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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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A model was developed to describe the effects of temperature and leaf wetness duration in controlled-environment experiments 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 percentage leaf area with sporulation, and included the parameters maximum percentage leaf area with sporulation (c); maximum rate of increase in percentage leaf area with sporulation (r); and latent period (l, the time from inoculation until the leaf area with sporulation reached 37% of c). 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 described the progress with time in percentage leaf area with sporulation, including the effects of temperature and leaf wetness duration on the parameters c, r and l. It generally fitted well to the observed data. Latent periods in previously published experiments were predicted accurately by the model, but percentage leaf area with sporulation was not. Assuming a great number of conidia were dispersed and infection occurred when there was 2 mm h^{-1} rain for 0·5 h, 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 1998/99 and 1999/2000. The predictions agreed with the observations.

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 components of disease progress, and are affected by a complex interaction between temperature and leaf wetness duration. The spatial pattern of light leaf spot in crops (Fitt *et al*., 1998; Evans *et al*., 1999) 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 that germinated increased as temperature increased from 5 to 20°C, or as wetness duration increased from 12 to 80 h (Figueroa *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 (Figueroa *et al*., 1995b; Gilles *et al*., 2000). At 12–20°C, a minimum leaf wetness duration of approximately 6 h was required for infection, and increasingly longer minimum leaf wetness durations of ≈10, ≈16 or ≈24 h were required for infection when temperature decreased to 8, 6 or 4°C, respectively. At temperatures ≥24°C, infection did not occur.

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After infection has occurred, the latent period of *P. brassicae* is also affected by temperature. Figueroa *et al*. (1995a) fitted a negative linear relationship between the length of the latent period in days and temperature in the range 5–15°C, although this is an approximation of a nonlinear 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 first sporulation, at lower temperatures (6 and 8°C; Gilles *et al*., 2000). Gilles *et al*. (2000) also found that the percentage leaf area with sporulation could be directly related to the number of conidia produced per leaf; was greatest on leaves incubated at approximately 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 percentage leaf area with sporulation and the number of conidia produced.

We report the development of a model that describes the effects of temperature and leaf wetness duration on the progress of light leaf spot severity with time; the testing of this model on controlled-environment data; and its application to predict the progress of light leaf spot under natural conditions.

Materials and methods

Experimental data

Data for progress with time after inoculation of percentage leaf area with sporulation were obtained from an experiment described by Gilles *et al*. (2000), in which leaves of oilseed rape plants at the four- to five-leaf growth stage (GS 1.4–1.5; Sylvester-Bradley & Makepeace, 1985) were inoculated with $0{\cdot}5 \times 10^6$ conidia mL^{-1} 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 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 (the cabinets), and the effects of leaf wetness duration and its interaction with temperature were tested on the subplots (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, leaf surfaces dried within 0·5 h. The different temperature treatments were applied to the different cabinets in

Table 1 Parameters of the model describing effects of temperature and leaf wetness duration on light leaf spot disease progress

Equations	Parameter	Estimate	Standard error
Percentage leaf area with sporulation, s			
$s(t) = c\ \exp{-}\exp(-(2.72/c)(t - l))$	c		
	r	see below	
	l		
Maximum percentage leaf area with sporulation, c			
$c(W) = m_c\ \exp{-}\exp(-q_c(W - v_c))$	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
Maximum rate of increase in percentage leaf area with sporulation, r			
$r(W) = m_r\ \exp{-}\exp(-q_r(W - v_r))$	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
Latent period, l			
$l(W, T) = d_1 - d_2T + d_3T^2 - d_4W$	d_1	48·25	3·460
	d_2	3·87	0·584
	d_3	0·11	0·022
	d_4	0·056	0·0149

Percentage 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 percentage 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). m_c, v_c, m_r *and* v_r depended on temperature (T). l was described by a polynomial in T and W.

each of the series of four experiments. However, as certain temperatures could not be obtained in all cabinets, randomization of temperature treatments across cabinets was not complete. Not all the treatments were repeated in all four experiments, giving an unbalanced design for analysis (Table 1, Gilles *et al.*, 2000). The percentage leaf area with sporulation was estimated visually on leaves 3 and 4 of each individual plant.

Percentage 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 percentage leaf area with sporulation (s) with time (t) as the explanatory variable. A re-parameterization of the function as $m = l$ and $b = 2.72r/c$ was used to rewrite the Gompertz function as:

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

to describe disease development in terms of the biologically meaningful parameters latent period (l); maximum rate of increase in sporulation (r); and maximum percentage leaf area with sporulation (c) (Fig. 1). The constant value 2·72 is the base value of the natural logarithm. The parameter for l in this function estimated latent period as the time from inoculation until the time when 37% of the maximum percentage leaf area with sporulation was reached. This was the point of inflection of the Gompertz curve. At this time the increase in percentage 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

$$\frac{\left(\frac{1}{\mathrm{se}_1}c_1 + \frac{1}{\mathrm{se}_2}c_2 + \ldots + \frac{1}{\mathrm{se}_8}c_8\right)}{\left(\frac{1}{\mathrm{se}_1} + \frac{1}{\mathrm{se}_2} + \ldots + \frac{1}{\mathrm{se}_8}\right)}.$$

As the experimental design was unbalanced, the weighted average values of l, r and c for each treatment were analysed by residual maximum likelihood analysis (REML in GENSTAT 5 Release 4·1), with temperature × 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.

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

Response surface methods were used to obtain expressions for parameters c, r and l in terms of both temperature and leaf wetness duration. For both maximum percentage 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

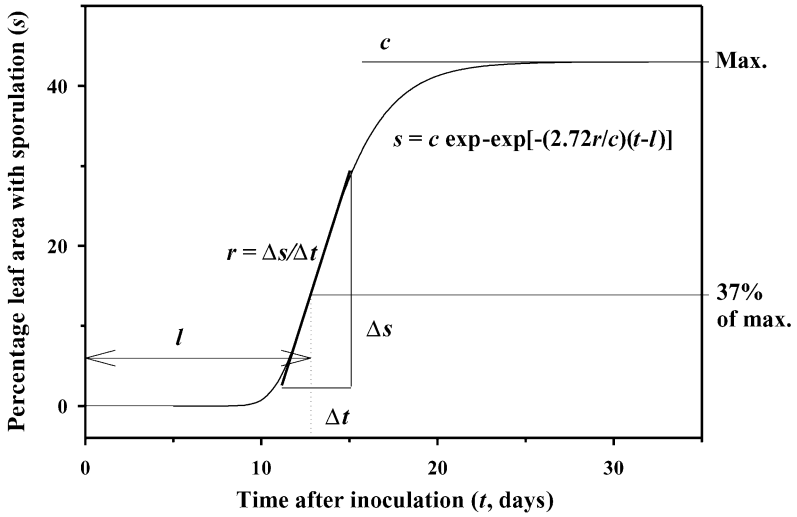

Figure 1 Percentage leaf area with sporulation (s) on oilseed rape leaves inoculated with conidia of *Pyrenopeziza brassicae* (light leaf spot) described as a function of time (t) with a Gompertz function ($s = c\ \exp{-}\exp\ [(-(2.72r/c)(t - l)]$; 2·72 is the base value of the natural logarithm). The function was based on the parameters maximum percentage leaf area with sporulation (c); maximum rate of increase in percentage 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 percentage leaf area with sporulation was reached.

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 (percentage 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 described approximately 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*., 2000) 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 overestimated the actual minimum leaf wetness duration required for infection. The parameter q was related to the maximum rate of increase in c (q_c) or r (q_r) with increasing leaf wetness duration (the point of inflection of the Gompertz curve).

The relationship between latent period and temperature over the range 4–20°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):

$$l(W,T) = d_1 - d_2T + d_3T^2 - d_4W \quad (3)$$

with parameters d_1, d_2, d_3 and d_4. Finally, the functions describing progress with time in the percentage 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 single model.

Testing the model

The predictions made by the model of percentage 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 ($\approx 2 \times 10^6$ conidia mL^{-1}) in controlled-environment cabinets at 12 or 18°C and with 16 or 48 h of leaf wetness after inoculation. The 'lesions' that were observed on the plants in controlled environments were not necrotic lesions (as observed in affected crops), but circular areas on a leaf that remained green during leaf senescence (Gilles *et al*., 2000). For each treatment, the numbers of 'lesions' were counted on three leaves on each 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 percentage leaf area with sporulation, by using a linear relationship between the percentage 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, $P = 0{\cdot}001$; Gilles *et al*., 2000). The values estimated from the experiment of Figueroa *et al*. (1995b) were compared with the values predicted by the model.

The latent period predictions were also compared with observed light leaf spot progress under natural conditions. The progress with time in percentage 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 stem and pod debris between the pots. The debris was 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

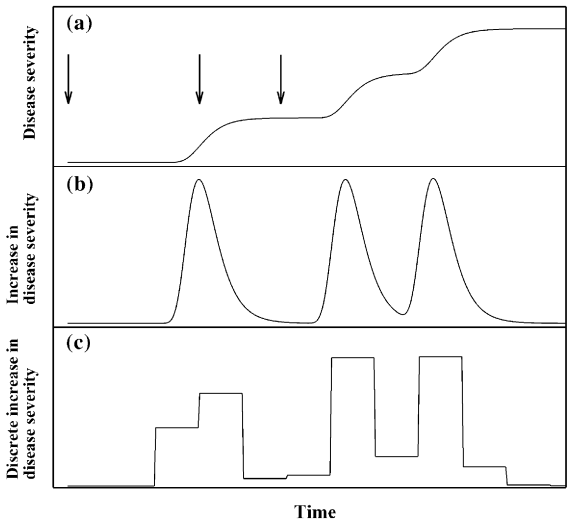

Figure 2 Schematic diagram of progress with time in disease severity for a polycyclic disease epidemic (a) to illustrate large increases in disease severity caused by three infection events (arrows) (b), and resulting increases in disease severity measured by assessments at discrete intervals (c).

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 there had been insufficient moisture for them to mature (Gilles & Fitt, 1999).

The percentage 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 percentage leaf area affected by light leaf spot (s), including the percentage 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 (percentage 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. For light leaf spot epidemics propagated by splash-dispersed conidia, such an increase in disease severity is likely to occur after a rain event that 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 percentage leaf area affected by light leaf spot was calculated by dividing the difference in the percentage 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 that 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 assessed was small (on average there were 5·1–5·6 leaves per plant in 1998/99, 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 percentage leaf area affected by light leaf spot between successive assessment dates was divided by the number of days between successive assessment dates ($t_a - t_{a-1}$):

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

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

Data from these two pot experiments were used to test the model predictions for progress of light leaf spot. The derived expression for latent period in terms of wetness duration and temperature (Eqn 3) was used to predict the time from a rain shower, which dispersed conidia by splash, until the increase in percentage leaf area with sporulation due to the resulting infection was maximum (steepest slope in the Gompertz curve). These predicted latent periods are actually overestimates 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 rain h^{-1} 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 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 percentage leaf area with light leaf spot was predicted was compared with the time when there was a large increase in percentage 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 that occurred before sporulation of *P. brassicae* was first observed were not used for predictions.

As 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.*, 2000), plants were inoculated with concentrated conidial suspensions ($0{\cdot}5 \times 10^6$ conidia mL^{-1}), 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 percentage 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 Eqn 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

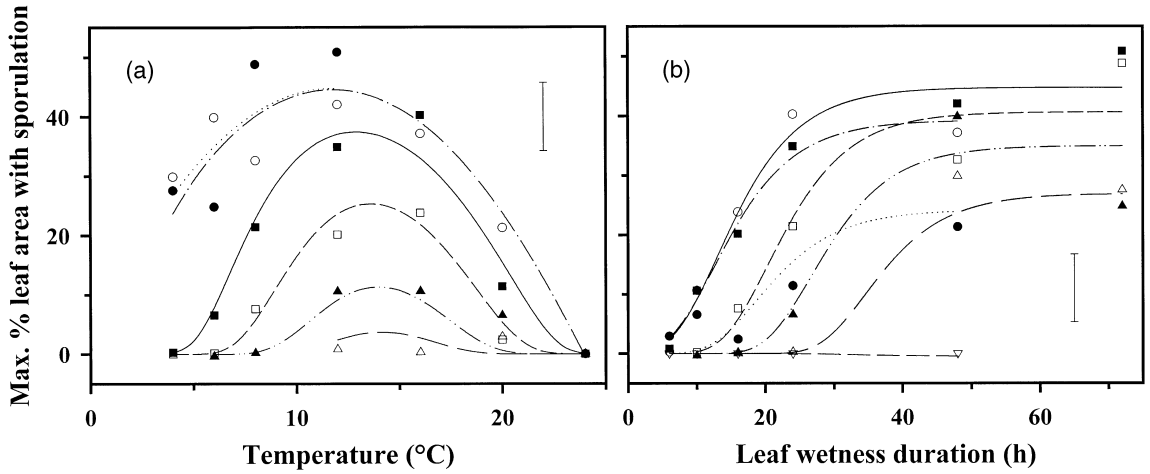

Figure 3 Effects of temperature and leaf wetness duration on the maximum percentage leaf area with sporulation (*c*) on oilseed rape leaves after inoculation with conidial suspensions of *Pyrenopeziza brassicae* (light leaf spot). Comparison between the mean values of *c* estimated by REML analysis (points) or predicted by the model (lines) (see Table 1). (a) Effects of temperature at 6 (△,– –); 10 (▲,–···–); 16 (□,– – –); 24 (■,——); 48 (○,–·–); or 72 (●,·····) h leaf wetness. (b) Effects of leaf wetness duration at 4 (△,– –); 6 (▲,–···–); 8 (□,– – –); 12 (■,——); 16 (○,–·–); 20 (●,·····); or 24 (▽,– –) °C. Error bars (23 d.f.) are twice the standard error of the estimated mean values of *c*.

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 using Eqn 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

Modified Gompertz functions (Eqn 1; Table 1) fitted the data for the progress with time (t; days) of percentage leaf area with sporulation (s) on oilseed rape leaves inoculated with *P. brassicae* conidia. Estimates of c, l and r were obtained for each temperature and leaf

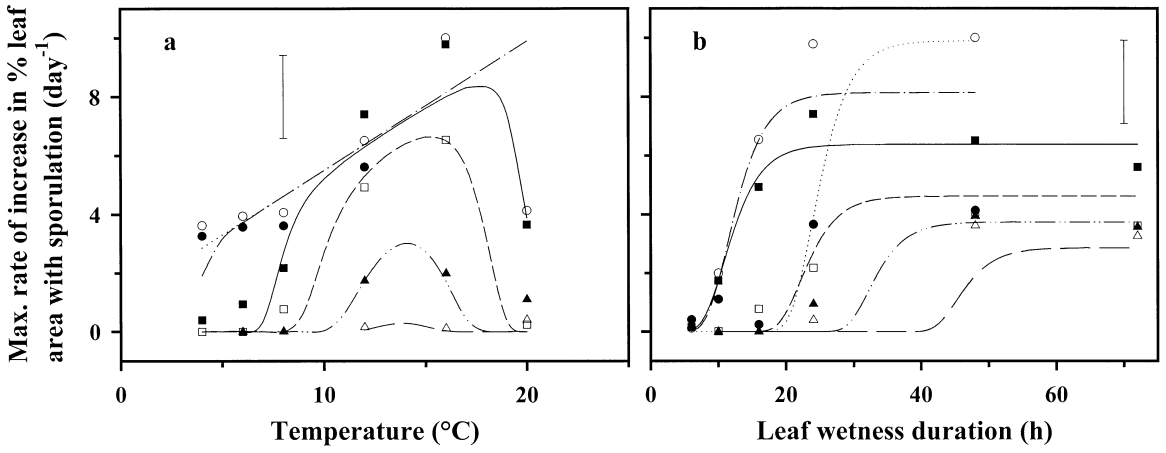

Figure 4 Effects of temperature and leaf wetness duration on the maximum rate of increase in percentage leaf area with sporulation (*r*) on oilseed rape leaves after inoculation with conidial suspensions of *Pyrenopeziza brassicae* (light leaf spot). Comparison between the mean values of *r* estimated by REML analysis (points) or predicted by the model (lines) (see Table 1). (a) Effects of temperature at 6 (△,– –); 10 (▲,–···–); 16 (□,– – –); 24 (■,——); 48 (○,–·–); or 72 (●,·····) h of leaf wetness. (b) Effects of leaf wetness duration at 4 (△,– –); 6 (▲,–···–); 8 (□,– – –); 12 (■,——); 16 (○,–·–); or 20 (●,·····)°C. Error bars (19 d.f.) are twice the standard error of the estimated mean values of *r*.

wetness duration treatment. Another Gompertz function (Eqn 2; Fig. 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 ($P = 0{\cdot}011$ and $P < 0{\cdot}001$, respectively), which were therefore incorporated into the model:

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

A modified Gompertz function (Eqn 2; Fig. 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 ($P = 0{\cdot}017$). 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 ($P = 0{\cdot}26$). 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:

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

The minimum leaf wetness duration required for infection on values of r was fitted by this model, except at a wetness duration of 72 h. However, in this model the maximum of r increased linearly with temperature, whereas the data showed that r decreased when temperatures increased above 16°C. This linear relationship between the maximum of r and temperature also determined the asymptote at each temperature, above which r could not increase (Fig. 4a,b). 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; Fig. 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 light leaf spot progress over 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 (Fig. 6), but not at all temperatures and leaf wetness durations. At 6°C/48 h leaf wetness duration, for example, the latent period was overestimated, and at 20°C/48 h leaf wetness duration the maximum percentage leaf area with sporulation was overestimated. From the REML analysis, there was no evidence of any significant variation between cabinets for 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 se 0·92). This difference is large in relation to temperature differences for this parameter (Fig. 4a). As temperature effects would be confounded with cabinet effects in an experiment without repeated randomized 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).

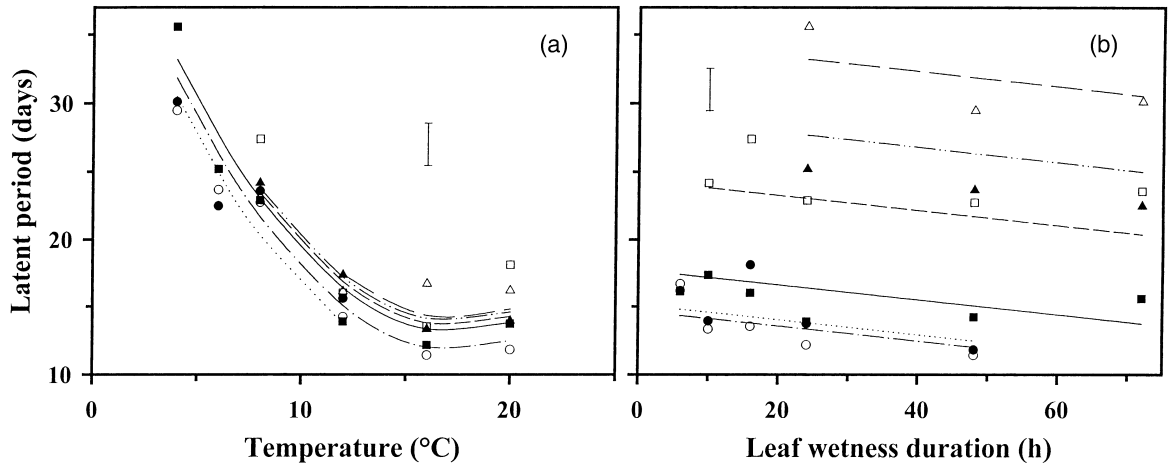

Figure 5 Effects of temperature and leaf wetness duration on the latent period (l) of *Pyrenopeziza brassicae* (light leaf spot) after inoculation of oilseed rape leaves with conidial suspensions. The latent period was estimated as the time from inoculation until the time when 37% of the maximum percentage leaf area with sporulation was reached. Comparison between the mean values of l estimated by REML analysis (points) or predicted by the model (lines); see Table 1. (a) Effects of temperature at 6 (△,– –); 10 (▲,– · · –); 16 (□,– – –); 24 (■,——); 48 (○,– · –); or 72 (●,· · · · ·) h leaf wetness. (b) Effects of leaf wetness duration at 4 (△,– –); 6 (▲,– · · –); 8 (□,– – –); 12 (■,——); 16 (○,– · –); or 20 (●,· · · · ·)°C. Error bars (16 d.f.) are twice the standard error of the estimated mean values of l.

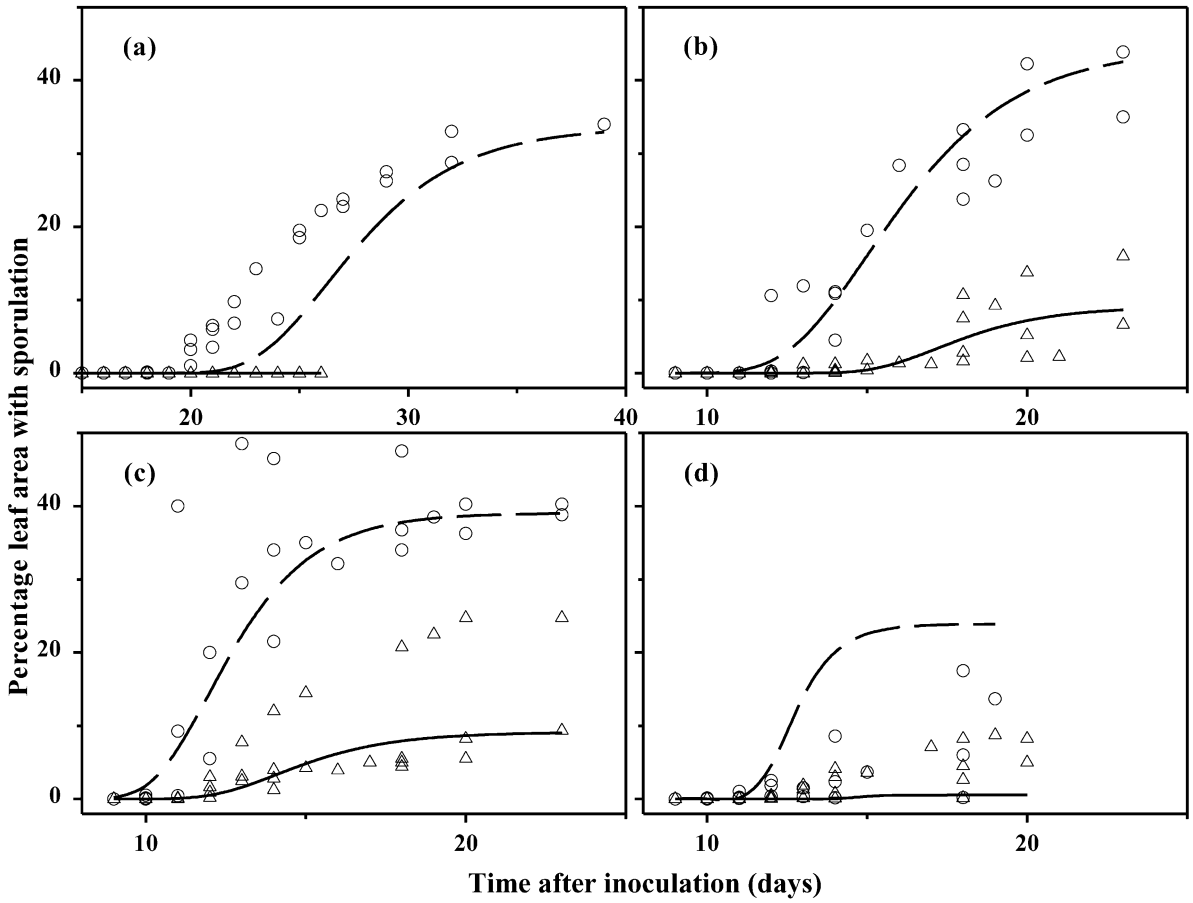

Figure 6 Predictions made by the model of progress with time (days after inoculation) in percentage leaf area with sporulation on oilseed rape (cv. Bristol) plants inoculated with conidial suspensions of *Pyrenopeziza brassicae* (light leaf spot) at (a) 6; (b) 12; (c) 16; or (d) 20°C, and with 10 (solid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

Testing of the model

The model gave a good prediction of the latent period of *P. brassicae* in the independent data for percentage leaf area with sporulation estimated from experiments of Figueroa *et al.* (1995b) (Fig. 7). The model did not fit the data for maximum percentage leaf area with sporulation.

To test the latent period equation component of the model with data from the pot experiments in the 1998/99 and 1999/2000 seasons, daily average temperatures were input into Eqn 5 with $W = 6$:

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

When these daily values (Eqn 10) were summed until they reached a value of 1 (Eqn 6), the model gave, on average, good predictions of the dates, with the greatest daily increases in percentage leaf area affected by light leaf spot in relation to previous occurrences of 'heavy' rain (0·5 h in duration) after sporulation was first observed (Fig. 8). Periods of 'light' rain were not often related to subsequent maxima in the increase in percentage leaf area affected by light leaf spot. In 1998/99, 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 'heavy' rain events on 24 and 28 November 1998. It is likely that the increase in disease severity in mid-December was associated with rainfall in mid-November, although this was not modelled as conidia were not observed until 16 November. In 1999/2000, there was one large maximum in the increase in disease severity (28 November–3 December 1999) and two smaller 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. There were no other definite maxima in the increase in disease severity in the period considered, and no other 'heavy' rain events. The

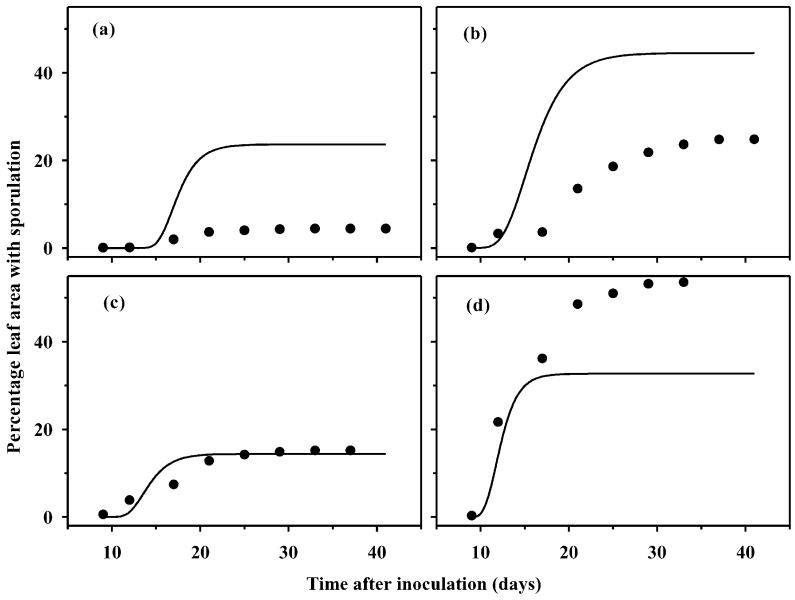

Figure 7 Comparison of values predicted by the model (lines) with observed values (points). Data from Figueroa *et al.* (1995b) for progress with time after inoculation in percentage leaf area with sporulation of oilseed rape (cv. Cobra) leaves inoculated with *Pyrenopeziza brassicae* conidial suspensions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

predictions for the time from splash-dispersal events until the greatest increases in percentage 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 6–13°C) to a 30-day period following the 'heavy' rainfall on 24 November 1998 (when average temperatures were 1–11°C). Thus the observed maximum increases in disease

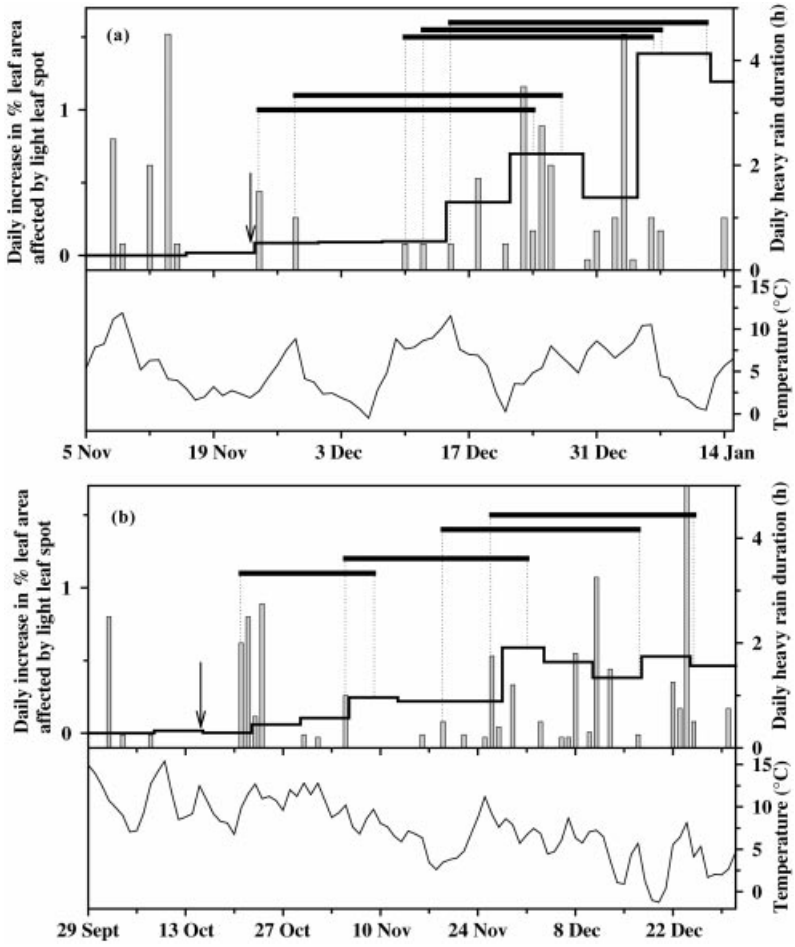

Figure 8 Daily increase in percentage leaf area affected by light leaf spot (*Pyrenopeziza brassicae*) (thin line in discrete steps) observed in weekly intervals on oilseed rape plants in pots outdoors in autumn/winter of seasons (a) 1998/99; (b) 1999/2000. Following 'heavy' rainfall events (≥2 mm rain h^{-1}; vertical bars) of ≥0·5 h after sporulation had started (arrows), the length of the latent period (*l*, thick horizontal bars) was predicted by summing the values for daily fulfilment of latent period predicted by the model until they reached 1. Daily average temperature (below) was input for the model.

severity during the experimental periods in both seasons could all be predicted by the model as associated with 'heavy' rain events that 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 the development of light leaf spot on oilseed rape after infection by *P. brassicae* conidia. Testing the model (Table 1) with data from an independent experiment (Figueroa *et al*., 1995b) suggested that the model predicts latent period (from infection to sporulation) well, but not percentage leaf area with sporulation (Fig. 7). The effects of temperature on latent period have successfully been described by nonlinear functions for other pathogens, such as *Bremia lactucae* on lettuce (Scherm & 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 plant pathogens. The decrease in the latent period of *P. brassicae* with increasing 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 produce conidia earlier. The model predictions for percentage 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 ($\approx 2 \times 10^6$ instead of $\approx 0{\cdot}5 \times 10^6$ conidia mL^{-1}) were used in the experiment of Figueroa *et al*. (1995b), by comparison with the experiment of Gilles *et al*. (2000) on which the model is based. Thus to predict disease severity quantitatively, the effects of factors such as cultivar, pathogen isolate and inoculum density need to be understood.

Further evidence that the model accurately predicts the latent period of *P. brassicae* was provided by 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 percentage leaf area affected by light leaf spot occurred in the autumn/winter of the 1998/99 and 1999/2000 seasons. Results suggested that 'heavy' rain showers (≥ 2 mm rain h^{-1} for $\geq 0{\cdot}5$ h) contributed more to splash dispersal of conidia than 'light' rain showers. The predictions of the dates of the greatest increases in percentage leaf area affected by light leaf spot agreed with observed dates 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 the occurrence of maxima in increase in percentage leaf area affected by light leaf spot in the pot experiments outdoors provides evidence 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 an important component of crop-specific forecasts of light leaf spot severity.

The accuracy of latent period predictions might be improved by predicting latent period in hours instead of days, and by using hourly instead of daily average temperatures as input into the latent period model. As the relationship between temperature and latent period is nonlinear, the latent period predicted by using average temperatures must differ from that predicted by using fluctuating temperatures for the same period (Kaufmann, 1932; Scherm & van Bruggen, 1994; Xu, 1996). 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 might 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. Ultimately these models, describing effects of temperature and wetness duration on the 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 disease severity and epidemic progress.

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