# MODELLING THE DYNAMICS OF INFECTIONS CAUSED BY SEXUAL AND ASEXUAL SPORES DURING *PLASMOPARA VITICOLA* EPIDEMICS

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## SUMMARY

*Plasmopara viticola* epidemics on grapevines are caused by both primary and secondary infections that overlap for part of the season. To date, this complexity has not been incorporated into models for grape downy mildew infection forecasting. A conceptual model was developed that links the quantitative aspects of both sexual and asexual stages of the *P. viticola* life cycle in a biologically coherent framework. Van der Plank's logistic equation was integrated with a time step of 1 day; the resulting rates of disease increase were calculated separately for primary and secondary infections, then linked together. Key model parameters included oospore density and development, infection efficiency for primary infections, ability of the lesions to reproduce asexually, infection efficiency for secondary infections, and carrying capacity of the host leaves. Model structural adequacy procedures (MSA) were used to estimate model parameters, and the model outputs were compared with real data not used in model development and parameterization. Data used for validation were taken from published studies of 23 vineyards, where lesions had been previously genetically analyzed and distinguished as primary (caused by singleton genotypes) and secondary (caused by clonal genotypes). The 23 vineyards had experienced epidemics characterised by a low to high level of clonality. The estimated model parameters were biologically plausible and consistent with previous knowledge, and the model accurately mimicked the variability of the 23 real epidemics. The model represents a coherent mathematical structure for developing a simulator for downy mildew epidemics in the vineyard.

*Key words:* polycyclic diseases, primary and secondary infections, model, grapevine, downy mildew.

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## INTRODUCTION

Many polycyclic fungal pathogens exhibit polymorphism in their spore forms, with sexual and asexual reproduction. Forms associated with sexual reproduction often serve to bridge adverse and/or non-host periods and are usually responsible for primary infections. Dispersal units associated with asexual reproduction form a secondary inoculum that is responsible for the succession of infection cycles during the host growing season (Agrios, 1988). These two kinds of spores often have different ecological requirements and have different epidemiological characteristics (Rapilly, 1991).

Downy mildew epidemics on grapevine, caused by the oomycete *Plasmopara viticola* Berl. et de Toni, are typical examples of polycyclic diseases caused by pathogens with dimorphic reproductive forms, i.e., with both sexual and asexual spores, which cause primary and secondary infections, respectively. Oospores are the sexual spores (Wong *et al.*, 2001) that form within the affected leaf tissue from grape ripening until leaf fall. These spores survive through the winter in the leaf litter or in the soil (Galbiati and Longhin, 1984; Gehmann, 1987). Oospores are the sole relevant source of inoculum for primary downy mildew infections (Galbiati and Longhin, 1984). They germinate gradually during the next spring (Park *et al.*, 1997; Rossi *et al.*, 2008a) and also during the spring(s) that follow (Kennelly *et al.*, 2007). Germinating oospores form sporangia, which release zoospores. These zoospores are dispersed by rain splashes from the soil to the grape plants. Once deposited on the leaves, zoospores cause primary infections, and initial disease symptoms are visible as lesions in the form of "oil spots". After a period of latency, the pathogen repeatedly produces sporangia on lesions (Kennelly *et al.*, 2007) that are dispersed in the vineyard onto new host tissues. Sporangia contain asexually produced zoospores, which cause secondary infections. Lesions actively contribute to the epidemic for an infectious period before becoming old and sterile (Kennelly *et al.*, 2007).

In the traditional concept of the pathogen life cycle, a downy mildew epidemic begins with a relatively small number of germinating oospores, and the explosive increase of the epidemic is ensured by massive clonal mul-

tiplication causing secondary infections (Blaeser and Weltzien, 1979; Lafon and Clerjeau, 1988). Recent studies carried out using polymorphic microsatellite markers for *P. viticola* (Gobbin *et al.*, 2003) showed, however, a continuous input of new genotypes into the epidemic during a prolonged period (May to August) (Rumbou and Gessler, 2004; Gobbin *et al.*, 2005; Kennelly *et al.*, 2007). Therefore, oospores play a key role in the development of downy mildew epidemics: in addition to triggering them, they actively contribute to the progress of the epidemics for part of the host growing season when both sexual and asexual spore types are present.

To date, this complexity (i.e. oospore contribution to both onset and development of downy mildew epidemics) has not been incorporated in models for grape downy mildew. Some of these models consider only particular aspects of the sexual cycle (Hill, 2000; Tran Manh Sung *et al.,* 1990; Rouzet and Jacquin, 2003) or the seasonal dynamics of the oosporic infections (Rossi *et al.*, 2008b). Other models account for the secondary infection cycles with no attention for primary infections. Hill (1990) quantitatively simulated the development of an epidemic over different subsequent secondary cycles starting from a single date of primary infection; the number of primary lesions at the time of disease onset had to be established by the user based on his previous experience (between 10 and 500 lesions/hectare). Blaise and Gessler (1992b) computed the development of the epidemic from a theoretical primary inoculum, expressed as diseased leaf area, and then adjusted this value by reporting the observed disease severity in the vineyard as soon as favourable conditions for infection had occurred. Magnien *et al.* (1991), Magarey *et al.* (1991), and Orlandini *et al.* (1993) followed similar approaches, e.g., their modelled downy mildew epidemics develop asexually, starting from the day when the first symptoms appear in the vineyard or when favourable conditions for primary infection occur. Park *et al.* (1997) accounted for repeated primary and secondary infections by providing an estimate of the infection periods based on the two infection types, but they did not produce a model linking primary and secondary infections to epidemic development.

Thus, a model linking in a biologically coherent framework the quantitative aspects of both sexual and asexual stages of the *P. viticola* life cycle has not been developed. In the current work, a conceptual model capable of simulating plant disease epidemics caused by pathogens with dimorphic spores with repeated primary and secondary infection cycles is elaborated. This model was applied to infections by *P. viticola* to grapevine leaves and was validated for its ability to reproduce the temporal dynamics of downy mildew lesions caused by both sexual and asexual spores.

## MATERIALS AND METHODS

**The model.** The model was developed following the approach and, when possible, the terms used by Van der Plank (1963, 1982) and Campbell and Madden (1990). The relational diagram of the model is shown in Figure 1, and variables and parameters used are listed in Table 1.

The basic equation for developing the model was the logistic equation for polycyclic diseases (Campbell and Madden, 1990):

$$y_t = 1/\{1 + [(1 - y_0)/y_0] \cdot \exp(-r \cdot t)\}$$

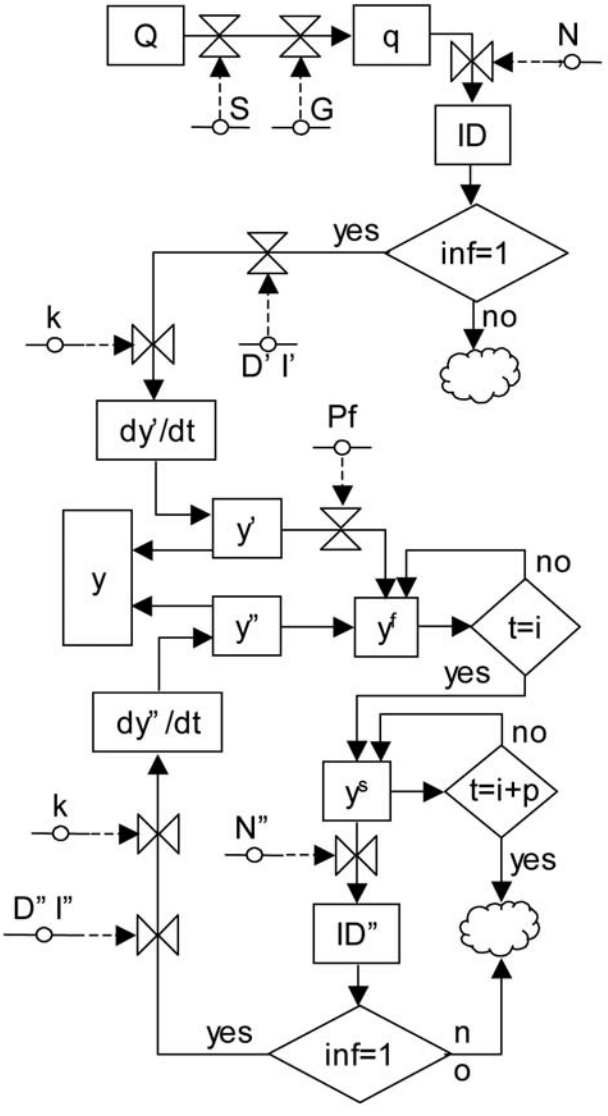


**Fig. 1.** Relational diagram of the model predicting primary and secondary infection cycles of *Plasmopara viticola*. Legend: □ state variable; –► flux and direction of states; ––► flux and direction of information; –○– parameter; ◇ switch; ⬭ outgoing variable; ⋈ valve in a flux (rate) (see Table 1 for acronym explanations); inf is a dichotomic variable, with values 1 when there is infection and 0 when there is no infection.

**Table 1.** Description of variables and parameters used in the model for grapevine downy mildew.

| Variable, parameter | Description | Dimension |
|---|---|---|
| a b | Parameters of equation [7] | number |
| c, d | Parameters of equation [9] | number |
| D', D" | Dispersal efficiency of primary and secondary inoculum, respectively | proportion |
| DOY | Day of the year | number |
| dy', dy" | New host sites occupied by primary and secondary lesions, respectively | n/$m^2$ soil |
| e, f | Parameters of equation [10] | number |
| G | Proportion of germinated oospores | proportion |
| i | Infectious period | n of days |
| I', I" | Infection efficiency of primary and secondary inoculum, respectively | proportion |
| ID', ID" | Amount of primary and secondary inoculum | n/$m^2$ soil |
| k | Variable accounting for the carrying capacity of the host | number |
| K | Total host sites | n/$m^2$ soil |
| km, kl | Proportion of main and lateral shoots at the end of the season | proportion |
| $K_m$, $K_l$ | Host sites on main and lateral shoots, respectively | n/$m^2$ soil |
| $K_{max}$ | Carrying capacity of the host plant (n of host sites) | n/$m^2$ soil |
| LAI | Maximum leaf area index | $m^2$ leaf/$m^2$ soil |
| LS | Lesion size (leaf area occupied by one lesion) | $cm^2$ |
| N' | Zoospores produced per oospore | n/oospore |
| N" | Sporangia produced on a sporulating secondary lesion | n/ lesion |
| or | Time required for new leaves to acquire ontogenic resistance | n of days |
| p | Latent period | n of days |
| Pf | Proportion of the oospore-derived lesions that are fertile | proportion |
| q | Germinated oospores | n/$m^2$ soil |
| Q | Oospores produced by the previous epidemic | n/$m^2$ soil |
| R | Number of daughter lesions per infectious mother lesion (progeny/parent ratio) | n/lesions |
| S | Survival rate of oospores | proportion |
| t | Time | DOY |
| $t_0$ | Time of the first seasonal infection | DOY |
| $t_{00}$ | Time of oospore formation | DOY |
| $t_{bb}$ | Time after bud break | n of days |
| $t_{inf}$ | Time of infection event | DOY |
| y | Total lesions | n/$m^2$ soil |
| y', y" | Primary and secondary lesions, respectively | n/$m^2$ soil |
| $y^f$ | Primary lesions capable of producing secondary lesions (i.e., fertile) | n/$m^2$ soil |
| $y^s$ | Actively sporulating lesions | n/$m^2$ soil |

where: $y_t$ = amount of disease at time t (the proportion of host tissue affected, including latent, infectious, and removed diseased tissue; equivalent to disease severity); $y_0$ = initial disease level; r = apparent infection rate; t = time (in days).

In particular, the model was developed based on the numerical solving of Van der Plank's equation by using an Euler integration method with a time step of 1 day (Teng, 1981), according to the scheme: y at time t+dt is equal to y at time t plus the rate of increase at time t multiplied by dt (dy/dt, or absolute rate of disease increase). This integration gives the new values of y, and

the calculation procedure is repeated (De Witt, 1993).

From a biological point of view, the term dy/dt expresses the rate of disease increase due to an infection cycle. Because of the presence of two spore forms, dy/dt is calculated separately for primary and secondary infections by introducing the terms dy'/dt and dy"/dt, respectively, as follows:

$$y_t = y'_t + y''_t \quad [1]$$

with:

$$y'_t = \sum_{t=t_0}^{t_{inf}} (dy'_t/dt) \quad [2]$$

$$y''_t = \sum_{t=t_0+p}^{t_{inf}} (dy''_t/dt) \quad [3]$$

where: y = total downy mildew lesions (n/m$^2$ soil); y' = primary lesions (n/m$^2$ soil); y" = secondary lesions (n/m$^2$ soil); dy' and dy" = new primary and secondary lesions, respectively (n/m$^2$ soil); t = time step (1 day); $t_0$ = time of the first seasonal infection (day of the year, DOY); $t_{inf}$ = time of any infection event during the season (DOY); p = latent period for the first seasonal infection (n of days).

Based on the differential-difference equation of Van der Plank (1963), the term dy'/dt of equation [2] (i.e. disease increase due to the primary oospore-derived infections) is written as:

$$dy'/dt = ID'_t \cdot D'_t \cdot I'_t \cdot k_t \quad [4]$$

where: ID' = amount of the primary inoculum (i.e., the number of zoospores/m$^2$ soil); D' = dispersal efficiency of the primary inoculum (i.e., the proportion of zoospores reaching the leaf surface with rain-splashed droplets); I' = infection efficiency of zoospores (i.e., the proportion of zoospores causing new lesions on leaves); k = variable accounting for the carrying capacity of the host.

ID' is calculated as a function of the number of germinated oospores at time t (q, n/m$^2$ soil) and the number of zoospores produced by each oospore ($N'_t$/oospore) as follows:

$$ID'_t = q_t \cdot N'_t \quad [5]$$

with:

$$q_t = Q \cdot \sum_{t_{00}}^{t} S_t \cdot G_t \quad [6]$$

where: Q = number of oospores produced by the previous epidemic (n/m$^2$ soil) at time $t_{00}$ (Q represents the initial condition of the system); $t_{00}$ = time when oospores have been produced by the previous epidemic (DOY); S = survival rate of oospores on each day between $t_{00}$ and t; G = proportion of germinated oospores.

G is calculated as:

$$G_t = \int_{t_{inf}}^{t} \exp[-a \cdot \exp(-b \cdot t)] \quad [7]$$

where: $t_{inf}$ = time of the previous infection event (DOY); a and b = parameters of a Gompertz equation that estimates the germination of oospores over time (Rossi *et al.*, 2008a).

The term dy"/dt of equation [3], which is the disease increase due to the secondary asexual infections, is written as:

$$dy''/dt = ID''_t \cdot D''_t \cdot I''_t \cdot k_t \quad [8]$$

where: ID"= amount of the secondary inoculum on the infectious lesions (i.e., n of sporangia/m$^2$ soil); D"= dispersal efficiency of the secondary inoculum (i.e., the proportion of sporangia reaching the leaf surface); I" = infection efficiency of the secondary inoculum (i.e., the proportion of sporangia establishing new infections).

ID" is calculated as:

$$ID''_t = y_t^s \cdot N''_t \quad [9]$$

$$y_t^s = y_{t-p} - y_{t-i-p} \quad [10]$$

where: $y^s$ = actively sporulating lesions (n/m$^2$ soil); p and i = latent and infectious periods, respectively (n of days); N" = number of sporangia produced per sporulating lesion (n/lesion).

In the early stage of the epidemic, ID" is composed of sporangia produced only on the proportion (Pf) of the primary lesions that are fertile ($y^f$, i.e., capable of producing secondary lesions, Gobbin *et al.*, 2005); later, sporangia are also produced by the secondary lesions, and these become prevalent in the last phases of the epidemic.

The variable k in equations [4] and [8] accounts for the carrying capacity of the host compared to the potential infections caused by the pathogen, where the carrying capacity is the maximum number of lesions that can be sustained on the host (Seem, 1988) (Fig. 2a). To determine k, the model calculates the total number of the host sites ($K_{max}$, expressed as n of sites/m$^2$ soil) and divides them into sites on main and lateral shoots ($K_m$ and $K_l$, respectively, n/m$^2$ soil) as a function of time after bud break ($t_{bb}$, in days):

$$K_{max} = (LAI \cdot 10000)/LS \quad [11]$$

$$K_{m_t} = (K_{max} \cdot km)/[1 + \exp(c - d \cdot t_{bb})] \quad [12]$$

$$K_{l_t} = (y_{max} \cdot kl)/[1 + \exp(e - f \cdot t_{bb})] \quad [13]$$

$$K_t = K_{m_t} + K_{l_t};\ K_t \le K_{max} \quad [14]$$

where: LAI = maximum leaf area index (m$^2$ leaf/m$^2$ soil); LS = lesion size, i.e., the leaf area occupied by one lesion (cm$^2$); km and kl = proportions of main and lateral shoots in the plant canopy at the end of the season, respectively, with km+kl=1; c to f = parameters of the logistic equations that estimate leaf growth over time after bud break.

After determining $K_t$, the model considers that only young leaves are receptive to infection (Gehman, 1987) because of ontogenic resistance (resistance acquired by age; Develey-Rivière and Galiana, 2007). The model al-

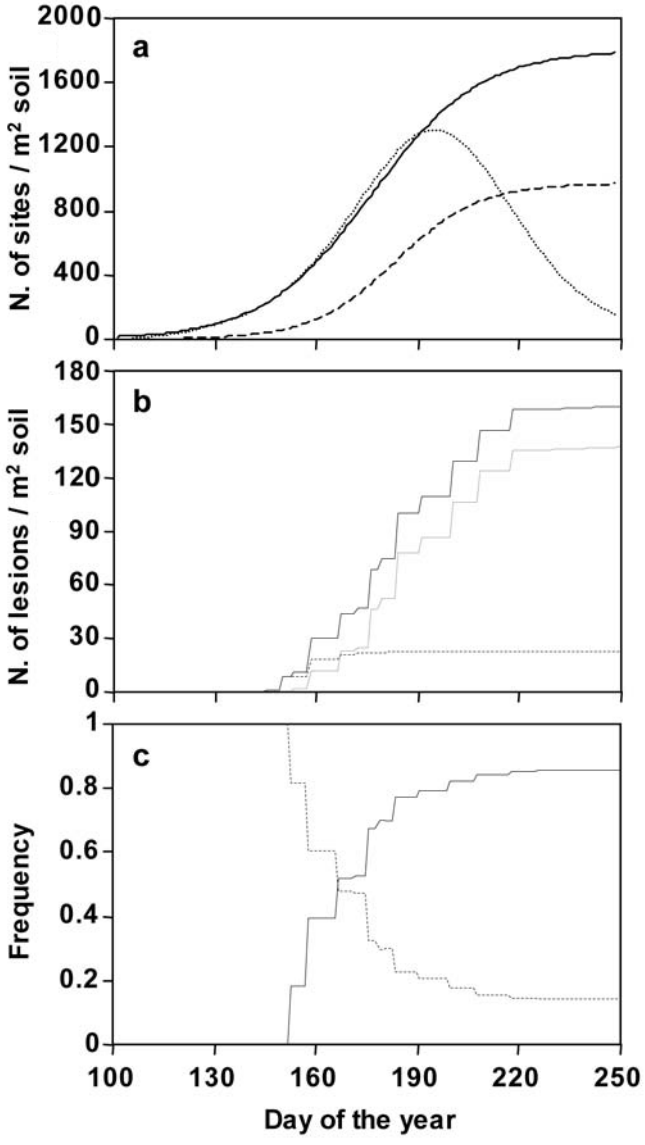


**Fig. 2.** Examples of model output: a, carrying capacity for *Plasmopara viticola* infections of the grapevine canopy, expressed as total number of sites in leaves on main (–) and lateral (---) shoots, and sites receptive to infection (····); b, number of primary oosporic-derived (---), secondary clonal (····), and total (–) downy mildew lesions; c, proportion of the primary (---) and secondary (–) lesions over the total lesions.

so considers that, at any time during the epidemic, a portion of these leaves is already infected. Therefore, $K_s$, the number of host sites susceptible to infection (i.e., receptive and infection-free), is calculated as:

$$K_{st} = \int_{t-or}^{t} (K_t - y_t) \quad [15]$$

where: or = time needed for leaves to acquire ontogenic resistance (n of days).

After $K_{st}$ is determined, k is calculated as:

$$\text{if } dy/dt \leq K_{st-1} \text{ then } k_t = 1 \quad [16]$$

$$\text{if } dy/dt > K_{st-1} \text{ then } k_t = K_{st-1}/(dy/dt) \quad [17]$$

where: $dy/dt = dy'/dt + dy''/dt$ [18]

**Model evaluation**. The "model structural adequacy procedures" (MSA) (Taper *et al.*, 2008) were used to evaluate the adequacy of the model structure for fulfilling the specific purpose of the model, i.e., estimating the dynamics over time of primary and secondary infections during a grape downy mildew epidemic. Evaluation followed three steps. In the first, a data set of realistic size was generated from the model and the model output was calculated. In the second step, the data generated were used to estimate the parameters for the model, while in the third step, the model output was calculated using these estimated parameters and was compared with "true" data (Taper *et al.*, 2008).

*Generation of a realistic data set*. To generate a realistic data set for the variable of interest, i.e., primary versus secondary infections, the model was operated 500 times by changing initial conditions and model parameters within a range of biologically plausible values taken from the literature or based on the author's experience and judgement. The numbers of primary and secondary lesions were calculated for each model run, and the relative contribution of primary lesions to total lesions (ratio y'/y) was determined for each day of the simulated epidemics. Initialization and parameterization of the model for these simulations were performed as described in the following paragraphs.

To calculate the amount of the primary lesions (equations [5] to [7]), Q ranged between 100 to 30,000 oospores per $m^2$ soil; these values were calculated from the number of oospores formed in leaf samples with light and severe symptoms of downy mildew (T. Caffi, unpublished information). S and N' were kept constant: S = 1, i.e., the model assumed that all oospores survive during winter; N' = 7, i.e., an average of seven zoospores was produced by each sporangium (Lalancette *et al.*, 1987). Parameters for equation [7] were calculated using a non-linear regression analysis on published data: a = 173.6, b = 0.119 (Rossi *et al.*, 2008a). Occurrence of an infection event was simulated using a random number generator for a Bernoulli distribution, with probability = 0.225 (i.e., an average of about seven infection events per month, Blaise and Gessler, 1990). For each infection event, D' and I' were generated using a random number generator for normal distributions with $\mu = 0.05$ and $\sigma = 0.025$, and $\mu = 0.1$ and $\sigma = 0.05$. When $\sigma = 0.05$, 5% of the rain splashes from soil reach the grape leaves (Cursano, 2008); when $\mu = 0.1$, 0.1 lesions are produced per zoospore on average. This parameterisation for I' is higher than the value of 0.06 found by Lalancette *et al.* (1987) for *V. labrusca* because downy mildew resistance is greater in *V. labrusca* than in *V. vinifera* (Lalancette *et al.*, 1988a).

To calculate secondary lesions, Pf ranged between 0.01 and 0.40 (D. Gobbin, personal communication),

latent and infectious periods were kept constant at p = 8 and i = 15 days, respectively (Müller and Sleumer, 1934; Kennelly *et al.*, 2007). To simplify the model use, the product ID"·D"·I" (see equation [8]) was considered as R, which is the number of daughter lesions per infectious mother lesion [or progeny/parent ratio (Van der Plank, 1982) or basic reproduction number (Diekmann *et al.*, 1990)]. The value of R ranged between 0.1 and 50, the highest value being about 10 times the value calculated by Gessler and Blaise (1992).

To calculate the carrying capacity of the host, the maximum LAI of the grapevine plants was kept at 2.5 $m^2$ leaf/$m^2$ soil, which is the normal LAI for curtain-trained vines (Poni *et al.*, 2006). The lesion size was LS = 9 $cm^2$, which is the approximate maximum size of a single downy mildew lesion (Galet, 1977). Therefore, $K_{max}$ = (2.5·10,000)/9 = 2,778 lesions/$m^2$ soil (equation [8]). The proportion of main and lateral shoots in the plant canopy at the end of the season was km = 0.65 and kl = 0.35, respectively (Palliotti *et al.*, 2000). Parameters for equations [9] and [10] were calculated using a non-linear regression analysis based on published data: c = 4.77, d = 0.063, e = 6.80, f = 0.082 (Palliotti *et al.*, 2000). The time needed for new leaves to acquire ontogenic resistance was "or" = 30 days, which is about half of the longevity of leaves (Wermelinger and Koblet, 1990).

As a consequence of this model setting, the number of host sites progressed as shown in Fig. 2a, the number of primary and secondary lesions, and the relative contribution of primary lesions to total lesions (ratio y'/y), progressed as shown in Fig. 2b and 2c, respectively, for one simulation run.

*Estimation of model parameters*. Estimates of the initial conditions (i.e., Q) and the model parameters Pf and R were drawn from the previously described data set.

*Comparison with real data.* The ratio y'/y was calculated by running the model using the estimated initial conditions and parameters (see Results for the estimated initial conditions and parameter values). These model outputs were compared with real data not used in model development and parameterization. Real data were taken from the literature (Gobbin *et al.*, 2005; Rumbou and Gessler, 2004, 2006) based on studies of 23 vineyards grown in different years (2000 to 2002) and locations across Europe. A total of 6,246 downy mildew lesions were taken from the selected vineyards at different times, between the first lesions noticed (as soon as possible) and the appearance of the mosaic lesion pattern (late in the epidemic) that impeded the collection of distinct lesions; 86 leaf samples were collected in total (Table 2). All or part of these lesions, depending on the vineyard, were genetically analysed in the original publications. Lesions in a vineyard caused by singleton genotypes (i.e., genotypes found only once) were considered derived from sexual reproduction (primary lesions) while those caused by genotypes found two or more times were considered clonal (secondary lesions). Details on methodologies are available in the cited papers (Gobbin *et al.*, 2005; Rumbou and Gessler, 2004, 2006). Based on genetic analyses, the lesions sampled in each vineyard during each sampling time were split into primary and secondary lesions, and the proportion of primary over the total lesions was calculated.

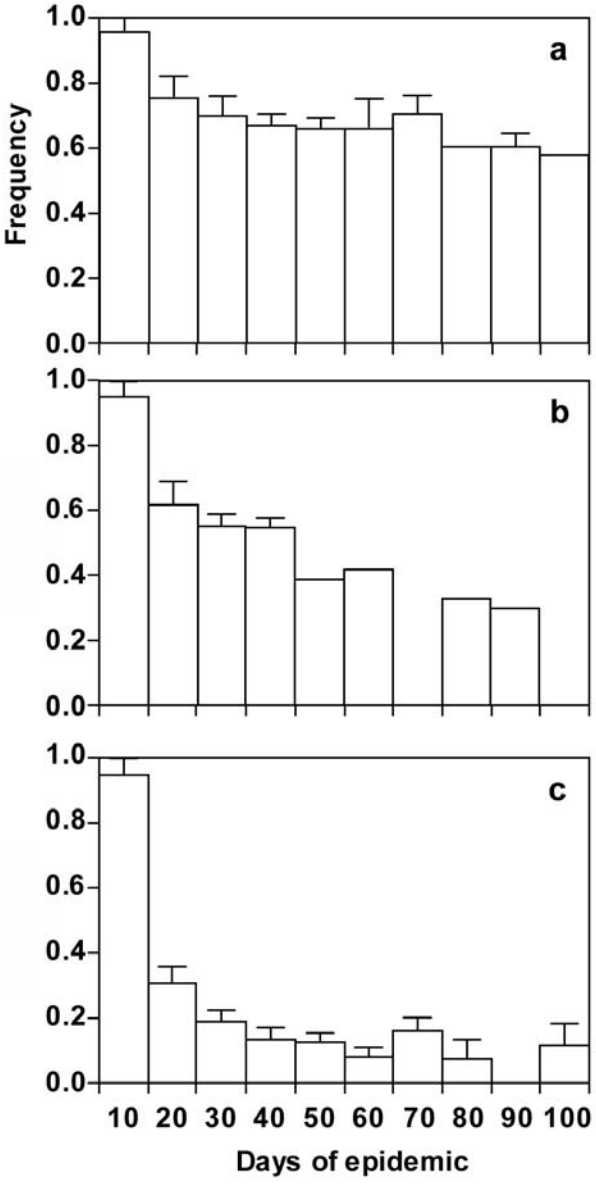


**Fig. 3.** Proportion of the downy mildew lesions caused by singleton *Plasmopara viticola* genotypes over total lesions in 23 vineyards with epidemics characterized by low (a), intermediate (b), and high (c) level of clonality (see Table 2 for vineyard characteristics). Bars are averages of 5 (a), 5 (b), and 13 (c) vineyards, and whiskers are the standard errors.

## RESULTS

**Characteristics of downy mildew epidemics**. Downy mildew epidemics in the 23 vineyards had different characteristics. The epidemics were grouped into three types based on the proportion of sexual vs. clonal lesions (Table 2). In the epidemics characterised by a low level of clonality (LC) (Fig. 3a), the proportion of lesions caused by singleton genotypes (i.e., from sexual reproduction) slowly decreased over time but was greater than 0.5 all season long. At vineyard "erb", for instance, the ratio y'/y was 0.78 after 2 weeks and 0.56 after 11 weeks (data not shown). The LC type of epidemic was observed in five European vineyards (Table 2).

In epidemics with an intermediate level of clonality (IC) (Fig. 3b), the lesions from sexual reproduction decreased to two-thirds of the total lesions 20 days after disease onset and continued to decrease, representing about one-third of total lesions at day 90. The IC type of epidemic was observed in five European vineyards (Table 2).

The epidemics with a high level of clonality (HC) (Fig. 3c) were characterised by a sudden decline in sex-

**Table 2.** Description of vineyards used for model validation, total number of downy mildew lesions examined in each vineyard, number of samples, sampling strategy, and type of epidemic.

| Vineyard[1] | | | Year | N. of lesions[2] | N. of samples | Sampling strategy[3] | Type of epidemic[4] |
|---|---|---|---|---|---|---|---|
| Aghialos | GR | agf | 2001 | 302 | 5 | T | HC |
| Aghialos | GR | ags | 2002 | 330 | 4 | T | HC |
| Biasca | CH | bia | 2000 | 314 | 4 | T/P | LC |
| Blanquefort | FR | bla | 2000 | 557 | 5 | T/P | LC |
| Bommes | FR | bom | 2001 | 193 | 4 | T/P | HC |
| Carpineta | IT | car | 2001 | 124 | 2 | T | IC |
| Cugnasco | CH | cum | 2001 | 484 | 4 | T/P | HC |
| Cugnasco | CH | cut | 2001 | 178 | 3 | T/P | HC |
| Erbach | D | erb | 2000 | 287 | 10 | T | LC |
| Gaillac | F | gai | 2000 | 47 | 2 | P | IC |
| Geisenheim | D | gei | 2000 | 206 | 4 | T | HC |
| Kephalonia | GR | kep | 2001 | 127 | 3 | T | HC |
| Lefkada | GR | lef | 2001 | 525 | 3 | P | HC |
| Lorch | D | lor | 2000 | 361 | 5 | T | IC |
| Navicello | IT | nac | 2000 | 572 | 4 | T | LC |
| Navicello | IT | nam | 2000 | 163 | 5 | T/P | LC |
| Perroy | CH | per | 2000 | 325 | 3 | P | HC |
| Stäfa | CH | sta | 2000 | 328 | 3 | P | IC |
| Tesero | IT | tes | 2000 | 97 | 3 | T | HC |
| Vädenswil | CH | wad | 2000 | 127 | 3 | P | IC |
| Vädenswil | CH | was | 2002 | 111 | 2 | T | HC |
| Vic | CH | vic | 2000 | 336 | 2 | T | HC |
| Zakynthos | GR | zak | 2001 | 152 | 2 | P | HC |

[1] Vineyard name, country, and abbreviation. Further details on the vineyards are in Gobbin *et al.* (2005), Rumbou and Gessler (2004 and 2006). [2] Total number of downy mildew lesions examined. [3] T, total sampling of the downy mildew lesions; P, partial sampling; T/P, total sampling in the first samples and then partial sampling. [4] LC, IC, and HC, epidemics characterised by low, intermediate, and high level of clonality, which is >0.50, 0.33 to 0.50, <0.33 of total lesions caused by singleton genotypes (i.e., from sexual reproduction).

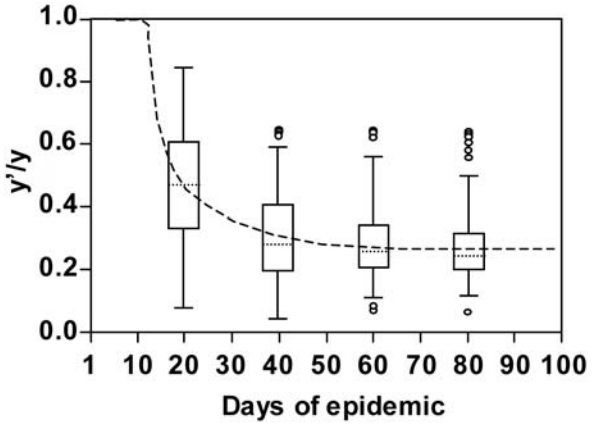


**Fig. 4.** Relative contribution of primary downy mildew lesions to total lesions (ratio y'/y) simulated by the model. The model was operated 500 times by changing initial conditions and model parameters within a range of biologically plausible values. The line (---) is the average of the 500 simulations; the box plots show the data distribution at 20, 40, 60, and 80 days of the simulated epidemics. The box includes 50% of the data, the segment (--) is the median, the whiskers extend to maximum and minimum values, while points are outliers.

ual lesions, which represented less than one-third of the total lesions within 20 to 30 days after the beginning of the epidemic. After day 30, the ratio y'/y further declined or remained almost unchanged. In some vineyards (like agf, was, and cum), sexual lesions represented less than 1% of total lesions at day 100 (data not shown). The HC type of epidemic was observed in 13 of 23 vineyards; all Greek vineyards and some European vineyards had this type of epidemic (Table 2).

**Simulation of downy mildew epidemics with biologically plausible model parameters**. The 500 model simulations performed by changing the values of both initial conditions and model parameters produced the distributions of y'/y given in Fig. 4. The average of these simulations steeply decreases in the first 40 days of the epidemic, from the initial value of 1.0 (i.e., only primary lesions are present at disease onset) to 0.32 (i.e., 32% of the downy mildew lesions present in the vineyard are caused by inoculum from sexual reproduction). After day 40, the average progressively declined to a final value of 0.27 after 80 days. Model simulations showed a higher variability in the early than in the later stages of the epidemic: the relative contribution of primary lesions to total lesions ranged from 0.85 to 0.08 after 20 days and from 0.50 to 0.12 after 80 days (with some outliers). The data distribution also had a progressively positive skew.

Three groups of simulations were selected whose values of y'/y at 20 days after the first seasonal infection were: (i) in the 1st quartile of the data distribution; (ii) within the 2nd and 3rd quartile; and (iii) in the 4th quartile (Fig. 4). Average and standard error of the values of Q, Pf, and R used for these simulation runs were: (i) Q = 350±19.1 oospores/m$^2$ soil, Pf = 0.45± 0.015, and R = 19.5±1.2; (ii) Q = 2100 ± 310 oospores/m$^2$ soil, Pf = 0.31±0.012, and R = 12.3 ±1.1; (iii) Q = 25,000±2,712 oospores/m$^2$ soil, Pf = 0.18±0.009, and R = 6.5±0.9.

**Comparison of model outputs with real data**. The 99% confidence intervals of Q, Pf, and R of each of the three groups mentioned in the previous paragraph were used as estimated parameters of the model for comparing model outputs with real data in the vineyards with

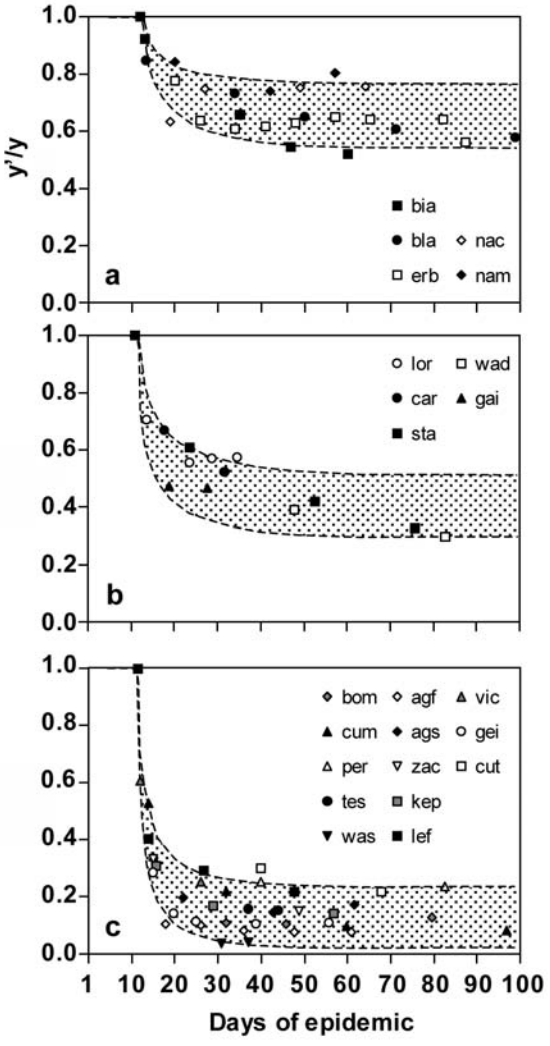


**Fig. 5.** Comparison between the relative contribution of primary downy mildew lesions to total lesions (ratio y'/y) simulated by the model and observed in 23 vineyards with epidemics characterized by low (a), intermediate (b), and high (c) level of clonality. The lower and upper curves in each panel indicate the predicted outcome (shaded area) using 99% confidence intervals for the parameters Q, Pf, and R. These values were: (a) Q = 18,000, Pf = 0.16, R = 4.2 (lower curve), Q = 32,000, Pf = 0.20, R = 9.5 (upper curve); (b) Q = 1300, Pf = 0.28, R = 4.2 (lower curve), Q = 2,900, Pf = 0.34, R = 15.1 (upper curve); (c) Q = 300, Pf = 0.41, R = 16.4 (lower curve), Q = 400, Pf = 0.49, R = 22.6 (upper curve). Points are the real data collected in the vineyards (see Table 2 for vineyard characteristics).

HC, IC, and LC epidemics. These values were: Q = 18,000, Pf = 0.16, R = 4.2 (upper curve), and Q = 32,000, Pf = 0.20, R = 9.5 (lower curve) for the LC epidemic; Q = 1,300, Pf = 0.28, R = 4.2 (lower curve), and Q = 2,900, Pf = 0.34, R = 15.1 (upper curve) for the IC epidemics; Q = 300, Pf = 0.41, R = 16.4 (lower curve), and Q = 400, Pf = 0.49, R = 22.6 (upper curve) for the HC epidemics.

The percentage of real data points that fell within the predicted outcome (i.e., below the upper curve and above the lower curve) was 89% for LC epidemics (Fig. 5a), 93% for IC epidemics (Fig. 5b), and 96% for HC epidemics (Fig. 5c). The absolute differences of calculated minus observed proportions were always < 0.05.

## DISCUSSION

The aim of this work was to develop a conceptual model capable of linking sexual and asexual infection cycles during an epidemic caused by the dimorphic pathogen *P. viticola* on grapevine leaves. To be conceptually valid, theories and assumptions underlying this model must be correct, or justifiable, and the model representation of the system, its structure, and relationships (logic, mathematical, and causal relationships) must be reasonable (Rykiel, 1996). Each component of the model presented here was developed to be consistent with the details of the *P. viticola* life cycle. The model as a whole was developed starting from the logistic equation, which is widely accepted and used in plant epidemiology for describing the progress of epidemics caused by polycyclic pathogens (Van der Plank, 1963; Campbell and Madden, 1990; Kranz, 2003). This equation was solved using an Euler integration method for primary (dy'/dt) and secondary (dy"/dt) infections, separately, and the resulting rates of disease increase were logically linked together. The low accuracy of the Euler algorithm (Dent and Blackie, 1979) should be not a problem in this model because the required precision does not exceed $10^{-3}$ (Blaise and Gessler, 1992a). A system of equations was developed to calculate dy'/dt and dy"/dt based on the differential-difference equation of Van der Plank (1963). A reason for using this approach is that, in simplest terms, it includes the essential elements that influence disease development: the multiplication potential of the pathogen, colonisable host tissue, and past history of the epidemic expressed through the infectious tissue (Blaise and Gessler, 1992a).

Madden *et al.* (2007) developed general equations for incorporating in the H-L-I-R (Healthy-Latent-Infectious-Removed) epidemic model the non-instantaneous initiation of the epidemic due to primary infections that occur over an extended period of time. This approach was mainly based on the work of Gilligan and Kleczkowski (1997) on soil-borne fungi. Both models (Van der Plank's and H-L-I-R) have the same levels of biological detail and realism (Madden *et al.*, 2007). The advantage of using the Van der Plank's equation for developing this model is that this equation expresses the disease as a proportion of the plant tissue and the progress of the epidemic is regulated by latent and infectious periods. In contrast, H-L-I-R uses densities of individuals in each of the four stages and probabilities that, in each time unit, each individual advances from one stage to the next. Insufficient data are available for parameterisation of the model using the H-L-I-R approach.

In apparent contrast with the use of the logistic equation, the progress of downy mildew epidemics under field conditions due to the sum of primary and secondary infections has been fitted using the equation of Gompertz (Liberati and Vercesi, 1999). This equation has a positive asymmetry and an earlier inflection point than the logistic, so that the absolute rate of disease increase approaches the inflection point more rapidly and declines more slowly. From a biological point of view, this asymmetry is due to the primary infections that contribute to disease severity in the first phase of epidemics (Rossi *et al.*, 2008a). Actually, there is no real difference between the logistic equation used in the current model and the Gompertz's one used to fit field data. This is because the sum of y' and y" shows a positive asymmetry due to the contribution of dy'/dt when Q is sufficiently high, and because opportunities for infection ($t_{inf}$) occur more frequently in spring than in summer.

The conceptual model developed in this work can therefore be considered scientifically acceptable because each model component and the model as a whole is logically consistent and biologically realistic (Oderwald and Hans, 1993). Conceptual validity, however, does not guarantee that the model will accurately predict reality (Rykiel, 1996).

An operational validation was then carried out to investigate whether model output reasonably mimics the real system. Validation usually requires an independent data set not used in model building or in estimating model parameters (i.e., calibration). Independence was difficult to apply for this model because of the lack of suitable data. Visual assessment of disease progress in the vineyard is not suitable for validation purposes because it is impossible to distinguish primary from secondary downy mildew lesions by visual inspection. Therefore, only the few works based on the genetic analysis of single lesions are suitable (Gobbin *et al.*, 2005; Rumbou and Gessler, 2004, 2006).

The model structural adequacy (MSA) procedures (Taper *et al.*, 2008) were used for validating the model because MSA makes it possible to estimate model parameters irrespective of observed data. In particular, the initial condition Q and the model parameters Pf and R, which are the main driving variables for producing pri-

mary and secondary infections, respectively, were estimated from a data set generated by the model itself by changing initial conditions and model parameters within a range of biologically plausible values taken from the literature or based on the author's experience and judgement. These estimated values were then used to operate the model, and the output was compared with the set of independent data taken from the literature (Gobbin *et al.*, 2005; Hug, 2005; Rumbou and Gessler, 2004, 2006). Oosporic-derived lesions collected in 23 vineyards were distinguished from the clonal lesions based on genetic analyses. This produced an accurate picture of the population structure of downy mildew lesions in each vineyard, even though the possibility of errors cannot be excluded. Such errors include immigration of new genotypes from the neighbouring vineyards, sampling procedure, and timing. These could potentially lead to an overestimation of singleton genotypes (D. Gobbin, personal communication).

The model accurately reproduced the variability of the real system. The model mimicked both those downy mildew epidemics characterized by a high degree of clonality (with most seasonal lesions caused by a few dominant genotypes) and those with a prevalence of oosporic-derived lesions.

The epidemics used in this work occurred in European vineyards. Downy mildew became endemic in Europe after its introduction in the 1880s (Lafon and Bulit, 1981), and the more or less even frequency of P1 and P2 mating types in European vineyards (Scherer and Gisi, 2006) demonstrates that sexual recombination is frequent and, consequently, that the genetic diversity is high (Gobbin *et al.*, 2003). Nevertheless, the population structure of primary and secondary lesions in other grape-growing areas is consistent with the model outputs. In Australia, the absence of oospores in many vineyards (Killigrew *et al.*, 2005) suggests that epidemics are dominated by highly adapted asexual clonal lineages (Koopman *et al.*, 2007). Hug (2005) found that in Western Australia contribution of primary lesions rapidly decreases to less than 1% of total lesions, as in the lower curve of the model simulations for HG epidemics (Fig. 4c). Similarly, in four vineyards of South Africa, the frequency of sexual lesions dropped rapidly to low values (12 to 44%) after the seasonal onset of disease (Koopman *et al.*, 2007); similar drops were produced by model simulations for IC and HC epidemics in the current study. In New York, unique genotypes represented most of the lesions in the early stages of the epidemic when, by day 12, the percentage of lesions with unique genotypes appeared to stabilize at about 50% (Kennelly *et al.*, 2005). The same occurred in the model simulations for the IC epidemics in the current study.

The estimated values of the initial condition Q ranged from 300 to about 32,000 oospores/$m^2$ soil. Unfortunately, only a few data are available for comparing these Q values with the actual densities of oospores in the vineyard soils. Burruano (1981) found a maximum of about 180 oospores/$cm^2$ of oospore-producing leaf tissue; Vercesi *et al.* (2002) found an average number of 175 and 308 oospores/$cm^2$ of leaf fragments rich in oospores, in two different years, with a maximum of 900. Tran Manh Sung *et al.* (1990) found > 1000 oospores/$cm^2$ leaf. In the work of Burruano (1981), production of *P. viticola* oospores occurred in 10 to 100 $mm^2$ out of 300 $cm^2$ of leaf samples showing mosaic-like downy mildew symptoms, which is 0.03 to 0.33% of the affected leaf tissue. The number of oospores produced in this kind of oospore-producing tissue ranged between 100 to 700 per $mm^2$. If a possible LAI for grapevine is 2.5 $m^2$ leaf/$m^2$ soil (Poni *et al.*, 2006), then the theoretical maximum leaf area supporting oospore production (i.e., when disease severity is 100%) is 7.5 to 82.5 $mm^2/m^2$ soil (25,000 $mm^2$ leaf/$m^2$ soil x 0.03% and 0.33%, respectively), which is 750 (7.5 $mm^2$ leaf/$m^2$ soil x100 oospores/$mm^2$ leaf) to 57,750 oospores $m^2$ soil (82.5 $mm^2$ leaf/$m^2$ soil x 700 oospores/$mm^2$ leaf). Therefore, the estimated values of Q are plausible. Also, these Q estimates were consistent with the actual situation in the sampled vineyards. Oospore densities were lower in vineyards with a high level of clonality and higher in those with a large contribution of the oospore-derived lesions to the epidemic.

The estimated values of the parameter Pf (proportion of the oospore-derived lesions that are fertile) ranged from 0.16 to 0.49, which are consistent with the frequency of sexual lesions able to produce secondary lesions calculated from the raw data of Gobbin *et al.* (2005), that ranged from 11 to 39%. Also, Koopman *et al.* (2007) showed that only a few genotypes (3 to 28%) were able to reproduce asexually. The limited number of *P. viticola* genotypes able to produce daughter lesions may be due to lower levels of asexual spore production in the field than in the laboratory (Reuveni, 2003), or to the high sensitivity of sporangia to unfavourable environmental conditions (Kast and Stark-Urnau, 1999), or to the genetic background of these genotypes. Kast (2004) and Kast *et al.* (2001) showed that *P. viticola* isolates differ in virulence and that not all field lesions produce sporangia under optimum laboratory conditions.

The estimated values of the parameter R (number of daughter lesions originated from a mother sporulating lesion) ranged from 4.2 to 22.6. This epidemiological parameter has been widely used in ecology and animal and human epidemiology, but has received far less attention in the plant pathology literature (van den Bosch *et al.*, 2008). The value calculated by Gessler and Blaise (1992) was 4.7, lower than the values estimated in this work. This is not surprising because Gessler and Blaise (1992) calculated an average value from disease severity data, which included all downy mildew lesions. Estimates in the present work, however, referred only to

those lesions able to reproduce asexually, which was Pf = 0.16 to 0.49 of total lesions. Therefore, Gessler and Blaise (1992) values are of the same magnitude as those estimated in this work.

In conclusion, the accuracy of the model predictions for different epidemics demonstrates the logical correctness of the model and the adequacy of its description of biological mechanisms (Caswell, 1976; Dent and Blackie, 1979; Fleming and Shoemaker, 1992).

The model developed in this work is not a simulator for downy mildew epidemics in the vineyard but represents a coherent mathematical structure for such a simulator. The next step for creating a simulator is the development of equations that make the estimation of the model parameters for each vineyard and each epidemic possible (Rossi *et al.*, 1997). Some model parameters that depend on the host plant (e.g., LAI, km, and kl) can be estimated based on vineyard characteristics (plant density, trellising system, etc.) or by using simulators for plant growth (Wermelinger *et al.*, 1991; Poni *et al.*, 2006). Other model parameters that depend on the effect of weather conditions on the pathogen can be estimated using equations already developed and included into simulation models. These consider either primary (Rossi *et al.*, 2008b) or secondary infections (Blaeser and Weltzien, 1979; Lalancette *et al.*, 1988a, 1988b; Hill, 1989; Kennelly *et al.*, 2007).

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