Spatial Aspects of Light Leaf Spot (*Pyrenopeziza brassicae*) Epidemic Development on Winter Oilseed Rape (*Brassica napus*) in the United Kingdom

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ABSTRACT

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In microplot experiments in 1998–99 and 1999–2000, the start of light leaf spot epidemics could be predicted from weather data, using empirical equations for *Pyrenopeziza brassicae* apothecial (ascospore) development, ascospore infection criteria, and the latent period of *P. brassicae*. The dates when *P. brassicae* sporulation was first observed fitted predictions and initial spread of light leaf spot from an inoculum source was mostly in the prevailing wind direction, with differences between the two growing seasons attributable to differences in wind patterns. Subsequent secondary spread of disease could be predicted

using temperature and rainfall data, and observations fitted predicted dates. In both 1998–99 and 1999–2000, initial spatial patterns of observed disease in January were random, because data were not significantly different from a binomial distribution ($P = 0.18$). Analysis of spatial data from samples in February and March indicated aggregation, because data fit was significantly different from a binomial distribution ($P \leq 0.026$). These data were described by a beta-binomial distribution, suggesting that the spatial distribution of light leaf spot becomes aggregated as secondary spread occurs. The importance of wind-dispersed ascospores in initiating epidemics and rain-splashed conidia in secondary localized spread in relation to strategies for sampling winter oilseed rape crops in the United Kingdom to assess light leaf spot is discussed.

Additional keywords: ascospore, phoma leaf spot, spatial pattern.

Winter (autumn-sown) oilseed rape (*Brassica napus*, also known as rapeseed or canola) is an important crop worldwide (mean 1990–2001 production, 32,480 billion tonnes per annum, FAO database). The annual average value of the crop in the United Kingdom is £350 million (currently \$550 million) (Department for Environment, Food and Rural Affairs, UK). Oilseed rape is the third most important arable crop in the United Kingdom, after wheat and barley, and provides growers with an economic alternative to cereals within standard arable rotational practice. Currently, the major diseases causing annual yield loss in oilseed rape are light leaf spot (*Pyrenopeziza brassicae*, polycyclic) and phoma stem canker (*Leptosphaeria maculans*, monocyclic). Seasonal yield losses caused by light leaf spot were estimated to range from £13 to £40 million in the United Kingdom over harvest years 1987 to 2001 (7). However, the severity of light leaf spot differs between seasons, between different regions of the United Kingdom and between individual crops within a region (9). For effective control of light leaf spot, fungicides need to be applied in the autumn (fall) (4). However, *P. brassicae* infections are often symptomless at this time and there is a need to understand epidemic initiation to optimize timing of fungicide applications.

Field observations suggest that epidemics of light leaf spot show “patchiness” (6), but the spatial development of the disease has not been fully investigated. It has been suggested that wind-blown sexual ascospores initiate epidemics and that “patches” of disease develop around the resultant foci after secondary infection

by rain-splashed asexual conidia in the winter (1,9,24). Mature apothecia of *P. brassicae* have been observed in the United Kingdom and Germany in late summer or the fall on stem and pod debris from harvested crops, and in spring on leaf debris under crop canopies (9,19,23). However, a role in epidemic initiation also has been suggested for asexual conidia from late-harvested crops or from oilseed rape plants that arise from seeds spilt at harvest (21,23), vegetable brassicas (2,22,28), or cruciferous weeds (21). There is a need to determine whether light leaf spot epidemics are initiated by airborne ascospores or rain-splashed conidia of *P. brassicae*.

Under controlled conditions, the maturation of *P. brassicae* apothecia (ascospores) and ascospore infection of oilseed rape leaves both were dependent on temperature and wetness (8,10, 11). The latent period (time from initial infection event to the production of conidia), production of conidia, and conidial infection conditions also were influenced by temperature or wetness (12,14). Evidence from this work, and observations of epidemics in crops, were used to propose the roles of ascospores and conidia in epidemic initiation and development (13). This article describes experiments to investigate changes in the spatial distribution of light leaf spot infections, with epidemics initiated early in the growing season (in fall) in relation to meteorological data.

MATERIALS AND METHODS

Experiment design. Large fungicide treatment experiments (123 by 66 m) of winter oilseed rape (cv. Apex) were sown on 26 August 1998 and 27 August 1999 in two fields (Great Knott I and Great Knott II, respectively) at Rothamsted, England, UK. Each season, the large experiment was inoculated with stem debris (on 6 November 1998 and 12 October 1999) from a previous winter

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oilseed rape crop which was infected with *P. brassicae*. On 27 November 1998, four sets of three microplots (0.5 by 0.5 m) were marked out in unsprayed crop areas adjacent to unsprayed control plots (20 by 3 m, two on the west and two on the east of the large experiment). On 18 October 1999, eight sets of three microplots (0.5 by 0.5 m) were marked out in unsprayed areas adjacent to unsprayed control plots (20 by 3 m) with two sets each to the north, south, east, and west of the large experiment. Each set of three microplots was in a row; the first microplot started 1 m from the end of a control plot in the large experiment or edge of the inoculated area, the second a further meter from that point, and the third a meter from that point. Ten plants in each microplot were tagged with string and plastic labels. Plants were chosen in pairs, with the five sets of two plants forming an "X" shape within the microplot.

During both 1998–99 and 1999–2000, weather parameters were recorded using a Campbell 21x datalogger (Campbell Scientific,

Logan, UT). Maximum and minimum temperature, percent relative humidity, rainfall (millimeters per day), and simulated leaf wetness (leaf wetness sensor; Delta T Devices, Burwell, Cambridge, UK) were recorded using an automated weather station (Delta T Devices) at crop canopy height (0.25 m). Wind data (direction at 9:00 a.m. [Greenwich Mean Time] recorded by a Dines anemograph and wind run [kilometers per day] recorded with an anemometer) were collected at the main Rothamsted meteorological site, 0.5 km from the experimental site.

Disease assessment methods. In 1998–99, the number of leaves per plant and the number of leaves with "infections" (described below) of light leaf spot were assessed every second week from 7 December 1998 (growth stage [GS] 1.1 to 1.5) (29) until 15 March 1999 (GS 1.10 to 1.15). In 1999–2000, the number of leaves per plant and the number of leaves with "infections" of light leaf spot were assessed every second week from 18 October 1999 (GS 1.0 to 1.3) to 16 March 2000 (GS 1.10 to 1.15). For

Fig. 1. Wind patterns at Rothamsted for **A**, 1 August 1998 to 31 March 1999 and **B**, 1 August 1999 to 31 March 2000. Data represent accumulated wind runs (measured in kilometers per day) over 10-day periods in each of eight directions (45-degree segments) and are plotted as contour lines. Vertical lines indicate the predicted times of first ascospore release.

light leaf spot assessments, an “infection” was considered to be an infected area of leaf with visible white spore pustules (acervuli containing conidia) (27) because necrotic lesions do not tend to form in winter oilseed rape crops until late in the season (12). After mid-March, “natural disease spread” could not be assessed accurately due to the possibility that assessors had spread conidia by contact during previous assessments. At each sample date, the disease assessments formed a cluster sample of 24 (*N*) clusters of 10 (*n*) plants.

Description of epidemic progress in time and space. For both seasons, the progress (cycles) of the light leaf spot epidemics were predicted from meteorological data using models developed previously at Rothamsted (14). There was no oilseed rape stem debris on the microplot experimental area (previous crops: Great Knott I [1998–99], spring barley, 1998, winter oats, 1997; Great Knott II [1999–2000], set-aside [fallow], 1999, winter pea, 1998). It was assumed that epidemics were initiated by ascospores produced on infected stems scattered on the adjacent large fungicide experiment (i.e., this local inoculum source was more important than any background inoculum from more distant sources). The debris had been stored dry in a barn until the field was inoculated and meteorological data were available; therefore,

the time (*t*) from inoculation until 50% of the maximum number of *P. brassicae* apothecia matured was estimated using the equation of Gilles et al. (11): $t(T) = 7.6 + 55.8(0.839)^T$ (equation 1), where *T* = temperature (recorded hourly) during periods when the debris was wet (estimated with a leaf wetness sensor). The contribution to the maturation process of a period of 1 h at temperature *T* when the debris was wet is shown as $1/(24t(T))$. The hourly progress of maturation (depending on *T*) was added until the sum equaled 1. The time until maturation was then the total number of hours from inoculation needed to reach a value of 1. Ascospore release was considered to take place over a period of 3 to 4 days following 50% maturation. Wind data (direction and wind run) were analyzed to predict the predominant direction of dispersal of the airborne ascospores across the field. Distributions of wind directions from August to March are shown in Figure 1. Each contour plot is based on 24 times eight values representing wind run, accumulated over 10-day periods and 45-degree segments. Vertical bars indicate the expected time of ascospore release.

Conditions for infection by *P. brassicae* ascospores were described by an equation developed to predict maximum percentage leaf area with *P. brassicae* sporulation (*c*) (14), which depends on *T* and daily hours of leaf wetness (*W*) at the time of infection:

TABLE 1. Comparison between predicted (from weather data, using empirical equations) and observed development of light leaf spot (*Pyrenopeziza brassicae*) epidemics on oilseed rape at Rothamsted in 1998–99 and 1999–2000^a

Development	1998–99		1999–2000	
	Predicted	Observed	Predicted	Observed
Plots inoculated	...	6 Nov 98	...	12 Oct 98
First ascospore release (equation 1)	18 Dec 98	...	12 Nov 99	...
Infection criteria fulfilled (equation 2)	...	after 21 Dec 98	...	after 12 Nov 99
Latent period	28 days	...	24 days	...
First sporulation (conidia)	19 Jan 99	1 Feb 99	6 Dec 99	6 Dec 99
Rain splash events	...	19, 20, 23, 25, and 26 Jan 99	...	6, 8, and 10 Dec 99
Latent period	30 days	...	37 days	...
First secondary sporulation	23 Feb 99	2 Mar 99	15 Jan 00	17 Jan 00

^a Predictions were approximate using meteorological data available. Observations were made every 2 weeks during the growing season from 7 December 1998 to 15 March 1999 and 18 October 1999 to 16 March 2000.

Fig. 2. Diagrammatic representation of light leaf spot epidemic development on 10 tagged plants (represented by boxes) per plot in four sets of three microplots on the west (two sets) and east (two sets) sides of an inoculated field experiment (represented by solid vertical bar) at Rothamsted during the 1998–99 growing season. Uninfected plants (white boxes), plants with at least one sporulating area (shaded boxes), and plants with >1 sporulating area (black boxes) at four sampling dates: A, 1 February, B, 15 February, C, 2 March, and D, 15 March 1999.

Fig. 3. Diagrammatic representation of light leaf spot epidemic development on 10 tagged plants (represented by boxes) per plot in eight sets of three microplots on the north, east, south, and west sides (two sets per side) of an inoculated winter oilseed rape field experiment (represented by solid horizontal bar) at Rothamsted during the 1999–2000 growing season. Uninfected plants (white boxes), plants with at least one sporulating area (shaded boxes), and plants with >1 sporulating area (black boxes) at six sampling dates: **A**, 6 December 1999 to 4 January 2000, **B**, 17 January, **C**, 31 January, **D**, 15 February, **E**, 1 March, and **F**, 16 March 2000. For missing observations (for example, if the tagged plant died), no boxes are drawn.

$c(T, W) = (3.65 + 7.02T - 0.3T^2)\exp(-\exp\{-0.15[W - (55.47 - 6.08T + 0.21T^2)]\})$ for $W \geq 6$ and $c(T, W) = 0$ for $W < 6$ (equation 2). After infection, visible sporulation was predicted after a latent period described as a function of temperature recorded hourly during this period by the equation (14) $l(T) = 48.0 - 3.87T + 0.117T^2$ (equation 3). The equations used to calculate infection criteria and latent period were developed from data for light leaf spot infection by conidia (11,14). However, Karolewski et al. (18) recently demonstrated that the infection criteria and latent period for ascospores were similar to those for conidia at a range of temperatures (10, 12, 16, and 20°C) and leaf wetness durations (7, 9, 16, 24, 48, and 72 h). In both seasons, the dates when the first sporulation of *P. brassicae* was expected were predicted from weather data, using equations 1 (first predicted ascospore release),

2 (first dates when infection conditions occurred after ascospore release), and 3 (latent period after first predicted infections).

Rain-splash dispersion of conidia was expected to cause the next infection events. Rain splash was considered to occur on days with recorded rainfall exceeding 1 mm. Dates when the first secondary sporulation of *P. brassicae* was expected were predicted from predicted first dates for secondary inoculum dispersal (first rainfall after predicted sporulation), using the latent period equation (equation 3). To compare disease development in time and space predicted from meteorological data with observed epidemic progress in microplots, maps indicating the amount of light leaf spot on each tagged plant in each plot were drawn for each assessment date in the two seasons. Increase in disease in plots that already were infected was considered to be caused by

Fig. 4. Frequency distributions of winter oilseed rape plants with *Pyrenopeziza brassicae* sporulation per microplot at Rothamsted during the 1998–99 growing season. Each row represents an assessment of 10 plants in each of 12 microplots. **A, E, I, and M,** Observed distribution; **B, F, J, and N,** fitted binomial distribution; **C, G, K, and O,** fitted beta-binomial distribution; and **D, H, L, and P,** fitted binomial distribution with side effect for **A, B, C, and D,** 1 February; **E, F, G, and H,** 15 February; **I, J, K, and L,** 2 March; and **M, N, O, and P,** 15 March 1999. Statistics are presented in Table 2.

splash-dispersed conidia. New light leaf spot infections on previously unaffected plots were assumed to be caused either by incoming airborne ascospores or through splash-dispersed conidia from infected neighboring plots.

Quantitative description of the spatial data. According to recent work on light leaf spot epidemics (9,13), the spatial distribution of the disease was expected to be random initially, and to be influenced systematically by wind directions during ascospore release, with epidemic progress within the microplots proportional to the incidence of infection. To analyze the spatial aggregation pattern of the disease quantitatively, different distributions were fitted to the data for both seasons. If infections are randomly distributed, mean disease incidence (proportion of plants affected) should be constant over all plots, and a binomial model can be used to describe the frequency (number of plots) distribution of incidence of light leaf spot (percent plants affected).

A generalized linear model with intercept term, logit link function, and binomial error distribution was used to test the null hypothesis that infections were randomly distributed (3,25). The binary disease incidence data (plant infected, yes or no) were summed over the 10 plants observed in each plot. Twice the scaled sum of the natural logarithms of the likelihoods (residual deviance) approximately follows a χ^2 distribution with degrees of freedom equal to number of plots minus one, under the null hypothesis.

To account for deviations from randomness caused by differences between plot disease levels depending on their location relative to the large experiment, "side" was included as an optional factor in the generalized linear model. For the 1998–99 experiment, there were two possible levels for side (east and west) and for the 1999–2000 experiment, there were four sides (east, west, north and south). To assess whether the side effect was significant, the differences in deviances of the models with or without the side effect were compared with a χ^2 distribution with 1 degree of freedom (df) for 1998–99 and 4 df for 1999–2000.

However, cycles of multiplication of a randomly distributed pathogen may cause differences in incidence between plots through aggregation, and it was hypothesized that this additional spatial variation could be described by a beta distribution; this would produce a beta-binomial distribution to describe the observed pattern of disease incidence. Therefore, the log-likelihood was maximized as a nonlinear function of the parameters. The likelihood that this particular number (out of 10) of infected plants occurred under the assumption of a beta-binomial distribution was derived for each plot. The corresponding residual deviance approximately follows a χ^2 distribution with degrees of freedom equal to number of plots minus two. The difference in deviances between the beta-binomial model and the binomial model without side effect was compared with a χ^2 distribution with 1 df. In addition,

the observed and estimated distributions of incidence (binomial, beta-binomial, binomial with side) were plotted for each assessment date and season to assess the goodness of fit visually. All statistical analyses were done with GenStat 5 (GenStat for Windows release 4.2, USN International Ltd., Oxford, UK) (26)

RESULTS

Description of epidemic progress in time and space. By inserting observed hourly temperatures (during periods of wetness) into equation 1, it was predicted that apothecia (ascospores) would mature on stem debris inoculum by 18 December 1998, 42 days after inoculation, and by 12 November 1999, 31 days after inoculation, in the 1998–99 and 1999–2000 seasons, respectively (Table 1). Wind records for Rothamsted at the predicted time of ascospore release in 1998 (indicated by a vertical line in Fig. 1A) show that winds were mainly from the southwest. However, the wind direction during the predicted period of ascospore release in 1999 was mainly from the north and northwest (distribution of wind run along line in Fig. 1B).

In 1998–99, on the days immediately after the first predicted ascospore release (18 December), temperatures were below 4°C and leaves were dry during daytime. Therefore, little disease progress was expected because infection criteria were not fulfilled (equation 2). From 21 December onward, conditions were more favorable for infection, with temperatures above 8°C and leaf wetness during the day. *P. brassicae* sporulation on leaves, the first visible sign of the epidemic, was expected after a latent period (equation 3) of 28 days (19 January 1999). In 1999–2000, during the period following the first predicted ascospore release (12 November 2000), temperatures were between 5 and 10°C and leaf wetness duration was generally short. According to equation 2, low incidences of primary infection were predicted. Sporulation was expected after a latent period of 24 days (6 December 1999). These predictions fitted with dates of the field observations of the first sporulation in both seasons. In 1998–99, light leaf spot was first observed on 1 February 1999 (Fig. 2A) on four different plants. In 1999–2000, the first two plants with light leaf spot were observed on 6 December 1999.

During both seasons, rain events to disperse conidia occurred frequently after the predicted date for sporulation (e.g., 19, 20, 23, 25, and 26 January 1999 in 1989–99 and 6, 8, and 10 December 1999 in 1999–2000). Thus, it was predicted that secondary infections would produce new sporulation after further latent periods (equation 3) on approximately 23 February 1999 and 15 January 2000, respectively. Disease assessments from 2 March 1999 (Fig. 2C) showed there had been a rapid increase in disease incidence, especially on eastern plots. In 1999–2000, no additional plants with sporulation were observed until 4 January 2000. By 17 Janu-

TABLE 2. Goodness of fit of binomial (without and with side effect) and beta-binomial models to observed data and comparison of binomial model without and with side effect and beta-binomial model for number (out of 10) of plants with light leaf spot (*Pyrenopeziza brassicae* sporulation) in each of 12 microplots of a winter oilseed rape field experiment done at Rothamsted during the 1998–99 growing season^a

1999									
Model	df	1 February		15 February		2 March		15 March	
		Deviance	P value	Deviance	P value	Deviance	P value	Deviance	P value
Binomial	11	15.05	0.180	15.92	0.144	...	...	...	...
Beta-binomial	10	13.64	0.190	14.56	0.149	...	...	...	...
Binomial with side	10	14.84	0.138	12.87	0.231	...	...	...	...
Binomial versus beta-binomial	1	1.41	0.235	1.36	0.243	...	...	...	...
Binomial versus binomial with side	1	0.21	0.647	3.05	0.081	...	...	...	...
Binomial	11	...	...	...	...	48.77	<0.001	76.45	<0.001
Beta-binomial	10	...	...	...	...	29.56	0.001	34.87	<0.001
Binomial with side	10	...	...	...	...	28.50	0.002	26.95	0.003
Binomial versus beta-binomial	1	...	...	...	...	19.21	<0.001	41.58	<0.001
Binomial versus binomial with side	1	...	...	...	...	20.27	<0.001	49.50	<0.001

^a Residual deviances (3) of fitted models (binomial, beta-binomial, binomial with side) and differences between deviances for comparisons between models (binomial versus beta-binomial, binomial versus binomial with side) were compared with a χ^2 distribution.

ary 2000 (Fig. 3B), there were increases in disease incidence on plants in close proximity to previously infected plants. Increased incidence of light leaf spot near sporulating plants was observed in the following assessments (after another latent period). Additionally, the disease had spread to plots that had not already been affected (Figs. 2D and 3C to E).

According to the observed wind distributions at the time of ascospore release, infection was predicted to occur more often on eastern plots in 1998–99 and on southern plots in 1999–2000, in the corresponding downwind directions. On 15 March 1999, disease assessments showed a consistently higher incidence of light leaf spot in eastern (80 to 100%) than in western (20 to 40%) plots (Fig. 2D), indicating a large effect of the prevailing west to south-westerly wind. The two maxima in the observed frequency distribution of plot disease incidence (Fig. 4J) reflect this observation. In the 1999–2000 season, only plants located south of the large experiment were affected before 4 January 2000. By 16 March

2000, light leaf spot had spread over all plots, with 60 to 100% of plants per plot showing symptoms (Fig. 3F).

Quantitative description of the spatial data. In February of the 1998–99 season, early in the progress of the epidemic, the frequency distributions of plot disease incidence imply that the spatial distribution of light leaf spot was random over all plots. A binomial distribution (Fig. 4B and F) could be used to describe the observed distribution of disease incidence (Fig. 4A and E) because the mean deviances (= deviance divided by degrees of freedom) for samples on 1 and 15 February did not significantly exceed 1 (compare the residual deviances to a χ^2 distribution with 11 df ($P = 0.144$, $P = 0.180$) (Table 2).

However, during March, as disease incidence increased, large differences in incidence between western and eastern plots (Fig. 4I and M) resulted in a significant lack of fit for the binomial distribution (Fig. 4J and N). The mean deviances for 2 and 15 March 1999 were 4.43 and 6.95, respectively, and the residual deviances

Fig. 5. Frequency distributions of winter oilseed rape plants with *Pyrenopeziza brassicae* sporulation per microplot at Rothamsted during the 1999–2000 growing season. Each row represents an assessment of 10 plants in each of 24 microplots. **A, E, I, and M,** Observed distribution; **B, F, J, and N,** fitted binomial distribution; **C, G, K, and O,** fitted beta-binomial distribution; and **D, H, L, and P,** fitted binomial distribution with side effect for **A, B, C, and D,** 31 January; **E, F, G, and H,** 15 February; **I, J, K, and L,** 1 March; and **M, N, O, and P,** 16 March 2000. Statistics are presented in Table 3.

were significantly greater than expected if infection was randomly distributed across plots ($P < 0.001$). Introducing the factor “side” into the model (Fig. 4L and P) reduced the deviances of both March samples significantly ($P < 0.001$) and there was also evidence for aggregation within each side, with mean deviances of 2.85 and 2.69, respectively. The beta-binomial distribution without side effect (Fig. 4K and O) fitted the March data significantly ($P < 0.001$) better than the binomial without side effect (Fig. 4J and N) and gave a more accurate estimate of the amount of variation in the data. However, the mean deviance still significantly exceeded 1. The histograms show a reasonable agreement between observed (Fig. 4I) and predicted (Fig. 4K) distributions for the 2 March data. However, it was not possible to reproduce the two maxima of the distribution of the 15 March observation (Fig. 4M) by fitting a single beta-binomial distribution and there were not enough observations (i.e., the number of plots was too small) to estimate individual beta-binomial distributions for each side.

Results for the 1999–2000 season suggest that substantial aggregation of infected plants was present by 31 January 2000 (Fig. 5A). Before this date, incidences of infection were too small to cause a significant lack of fit for the binomial distribution (6 December 1999 to 4 January 2000, $P > 0.999$; 17 January 2000, $P = 0.547$) (Table 3). From 31 January onward, disease incidence (Fig. 5A, E, I, and M) was significantly nonrandomly distributed across the plots ($P = 0.004$, $P < 0.001$, $P = 0.004$, $P = 0.026$) (Table 3). The beta-binomial distribution (Fig. 5C, G, and K) accounted for some of the additional variation in the data and fitted the disease data of 31 January ($P < 0.001$), 15 February ($P = 0.002$), and 1 March ($P = 0.048$) significantly better than the binomial distribution (Fig. 5B, F, and G). However, both distributions (Fig. 5N and O) sufficiently described the observed spatial patterns in incidences in mid-March, by which time light leaf spot incidence was high in most plots (Fig. 5M). There was no evidence for differences in disease levels between sides relative to the large experiment (Fig. 5D, H, L, and P).

DISCUSSION

These results provide field evidence that light leaf spot epidemics are initiated by ascospores, as suggested by Gilles et al. (9,13) and Papastamati et al. (14). The distribution of initial infections during both seasons, particularly downwind of the inoculum source, suggests that initial infections are caused by windborne ascospores. The use of dry ascospore inoculum provided the opportunity to gather data to examine this hypothesis. Using existing models (8), in conjunction with information on inoculum, temperature, and rainfall, it was possible to show that epidemic develop-

ment under field conditions matched the hypothesized development of the epidemic (8,11). Work by McCartney and Lacey (23) suggests that ascospores can be produced throughout this intercrop period, even if in low numbers. Under natural conditions, initial ascospore infections may take place early in crop growth and, due to the symptomless nature of the early pathogen growth (5), the first two or three cycles of conidial infection may not be detected in winter oilseed rape crops.

The results also demonstrate the importance of splash dispersal of conidia in secondary spread and the subsequent development of polycyclic light leaf spot epidemics. The clear deviation from a random distribution of disease incidence indicates aggregation in the distribution that can be plausibly explained only by a secondary mode of dispersal that multiplies the disease locally. The spatial distributions (Figs. 2 and 3) suggest that the first secondary increase in disease incidence at the beginning of the epidemic was observed mainly around those plants which were infected initially. The experiments were inoculated at known times; therefore, the latent periods to the production of new conidial inoculum could be measured to validate the latent period models of Gilles et al. (12) under field conditions. The importance of splash dispersal from plants with primary infections onto neighboring plants in epidemic development was confirmed by calculation of latent periods to new infections on plants with primary infections or neighboring plants.

The results also show how an understanding of the spatial dynamics of epidemic progress can have practical consequences. For example, aggregation of disease has a consequence for the protocol for sampling crops to estimate disease incidence (15,16,20). To estimate disease incidence with the same accuracy as for randomly distributed disease, larger numbers of small samples are required. The cluster sampling technique employed gave detailed information at a level to take account of both the “randomness” of initial infections and the aggregated nature of the developing light leaf spot epidemic. However, in practice, it is difficult to take the numbers of samples required to sample oilseed rape crops effectively to assess incidence of aggregated light leaf spot (17).

The factor of most concern to the UK farmers is that *P. brassicae* often produces symptomless infections during winter (late October through January) which develop sporulating pustules in spring, allowing a rapid increase in disease (5). If, as suggested by data collected, the infections are dependent on inoculum whose dispersal is influenced by local conditions, sampling procedures for the detection of light leaf spot early in the season need to take account of the low levels of observed infection. Samples in spring (March to April) to determine the need for a second fungicide treatment must account for aggregation, which depends on initial

TABLE 3. Goodness of fit of binomial (without and with side effect) and beta-binomial models to observed data and comparison of binomial model without and with side effect and beta-binomial model for number (out of 10) of plants with light leaf spot (*Pyrenopeziza brassicae* sporulation) in each of 24 microplots of a winter oilseed rape field experiment done at Rothamsted during the 1999–2000 growing season^a

		2000 ^c											
		4 January		17 January		31 January		15 February		1 March		16 March	
Model ^b	df	Dev	P	Dev	P	Dev	P	Dev	P	Dev	P	Dev	P
Bi	23	6.38	0.999	21.56	0.547	44.72	0.004	...	...	...	...	...	...
Beta-bi	22	...	...	14.68	0.876	31.76	0.082	...	...	...	...	...	...
Bi-w	20	3.34	>0.999	14.16	0.822	36.94	0.012	...	...	...	...	...	...
Bi vs. beta-bi	1	...	...	6.88	0.009	12.96	<0.001	...	...	...	...	...	...
Bi vs. bi-w	3	3.04	0.385	7.4	0.060	7.78	0.051	...	...	...	...	...	...
Bi	23	...	...	...	...	...	...	56.22	<0.001	44.72	0.004	37.86	0.026
Beta-bi	22	...	...	...	...	...	...	46.34	0.002	40.82	0.009	37.04	0.023
Bi-w	20	...	...	...	...	...	...	51.78	<0.001	42.56	0.002	26.54	0.149
Bi vs. beta-bi	1	...	...	...	...	...	...	9.88	0.002	3.9	0.048	0.82	0.365
Bi vs. bi-w	3	...	...	...	...	...	...	4.44	0.218	2.16	0.540	11.32	0.010

^a Residual deviances (3) of fitted models (binomial, beta-binomial, binomial with side) and differences of deviances for comparisons between models (binomial versus beta-binomial, binomial versus binomial with side) were compared with a χ^2 distribution.

^b Bi = binomial, beta-bi = beta-binomial, and w = with side.

^c Dev = deviance.

ascospore-derived infection rates, and the temperature and weather factors which affect latent period and splash efficiency. Thus, the results from this experiment may be used to improve forecasting methods to assess risk from light leaf spot (9).

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LITERATURE CITED

- Amelung, D., and Daebeler, F. 1991. Occurrence of fertile apothecia and the epidemiology of *Pyrenopeziza brassicae* Sutton & Rawlinson (anamorph: *Cylindrosporium concentricum* Grev.) in the German Democratic Republic. IOBC wprs Bull. 14:147-150.
- Cheah, L. H., and Hartill, W. F. T. 1985. Disease Cycle in New-Zealand of Light Leaf-Spot of Brassicas, Induced by *Pyrenopeziza brassicae*. N. Z. J. Agric. Res. 28:567-573.
- Collet, D. 1991. Modelling Binary Data. Chapman & Hall, London.
- Figuerola, L., Shaw, M. W., Fitt, B. D. L., McCartney, H. A., and Welham, S. J. 1994. Effect of previous cropping and fungicides timing on the development of light leaf spot (*Pyrenopeziza brassicae*), seed yield and quality of winter oilseed rape (*Brassica napus*). Ann. Appl. Biol. 124:221-239.
- Fitt, B. D. L., Doughty, K. J., Gilles, T., Gladders, P., Steed, J. M., Su, H., and Sutherland, K. G. 1998. Methods for assessment of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. Ann. Appl. Biol. 133:329-341.
- Fitt, B. D. L., Doughty, K. J., Gladders, P., Steed, J. M., and Sutherland, K. G. 1998. Diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. Ann. Appl. Biol. 133:155-166.
- Fitt, B. D. L., Gladders, P., Turner, K. J., Sutherland, K. G., Welham, S. J., and Davies, J. M. L. 1997. Prospects for developing a forecasting scheme to optimise use of fungicides for disease control on winter oilseed rape in the UK. Asp. Appl. Biol. 48:135-142.
- Gilles, T., Ashby, A. M., Fitt, B. D. L., and Cole, T. 2001. Development of *Pyrenopeziza brassicae* apothecia on agar and oilseed rape debris. Mycol. Res. 105:705-714.
- Gilles, T., Evans, N., Fitt, B. D. L., and Jeger, M. J. 2000. Epidemiology in relation to methods for forecasting light leaf spot (*Pyrenopeziza brassicae*) severity on winter oilseed rape (*Brassica napus*) in the UK. Eur. J. Plant Pathol. 106:593-605.
- Gilles, T., and Fitt, B. D. L. 1999. Infectivity of ascospores of *Pyrenopeziza brassicae* on leaves and factors affecting maturation of apothecia on debris of oilseed rape. Asp. Appl. Biol. 56:61-66.
- Gilles, T., Fitt, B. D. L., and Jeger, M. J. 2001. Effects of environmental factors on development of *Pyrenopeziza brassicae* (light leaf spot) apothecia on oilseed rape debris. Phytopathology 91:392-398.
- Gilles, T., Fitt, B. D. L., Kennedy, R., Welham, S. J., and Jeger, M. J. 2000. Effects of temperature and wetness duration on conidial infection, latent period and asexual sporulation of *Pyrenopeziza brassicae* on leaves of oilseed rape. Plant Pathol. 49:498-508.
- Gilles, T., Fitt, B. D. L., McCartney, H. A., Papastamati, K., and Steed, J. M. 2001. The roles of ascospores and conidia of *Pyrenopeziza brassicae* in light leaf spot epidemics on winter oilseed rape (*Brassica napus*) in the UK. Ann. Appl. Biol. 138:141-152.
- Gilles, T., Fitt, B. D. L., Welham, S. J., Evans, N., Steed, J. M., and Jeger, M. J. 2001. Modelling the effects of temperature and wetness duration on development of light leaf spot on oilseed rape leaves inoculated with *Pyrenopeziza brassicae* conidia. Plant Pathol. 50:42-52.
- Hughes, G., and Madden, L. V. 1992. Aggregation and incidence of disease. Plant Pathol. 41:657-660.
- Hughes, G., and Madden, L. V. 1995. Some methods allowing for aggregated patterns of disease incidence in the analysis of data from designed experiments. Plant Pathol. 44:927-943.
- Hughes, G., Madden, L. V., and Munkvold, G. P. 1996. Cluster sampling for disease incidence data. Phytopathology 86:132-137.
- Karolewski, Z., Evans, N., Fitt, B. D. L., Todd, A. D., and Baierl, A. 2002. Sporulation of *Pyrenopeziza brassicae* (light leaf spot) on oilseed rape (*Brassica napus*) leaves inoculated with ascospores and conidia at different temperatures and wetness durations. Plant Pathol. 51:654-665.
- Lacey, M. E., Rawlinson, C. J., and McCartney, H. A. 1987. First record of the natural occurrence in England of the teleomorph of *Pyrenopeziza brassicae* on oilseed rape. Trans. Br. Mycol. Soc. 89:135-140.
- Madden, L. V., and Hughes, G. 1999. Sampling for plant disease incidence. Phytopathology 89:1088-1103.
- Maddock, S. E., and Ingram, D. S. 1981. Studies of survival and longevity of the light leaf spot pathogen of brassicas, *Pyrenopeziza brassicae*. Trans. Br. Mycol. Soc. 77:153-159.
- Maddock, S. E., Ingram, D. S., and Gilligan, C. A. 1981. Resistance of cultivated brassicas to *Pyrenopeziza brassicae*. Trans. Br. Mycol. Soc. 76:371-382.
- McCartney, H. A., and Lacey, M. E. 1990. The production and release of ascospores of *Pyrenopeziza brassicae* on oilseed rape. Plant Pathol. 39:17-32.
- McCartney, H. A., Lacey, M. E., and Rawlinson, C. J. 1986. Dispersal of *Pyrenopeziza brassicae* spores from an oil-seed rape crop. J. Agric. Sci. 107:299-305.
- McCullagh, P., and Nelder, J. 1989. Generalized Linear Models. 2nd ed. Chapman & Hall, London.
- Payne, R. W., Lane, P. W., Digby, P. G. N., Harding, S. A., Leech, P. K., Morgan, G. W., Todd, A. D., Thompson, R., Tunnicliffe-Wilson, G., Welham, S. J., and White, R. P. 1993. Genstat 5 Release 3 Reference Manual. Clarendon Press, Oxford, UK.
- Rawlinson, C. J., Sutton, B. C., and Muthyalu, G. 1978. Taxonomy and biology of *Pyrenopeziza brassicae* sp. nov. (*Cylindrosporium concentricum*), a pathogen of winter oilseed rape (*Brassica napus* ssp. *oleifera*). Trans. Br. Mycol. Soc. 71:425-439.
- Staunton, W. P. 1967. Studies on light leaf spot (*Gleospodium concentricum*) of Brassicae. Irish J. Agric. Res. 6:203-211.
- Sylvester-Bradley, R., and Makepeace, R. J. 1985. Revision of a code for stages of development in oilseed rape (*Brassica napus* L.). Asp. Appl. Biol. 10:395-400.