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FULL RESEARCH PAPER

Development of *Peronospora parasitica* epidemics on radish as modelled by the effects of water vapour saturation deficit and temperature

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Abstract Epidemics of *Peronospora parasitica* are strongly affected by temperature and air moisture, and the interaction of these factors. Because a significant percentage of radish plants are grown in greenhouses, it may be possible to influence epidemics by altering the greenhouse climate. The objective of this study was to test the hypothesis that epidemics of *P. parasitica* can be modelled by the effects of air temperature and moisture in the greenhouse. Such a model could then be used to analyse greenhouse climate control strategies with regard to managing downy mildew. Five radish crops were grown under greenhouse conditions with set-points for heating and ventilation intended to obtain favourable conditions for disease development during the first part of the growing cycle. Subsequent to this first phase, unfavourable conditions were set until harvest. Disease incidence was measured once a week until the radishes reached marketable size. In addition, experiments were carried out in growth chambers in which inoculated plants were subjected to air temperatures between 8 and 27°C, and disease incidence and sporulation intensity were measured. Data from these two experiments were then used to estimate model parameters. In this model, the interactions of air temperature (T) and water vapour saturation deficit (SD) were adequately described by a multiplicative relationship. The simulated epidemics by the fitted model were highly correlated with the observed epidemics ($r = 0.91$, $R^2 = 0.83$, $n = 29$). Parameter estimates indicated that T of ca. 20°C and SD < 0.03 hPa resulted in the highest rates of disease development and that the rate was zero when SD > 2.0 hPa. Both experimental data and simulations showed that epidemics of *P. parasitica* can be effectively controlled by managing the greenhouse climate.

Keywords Downy mildew · Model · Greenhouse climate control

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Introduction

Downy mildew diseases are polycyclic. Each step of the life cycle, i.e. conidial germination, infection, latency and sporulation, is influenced by climatic conditions. Conidia of *Peronospora parasitica*, the cause of downy mildew of radish (*Raphanus sativus*), can germinate in temperatures

ranging from 5 to 30°C. However, different studies have shown significant differences in optimum temperatures for germination: for example, 6–8°C (Felton & Walker, 1946), 13°C (Hartmann, Sutton, & Procter, 1983), 20°C (Bonnet & Blanchard, 1987), and 15–25°C (Achar, 1998). Germination of conidia of *P. parasitica* commenced after 1 h under favourable conditions and the maximum proportion of germination was obtained after 5 h (Weis, 1994). Conidia germinate when exposed to free water but not at an atmospheric water potential ψ of 0, –30, –60, –90 bar within 24 h (Hartmann et al., 1983). In a study by Achar (1998), no infection occurred after an incubation period of 6 h at 100% relative humidity (RH) at any temperature, although proportion of germination was high. After incubation periods of 12 and 24 h at 20–25°C proportions of infection were approximately 60%. Prolonged incubation periods of 48 h increased proportions of infection from 50 to 90% at 15°C, and from 60 to 100% at 25°C (Achar, 1998). The time between infection and the appearance of lesions or the production of new inoculum depends on both the genotype (3–10 days; Bonnet & Blancard, 1987) and the plant organ (5 days for cotyledons, up to 7 days for true leaves of radish; Weis, 1994). The most rapid development of disease in radish occurred at temperatures of 20–24°C (Lakra, 2001). Sporulation of *P. parasitica* is affected by temperature, humidity, and light. Sporulation can occur overnight in a temperature range from 4 to 24°C (Felton & Walker, 1946). Maximum sporulation intensities were found between 12 and 16°C, and generally sporulation intensity increased with a prolonged duration of favourable moist conditions (Felton & Walker, 1946; Hartmann et al., 1983; Jang & Safeeulla, 1990). Moisture requirements for sporulation of *P. parasitica* were identified by Klodt-Bussmann (1995) and Hartmann et al. (1983) as ≥98% and ≥95.5% RH at 18°C, respectively.

Plant disease models aim to understand the main determinants of epidemic development in order to develop sustainable approaches for strategic and tactical management of diseases (van Maanen & Xu, 2003). Several versions of a forecaster for *P. destructor* on onions were developed (DOWNCAST, Jesperson and Sutton, 1987; deVisser, 1998; ZWIPERO, Friedrich, Leinhos, & Lopmeier, 2003; MILIONCAST, Gilles et al., 2004) to predict the risk of sporulation and infection. A model for predicting infection periods of *Bremia lactucae* (the cause of downy mildew of lettuce), based on prognosis of leaf wetness in the morning (Scherm et al., 1995), was updated by Wu et al. (2002), who demonstrated that development and survival of conidia need not be considered. The common aim of these forecasting systems was the improvement of fungicide application schemes. For downy mildew of cruciferous crops, caused by *P. parasitica*, only the model of Fink and Kofoet (2005) is available. A significant proportion of radishes are grown in greenhouses from autumn to spring in north and central Europe, which renders it possible to influence the epidemic by managing the greenhouse climate, via manipulating heating, ventilation and fogging. The objective of this study was to test the hypothesis that the epidemic of *P. parasitica* can be modelled by the effects of air temperature and moisture in the greenhouse. Such a model could be used, in combination with further experimentation, to analyse greenhouse climate control strategies with regard to managing downy mildew.

Materials and methods

Inoculum

Radish from the susceptible cv. Sirri was sown in a horticultural substrate (Fruhsdorfer Type P) in either pots of 8 cm diameter (one plant per pot) or containers of size 46 × 28 × 6 cm (70 plants per container). Plants were grown in a greenhouse at a day temperature of 15–20°C and a night temperature of 10–15°C. Plants were inoculated using a chromatography sprayer with an inoculum density of 10^5 conidia ml^{-1} when plants reached growth stage BBCH 12 (Hack et al., 1992) i.e. when the second true leaf was unfolded. To provide optimum conditions for infection, plants were then placed for 24 h under high humidity conditions under a plastic sheath, which was sprayed inside with water until run-off occurred. Subsequently, the plants were placed in a greenhouse (day temperature of 15–20°C and night temperature of 10–15°C) under low humidity

conditions to avoid spontaneous sporulation. Three to six days later, all plants were placed overnight under saturated humidity conditions to induce the sporulation of *P. parasitica*. The freshly produced conidia were used to inoculate plants grown in containers for the climate chamber experiments and plants grown in pots for the greenhouse experiments.

Experiments

In all experiments the inoculum was applied with a chromatography sprayer as described above. Climate chamber experiments were carried out in a 1.25×0.75 m chamber (VB1014, Voetsch, Germany). When the plants reached growth stage BBCH 12, four containers with 70 plants per container were placed in each climate chamber. Light was switched on for 16 h with a light intensity of 400 µmol m^{-2} s^{-1} (designated 'day') followed by 8 h without light (designated 'night'). After inoculation, the plants were incubated for 24 h in the dark at the 'day' temperature shown in Table 1. After incubation, the plants were cultivated with 'day' and 'night' temperature set-points, shown in Table 1. The experiments were conducted in two climate chambers. Sporulation was induced over night under high humidity conditions under a plastic sheath, 2–3 times for each inoculation only, because after twofold sporulation the intensity has been shown to decrease. Disease incidence and sporulation intensity were measured for a period of up to 6 days (see Fig. 4). Sporulation intensity was classified and a sporulation intensity index (SI) was calculated. A mean score derived from plants scored as 0, 0.025, 0.15, 0.375, 0.75 corresponded to 0, <5, <25, <50, >50% leaf area affected.

Table 1 Temperature set-points in growth chamber experiments

Treatment number	'Day' temperature °C	'Night' temperature °C	Average temperature °C
1	10	5	8.3
2	10	10	10.0
3	15	5	11.7
4	15	10	13.3
5	20	10	16.7
6	20	15	18.3
7	25	15	21.7
8	25	20	23.3
9	30	20	26.7

Greenhouse experiments were carried out in an 18×7 m greenhouse at the Institute of Vegetable and Ornamental Crops, Grossbeeren, Germany. The climatic conditions in the greenhouse were adjusted to either promote (type I) or reduce (type II) the downy mildew epidemic. Because the greenhouse controller did not allow programming of set points for water vapour saturation deficit (SD) directly, we changed SD by manually adjusting set points of heating, ventilation and fogging systems depending on outside air moisture and temperature. Under type I conditions, the SD was low between 21.00 and 09.00 h to induce sporulation of *P. parasitica* and to favour infection. SD was increased between 09.00 and 13.00 h to promote the liberation and dissemination of the conidia, and decreased again between 13.00 and 17.00 h to favour conditions for infection, and increased again between 17.00 and 21.00 h. Under type II conditions, the sporulation-infection process was hindered by the increased SD from 00.00 to 24.00 h. Since temperature and moisture inside a greenhouse are strongly influenced by outside conditions, the actual inside temperature and SD varied both within and between experiments. The resulting actual conditions are shown in Fig. 1. The temperature and air moisture were measured psychrometrically in the middle of the greenhouse, 0.20 m above ground, by a psychrometer (Model 1.1130.10.000, Adolf Thies GmbH & Co. KG Göttingen, Germany).

Five sets of radish, cv. Sirri, were sown in greenhouse soil (loamy sand). Although the same number of seeds was sown in all sets, plant densities varied between sets due to different proportions of emergence (198, 177, 138, 202 and 152 plants m^{-2}, respectively). The plants were inoculated when they reached growth stage BBCH 10 i.e. when cotyledons were unfolded, which occurred on 15 September 2001, 16 November 2001, 22 February 2002, 29 April 2003, and 6 June 2003, respectively. Twenty-seven infected potted plants were then placed in the greenhouse

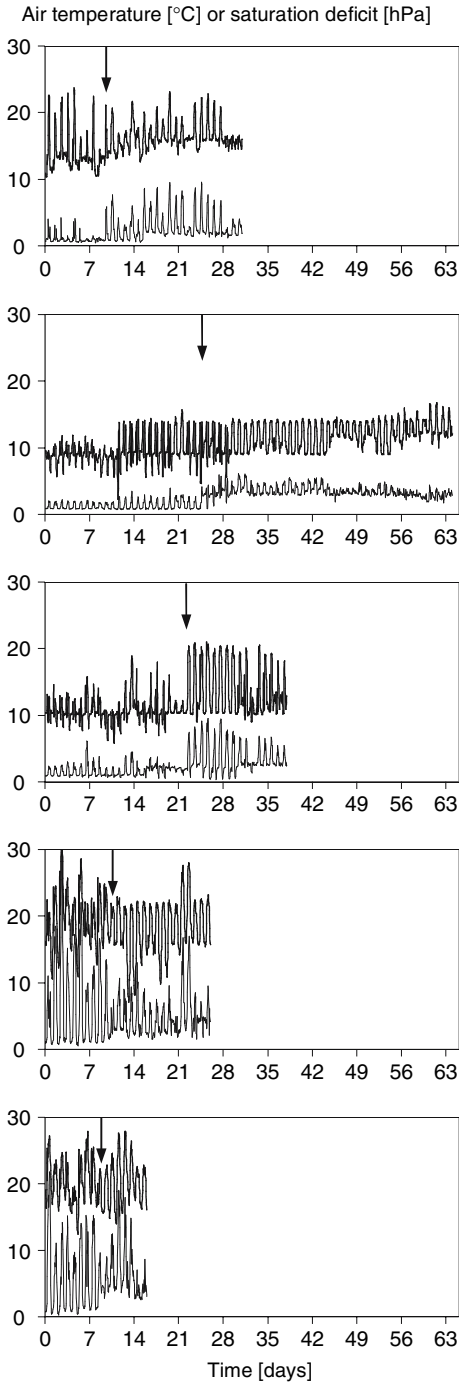

Fig. 1 Hourly averages of air temperature (upper line) and water vapour saturation deficit (lower line) for five data sets in the greenhouse. Day 1 is the day on which inoculated plants were put into the greenhouse. Arrows indicate the change of climatic conditions

2×2 m apart on 18×6 m grid. Inoculation was repeated for sets four and five (on 2 May 2003 and 16 June 2003, respectively) since sporulation on the potted plants was sparse. The plants were initially grown under type I climatic conditions (favourable for epidemic development), and then the set-points were altered to type II conditions on the dates shown in Fig. 1. When necessary, greenhouse plots were irrigated at noon to reduce the influence of irrigation on the sporulation of the downy mildew. Except for the lack of fungicide treatments, crop husbandry was carried out as in commercial practice. Disease incidence was assessed once a week by counting the number of plants with symptoms of *P. parasitica* in every second row. For each assessment 5,300–7,800 plants were scored, depending on plant density. Each set of plants was assessed 4–8 times because the experiments were terminated when radish plants reached marketable size (20 mm diameter), which took 15–64 days, depending on temperature and irradiance.

Model description

The model is based on a generic model described by Xu and Ridout (1998). Fink and Kofoet (2005) showed that this model approach adequately describes the epidemic of *P. parasitica* on radish. Until now, however, the model had only been parameterised for climatic conditions, which were not limiting for *P. parasitica*. For the present study we added two functions to the model to allow for limiting effects of air moisture and temperature. Disease progress is modelled on a rectangular grid of adjacent units. A unit in our model is equivalent to a quadrate of 1×1 m, containing a fixed number of plants, which is determined by the plant density of each experiment. Plants are either healthy, latent infected, or symptomatically diseased, i.e. the model describes disease incidence and not disease severity. Model runs were initialised by setting a number of initially diseased plants (IDP) in those quadrates of the grid that corresponded to the quadrates where the inoculated potted plants were put in

the greenhouse. Diseased plants produce infectious units that infect healthy plants ('successful spores'), according to a Poisson process with a rate parameter λ, where λ is the mean sporulation rate.

The spread of disease and the description of the corresponding part of the model were adopted from Xu and Ridout (1998): in each time step, here taken as 1 day, ''spores travel in straight lines of random orientation, determined by a uniform random number generator on the interval (0°, 360°). The dispersal distance varies randomly according to a half-Cauchy distribution (Stewart et al., 1995) with median dispersal distance parameter μ. With this distribution, the dispersal distance (d) of a particular spore is simulated by taking d = μtan (πx/2), in which x is a uniform random number on the interval (0,1)''. If a new spore falls outside the simulated grid of 18×7 quadrates, it is ignored. Otherwise it increases the number of infected plants of the target quadrate. As the epidemic progresses, the number of plants already infected increases, reducing the probability of hitting a healthy plant. Hence, in order to determine whether a spore hits healthy plants in a quadrate, the model took random samples from a binomial distribution with a mean equal to the proportion of healthy plants within the quadrate. Hit plants stay in a latent period for a number of days (t_{lag}) and then change to the set of infectious plants. Sporulation stops when the infectious period is over, after a number of days determined by parameter t_i. Then plants return to the pool of non-infectious but symptomatic plants, and—as new and healthy leaves are being developed—can be infected again.

Random numbers were generated using the generator RAN1 (Press, Teukolsky, Vetterling, & Flannery, 1992). The Poisson process was simulated by the generator POIDEV (Press et al., 1992). The model has five actuating variables: the number of initially diseased plants [IDP (plants m^{-2})], the sporulation rate [λ (spores $plant^{-1}$ day^{-1})], the mean dispersal distance [μ (m)], the infectious period [t_i (day)], and the period between infection and first appearance of symptoms [t_{lag}, (day)]. IDP, μ, t_i and t_{lag} were estimated as parameters, whereas λ was modified by functions of air temperature (T) and air moisture (SD). These functions are described as follows.

Effects of water vapour saturation deficit

The effect of SD on the sporulation rate λ is modelled using a two-step approach. In the first step, the model uses the hourly average of SD as an input to assign a relative response to SD to each hour of a day.

$$c(SD) = \begin{cases} 1 & SD \leq SD_{\min} \\ 0 & SD \geq SD_{\max} \\ \frac{SD_{\max} - SD}{SD_{\max} - SD_{\min}} & else \end{cases} \quad (1)$$

where c(SD), SD_{min} and SD_{max} are the hourly relative responses to SD and two parameters describing two SD threshold values [hPa] for fungal development, respectively. Equation 1 generates a response that is characterised by two straight lines (Fig. 2). In the second step, hourly responses are multiplied by a scaling factor and then integrated over 24 h.

$$a(SD) = \begin{cases} 1 & a(SD) > 1 \\ \sum_{h=1}^{24} (c(SD) \cdot \lambda_{hour}) & else \end{cases} \quad (2)$$

where a(SD) and λ_{hour} are the daily relative responses to SD and a parameter related to the effect of SD on sporulation, respectively c(SD) is given in Eq. 1. Function a(SD) returns a value ranging from 0 to 1, which represents the relative daily response to SD. The implementation of the parameter λ_{hour} allows for the fact that the production of conidiophores and conidia takes a

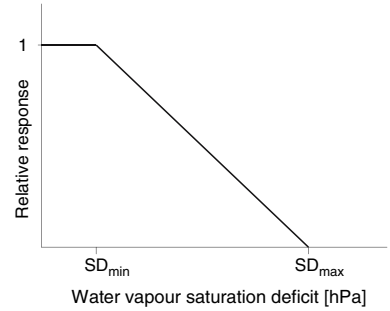

Fig. 2 Relative response to water vapour saturation deficit (Eq. 1)

certain number of hours, even under conditions that are optimum for the fungus. For instance, setting λ_{hour} to 0.2 means that at least 5 h with an hourly response of 1 (i.e. SD < SD_{min}) were required to return a relative daily response of 1.

Effects of air temperature

The effects of air temperature (T °C) were described by Eq. 3, which is well suited to describe the temperature response of plant pathogens (Hau, 1990). We added a scale parameter (pS1) because we used the function b(T) to estimate the temperature response of both the latent period (Eq. 5) and the sporulation rate (Eq. 6), which requires rescaling by changing pS.

$$b(T) = (T - T_{min})^{p1} \cdot (T_{max} - T)^{p2} \cdot pS1 \qquad (3)$$

where b(T), T_{min}, T_{max}, p1, p2 and pS1 are the relative responses to temperature (°C) for fungal development, minimum and maximum temperature two curve shape parameters and a rescaling parameter, respectively. Temperature responses were derived from growth chamber experiments, where we measured the proportion of symptomatic plants as affected by air temperature and time (t, day) after inoculation. The time course was described by:

$$DI(t,T) = \begin{cases} 0 & t \le t_{lag} \\ b(T) \cdot t & t > t_{lag} \\ & DI(t,T) < DI_{max} \\ DI_{max} & else \end{cases} \quad and \qquad (4)$$

where DI(t, T), t_{lag} and DI_{max} are disease incidence, lag time (day) and average maximum DI in growth chamber experiments, respectively; b(T) is given in Eq. 3. Equation 4 generates a time course that is characterised by two straight lines (Fig. 3). Parameter t_{lag} represents the lag time, i.e. the number of days after inoculation until the first plants show symptoms. DI_{max} allows for the fact that not all plants in the growth chamber experiments became diseased, although they were all inoculated. The slope of the regression line in Fig. 3 is determined by a function of temperature (Eq. 3).

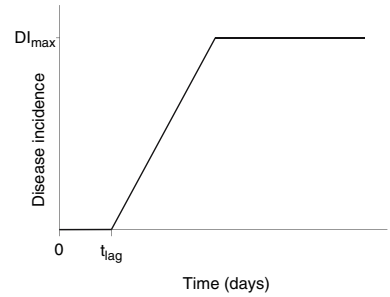

Fig. 3 Time-course of disease incidence (DI) generated by Eq. 4. All plants were inoculated at time t = 0

As shown in Fig. 4, disease incidence of a set of plants that were inoculated at the same date increases linearly over a period of time, i.e. plants vary in the period between infection and appearance of symptoms. For this reason, it is more appropriate to describe this period as a distribution rather than a fixed value. Therefore, we took the proportion of infected plants calculated in Eq. 4 as an estimate for the probability that a plant has become symptomatic:

$$pL(t,T) = \begin{cases} 0 & t \le t_{lag} \\ b(T) \cdot t & t > t_{lag} \\ & pL(t,T) < 1 \\ 1 & else \end{cases} \quad and \qquad (5)$$

where pL is the probability that the plant became symptomatic; b(T) and t_{lag} are given in Eqs. 3 and 4. To determine whether a plant has become symptomatic, the model took random samples from a binomial distribution with a mean equal to pL(t,T) (Eq. 5) for every day after a plant had been hit by a 'spore'. When the sample function returned 1, the respective day was taken as the day on which this plant became symptomatic. This approach generated the same time course (not shown) of diseased plants as measured in the growth chamber experiment (Fig. 4).

The relative responses to saturation deficit (Eq. 2) and to air temperature (Eq. 3) were used to modify the potential sporulation rate according to Eq. 6.

$$\lambda(T, SD) = \lambda_{pot} \cdot a(SD) \cdot b(T) \qquad (6)$$

where λ and λ_{pot} are sporulation rate [spores $plant^{-1}$ day^{-1}] and potential sporulation rate

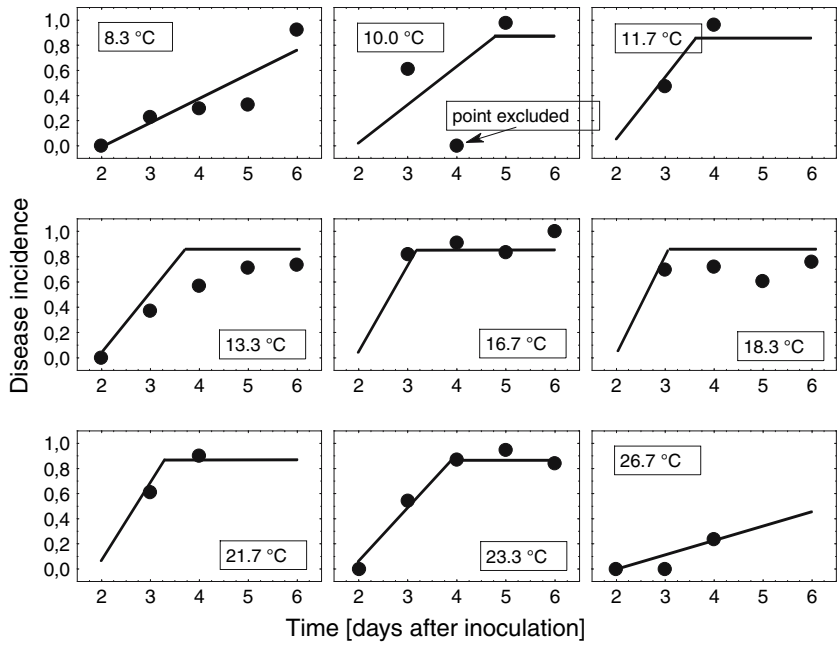

Fig. 4 Disease incidence in growth chamber experiments related to time after inoculation and mean air temperature. The dots denote measurements. Regression lines are calculated with Eq. 4; $r = 0.88$, $n = 32$. All plants were inoculated on day 0

[spores plant^{-1} day^{-1}], respectively; a(SD) and b(T) are given in Eqs. 2 and 3.

Parameter estimation

First, we estimated temperature response of DI as measured in the growth chamber experiments. Hence, parameters of Eqs. 3 and 4 were derived by fitting these equations to DI, using the 24 h average temperature in the growth chamber as independent variable (Table 1). The fitting was done by the non-linear regression procedure of the STASTICA programme (StatSoft, Tulsa, OK, USA). Second, we rescaled the temperature response function (Eq. 3) for use to estimate the effect of the temperature on the sporulation rate (Eq. 6). For this purpose, we retained all parameter values but removed the scaling parameter (pS1). This resulted in the same shape of the response curve. A new scaling parameter (pS2) was then calculated in such a way that the values returned by Eq. 3 ranged from 0 to 1, which was a requirement for use of this function in Eq. 6. Third, we used parameter estimates of the potential sporulation rate (λ_{pot}) and the mean dispersal distance (μ), as determined from independent data in a previous study (Fink & Kofoet, 2005), and estimated all other model parameters by fitting simulated mean disease incidence to disease incidence measured in the greenhouse experiments.

Thus, nine parameters were estimated by the least square regression approach, which was driven by an algorithm with an evolutionary optimisation strategy. This approach is described in detail by Schwefel (1981). In brief, we started with an arbitrarily chosen parameter set to calculate the result of the quality function, in our case the sum of squared residuals. Model runs were repeated 20 times for all data sets, and the sum of the squared residuals was calculated from these 20 runs. This was done to account for the stochastic character of the model, which causes a range of simulated disease progress curves even with the same set of model parameters. Parameters were varied randomly. If a new parameter set resulted in a better fit of the model, these parameters were taken as starting points for further random variation. The algorithm repeated this procedure 1000 times and displayed the parameters for the best fit and the corresponding coefficient of determination. Since the variance of disease incidence data increases with increasing disease incidence (Madden & Hughes, 1995), the model was fitted

to log-transformed data. Model and algorithms for parameter estimation were programmed in the computer language FORTRAN.

Results

Temperature response in growth chamber experiments

Peronospora parasitica infected the radish plants in a broad temperature range from 8.3 to 26.7°C mean temperature (Fig. 4). Independent of temperature, plants were without symptoms for the first two days after inoculation. Then the number of symptomatic plants increased with a temperature dependent rate. Although all plants were similarly inoculated, in most treatments a few plants did not show symptoms within the observation period. The average maximum disease incidence (DI_{max}, which was estimated by fitting Eq. 4 to data from the growth chamber experiment) was 0.819. The time course of DI was well described by Eq. 4 ($r = 0.88$, $n = 32$, Fig. 4). Parameter estimates are given in Table 2. Sporulation intensity (SI) of *P. parasitica* on radish leaves was influenced significantly by night and day temperature. Maximum SI of 0.05, 0.15, 0.23 and 0.11 were obtained at night temperatures of 5, 10, 15 and 20°C, respectively. At day temperatures of 10, 15, 20, 25 and 30°C, maximum SI of 0.06, 0.11, 0.26, 0.14 and 0.00 were obtained, respectively. Sporulation intensity was favoured by night temperatures of 15°C and day temperatures of 20°C. Sporulation was possible on successive days. The temperature response curve (Eq. 3) that was derived by fitting Eq. 4 to the growth chamber data is characterised by an almost linear increase up to the optimum temperature, which was estimated as 20°C (Fig. 5). Since the experimental data only ranged from 8.3 to 26.7°C, estimated responses outside that range should be considered as extrapolation.

Response to temperature and water vapour saturation deficit in greenhouse experiments

Temperature (T) and water vapour saturation deficit (SD) conditions varied widely in the greenhouse experiments. The model simulated the spread of disease over time well ($r = 0.91$, $R^2 = 0.83$, $n = 29$, Fig. 6). The epidemic was promoted by moderate T and low SD (e.g. the first part of set 1). Sporulation rates decreased with either suboptimal T (e.g. the first part of set 2) or suboptimal SD (e.g. the first part of set 4). Rates were close to zero—both at high and low T—when SD was high (e.g. the second parts of sets 2, 4 and 5). These interactions of T and SD were adequately described by the multiplicative connection of T and SD response functions in Eq. 6. After changing the climatic conditions from type I to II, disease incidence continued to increase for a few days, for instance in data sets 2, 4 and 5, although sporulation rates were almost zero. This was because some plants were infected shortly before the change and became symptomatic later. Disease-promoting conditions at the beginning of set 3 were followed by alternating dry and wet conditions for some days. The consequence, an alternating sporulation rate and a moderate increase in DI, was well described by the model (set 3, Fig. 6). The duration of the experiments differed significantly because the experiments were terminated when radish plants reached a marketable size (20 mm diameter), which took 15–64 days, depending on temperature and irradiance.

Discussion

Our hypothesis that the epidemic of *P. parasitica* on radish can be modelled by the effects of temperature (T) and moisture (SD) in the greenhouse was confirmed ($r = 0.91$, $R^2 = 0.83$, $n = 29$, $P < 0.0001$, Fig. 6a). Disease progress decelerated (e.g. in set 3) or even ceased completely (e.g. set 2) when the climate was changed to conditions unfavourable for *P. parasitica*.

The duration of the latent period is crucial for the number of disease cycles during crop growth and therefore significantly affects potential yield losses. In our growth chamber experiments, the first plants became symptomatic 3 days after inoculation (Fig. 4). The minimum latent period was independent of temperature, although the temperature did affect the number of days it took

Table 2 Model parameters

Parameter	Estimate	Standard error	Unit	Source	Definition
t_{lag}	1.93	0.31	day	Growth Chamber	Time lag between inoculation and appearance of first symptoms
T_{min}	1.15	0.57	°C	Growth Chamber	Minimum air temperature threshold that limits disease progress
T_{max}	26.9	0.69	°C	Growth Chamber	Maximum air temperature threshold that limits disease progress
p1	2.01	0.21	–	Growth Chamber	Shape parameter
p2	0.738	0.34	–	Growth Chamber	Shape parameter
DI_{max}	0.819	0.051	–	Growth Chamber	Average maximum disease incidence in growth chamber experiments
pS1	0.000445	0.00020	–	Growth Chamber	Shape parameter
pS2	0.000655	n.c.[a]	–	Growth Chamber	Shape parameter
SD_{min}	0.03077	n.c.	hPa	Greenhouse	Minimum saturation deficit threshold that does not limit disease progress
SD_{max}	2.054	n.c.	hPa	Greenhouse	Maximum saturation deficit threshold that limits disease progress
λ_{hour}	0.2074	n.c.	$hour^{-1}$	Greenhouse	Relative hourly response to saturation deficit
t_i	4	n.c.	day	Greenhouse	Period in which one new lesion can produce spores
IDP_1	156	n.c.	plants m^{-2}	Greenhouse, set 1	Number of initially infected plants, set 1
IDP_2	13	n.c.	plants m^{-2}	Greenhouse, set 2	Number of initially infected plants, set 2
IDP_3	7	n.c.	plants m^{-2}	Greenhouse, set 3	Number of initially infected plants, set 3
IDP_4	150	n.c.	plants m^{-2}	Greenhouse, set 4	Number of initially infected plants, set 4
IDP_5	114	n.c.	plants m^{-2}	Greenhouse, set 5	Number of initially infected plants, set 5
λ_{pot}	3.1	n.a.[b]	spores $plant^{-1}$ day^{-1}	Fink and Kofoet (2005)	Potential sporulation rate
μ	7.6	n.a.	m	Fink and Kofoet (2005)	Median spore dispersal distance

[a] n.c.—not calculated. The software used estimated parameters with an evolutionary optimisation algorithm and did not calculate standard errors of estimation.

[b] n.a.—not applicable. Parameters were taken from the literature (Fink & Kofoet, 2005), not estimated from experimental results.

until the latent period was over for all infected plants, which was up to 6 days at a low temperature (8.3°C). These results corresponded with the data of Bonnet and Blancard (1987), who reported three days as a minimum latent period. The latent period is not a fixed value, as suggested by Bonnet and Blanchard (1987) and Weis (1994) and as assumed in our first version of the model (Fink & Kofoet, 2005). Instead, it should be described as a distribution. In our experiments, the shortest period until most newly infected plants had produced spores was at a day temperature of 20°C and a night temperature of 10–15°C. Also the sporulation intensity was high when temperatures were in this range. Hence, our data corresponded well with the field data of Lakra (2001), who reported highest rates of disease development between 20 and 24°C.

The model parameter t_i (infectious period) was estimated as 4 days (Table 2), i.e. the fructification in one lesion can occur in four following nights. To our knowledge, the infectious period of *P. parasitica* is not reported in the literature. Deil (2003) showed that the sporulation of *P. farinosa* f.sp. *spinaciae* can occur over a period of 10 days, but the sporulation intensity was reduced after 2 days when more than 80% of the conidia had already been produced. Downy mildew epidemics are driven by 'successful spores', i.e. spores that have been produced and disseminated, infecting healthy plants. This requires both the availability of inoculum and favourable conditions for

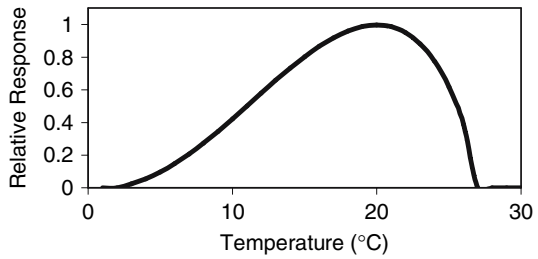

Fig. 5 Temperature response of *P. parasitica* derived by fitting Eq. 4 to the time-course of disease incidence in growth chamber experiments (Fig. 4). The line is calculated with Eq. 3 and parameters are shown in Table 2

infection. All single steps in the infection cycle are influenced by temperature. The estimates of the model parameters indicated an optimum mean temperature for epidemics of *P. parasitica* between 15 and 25°C, with minimum and maximum temperatures of 1.1 and 26.9°C, respectively. In our growth chamber experiments, infection occurred in a broad temperature range between 8.3 and 26.7°C, which corresponds with data for germination (5–30°C; Achar, 1998; Bonnet and Blanchard, 1987; Felton & Walker, 1946; Hartmann et al., 1983; Weis, 1994) and infection (12–24°C; Lakra, 2001). Sporulation occurred overnight and sporulation intensity was high at 15°C, corresponding with the results of 12–16°C reported by Felton and Walker (1946), Hartmann et al. (1983) and Jang and Safeeulla (1990).

The model parameter SD_{min}, i.e. the air moisture threshold that resulted in disease progress not just limited by moisture, was estimated as 0.03 hPa (Table 2). A water vapour saturation deficit of 0.03 hPa corresponds to a high RH of, for instance, 99.85% at 18°C. The parameter estimate agreed well with data from Hartmann et al. (1983) and Paul, Klodt-Bussman, Dapprich, Capelli, & Tewari (1998), who showed that a high RH is necessary for intensive sporulation. Our model suggests, however, that disease progress stopped only when SD was greater than 2.0 hPa (SD_{max}), which corresponds to approximately 90% RH at 18°C. This estimated moisture threshold differed from results of moisture chamber experiments, where sporulation was already inhibited when RH was 95.5% at 13°C and 18°C (Hartmann et al., 1983). These different results were probably caused by different experimental set-ups. Our experiments were carried out in a greenhouse and we measured SD on top of the canopy. In contrast to moisture chamber experiments, in which all plants are exposed to a uniform aerial environment, SD is not evenly distributed in a plant canopy but usually decreases with lower canopy layers. For this reason, we assumed that sporulation in lower canopy layers was possible until SD on top of the canopy was less than SD_{max}. The highest rate of disease development occurs if optimum conditions are realised on subsequent days. The estimate of the model parameter λ_{hour} (Eq. 2) indicated that approximately 5 h daily of optimum conditions for T and SD were required to yield the potential sporulation rate ($1 / \lambda_{hour}$ = 4.8 h; Table 2). Hildebrand and Sutton (1984a) reported that sporulation requires 4 h under optimum conditions and that intensity increased when the humid period was extended from 4 to 7 h. Other studies showed that germination of conidia of *P. parasitica* began after 1 h and that maximum values were reached after 5 h (Weis, 1994), and that infection of *P. destructor* occurred in some onion leaves within 4 h at temperatures of 6 to 22°C (Hildebrand and Sutton, 1984b). Hence, the estimate of model parameter λ_{hour} is in accordance with the data for the sporulation-infection cycle of downy mildew.

Transpiration and hence transpiration stress for organisms are related to SD rather than to RH. Air moisture requirements of pathogens should therefore be expressed in terms of SD if the response to air moisture is compared at different temperatures. This conclusion is supported by our model calculations, which gave a better fit to measurements when SD rather than RH was used to assess air moisture (data not shown).

The model describes the interactive effects of temperature, humidity and time on 'successful spores'. Parameter estimates for describing effects of air humidity and temperature on 'successful spores' corresponded well with previous work carried out on infection and sporulation. Sporulation and infection were not modelled as separate processes, since this would have required estimating even more model parameters from

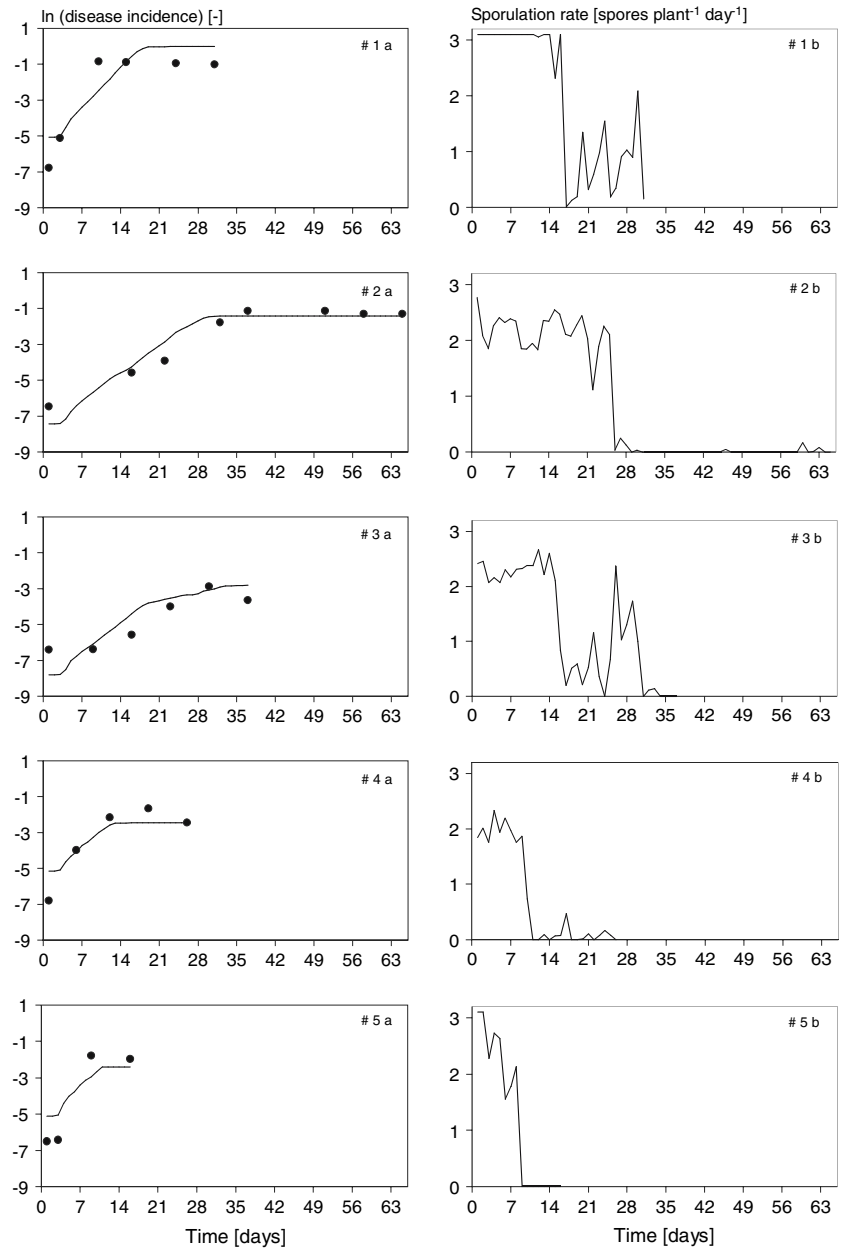

Fig. 6 (**a**) Disease incidence related to time after inoculation. Graph numbers refer to data sets, dots denote measurements, lines are simulations. (**b**) Simulated sporulation rate (Eq. 6) related to time

limited experimental data. Moreover, our objective was to develop a tool to evaluate climate control strategies, and not to predict single sporulation or infection events. The effects of T and SD on epidemic development can be quantified with this model, even when the climatic conditions are highly variable over time, which is the case in the greenhouse in contrast to climate chamber experiments. Overall the model described disease development in the greenhouse well ($r = 0.91$, $R^2 = 0.83$, Fig. 6). Regression analysis of measured and simulated DI revealed no significant bias (Fig. 7), i.e. the slope of the regression line was not significantly different from 1 and the intercept not significantly different from 0. The results of the study can be used to derive strategies to control the epidemic development of downy mildew on radish under greenhouse conditions by controlling climatic conditions.

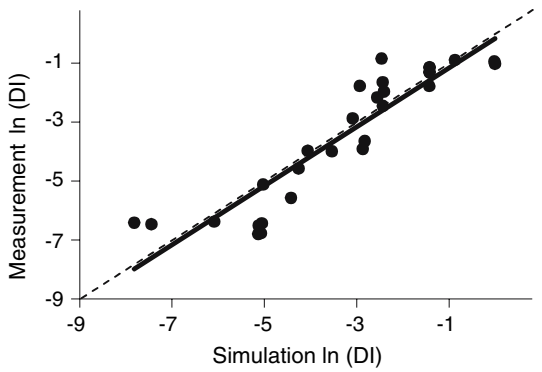

Fig. 7 Measured versus simulated disease incidence (DI) in the greenhouse experiments. Solid line is the regression line ($y = 1.0011x - 0.1579$, $r = 0.91$, $R^2 = 0.83$, $n = 29$, some dots are on top of each other), dashed line is the 1:1 line

In the present study all available data from growth chamber experiments, greenhouse experiments and from the literature were used for estimating model parameters, i.e. the model was not tested with independent data. We have presented the first steps towards a decision support system: (1) proof that the mildew epidemic can be affected by manipulating greenhouse climate and (2) that the effects of air moisture and temperature can be significantly described by our simple model. In a forthcoming study, the model with the current parameter estimates will be validated with independent data, to prove its robustness.

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