

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## Chapter 3

### The roles of ascospores and conidia of *Pyrenopeziza brassicae* in light leaf spot epidemics on winter oilseed rape (*Brassica napus*) in the UK

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#### Abstract

Debris of oilseed rape affected with light leaf spot was exposed outdoors from 23 September 1998 or from 26 August 1999, and air-borne ascospores of *Pyrenopeziza brassicae* were first collected nearby after 12 or 23 days, respectively. When winter oilseed rape was inoculated with such debris on 3 November 1998 or 12 October 1999, light leaf spot (assessed by production of *P. brassicae* conidia) was first observed on leaves on 6 January 1999 or 15 February 2000, respectively. In 1991/92, numbers of air-borne ascospores above a naturally infected crop were small from January to April and increased in June and July; *P. brassicae* conidia were first observed in February and the % plants with leaves, stems or pods with light leaf spot increased greatly in May and June. In 1992/93, in a crop inoculated with debris, numbers of air-borne ascospores were small from October to January and increased from April to June; light leaf spot was first observed on leaves in late November and on stems and pods in March and June 1993, respectively. In June 2000, on the ground under a crop with light leaf spot, numbers of petioles with apothecia decreased with increasing distance from the edge of pathways. Ascospores were c. 400 times more infective than conidia on oilseed rape (cv. Bristol) leaves. Lesions caused by conidia were more often located on leaf veins than lesions caused by ascospores.

**Keywords:** disease progress, oilseed rape debris, spore concentrations

## Introduction

Both ascospores and conidia of *Pyrenopeziza brassicae* have been observed during light leaf spot epidemics on winter oilseed rape in the UK, but the role of each spore type in the development of epidemics is not fully understood (Gilles *et al.*, 2000a). The occurrence of both mating types of *P. brassicae* and the genetic variation between isolates suggests that sexual reproduction occurs frequently in the UK (Majer *et al.*, 1998; Ball *et al.*, 1990). In the autumn, mature apothecia of *P. brassicae* have been observed on oilseed rape debris (Lacey *et al.*, 1987), indicating that ascospores may have a role in causing primary infections and initiating light leaf spot epidemics in newly sown winter oilseed rape crops. Ascospores have been identified as the primary inoculum for initiating epidemics of fungal diseases such as white leaf spot (*Mycosphaerella capsellae*) on winter oilseed rape (Inman *et al.*, 1999), ascochyta blight (*Didymella rabiei*) on chickpeas (Trapero-Casas *et al.*, 1996), septoria leaf blotch (*Mycosphaerella graminicola*) on winter wheat (Scott *et al.*, 1988), powdery mildew (*Uncinula necator*) on grapes (Pearson & Gadoury, 1987) and neck rot (*Sclerotinia squamosa*) on onions (Ellerbrock & Lorbeer, 1977). However, little work has been done to monitor numbers of air-borne *P. brassicae* ascospores in relation to the onset of light leaf spot epidemics in UK winter oilseed rape crops in the autumn and winter.

Large numbers of air-borne *P. brassicae* ascospores have been observed above UK winter oilseed rape crops in spring and summer at times when incidence of light leaf spot on stems was increasing (McCartney & Lacey, 1990). Since numbers of air-borne ascospores in spring were related to numbers of *P. brassicae* apothecia on leaf debris on the ground underneath crops (McCartney *et al.*, 1986), it was suggested that the debris was the source of these ascospores. The development of apothecia is affected by both temperature and wetness of the debris (Gilles & Fitt, 1999). Under a crop canopy there may be spatial variation in humidity, wetness of debris and other factors, which may affect development of apothecia and production of ascospores at different locations. However, the factors affecting the relationship between the occurrence of *P. brassicae* ascospores in spring and progress of light leaf spot epidemics on leaves, stems and pods are unclear.

A study of changes in the spatial pattern of light leaf spot with time has provided evidence that polycyclic light leaf spot epidemics initiated by air-borne ascospores are subsequently spread by splash-dispersed conidia (Evans *et al.*, 1999b). Early in the season, the distribution of light leaf spot was random, suggesting that lesions were initiated by air-borne ascospores; whereas subsequently it was aggregated, suggesting that secondary spread was over short distances by splash-dispersed conidia. Conidia are produced in large numbers in acervuli on leaves during the course of epidemics in the autumn and winter period (Rawlinson *et al.*, 1978). Ascospores and conidia of *P. brassicae* are very similar in appearance (Rawlinson *et al.*, 1978), but their relative effectiveness as inoculum for infecting oilseed rape leaves has not been fully investigated. There are considered to be two potentially damaging phases in light leaf spot epidemics on winter oilseed rape in the UK (Fitt *et al.*, 1998a; Gilles *et al.*, 2000a). Firstly, severe light leaf spot may kill leaves and even plants during the winter. Secondly, severe light leaf spot on pods in spring may greatly decrease yields. However, there is a need to investigate the importance of *P. brassicae* ascospores and conidia in these two phases of epidemics. This paper presents results of field and controlled environment experiments to investigate factors affecting the roles of ascospores and conidia in the development of light leaf spot epidemics in UK winter oilseed rape crops.

## Materials and methods

### *Release of ascospores and the onset of epidemics in autumn/winter*

In July 1998 and July 1999, after harvest at Rothamsted, debris of oilseed rape (cvs Bristol and Capitol) stems and pods affected by light leaf was baled and stored in a farm shed under dry conditions. This debris was used both to study the production of ascospores in the autumn and to inoculate the new winter oilseed rape experiments sown in the autumns of 1998 and 1999. To study ascospore production, samples of the oilseed rape debris were placed outdoors in trays around a 7-day recording volumetric spore sampler (Burkard Scientific Limited, Uxbridge, UK) (Hirst, 1952) on 23 September 1998 and 26 August 1999. Six trays (75 cm × 44 cm) filled with oilseed rape stem and pod debris were placed up to a distance of c. 1 m around the spore sampler, which was operated until 12 January in both seasons. Data for daily average temperature and rainfall were obtained from the Rothamsted meteorological station, which was located at a distance of 0.5 km from the experiments.

The spore sampler contained a rotating drum over which Melinex tape was wound. The tape was coated with a thin layer of wax by rotating the drum through a 5% solution of wax in hexane (w/v). The drum rotated once in 7 days and air-borne spores were drawn through an orifice of width ( $W$ ) 14 mm by a pump with an airflow rate ( $F$ ) of 10 l min<sup>-1</sup> so that they impacted onto the tape. The tape was replaced with a newly waxed tape every 7 days at 10.00 GMT. The exposed tape was cut into seven pieces, each containing the spores collected from 10.00 GMT until 10.00 GMT of the next day, and these pieces were mounted on microscope slides and stained with trypan blue in lactophenol. The trypan blue in lactophenol was prepared by dissolving 20 g phenol (Fisons Scientific Limited, Loughborough, UK) in 16 ml lactic acid (Fisher Scientific UK, Loughborough, UK) and 31 ml glycerol (BDH Laboratory Supplies, Poole, UK) and adding 0.16 g trypan blue (Sigma-Aldrich Company Limited, Poole, UK) dissolved in 20 ml distilled water. The slides were examined with a light microscope at 400× magnification by traversing each slide once along its length (corresponding to a 24 h sample) and counting the number of ascospores ( $N_c$ ). At this magnification, a microscope field width ( $f$ ) of 440 μm was observed. The daily average number of air-borne ascospores per m<sup>3</sup> of air ( $N$ ) was calculated with the equation

$$N = N_c \cdot \frac{W}{f} \cdot \frac{10^6}{1440F}. \text{ A slide with } P. \textit{ brassicae} \text{ ascospores, which had been released from } P.$$

*brassicae* apothecia, was used as a reference. Ascospores of *Unguicularia* cfr. *raripila*, a saprophytic fungus on oilseed rape debris, are almost identical to the ascospores of *P. brassicae* (Inman *et al.*, 1992); photographs of the ascospores of both species (Figures 4 and 5 in Inman *et al.*, 1992) were used to help to identify of *P. brassicae* ascospores.

Progress of light leaf spot (% plants affected) on winter oilseed rape was studied on cvs Bristol and Capitol during 1998/99 (Steed *et al.*, 1999) and on cv. Apex during 1999/2000. In 1998/99, the oilseed rape was sown on 26 August and the plants had emerged by 15 September. The experiment (Electronic Rothamsted Archive code 99/R/RAW/3) consisted of 60 sub-plots (3 m × 15 m) arranged in three randomised blocks, each with ten main plots (fungicide treatments) with cultivars as sub-plots. The crop was inoculated on 3 November with c. 5 kg per sub-plot of oilseed rape stem debris (cvs Bristol and Capitol) obtained after harvest in July 1998. Plots were treated with a number of different autumn and spring fungicide treatments, with tebuconazole applied as Folicur, or were untreated. In 1999/2000, the crop was sown on 27 August and the plants had emerged by 3 September. The experiment (code 00/R/RAW/4) consisted of 63 plots (3 m × 20 m), arranged in three randomised blocks. On 12 October, the crop was inoculated with c. 2 kg per plot of stem debris, obtained after harvest of winter oilseed rape (cvs Bristol and Capitol) in July 1999. Plots were treated with a

range of different autumn and spring fungicide (tebuconazole) sprays or were untreated. Incidence of light leaf spot (% plants with leaves affected) was assessed in the crops at c. monthly intervals from October/November onwards in both seasons. Samples of ten plants per plot were taken and incubated for 3-5 days in polyethylene bags at 9-14°C (Fitt *et al.*, 1998a) to stimulate production of conidia and facilitate assessment of light leaf spot. Since the presence on leaves of conidia in minute white pustules is the first symptom of light leaf spot, before lesions appear (Gilles *et al.*, 2000b), the disease assessments reflected the occurrence of *P. brassicae* conidia during epidemics.

#### *Air-borne ascospores and disease progress (conidial production)*

In the 1991/92 experiment (code 92/R/RAW/6), oilseed rape (cvs Capricorn and Falcon) was sown on 6 September and a light leaf spot epidemic developed naturally on this crop. The % plants with leaves, stems or pods affected by light leaf spot was assessed monthly in 48 plots. There were four randomised blocks, each with six main plots (fungicide treatments), with the two cultivars in sub-plots (3m x 21m). The fungicide treatments were autumn, winter, spring, or summer, or autumn plus spring plus summer applications and control plots were unsprayed. The summer sprays were with iprodione (as Rovral) and all other sprays were with prochloraz (as Sportak). In 1992/93 (code 93/R/RAW/5), oilseed rape (cv. Envoy) was sown on 26 August and the crop was inoculated on 16 October with stem debris, obtained after harvest of a crop affected by light leaf spot in the summer of 1992. The % plants with leaves, stems or pods affected by light leaf spot was assessed monthly in 66 plots (3m x 25m). Plots were untreated or treated with 21 different fungicide treatment regimes (either starting at the beginning of the season and stopping progressively later or starting progressively later and stopping at the end of the season) with a mixture of prochloraz (as Sportak 45) and iprodione plus thiophanate-methyl (as Compass). The % plants affected by light leaf spot reflected the occurrence of *P. brassicae* conidia during the epidemics, because conidia on all affected tissues (Rawlinson *et al.*, 1978). A 7-day recording volumetric spore sampler was used to estimate air-borne concentrations of *P. brassicae* ascospores in these winter oilseed rape crops from September to July in both 1991/92 and 1992/93. Daily average temperature and rainfall data were obtained from the Rothamsted meteorological station, which was situated at a distance of c. 1 km from both experiments.

#### *Occurrence of apothecia at different distances into a crop*

On 20 June 2000, petioles of senesced leaves were sampled at distances of 0.5, 1.0, 1.5 or 2.0 m into an uninoculated winter oilseed rape crop (cv. Apex) from the edge of a pathway at three locations which were known to have had severe light leaf spot on leaves earlier in the season. The petioles were sampled by placing a square metal frame (area 1 m<sup>2</sup>) in the crop and collecting all petioles from the ground within that frame. The number of petioles in each sample and the number of petioles with *P. brassicae* apothecia were counted. The data for numbers of petioles were transformed to natural logarithms to normalise the residuals. The transformed numbers of petioles and petioles with apothecia were regressed linearly on distance into the crop. Analysis of position and parallelism was done to determine if there were significant differences in slope or intercept between these two regression lines.

#### *Infectivities of conidia and ascospores*

##### Plant material

Oilseed rape (cv. Bristol) was sown in a glasshouse in compost (75% peat, 12% loam, 10% grit, 3% vermiculite; Petersfield Products, Cosby, Leicester) in pots (diameter 8 cm). After emergence (c. 5 days after sowing), the pots with seedlings (1 plant per plot) were transferred

to a controlled environment room (16°C, 80% relative humidity, 12 h light/12 h dark, 190  $\mu\text{E m}^{-2}\text{s}^{-1}$  light intensity at canopy level (supplied by fluorescent and tungsten lighting)). Pots were placed in trays (22 cm x 36.5cm, six pots per tray) that could be fitted with plastic covers. The plants were inoculated with either conidia or ascospores of *P. brassicae* after 3 weeks, when they had four or five true leaves (GS 1,4 to 1,5; Sylvester-Bradley & Makepeace, 1985).

#### Production of inoculum

Oilseed rape plants (cv. Bristol) at GS 1,4 to 1,5 were inoculated with a conidial suspension ( $0.5 \times 10^6$  conidia  $\text{ml}^{-1}$ ) of a *P. brassicae* isolate, obtained from light leaf spot lesions with sporulating *P. brassicae* on winter oilseed rape (cv. Bristol) grown at Rothamsted in 1997 and subsequently maintained on oilseed rape plants (cv. Bristol). Leaves were sprayed with conidial suspensions using an aerosol sprayer (Chrom Atomiser, Camlab, Cambridge) until drops ran off the leaf surfaces. Then the plants were covered with polyethylene bags for 24 h to ensure that infection occurred (Gilles *et al.*, 2000b). Leaves with light leaf spot lesions were taken from the plants after 2.5 weeks and shaken in distilled water to remove the conidia. A haemocytometer slide (Weber Scientific International Limited, Teddington, UK) was used to measure the concentration of conidia in the suspension, which was then diluted to produce conidial suspensions with  $10^5$ ,  $0.5 \times 10^5$ ,  $10^4$ ,  $0.5 \times 10^4$ ,  $10^3$ ,  $0.5 \times 10^3$ ,  $10^2$  or  $0.5 \times 10^2$  conidia  $\text{ml}^{-1}$ .

For the production of ascospores, plants (cv. Bristol) were inoculated with a conidial suspension, and leaves affected by light leaf spot were collected after 4.5 weeks (when they had senesced) and dried at 20°C for 1 day. The petioles of these leaves were wetted for 16 h in rain water and then placed in plastic boxes (12.5 cm x 8 cm), on three layers of filter paper (Whatman no. 1; Whatman International Limited, Maidstone, Kent, UK) wetted with 7 ml of rain water. The boxes were incubated at 13°C in continuous darkness for 16 days and sections of petioles with mature apothecia were collected and used as sources of ascospores.

#### Inoculation and assessment

For inoculation with either conidia or ascospores, six plants were placed in each tray. To estimate the number of spores deposited per unit leaf area, glass microscope slides were placed in between the plants at approximately the same height as the third and fourth leaves. The areas of the third and fourth true leaves were measured by holding a 2.1 cm x 2.1 cm grid on a transparent sheet over each leaf and counting the number of squares of the grid that were covered by the leaf. The inoculations with conidia were done by spraying in order of increasing concentration conidial suspensions onto the surfaces of the leaves with the aerosol sprayer. Each conidial suspension of a certain concentration was applied to the plants in a tray selected at random. The slides were removed and dried in air, and then the covers were fitted onto the trays for 48 h to ensure that leaf surfaces remained wet to favour infection. To inoculate leaves with ascospores, petioles with mature apothecia were attached with vaseline to the underside of the tray covers. The quantity of ascospore inoculum was varied by attaching different numbers of apothecia (from c. 5 to 100 apothecia) to the underside of the covers of the different trays, selected at random. The covers with attached apothecia were placed with a gap of 2 cm between the trays and the cover to allow air circulation and maintain a low humidity for 24 h and thereby stimulate ascospore release through natural drying of the petioles. After 24 h, petioles with apothecia and glass slides were removed, leaves were sprayed with distilled water and covers were fitted onto the trays to maintain leaf wetness for 48 h., as for conidial inoculation.

The microscope slides were stained with trypan blue before examination with a light microscope. The numbers of conidia or ascospores deposited were counted on an area of c.

350 mm<sup>2</sup> on each microscope slide and spore numbers per cm<sup>2</sup> calculated. The estimates of spore deposits were then multiplied by leaf areas, to estimate the number of spores deposited per leaf during inoculation. Two weeks after inoculation, the numbers of 'lesions' per leaf were counted on the third and fourth leaves. The 'lesions' were small concentric areas on the leaf surface in which white acervular conidiomata were visible (see Figure 1a in Gilles *et al.*, 2000b). This experiment was done twice. The numbers of 'lesions' per leaf (log<sub>10</sub>-transformed) were regressed on the numbers of spores deposited onto a leaf (log<sub>10</sub>-transformed) for both ascospores or conidia in each experiment. Analyses of position and parallelism were done to investigate whether the slopes and intercepts of the linear regression lines for number of 'lesions' per leaf on number of spores per leaf differed between conidia and ascospores or between experiments 1 and 2.

#### *Observations on conidia and ascospores of P. brassicae*

Conidia or ascospores were produced, the spore-forming structures, and the distributions of lesions on leaves inoculated with conidia or ascospores were examined. Thin sections were made through acervular conidiomata on infected oilseed rape leaves, mounted on microscope slides and stained with trypan blue in lactophenol. Conidia were inoculated onto leaf surfaces, then epidermal strips were obtained and mounted onto microscope slides stained with trypan blue in lactophenol and examined. Senesced oilseed rape leaf petioles with apothecia were collected from a winter oilseed rape crop in spring and examined. Air-borne ascospores, which were collected on Melinex tape by a spore sampler during the 1999/2000 season, were mounted on slides stained with trypan blue in lactophenol and examined.

The spatial patterns of lesions on oilseed rape leaves (cv. Bristol) were studied during the experiment in which leaves were inoculated with either conidia by spraying (to represent the deposition of conidia onto leaves by splash-dispersal) or ascospores by ejecting ascospores from apothecia (to represent the deposition of air-borne ascospores onto leaves under natural conditions). The total number of lesions, the number of lesions that were on a leaf vein, the number of lesions that were in the lower third of a leaf (the part of the leaf nearest to the petiole) and the number of lesions that were on the edge of a leaf were counted. From these counts, the proportions of lesions on a vein, on the lower third of a leaf and on the edge of a leaf were calculated for each spore type. A Student's t-test was done to investigate whether there were differences between conidial and ascospore inoculum in the proportions of lesions on different areas of a leaf.

## Results

### *Release of ascospores and the onset of epidemics in autumn/winter*

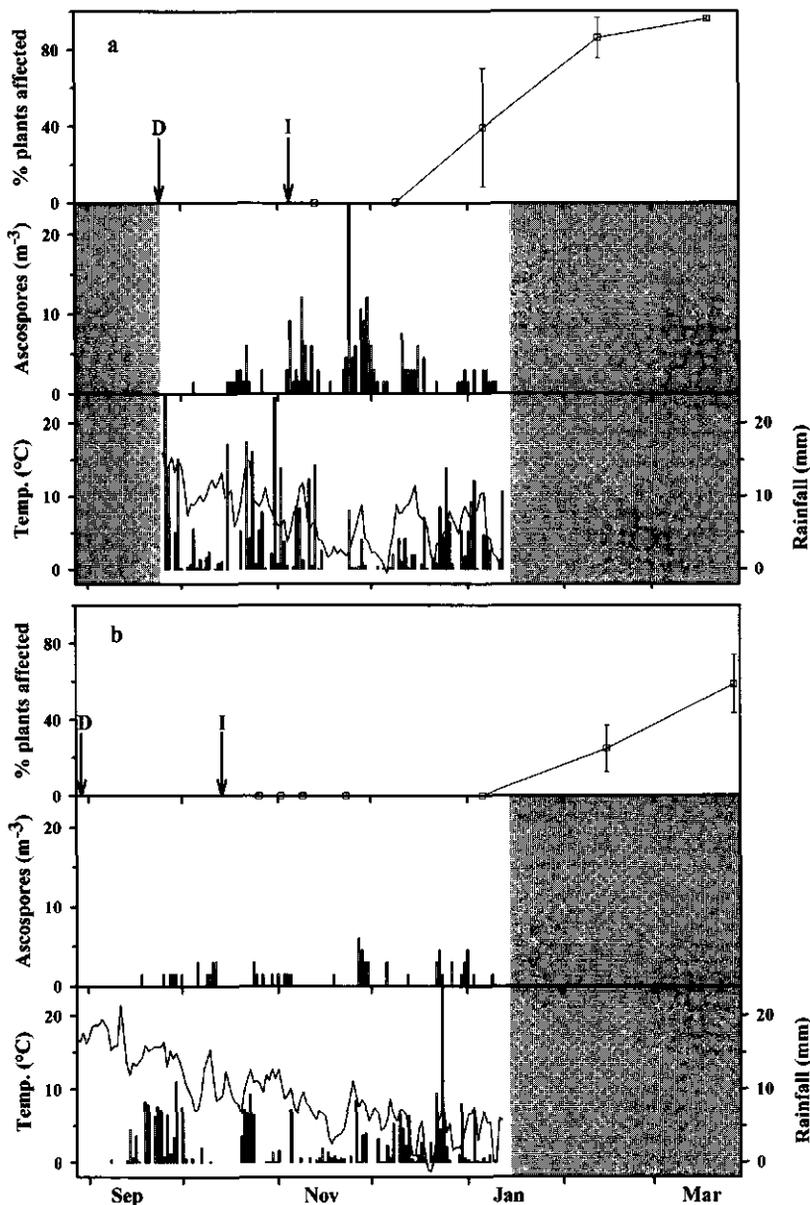
In 1998/99, air-borne ascospores were first collected on 5 October, 12 days after the oilseed rape debris had been placed outdoors (Figure 1a;). The numbers of ascospores tended to increase until 24 November (25 ascospores  $m^{-3}$ ) and then to decline. Light leaf spot (i.e. *P. brassicae* conidia) was first observed on leaves of winter oilseed rape (cvs Bristol and Capitol), inoculated with the debris from the same source on 3 November, on 6 January 1999 and increased to >80% plants affected between January and March 1999. In the period when air-borne ascospores were monitored, the highest daily average temperature (16.2°C) was on 24 September and the lowest temperature (-0.5°C) was on 6 December. Periods when rainfall was high were 26-30 September, 20 October - 13 November and 18 December - 14 January.

In 1999/2000, ascospores were first collected on 18 September, 23 days after debris was placed outdoors (Figure 1b). The numbers of ascospores collected were less in 1999/2000 (maximum 6 ascospores  $m^{-3}$ ) than in 1998/99. The first symptoms of light leaf spot (i.e. observations of *P. brassicae* conidia) on the winter oilseed rape (cv. Apex) inoculated with debris on 12 October were observed on 15 February 2000, but the % plants with light leaf spot had not reached to >60% by the end of March. Daily average temperatures ranged from 21.5°C on 11 September to -1.25°C on 20 December. Periods of high rainfall were 14 September - 1 October, 20-24 October and 8-25 December. Dry periods when little rainfall was observed were 26 August - 13 September and 2-19 October.

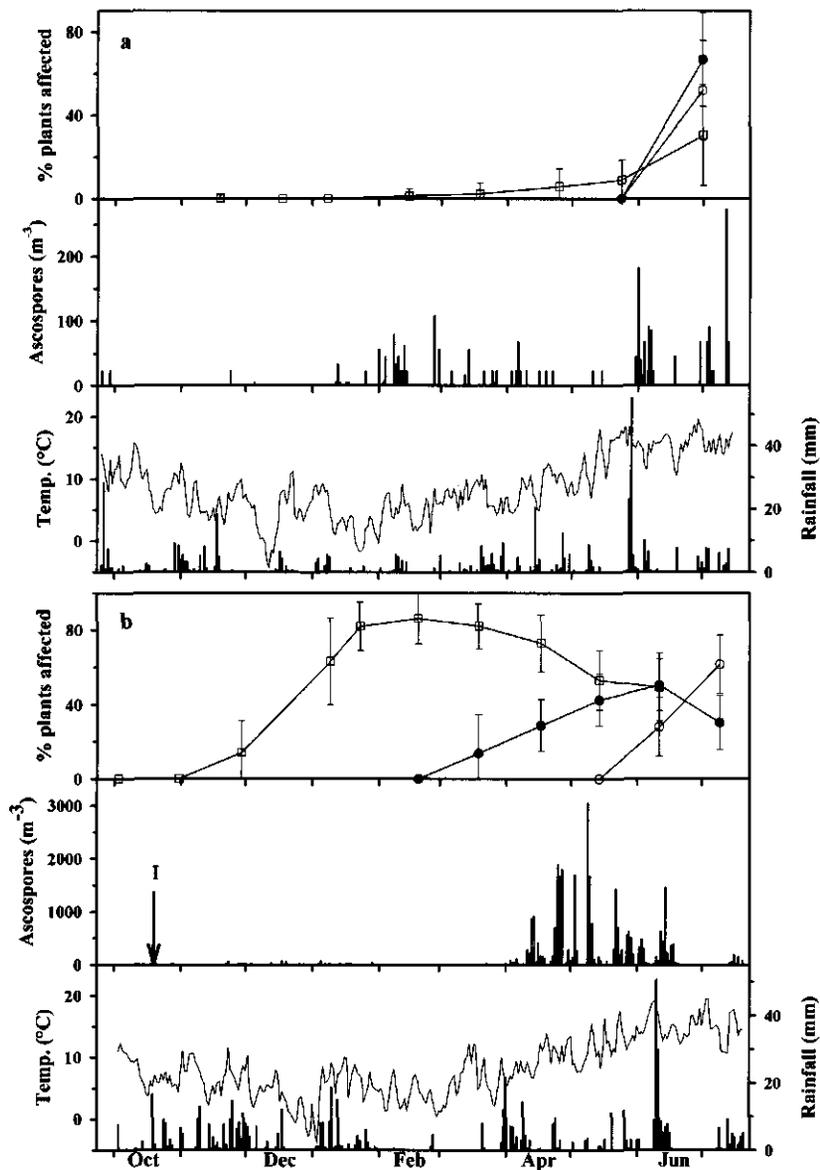
### *Air-borne ascospores and disease progress (conidial production)*

In 1991/92, ascospores of *P. brassicae* were collected in the naturally infected winter oilseed rape crop on occasions from September 1991 until July 1992 (Figure 2a). Few air-borne ascospores were collected on 24, 25 and 29 September, on 24 November and on 5 December. Ascospores were collected regularly during the period from 12 January to 22 April. From late June until mid-July, large numbers of ascospores (maximum 274 ascospores  $m^{-3}$ ) were collected frequently. A few plants with light leaf spot (*P. brassicae* conidia) on their leaves were first observed on 19 November, but none were observed on 18 December and 8 January. Plants with light leaf spot on leaves were observed on 15 February and the incidence of light leaf spot increased until the last assessment on 1 July 1992. The % plants with stems or pods affected by light leaf spot increased rapidly between 24 May and 1 July. There was most rain in the periods 24 -30 September, 29 October - 4 November, 22 - 30 March and 28 May - 6 June. Daily average temperatures generally decreased from mid-October (15.8°C on 10 October) to mid-December (-4.4°C on 12 December) and then increased in spring and summer (19.6°C on 29 June).

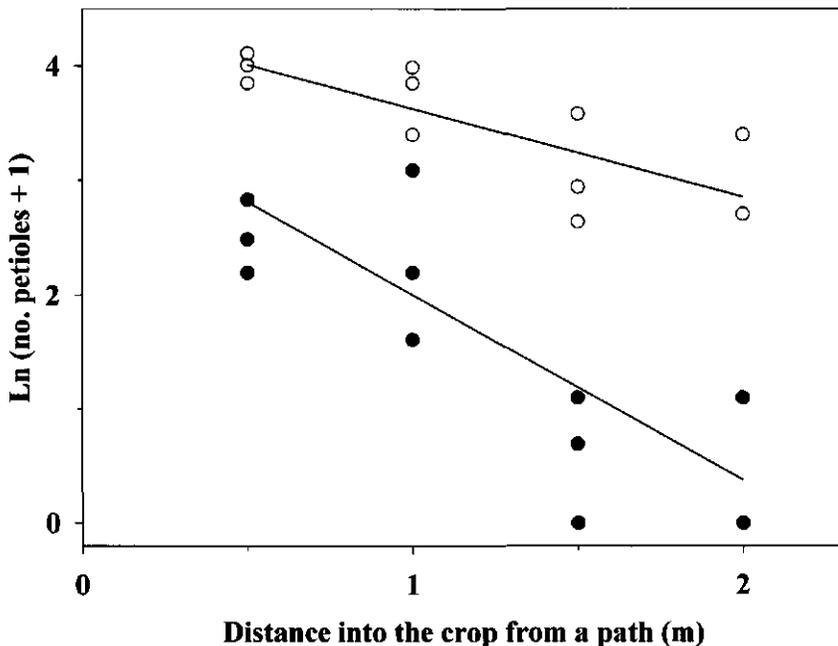
In 1992/93, when plots were inoculated on 16 October, air-borne ascospores were first collected on 28 October. Two distinct periods of ascospore release were observed. Small numbers of ascospores were collected regularly between 19 November and 29 January. Large numbers of air-borne ascospores were collected between 1 April and 20 June (maximum 3060 ascospores  $m^{-3}$ ). Light leaf spot symptoms were first observed on 29 November and the % plants with leaves affected then increased to >80% by February. Light leaf spot symptoms were first observed on stems on 19 March and on pods on 11 June and the % plants with light leaf spot on stems or pods increased until June or July, respectively. In 1992/93 there was more rainfall than in the previous season. Most rainfall occurred in the periods 19 October - 3 December, 5 - 27 January, 30 March - 12 April and 9 - 16 June. Daily average temperatures generally decreased from October (12.4°C on 4 October) to January (-3.9°C on 3 January) and then increased in spring and summer (19.5°C on 3 July).



**Figure 1** Progress with time in % plants with leaves affected by light leaf spot (producing *P. brassicae* conidia) in winter oilseed rape at Rothamsted in autumn/winter of 1998/99 (cvs Bristol and Capitol) (a) and 1999/2000 (cv. Apex) (b); plots had been inoculated with light leaf spot infected oilseed rape debris on 3 November 1998 or on 12 October 1999, respectively (arrows with 'I'). In each season, air-borne ascospores of *P. brassicae* were collected near a concentrated source of debris, which had been placed outdoors under natural conditions on 25 September 1998 or on 28 August 1999 (arrows with 'D'). The values for % plants with leaves affected by light leaf spot the means of 60 (1998/99) or 63 plots (1999/2000) ( $\square$ ) presented with their standard errors (vertical error bars). Data for daily average temperature (lines) and daily rainfall were obtained from a meteorological station at a distance of 0.5 km from the debris. In both seasons, the same sources of debris were used to inoculate the winter oilseed rape crops and to monitor air-borne *P. brassicae* ascospores.



**Figure 2** Progress with time in % plants with leaves (□), stems (●) or pods (○) affected by light leaf spot (producing *P. brassicae* conidia) in winter oilseed rape in 1991/92 (cvs Capricorn and Falcon) (a) and in 1992/93 (cv. Envol) (b). The values are means of 48 (1991/92) or 66 (1992/93) plots presented with their standard errors (vertical error bars). Concentrations of air-borne ascospores within these crops are presented in relation to the daily average temperature (line) and daily rainfall. In 1991/92, a natural light leaf spot epidemic occurred on the crop; in 1992/93, the crop was inoculated with infected oilseed rape debris on 16 October 1992.



**Figure 3** The number of senesced petioles  $m^{-2}$  (○) and the number of senesced petioles with apothecia (●) at different distances into a winter oilseed rape (cv. Apex) crop from a pathway at sites of three patches affected with light leaf spot within the crop at Rothamsted on 20 June 2000. The numbers of petioles ( $\ln$  transformed) were regressed on distance into the crop ( $d$ ) (lines), for both the total number of petioles ( $p$ ) ( $\ln(p + 1) = 4.39 - 0.77d$ ) and the number of petioles with apothecia ( $p_a$ ) ( $\ln(p_a + 1) = 3.63 - 1.62d$ ). Both lines accounted for 85.4% of the variance.

#### *Occurrence of apothecia at different distances into a crop*

The density of petioles underneath a crop decreased from, on average, c. 53 petioles  $m^{-2}$  to c. 19 petioles  $m^{-2}$  as the distance into the crop from the edge of a pathway increased from 0.5 to 2.0 m (Figure 3). The number of petioles with apothecia decreased from, on average, c. 12 petioles with apothecia  $m^{-2}$  to less than 1 petiole with apothecia  $m^{-2}$  when the distance into the crop increased from 0.5 to 2.0 m. The decreases with distance in numbers of petioles and numbers of petioles with apothecia were fitted by a negative exponential equation, since linear regressions of  $\ln$ (numbers of petioles) on distance accounted for 85% of the variance. Analysis of position and parallelism suggested that the rate of decrease with distance into the crop in the number of petioles with apothecia was greater than the rate of decrease in total number of petioles ( $P = 0.033$ ).

### *Infectivities of conidia and ascospores*

In both experiments 1 and 2, the ranges of densities of ascospores per leaf obtained by exposing the leaves to different numbers of apothecia were small, and two distinct groups of ascospore data were obtained (Figure 4). For conidia, a wide range of different spore densities per leaf was obtained in both experiments. However, analysis of position and parallelism found no evidence for differences between experiments 1 and 2. Therefore, it was assumed that there was no experiment effect for the data for ascospores. The transformed values for number of 'lesions' per leaf ( $l$ ) ( $\log_{10}(l + 1)$ ) were regressed on the transformed values for number of spores per leaf ( $s$ ) ( $\log_{10}(s + 1)$ ). Analysis of position and parallelism suggested that there was no difference in the slope between the regression lines for conidia and ascospores, but that the values of the intercept differed between them. A small positive value of the constant (0.38), obtained after fitting the regression line to the data for ascospores, suggests that infections might occur in the absence of spores. Therefore, the regression line for ascospores was forced through the origin on the logarithmic scale ( $\log_{10}(l+1) = 0$  when  $l = 0$ ) by omitting the constant. The regression lines fitted to describe the linear relationships between numbers of 'lesions' on a leaf and numbers of spores applied to a leaf were, for conidia:

$$\log_{10}(l + 1) = -1.45 + 0.56 \log_{10}(s + 1)$$

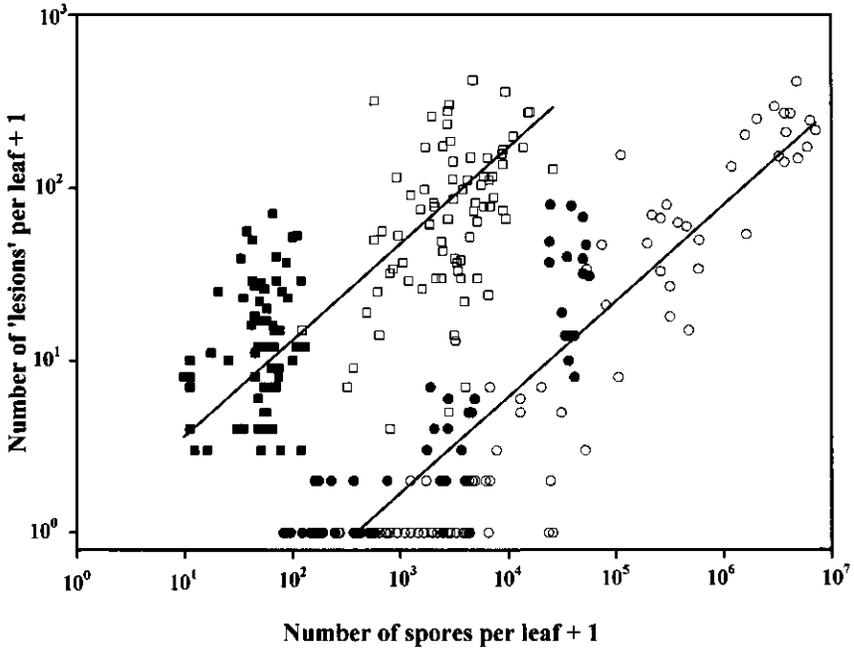
and for ascospores:

$$\log_{10}(l + 1) = 0.56 \log_{10}(s + 1)$$

These regression lines suggest that, on average, *c.* 400 times as many conidia as ascospores need to be applied to a leaf to cause the same number of 'lesions'.

### *Observations on conidia and ascospores of P. brassicae*

Conidia were produced in acervular conidiomata beneath the cuticle of living leaves (Figure 5a). The conidiomata ruptured through the cuticle to expose the conidia to rain for dispersal. The conidia were produced in small pustules and appeared to be adhering to each other; these pustules are the small white spots which are the diagnostic characteristic of *P. brassicae*. Ascospores were produced in discoid apothecia on senesced leaf petioles (Figure 5b). Conidia (Figure 5c) did not differ from ascospores (Figure 5d) in their morphology. Both spore types are cylindrical, 0-1 septate, with length *c.* 8-15  $\mu\text{m}$  and width *c.* 2-3  $\mu\text{m}$ . 'Lesions' caused by conidia were often distributed along the veins of a leaf (Figure 5e), whereas 'lesions' caused by ascospores were more randomly distributed across a leaf surface (Table 1; Figure 5f). There were no differences between ascospores and conidia in the distribution of 'lesions' they caused on the lower third of a leaf or on the edge of a leaf.



**Figure 4** The number of observed 'lesions' per leaf ( $\log_{10}$ -transformed) in relation to the number of ascospores (■, □) or conidia (○, ●) applied per leaf ( $\log_{10}$ -transformed) in experiments 1 (□, ○) and 2 (■, ●). Linear regression of numbers of 'lesions' per leaf ( $l$ ) on numbers of spores per leaf ( $s$ ) (lines) fitted data for both ascospores ( $\log_{10}(l+1) = 0.56 \log_{10}(s+1)$ ) and conidia ( $\log_{10}(l+1) = -1.45 + 0.56 \log_{10}(s+1)$ ). Both regression lines accounted for 78.2% of the variance.

**Table 1.** Fraction of lesions caused by *P. brassicae* ascospores or conidia that were located on a leaf vein, at the lower third of a leaf or at the leaf edge after inoculations of oilseed rape (cv. Bristol) leaves with either conidia or ascospores. A Student's t-test was used to test for differences in the fraction of lesions at a specific location on a leaf between lesions caused by either ascospores or conidia.

Spore type	Fraction of lesions at a specific location on a leaf		
	Veins	Lower 1/3	Edge
Ascospores	0.25	0.30	0.13
Conidia	0.48	0.34	0.14
t-test	-4.88	-0.66	-0.24
probability	<0.001	0.51	0.81

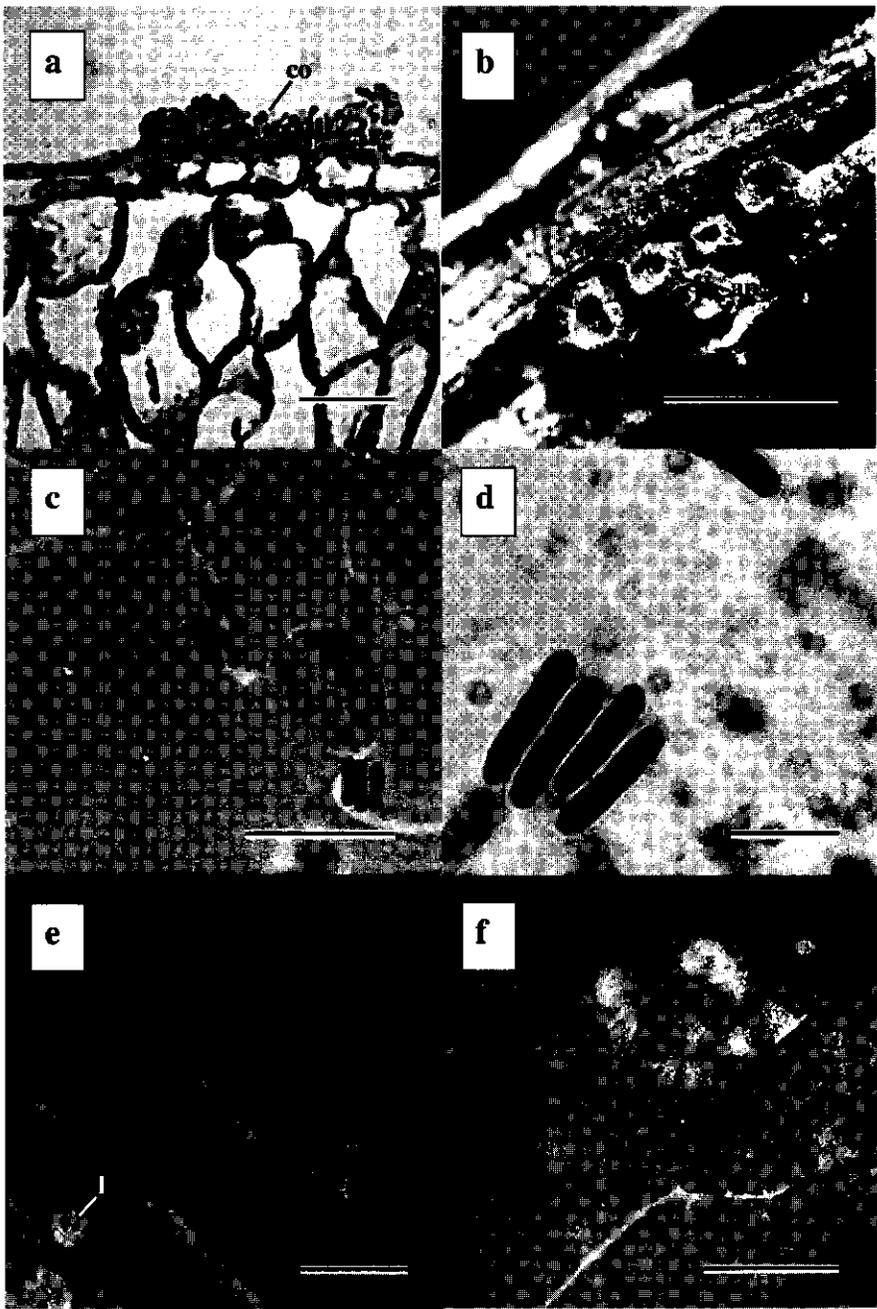
## Discussion

These results confirm that both ascospores and conidia of *P. brassicae* contribute to progress of light leaf spot epidemics on winter oilseed rape in the UK and that air-borne ascospores produced on stem and pod debris from previously affected crops play an important role in initiating light leaf spot epidemics in autumn (Table 2). The occurrence of air-borne ascospores both near infected oilseed rape debris and within winter oilseed rape crops in autumn, together with observations of mature apothecia on such debris, suggest that the primary infections are likely to be caused by ascospores. Ascospores are wind-dispersed (McCartney *et al.*, 1986), and our results demonstrate they are highly infective to oilseed rape leaves, and hence small numbers of ascospores may cause infections in oilseed rape crops at a considerable distance from their source. By contrast, conidia only travel short distances by splash dispersal (Fatemi & Fitt, 1983) and are less infective, despite their morphology being similar to that of *P. brassicae* ascospores. The random distribution of light leaf spot lesions in crops at the start of the season (Evans *et al.*, 1999b) provides further support for the conclusion that they are initiated by wind-dispersed ascospores. The role of ascospores released from previous crop debris in initiating light leaf spot epidemics may explain why forecasts of light leaf spot severity in the new season can be based on survey data for the occurrence of the disease at the end of the previous season (Gilles *et al.*, 2000a).

The release of *P. brassicae* ascospores soon after stored-dry debris was exposed to natural conditions may explain why light leaf spot epidemics are generally more serious in Scotland than in southern England (Gilles *et al.*, 2000a). There is evidence that *P. brassicae* apothecia mature and release ascospores within a few weeks at summer/autumn temperatures, providing the debris remains wet (Gilles & Fitt, 1999). In southern England there may be an interval of 2 months between harvest in July and emergence of the new crop in September and it seems likely that the main period of *P. brassicae* ascospore release occurs in August before emergence, unless it is delayed by dry weather. However, in Scotland crops are harvested later and planted earlier, so the emergence of new crops may coincide with the main period of ascospore release.

These experiments provide evidence that the splash-dispersed conidia of *P. brassicae* are important in the secondary spread of light leaf spot during the autumn and winter. White pustules of conidia may be observed on oilseed rape leaves as early in the season as November (e.g. in 1992). The change in the distribution of light leaf spot from a random to an aggregated pattern provided support for the role of conidia, dispersed over short distances by splash, in secondary disease spread (Evans *et al.*, 1999b). When cycles of conidial dispersal, infection and sporulation occur rapidly in relation to plant growth then light leaf spot can cause stunting and death of plants in the winter (Fitt *et al.*, 1998a).

The relative importance of ascospores and conidia in the spread of light leaf spot from leaves to stems and pods after stem extension in spring is less clear. Large numbers of air-borne ascospores were collected in spring and early summer and are thought to be released from apothecia on leaf debris underneath the crops (McCartney & Lacey, 1990). The incidence of stem and pod disease in the spring of 1992 and the incidence of pod disease in the spring of 1993 increased after large numbers of air-borne ascospores were observed, suggesting that the release of ascospores from leaf debris in spring could contribute to stem and pod infections. However, in 1992/93, stem lesions were observed on 19 March after a c. 7-week period when no air-borne ascospores were collected, but when conidia were produced in large numbers on leaves because of the high disease incidence. This suggests that conidia, which are moved up the crop canopy by rain-splash (Pielaat, 2000), also contribute to stem and pod disease.



**Figure 5** Section through an oilseed rape leaf infected with *P. brassicae* illustrating the production of conidia (co) in an acervulus (a, scale bar is 200  $\mu$ m). Mature apothecia (ap) in which ascospores are produced, on a senesced oilseed rape leaf petiole (b, scale bar 1 mm). Conidia on an oilseed rape leaf surface (c, scale bar 50  $\mu$ m). Air-borne ascospores, which were collected on a Melinex tape with a spore sampler (d, scale bar 10  $\mu$ m). Lesions (l) on oilseed rape (cv. Bristol) leaves caused by conidia (e) or ascospores (f, scale bars both 20  $\mu$ m).

**Table 2.** Comparison between ascospores and conidia of *Pyrenopeziza brassicae* (light leaf spot): morphology, production, dispersal, infectivity and role in epidemics.

Factor	Ascospores	Conidia
Morphology	hyaline, cylindrical, septate, 13.5-15.5 x 2.5-3.0µm	hyaline, cylindrical, septate 10-16 x 3-4µm
Period of production (UK)	Maximum in spring; also in summer/autumn	Maximum in winter and early spring
Host tissue	Dead leaves (spring) Dead stems/pods (summer)	Living leaves, stems and pods
Fungal structure	Apothecia	Conidiomata (spores in sticky white mass)
Mode of dispersal	Wind-dispersal	Rain splash dispersal
Infectivity	very infective	not as infective
Role in epidemics	Primary inoculum? spread of disease to pods?	Secondary inoculum

The observation that numbers of apothecia on leaf debris underneath crops in spring decreased with increasing distance into the crop suggests that production of ascospores in spring may require specific conditions. The decrease in numbers of apothecia was, in part, the result of a decrease in number of petioles with increasing distance into the crop, which may have occurred because leaf petioles decayed more quickly when they remained wet for longer within the crop. The further decrease in the number of apothecia which matured on debris within the crop may have occurred because growth of competing saprophytic fungi and bacteria on this debris was greater than on the debris near pathways which experienced more wetting and drying. Differences between seasons in these environmental factors influencing maturation of apothecia and production of *P. brassicae* ascospores may account for the variation between seasons in the severity of light leaf spot epidemics on oilseed rape pods.

### Acknowledgements

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## Chapter 4

### Development of *Pyrenopeziza brassicae* apothecia on oilseed rape debris and agar

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#### Abstract

The development of apothecia of *Pyrenopeziza brassicae* (anamorph = *Cylindrosporium concentricum*) on oilseed rape debris and on compost malt agar was observed by scanning electron and light microscopy. On oilseed rape debris, apothecia of *P. brassicae* developed directly underneath the epidermis as small globular structures of dense mycelium, which protruded through the epidermis when they increased in size. The apices of erumpent immature apothecia depressed and then the disks expanded to expose the hymenia of mature apothecia containing dome-shaped asci interspersed by filiform paraphyses. On compost malt agar, *P. brassicae* hyphae of one mating type grew towards hyphae of the opposite mating type 3 to 4 days after inoculation of conidia onto agar surfaces. The subsequent development of apothecia on compost malt agar was similar to the development on oilseed rape debris.

## Introduction

Apothecia of *Pyrenopeziza brassicae* Sutton & Rawlinson, a discomycetous ascomycete which is the cause of light leaf spot on winter oilseed rape, were first observed on agar containing cabbage extract, and described as black sclerotium-like structures, by Thomson (1936) and Cabral (1940). These structures were later referred to as immature apothecia (Hickman, Schofield & Taylor 1955). *P. brassicae*, the teleomorph of *Cylindrosporium concentricum* Grev. (Greville 1823), was formally described from apothecia produced on 2% malt agar (Rawlinson, Sutton & Muthyalu 1978). The apothecia ranged in diameter from c. 0.2 to 0.8 mm, and the cylindrical ascospores ranged in length from 12.5 to 18.5  $\mu\text{m}$  and width from 2.5 to 3  $\mu\text{m}$ . The first reported observation of *P. brassicae* apothecia under natural conditions was in Ireland on debris of vegetable brassicas (Staunton & Kavanagh 1966). The apothecia were also observed on debris of vegetable brassicas in New Zealand (Cheah, Hartill & Corbin 1980; Cheah & Hartill 1985). The teleomorph was first reported on oilseed rape debris in the UK by Lacey, Rawlinson & McCartney (1987). Small apothecia were observed on the leaf lamina and ranged in diameter from 0.03 to 0.1 mm, and large apothecia were observed on leaf veins and petioles and ranged in diameter from 0.13 to 0.58 mm. The length of the ascospores released from these apothecia ranged from 7.5 to 18.8  $\mu\text{m}$  and their width ranged from 1.8 to 3.7  $\mu\text{m}$ . Small and large apothecia developed after more than 15 and 24 days, respectively, on leaves of a winter oilseed rape crop in April-May 1987 when average daily temperatures varied between 5.5 to 14.6°C (McCartney & Lacey 1990).

Molecular and genetic studies have provided evidence to indicate how the sexual development of *P. brassicae* occurs. *P. brassicae* is heterothallic and two mating types have been identified (Ilott, Ingram & Rawlinson 1984). Experiments on agar showed that these two mating types needed to interact within 7 days after conidial germination to initiate sexual development (Siddiq 1989; Ashby 1997). However, little is known about how the early development of apothecia on agar relates to development on oilseed rape debris. The mating type locus of *P. brassicae* was cloned and sequenced and comprises a single locus with two idiomorphs *MAT-1* and *MAT-2* (Singh & Ashby 1997; Singh & Ashby 1999). One of the genes present within the *MAT-1* idiomorph (*MAT-1-4*) encodes a putative metallothionein which may function as an environmental sensor *in planta*, recognising the onset of senescence and stimulating the fungus to proceed with sexual development (Singh & Ashby 1998; Singh, Dyer & Ashby 1999). The description of mature apothecia of *P. brassicae* on agar mentions the presence of an ectal excipulum consisting of *textura angularis* and a hymenium (Rawlinson *et al.* 1978). The hymenium, which contained asci and paraphyses, was said to be initially covered by a pseudo-epithecium, which later detached. Apothecial development has been described for other discomycete species (Corner 1929a & 1929b; Jayachandran, Willetts & Bullock 1987; O'Donnell, Fields & Hooper 1974; O'Donnell & Hooper 1978; Meyer & Luttrell 1986) but few descriptions of apothecial development *in planta* exist for fungi within the Pseudopezizeae, which includes *Pyrenopeziza*. Apothecial development of *Pseudopeziza trifolii* f.sp. *medicaginis-sativae* was initiated on masses of loosely woven vegetative hyphae near the centre of lesions on lucerne leaflets; apothecia developed within the leaflet tissues and were shown to be erumpent when mature (Meyer & Luttrell 1986).

There is evidence that *P. brassicae* ascospores could contribute to development of light leaf spot epidemics on winter oilseed rape in northern Europe. Genetic variation between field isolates of *P. brassicae* collected in the UK and France (Majer, Lewis & Mithen 1998) and observations of apothecia on oilseed rape debris in England (Lacey *et al.* 1987; McCartney & Lacey 1990) and Germany (Amelung & Daebeler 1991) suggest that sexual reproduction occurs frequently on oilseed rape debris throughout northern Europe. Mature apothecia, which released ascospores, were observed on stem debris from the previous winter oilseed rape crop in the autumn and on leaf debris from the current crop in spring. Ascospores were

more infective on oilseed rape leaves than conidia of *P. brassicae* (Gilles & Fitt 1999). The random spatial pattern of light leaf spot observed in winter oilseed rape crops at the start of epidemics in the autumn suggests that primary infections are caused by wind-borne ascospores released from apothecia on debris from previous crops (Evans *et al.* 1999b). The release of ascospores from apothecia on leaf debris under crop canopies in spring could contribute to upward spread of light leaf spot onto stems and pods. A detailed study of apothecial development both *in planta* and *in vitro* will improve understanding of the sexual development of *P. brassicae* in winter oilseed rape crops. Although mature apothecia of *P. brassicae* have been described by Rawlinson *et al.* (1978), the stages in development of these apothecia have not been described either *in planta* or *in vitro*. This paper presents a study of the development *P. brassicae* apothecia on oilseed rape debris and on agar by scanning electron and light microscopy.

## Materials and methods

### *P. brassicae* isolates

The single conidiospore isolates of *P. brassicae* JH26 (*MAT-1*) and PB23 (*MAT-2*), which were both isolated in 1983 from light leaf spot lesions on oilseed rape crops, at Ticknall, Derbyshire and in the ADAS northern region, respectively (Simons & Skidmore 1988), were used to study apothecial development on agar. These isolates were maintained on 3% MA (3% (w/v) malt extract (Oxoid), 1.2% (w/v) Difco Bacto Agar) at 18°C and were subcultured every 2 months. The interaction between the opposite mating types during the early stages of apothecial development on agar was studied by using the *P. brassicae* transformants JH26 pNOM 102/8 (*MAT-1*) and NH10 pNOM 102/18 (*MAT-2*). These transformants of isolates JH26 and NH10 constitutively expressed the  $\beta$ -glucuronidase (*GUS*) construct pNOM 102 (Roberts *et al.* 1989) and have been described by Ashby & Johnstone (1993). These transformed isolates were maintained on 3% MA with 16  $\mu\text{g ml}^{-1}$  hygromycin (Sigma). A mass mycelial isolate of *P. brassicae* (RES97), which was obtained from light leaf spot lesions with sporulating *P. brassicae* on leaves from a winter oilseed rape (cv. Bristol) crop at Rothamsted in 1997, was used to study apothecial development on leaf debris. This Rothamsted isolate was maintained on oilseed rape (cv. Bristol) plants by inoculating conidial suspensions at monthly intervals onto uninfected plants growing at 16°C. The isolate was sometimes stored for 2 to 3 weeks as a frozen conidial suspension at -20°C before use.

### Production of apothecia on agar and *GUS*-staining of *P. brassicae* isolates

A 1:1 mixture of conidial suspensions ( $10^6$  conidia  $\text{ml}^{-1}$ ) of the isolates JH26 and PB23 was inoculated onto a compost malt agar medium (CMM; Ashby & Johnstone 1993) or onto Melinex film (Agar Scientific Limited, Stansted, Essex, UK) covered with a thin layer of CMM (MFCMM) in Petri dishes. The inoculated Petri dishes were incubated at 16°C in continuous darkness. Thin squares (c. 7 mm  $\times$  7 mm  $\times$  2 mm) were cut from either CMM or MFCMM each day from 1 to 10 days and 15 and 46 days after inoculation and prepared for scanning electron microscopy (SEM). Reciprocal crosses on CMM between the field isolate JH26 and the transformant isolate NH10 pNOM102/18 and between the field isolate PB23 and the transformant isolate JH26 pNOM102/8 were stained after 4 days of incubation by using the substrate 5-bromo-4-chloro-3-indolyl  $\beta$ -D-glucuronide (X-gluc; Biosynth AG, Switzerland) (*GUS*-staining) and examined as described by Ashby & Johnstone (1993).

### *Production of apothecia on oilseed rape leaf debris*

Oilseed rape (cv. Bristol) plants were grown in trays in a controlled environment room built at Rothamsted (16°C, 12 h/12 h light/dark regime, 190  $\mu\text{E m}^{-2}\text{s}^{-1}$  light intensity at canopy level, fluorescent and tungsten lighting). After c. 3.5 weeks, the plants had developed four to five leaves (GS 1.4 to 1.5; Sylvester-Bradley & Makepeace 1985) and were inoculated with a conidial suspension ( $0.5 \times 10^6$  conidia  $\text{ml}^{-1}$ ) of isolate RES97. The conidial suspension was sprayed onto the plants with an aerosol sprayer (Chrom Atomiser, Camlab, Cambridge, UK) for c. 10 s until drops began to run off the leaves. The inoculated leaves were then enclosed in polyethylene bags for c. 24 h to maintain leaf wetness to ensure that infection by *P. brassicae* occurred (Gilles *et al.* 2000b). After inoculation, the plants were kept under the same conditions until 4.5 weeks after inoculation, when the infected leaves had senesced and were collected and dried at room temperature for 1 day. After 2 days, the dry senesced leaves were wetted for 16 h with rain water and placed in plastic boxes (12.5 cm  $\times$  8 cm) on three layers of filter-paper (Whatman no. 1, Whatman International Limited, Maidstone, Kent, UK) which had been wetted with 7 ml of rain water. These boxes were incubated in a controlled environment room at 13°C in continuous darkness and samples were taken every 2 to 3 days to observe apothecial development. After harvest (on 23 July 1997) of a winter oilseed rape (cv. Bristol) crop at Rothamsted, which was severely affected by light leaf spot, samples of stem debris were collected between 11 and 15 August 1997. Leaf petioles of senesced leaves were collected on 24 May 2000 from the ground under a winter oilseed rape (cv. Apex) crop at Rothamsted, which was affected by light leaf spot. These samples were examined microscopically for the presence of *P. brassicae* apothecia.

### *Microscopy and photography*

The samples cut from colonies of *P. brassicae* on agar were fixed with 2.5% glutaraldehyde in 50 mM PIPES buffer at pH 7.0 for c. 60 min, washed twice with the same buffer (15 min for each wash), post-fixed with 1%  $\text{OsO}_4$  in 50 mM PIPES buffer pH 7.0 for 60 min and washed with reverse osmosis water for 5 min. The samples were dehydrated by using a graded acetone series until they were in 100% acetone and then dried by critical point drying with liquid  $\text{CO}_2$ . The samples were then attached to aluminium stubs, which were treated with colloidal silver and sputter coated with 30 nm particles of gold-palladium (80-20) before observations were made with a JEOL 35 CA Scanning Electron Microscope (Multi Imaging Centre, Cambridge).

A Wild binocular dissecting microscope (Leica Microsystems Inc., Heerbrugg, Switzerland) with a Olympus OM-2 camera (Olympus Optical Company Limited, London, UK) attached to it was used to photograph *P. brassicae* apothecia on debris at different stages of their development on Ilford FP4 plus 125 DX 135 mm black & white film (Ilford Limited, Moberley, Cheshire, UK). For SEM, sections of debris with *P. brassicae* apothecia were attached to aluminium stubs (10 mm in diameter, 3 mm thick) with Tissue-Tek (Sakura Finetek, Torrance, California, USA) and secured into a stub holder. The stub holder with the sample was attached to a vacuum transfer device and this device was then secured onto a chamber containing liquid nitrogen which was part of a CT1500 cryo-transfer system (Oxford Instruments, Eynsham, Oxfordshire, UK). The stub holder was held in the vapour just above the liquid nitrogen for c. 30 s before the air in the chamber was pumped out to produce a vacuum. When the liquid nitrogen had begun to solidify, the tip of the stub holder was held in the solidifying nitrogen. As soon as the nitrogen had solidified, the stub holder was taken out of the nitrogen and enclosed under vacuum in the inner chamber of the vacuum transfer device. This device was then disconnected from the chamber containing the nitrogen and connected onto the preparation chamber where the sample was coated with 15 nm of gold

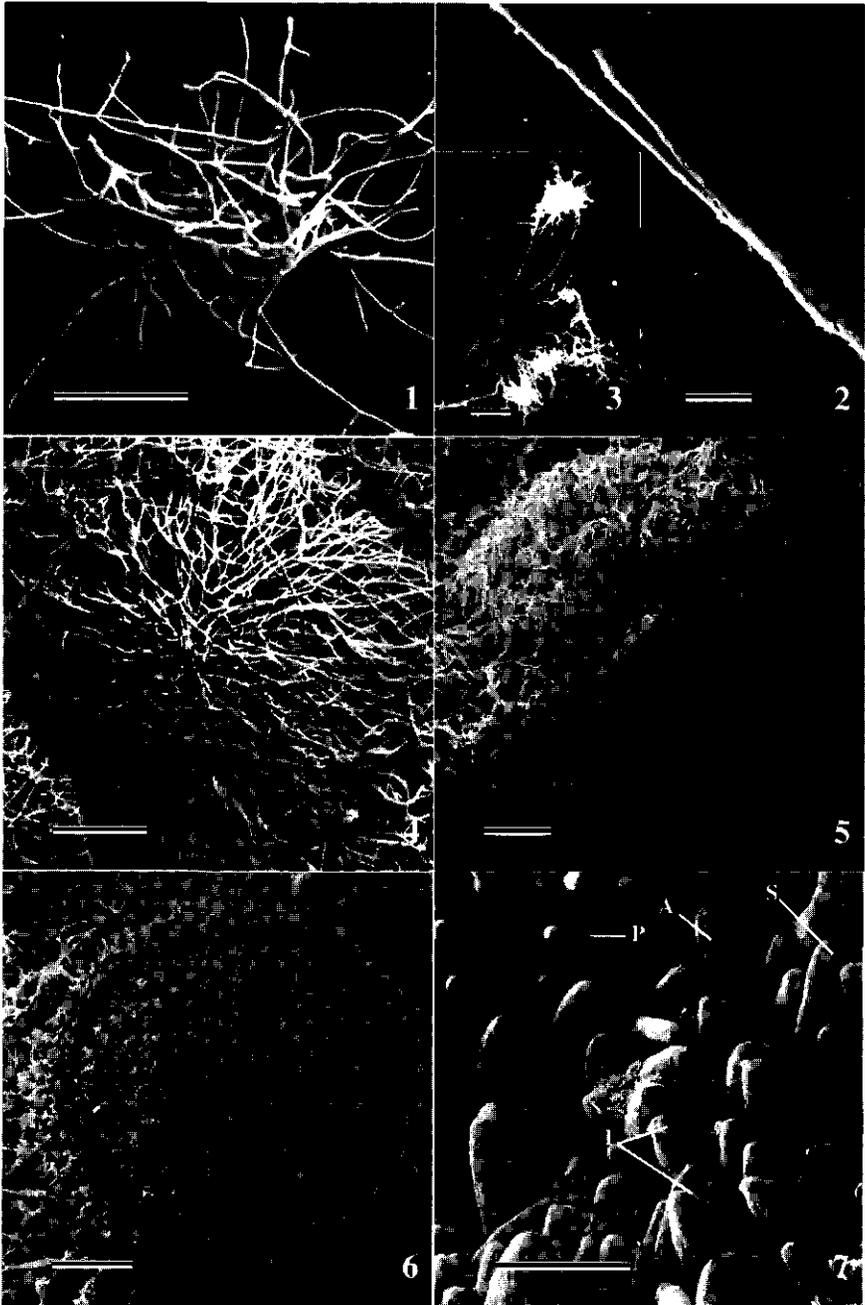
prior to examination with a Philips XL40 scanning electron microscope, which stored the images digitally.

Other samples of apothecia on oilseed rape debris were taken at the same time that samples were taken for SEM. These were fixed with 2% glutaraldehyde (Agar Scientific Limited) in a pH 7.0 phosphate buffer in small glass vials that were placed in a rotating wheel. After 2 days, the samples were washed two times in 70% ethanol for 2 h to remove the excess glutaraldehyde and then washed in 96% ethanol for 16 h to dry them. The samples were gradually transferred into resin by washing them in propyl alcohol (Agar Scientific Limited) for 1 h and in propyl alcohol:TAAB transmit resin (Agar Scientific Limited) (1:1, v/v) for 1 h before they were washed in TAAB transmit resin only for 4 h to completely permeate the tissues with resin. The samples permeated with resin were placed in tablet-shaped moulds filled with TAAB transmit resin and heated in an oven at 70°C for c. 16 h to harden the resin. The resin was prepared by mixing 4.4 g TAAB transmit resin, 9 g hardener TH1, 3 g hardener TH2 and 0.32 g accelerator. The characteristics of this resin are similar to those of Spurr resin (Spurr 1969). An Ultracut microtome (Reichert-Jung, Vienna, Austria) with glass knives was used to cut sections (c. 20 µm thick) of the samples embedded in resin. These sections were collected in water droplets on glass slides, which were heated for c. 2 to 3 minutes on a 60°C hot plate to evaporate the water and stretch the resin, and stained with 0.1% trypan blue (Sigma, Poole, UK) in lactophenol (BDH Microscopical Reagents, Poole, UK). The slides were examined with a Zeiss Axioskop light microscope (Carl Zeiss Limited, Welwyn Garden City, Hertfordshire, UK) and the images were recorded with a Xillix MicroImager digital camera (Xillix Technologies Corporation, Richmond, Canada) attached to the microscope. The contrast of the images was improved with an Openlab image analysis system on a Power Macintosh computer.

## Results

### *Development of Pyrenopeziza brassicae apothecia on agar*

*P. brassicae* hyphae ramified and formed clusters of mycelium 3-4 days after inoculation of CMM with conidia (Fig. 1). Long hyphae extended outwards from these clusters of mycelium. Close contact between long hyphae originating from different mycelial clusters was often observed (Fig. 2). GUS-staining of mycelium of a field isolate and a GUS-transformant isolate of the opposite mating type showed that the hyphae extending outwards from mycelial clusters grew towards mycelial clusters of the opposite mating type (Fig. 3). These clusters of mycelium increased in size to become larger concentric areas of dense mycelium (Fig. 4), which then developed into globular masses of dense mycelium (Fig. 5), before discoid apothecia were observed (Fig. 6). These developmental stages were observed at 10, 15 and 46 days after inoculation, respectively. The discoid apothecia ranged in diameter from c. 0.2 to 1.0 mm. The asci in the hymenium of the discoid apothecium were interspersed by paraphyses (Fig. 7). The shape of the apex of the asci ranged from dome-shaped to a more pointed shape and a slight indentation of the apex suggested the presence of a pore. A membranous film appeared to be present between the asci and paraphyses.



**Figs 1-7.** SEM observations of the development of *Pyrenopeziza brassicae* apothecia on compost malt agar incubated at 16°C. **Fig. 1.** Ramification of hyphae 3 days after inoculation of conidia onto the agar. Bar = 50 µm. **Fig. 2.** Contact (possibly anastomosis) between hyphae of separate mycelial clusters 3 days after inoculation. Bar = 10 µm. **Fig. 3.** Interaction and contact between hyphae of the natural isolate JH26 (white colour) and the transformant isolate NH10 pNOM102/18 (grey colour) made visible after staining with X-gluc 4 days after inoculation. Bar = 100 µm. **Fig. 4.** A cluster of dense mycelium 10 days after inoculation. Bar = 100 µm. **Fig. 5.** A globular immature apothecium surrounded by dense mycelium 15 days after inoculation. Bar = 100 µm. **Fig. 6.** A mature discoid apothecium 46 days after inoculation. Bar = 100µm. **Fig. 7.** Ultrastructure of the hymenium of the mature apothecium (Fig. 6) with asci (A), paraphyses (P) and spores (S). The asci have a slight indentation at the apex (I). Bar = 10 µm.

#### *Apothecial development on oilseed rape debris*

On some of the senesced leaves, conidiomata were observed to produce *P. brassicae* conidia after 2 days of incubation at 12°C (Fig. 8). Subsequently, production of conidia ceased and conidiomata turned black. *P. brassicae* appeared to grow saprophytically outwards from infected areas (Fig. 9), which had turned a dark-grey colour after 4-5 days of incubation. Small, dark globular structures had developed on the edge of these infected areas after 5 to 7 days of incubation (Fig. 10). On close observation (Fig. 11), these globular structures appeared to develop within the leaf lamina before they protruded outwards through the tissue when they increased in size. The apex of these globular structures then depressed inwards before the disk expanded to expose the hymenium after 16 days of incubation (Fig. 12). The expanded apothecial disks consisted of a flat to convex hymenium and ranged in diameter from 0.2 to 1.3 mm and the ascospores ranged in length from 8.0 to 14.5 µm and in width from 2.5 to 3.0 µm. In August 1997 at Rothamsted, on stem debris from a harvested winter oilseed rape (cv. Bristol) crop, which had been severely affected by light leaf spot, apothecia were frequently observed at the edge of light leaf spot lesions. All the apothecia observed in August 1997 had already released their ascospores and were cupular structures, which possibly consisted of the remains of the hymenial cell layers (Fig. 13). These apothecia ranged in diameter from 0.5 to 1.0 mm. On the leaf petioles collected on 24 May 2000 from under a winter oilseed rape crop affected by light leaf spot at Rothamsted numerous apothecia were observed which ranged in diameter from 0.2 to 0.5 mm and contained ascospores ranging in length from 8.2 to 13.8 µm and in width from 2.7 to 3.5 µm.

The SEM images of infected leaf petioles at different times showed that after 3 days of incubation mycelium protruded through the epidermis, which bulged outwards (Fig. 14). Distinct gaps were observed in the epidermal wall where the mycelium had protruded through the epidermis. After 6 days of incubation, globular immature apothecia consisting of dense mycelium protruded through the epidermis (Fig. 15). The apex of these immature apothecia depressed after 10 days of incubation (Fig. 16) before the disks expanded (Figs. 18 and 20) after 12-14 days of incubation to expose the hymenium of the cupular apothecia. When the apex depressed, only the apices of paraphysis-like cells were observed (Fig. 17). Asci and ascospores were observed when the disks had started to expand (Figs. 19 and 21). After 14 days of incubation, gaps in the hymenium were observed and a membranous film appeared to be present between the asci and paraphyses (Fig. 21). The gaps in the hymenium suggested that some asci had already released their ascospores.

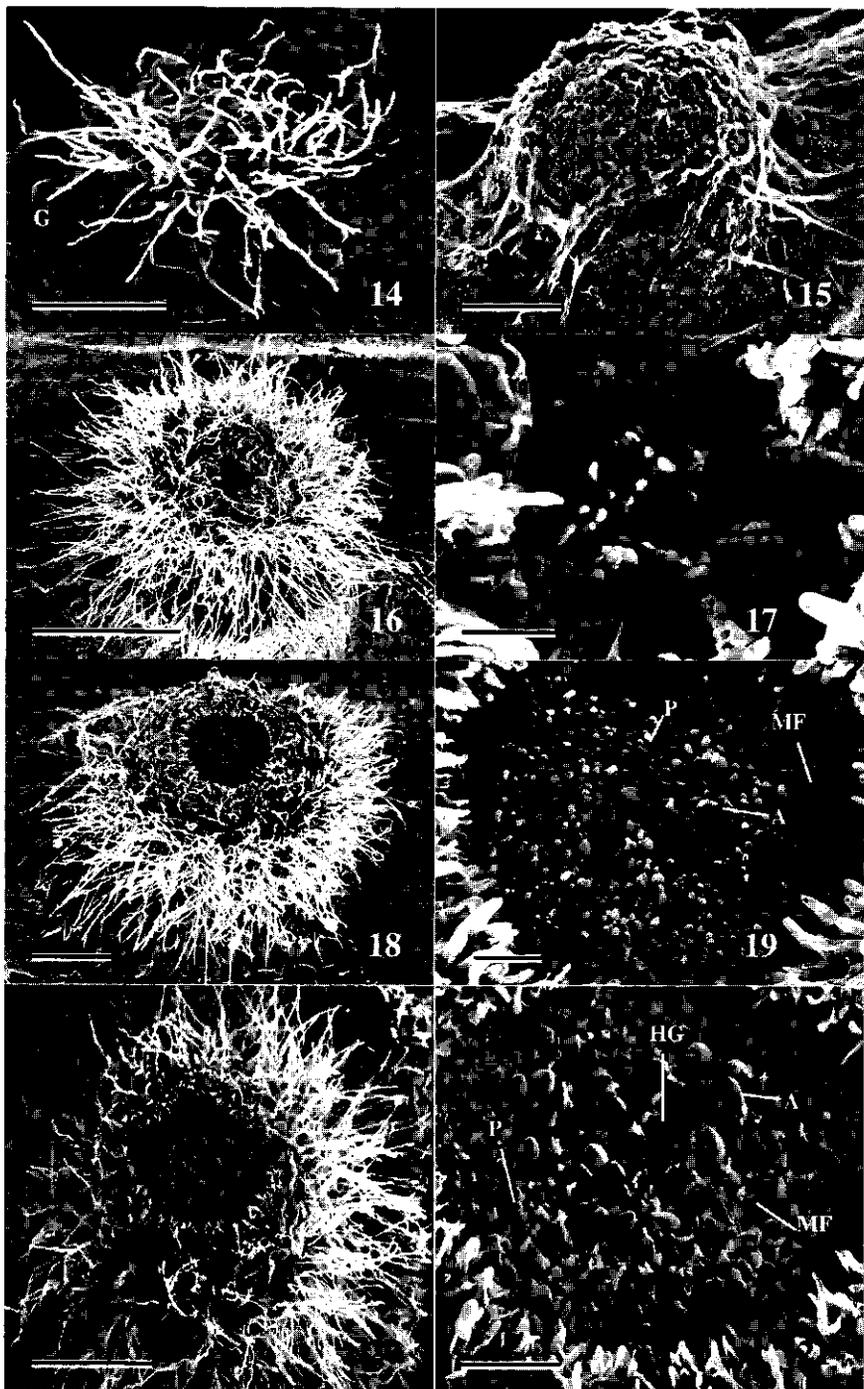


**Figs 8-13.** Observations under a stereo-microscope of oilseed rape (cv. Bristol) debris infected with *Pyrenopeziza brassicae* isolate RES97 and incubated at 12°C. **Fig. 8.** Production of conidia in conidiomata (CM) on a central leaf vein after 2 days of incubation. Bar = 2 mm. **Fig. 9.** Saprophytic growth (SG) of *P. brassicae* outwards from affected areas on a leaf lamina after 4 days of incubation. Bar = 5 mm. **Fig. 10.** Small globular structures (GS) on edges of areas affected by *P. brassicae* on a leaf lamina after 5 days of incubation. Bar = 5 mm. **Fig. 11.** Erumpent immature apothecia (IA) on a leaf lamina after 7 days of incubation. Bar = 0.5 mm. **Fig. 12.** Mature discoid apothecia on a leaf petiole after 16 days of incubation. Bar = 2 mm. **Fig. 13.** An apothecium, which had released its ascospores, on stem debris of a winter oilseed rape (cv. Bristol) crop on 15 August 1997 at Rothamsted. Bar = 2 mm.

Microscopic observations of sections of infected leaf petiole debris after different times of incubation at 12°C showed that small globular structures consisting of dense mycelium developed underneath the epidermis after 4 days of incubation under wet conditions (Fig. 22). After 7 days of incubation, the globular structures had increased in size and protruded through the epidermis. Within these structures, cells had started to differentiate to produce an ectal excipular layer consisting of *textura angularis* and a medullary excipular layer consisting of *textura intricata* (Fig. 23). The globular structures extended upwards after 10 days of incubation (Fig. 24) before disk expansion occurred after 16 days of incubation. At this mature stage, the initial cupular shape of apothecia had become more lenticular. Detailed observations of the different tissue layers in a mature apothecium (Fig. 25) showed that a hypothecium, an ectal excipulum, a medullary excipulum (Fig. 26) and a hymenium (Fig. 27) could be distinguished. Underneath the apothecia, hyphae grew as excrecent cortical cells surrounding the ectal excipulum, which consisted of *textura angularis* type tissue, and the medullary excipulum, which consisted of *textura intricata*. Within the hymenial layer, asci containing cylindrical ascospores were interspersed with filiform paraphyses.

## Discussion

These observations of development of *P. brassicae* apothecia on oilseed rape debris and on CMM agar suggest that apothecial development is similar on both substrates. The observed development of mycelial aggregates on the agar was comparable to the observed saprophytic growth of dense mycelium outwards from areas on leaf laminae infected by *P. brassicae* (Fig. 28). These mycelial aggregates can be compared with the masses of loosely woven hyphae of *Pseudopeziza trifolii* f.sp. *medicaginis-sativae*, a closely related fungus, observed within lesions on alfalfa leaflets (Meyer & Luttrell 1986). The processes of apothecial development from globular structures, with apical depression followed by disk expansion, were identical on oilseed rape debris and agar. The diameters of *P. brassicae* apothecia on incubated oilseed rape leaf debris and on agar were comparable to the diameters of apothecia in the type description, which was based on a *P. brassicae* culture on agar (Rawlinson *et al.* 1978). However, the largest apothecia on leaf veins and petioles collected from under winter oilseed rape crops in May-July 1986 (Lacey *et al.* 1987) or on 24 May 2000 were smaller than the apothecia on incubated oilseed rape debris or on agar. The apothecia produced under natural conditions may have been smaller than those produced on incubated leaf petioles, because natural debris is exposed to alternating wet and dry periods, whereas incubated leaf petioles were continuously wet. Nevertheless, the sizes of ascospores produced on incubated oilseed rape leaf debris were within the range of sizes of the ascospores that were released from the apothecia collected from under oilseed rape crops.





**Figs 14-21.** SEM observations of development of *Pyrenopeziza brassicae* apothecia on senesced oilseed rape (cv. Bristol) leaf petioles infected with isolate RES97 and incubated at 12°C. **Fig. 14.** Mycelium protruding through gaps (G) in the epidermis, which is bulging outwards, after 3 days of incubation. Bar = 100 µm. **Fig. 15.** An erumpent immature apothecium after 6 days of incubation. Bar = 50 µm. **Figs 16-17.** Immature apothecium after 10 days of incubation. **Fig. 16.** Apical depression. Bar = 200 µm. **Fig. 17.** Paraphysis-like cells within the apical depression. Bar = 10 µm. **Figs 18-19.** Apothecium after 12 days of incubation. **Fig. 18.** Expansion of the disk. Bar = 100 µm. **Fig. 19.** Hymenium with an ascus (A), paraphyses (P) and a membranous film (MF) at the edge of the hymenium. Bar = 20 µm. **Figs 20-21.** Apothecium after 14 days of incubation. **Fig. 20.** Exposure of the hymenium by disk expansion. Bar = 100 µm. **Fig. 21.** Hymenium with asci (A), paraphyses (P), gaps in the hymenium (HG) and the remains of a membranous film (MF) between asci and paraphyses.

**Figs 22-27.** Light microscopic observations of sections of senesced oilseed rape (cv. Bristol) leaf petioles infected with *Pyrenopeziza brassicae* isolate RES97 and incubated at 12°C. **Fig. 22.** The development under the epidermis of a small globular structure consisting of dense mycelium after 4 days of incubation. Bar = 200 µm. **Fig. 23.** An erumpent immature apothecium consisting of ectal (EE) and medullary excipula (ME) after 7 days of incubation. Bar = 300 µm. **Fig. 24.** An immature apothecium extending longitudinally after 10 days of incubation. Bar = 200 µm. **Figs 25-27.** A mature apothecium with a fully expanded disk after 16 days of incubation. **Fig. 25.** An overview of the apothecium. Bar = 500 µm. **Fig. 26.** Details of mycelial hyphae (MH), the ectal excipulum (EE) consisting of *textura angularis* and the medullary excipulum (ME) consisting of *textura intricata*. Bar = 50 µm. **Fig. 27.** Details of the hymenium with asci (A) containing ascospores (AS) and filliform paraphyses (P). Bar = 50 µm.

The scanning electron and light microscopic observations of apothecial development on oilseed rape debris and on agar improve our understanding of the sexual development of this discomycete fungus. Observations of early stages in the development *P. brassicae* apothecia on agar suggest that sexual development is initiated by growth of hyphae towards those of the opposite mating type, followed by contact between hyphae of the opposite mating types (Fig. 28). Anastomosis and the exchange of nuclei might occur between hyphae of the opposite mating types. However, typical ascogonial coils were not observed, although they have been observed early in the apothecial development of *P. trifolii* f.sp. *medicaginis-sativae* on alfalfa (Meyer & Luttrell 1986). Previous studies on sexual development of *P. brassicae* revealed that there is a period of time within which sexual development can be initiated. Treatment of *P. brassicae* cultures with the lipoidal sex factor (SF), a morphogenic factor known to affect the development of sexual structures (Siddiq *et al.* 1989; Siddiq, Johnstone & Ingram 1990), demonstrated that the sex factor functioned as a morphogen between 3 and 6 days after the start of incubation of conidia of a single mating type of *P. brassicae* (Siddiq 1989). Similarly, when plates were inoculated with conidia of the two mating types, the initiation of sexual reproduction of *P. brassicae* could occur up to 7 days after inoculation, after which time the fungus was unable to reproduce sexually (Ashby 1997).

It is likely that the interaction and exchange of nuclei between hyphae of opposite mating types also occurs *in planta*, but no direct observations of hyphal interactions *in planta* were made in this study. Therefore, it is not known whether this hyphal interaction occurs shortly after inoculation of living leaf tissues with conidia of opposite mating types or after senescence of the infected leaf tissues. It is more likely that onset of sexual development occurs in living tissues, since the rate of development of *P. brassicae* apothecia on debris would then be similar to the rate of their development on agar. For example, after inoculation of oilseed rape leaves, *P. brassicae* is present in the living leaf tissues for 32 days at 16°C until senescence and a further 16 days in the senesced leaf tissues incubated at 12°C until mature apothecia are produced (thus 48 days in total) and on agar, mature apothecia were observed 46 days after inoculation of the agar with a mixture of conidia of the opposite

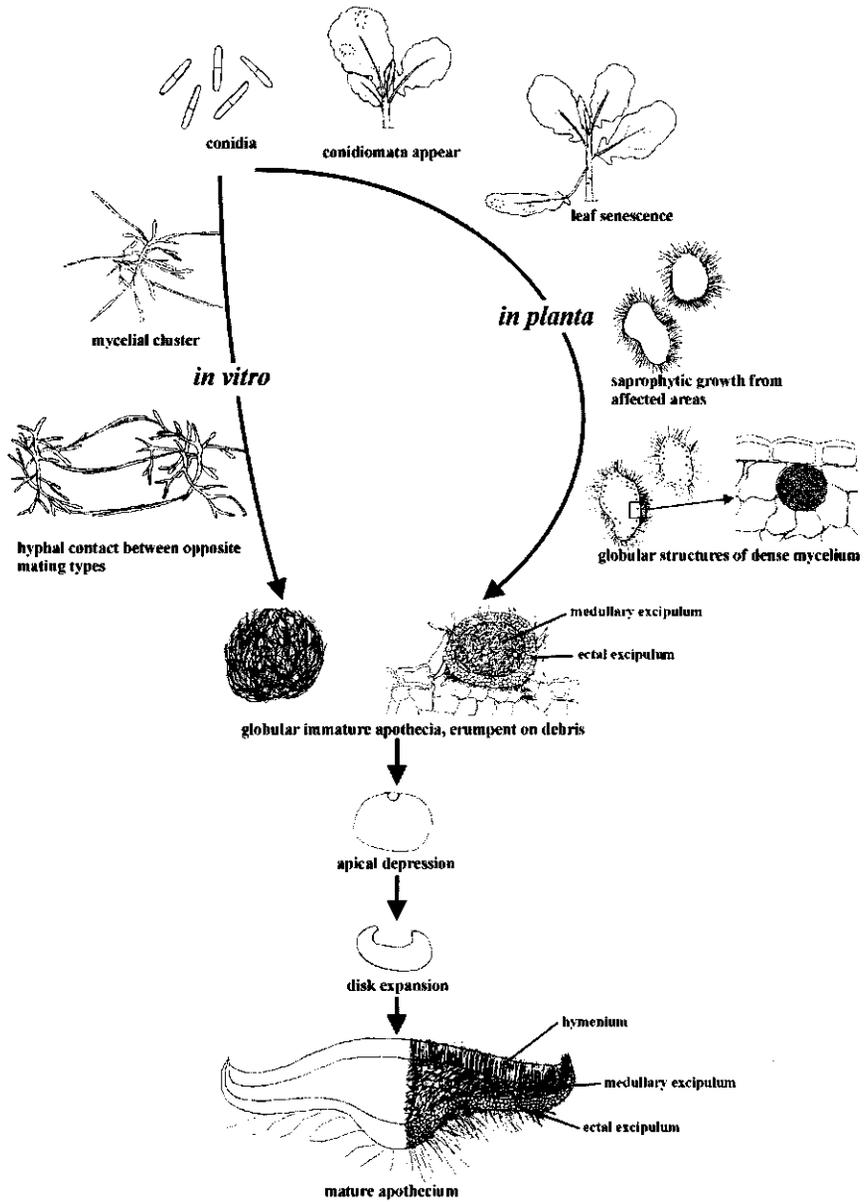


Fig. 28. Schematic drawing of the development of *Pyrenopeziza brassicae* apothecia *in vitro* on compost malt agar medium and *in planta* on oilseed rape leaves (not to scale).

mating types and incubation at 16°C. Under natural conditions, apothecia were observed 20 to 25 days after harvest of a winter oilseed rape crop at Rothamsted in 1997 or at least 24 days after senescence of leaves in a crop in spring (McCartney & Lacey 1990), which is comparable to the observation of mature apothecia on senesced leaves after 16 days of incubation. However, observations of saprophytic growth shortly after incubation of senesced infected leaf tissues suggest that hyphal interaction could also occur after the onset of senescence. This supports the hypothesis of Singh & Ashby (Singh & Ashby 1998; Singh *et al.* 1999) that the metallothionein encoded by the *MAT-1* idiomorph may function as an environmental sensor of senescence *in planta*. However, this hypothesis, which would mean that apothecial development on oilseed rape debris is faster than the development on agar, remains to be tested.

The SEM observations suggest that asci develop after apical depression and, thereby, suggest a hemi-angiocarpic development of the apothecia (Corner 1929b). The observations by SEM of the hymenium of mature apothecia suggest that a membranous film is present between the asci and paraphyses on the hymenium. Jayachandran, Willetts & Bullock (1987) made similar observations of the presence of a membranous film across the hymenium of the apothecia of *Sclerotinia trifoliorum*. Rawlinson *et al.* (1978) mentioned the presence of a pseudo-epithecium, which could be the same as the membranous film observed in this study. A protein involved in sexual morphogenesis of *P. brassicae* was recently purified and designated as SFI1 (Ashby 1997). Immuno-gold microscopical studies have shown that SFI1 is localised within the hyphal wall and inter-hyphal space occupied by fibrillar proteins within the developing ascocarp. A role for SFI1 in coordinating hyphal aggregation during apothecial development was, therefore, proposed, but it is also feasible that SFI1 plays a role in the development of this membranous film. This membranous film may have a role in reducing water loss from an expanded apothecial disk.

### **Acknowledgements**

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## Chapter 5

### Modelling effects of temperature and wetness on the development of apothecia of *Pyrenopeziza brassicae* (light leaf spot) on oilseed rape debris

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#### Abstract

The development of *P. brassicae* apothecia was studied on petiole debris from artificially infected oilseed rape leaves, incubated at temperatures from 6 to 22°C under different wetness regimes, and in 16 h light/8 h dark or continuous darkness. There was no difference between light treatments in numbers of apothecia that developed. Mature apothecia developed at temperatures from 6 to 18 but not at 22°C. The rate of apothecial development decreased as temperature decreased from 18 to 6°C; mature apothecia were first observed after *c.* 5 days at 18°C and after *c.* 14 days at 6°C. An interruption in wetness of the petiole debris for 4 days after 4, 7, or 10 days of wetness delayed the time to observation of the first mature apothecia for *c.* 4 days and decreased the number of apothecia produced (by comparison with continuous wetness). Models were fitted to estimates of the wetness duration (days) for 50% of the maximum number of apothecia to develop ( $t_1$ ; model 1,  $t_1 = 8.0 + 68.6(0.805)^T$ ) and the time until the number of apothecia had subsequently decreased to 50% of the maximum ( $t_2$ ; model 2,  $t_2 = 25.8 + 543(0.687)^T$ ) at temperatures ( $T$ ) from 6 to 18°C. Estimates of  $t_1$  and  $t_2$  obtained from independent experiments, in which naturally infected pod debris was incubated at temperatures from 5 to 17°C, corresponded well with the values of  $t_1$  and  $t_2$  predicted by the models. The differences between the values of  $t_1$  predicted by model 1 and the dates when mature apothecia were first observed on pod debris incubated under natural conditions in a field plot were only 1 to 10 days. However, apothecia decayed more quickly on the pod debris incubated under natural conditions than predicted by model 2.

Keywords: ascospores, debris wetness sensor, forecasting, exponential model

## Introduction

*Pyrenopeziza brassicae*, the cause of light leaf spot on winter oilseed rape, develops apothecia (a discoid ascoma) during its sexual stage on senescent infected oilseed rape plant tissues. Apothecia of *P. brassicae* have been observed on winter oilseed rape debris in the UK (Lacey *et al.*, 1987) and Germany (Amelung & Daebeler, 1991). Both mating types of *P. brassicae* are present in the UK (Ball *et al.*, 1990) and sexual reproduction of *P. brassicae* is probably frequent in north-west Europe (Majer *et al.*, 1998). Mature apothecia have been observed in late summer or autumn on stem and pod debris from harvested crops, and in spring on leaf debris under crop canopies (McCartney & Lacey, 1990; Lacey *et al.*, 1987). The wind-dispersed ascospores of *P. brassicae* (McCartney *et al.*, 1986), released from apothecia in autumn, are thought to cause primary infections which initiate light leaf spot epidemics. This suggestion is supported by the observation that light leaf spot lesions in a crop are randomly distributed early in the season (Evans *et al.*, 1999b), which indicates dispersal by wind. Ascospores released from apothecia on leaf debris under crop canopies in spring are thought to be a cause of infections on stems and pods (Gilles *et al.*, 2000a). More information about the conditions which favour maturation of apothecia is needed to improve understanding of the role of *P. brassicae* ascospores in light leaf spot epidemics.

There is little information about the effects of temperature and debris wetness on the development of *P. brassicae* apothecia. These apothecia develop until they are mature when their hymenium expands and they release their ascospores, after ascospore release they decay (Gilles & Fitt, 1999). A decrease in the numbers of air-borne ascospores of *P. brassicae* collected when the weather became dry suggested indirectly that dry weather decreases the number of apothecia maturing (McCartney & Lacey, 1990) and that wetness is required for development of *P. brassicae* apothecia on debris. There is also evidence that the amount of rainfall affects the development of apothecia of *Sclerotinia sclerotiorum* (sclerotinia stem rot on oilseed rape) on oilseed rape debris in Sweden (Nordin *et al.*, 1992) and the development of apothecia of *Pseudopezizula tracheiphila* ('rotbrenner' on grape) on grapevine leaf debris (Reiss & Zinkernagel, 1997). Effects of temperature on apothecial development have been demonstrated for *Whetzelinia sclerotiorum* (white mould on phaseolus beans); apothecia did not develop on sclerotia above or below the upper or lower threshold temperatures, respectively (Abawi & Grogan, 1975). The rate of apothecial development and length of time when apothecia of *Blumeriella jaapii* (cherry leaf spot) are present on senesced cherry leaves were also affected by temperature (Garcia & Jones, 1993). This paper reports the development of a model to describe effects of temperature and wetness duration on the maturation of apothecia of *P. brassicae* on oilseed rape debris.

## Materials and methods

### *Effects of light, temperature and wetness on the development of apothecia*

Apothecia were produced on leaf petiole debris, collected after senescence of oilseed rape (cv. Bristol) leaves inoculated with *P. brassicae* isolate RES97, according to the method described by Gilles *et al.* (2000b). After collection, the petioles were dried in air at 20°C for 1 day and kept dry for 2 days before being soaked in rain water for 16 h. The wetted petioles were placed in plastic boxes (12.5 cm × 8 cm), with seven petioles per box, on three layers of filter paper (Whatman no. 1; Whatman International Limited, Maidstone, Kent, UK), which had been wetted with 7 ml of rain water. To test the effects of light on the development of mature apothecia, one box with petioles was incubated in a controlled environment cabinet at 16°C (+1.5°C during the light period) with a daily regime of 16 h light/8 h dark (190 µE m<sup>-2</sup>s<sup>-1</sup> light intensity, fluorescent and tungsten lighting) and another box was incubated in the same

cabinet in continuous darkness inside an enclosed box. The number of mature apothecia per length of petiole was counted by observation of the petioles through a binocular dissecting microscope (Olympus Optical Company Limited, London, UK) at 14× magnification, after 25 days of incubation. Apothecia were recorded as mature when the light grey coloured hymenium was visible, because previous observations of developing apothecia suggested that ascospores are released at this stage (Gilles *et al.*, unpublished). The experiment was done three times to replicate the alternating light/dark or continuous darkness treatments.

Effects of temperature and wetness duration on the development of mature apothecia of *P. brassicae* were tested by incubating artificially infected petioles on wet filter paper in the plastic boxes in temperature-controlled rooms at 6(5.8-6.2)°C, 9(9.1-9.9)°C, 13(12.0-13.5)°C, 17(16.7-17.4)°C, 18(17.7-18.4)°C or 22(21.9-22.7)°C. At each temperature, apothecial development was assessed by counting the number of mature apothecia per length of petiole on 20 petioles in five boxes (four petioles per box) after different times of incubation. The experiment was done two times to replicate the temperature treatments; the rooms remained at the same temperatures in both experiments.

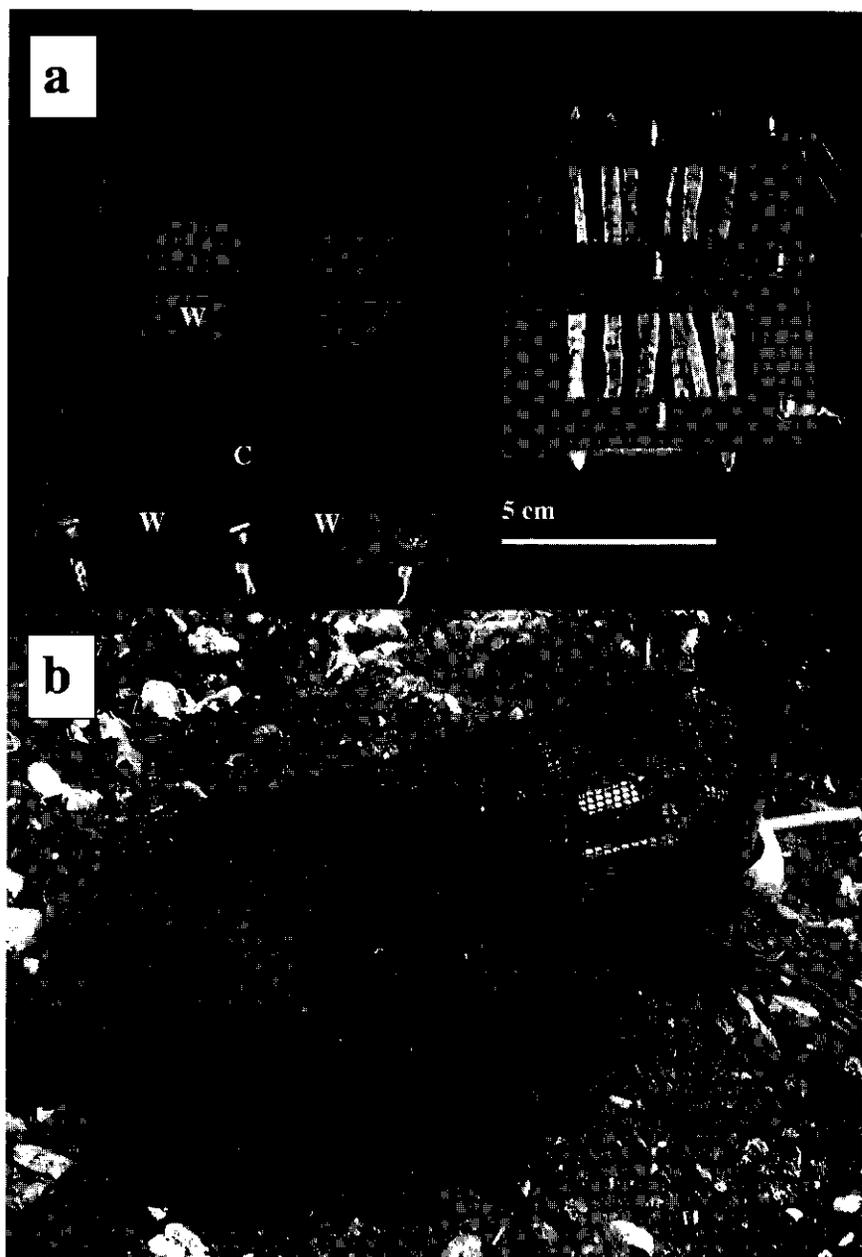
The effects of an interruption in wetness on the development of *P. brassicae* apothecia were tested on artificially infected petioles, incubated in plastic boxes on wet filter paper in a temperature-controlled room at 13(12.0-13.5)°C. After 4, 7 or 10 days of wetness, sets of three boxes containing 20 petioles per box were taken out of the temperature-controlled room, their lids were removed and they were placed in an air-flow cabinet (Microflow Limited, Fleet, UK) for 3 h to dry the petioles. The boxes were then closed and replaced in the temperature-controlled room at 13°C. After another 4 days, the dried petioles in the boxes were wetted again by adding 15 ml of rain water to the filter paper in the boxes. The petioles in another set of three boxes were kept under continuous wetness as a control treatment. The number of apothecia per length of petiole was counted after different times of incubation. This experiment was done three times to replicate the interrupted wetness treatments; each treatment was applied to three boxes selected at random from the 12 boxes containing petioles in each experiment.

#### *Modelling effects of temperature and wetness duration on maturation of apothecia*

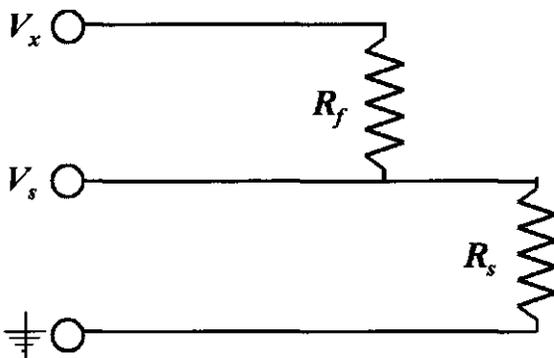
The data for the changes with time under continuous wetness in numbers of apothecia of *P. brassicae* per length of petiole at 6, 9, 13, 17 or 18°C were used to construct a model describing the changes with wetness duration in numbers of mature apothecia as a function of temperature. With increasing time, the numbers of mature apothecia increased to a maximum and then decreased to zero. From these data, the times of continuous wetness until the number of apothecia had increased to 50% of the maximum number of apothecia ( $t_1$ ) and until the number of apothecia had subsequently decreased to 50% of the maximum ( $t_2$ ) were calculated. If the times  $t_1$  or  $t_2$  were between two times at which the numbers of apothecia had been counted, linear interpolation was used to estimate their values. Two values of  $t_1$  and  $t_2$  were estimated at each temperature from the data for numbers of apothecia on petioles for the two experiments in which effects of temperature had been tested. With the statistical software Genstat 5™ Release 4.1 (Payne *et al.*, 1993), an exponential function of the form:

$$t_i = a + br^T \quad (i = 1, 2) \quad (1)$$

was fitted to the estimates of  $t_1$  (in days) to describe the effects of temperature ( $T$  in °C) and wetness duration on development of mature apothecia (model 1). The same function was also fitted to the estimates of  $t_2$  (in days) to describe the effects of temperature on the time under continuous wetness for decay of apothecia (model 2).



**Figure 1** Pod debris wetness sensor made from two epoxy glass laminate boards coated with three strips of copper (C), set up to measure the resistance of 12 half pods (a) (scale bar  $x$  cm) and operating in field conditions (b). In between the strips of copper the boards were waxed (W) to prevent capillary flow of water to make contact between these strips.



**Figure 2** Half-bridge electrical circuit to measure the resistance through a 'debris wetness sensor' ( $R_s$ ) with a data logger by putting an excitation voltage ( $V_x$ ) across a reference resistor ( $R_f$ ), which was in series with the debris wetness sensor. The residual voltage across the debris wetness sensor ( $V_s$ ) was measured in relation to the excitation voltage  $V_x$ ; the value recorded by the data logger was  $x$  ( $x = V_s/V_x$ ). The electrical resistance of the debris wetness sensor  $R_s = x R_f / (1 - x)$ .

#### *A method to measure wetness of pod debris*

The models describing the effects of temperature and wetness duration on the development or decay of mature apothecia of *P. brassicae* are based on data for changes in numbers of mature apothecia with time under continuous wetness. Therefore, to test the use of this model to predict the occurrence of mature apothecia on debris under natural conditions, a method was needed to measure when debris is wet under natural conditions. To measure the electrical resistance of pod debris, a device was made of two 10 cm × 8 cm epoxy glass laminate boards coated with copper (RS Components, Corby, UK) from which two 2.5 cm × 5 cm sections were cut out (Figure 1a). On the two shorter sides of the cut-out sections, the copper was removed, leaving three strips of copper across the width of the boards. Where the copper was removed the boards were waxed to prevent capillary flow of water between the strips of copper. Two boards were fixed on top of each other with bolts, and between these boards oilseed rape debris (12 half pods) were clamped so that they made contact with the copper strips at both ends. An excitation pole of 5000 mV ( $V_x$ ) from a data logger (21× micrologger; Campbell Scientific Limited, UK) was connected via a 15 kΩ reference resistor ( $R_f$ ) to the central copper strip on this pod debris wetness sensor. The copper strips at both ends of the sensor were connected to the earth of the data logger (half bridge electrical circuit; Figure 2). The residual voltage from the pod debris wetness sensor ( $V_s$ ) was measured in relation to the excitation voltage ( $V_x$ ) as  $x$  ( $x = V_s/V_x$ ), recorded by the data logger and used to calculate the electrical resistance of the sensor ( $R_s$ ) with the equation:  $R_s = \frac{xR_f}{(1-x)}$  (Anonymous, 1984).

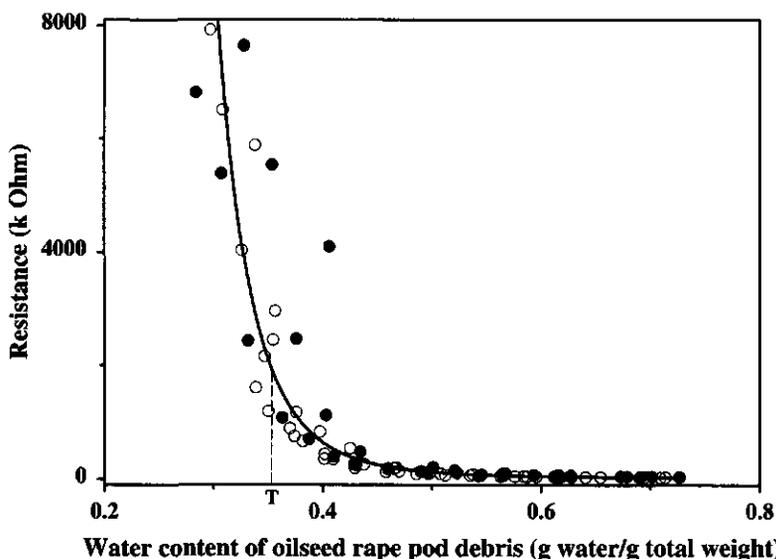
However, to estimate the water content of the pod debris, it was necessary to relate the electrical resistance to the water content of the pod debris.

To study the relationship between the water content of 12 half pods and the electrical resistance of the debris wetness sensor, half pods were collected on 30 July 1999 from the ground after harvest of a winter oilseed rape crop (cv. Bristol) at Rothamsted, which had been severely affected by light leaf spot. They were wetted in distilled water for 1 h. Excess

surface water on these half pods was then removed with absorbent paper, before the half pods were clamped into four pod debris wetness sensors (12 half pods per sensor), which were connected to a data logger (21× micrologger; Campbell Scientific Limited). At c. 5 min intervals, the debris wetness sensors were disconnected and their weight was measured on a balance (Model AT100, Mettler-Toledo Limited, Leicester, UK). Directly after each weight measurement, the sensors were reconnected to the data logger and their electrical resistance was measured. These measurements continued until the measured resistance was greater than  $8 \times 10^4$  k $\Omega$ . The dry weights of the four sets of 12 half pods and the dry weights of the four sensors without the half pods were then measured, and these measurements were used to calculate the water content of the four sets of 12 half pods during the experiment. These measurements were repeated once with four different sets of 12 half pods collected after harvest of the crop. A function was fitted to the data for electrical resistance ( $R$ , measured in k $\Omega$ ) in relation to the water content of the 12 half pods ( $W$ , measured in g water/g total weight) (Figure 3):

$$\log R = 3.29 + 24.09(52.69)^{\log(1-W)} \quad (2)$$

The data for  $R$  and  $1-W$  (values of  $W$  increased from 0 to an asymptote at 1 (complete saturation of debris with water) were log-transformed to normalise the residuals. Equation 2 accounted for 93% of the variance in the data. With this equation, the water content of the 12 half pods in the sensor was estimated from the measured electrical resistance through the debris wetness sensor.



**Figure 3** The electrical resistance ( $R$ ) of oilseed rape pod debris (12 half pods) measured by a debris wetness sensor (in k $\Omega$ ) in relation to their water content ( $W$ , in g water/g total weight). The data were obtained from measurements with four wetness sensors during an experiment (O) which was repeated with different pods (●). A function (line;  $\log R = 3.29 + 24.09(52.69)^{\log(1-W)}$ ) was fitted to these data. It was assumed that apothecial development and apothecial decay did not continue on debris below a threshold water content ( $T$ ) of 0.35 g water/g total weight.

### Validation of the model

Naturally infected pod debris was collected on 30 July 1999 after harvest of the winter oilseed rape crop at Rothamsted, and half pods were incubated in plastic boxes on wetted filter paper in temperature-controlled rooms at 5(5.0-5.8)°C, 6(6.2-6.9)°C, 10(10.2-10.6)°C or 17(17.0-17.7)°C. Each box contained 20 half pods and one box was placed in each temperature-controlled room. The experiment was done three times to replicate the temperature treatments; rooms remained at the same temperatures throughout the three experiments. The total number of pods was counted after different times of incubation and  $t_1$  and  $t_2$  were estimated by interpolation between the nearest times of observation. Thus, three estimates for  $t_1$  or  $t_2$  were obtained at each temperature. The values of  $t_1$  and  $t_2$  predicted by models 1 and 2 were compared with the values for  $t_1$  and  $t_2$  measured from this experiment to validate the models.

Naturally infected pod debris, collected on 30 July 1999 after harvest of the winter oilseed rape crop at Rothamsted, was dried at room temperature for 2 days and stored at 4°C. At seven different times during the autumn/winter of the 1999/2000 season, two trays (22 cm × 16.5 cm, 8 cm deep; four holes in the bottom of each tray to drain the excess water) covered with plastic netting (mesh size 3 mm × 3 mm; to prevent pods from being blown away by wind) and containing 30 half pods were half-buried in the ground of a field plot at Rothamsted. The numbers of mature apothecia on this debris exposed outdoors were counted under a binocular dissecting microscope (Olympus) at 14 × magnification in the laboratory at regular intervals from 19 November 1999 until 3 May 2000. As a control (to determine the ability of these pods to produce mature apothecia), some of this pod debris was incubated in a temperature-controlled room at 13(12.0-13.5)°C in plastic boxes on wetted filter paper at the same time as pod debris was placed in trays outdoors.

In the field plot, four debris wetness sensors with 12 half pods clamped to each sensor were placed in pots covered with netting (mesh size 3 mm × 3 mm) and half-buried in the ground to create conditions comparable to those of the pod debris half-buried in the trays covered with netting (Figure 1b). The electrical resistance of the wetness sensors was measured every 10 min and hourly averages were recorded with a 21× micrologger (Campbell Scientific Limited). Hourly measurements of temperature were made with a Tinytalk II temperature data logger (Gemini Data Loggers Limited, Chichester, UK). Hourly rainfall data were obtained from the Rothamsted meteorological station, which was a distance of 50 m from this field plot.

For validation of models 1 and 2, the equations were modified to give hourly rate functions:

$$1/t_1 = 1/\left[192.1 + 1646(0.805)^T\right] \quad (3)$$

$$1/t_2 = 1/\left[619.9 + 13043(0.687)^T\right] \quad (4)$$

Hourly average temperatures ( $T$ ) were input into these equations to predict the hourly rates of apothecial development ( $1/t_1$ ) or hourly rates of apothecial decay ( $1/t_2$ ). These hourly rate values were summed over periods when the pod debris water content was above the threshold value of 0.35 g water/g total weight (Figure 2) and temperature was above 0°C until the value 1 was reached, which was at times  $t_1$  or  $t_2$ , respectively. It was assumed that the apothecia of *P. brassicae* did not develop or decay when the pod debris water content was ≤ 0.35 g water/g total weight or when temperature was ≤ 0°C. The predicted values of  $t_1$  and  $t_2$  were compared with observed dates of appearance of mature apothecia on pod debris which had been placed outdoors at different times during the autumn/winter of 1999/2000.

## Results

### *Effects of light, temperature and wetness on the development of apothecia*

Under alternating 16 h light/8 h darkness, an average of 2.30 apothecia per cm length of petiole were produced; in continuous darkness, an average of 2.27 apothecia per cm length of petiole were produced. Thus, the number of apothecia produced in alternating light/dark conditions did not differ significantly from the number produced in continuous darkness (sed = 0.796, df = 2). Mature apothecia developed under continuous wetness at temperatures from 6 to 18°C but not at 22°C (Figure 4). The length of the period of wetness required until the first mature apothecia were observed increased from c. 5 to c. 14 days when temperature decreased from 18 to 6°C. Furthermore, the time period over which mature apothecia were observed (i.e. from the first to the last apothecia) increased from c. 27 to c. 82 days when temperature decreased from 18 to 6°C.

When a 4-day interruption in wetness was applied after 4 or 7 days of continuous wetness at 13°C, mature apothecia were first observed after 14 days of incubation (Figure 5). When the 4-day interruption in wetness was applied after 10 days of wetness, mature apothecia had already been observed. Under continuous wetness, mature apothecia were first observed after 10 days of incubation. The maximum number of mature apothecia occurred after 18 to 22 days of incubation for all treatments, except where there was a 4-day interruption in wetness after 4 days of wetness; in this case the maximum occurred after 16 to 22 days of incubation. After a 4-day interruption in wetness, fewer mature apothecia developed than when petioles were incubated under continuous wetness.

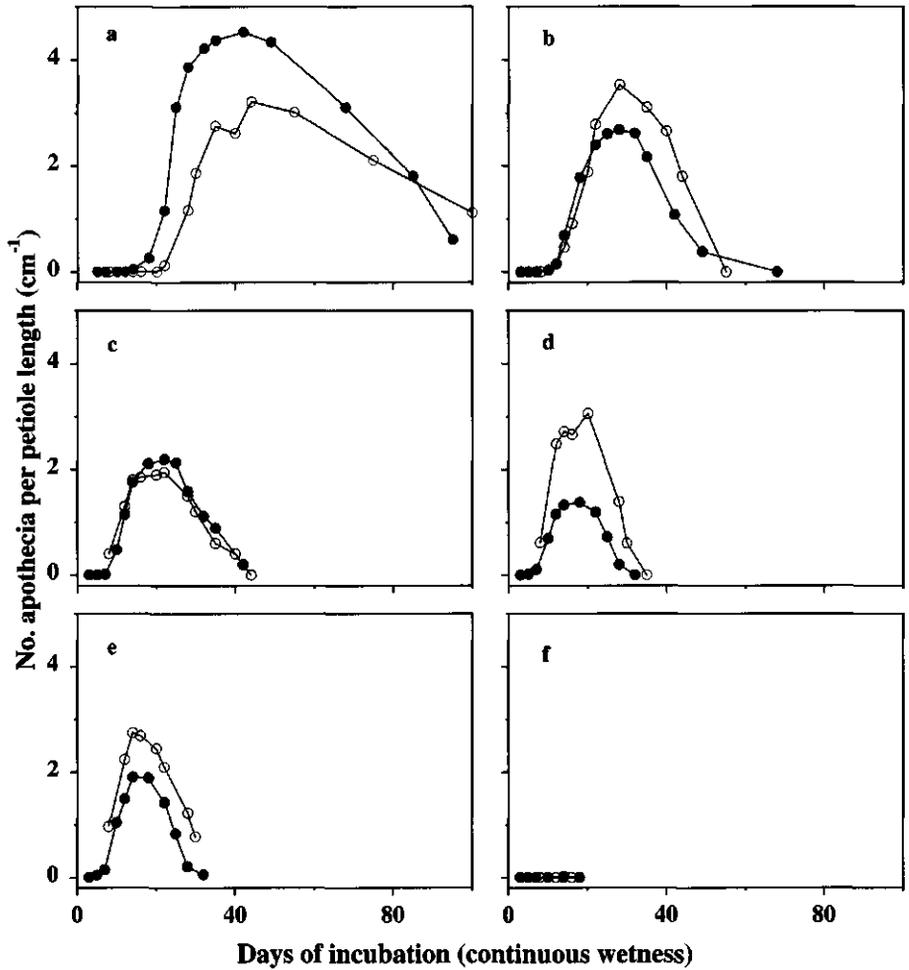
### *Modelling effects of temperature and wetness duration on maturation of apothecia*

Equation 1 was fitted to the data for  $t_1$  (in days) or  $t_2$  (in days) to obtain parameter estimates for models 1 and 2:

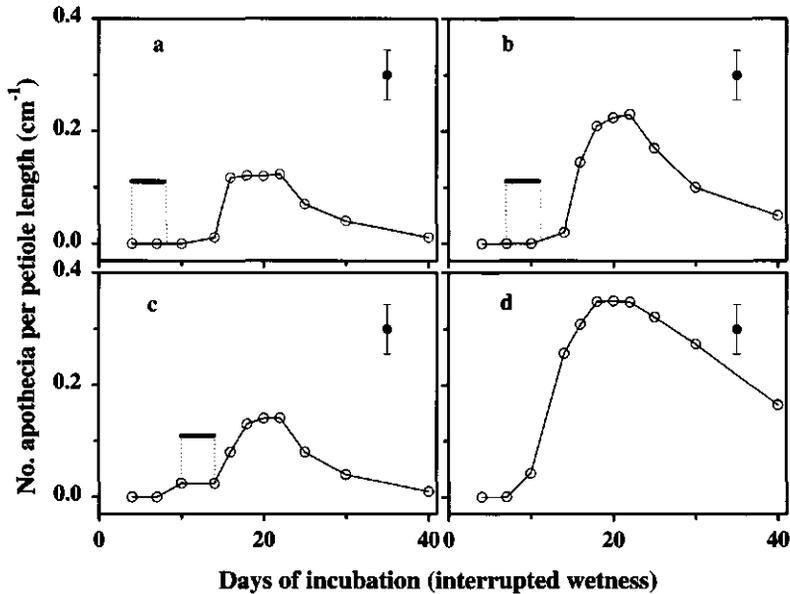
$$t_1 = 8.0 + 68.6(0.805)^T \quad (\text{model 1})$$

$$t_2 = 25.8 + 543(0.687)^T \quad (\text{model 2})$$

which describe the effects of temperature on the wetness periods required until 50% of apothecia were mature (model 1) or 50% of apothecia had decayed (model 2). Model 1 and model 2 fitted well to the data and accounted for 93% and 98 % of the variance, respectively (Figure 6). The difference between  $t_1$  and  $t_2$ , an estimate of the length of time when mature apothecia were present, increased when temperature decreased from 18 to 6°C.



**Figure 4** Numbers of mature apothecia of *P. brassicae* (light leaf spot) per length of leaf petiole debris (cm<sup>-1</sup>) after different times of incubation under continuous wetness at 6 (a), 9 (b), 13 (c), 17 (d), 18 (e) or 22°C (f) in two experiments (○, ●). The leaf petiole debris had been obtained from artificially infected oilseed rape plants (cv. Bristol).

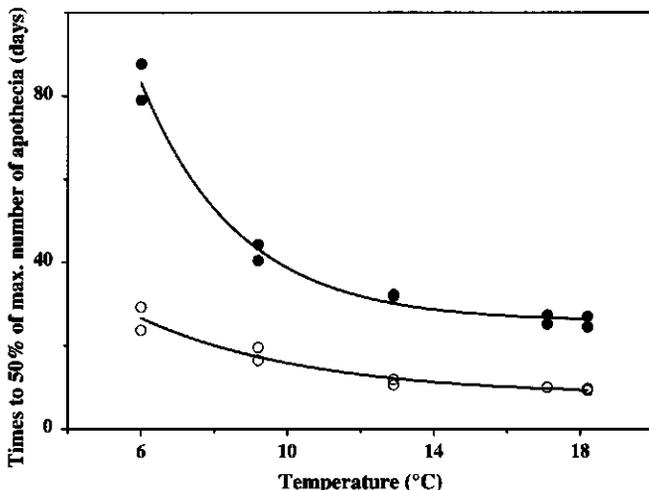


**Figure 5** Changes with time in numbers of mature apothecia per length of leaf petiole debris ( $\text{cm}^{-1}$ ), which had received a 4-day interruption (=) in continuous wetness after 4 (a), 7 (b) or 10 days of wetness (c) or was under continuous wetness (d) at  $13^{\circ}\text{C}$ . The leaf petiole debris was obtained from artificially infected oilseed rape plants (cv. Bristol). The data points are estimated mean values (three experiments). The vertical error bars are the standard errors of the differences between mean values (52 df).

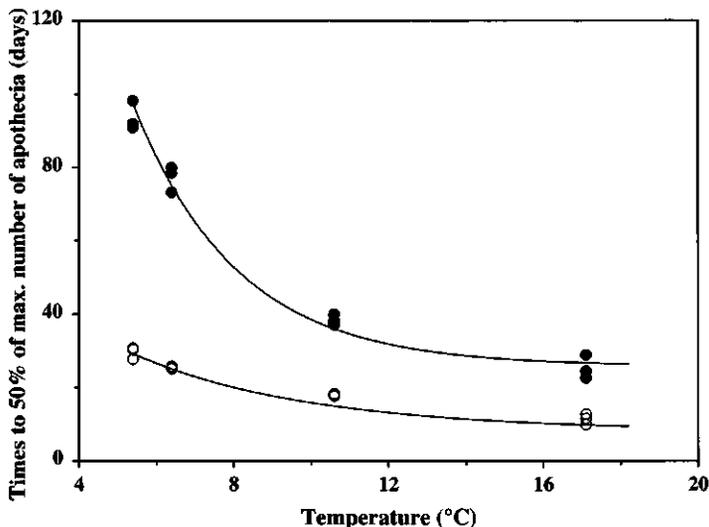
#### Validation of the model

The estimates of  $t_1$  and  $t_2$  obtained from the experiments in which naturally infected pod debris was incubated in temperature-controlled rooms at 5, 6, 10 or  $17^{\circ}\text{C}$  agreed well with the values predicted by the models for these temperatures (Figure 7). Furthermore, the values of  $t_1$  predicted by model 1 corresponded very well with the times when the first mature apothecia were observed on naturally infected pod debris under outdoor conditions (Figure 8). The values of  $t_1$  predicted by model 1 were just before the date when apothecia were first observed for pod debris placed outdoors on 19 November 1999, 26 November 1999, 10 December 1999 or 7 January 2000. The predicted values of  $t_1$  were directly after the date when apothecia were first observed for pod debris placed outdoors on 3 December 1999, 1 February 2000 or 17 February 2000 (Figure 8, Table 1). The difference between the values of  $t_1$  predicted and the dates when mature apothecia were first observed was only 1 to 10 days for the pod debris placed outdoors on seven different occasions during the autumn/winter of 1999/2000. The values of  $t_2$  predicted by model 2 did not correspond well with the dates when mature apothecia were last observed; the last mature apothecia had always decayed several weeks earlier than the predicted dates ( $t_2$ ). The numbers of mature apothecia observed on the pod debris placed outdoors on 19 November 1999, 26 November 1999 and 10 December 1999

decreased greatly between 31 January 2000 and 8 February 2000 (Figure 8a,b,c,d). A heavy rain shower occurred during the night of 1/2 February and the pod debris wetness greatly decreased on 8 February 2000. During periods when there was frequent wetting and drying of the pod debris (e.g. 8 to 26 February 2000), the numbers of mature apothecia observed were much smaller than during periods of more continuous wetness (e.g. 4 to 25 January 2000). The pod debris that was incubated under continuous wetness at 13°C at the same time that pod debris was placed outdoors produced larger numbers of apothecia.



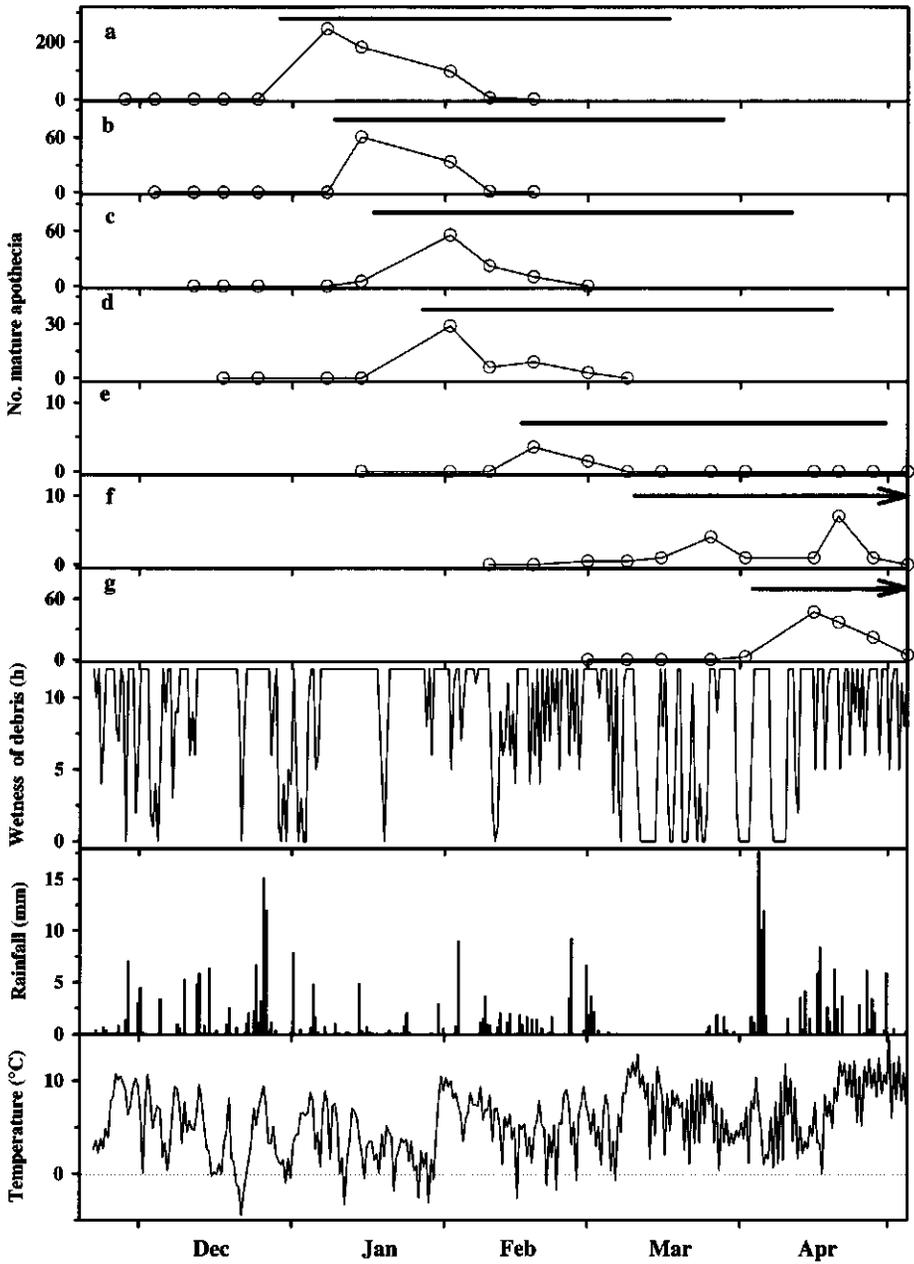
**Figure 6** Modelling effects of temperature on days of incubation under continuous wetness until the number of apothecia of *P. brassicae* had increased to 50% of the maximum ( $t_1$ , ○) and until the number had subsequently decreased to 50% of the maximum ( $t_2$ , ●) on petiole debris from artificially inoculated leaves (cv. Bristol). From the observed data for the numbers of apothecia,  $t_1$  or  $t_2$  were estimated by linear interpolation between observations on two successive times when numbers were above and below 50% of the maximum number of apothecia. Exponential decay functions (lines) were fitted to these estimates for  $t_1$  ( $t_1 = 8.0 + 68.6(0.805)^T$ ; model 1 accounted for 93% of the variance in the data) or  $t_2$  ( $t_2 = 25.8 + 543(0.687)^T$ ; model 2 accounted for 98% of the variance in the data).



**Figure 7** Validating models of effects of temperature on days of incubation under continuous wetness until the number of apothecia of *P. brassicae* had increased to 50% of the maximum ( $t_1$ ) and until the number had subsequently decreased to 50% of the maximum ( $t_2$ ) with naturally infected oilseed rape (cv. Bristol) pod debris. Debris was incubated at 5, 6, 10 or 17°C and changes with time in numbers of mature apothecia were observed. Estimates of  $t_1$  (○) or  $t_2$  (●) were obtained from these data by linear interpolation between observations on two successive observations times when numbers were above and below 50% of the maximum number of apothecia. These estimates were compared with values of  $t_1$  predicted by model 1 (line;  $t_1 = 8.0 + 68.6(0.805)^T$ ) or values of  $t_2$  predicted by model 2 (line;  $t_2 = 25.8 + 543(0.687)^T$ ).

### Discussion

These results suggest that temperature and wetness both affect the maturation of apothecia of *P. brassicae* on oilseed rape debris. The experiments on apothecial development under controlled temperature conditions suggest that apothecia can develop to maturity at temperatures from 5 to 18°C but not at or above 22°C. The rates of both apothecial development and apothecial decay were greatest at c. 17-18°C and decreased as temperature decreased to 6°C or increased to 22°C. Thus, at 18°C apothecia matured after only 5 days but were present on debris for only 27 days, whereas at 6°C apothecia first matured after 14 days, but were present for 82 days. There was a similar effect of temperature on the rates of development and decay of apothecia of *Blumeriella jaapii* on debris of cherry leaves (Garcia & Jones, 1993). However, in these experiments when *P. brassicae* apothecia were present on debris for long periods the debris remained wet during the entire period whereas apothecia on debris under natural conditions are exposed to wetting and drying.



**Figure 8** Numbers of mature apothecia on oilseed rape pod debris (○), which had been naturally infected with *P. brassicae* the previous season, and was placed on a field plot on 19 November 1999 (a), 26 November 1999 (b), 3 December 1999 (c), 10 December 1999 (d), 7 January 2000 (e), 1 February 2000 (f) or 17 February 2000 (g). The water content of debris was measured with a 'debris wetness sensor' and the number of hours when the hourly average water content was above a threshold of 0.35 g water/g total water content were summed to give the debris wetness duration (in h) per half day (from 8:00 to 20:00 and from 20:00 to 8:00) in relation to total rainfall (in mm) and average temperature (in °C). Models 1 and 2 were used to predict the times of incubation ( $t_1$ ) until 50% of the maximum number of mature apothecia developed ( $t_1$ ;

$$1/t_{1,h} = 1/\left[192.1 + 1646(0.805)^T\right], \text{ with } t_1 \text{ defined as } \sum_{i=0}^{t_1} \left(1/t_{1,h}\right), \text{ and until 50\% of the maximum number of apothecia had decayed } (t_2; 1/t_{2,h} = 1/\left[619.9 + 13043(0.687)^T\right], \text{ with } t_2 \text{ defined as } \sum_{i=0}^{t_2} \left(1/t_{2,h}\right), \text{ and the time interval between } t_1 \text{ and } t_2 \text{ (thick horizontal bar) was calculated.}$$

**Table 1** The last date of observation before the first mature apothecia of *P. brassicae* were observed on natural infected oilseed rape pod debris that was placed outdoors at different times during the autumn and winter of the 1999/2000 season, the date of observation when mature apothecia were first observed in comparison with model predictions of the time to development of 50% of the maximum number of mature apothecia ( $t_1$ ), and the date when mature apothecia were last observed in comparison with model predictions of the time to 50% decay of the maximum number of mature apothecia ( $t_2$ ).

Placed outside	Appearance mature apothecia		Disappearance mature apothecia	
	observed	predicted	observed	predicted
19 Nov	6 Jan	27 Dec	17 Feb	15 Mar
26 Nov	13 Jan	7 Jan	17 Feb	26 Mar
3 Dec	13 Jan	15 Jan	17 Feb	9 Apr
10 Dec	31 Jan	25 Jan	28 Feb	17 Apr
7 Jan	17 Feb	14 Feb	28 Feb	28 Apr
1 Feb	7 Mar	8 Mar	26 Apr	> 4 May
17 Feb	31 Mar	1 Apr	3 May	> 4 May

The experiments in which incubated petioles were exposed to a 4-day interruption in wetness suggest that intermittent dry periods delay the maturation of apothecia, probably because the rate of apothecial development within dry debris is very small, and decrease the number of mature apothecia produced. This conclusion is supported by the observations on apothecial development on pods exposed outdoors, because smaller numbers of apothecia were observed from 8 to 26 February 2000, when the pod debris was frequently dry, than in the period from 4 to 25 January 2000, when the debris was almost continuously wet. Similarly, dry weather delayed maturation of perithecia of *Guignardia citricarpa* (citrus black spot) on senesced citrus leaves (Lee and Huang, 1973). The decrease in the numbers of apothecia on pods outdoors between 31 January and 8 February 2000, coinciding with heavy rainfall on 1-2 February followed by drying of the debris, suggests that wetting and drying may initiate ascospore release and subsequent apothecial decay. This conclusion is supported by observations on the effects of rain showers and subsequent dry periods on numbers of airborne *P. brassicae* ascospores (McCartney & Lacey, 1990). Under controlled conditions, no evidence was found that light effects the development of apothecia, but this does not exclude a possible effect of daylength or exposure to light on *P. brassicae* on living oilseed rape tissues prior to tissue senescence.

The experiments to validate the models to describe effects of temperature and wetness duration on apothecial development suggest that model 1 may be a useful tool to predict the first appearance of mature apothecia on infected oilseed rape debris, and thus the release of *P.brassiccae* ascospores to initiate light leaf spot epidemics. The observations on apothecial maturation on naturally infected pod debris exposed outdoors conditions at seven different times during the autumn/winter of 1999/2000 suggest that model 1 accurately predicts the date of appearance of mature apothecia on pod debris. Whereas model 2 accurately predicted the decay of the apothecia on naturally infected pods incubated at different temperatures under continuous wetness, it did not accurately predict the rate of apothecial decay when pods were exposed to natural conditions. The numbers of apothecia on pod debris put outdoors on 19 or 26 November, 3 or 10 December 1999 decreased from 31 January 2000 onwards, which was much earlier than predicted by model 2. This decrease in numbers of apothecia coincided with a few weeks when intermittent periods of wetting and drying of pod debris occurred. This suggests that rapid wetting and drying of pod debris causes apothecia to release their ascospores (McCartney & Lacey, 1990) and decay more quickly than predicted by model 2, which is based on apothecial development under continuous wetness.

If ascospores are important in initiating light leaf spot epidemics in the autumn in the UK (Evans *et al.*, 1999b; Gilles *et al.*, 2000a), then model 1 could be useful as a component of models to describe progress of light leaf spot epidemics (Papastamati *et al.*, 1999) and of forecasting systems to guide control strategies. Previous work suggests that inoculum is an important component of systems to forecast the severity of light leaf spot epidemics in the UK (Welham *et al.*, 1999). Model 1 could be used to predict the first appearance of mature apothecia on debris left after harvest of infected crops, and thus when *P.brassiccae* ascospores will be released. For forecasting the development of light leaf spot epidemics, it is less important that model 2 cannot accurately be used to predict the length of time over which ascospores are released. During a dry summer apothecia will develop slowly, whereas during a wet summer apothecia will develop rapidly. Based on this information, growers can then decide whether they need to plough in the crop debris to decrease the primary inoculum of air-borne ascospores in the autumn, to delay sowing to avoid the presence of air-borne ascospores when the new crop emerges or to apply a fungicide spray in the autumn to control light leaf spot before the appearance of symptoms (Fitt *et al.*, 1998a). Furthermore, during the period of stem extension in early spring, model 1 could be used to assess the risk that ascospores will spread light leaf spot onto the pods (Gilles *et al.*, 2000a). In combination with a yield loss model based on incidence of light leaf spot at early flowering (Su *et al.*, 1998), this may help to guide decisions about application of fungicide sprays in spring. However, model 1 is based on measurements of temperature and debris wetness. Whilst it would not be difficult for growers to obtain temperature data, further research is needed to develop a simple method of measuring debris wetness on farms or to relate debris wetness to rainfall and other meteorological factors which can be routinely measured.

### Acknowledgements

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## Chapter 6

### Effects of temperature and wetness duration on conidial infection, latent period and asexual sporulation of *Pyrenopeziza brassicae* on leaves of oilseed rape

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#### Abstract

Experiments in controlled environments were done to determine the effects of temperature and leaf wetness duration on infection of oilseed rape leaves by conidia of the light leaf spot pathogen, *Pyrenopeziza brassicae*. Visible spore pustules developed on leaves of cv. Bristol inoculated with *P. brassicae* conidia at temperatures from 4 to 20°C, but not at 24°C; spore pustules developed when the leaf wetness duration after inoculation was longer than or equal to c. 6 h at 12-20°C, c. 10 h at 8°C, 16 h at 6°C or 24 h at 4°C. On leaves of cvs Capricorn or Cobra, light leaf spot symptoms developed at 8 and 16°C when the leaf wetness duration after inoculation was greater than 3 h or 24 h, respectively. The latent period (i.e. the time period from inoculation to first spore pustules) of *P. brassicae* on cv. Bristol was, on average, c. 10 days at 16°C when leaf wetness duration was 24 h, and increased to c. 12 days as temperature increased to 20°C and to c. 26 days as temperature decreased to 4°C. At 8°C, an increase in leaf wetness duration from 10 to 72 h decreased the latent period from c. 25 to c. 16 days; at 6°C, an increase in leaf wetness duration from 16 to 72 h decreased the latent period from c. 23 to c. 17 days. The numbers of conidia produced were greatest at 12-16°C, and decreased as temperature decreased to 8°C or increased to 20°C. At temperatures from 8 to 20°C, an increase in leaf wetness duration from 6 to 24 h increased the production of conidia. There were linear relationships between the number of conidia produced on a leaf and the proportion of the leaf area covered by lesions (both log<sub>10</sub>-transformed) at different temperatures.

**Keywords:** conidial production, cultivar, disease forecasting, disease progress curves, infection criteria, light leaf spot

## Introduction

Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is a damaging polycyclic disease of winter oilseed rape. Seasonal yield losses caused by light leaf spot have been estimated to range from £13M to £40M in the UK over the harvest years 1987 to 1995 (Fitt *et al.*, 1997). The severity of the disease varies not only between seasons, but also between regions in the UK and between individual crops within a region (Fitt *et al.*, 1996; Hardwick & Turner, 1995). Seasons, regions and individual crops which are at risk of developing severe light leaf spot epidemics could be predicted if the effects of weather factors on the spread and multiplication of *P. brassicae* were better understood. Infection of oilseed rape leaves by conidia is thought to play an important role in the development of light leaf spot epidemics (Rawlinson *et al.*, 1978). The patchy distribution of light leaf spot, frequently observed in crops in spring (Fitt *et al.*, 1998a), suggests that during autumn and winter *P. brassicae* spreads over short distances from initial infections by means of splash-dispersal of conidia during rain showers (Fatemi & Fitt, 1983). However, after dispersal by rain-splash these conidia can infect leaves of oilseed rape plants only if the environmental conditions are suitable for infection.

The success of infection of oilseed rape leaves by conidia of *P. brassicae* is affected by temperature and leaf wetness duration. In controlled environment experiments, the greatest number of lesions was produced at an optimum temperature of *c.* 15°C (Figueroa *et al.*, 1995a). At 12 or 18°C, infections by conidia were successful only when the leaf wetness duration was more than 16 h (Figueroa *et al.*, 1995b). However, temperatures are frequently much lower than this in the UK in late autumn, winter and early spring when light leaf spot is spreading. Therefore, a more precise knowledge of the temperature and leaf wetness duration boundary conditions for infection over the appropriate temperature range is needed to define the infection criteria for *P. brassicae*. This knowledge could be used as a component of a scheme for predicting the development of severe light leaf spot epidemics. In the experiments of Figueroa *et al.* (1995a), temperature also affected the latent period of *P. brassicae* (*i.e.* the time between infection of a leaf and the appearance of the first spore pustules), which was shortest at *c.* 15°C. The length of the latent period determines the rate at which infected plants become infectious and thus become sources of conidial inoculum themselves. Temperature would, therefore, be expected to influence the number of generations of asexual reproduction that can occur during a winter oilseed rape growing season, and thus the severity of light leaf spot in crops.

When conidia have infected oilseed rape leaves successfully, the numbers of conidia produced on these infected leaves affect the probability that new infections can occur after splash-dispersal. If more conidia are produced, more conidia can be dispersed during rain showers, and this increases the probability that new infections can occur (Zadoks & Schein, 1979). In the experiments of Figueroa *et al.* (1995a), the production of conidia per leaf area was affected by temperature and was greatest at *c.* 15°C, but the effects of leaf wetness duration on production of conidia were not investigated. Knowledge of the effects of temperature and leaf wetness duration on asexual sporulation could be used to predict the numbers of conidia produced in infected crops and, therefore, the numbers of new infections after each rain shower. This paper reports research to define the temperature and leaf wetness duration boundary conditions for infection of oilseed rape leaves by conidia and to investigate the effects of temperature and leaf wetness duration on the latent period and the asexual sporulation of *P. brassicae*.

## Materials and methods

### *Plant material*

At IACR-Rothamsted, oilseed rape (cv. Bristol) plants, which are susceptible to *P. brassicae* (Anonymous, 1997), were grown in compost (75% peat, 12% loam, 10% grit, 3% vermiculite; Petersfield Products, Cosby, Leicester) in 8 cm diameter pots in a controlled environment room (built at Rothamsted; 16°C, 12 h light/12 h dark, 190  $\mu\text{E m}^{-2}\text{s}^{-1}$  light intensity at canopy level (fluorescent and tungsten lighting), 80% relative humidity). After c. 25 days, the plants had developed four to five true leaves and were transferred to controlled environment cabinets with the same lighting and humidity conditions at 4, 6, 8, 12, 16, 20 or 24°C and left for two days to equilibrate before they were inoculated. Gallenkamp 600H and Gallenkamp Sanyo Fitotron cabinets were used to maintain the temperature at a constant 4, 6 or 8°C (+1.0°C during the light period) and other cabinets, built at Rothamsted, were used to maintain the temperature at a constant 12, 16, 20 or 24°C (+1.0 to 1.5°C during the light period).

At HRI-Wellesbourne, oilseed rape (cvs. Cobra or Capricorn) plants, which are moderately resistant or moderately susceptible, respectively, to *P. brassicae* (Anonymous, 1997), were grown in a glasshouse (10°C night/ 14°C day) in Hassey 308 trays (LBG, Badsey, Evesham) divided into twelve cell units filled with Fisons F2 compost (LBG, Badsey, Evesham). When the plants had developed three true leaves, they were transferred from the glasshouse to cabinets (Sanyo Gallenkamp SGC970/C/R0-HFL/Rotronic; 12 h light/12 h dark period, c. 200  $\mu\text{E m}^{-2}\text{s}^{-1}$ , 75-85% relative humidity) at a constant 8 or 16°C. The plants were left for 12 h to equilibrate before they were inoculated.

### *Inoculation of oilseed rape leaves with conidia of P. brassicae*

The oilseed rape (cv. Bristol) plants were inoculated with conidial suspensions ( $0.5 \times 10^6$  conidia per mL) of a field isolate of *P. brassicae* with an aerosol sprayer (Chrom Atomiser, Camlab, Cambridge) until drops had begun to run off the leaves. The field isolate of *P. brassicae* was derived from sporulating infected leaves of a winter oilseed rape (cv. Bristol) crop at Rothamsted in May 1997 and subsequently maintained on oilseed rape plants. Directly after inoculation, the plants were enclosed in polyethylene bags to maintain continuous leaf wetness. When the polyethylene bags were removed after 6, 10, 16, 24, 48 or 72 h, the leaf surfaces dried within 0.5 h. All inoculations were done at the start of the dark period in the cabinets. To ensure that conidia were not washed off the leaves, the pots with inoculated plants were placed in trays and watered from underneath by filling the trays with water.

At HRI-Wellesbourne, oilseed rape plants were inoculated with  $10^4$  spores per mL of an isolate obtained from a winter oilseed rape (cv. Capricorn) crop. In each cabinet, every 9.5 minutes a fine mist of water was sprayed for 30 seconds to keep the relative humidity at >95%. The plants were taken out of the humid cabinets after 3, 18, 24, 30, 42, 54, 72, 90, 114 or 168 h and leaf surfaces were dried in an air-flow cabinet before the plants were transferred to other cabinets (Sanyo Gallenkamp SGC970/C/R0-HFL/Rotronic; 12 h light/12 h dark period, c. 200  $\mu\text{E m}^{-2}\text{s}^{-1}$ , 75-85% relative humidity) at 8 or 16°C. This produced the different leaf wetness duration treatments.

### *Experimental design*

The experiment at IACR-Rothamsted was repeated four times over a period of six months. However, not all temperature treatments were repeated four times, because there were not enough cabinets available in each experiment (Table 1). The temperature treatments were randomised over the available cabinets, and leaf wetness duration treatments were randomised over trays of four plants in each experiment. Thus, the series of experiments was arranged in a

split-plot design with cabinets at different temperatures as the main plots replicated in time and wetness duration treatments as the sub-plots. At HRI-Wellesbourne, the temperatures 8 and 16°C were assigned to two cabinets. The Hassey 308 trays with twelve cell units for each cultivar were randomised within each cabinet and the two replicates were randomly assigned to each wetness duration and cultivar. The experiment with two replicates of wetness duration and cultivar was repeated a second time to replicate temperature treatments.

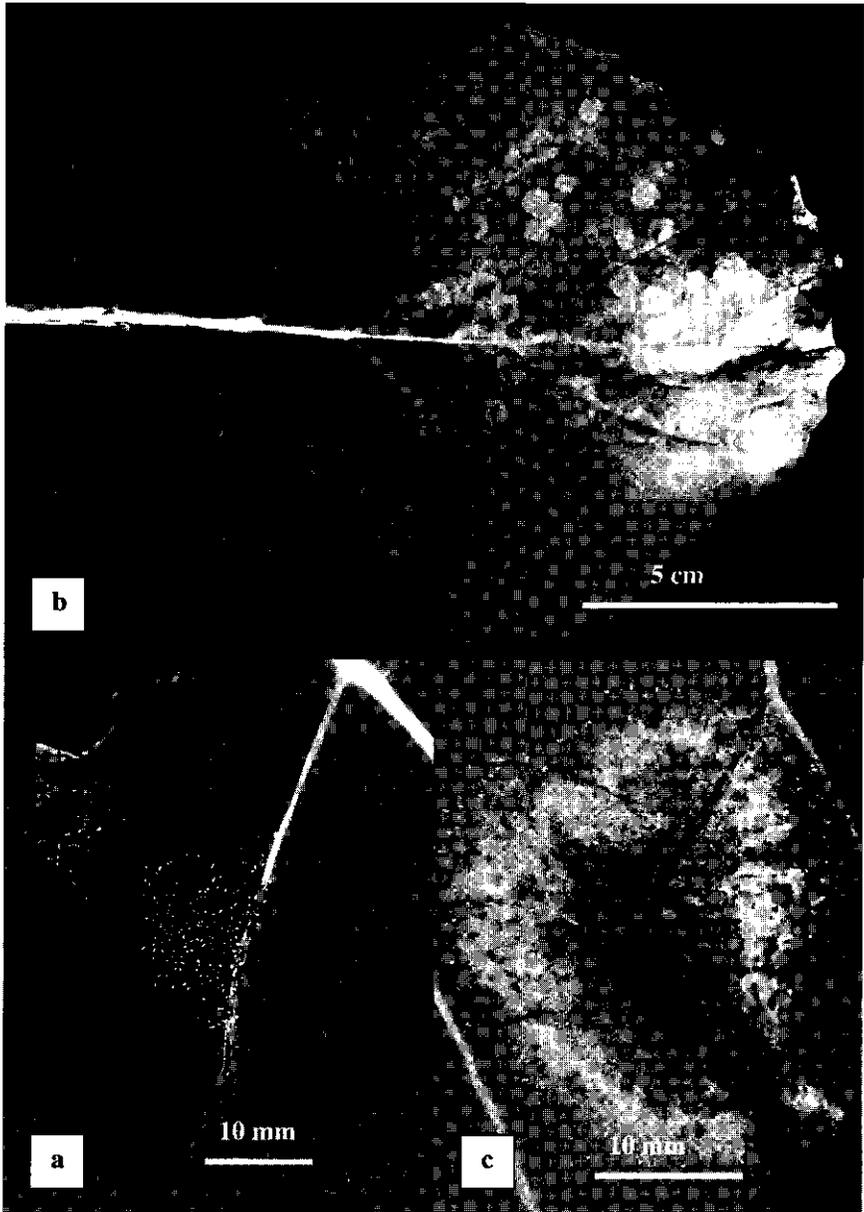
**Table 1** The temperature (Temp) and leaf wetness duration (LWD) treatments used in four experiments at IACR-Rothamsted investigating the conditions for the infection of oilseed rape (cv. Bristol) leaves by conidia of *P. brassicae*. In each experiment (Exp), the temperature treatments were randomised over the cabinets A, B, C, D, E, F and GA.

LWD (h)	6				10				16				24				48				72								
	Exp				Exp				Exp				Exp				Exp				Exp								
Temp (°C)	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
4												C				C				C				C				C	
6					B	-	-	-	B	B	C	-	-	B	C	-	B	B	C	-	-	B	C	-					
8					A	C	A	B	A	C	A	B	-	C	A	B	A	C	A	B	-	C	A	B					
12		F	F	D	E	F	F	D	E	F	F	D	E	-	F	D	E	F	-	D	E	-	-	D	E				
16		-	E	F	D	D	E	F	D	D	E	F	D	-	E	F	D	D	-	F	D								
20		-	D	G	F	E	D	G	F	E	D	G	F	-	D	G	F	-	-	G	F								
24		-	G	E	-	-	G	E	-	-	G	E	-	-	G	E	-	-	G	E	-								

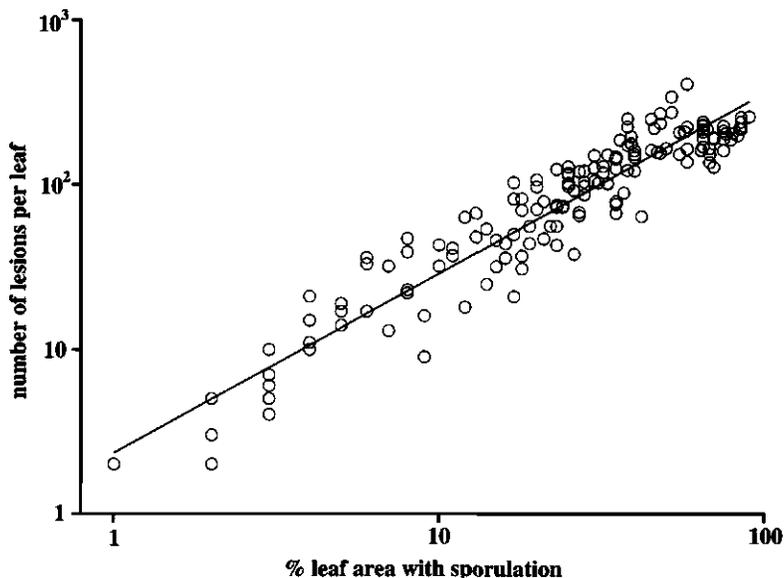
A Cabinets A and B were Gallenkamp 600H cabinets, cabinet C was a Gallenkamp Sanyo cabinet, and cabinets D, E, F and G were cabinets built at Rothamsted.

#### Assessment of light leaf spot

In experiments at IACR-Rothamsted, the severity of light leaf spot on oilseed rape was assessed at regular intervals by estimating visually the percentage leaf area with *P. brassicae* sporulation on leaves three and four. Sporulation, visible as white spore pustules (acervular conidiomata; Rawlinson *et al.*, 1978) in circular areas on the leaves (Fig. 1a), was the first visible sign of disease on the inoculated leaves. However, these circular areas of pustules on a leaf were often merged and could not be counted individually. The assessment of the severity of disease was, therefore, a visual estimate of % leaf area covered by sporulation. To estimate % leaf area with sporulation more accurately, a computer simulation training exercise was done (Nutter, 1997). Later, when leaves became senescent and began to turn a yellow or purple colour, individual 'lesions' became visible as green circular areas (Fig. 1b) and could be counted. The circular green islands on senescent leaves are referred to as 'lesions' in this paper. In two experiments, both % leaf area with sporulation was estimated and individual 'lesions' were counted on senescent leaves on which the 'lesions' were visible as separate individual 'lesions'. There was a linear relationship between the number of 'lesions' per leaf ( $n$ ) and % leaf area with sporulation ( $s$ ) (Fig. 2):  $\log_{10} n = 0.37 + 1.09 \log_{10} s$  (90.1% of the variance accounted for). This suggests that the visual assessment of % leaf area with sporulation was as appropriate a measurement of disease severity as the count of numbers of 'lesions'. On winter oilseed rape crops, white pustules are also the first symptoms of light leaf spot infection, but are usually difficult to see in the field (Fitt *et al.*, 1998a). The 'green islands' seen in controlled environment experiments, are not usually observed in crops. In crops, necrotic lesions are usually the first easily observed symptoms of light leaf spot (Fig. 1c). However, these necrotic lesions have not been reproduced in the controlled environment experiments at Rothamsted.



**Figure 1** Symptoms of light leaf spot on oilseed rape (cv. Bristol) leaves. On leaves inoculated with *P. brassicae* in controlled environment cabinets, the minute white spore pustules in small circular areas (a) were the first symptoms of infection to appear. The circular areas with sporulation became visible as circular 'green islands' when leaves senesced (b). In winter oilseed rape crops, necrotic lesions are generally observed some time after the white spore pustules become visible. For example, a circular necrotic lesion (c) surrounded by white pustules developed on a leaf of an oilseed rape (cv. Bristol) crop in February 1998 at Rothamsted.



**Figure 2** Relationship between the number of light leaf spot 'lesions' per leaf ( $n$ ,  $\log_{10}$ -scale) and the % leaf area with *P. brassicae* sporulation ( $s$ ,  $\log_{10}$ -scale) in controlled environment experiments at IACR-Rothamsted in which oilseed rape (cv. Bristol) leaves were inoculated with conidial suspensions of *P. brassicae*. The linear regression  $\log_{10} n = 0.37 + 1.09 \log_{10} s$  accounted for 90.1% of the variance ( $P < 0.001$ ).

In experiments at HRI-Wellesbourne, the severity of light leaf spot on oilseed rape plants was assessed 28 days after inoculation by counting the numbers of lesions per leaf on leaves one and two. Both 'green island'-type lesions (Fig. 1b) and necrotic lesions were observed and both types of lesion were included. As leaf wetness duration increased, the numbers of lesions per leaf had increased to a maximum by c. 90 h of leaf wetness duration on both cultivars. A mean maximum number of lesions per leaf was, therefore, calculated as an average of the numbers of lesions per leaf observed at leaf wetness durations of 90, 114 and 168 h.

The minimum leaf wetness duration required for infection is defined as the shortest leaf wetness duration at which infections occur. The minimum leaf wetness duration required for infection was estimated from assessments of % leaf area with sporulation on oilseed rape (cv. Bristol) at 4, 6, 8, 12, 16 or 20°C in the experiment at Rothamsted and from the numbers of lesions counted on Capricorn or Cobra at 8 or 16°C in the experiments at Wellesbourne.

The total number of conidia produced per leaf of cv. Bristol during asexual sporulation was measured by washing conidia off infected leaves (leaves three or four) when the leaves had begun to senesce (leaves had turned yellow). The number of conidia washed from a senescent leaf was considered to be an estimate of the total number of conidia produced during the infectious period, as the conidia, produced in mucilage, are not dispersed from the leaf surface

in the absence of water. The conidia were removed by shaking each leaf in 8 mL of a 1% solution of polyoxyethylene (20) sorbitan monolaurate (Tween 20; Merck, Poole) in a 30 mL glass vial. The concentrations of conidia in the leaf washings were counted with a haemocytometer. Conidial production on leaves was measured in experiments two and four at Rothamsted (Table 1). Latent period, infectious period, leaf size and the proportion of the leaf area covered by 'lesions' were also assessed, because these factors may affect conidial production. Latent period was estimated by recording the time period from inoculation of plants with conidia until the first spore pustules were observed on the leaf surface. In this experiment, infectious period was estimated as the time period from first sporulation until senescence of the leaf. It has never been investigated whether *P. brassicae* can continue to sporulate on senesced leaf tissues. The actual infectious period could, therefore, be longer than estimated in this experiment. Leaf area was estimated by placing over a leaf a transparent sheet with a 4.5 cm<sup>2</sup> grid and counting the number of squares covered.

### Data analysis

Data from the Rothamsted experiment for maximum % leaf area with sporulation, latent period and the number of conidia produced per leaf were analysed by residual maximum likelihood analysis ('REML' in Genstat 4.1), because of the unbalanced design (Table 1) (Payne *et al.*, 1993). The data for the maximum % leaf area with sporulation, the latent period and the number of conidia produced per leaf were logit-, log<sub>10</sub>- and log<sub>10</sub>-transformed, respectively, to stabilise the variance. Values of logit(maximum % leaf area with sporulation + 0.1), log<sub>10</sub>(latent period) and log<sub>10</sub>(conidia per leaf) at each temperature (*temp*) and leaf wetness duration (*lwd*) were predicted by fitting a model with the factors temperature and leaf wetness duration (*temp* × *lwd*) as the fixed effects and the factors experiment, cabinet, plant and leaf, describing the data structure, as the random effects. This adjusted the means for systematic experiment, cabinet, plant and leaf effects. Wald tests were used to test the fixed model terms. Where evidence of treatment differences was found, an approximation of the least significant difference between means was calculated by doubling the standard error of differences between means. An analysis of variance was done to calculate the standard error of differences between mean values of maximum number of lesions on leaves of cvs Capricorn or Cobra from the Wellesbourne data.

Log<sub>10</sub>(conidia per leaf) was regressed on log<sub>10</sub>(latent period), infectious period, leaf size or log<sub>10</sub>(proportion of leaf area covered by 'lesions') to investigate the relationships between these factors and conidial production on leaves of cv. Bristol in the Rothamsted experiment. Analyses of position and parallelism were done to investigate whether regression lines differed between different temperatures and different leaf wetness durations.

Gompertz curves ( $s = c \exp\left[-\exp\left[-\left(\frac{r \cdot e}{c}\right)(t - l)\right]\right]$ ) were fitted to scaled data for %

leaf area with sporulation (*s*) to describe the disease progress with days after inoculation (*t*) on cv. Bristol at Rothamsted. The data for % leaf area with sporulation were scaled to the predicted maximum % leaf area with sporulation for each temperature and leaf wetness duration. This scaling was used to give comparable data sets, so that Gompertz curves could be fitted simultaneously to data for the four experiments. By fitting the Gompertz curves, the parameters maximum % leaf area with sporulation (*c*), maximum rate of increase in % leaf area with sporulation per day (*r*), and day on which the rate of increase in % leaf area with sporulation was maximum (*l*) were estimated for each temperature and leaf wetness duration.

## Results

### *Infection of oilseed rape leaves by conidia of P. brassicae*

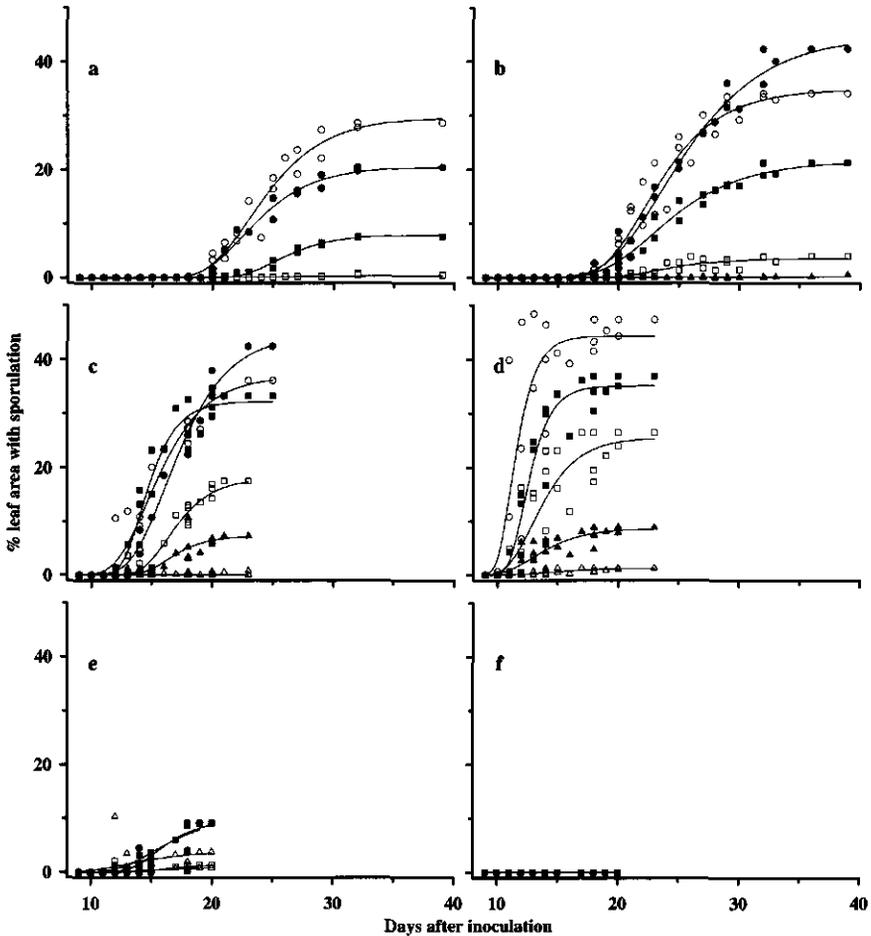
In the experiment at Rothamsted, pustules were observed on oilseed rape leaves (cv. Bristol) inoculated with conidia of *P. brassicae* at 4 to 20°C (Fig. 3), but were not observed on leaves inoculated at 24°C. Spore pustules were observed only after a leaf wetness duration of  $\geq 6$  h at 20, 16 or 12°C; this increased to  $\geq 10$  h,  $\geq 16$  h or  $\geq 24$  h at 8, 6 or 4°C, respectively (Table 2). The disease progress at each temperature/leaf wetness duration (LWD) was fitted well by the Gompertz function ( $\geq 72\%$  of the variance accounted for), except at 20°C/10 h LWD (only 24.5% of the variance accounted for). For the treatments 6°C/16 h LWD, 8°C/10 h LWD and 12°C/6 h LWD, the % leaf area with sporulation was near 0% and Gompertz curves could not be fitted to these data. The % leaf area with sporulation was, on average, greatest at 16°C (Fig. 4) and, on average, decreased as temperature increased to 20°C or decreased to 4°C. However, a decrease in temperature did not affect the % leaf area with sporulation if there was a long leaf wetness duration after inoculation. For example, the % leaf area with sporulation at 16°C/24 or 16 h LWD, 12°C/72, 48 or 24 h LWD, 8°C/72, 48 or 24 h LWD, 6°C/72 or 48 h LWD or 4°C/72 or 48 h LWD did not differ significantly from the % leaf area with sporulation at 16°C/48 h LWD. The % leaf area with sporulation, on average, decreased as leaf wetness duration decreased.

### *Latent period of P. brassicae*

The latent period of *P. brassicae* was, on average, shortest at 16°C and increased as temperature increased to 20°C (except at 6 h LWD) or decreased to 4°C. At 24 h leaf wetness duration after inoculation, for example, latent period was c. 10 days at 16°C (Fig. 5) and increased to c. 12 days as temperature increased to 20°C, or increased to 12, 17, 20 or 26 days as temperature decreased to 12, 8, 6 or 4°C, respectively. At 6 and 8°C, leaf wetness duration also affected the latent period. At 8°C, the latent period decreased from c. 25 to c. 16 days as leaf wetness duration increased from 10 h to 72 h; at 6°C, the latent period decreased from c. 23 to c. 17 days as leaf wetness duration increased from 16 to 72 h.

### *Effects of cultivar*

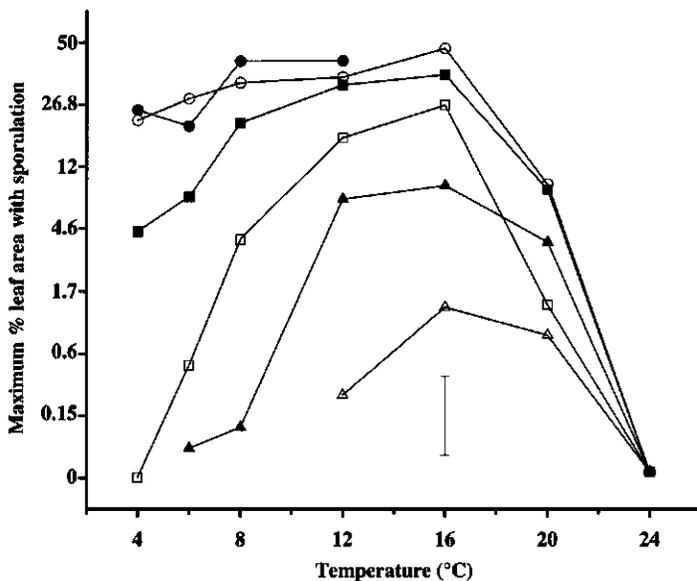
In the experiments at Wellesbourne, light leaf spot lesions were observed at 28 days after inoculation on some leaves of cv. Capricorn at 8 and 16°C when the leaf wetness duration was 3 h, but were not observed on cv. Cobra with leaf wetness durations less than 24 h (Table 2). The maximum number of lesions on cv. Capricorn was 3.5 or 3.8 lesions per leaf at 8 or 16°C, respectively, and on cv. Cobra was 1.3 or 1.1 lesions per leaf at 8 or 16°C, respectively (SED(435 df) = 0.27). Thus, the maximum number of lesions per leaf differed between cvs Capricorn and Cobra, but did not differ between temperatures of 8 and 16°C.



**Figure 3** Increase in % leaf area with sporulation ( $s$ ) with time (days after inoculation;  $t$ ) on oilseed rape (cv. Bristol) leaves inoculated with  $0.5 \times 10^6$  conidia per mL of *P. brassicae* at 6(a), 8(b), 12(c), 16(d), 20(e) or 24°C(f). Directly after inoculation leaves were kept wet for 6( $\Delta$ ), 10( $\blacktriangle$ ), 16( $\square$ ), 24( $\blacksquare$ ), 48( $\circ$ ) or 72 h( $\bullet$ ). To describe the increase in % leaf area with sporulation, Gompertz curves ( $s = c \exp\left\{-\exp\left[-\left(\frac{r-e}{c}\right)(t-l)\right]\right\}$ ) were fitted to the data at each temperature and leaf wetness duration. The parameters  $c$ ,  $r$  and  $l$  are the maximum % leaf area with sporulation, the maximum rate of increase in % leaf area with sporulation per day, and the day on which the rate of increase in % leaf area with sporulation is maximum, respectively.

**Table 2** Estimates of the minimum leaf wetness duration required for infection obtained by assessing sporulation on oilseed rape (cv. Bristol) plants inoculated at IACR-Rothamsted with  $0.5 \times 10^6$  conidia of *P. brassicae* per mL at 4, 6, 8, 12, 16 or 20°C or by assessing lesion development on oilseed rape (cvs Capricorn or Cobra) plants inoculated at HRI Wellesbourne with  $10^4$  conidia per mL at 8 or 16°C.

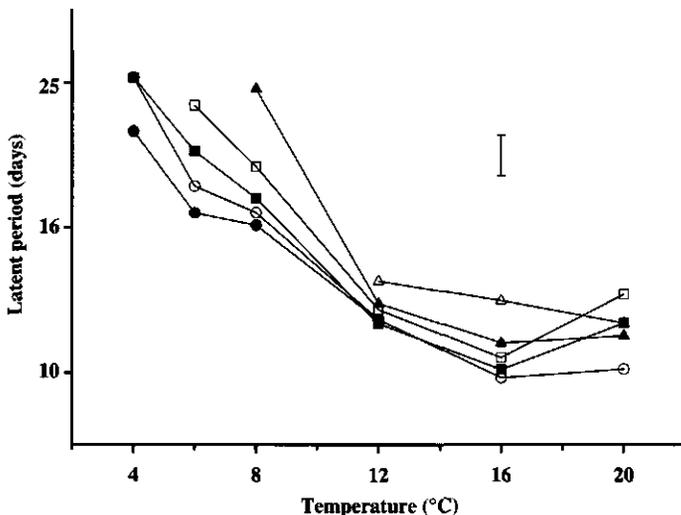
Cultivar	Temp (°C)	LWD (h)	No. leaves affected/total no. leaves	Min LWD
Bristol	4	16	0/8	16h<LWD≤24h
		24	6/7	
	6	10	0/10	10h<LWD≤16h
		16	11/18	
		24	7/8	
	8	10	4/26	LWD≤10h
		16	25/26	
		24	16/16	
	12	6	7/16	LWD≤6h
		10	25/26	
		16	25/26	
		24	16/16	
	16	6	9/16	LWD≤6h
		10	26/26	
		16	26/26	
		24	16/16	
20		6	10/16	
10	23/26			
16	21/26			
24	14/14			
Capricorn	8	0	0/40	0h<LWD≤3h
		3	4/40	
		18	11/40	
		24	23/40	
	16	30	23/40	0h<LWD≤3h
		0	0/40	
		3	15/40	
		18	12/40	
Cobra	8	24	19/38	18h<LWD≤24h
		30	19/39	
		0	0/40	
		3	0/40	
	16	18	0/40	18h<LWD≤24h
		24	3/40	
		30	4/40	
		0	0/40	
3		0/40		
18		0/40		
24	6/40	14/40		
	30			



**Figure 4** Maximum % leaf area with sporulation ( $s$ ) (y-axis:  $\text{logit}(s + 0.1)$ -scale) on oilseed rape (cv. Bristol) leaves inoculated with a *P. brassicae* conidial suspension of  $0.5 \times 10^6$  spores per mL at 4, 6, 8, 12, 16, 20 or 24°C. Directly after inoculation leaves were kept wet for 6(Δ), 10(▲), 16(□), 24(■), 48(○) or 72 h(●). The data points are predicted means, which have been corrected for systematic cabinet and experiment effects. The vertical bar is two times the standard error of the difference between means.

### Production of conidia

In the experiment at Rothamsted, the greatest numbers of conidia per leaf were produced at 12 to 16°C, and smaller numbers were produced at 8 or 20°C (Fig. 6a). The numbers of conidia produced per leaf increased as leaf wetness duration increased from 6 to 24 h (Fig. 6b). Regression analysis suggested that an increase in the length of the latent period ( $q$ ) was associated with a decrease in the numbers of conidia produced per leaf ( $p$ ) ( $\log_{10} p = 10.7 - 1.48 \log_{10} q$ ;  $P < 0.001$ , 16.4% of the variance accounted for) and that an increase in the length of the infectious period ( $i$ ) was associated with an increase in the numbers of conidia produced per leaf ( $\log_{10} p = 5.66 + 0.075i$ ;  $P < 0.001$ , 12.4% of the variance accounted for) (Fig. 7). However, both linear regressions explained little of the variation in the data. The numbers of conidia produced per leaf were not related to the size of the leaves. Linear relationships were observed between the numbers of conidia produced per leaf ( $\log_{10}$ -transformed) and the proportion of the leaf area covered by 'lesions' ( $f$ ) ( $\log_{10}$ -transformed). Analysis of position and parallelism suggested that the variation in the data was best explained by two non-parallel lines; one for 8, 12 and 16°C grouped ( $\log_{10} p = 8.10 + 1.24 \log_{10} f$ ;  $P < 0.001$ ) and one for 20°C ( $\log_{10} p = 7.44 + 1.59 \log_{10} f$ ;  $P = 0.025$ ), respectively (82.8% of the variance accounted for).

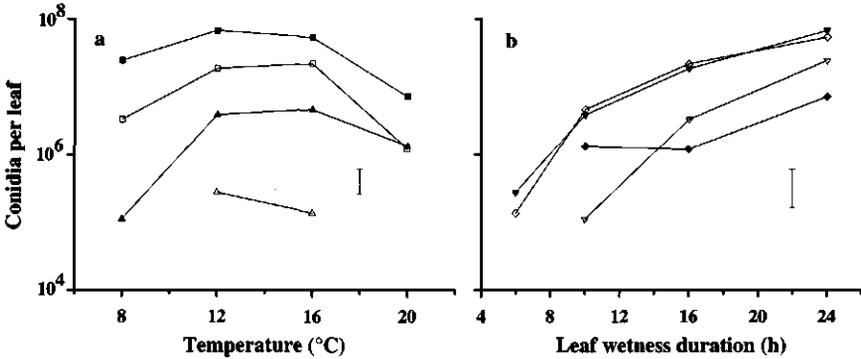


**Figure 5** Number of days from inoculation with conidia of *P. brassicae* to first sporulation (latent period; log<sub>10</sub>-scale) on oilseed rape (cv. Bristol) leaves at 4, 6, 8, 12, 16 or 20°C. Directly after inoculation, leaves were kept wet for 6(Δ), 10(▲), 16(□), 24(■), 48(○) or 72 h(●). The data points are predicted means, which have been corrected for systematic cabinet and experiment effects. The vertical bar is two times the standard error of the difference between means.

## Discussion

The results of the infection experiments at Rothamsted help to define the temperature and leaf wetness duration boundary conditions for the infection of oilseed rape leaves by conidia of *P. brassicae*. The results suggest that successful infections on cv. Bristol can occur from 4 to 20°C, but not at or above 24°C (Fig. 3). However, temperatures of c. 24°C or greater do not occur frequently in the autumn, winter or spring when light leaf spot is spreading in winter oilseed rape in the UK. Such temperatures, therefore, only rarely limit infection. At temperatures of 4 to 20°C, infections are likely to be successful only if leaf wetness duration is longer than a minimum length of time. The minimum leaf wetness duration required for infection is temperature-dependent, being c. 6 h, at 12–20°C, and increasing to c. 10 h, 10–16 h, or 16–24 h as temperature decreases to 8, 6 or 4°C, respectively (Table 2). Under natural conditions, if leaf wetness duration is shorter than the minimum required for infection, conidia may survive (Maddock & Ingram, 1981) to infect leaves when leaf wetness duration becomes sufficient, as for *Mycosphaerella pinodes* on pea seedlings (Roger *et al.*, 1999). Measurements of leaf wetness duration using wetness sensors (McCartney & Lacey, 1990) in winter oilseed rape crops at Rothamsted in the 1990/91 and 1991/92 seasons (Figures 4 and 5 in Figueroa *et al.*, 1995b) suggest that, during the UK growing season, leaf wetness duration is frequently longer than the minimum leaf wetness duration required for infection of cv. Bristol. However, the minimum leaf wetness duration required for infection can differ between cultivars, as the results of the experiments at Wellesbourne suggest. The occurrence

and duration of periods during a growing season when conditions are suitable for infection, therefore, also depend on the cultivar sown. Thus, leaf wetness duration is more likely to be a factor limiting infection by conidia of *P. brassicae* for oilseed rape cultivars requiring long leaf wetness durations for infection.



**Figure 6** The effects of temperature (a) and leaf wetness duration (LWD) (b) on the total number of conidia produced per leaf (log<sub>10</sub>-scale) of oilseed rape (cv. Bristol) inoculated with 0.5 × 10<sup>9</sup> conidia per mL of *P. brassicae* at 6(Δ), 10(▲), 16(□) or 24 h LWD(■), and 8(▽), 12(▼), 16(◇) or 20°C(◆), respectively. The total number of conidia produced per leaf was the number of conidia produced between infection and senescence of a leaf. The data points are predicted means, which have been corrected for systematic cabinet and experiment effects. The vertical bars are two times the standard error of the difference between means.

The results of the Rothamsted experiment suggest that not only temperature but also leaf wetness duration affects the latent period of *P. brassicae*. These results suggest that the latent period is shortest at c. 16°C, and increases as temperature increases from 16 to 20°C or as temperature decreases from 16 to 4°C (Fig. 4), and are supported by the results of Figueroa *et al.* (1995a) at 5 to 20°C. Furthermore, these results suggest that the latent period decreases as leaf wetness duration increases at colder temperatures (6–8°C). The maximum number of asexual generations that could occur in an infected oilseed rape crop during an average season can be estimated by using 30-year averages of UK monthly temperatures (Table 3). On average, it has been estimated that c. 17 asexual generations can occur from early October until late June in an average UK growing season. This estimate is based on the assumptions that spores are dispersed instantly when a plant becomes infectious and instantly infect the new plants on which they are deposited. However, in winter oilseed rape crops, the number of asexual generations is likely to be less than this estimate, due to periods without rainfall when conidia are not dispersed from infectious plants and periods when dispersed conidia cannot infect plants due to sub-optimal infection conditions.

The Rothamsted results suggest that the production of conidia is greatest at c. 12–16°C and decreases when temperature decreases or increases, and that the number produced increases when leaf wetness duration increases from 6 to 24 h (Fig. 6). In a crop, an increase in the production of conidia would be expected to increase the number of new lesions produced after each splash-dispersal event (Zadoks & Schein, 1979). The temperature and leaf wetness duration during conidial infection of oilseed rape crops could thus affect the number of new

infections in the next asexual generation of *P. brassicae* and thereby affect the epidemic progress in crops.

**Table 3** Estimated number of asexual generations of *P. brassicae* that can occur during a winter oilseed rape growing season when primary infection in the crop has occurred at the end of September, under the condition that conidia are dispersed directly after plants become infectious and the dispersed conidia infect plants directly.

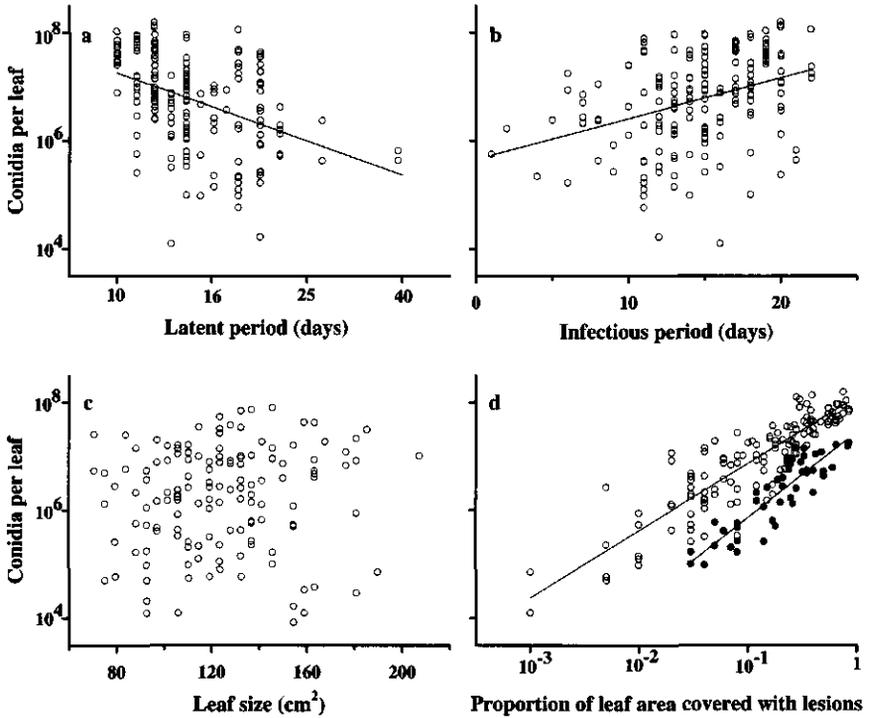
month	average temp <sup>a</sup>	latent period <sup>b</sup>	asexual generations
Oct	10.5	13.5	2.3
Nov	6.5	16.4	1.8
Dec	4.5	21.8	1.4
Jan	4.0	21.8	1.4
Feb	4.0	21.8	1.3
Mar	6.0	16.4	1.9
Apr	8.0	15.6	1.9
May	11.0	13.5	2.3
Jun	14.0	11.0	2.7

<sup>a</sup> 30-year averages of UK temperatures from 1961 to 1990 (data source Meteorological Office, <http://www.met-office.gov.uk/sec3/1998/temp.html>)

<sup>b</sup> the lengths of the latent period were estimated from Fig. 4

The analysis of the conidial production data for the Rothamsted experiment suggests that the number of conidia produced on a leaf is related to the proportion of the leaf area covered by 'lesions' (Fig. 7d). Although this relationship was derived in a controlled environment experiment, a similar relationship may apply between % leaf area affected with light leaf spot in a crop and the number of conidia produced in that crop. Fitt *et al.* (1998b) have found that % leaf area affected with light leaf spot in a crop is linearly related to % leaves affected or % plants affected in that crop. Therefore, assessment data for % leaves or % plants in a crop affected by light leaf spot could also be related to the number of conidia produced in that crop. If such a relationship exists, then this could be used to predict the increase in disease severity after each splash-dispersal of asexual spores.

The results of the controlled environment experiments done at Rothamsted provide evidence that temperature and leaf wetness duration affect the success of an infection, the latent period and the asexual sporulation of *P. brassicae*. However, caution must be taken in generalising the results on the effects of temperature and leaf wetness duration on oilseed rape (cv. Bristol), when considering other cultivars or plants at other developmental stages. The Wellesbourne experiments with cvs Capricorn and Cobra suggested that the minimum leaf wetness duration required for infection and the number of lesions produced might differ between cultivars. Figueroa *et al.* (1995a) also found differences between oilseed rape cultivars in the severity of light leaf spot and the production of conidia on leaves. Experiments in New Zealand on cauliflower showed that leaf age could also affect infection by conidia (Hartill & Cheah, 1984). Thus, the relationships between temperature, leaf wetness duration and success of infection, length of the latent period and number of conidia produced per leaf require further experimental study under natural conditions in crops of different cultivars at different developmental stages. Eventually, weather parameters such as temperature and leaf wetness duration could be incorporated into forecasting models to predict the risk of severe light leaf spot epidemics in different seasons, in different regions and for individual crops.



**Figure 7** The effects of latent period ( $q$ ;  $\log_{10}$ -scale) (a), infectious period ( $i$ ) (b), leaf size (c) and proportion of the leaf area covered by 'lesions' ( $f$ ;  $\log_{10}$ -scale) (d) on the total number of conidia produced per leaf ( $p$ ;  $\log_{10}$ -scale) of oilseed rape (cv. Bristol) inoculated with  $0.5 \times 10^8$  conidia per mL of *P. brassicae*.  $\log_{10}(\text{conidia per leaf})$  regressed linearly on  $\log_{10}(\text{latent period})$  ( $\log_{10} p = 10.7 - 1.48 \log_{10} q$ ;  $P < 0.001$ , 16.4% of variance accounted for), on infectious period ( $\log_{10} p = 5.66 + 0.075i$ ;  $P < 0.001$ , 12.4% of variance accounted for), and on  $\log_{10}(\text{proportion of the leaf area covered by 'lesions' at 8, 12 or 16}^\circ\text{C})$  ( $\log_{10} p = 8.10 + 1.24 \log_{10} f$ ;  $P < 0.001$ ) or at  $20^\circ\text{C}$  ( $\bullet$ ) ( $\log_{10} p = 7.44 + 1.59 \log_{10} f$ ;  $P = 0.025$ ) (82.8% of variance accounted for).

**Acknowledgements**

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## Chapter 7

### Modelling the effects of temperature and wetness duration on development of light leaf spot on oilseed rape leaves inoculated with *Pyrenopeziza brassicae* conidia

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#### Abstract

A model was developed to describe the effects of temperature and leaf wetness duration on the development of light leaf spot on oilseed rape (cv Bristol) leaves inoculated with *Pyrenopeziza brassicae* conidial suspensions. A Gompertz function was used to describe the progress with time in % leaf area with sporulation, and included the parameters maximum % leaf area with sporulation ( $c$ ), maximum rate of increase in % leaf area with sporulation ( $r$ ) and latent period ( $l$ ; estimated as the time from inoculation until the time when 37% of maximum % leaf area with sporulation was reached). The effects of leaf wetness duration on  $c$  and  $r$  were also described with Gompertz functions, which included the parameters minimum leaf wetness duration ( $v_c$  or  $v_r$ ) and maximum of  $c$  ( $m_c$ ) or maximum of  $r$  ( $m_r$ ). The effects of temperature on  $m_c$ ,  $v_c$  and  $v_r$  were described by quadratic functions, and the effect of temperature on  $m_r$  was described by a linear function. The combined model, describing the progress with time in % leaf area with sporulation and including the effects of temperature and leaf wetness duration on the parameters  $c$ ,  $r$  and  $l$ , generally fitted well to the observed data for progress with time in % leaf area with sporulation in controlled environment experiments at different temperatures and leaf wetness durations that were used to estimate the model parameters. The latent periods predicted by the model fitted well to latent periods observed on oilseed rape (cv Cobra) in an independent experiment, but model predictions for % leaf area with sporulation did not fit well to the observed data. The model estimates for latent period were used to predict the dates when large increases in light leaf spot severity occurred in experiments at Rothamsted on winter oilseed rape (cv Bristol) under natural conditions. In the autumn/winter of the 1998/99 and 1999/2000 seasons, the occurrence of the greatest daily increases in % leaf area affected by light leaf spot were successfully predicted, using latent periods derived with the model and starting from dates when there was  $\geq 2$  mm of rain per h for  $\geq 0.5$  h.

**Keywords:** asexual generations, disease forecasting, disease progress, latent period, polycyclic disease, splash dispersal

## Introduction

Models describing the effects of weather factors on stages in the life cycle of *Pyrenopeziza brassicae* are needed as a basis for developing crop-specific forecasts of light leaf spot severity to guide farmers making decisions about control of the disease in winter oilseed rape (*Brassica napus*) crops in the UK (Fitt *et al.*, 1996; Welham *et al.*, 1999). Infection by conidia, the latent period and asexual sporulation of *P. brassicae* are important biological processes that contribute to progress with time in light leaf spot severity in winter oilseed rape crops and are affected by a complex interaction between the weather factors temperature and leaf wetness duration. The spatial pattern of light leaf spot in crops (Evans *et al.*, 1999b; Fitt *et al.*, 1998a) suggests that, after primary infections by the wind-dispersed ascospores in the autumn (Gilles & Fitt, 1999), light leaf spot is spread by successive cycles of conidial infections following splash dispersal of conidia over short distances during rain showers (Fatemi & Fitt, 1983). Light leaf spot is thus a polycyclic disease. Infection occurs as conidia germinate and directly penetrate the leaf cuticle by means of germ tubes (Rawlinson *et al.*, 1978). In experiments, the proportion of conidia which germinated increased as temperature increased from 5 to 20°C or as wetness duration increased from 12 to 80 h (Figuroa *et al.*, 1995a). For infections to be successful, the leaf wetness duration needed to be longer than a certain minimum, which was affected by temperature (Figuroa *et al.*, 1995b; Gilles *et al.*, 2000b). At 12 to 20°C, a minimum leaf wetness duration of *c.* 6 h was required for infection, and increasingly longer minimum leaf wetness durations of *c.* 10, *c.* 16 or *c.* 24 h were required for infection when temperature decreased to 8, 6 or 4°C, respectively. At temperatures  $\geq 24^\circ\text{C}$  infection did not occur.

After infection has occurred, the latent period of *P. brassicae*, which can be considered as the time from infection until the first sporulation, is also affected by temperature. Figuroa *et al.* (1995a) fitted a negative linear relationship between the length of the latent period in days and temperature in the range 5 to 15°C (Figuroa *et al.*, 1995a), although this is an approximation of a non-linear relationship. It was also suggested that an increase in leaf wetness duration may decrease the length of the latent period, estimated as the time from inoculation until sporulation, at lower temperatures (6 and 8°C; Gilles *et al.*, 2000b). Gilles *et al.* (2000b) also found that the % leaf area with sporulation could be directly related to the number of conidia produced per leaf, was greatest on leaves incubated at *c.* 16°C, and decreased as temperature increased to 24°C or decreased to 4°C. An increase in leaf wetness duration after inoculation from 6 to 24 h also increased the % leaf area with sporulation and the number of conidia produced. This paper reports the development of a model which describes the effects of temperature and leaf wetness duration on the progress with time in light leaf spot severity, the testing of this model on controlled environment data and an application of the latent period equation to predict the progress of light leaf spot under natural conditions.

## Materials and methods

### *The experimental data*

Data for progress with time (days) after inoculation in % leaf area with sporulation were obtained from an experiment described by Gilles *et al.* (2000b), in which leaves of oilseed rape plants at the 4-5 leaf growth stage (GS 1,4 to 1,5; Sylvester-Bradley & Makepeace, 1985) were inoculated with  $0.5 \times 10^6$  conidia per mL of *P. brassicae* and kept in controlled environment cabinets at 4, 6, 8, 12, 16, 20 or 24°C with 6, 10, 16, 24, 48 or 72 h of leaf wetness after inoculation. Trays containing four plants in pots were placed in these cabinets. The experimental design was a split-plot design, in which the effects of temperature were tested on the main plots (i.e. the cabinets) and the effects of leaf wetness duration and its interaction with temperature were tested on the sub-plots (i.e. the trays within the cabinets). The different leaf wetness duration treatments were randomly applied to the trays within each cabinet. Directly after inoculation, the plants were enclosed in polyethylene bags to maintain continuous wetness; when the bags were removed after wetness treatments were finished, leaf surfaces dried within 0.5 h. The different temperature treatments were applied to the different cabinets in each of the series of four experiments. However, since certain temperatures could not be obtained in all cabinets, randomisation of temperature treatments across cabinets was not complete. Not all the treatments were repeated in all the four experiments, giving an unbalanced design for analysis (Table 1, Gilles *et al.*, 2000b). The % leaf area with sporulation was estimated visually on leaves three and four of each individual plant.

### *% leaf area with sporulation as a function of time*

In each experiment, each temperature and leaf wetness duration treatment combination was applied to a tray with four plants, on each of which two leaves were assessed at different times. Thus, eight time series were obtained for each treatment combination in each experiment. The Gompertz function with zero intercept,  $s = c \exp\{-\exp[-b(t-m)]\}$ , as implemented in the statistical software Genstat 5™ Release 4.1 (Payne *et al.*, 1993; Oude Voshaar, 1994), with unknown parameters  $b$ ,  $m$  and  $c$  was fitted to the data for % leaf area with sporulation ( $s$ ) with time ( $t$ ) as the explanatory variable. A re-parameterisation of the function as  $m = l$  and  $b = \frac{2.72r}{c}$  was used to rewrite the Gompertz function as

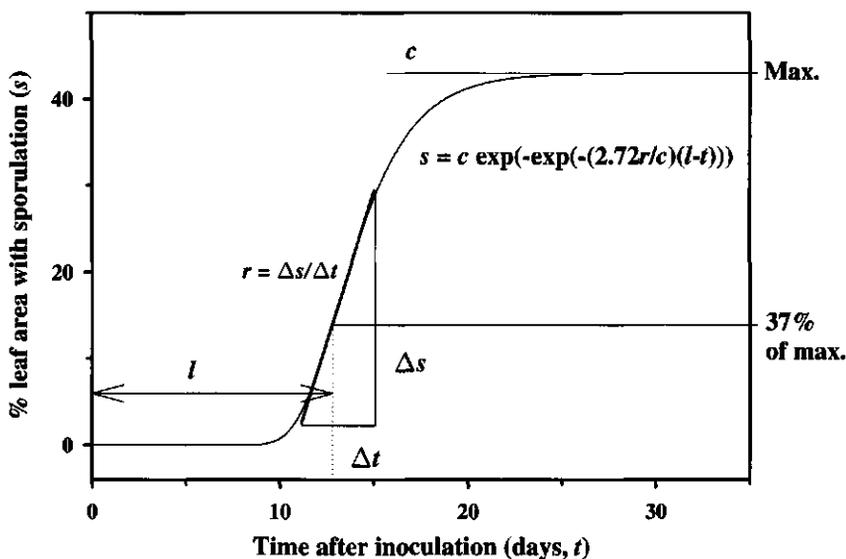
$$s = c \exp\left\{-\exp\left[-\left(\frac{2.72r}{c}\right)(t-l)\right]\right\} \quad (1)$$

to describe the disease development in terms of the biologically meaningful parameters latent period ( $l$ ), maximum rate of increase in sporulation ( $r$ ) and maximum % leaf area with sporulation ( $c$ ) (Figure 1). The constant value 2.72 is the base value of the natural logarithm. The parameter for latent period ( $l$ ) in this function estimated latent period as the time from inoculation until the time when 37% of the maximum % leaf area with sporulation was reached. This was the point of inflection of the Gompertz curve. At this time the increase in % leaf area with sporulation was greatest.

Estimates and approximate standard errors for  $c$ ,  $r$  and  $l$  were obtained from the Gompertz function fitted to data for disease progress with time. The standard errors (se) were used to give weighting to the estimated parameters in calculating the average treatment values of these parameters for each experiment. For example, for parameter  $c$ , the average was

calculated as:  $\frac{\left(\frac{1}{se_1}c_1 + \frac{1}{se_2}c_2 + \dots + \frac{1}{se_8}c_8\right)}{\left(\frac{1}{se_1} + \frac{1}{se_2} + \dots + \frac{1}{se_8}\right)}$ . Since the experimental design was unbalanced,

the weighted average values of  $l$ ,  $r$  and  $c$  for each treatment were analysed by residual maximum likelihood ('REML' in Genstat 5 Release 4.1) analysis, with temperature  $\times$  leaf wetness duration as fixed effects and experiment/cabinet as random effects, to predict the mean values of  $l$ ,  $r$  and  $c$ . This adjusted the predicted mean values of  $l$ ,  $r$  and  $c$  for systematic experiment and cabinet effects. The residual values of the predicted mean values of  $l$ ,  $r$  and  $c$  were approximately normally distributed with equal error variance and transformations were not needed. This REML analysis was also used to assess the potential effect of differences between cabinets on the results if these effects had not been eliminated.



**Figure 1** The % leaf area with sporulation ( $s$ ) on oilseed rape leaves inoculated with conidia of *Pyrenopeziza brassicae* (light leaf spot) described as a function of time ( $t$ ) with a Gompertz function ( $s = c \exp(-\exp(-\frac{2.72r}{c})(t-l))$ ); 2.72 is the base value of the natural logarithm). The

function was based on the parameters maximum % leaf area with sporulation ( $c$ ), maximum rate of increase in % leaf area with sporulation ( $r$ ) and latent period ( $l$ ). The latent period was estimated as the time from inoculation until the time when 37% of the maximum % leaf area with sporulation was reached.

### Functions to describe effects of temperature and leaf wetness duration on the parameters $c$ , $r$ and $l$

Response surface methods were used to obtain expressions for the parameters  $c$ ,  $r$  and  $l$  in terms of both temperature and leaf wetness duration. For both maximum % leaf area with sporulation ( $c$ ) and maximum rate of increase in sporulation ( $r$ ), examination of the predicted means from REML analysis indicated that as leaf wetness duration ( $W$ ) increased there were sigmoidal increases in values of these parameters to a maximum ( $m$ ) starting from a minimum leaf wetness duration ( $v$ ), which differed between temperatures. Therefore, Gompertz functions of the form  $m \exp\{-\exp[-q(W-v)]\}$  were fitted to the weighted average values of  $c$  at temperatures from 4 to 24°C and to the weighted average values of  $r$  at temperatures from 4 to 20°C (% leaf area affected was 0 at 24°C; thus  $r$  could not be estimated). Analysis of position and parallelism was used to investigate whether the parameters  $m$  and  $v$  differed between temperatures and a constant value for  $q$  was fitted for all temperatures. Where there was evidence that different parameters were required for different temperatures, the estimated parameters were approximately described as either linear or quadratic functions of temperature, leading to functions of the form

$$r(t) = m(T) \exp\{-\exp[-q(W-v(T))]\} \quad (2)$$

An identical equation was used to describe  $c(T)$ . This function estimated the minimum leaf wetness duration required for infection ( $v$ ) (Gilles *et al.*, 2000b) as the leaf wetness duration at which the disease severity reached 37% of the maximum value for  $c$  ( $m_c$ ) or  $r$  ( $m_r$ ). The parameter  $v$ , therefore, over-estimated the actual minimum leaf wetness duration required for infection. The parameter  $q$  was related to the maximum rate of increase in  $c$  or  $r$  with increasing leaf wetness duration (the point of inflection of the Gompertz curve).

The relationship between latent period and temperature over the range 4 to 20°C was approximately quadratic. Therefore, a function with linear and quadratic temperature terms was fitted to the weighted average values of  $l$ . Analysis of position and parallelism was used to determine if separate parameters were required to describe the pattern for different wetness durations, and differences in intercept due to wetness were described by a linear form. Thus, the latent period ( $l$ ) was described as a function of wetness duration ( $W$ ) and temperature ( $T$ ):

$$l(W, T) = d_1 - d_2 T + d_3 T^2 - d_4 W \quad (3)$$

with parameters  $d_1$ ,  $d_2$ ,  $d_3$  and  $d_4$ . Finally, the functions describing the progress with time in the % leaf area with sporulation and the functions describing the relationships between parameters  $c$ ,  $r$  and  $l$  and temperature and leaf wetness duration were combined into a model describing the effects of temperature and leaf wetness duration on the progress with time in light leaf spot severity on oilseed rape (cv. Bristol) leaves.

### Testing of the model

The predictions made by the model of progress with time after inoculation with conidia in % leaf area with sporulation were compared with data obtained from an independent experiment described by Figueroa *et al.* (1995b). In that experiment, the numbers of lesions were counted on leaves of oilseed rape (cv. Cobra) plants that were inoculated at the six-leaf stage with *P. brassicae* conidial suspensions (c.  $2 \times 10^6$  conidia per mL) in controlled environment cabinets at 12 or 18°C and with 16 or 48 h of leaf wetness after inoculation. The 'lesions' which were observed on the plants in controlled environments were not necrotic lesions, as observed in affected crops, but circular areas on a leaf which remained green during leaf senescence (Figure 1, Gilles *et al.*, 2000b). For each treatment, the numbers of 'lesions' were counted on three leaves per plant of three plants. The leaf wetness duration treatments were done three times. The temperature treatments were not replicated. The average values for the numbers of

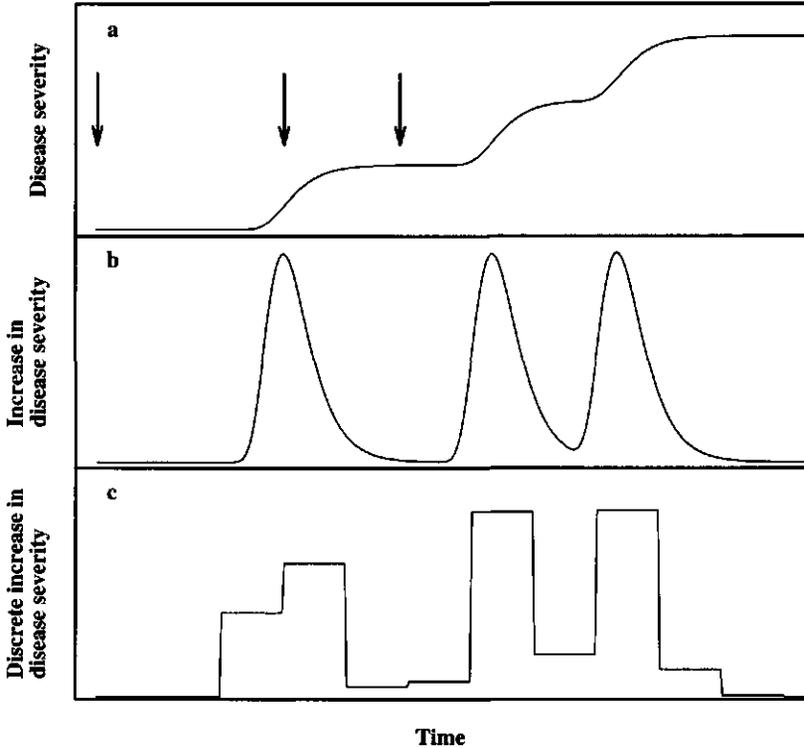
'lesions' per leaf obtained were converted to values for % leaf area with sporulation by using a linear relationship between the % leaf area with sporulation ( $s$ ) and the number of 'lesions' per leaf ( $n$ ):  $\log_{10} n = 0.37 + 1.09 \log_{10} s$  (90.1% of the variance accounted for,  $P = 0.001$ ; Gilles *et al.*, 2000b). The values estimated from the experiment of Figueroa *et al.* (1995b) were compared with the values predicted by the model for % leaf area with sporulation for *P. brassicae* conidial infections at 12 or 18°C with 16 or 48 h of leaf wetness after inoculation.

The latent period predictions were also compared with observed light leaf spot progress under natural conditions. The progress with time in % leaf area affected by light leaf spot was studied in detail on groups of 24 oilseed rape (cv. Bristol) plants grown in 12.5 cm pots (one plant per pot) outdoors during the autumn and winter of the 1998/99 and 1999/2000 seasons. In 1998, the seeds were sown in the glasshouse on 23 September and plants in pots were moved outside on 20 October. The plants were inoculated on 21 October by spreading between the pots stem and pod debris, which had been obtained after harvest of a winter oilseed rape crop affected with light leaf spot at Rothamsted in the summer of 1998. On 17 November, more debris was spread between the plants. In 1999, seeds were sown in the glasshouse on 11 August. Debris, which had been obtained after harvest of an affected crop at Rothamsted in the summer of 1999, was spread outside on 27 August before the plants in pots were moved outside on 15 September. The period between 27 August and 15 September 1999 was very dry and ascospores, therefore, were expected to have been immature when the plants were placed near the debris, because periods for which the debris had been wet had been insufficient for them to mature (Gilles & Fitt, 1999).

The % leaf area affected by light leaf spot was assessed approximately weekly on each individual leaf of each plant. Development of new leaves with time was assessed by marking leaves with small metal rings folded around the petioles when they emerged. A leaf was considered to have emerged when its petiole started to elongate and could be ringed. At this stage, the leaf lamina had unfolded, but was still small and increasing in size. The senescence of these marked leaves was also assessed. The % leaf area affected by light leaf spot ( $s$ ), including the % leaf area that was lost due to leaf senescence, was estimated for all leaves in each assessment. These data were used to calculate the increases in disease severity (% leaf area affected) between each assessment in order to identify occasions when there were large increases in severity (Fig. 2). For a polycyclic disease, the observed disease severity is the sum of disease caused by each individual infection event. A large increase in disease severity between assessment dates is likely to be the result of many individual infection events which have occurred at the same time. For light leaf spot epidemics propagated by splash-dispersed conidia, such an increase in disease severity is likely to occur after a rain event which is able to disperse the conidia widely and maintain leaf wetness duration for a sufficient length of time for infections to occur. The increase in % leaf area affected by light leaf spot was calculated by dividing the difference in the % leaf area affected summed over all leaves between assessment date  $a$  ( $s_a$ ) and the previous assessment date  $a-1$  ( $s_{a-1}$ ) by the total number of leaves on the 24 plants on assessment date  $a$  ( $p_a$ ). Since this increment in disease resulted from infections which occurred before date  $a$ ,  $s_a - s_{a-1}$  should actually have been divided by the number of leaves that were present at the times when infections occurred. The exact times when infections occurred were not known in these experiments. Nevertheless, dividing  $s_a - s_{a-1}$  by the number of leaves at assessment date  $a$  is acceptable, because the variation in the number of leaves over the periods that were assessed was small (on average, there were 5.1–5.6 leaves per plant in 1998/1999 and 4.2–4.8 leaves per plant in 1999/2000). As the intervals between assessments were not always exactly 7 days, the increase in % leaf area affected by light leaf spot between successive assessment dates was divided by the number of days between successive assessment dates ( $t_a - t_{a-1}$ ).

$$\frac{(s_a - s_{a-1})}{Pa(t_a - t_{a-1})} \quad (4)$$

to calculate the daily increase in % leaf area affected by light leaf spot.



**Figure 2** Schematic diagram of progress with time in disease severity for a polycyclic disease epidemic (a) to illustrate large increases in disease severity caused by three infection events (arrows) (b) and resulting increases in disease severity measured by assessments at discrete intervals (c).

Data from these two pot experiments were used to test the model predictions of progress of light leaf spot. The derived expression for latent period in terms of wetness duration and temperature (equation 3) was used to predict the time from a rain shower, which dispersed conidia by splash, until the increase in % leaf area with sporulation as a result of the resulting infection, was maximum (steepest slope in the Gompertz curve). These predicted latent periods are actually over-estimates of the latent period, because they include both the time from infection to sporulation (latent period) and the time from conidial dispersal to germination and infection. However, in this paper these predicted times are referred to as latent period predictions. These comparisons were done with predicted latent periods starting with conidial dispersal, either from all rain periods or from only 'heavy' rain periods. To

distinguish a 'heavy' rain period from other periods of rain, an arbitrary value of  $\geq 2$  mm of rain per h for rain intensity and an arbitrary value of  $\geq 0.5$  h for rain duration were chosen. The rain intensity and rain duration were read from daily rain intensity charts, which were obtained from the Rothamsted meteorological station, less than 0.5 km from the experiment. Data for daily average temperature were also obtained from this meteorological station. The time from a rain period until the time when a maximum in the increase in % leaf area with light leaf spot was predicted was compared with the time when there was a large increase in the % leaf area affected by light leaf spot on the leaves of the plants outdoors. When there were periods of 'heavy' rain on successive days, only the first period of rain after a few days without rain was used to predict the large increase in disease. Rainfall events which occurred before the first sporulation of *P. brassicae* occurred were not used for predictions.

Since leaf wetness duration ( $W$ ) was not measured on plants in the pot experiments in the 1998/99 and 1999/2000 seasons, an arbitrary value for  $W$  had to be chosen to insert into the model. In the controlled environment experiments on which the model is based (Gilles *et al.*, 2000b), plants were inoculated with concentrated conidial suspensions ( $0.5 \times 10^6$  conidia per mL) and leaf wetness duration was considered to limit the proportion of these conidia that could germinate and infect a leaf, because a decrease in leaf wetness duration was found to decrease the % leaf area with sporulation. In the 1998/99 and 1999/2000 experiments with plants in pots outdoors, it is likely that numbers of infections were limited by the small numbers of conidia deposited on the surface of a leaf after splash-dispersal by rain. A short leaf wetness duration of 6 h was, therefore, fitted into equation 3 to reflect the limitation of infections by small numbers of conidia available in the pot experiments. The actual wetness durations on leaves of these pot plants outdoors following rain events causing splash-dispersal of conidia were assumed to have been sufficiently long for infections to occur.

For each day following a rainfall event ( $t$ ), the daily fulfilment of the latent period was calculated as the reciprocal of the latent period ( $l_t$ ) predicted by equation 3 by inputting into the equation daily average temperatures ( $T_t$ ) and 6 h for leaf wetness duration ( $W = 6$ ),

$$\frac{1}{l_t} = \frac{1}{d_1 - d_2 T + d_3 T^2 - d_4 W} \quad (5)$$

The length of the latent period ( $l$ ) was predicted by summing the values for daily fulfilment of the latent period until the value 1 was reached:

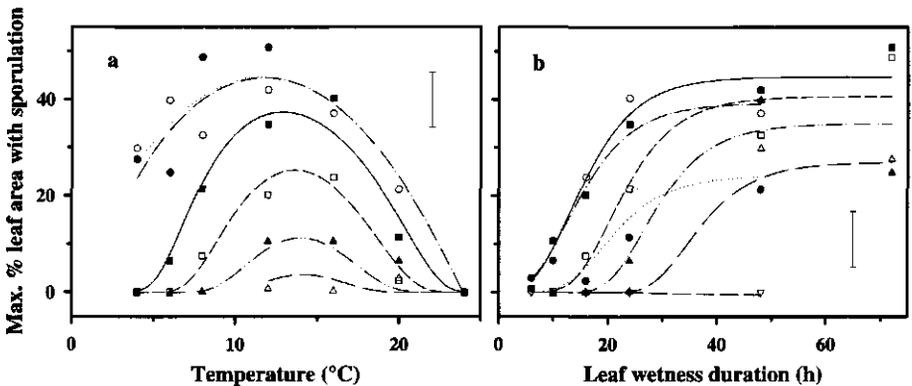
$$\sum_{t=1}^l \frac{1}{l_t} = 1 \quad (6)$$

## Results

### The model

A modified Gompertz function (equation 1; Table 1) fitted the data for the progress with time ( $t$ ; days) in % leaf area with sporulation ( $s$ ) on oilseed rape leaves inoculated with *P. brassicae* conidia and estimates of  $c$ ,  $l$  and  $r$  were obtained for each temperature and leaf wetness duration treatment. Another Gompertz function (equation 2, Figure 3) fitted the effects of leaf wetness duration ( $W$ ) on the weighted average values of  $c$  and gave estimates of  $v_c$  and  $m_c$  for each temperature and a value for the constant  $q_c$ . The effects of temperature ( $T$ ) on both  $v_c$  and  $m_c$  were fitted by quadratic relationships ( $P = 0.011$  and  $P < 0.001$ , respectively), which were, therefore, incorporated into the model:

$$c = (3.65 + 7.02T - 0.30T^2) \exp\left\{-\exp\left\{-0.15\left[W - (55.47 - 6.08T + 0.21T^2)\right]\right\}\right\} \quad (7)$$



**Figure 3** Effects of temperature and leaf wetness duration on the maximum % leaf area with sporulation ( $c$ ) on oilseed rape leaves after inoculation with conidial suspensions of *Pyrenopeziza brassicae* (light leaf spot). Comparison between the mean values of  $c$  estimated by REML analysis (points) or predicted by the model (lines) (see Table 1): (a) effects of temperature at 6 ( $\Delta$ , —), 10 ( $\blacktriangle$ , - - -), 16 ( $\square$ , - - -), 24 ( $\blacksquare$ , —), 48 ( $\circ$ , —) or 72 h ( $\bullet$ , - - -) of leaf wetness and (b) effects of leaf wetness duration at 4 ( $\Delta$ , —), 6 ( $\blacktriangle$ , - - -), 8 ( $\square$ , - - -), 12 ( $\blacksquare$ , —), 16 ( $\circ$ , - - -), 20 ( $\bullet$ , - - -) or 24°C ( $\nabla$ , —). The error bars (23 d.f.) are two times the standard error of the estimated mean values of  $c$ .

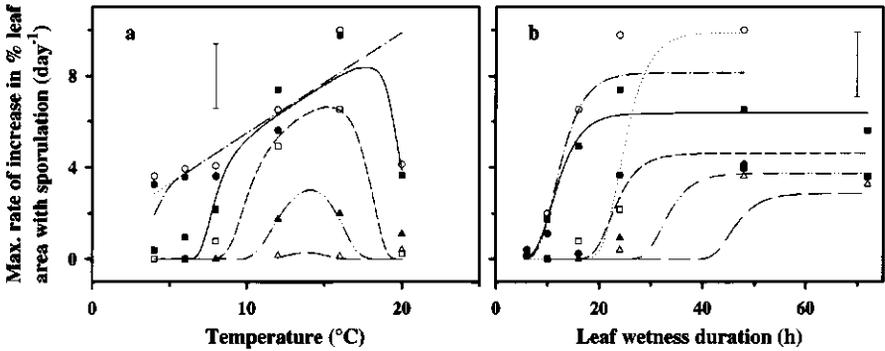
A modified Gompertz function (equation 2, Figure 4) fitted the effects of leaf wetness duration ( $W$ ) on the weighted average values for  $r$  and gave estimates for  $v_r$  and  $m_r$  for each temperature and a value for the constant  $q_r$ . The use of a quadratic temperature term ( $T$ ) to describe the minimum leaf wetness duration required for infection ( $v_r$ ) as a function of temperature improved the model ( $P = 0.017$ ). However, adding a quadratic temperature term to the function to describe the maximum of  $r$  ( $m_r$ ) as a function of temperature did not improve the model ( $P = 0.26$ ). Therefore, both linear and quadratic temperature terms were used in the model to describe the effect of temperature on  $v_r$ , and a linear temperature term was used to describe the effect of temperature on  $m_r$ ,

$$r = (1.10 + 0.44T) \exp\left\{-\exp\left\{-0.32\left[W - (80.22 - 10.23T + 0.37T^2)\right]\right\}\right\} \quad (8)$$

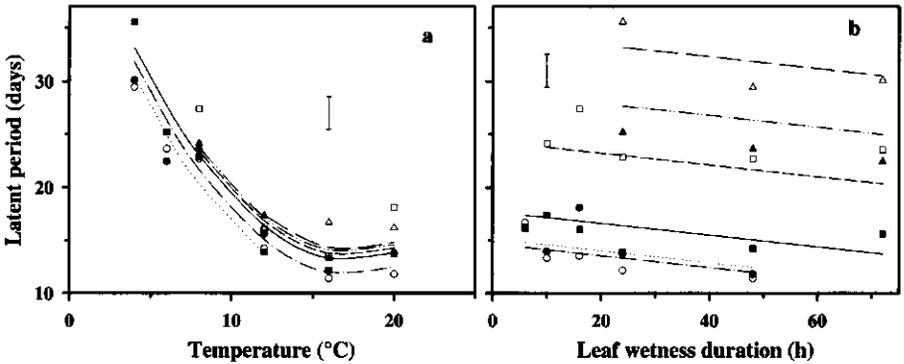
The effect of the minimum leaf wetness duration required for infection on  $r$  was fitted by this model except at a wetness duration of 72 h, but the decrease in the maximum of  $r$  for an increase in temperature from 16 to 20°C was not fitted by this function, because the temperature term for  $m_r$  was linear. In this model, the maximum of  $r$  increased linearly with temperature, whereas the observed data suggested that  $r$  decreased when temperatures increased above 16°C (Figure 4b). This linear relationship between the maximum of  $r$  and temperature also determined the asymptote at each temperature, above which  $r$  could not increase (Figure 4a). Thus, the model was only able to provide an approximate description of these data. The effect of temperature on the latent period was, in general, fitted well by a quadratic function. The effects of leaf wetness duration were fitted well by a negative linear function (Table 1, Figure 5) and estimates for  $d_1$ ,  $d_2$ ,  $d_3$  and  $d_4$  were obtained:

$$l = 48.25 - 3.87T + 0.11T^2 - 0.056W \quad (9)$$

The model, combining the Gompertz function describing the light leaf spot progress with time with the functions describing the effects of temperature and leaf wetness duration on the parameters  $c$ ,  $r$  and  $l$ , generally fitted well to the observed data for progress with time in % leaf area with sporulation (Figure 6), but did not fit at all temperatures and leaf wetness durations. At 6°C/48 h leaf wetness duration, for example, the latent period was over-estimated, and at 20°C/48 h leaf wetness duration the maximum % leaf area with sporulation was over-estimated. Nevertheless, the model, in general, described well the effects of temperature and leaf wetness duration on the parameters  $c$ ,  $r$  and  $l$ , with the exception of  $r$  at 20°C/48 h leaf wetness duration (Figures 3, 4, 5). From the REML analysis, there was no evidence for any significant variation between cabinets for the parameters  $c$  and  $l$ . There was evidence for significant variation between cabinets for parameter  $r$  with the cabinet effects ranging from -0.77 to 1.37 (average s.e. 0.92). This difference is large in relation to temperature differences for this parameter (Fig. 5b). Since temperature effects would be confounded with cabinet effects in an experiment without repeated randomised cabinet runs, this demonstrates that cabinet effects could severely bias the assessment of temperature effects in an experiment where the same cabinets are used for each temperature in different runs (replicates in time).



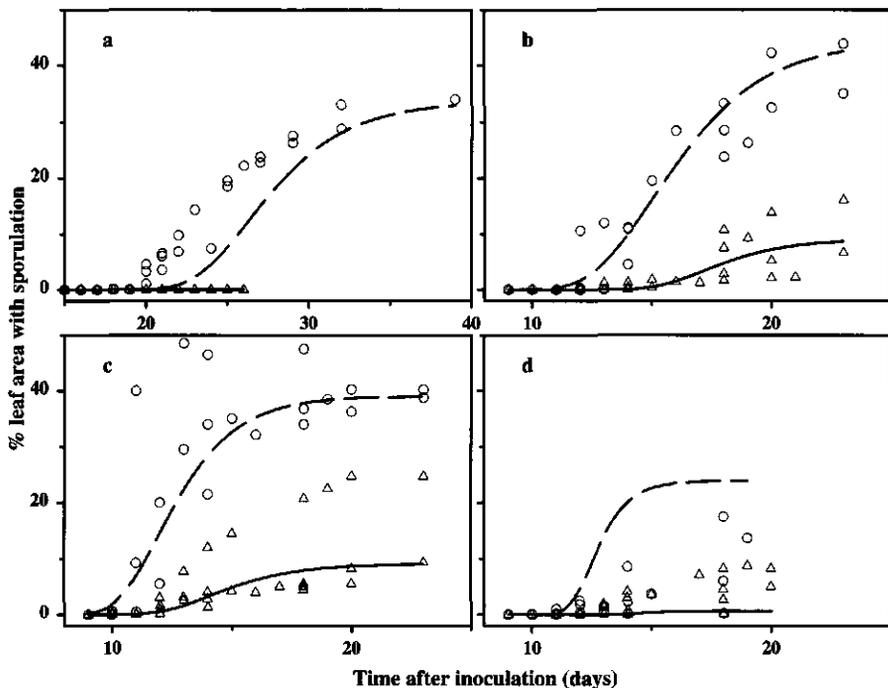
**Figure 4** Effects of temperature and leaf wetness duration on the maximum rate of increase in % leaf area with sporulation ( $r$ ) on oilseed rape leaves after inoculation with conidial suspensions of *Pyrenopeziza brassicae* (light leaf spot). Comparison between the mean values of  $r$  estimated by REML analysis (points) or predicted by the model (lines) (see Table 1): (a) effects of temperature at 6 ( $\Delta$ , —), 10 ( $\blacktriangle$ , - - -), 16 ( $\square$ , - - -), 24 ( $\blacksquare$ , —), 48 ( $\circ$ , —) or 72 h ( $\bullet$ , ····) of leaf wetness and (b) effects of leaf wetness duration at 4 ( $\Delta$ , —), 6 ( $\blacktriangle$ , - - -), 8 ( $\square$ , - - -), 12 ( $\blacksquare$ , —), 16 ( $\circ$ , —) or 20°C ( $\bullet$ , ····). The error bars (19 d.f.) are two times the standard error of the estimated mean values of  $c$ .



**Figure 5** Effects of temperature and leaf wetness duration on the latent period ( $l$ ) of *Pyrenopeziza brassicae* (light leaf spot) after inoculation of oilseed rape leaves with conidial suspensions. The latent period was estimated as the time from inoculation until the time when 37% of the maximum % leaf area with sporulation was reached. Comparison between the mean values of  $r$  estimated by REML analysis (points) or predicted by the model (lines) (see Table 1): (a) effects of temperature at 6 ( $\Delta$ , —), 10 ( $\blacktriangle$ , - - -), 16 ( $\square$ , - - -), 24 ( $\blacksquare$ , —), 48 ( $\circ$ , —) or 72 h ( $\bullet$ , ····) of leaf wetness and (b) effects of leaf wetness duration at 4 ( $\Delta$ , —), 6 ( $\blacktriangle$ , - - -), 8 ( $\square$ , - - -), 12 ( $\blacksquare$ , —), 16 ( $\circ$ , —) or 20°C ( $\bullet$ , ····). The error bars (16 d.f.) are two times the standard error of the estimated mean values of  $c$ .

**Table 1** Components of a model describing effects of temperature and leaf wetness duration on progress with time in % leaf area with sporulation on oilseed rape (cv. Bristol) leaves inoculated with *Pyrenopeziza brassicae* conidial suspensions. The % leaf area with sporulation ( $s$ ) was described as a Gompertz function of time ( $t$ ), with parameters  $c$  (max. % leaf area with sporulation),  $r$  (max. rate of increase in % leaf area with sporulation) and  $l$  (latent period).  $c$  and  $r$  were described as Gompertz functions of leaf wetness duration ( $W$ ), with parameters  $m_c$  (max. of  $c$ ) or  $m_r$  (max. of  $r$ ) and  $v_c$  or  $v_r$  (min. leaf wetness duration required for infection).  $v_c$  or  $v_r$  estimated min. leaf wetness duration required for infection as the wetness duration at which 37% of the max. % leaf area with sporulation ( $v_c$ ) or 37% of the max. rate of increase in % leaf area with sporulation ( $v_r$ ) was reached.  $m_c$ ,  $v_c$ ,  $m_r$  and  $v_r$  were described as functions of temperature ( $T$ ). Effects of temperature and leaf wetness duration on latent period were described in a function with linear and quadratic temperature ( $T$ ) and linear leaf wetness duration ( $W$ ) terms.

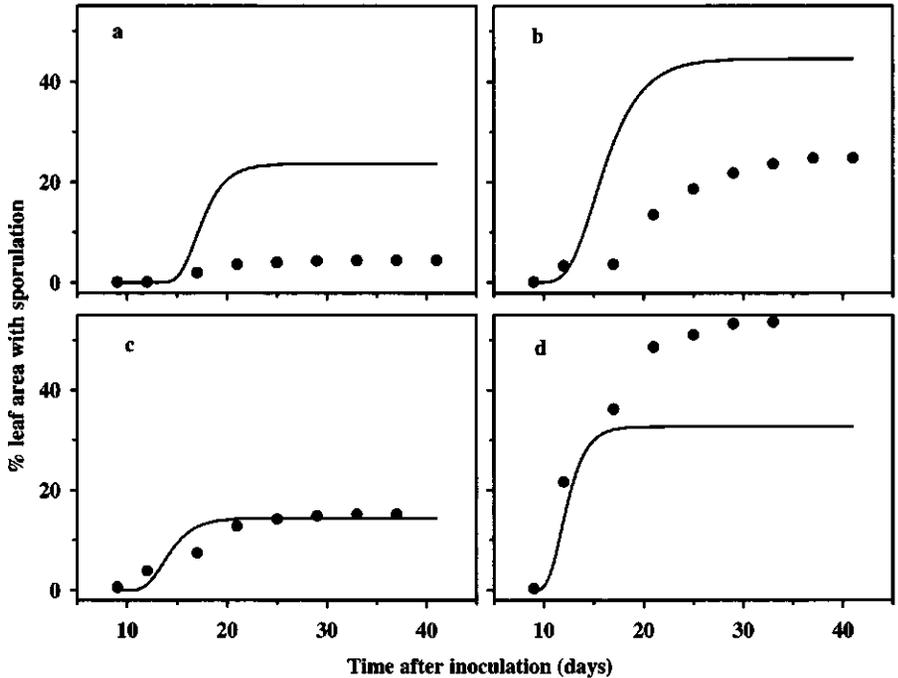
Equations	Parameter	Estimate	Standard error
<b>% leaf area with sporulation</b>			
$s(t) = c \exp(-\exp(-\frac{2.72r}{c}(t-l)))$			
<b>Maximum % leaf area with sporulation</b>			
$c(W) = m_c \exp(-\exp(-q_c(W-v_c)))$	$q_c$	0.15	0.029
$m_c(T) = a_1 + a_2T - a_3T^2$	$a_1$	3.65	7.380
	$a_2$	7.02	1.220
	$a_3$	0.30	0.045
$v_c(T) = a_4 - a_5T + a_6T^2$	$a_4$	55.47	4.160
	$a_5$	6.08	0.641
	$a_6$	0.21	0.21
<b>Maximum rate of increase in % leaf area with sporulation</b>			
$r(W) = m_r \exp(-\exp(-q_r(W-v_r)))$	$q_r$	0.34	0.126
$m_r(T) = b_1 + b_2T$	$b_1$	0.70	1.00
	$b_2$	0.44	0.081
$v_r(T) = b_3 - b_4T + b_5T^2$	$b_3$	80.62	6.960
	$b_4$	10.21	0.934
	$b_5$	0.37	0.027
<b>Latent period</b>			
$l(W, T) = d_1 - d_2T + d_3T^2 - d_4W$	$d_1$	48.25	3.460
	$d_2$	3.87	0.584
	$d_3$	0.11	0.022
	$d_4$	0.056	0.0149



**Figure 6** Predictions made by the model of progress with time (days after inoculation) in % leaf area with sporulation on oilseed rape (cv. Bristol) plants inoculated with conidial suspensions of *Pyrenopeziza brassicae* (light leaf spot) at (a) 6, (b) 12, (c) 16 or (d) 20°C and with 10 (—) or 48 h (—) of leaf wetness after inoculation. The model parameters are shown in Table 1. For comparison, the observed data for progress with time in % area with sporulation on oilseed rape leaves for each experiment at these temperatures with 10 ( $\Delta$ ) or 48 h ( $\circ$ ) leaf wetness duration after inoculation are shown.

### Testing of the model

The model gave a good prediction of the latent period of *P. brassicae* for the independent data for % leaf area with sporulation on oilseed rape (cv Cobra) leaves estimated from experiments of Figueroa *et al.* (1995b) (Figure 7). Although the model prediction of the maximum % leaf area with sporulation fitted well to the observed data for 18°C/16 h leaf wetness duration, predictions of maximum % leaf area with sporulation for 18°C/48 h leaf wetness duration, 12°C/16 h leaf wetness duration and 12°C/48 h leaf wetness duration did not fit well to the observed data.



**Figure 7** Comparison of values predicted by the model (—) with observed values (●; data from Figueroa *et al.*, 1995b) for progress with time after inoculation in % leaf area with sporulation of oilseed rape (cv Cobra) leaves inoculated with *Pyrenopeziza brassicae* conidial suspensions at 12 (a,b) or 18°C (c,d) and with 16 (a,c) or 48 h (b,d) of leaf wetness after inoculation. The model and estimates of model parameters are shown in Table 1.

To test the latent period equation component of the model with data from the pot experiments in the 1998/1999 and 1999/2000 seasons, daily average temperatures were inputted into equation 5 with  $W = 6$ :

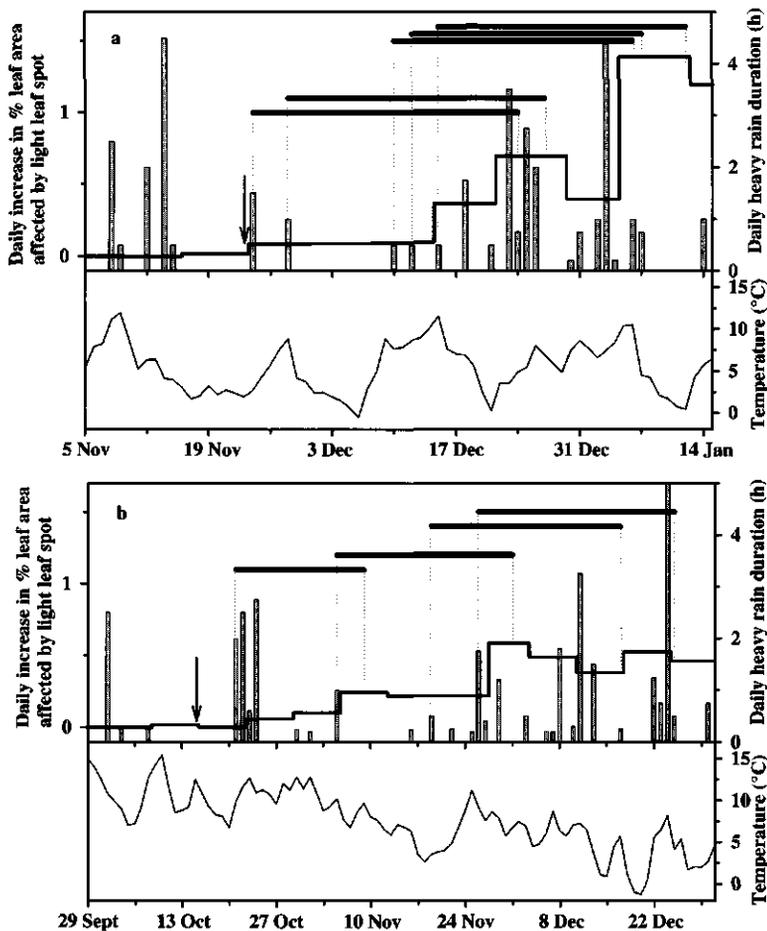
$$\frac{1}{l_t} = \frac{1}{47.91 - 3.87T_t + 0.11T_t^2} \quad (10)$$

When these daily values were summed until they reached a value of 1 (equation 6), the model gave, on average, good predictions of the dates with the greatest daily increases in % leaf area affected by light leaf spot in relation to previous occurrences of 'heavy' rain (Figure 8). The periods of 'light' rain were not often related to subsequent maxima in the increase in % leaf area affected by light leaf spot. When disease increases were predicted with equation 10 following 'heavy' rain showers of  $\geq 0.5$  h in duration when sporulation had been observed, the local maxima in increase in disease severity were well predicted. For the 1998/99 season, the largest increase in light leaf spot severity occurred in the period 5-12 January 1999. This increase corresponded to the predicted increase in disease severity associated with 'heavy' rain events on 10, 12 and 15 December 1998. A smaller maximum occurred in the period 22-29 December 1998, corresponding to two 'heavy' rain events on 24 and 28 November 1998. It is likely that the increase in mid-December was associated with rainfall in mid-November,

although this was not modelled since conidia were not observed until 16 November. There were no other definite maxima in increase in disease severity in the period considered and no other heavy rain events of  $\geq 0.5$  h in duration when sporulation had been observed. For the 1999/2000 season, there was one clear maximum in increase in disease severity (28 November-3 December 1999) and two smaller local maxima (6-12 November and 18-24 December 1999). These all either include or almost include the predicted periods of increase associated with previous 'heavy' rain events of  $\geq 0.5$  h when sporulation had been observed. The predictions for the time from splash-dispersal events until the greatest increases in % leaf area affected by light leaf spot ranged from a 19 day period following the 'heavy' rainfall on 21 October 1999 (when daily average temperatures were 6 to 13°C) to a 30 day period following the 'heavy' rainfall on 24 November 1998 (when average temperatures were -1 to 11°C). Thus the observed maximum increases in disease severity during the experimental periods in both seasons could all be maxima predicted by the model associated with 'heavy' rain events which occurred after the *P. brassicae* spore pustules had first been observed on leaves.

## Discussion

This work shows that a model can be constructed to describe the effects of temperature and leaf wetness duration on progress with time in % leaf area with sporulation of *P. brassicae* following a series of conidial infections on oilseed rape. Testing of the combined model (Table 1) with data from an independent experiment (Figueroa *et al.*, 1995b) suggested that the model predicts latent period well, but that predictions for % leaf area with sporulation are inconsistent (Figure 7). The effects of temperature on latent period have successfully been described by non-linear functions for other pathogens, for example *Bremia lactucae* on lettuce (Scherin & van Bruggen, 1994) and *Sphaerotheca pannosa* on rose (Xu, 1999). However, there has been little work on the effects of leaf wetness duration on the latent period of fungal pathogens. The decrease in the latent period of *P. brassicae* for an increase in leaf wetness duration could have been caused by an increase in the number of conidia that could germinate, penetrate and infect a leaf. An increase in the number of conidia infecting a leaf is likely to cause an increase in fungal biomass within the leaf. This results in greater competition for assimilates within the leaf between individual fungi, and may, therefore, stimulate *P. brassicae* to regenerate earlier by producing conidia. The model predictions for % leaf area with sporulation may not have fitted the data of Figueroa *et al.* (1995b) because a different oilseed rape cultivar (cv Cobra instead of cv Bristol), more mature plants (GS 1,6 instead of GS 1,4/1,5), inoculum of a different isolate of *P. brassicae* (a mixture of three monosporic isolates instead of a field isolate) and a different concentration of conidia in suspension (c.  $2 \times 10^6$  conidia per mL instead of c.  $0.5 \times 10^6$  conidia per mL) were used in the experiment of Figueroa *et al.* (1995b), by comparison with the experiment of Gilles *et al.* (2000b) on which the model is based. Thus, to predict disease severity quantitatively, the effects of factors like cultivar, pathogen isolate and inoculum density need to be understood.



**Figure 8** Daily increase in % leaf area affected by light leaf spot (*Pyrenopeziza brassicae*) (thick lines) observed on oilseed rape plants in pots outdoors in the autumn/winter of the seasons (a) 1998/1999 and (b) 1999/2000. The daily increase in % leaf area affected by light leaf spot was calculated by dividing the difference between the sum of % leaf affected for an assessment ( $s_a$ ) and the previous assessment ( $s_{a-1}$ ) by the total number of leaves on the pot plants ( $p_a$ ) and the number of days between assessments ( $t_a - t_{a-1}$ ):  $\frac{(s_a - s_{a-1})}{p_a(t_a - t_{a-1})}$ . Between successive assessments, the daily increase in % leaf area affected by light leaf spot is presented as a horizontal line (==). Following 'heavy' rainfall events ( $\geq 2$  mm of rain per h; vertical bars) of  $\geq 0.5$  h when sporulation had been observed (arrows indicate when sporulation was first observed), the daily fulfilment of latent period ( $\frac{1}{l_t}$ ) was calculated by the equation:  $\frac{1}{l_t} = \frac{1}{47.91 - 3.87T_t + 0.11T_t^2}$ , using daily average temperature ( $T_t$ ; —, bottom graph) for the days following this rainfall event ( $t$ ). The length of the latent period ( $l_t$ ; ■) predicted by cumulating the daily fulfilment of latent period until the value 1 was reached ( $\sum_{i=1}^l \frac{1}{l_t} = 1$ ). This equation predicted the time from a rainfall event until the time when a maximum in daily increase in % leaf area with sporulation occurred.

Further evidence that the model accurately predicts the latent period of *P. brassicae* was provided by the experiments with pot plants under natural conditions in two seasons. Equations 4 and 5, which were derived from the latent period function in the model, were successful in predicting the times when maxima in the increase in % leaf area affected by light leaf spot occurred in the autumn and winter in the 1998/99 and 1999/2000 seasons. Results suggested that 'heavy' rain showers ( $\geq 2$  mm of rain per h for  $\geq 0.5$  h) contribute more to splash dispersal of conidia than 'light' rain showers. The predictions of the dates of occurrence of the greatest increases in % leaf area affected by light leaf spot were related to observed occurrences of large increases if latent periods started only at 'heavy' rain periods, but not if they started at all rain periods. Similarly, Gottwald *et al.* (1989) found that the occurrence of wind-blown rain storms coincided with the spread of the splash-dispersed bacterium *Xanthomonas campestris* pv. *citri* (citrus canker) in citrus orchards. The relation between 'heavy' rainfall and occurrence of maxima in increase in % leaf area affected by light leaf spot in the pot experiments outdoors indicates that cycles of dispersal and infection by conidia contribute to disease progress during autumn/winter in the UK, although ascospore infections cannot be excluded (McCartney & Lacey, 1990). The prediction of disease progress by cycles of conidial infections is, therefore, important for crop-specific forecasts of light leaf spot.

The accuracy of latent period predictions might be improved by predicting latent period in hours instead of days and by using hourly average temperatures instead of daily average temperatures as input into the latent period model. Since the relationship between temperature and latent period is non-linear, the latent period predicted by using average temperatures must differ from the latent period predicted by using fluctuating temperatures for the same period (Xu, 1996; Scherm & van Bruggen, 1994; Kaufmann, 1932). The use of daily average temperatures adjusts for variation in temperature between days. However, the diurnal fluctuations in temperature in one day can often be greater than the fluctuations in average temperature between days. Therefore, using hourly average temperatures will improve the accuracy of the latent period prediction. Further knowledge of the effects of very low temperatures on the latent period of *P. brassicae* could also improve latent period predictions. Observed latent periods when daily average temperatures were below 1°C suggested that latent period was shorter than expected at these temperatures (Figueroa *et al.*, 1995b). Frosts may accelerate the appearance of light leaf spot symptoms so that spore pustules are detected earlier than under frost-free conditions. Further research is needed to investigate the effects of temperatures below 1°C on the *P. brassicae* latent period and appearance of light leaf spot symptoms. Ultimately, these models, describing effects of temperature and wetness duration on development of light leaf spot, could be incorporated into a scheme for forecasting the severity of light leaf spot epidemics to provide farmers with accurate crop-specific forecasts of light leaf spot severity and epidemic progress.

### Acknowledgements

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## Chapter 8

### Prospects for forecasting light leaf spot severity in the UK – A general discussion

Knowledge of the effects of weather on light leaf spot epidemics is required to develop systems which can forecast the severity of these epidemics on individual oilseed rape crops (Chapter 2). The severity of light leaf spot epidemics varies between and during seasons, between regions in the UK, and between crops within each region (Fitt *et al.*, 1996; Hardwick & Turner, 1995). An existing risk forecasting system can predict the seasonal and regional variation in % plants with light leaf spot on leaves in March based on the incidence of crops with pod disease in July of the previous season and regional averages of summer temperature and winter rainfall (Gladders *et al.*, 1995; Fitt *et al.*, 1996; Welham *et al.*, 1999). This risk forecasting system has been modified to include crop specific factors such as cultivar, sowing date and whether or not a fungicide spray is applied in the autumn (Fitt *et al.*, 1996), but these additional factors explain only a small amount of the variation between crops within a region. The great variation in light leaf spot severity between regions with different climates in the UK suggests that light leaf spot epidemics are mostly driven by prevailing weather. Therefore, an improved knowledge of the light leaf spot epidemic cycle and how stages in the epidemic are affected by weather could explain the variation in disease severity between individual crops and the variation in disease severity with time during a season. The light leaf spot epidemic cycle, the effects of weather factors on stages in the epidemic cycle and the application of models describing these effects in forecast systems, are now discussed.

#### The light leaf spot epidemic cycle

Light leaf spot epidemics on winter oilseed rape are initiated mainly by infections caused by wind-dispersed ascospores of *P. brassicae*, which are produced during the sexual stage of the fungus on debris from previous crops in late summer and autumn. Experiments in controlled environments suggested that the wind-dispersed ascospores of *P. brassicae* are very infective to oilseed rape leaves (Chapter 3). Apothecia of *P. brassicae*, in which the ascospores are produced, develop on debris in late summer and autumn; they were observed on stem debris in August (Chapter 4) and on pod debris during autumn and winter (Chapter 5). These observations confirm earlier observations of apothecia in late summer and autumn by Lacey *et al.* (1987). Ascospores of *P. brassicae* are, therefore, expected to be released during late summer, autumn and winter. This is confirmed by the sampling of air-borne spores, indicating that ascospores of *P. brassicae* are released in small numbers from previous crop debris during autumn and winter (Chapter 3). Although *P. brassicae* ascospores were sampled in small numbers per unit volume of air within crops, when integrated over time, substantial numbers of highly infective ascospores are deposited onto crops.

In southern regions of the UK, the period between harvest and emergence of newly sown crops can be *c.* 2 months (Chapter 2). When summer weather conditions are wet and temperatures mild (12-18°C), apothecia of *P. brassicae* can develop within 10 days (Chapter 5). Therefore, in summers with high rainfall, most ascospore release may occur before newly sown crops emerge in southern regions of England. However, in northern regions of England and in Scotland, the period between harvest and emergence of newly sown crops is shorter and in certain seasons in Scotland the previous crop can still be present when newly sown

crops are emerging (Sutherland *et al.*, 1995). Therefore, ascospores of *P. brassicae* are often released with the emergence of newly sown crops have emerged in northern regions. Furthermore, in these regions, splash-dispersed conidia can also cause primary infections in newly sown winter oilseed rape at short distances from a late harvested winter oilseed rape crop. This may explain why light leaf spot is more prevalent in northern England and in Scotland than in southern England (Welham *et al.*, 1999).

In late autumn, winter and spring, secondary spread of light leaf spot occurs by splash dispersal of conidia. Conidia are produced in large numbers on all infected tissues (Rawlinson *et al.*, 1978) after a period of latency of *P. brassicae* (Chapter 6; Figueroa *et al.*, 1995a). Conidia are not as infective as ascospores of *P. brassicae* (Chapter 3), but their dispersal by rain-splash (Fatemi & Fitt, 1983) from infected plant tissues, on which they are produced in large numbers, leads to infections at short distances from the source. This is confirmed by the observation that the pattern of light leaf spot in a crop became more aggregated as the season progressed (Evans *et al.*, 1999). A maximum of 17 asexual generations were estimated to occur from early October to late June, using calculations of latent period based on 30-year monthly average temperatures (Chapter 6). During the infectious period, conidia can be dispersed more than once, but more information about the rate of conidial production is needed for an understanding of replenishment of conidia after dispersal of conidia during a rain shower.

In spring and summer, light leaf spot spreads onto the stems, flowers and pods of oilseed rape crops (Rawlinson *et al.*, 1978), with both ascospores and conidia produced. However, it is uncertain whether the light leaf spot lesions on stems, flowers and pods are caused mainly by ascospores, mainly by conidia or equally by both spore types. In late winter, spring and summer, ascospores are released in large numbers from apothecia, which develop on infected leaf debris beneath crops (Chapter 3; McCartney & Lacey, 1990). It is thought that these wind-dispersed and highly infective ascospores contribute to stem and pod infections in crops. The observation of stem lesions in March 1993 when no air-borne ascospores were collected, and when conidia were produced in large numbers indicates that conidia can contribute to stem infections. It is not clear whether these stem infections arose earlier in the season on meristematic tissues of plants at the rosette-stage (Chapter 2; Rawlinson & Cayley, 1984) or whether conidia, splashed upwards in the crop (Pielaat, 2000), caused these lesions.

### **Forecasting light leaf spot epidemics**

Since ascospores have now been identified as primary inoculum in late summer and autumn and conidia as secondary inoculum in autumn, winter and spring models have been developed to predict (i) the time to apothecial development and (ii) the time to the maximum increase in disease severity following dispersal of conidia.

#### *Predicting apothecial development*

A model describing the effects of temperature and wetness of debris on apothecial development was successful in predicting the time to first appearance of mature apothecia of *P. brassicae* (Chapter 5). The first appearance of mature apothecia is thought to correspond with the first release of air-borne ascospores, but further work is needed to relate the presence of mature apothecia and weather conditions to ascospore release. In a forecasting system, this model could be used to predict the first appearance of mature apothecia after harvest of winter oilseed rape crops. Based on this information, growers can sow later if the model predicts that apothecia will mature early, which will often occur in a wet summer. Advice can be given to sow crops later to avoid the air-borne ascospores and to plough in debris early to remove the

inoculum source. When debris is incorporated into the soil by ploughing, apothecia do not develop, probably because they are readily overgrown by other saprophytes (Cheah & Hartill, 1985). If the model predicts that apothecia will mature late, which will often occur during a dry summer, then fields of harvested oilseed rape can be ploughed later, but still before predicted maturation of apothecia. The inputs for the apothecial model are measurements of temperature and wetness of debris. Temperature can be measured routinely on farm, but debris wetness sensors are not readily available and need to be specially made. Debris wetness could be measured by farm advisors in their regions and these data can then be used by oilseed rape growers within each region. However, debris wetness is likely to vary greatly because of differences in local weather. Therefore, by using data for debris wetness measured at a regional level as input, the model won't predict the variation within a region in the time to maturation of apothecia as a result of variation in debris wetness.

#### *Predicting the time to the maximum increase in disease severity following dispersal of conidia*

A model for latent period of *P. brassicae* was successful in predicting the times when the greatest increases in severity of light leaf spot occurred after 'heavy' rain events ( $\geq 2$  mm of rain  $\text{h}^{-1}$  for 0.5 h) (Chapter 7). The predictions by this model could indicate whether many conidial infections have occurred in autumn, winter and spring and could be used to advise farmers on the need for a second fungicide spray application in spring. In testing this model, 'heavy' rain events were assumed to disperse large numbers of conidia. However, the relationship between the intensity of rain or rain impact and dispersal of conidia of *P. brassicae* needs further investigation before this model can be used with confidence. A similar study investigated the relationship between the rainfall impact force and splash-dispersal of conidia of *Mycosphaerella graminicola* in winter wheat (Lovell *et al.*, 1999). Progress of light leaf spot as a result of conidial infections could probably be described as a function of the number of rain showers above a certain rainfall impact level.

#### **Light leaf spot on other crops and in other countries**

*P. brassicae* occurs not only on winter oilseed rape (*Brassica napus*) in the UK, but is also prevalent on oilseed rape and vegetable brassicas in the cool and wet climates of north-west Europe and New Zealand (Amelung & Daebeler, 1991; Staunton, 1967; Cheah & Hartill, 1985). Thus, light leaf spot can become an important disease in other countries with a cool and wet climate where brassicas are cultivated. However, the opposite mating types of *P. brassicae* need to be present, because ascospores are an important primary inoculum of light leaf spot. Since the models predicting apothecial maturation and the time to maximum increase in disease severity following conidial dispersal are based on relationships with weather factors, these models can be used anywhere where light leaf spot is an important disease. However, it is uncertain whether isolates obtained from different locations behave differently when exposed to different weather conditions.

## Summary

Forecasts of the severity of light leaf spot of winter oilseed rape are needed to help growers with their decisions on fungicide applications at times when sprays are needed to control light leaf spot, but the disease is difficult to diagnose. A thorough understanding of stages in development of *Pyrenopeziza brassicae* contributing to light leaf spot epidemics on winter oilseed rape and how these stages in development are affected by weather factors can be used to develop models, which can predict epidemic progress under different weather conditions. A risk forecast system is currently in use, which on a regional level predicts leaf disease incidence in March based on a regression with pod disease incidence in July, summer temperatures and winter rain. This regional forecast system can be made more specific for individual winter oilseed rape crops by incorporating models which can predict the effects of weather conditions on stages in development of *P. brassicae*. The main aims of this research project were firstly to investigate the light leaf spot epidemic cycle and secondly to identify stages in the development of *P. brassicae*, which are critical for epidemic progress and investigate how these stages in development are affected by weather factors.

Air-borne ascospores of *P. brassicae* released from stem and pod debris from previous winter oilseed rape crops are the main primary inoculum initiating light leaf spot epidemics on winter oilseed rape in the UK. Secondary spread of light leaf spot occurs by conidia, which are dispersed over short distances by splash during rain showers. Several cycles of conidial dispersal, infection and sporulation can occur during late autumn, winter and spring. In spring and summer, both ascospores and conidia are present when stem, flower and pod infections occur. Ascospores are produced on infected leaf debris underneath crops and ascospores, which are dispersed upwards in crops by wind, could cause infections of stems, flowers and pods. Conidia can be dispersed upwards in crops by rain splash and thereby cause infections at a higher canopy levels in crops. Also, it has been suggested that conidia can cause latent infections of primordia during late winter, which become apparent after stem extension in spring. Thus, light leaf spot epidemics are polycyclic and both wind-dispersed ascospores and splash-dispersed conidia contribute to disease progress.

The sexual stage of *P. brassicae* develops only after senescence of oilseed rape tissues. After a short phase of saprophytic growth on the senesced tissues, globular structures of immature apothecia develop. The apex of a globular structure depresses before the disk expands and asci are able to release ascospores. Apothecia develop to maturity at temperatures from 6 to 18°C when debris is wet, but do not develop at or above 22°C. As temperature decreases from 18 to 6°C the rate of apothecial development decreases, but the time to apothecial decay increases and thereby increases the duration of time over which mature apothecia are present on debris. An interruption in wetness delays apothecial development and decreases the number of mature apothecia, but does not inhibit apothecial development. The effects of temperature on the time to apothecial development and the time to apothecial decay have been described in models. The model describing the effects of temperature on apothecial development successfully predicted the time to the first observation of mature apothecia on pod debris, which was incubated under natural conditions in a field plot. The model describing the effects of temperature on apothecial decay over-predicted the time when apothecia were present on the pod debris outdoors. It is suggested that rapid wetting and drying of debris causes apothecia to release their ascospores and when the ascospores have been released apothecia decay.

Infection by conidia of *P. brassicae* is affected by temperature and leaf wetness duration. On oilseed rape (cv. Bristol), infections can occur at temperatures ranging from 4 to 20°C, but not at or above 24°C. Infections are successful only if leaf wetness duration is longer than a

minimum length of time. The minimum leaf wetness duration, which is required for infection, is temperature-dependent. At temperatures ranging from 12 to 20°C, the minimum leaf wetness duration required for infection is 6 h, and increases to 10, 10-16 or 16-24 h as temperature decreases to 8, 6 or 4°C, respectively. Measurements of leaf wetness duration in previous field experiments suggest that leaf wetness duration in the UK is frequently longer than the minimum leaf wetness duration required for infection. Thus, leaf wetness duration is often not a factor limiting to infection. The latent period of *P. brassicae* is shortest at c. 16°C and increases as temperature increases from 16 to 20°C or as temperature decreases from 16 to 4°C. Leaf wetness duration also affected latent period at 6 and 8°C; latent period of *P. brassicae* decreased when leaf wetness duration increased.

The effects of temperature and leaf wetness duration on light leaf spot development following single conidial infections in controlled environments were described in a model with the parameters maximum disease severity, maximum rate of increase in disease severity and latent period (time from inoculation to 37% of the maximum disease severity; this corresponds with the steepest slope of the Gompertz curve). This model predicted well the latent period of an independent data set for disease severity with time after conidial infections in controlled environments at 12 or 18°C after 16 or 48 h leaf wetness duration. However, levels of disease severity were not predicted well by the model. The part of the model describing the effect of temperature on the latent period of *P. brassicae* was tested with data for outdoor pot plants showing increases in disease severity in relation to rainfall events. The model predictions for a latent period following 'heavy' rainfall events ( $\geq 2$  mm of rain  $\text{h}^{-1}$ ) for  $> 0.5$  h corresponded well with observed maxima in the increase in disease severity, but less 'heavy' rain events did not correspond well with the observed maxima in the increase in disease severity.

The models predicting the effects of weather on the time to development of mature apothecia of *P. brassicae* and the time to the greatest increase in disease severity following conidial infection events can now be incorporated in systems forecasting light leaf spot severity on winter oilseed rape. By incorporating these models based on relationships with weather in the existing regional risk forecasting system, the effects of local variation in weather on epidemic progress can be predicted, and forecasts can become more specific to individual winter oilseed rape crops. For the model predicting the time to development of mature apothecia measurements of temperature and debris wetness are input; for the model predicting the time to the greatest increase in disease severity following conidial infection events measurements of temperature and rain intensity are input. Although temperature is measured by many growers, rain intensity and debris wetness are usually not measured by growers. Rain intensity and debris wetness can probably be measured by farm advisors for several farmers within a region. Therefore, although the newly developed models have the potential to predict the development of *P. brassicae* at an individual crop level, this potential is often not obtainable because of the lack of equipment to measure specific weather parameters.

## Samenvatting

Voorspellingen voor 'light leaf spot' in winterkoolzaad zijn noodzakelijk om landbouwers hulp te bieden bij het maken van beslissingen over het gebruik van fungiciden om de ziekte, welke vaak moeilijk waar te nemen en te herkennen is, te bestrijden. Een diepgaande kennis van de ontwikkelingsstadia van *Pyrenopeziza brassicae*, die bijdragen aan epidemieën van 'light leaf spot' in winterkoolzaad, en hoe deze ontwikkelingsstadia beïnvloed worden door weersfactoren kan gebruikt worden voor de ontwikkeling van modellen, die de voortgang in de epidemie onder diverse weersomstandigheden kunnen voorspellen. Momenteel wordt een systeem gebruikt dat waarschuwt voor het risico dat een zware epidemie zich ontwikkelt. Dit systeem voorspelt per regio de hevigheid van aantasting van bladeren door 'light leaf spot' in maart met behulp van een model dat gebaseerd is op een relatie tussen de hevigheid van aantasting van de peulen in juli, temperaturen in de zomer en regenval in de winter en de hevigheid van aantasting in maart. Dit regionaal voorspellingsstelsel voor 'light leaf spot' kan gespecificeerd worden tot een voorspelling voor individuele winterkoolzaad gewassen door het toevoegen van modellen, die de effecten van weersfactoren op ontwikkelingsstadia van *P. brassicae* kunnen voorspellen. De hoofddoelen van dit onderzoeksproject waren om ten eerste de cyclus van de 'light leaf spot' epidemie te bestuderen en ten tweede om de effecten van weersfactoren op de ontwikkelingsstadia van *P. brassicae*, die een voorname bijdrage leveren aan de ontwikkeling en voortgang van een epidemie, te bestuderen.

Wind-verspreide ascosporen van *P. brassicae* zijn het voornaamste primaire inoculum voor het veroorzaken van de eerste infecties in winterkoolzaad en worden geproduceerd op stengel- en peulresten van het gewas. Secundaire verspreiding van 'light leaf spot' vindt plaats door spatverspreiding van conidiën in druppels gedurende regenbuien. Gedurende de late herfst, winter en het voorjaar kunnen meerdere cycli van spatverspreiding van conidiën, infectie en sporulatie voorkomen. In het voorjaar en de zomer zijn ascosporen en conidiën beide aanwezig wanneer stengels, bloemen en peulen geïnfecteerd worden. Ascosporen worden geproduceerd op geïnfecteerde bladresten, die zich onder het gewas bevinden. Deze ascosporen worden door wind omhoog in het gewas verspreid, waar zij stengels, bloemen en peulen kunnen infecteren. Conidiën kunnen in het gewas omhoog verspreid worden door het verticaal omhoog spatten van conidien gedurende regenbuien. Ook is het mogelijk dat conidiën primordia infesteren aan het einde van de winter. Deze infecties blijven latent aanwezig totdat strekking van het gewas plaatsvindt in het voorjaar. De epidemieën van 'light leaf spot' zijn dus polycyclisch en zowel windverspreide ascosporen als spatverspreide conidiën dragen bij aan de voortgang van de ziekte.

Het seksuele stadium van *P. brassicae* ontwikkelt zich alleen na het afsterven van weefsels van koolzaad. Na een kortstondige fase waarin de schimmel saprofytisch groeit op afgestorven weefsels, worden globulaire structuren, de onrijpe apotheciën, ontwikkeld. De top van zo'n globulaire structuur zinkt in, waarna de schijf van het apothecium zich ontvouwt en de eerste vlucht van ascosporen kan gebeuren. Apotheciën ontwikkelen zich bij temperaturen van 6 tot 18°C wanneer de gewasresten nat zijn, maar ontwikkelen zich niet bij 22°C of hoger. Wanneer de temperatuur afneemt van 18 tot 6°C neemt zowel de snelheid van ontwikkeling van apotheciën als de snelheid van afbraak van apotheciën af. Een onderbreking van de natheid van gewasresten met een periode waarin de gewasresten droog zijn vertraagt de ontwikkeling van apotheciën en vermindert het aantal apotheciën dat zich ontwikkelt, maar hindert de verdere ontwikkeling van apotheciën niet. De effecten van temperatuur op de tijdsduur tot ontwikkeling van apotheciën en de tijdsduur tot afbraak van apotheciën zijn beschreven in modellen. Het model dat de effecten van temperatuur op de ontwikkeling van apotheciën beschrijft was succesvol in het voorspellen van de tijd wanneer apotheciën voor

het eerst gezien werden op peulresten, die onder natuurlijke omstandigheden in een veld geïncubeerd waren. Het model dat de effecten van temperatuur op de afbraak van apotheciën beschrijft gaf een te late voorspelling van de waargenomen afbraak van apotheciën. Het is mogelijk dat apotheciën gestimuleerd worden om hun ascosporen vrij te laten door het snel afwisselen van periodes waarin de gewasresten nat of droog zijn. Zodra alle ascosporen vrijgelaten zijn vindt afbraak van de apotheciën plaats.

Infectie door conidiën van *P. brassicae* wordt beïnvloedt door temperatuur en bladnat-periode. *P. brassicae* kan koolzaad (cv. Bristol) infekteren bij temperaturen van 4 tot 20°C, maar niet bij 24°C of hoger. Infecties zijn alleen succesvol als de bladnat-periode langer is dan een minimale duur. De minimale bladnat-periode, waarbij infectie kan plaatsvinden, is afhankelijk van de temperatuur. Van 12 tot 20°C, is de minimale bladnat-periode voor infectie 6 h, en neemt toe tot 10, 10-16 of 16-24 h wanneer de temperatuur daalt tot 8, 6 of 4°C, respectievelijk. Metingen van bladnat-periode in voorgaande veldproeven suggereren dat de bladnat-periode in het Verenigd Koninkrijk voornamelijk van langere duur is dan de minimale bladnat-periode die nodig is voor infectie. Bladnat-periode is daarom niet vaak een limiterende factor voor infectie. De duur van de latentie-periode van *P. brassicae* is het korst bij c. 16°C en neemt af zodra de temperatuur toeneemt van 16 naar 20°C of als de temperatuur afneemt van 16 naar 4°C. De bladnat-periode beïnvloedde de latentie-periode bij 6 en 8°C; de duur van de latentie-periode nam af wanneer de bladnat-periode toenam bij deze temperaturen.

De invloeden van temperatuur en bladnat-periode op de ontwikkeling van 'light leaf spot' na een enkele infectie gebeurtenis met conidiën in klimaatkamers zijn beschreven in een model met de parameters maximum percentage aantasting van het bladoppervlak, maximum toename in aantasting en latentie-periode (de tijdsduur van inoculatie tot 37% van de maximale aantasting; dit komt overeen met de grootste toename van de Gompertz-curve). Dit model gaf een goede voorspelling van de latentie-periode die waargenomen was in een voorgaand onafhankelijk experiment, waarin de toename in aantasting na infecties door conidiën bij 12 of 18°C en na 16 of 48 h bladnat-periode was bestudeerd. Het model gaf geen goede voorspelling van het niveau van aantasting. Het deel van het model dat de invloeden van temperatuur op de latentie-periode beschrijft is getest met data voor toename in aantasting door 'light leaf spot' op potplanten buiten onder natuurlijke omstandigheden. De modelvoorspellingen van latentie-periodes gaven een goede voorspelling van maxima in de toename van aantasting na zware regenbuien ( $\geq 2$  mm regen  $h^{-1}$  voor  $> 0.5$  h), maar niet na minder zware regenbuien.

De modellen, die voorspellingen geven van weereffecten op de ontwikkelingssnelheid van apotheciën van *P. brassicae* en de tijdsduur tot de grootste toename in aantasting na een infectie door conidiën, kunnen nu gebruikt worden in systemen voor het voorspellen van 'light leaf spot' in winterkoolzaad. Door het toevoegen van deze modellen aan het bestaande regionale waarschuwingssysteem voor 'light leaf spot' epidemiën, kan dit systeem beter de effecten van lokale variaties in weerscondities op deze epidemiën voorspellen. De voorspellingen van aantasting door 'light leaf spot' kunnen dan meer specifiek worden voor individuele gewassen. Metingen van temperatuur en natheid van gewasresten zijn input voor het model dat de ontwikkelingssnelheid van apotheciën voorspelt; metingen van temperatuur en intensiteit van regenval zijn input voor het model dat de tijdsduur tot de grootste toename in aantasting na een infectie door conidiën voorspelt. Temperatuur wordt door de meeste landbouwers gemeten, maar intensiteit van regenval en natheid van gewasresten niet. Intensiteit van regenval en natheid van gewasresten kunnen waarschijnlijk gemeten worden door landbouw adviseurs voor verscheidene landbouwers in een regio. Ondanks dat de nieuw ontwikkelde modellen voorspellingen kunnen geven van de voortgang van 'light leaf spot' epidemiën voor individuele winterkoolzaad gewassen gebaseerd op de lokale

weersomstandigheden zijn deze voorspellingen voor individuele gewassen vaak nog niet mogelijk vanwege het gebrek aan apparatuur voor het meten van specifieke weersparameters.

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## Curriculum vitae



Tijs Gilles werd geboren op 18 Juni 1972 in 's Gravenhage. Na zijn lagere school periode van 1978 to 1984 aan de Snijdersschool in Rijswijk heeft hij zijn middelbare voorbereidend wetenschappelijke opleiding (VWO) opleiding gevolgd aan het Huigens Lyceum in Voorburg van 1984 tot 1990. In september 1990 is hij begonnen aan zijn universitaire opleiding in de richting plantenveredeling (toenmalig T13) aan de Landbouw Universiteit Wageningen (nu Universiteit Wageningen). Gedurende deze opleiding heeft hij zich gespecialiseerd in de klassieke plantenveredeling met verscheidene bijvakken in de fytopathologie. Een afstudeervak op de Vakgroep Fytopathologie was besteed om de fysio-specifieke specialisatie van *Peronospora parasitica* (valse meeldauw) isolaten op bloemkool cultivars te bestuderen onder begeleiding van Herman Frinking. Een tweede afstudeervak onderzoek was uitgevoerd op het toenmalige Centrum voor Plantenveredeling en Reproductie Onderzoek (CPRO, nu Plant Research International) onder begeleiding van Dick Mastebroek en Harry Limburg. Dit onderzoek was gericht op het ontwikkelen van een techniek om voor de bloei te kunnen selecteren voor genotypen van gierstmelde (*Chenopodium quinoa*) met zaden met een laag saponinen gehalte. Na zijn afstuderen in september 1996 heeft hij voor korte tijd vrijwillig op het CPRO gewerkt aan het 'taggen' van resistentiegenen voor *P. parasitica* in *Arabidopsis thaliana* in de groep van Andy Pereira. In Juli 1997 is hij begonnen aan zijn promotie onderzoek op het 'Institute for Arable Crops Research – Rothamsted'. De promotor voor dit onderzoek is Mike Jeger (voormalig hoogleraar aan de Vakgroep Fytopathologie, nu hoogleraar aan Wye College in Kent, Engeland) en het onderzoek werd uitgevoerd onder directe begeleiding van Bruce Fitt. De resultaten van dit onderzoek zijn gepresenteerd in dit proefschrift. Hij heeft nu een voltijds baan als postdoc-onderzoeker aan 'Horticulture Research International' in Wellesbourne in Engeland binnen de 'epidemiology of leaf pathogens of vegetables'-groep van Roy Kennedy. Daar heeft hij als taak de epidemiën van valse meeldauw (*P. destructor*), *Botrytis spp.* in uien en roest (*Puccinia allii*) in prei te onderzoeken en voorspellingsmodellen voor deze ziekten te ontwikkelen en/of verbeteren.