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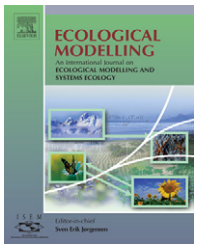

A mechanistic model simulating primary infections of downy mildew in grapevine

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ABSTRACT

A dynamic model for *Plasmopara viticola* primary infections on grapevine was elaborated according to a mechanistic approach. Development of the sexual stage of the pathogen was split into different state variables, in which changes from one state to another were regulated by rates depending on environmental conditions. The conceptual model was based on the definition of a primary inoculum season, a seasonal oospore (inoculum) dose, and its division into many coeval cohorts. Each cohort progresses along the primary infection cycle (production and survival of sporangia, release, survival and dispersal of zoospores, infection, appearance of disease symptoms) simultaneously, with a time step of one hour. The model was evaluated over a 12-year period (1995–2006) in Emilia-Romagna (Northern Italy) by comparing simulations with actual observations of the first seasonal symptoms of downy mildew in 43 vineyards. This data set was not used in model building and represents different environmental conditions, with both early and late primary infections. The model showed high sensitivity, specificity and accuracy. The achieved probabilities that an oospore cohort produce or not an infection were 0.994 and 0.999, respectively, while there was practically no probability that an oospore cohort produce infection when infection is not predicted by the model. Because of a small probability (<0.01) that a predicted infection is not a true infection, confidence in prediction of non-infections was higher than in prediction of infections. Most of the wrong positive predictions occurred in early season, when the host was in the earlier growth stages, or when the oospore germination was triggered by isolated weak rain events. Considering that neither calibration nor empirical adjustment of model parameters were necessary to obtain accurate simulation, it was concluded that this model produces a reasonable approximation of the primary infection processes underlying oospore development. The model showed that 86% of the oospore cohorts failed infection, because sporangia did not survive till zoospore release (5.6% of these cohorts), zoospores released from sporangia did not survive until dispersal (83.6%), or the zoospores that dispersed to leaves did not cause infection (10.8%).

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1. Introduction

Plasmopara viticola (Berk et Curt.) Berlese et de Toni is the causal agent of grapevine downy mildew, an important disease in all grape-growing areas characterized by frequent rain (Lafon and Clerjeau, 1988). This heterothallic fungus is dimorphic for its reproductive forms, with both sexual and asexual spores. This trait strongly influences the epidemiological behaviour of *P. viticola*, which is characterized by primary (sexual) and secondary (asexual) infection cycles that overlap for a part of the season.

Oospores represent the sexual stage of *P. viticola*, generated by fusion of an oogonium and antheridium (Wong et al., 2001) in host leaves (Berlese, 1898). Oospores are the sole relevant source of inoculum for primary downy mildew infections in the following season (Galbiati and Longhin, 1984). Oospores overwinter in the leaf debris above the vineyard ground or buried in soil; in spring they germinate in a macrosporangium that releases zoospores which are responsible for primary infections on grape leaves and clusters. After 5–18 days, depending on the temperature, the fungus produces sporangia containing asexually produced zoospores (Agrios, 1988). First infections are therefore followed by successive asexual cycles (Blaeser and Weltzien, 1979).

The disease is potentially destructive and requires protection with fungicides. In Northern Italy, 6–9 fungicide sprays are used to control the disease (Borgo et al., 2004). Some of these sprays are usually unnecessary given the sporadic occurrence of the disease. In order to identify high-risk periods and time fungicide sprays, weather-driven models have been proposed in France (Stryzik, 1983; Tran Manh Sung et al., 1990; Magnien et al., 1991), Germany (Hill, 1990), Switzerland (Blaise et al., 1999; Viret and Bloesch, 2002), Italy (Rosa et al., 1993), Australia (Magarey et al., 1991) and the USA (Park et al., 1997). However, models often fail in predicting the real development of epidemics, which restricts their use in practice (Vercesi et al., 1999a).

In the traditional conception of pathogen life cycle, a downy mildew epidemic begins with a restricted number of germinating oospores and the explosive increase of the epidemic is ensured by massive clonal multiplication causing secondary infections (Blaeser and Weltzien, 1979; Lafon and Clerjeau, 1988; Lalancette et al., 1988a,b; Blaise et al., 1999; Orlandini et al., 1993). Recent studies carried out using polymorphic microsatellite markers for *P. viticola* (Gobbin et al., 2003) demonstrated a continuous input of new genotypes to the epidemic during a prolonged period from May to August (Rumbou and Gessler, 2004; Gobbin et al., 2005; Kennely et al., 2007). Therefore, oospores play a key role in the development of downy mildew epidemics in addition to triggering them.

Hence, modelling of downy mildew epidemics can be significantly improved by taking into account quantitative aspects of both sexual and asexual stages. To date, only a few models in the literature take into consideration oospore-derived infections. Stryzik (1983) elaborated the EPI model based on data collected in the area of Bordeaux (France), which accounts for the effect of meteorological conditions during winter on oospore development. His conceptual model is based on the assumption that the presence of *P. viticola* in a vine-growing area is the result of an ecological adaptation of the fungus to the meteorological conditions of such an area. Therefore, model calculations are based on the differences between actual meteorological data and a 30-year climatic series. Tran Manh Sung et al. (1990) developed the POM model, based on daily rainfall beginning in September, to calculate DOM, the date when most oospores are mature, and consequently disease severity: the earlier the DOM, the more severe the disease is expected to be in spring. Hill (2000) developed the SIMPO predictor for estimating periods due to fast oospore germination based on daily data of temperature, relative humidity and precipitation. It calculates a daily index to determine the number of days needed for oospore germination to occur under favourable conditions. The DMCAST model uses the same parameters as the POM model, and predicts the date of primary infection when almost 3% of oospores are ready to germinate (Park et al., 1997).

These models have been validated under several environmental conditions in Italy, but none of them was accurate or robust enough to be used for scheduling fungicide applications against downy mildew (Egger et al., 1994; Vercesi et al., 1999a; Vercesi and Liberati, 2001; Caffi et al., 2007). As a consequence, the warning systems operating throughout Italy continue to adopt the simple and widely known "3–10" empiric rule (Rossi et al., 2000a), although it often indicated risk of infections that did not occur (Vercesi, 1995; Serra et al., 1998; Vercesi et al., 1999a). This rule is based on the simultaneous occurrence of the following conditions: (i) air temperature equal to or greater than 10 °C; (ii) vine shoots at least 10 cm in length; (iii) a minimum of 10 mm of rainfall in 24–48 h (Baldacci, 1947).

In order to reproduce the ecological behaviour of *P. viticola* oospore population and processes involved in the primary disease cycle, a new model based on a mechanistic approach was constructed.

2. Material and methods

2.1. Primary disease cycle processes

The model concerns five stages in the primary *P. viticola* cycle (Fig. 1). Oospores form within the affected leaf tissue (Arens, 1929) from ripening until leaf fall, and overwinter in the leaf litter above or buried in the soil (Galbiati and Longhin, 1984; Gehman, 1987). During the winter, the oospores reach morphological maturity (Vercesi et al., 1999b) but their germination may be prevented, even if environmental conditions are right, due to dormancy (Galet, 1977) regulated by environment, nutrient impermeability and endogenous inhibitors (Schweizer and Oliver, 1999). When dormancy is broken, oospores reach physiological maturation and are ready to germinate under favourable environment (Ronzon Tran Manh Sung and Clerjeau, 1989). Since the oospore field population is not coeval, germination occurs gradually during the current (Jermini et al., 2003) and even in the following (Arens, 1929) seasons.

Germinating oospores form sporangia that survive for a few hours to a few days (Blaeser and Weltzien, 1979) and release zoospores from the inside in the presence of water. Survival of the zoospores is strictly dependent on the presence of a film of

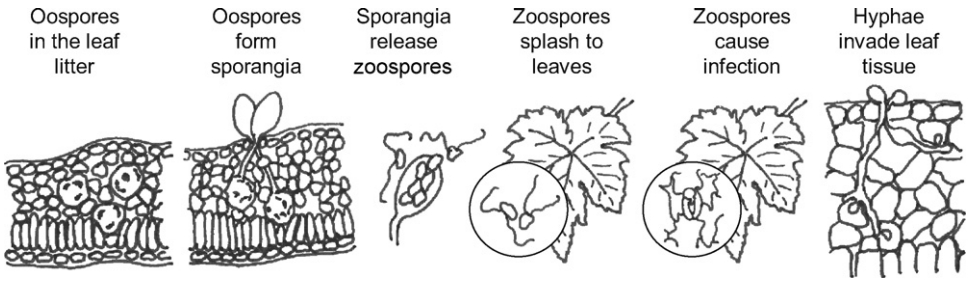

Fig. 1 – Sexual stages of the life cycle of *Plasmopara viticola*.

water. Viable zoospores on the soil surface are dispersed from the soil to the grape leaves by rain splashes. Once deposited on the leaves, zoospores swim towards the stomata, form a germ tube and penetrate the host. Infecting hyphae colonize the host tissue and the initial disease symptoms are noticed in the form of "oil spots".

2.2. Modelling approach

The model was elaborated according to principles of the "systems analysis" (Leffelaar and Ferrari, 1989). Previously described processes were separated into different stages and changes from one stage to another depend on environmental conditions, as shown in the relational diagram of the model (Fig. 2 and Table 1). Stages of the primary disease cycle are considered as state variables and rate variables or switches regulate changes in such variables. Influencing weather conditions are considered as parameters or intermediate variables.

2.3. Model description

The first state variable is set at leaf fall and consists of the oospore population that will germinate during the next grape-growing season. These oospores represent the seasonal oospore dose (SOD), with SOD = 1.

The second state variable is represented by the morphologically mature oospores (MMO): the change from SOD to MMO depends on the day of the year (DOY). Since oospores reach this stage in November, irrespective of the period of their formation (Vercesi et al., 1999b), the model assumes that the entire oospore population is in the MMO stage on the 1st of January (DOY = 1) at 01.00 h ($h = 1$, with h being the counter for the hours). Therefore, when $h = 1$, $MMO_h = SOD$.

The third state variable consists of the physiologically mature oospores (PMO). Oospores go from MMO to PMO at the end of dormancy, and this change is regulated by the rate variable DOR (progress of dormancy breaking), as follows:

$$PMO_h = MMO_h \times DOR_h \quad (1)$$

DOR depends on the physiological time regulating the breaking of dormancy (Rossi et al., 2007). In addition, exposure to low temperature during winter seems to play an important role in breaking dormancy (Galbiati and Longhin, 1984), but this effect is not included in the model because it is assumed that oospores must experience such a low temperature in the winter of temperate climates where *P. viticola* usually develops.

DOR is then calculated with a time step of 1 h using the following equation of Gompertz (adapted from Rossi et al., 2007) (Fig. 3):

$$DOR_h = \exp(-15.891 \exp(-0.653(HT_h + 1))) \quad (2)$$

where HT is the hydro-thermal time, calculated as:

$$HT_h = \sum_{h=1}^{\eta} \frac{M_h}{(1330.1 - 116.19T_h + 2.6256T_h^2)} \quad (3)$$

$$\text{when } T_h \le 0\,^{\circ}\text{C}, \quad \text{the ratio} \frac{M_h}{f(T_h)} = 0$$

where h is the subscript for hours, with $h = 1$ to the current hour η; T is the air temperature (in °C) at each hour; M is a dichotomic variable accounting for the moisture of the leaf litter holding oospores, as follows:

$$\begin{aligned} &\text{when } R_h > 0\,\text{mm} \quad \text{or} \quad VPD_h = 4.5\,\text{hPa}, \quad M_h = 1 \\ &\text{when } R_h = 0\,\text{mm} \quad \text{and} \quad VPD_h > 4.5\,\text{hPa}, \quad M_h = 0 \end{aligned}$$

where R is rainfall (in mm); VPD is the vapour pressure deficit (in hPa) calculated from T and relative humidity (RH, in %) following Buck (1981).

The HT is also used to calculate the length of the primary inoculum season (PIS): PIS starts and finishes when HT = 1.3 and 8.6, respectively (Fig. 3). These values correspond to the time when 3% and 97% of the total oospores forming the SOD have entered the PMO stage, respectively, according to Eq. (3).

The fourth state variable consists of the germinated oospores (GEO), i.e. oospores that have produced sporangia. The sporangia are formed at the end of a germination process, which is triggered by rainfall (Darpoux, 1943; Burruano et al., 1995) and its duration regulated by temperature (Laviola et al., 1986) and moisture (Rossi and Caffi, 2007). The model considers that oospores in the PMO stage begin germination when rainfall moistens the leaf litter and enter the GEO stage based on the variable GER (germination).

Events triggering oospore germination start with $R_{h=\varepsilon} \ge 0.2$ mm (where ε is the first hour of the event), last for the following wet hours irrespective of the presence of further rainfall over such a wet period, and finish with the first dry hour. During the PIS there are a number of events,

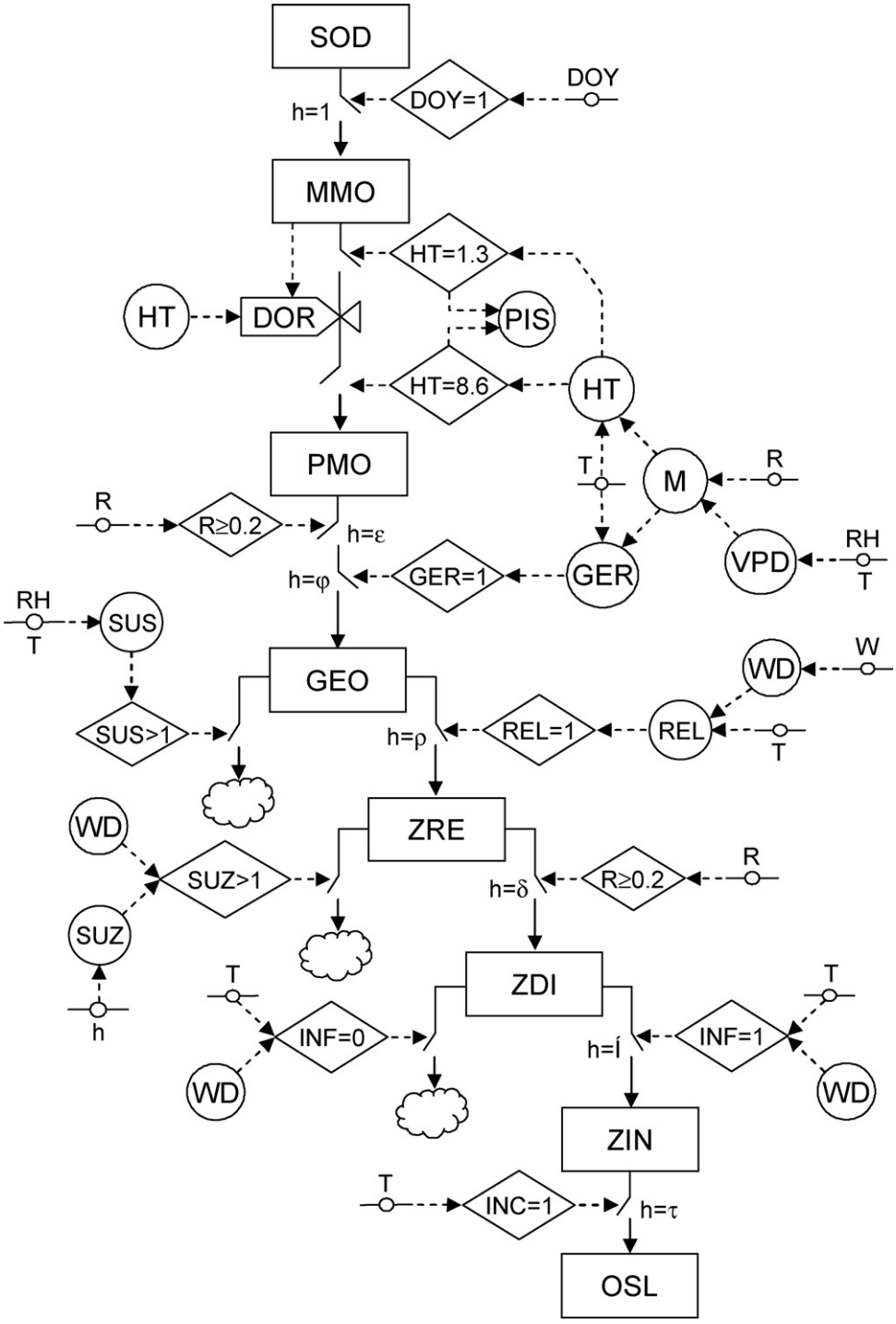

Fig. 2 – Relational diagram of the model simulating the sexual stage of *Plasmopara viticola*. Legend: (□), state variable; (—►), flux and direction of states; (– – ►), flux and direction of information; (–○–), parameter; (○), intermediate variable; (◊), switch; (☁), outgoing variable; (⋈), valve in a flux (rate) (see Table 1 for acronym explanation).

each lasting one to many hours; these events are defined by the subscript j, with $j = 1, 2, \ldots, J$, where J is the total number of events in the PIS (Fig. 3).

Each j^{th} event triggers germination of an oospore cohort, which is composed of oospores in PMO stage at hour ε; a cohort goes on in the model uniformly. Cohorts are defined by the subscript c, with $c = 1, 2, \ldots C$, where C is the total number of cohorts, and obviously $C = J$. Density of each cohort is defined as follows (Fig. 3):

$$\mathrm{PMO}_c = \int_{\varepsilon(j-1)}^{\varepsilon-1(j)} \mathrm{DOR}_h \tag{4}$$

Table 1 – List of variables used in the model

Acronym	Description	Unit
SOD	Seasonal oospore dose	Number (set at 1)
MMO	Morphologically mature oospores	Number (0–1)
PMO	Physiologically mature oospores	Number (0–1)
GEO	Germinated oospores (i.e. oospores with sporangia)	Number (0–1)
ZRE	Zoospores released from sporangia	Number (0–1)
ZDI	Zoospores dispersed from soil to leaves	Number (0–1)
ZIN	Zoospores causing infection	Number (0–1)
OSL	Oil spots on leaves	Number (0–1)
DOR	Progress of dormancy breaking in the oospore population	Proportion/h
GER	Germination of oospores (i.e. formation of sporangia)	Number
SUS	Survival of sporangia	Number
REL	Zoospore release	0 (no) or 1 (yes)
SUZ	Survival of zoospores	Number
INF	Infection by zoospores	0 (no) or 1 (yes)
INC	Incubation	0–1
PIS	Length of the primary inoculum season	Days
PMO_c	Density of the cth oospore cohort	Number (0–1)
h	Counter for the time (hours), with $h=1$ on 1st January, 01.00 h	h
η	Current hour	h
ε	Time when an oospore cohort begins germination	h
φ	Time when an oospore cohort ends germination	h
ρ	Time of the zoospore release	h
δ	Time of zoospore dispersal	h
ι	Time of infection	h
τ	Time of disease onset	h
j	Subscript for the oospore germination events	
J	Total oospore germination events in the PIS	
c	Subscript for the oospore cohort	
C	Total oospore cohorts	
DOY	Day of the year	Number
T	Air temperature	°C
R	Rainfall	mm
RH	Relative humidity	%
W	Presence of wetness	0 (no) or 1 (yes)
VPD	Vapour pressure deficit	hPa
WD	Wetness duration	h
HT	Hydro-thermal time	°C × h
M	Moisture of the leaf litter	0 (no) or 1 (yes)
T_{WD}	Average T over WD	°C

where $c=j$; $\varepsilon(j-1)$ is the first hour of the previous event; $\varepsilon-1(j)$ is the last hour before the current event. It means that at the ninth rain event ($j=9$) starts the germination of the ninth oospore cohort ($c=9$) (Fig. 3).

GER is calculated for each cohort starting from any event as follows:

$$\mathrm{GER}_h = \sum_{h=\varepsilon}^{\eta} \frac{M_h}{(1330.1 - 116.19T_h + 2.6256T_h^2)} \tag{5}$$

$$\text{when } T_h \le 0\,^\circ\mathrm{C},\quad \text{the ratio}\,\frac{M_h}{f(T_h)} = 0$$
$$\text{when } \mathrm{GER}_h = 1,\quad \varphi = \eta,\quad \mathrm{GEO}_\varphi = \mathrm{PMO}_c$$

where ε and φ are the hours when the germination process starts and ends, respectively; c is the subscript for the oospore cohort (Fig. 3).

Sporangia survive for some time, depending on T and RH (Blaeser and Weltzien, 1979). The model calculates the survival of sporangia (SUS) as follows:

$$\mathrm{SUS}_h = \sum_{h=\phi}^{\eta} \frac{1}{(24(5.67 - 0.47(T_h(1 - RH_h/100)) + 0.01(T_h(1 - RH_h/100))^2))} \tag{6}$$

and considers that:

$$\text{when } \mathrm{SUS}_h \le 1,\quad \mathrm{GEO}_h = \mathrm{GEO}_\varphi$$
$$\text{when } \mathrm{SUS}_h > 1,\quad \mathrm{GEO}_h = 0$$

The fifth state variable is represented by the zoospores released from viable sporangia (ZRE). Zoospore release occurs in the presence of water, with a suitable T (Ravaz, 1914; Galet, 1977). The variable REL (zoospore release) is a dichotomic variable accounting for this process: it assumes the values one (release) or zero (no release), according to the following rule:

$$\text{when } \mathrm{WD}_h \ge \exp\left(\frac{-1.022 + 19.634}{\mathrm{TWD}_h}\right),$$
$$\mathrm{REL}_h = 1,\quad \rho = h,\quad \mathrm{ZRE}_\rho = \mathrm{GEO}_h$$
$$\text{otherwise } \mathrm{REL}_h = 0$$

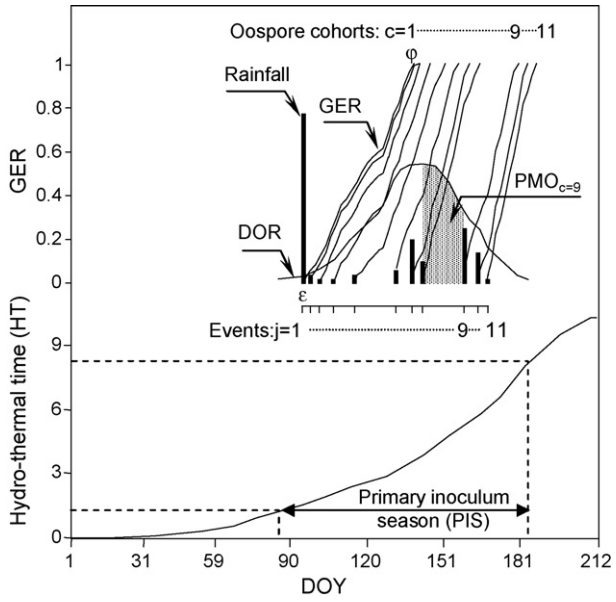

Fig. 3 – Schematic representation of how the model defines the oospore cohorts of *Plasmopara viticola*. The hydro-thermal time makes it possible to calculate the beginning and length of the primary inoculum season (PIS), the dynamic of oospore maturation by means of the rate DOR, the formation of the oospore cohorts c on any rain event j and the definition of their size (PMO$_c$). The variable GER determines the germination course of each cohort, with ε and φ being hours when a cohort starts and ends germination. DOY is the day of the year.

where WD is the duration of wetness (in hours), calculated as: $\sum_{h=\phi+1}^{\eta} W_h$; W is a dichotomic variable which is equal to one (wet hour) or zero (dry hour); TWD is the average T of this wet period; ρ is the time of zoospore release, i.e. the first hour from the beginning of WD when the above mentioned condition is satisfied.

Zoospores in the ZRE stage survive until the water triggering their release dries out. The model calculates the survival of zoospores (SUZ), as follows:

$$\mathrm{SUZ}_h = \frac{\sum_{h=1}^{\eta}(h-\rho)}{\sum_{h=\rho+1}^{\eta} W_h} \tag{7}$$

and considers that

$$\text{when } \mathrm{SUZ}_h > 1;\ \ \mathrm{ZRE}_h = 0$$

In Eq. (7) the numerator accumulates hours after zoospore release, and the denominator accumulates the correspondent hours of wetness; therefore, when the ratio is greater than one, the wet period is over and zoospores die.

The sixth state variable is represented by the zoospores dispersed (ZDI) from soil to grapevine leaves by rain splashes. The model assumes that any rainfall splashes the zoospores into the ZRE stage to leaves (Rossi et al., 2002), as follows:

$$\text{when } R_h \geq 0.2\,\mathrm{mm};\ \ \delta = h;\ \ \mathrm{ZDI}_\delta = \mathrm{ZRE}_\rho$$

where δ is the time of zoospore dispersal.

The seventh state variable is represented by zoospores causing infection (ZIN). The possibility that zoospores cause infection depends on a favourable combination of T and WD (Blaeser and Weltzien, 1979). The variable INF (infection) accounts for this process: it is a dichotomic variable which assumes the values one (infection) or zero (no infection), according the following rule:

$$\text{if } \mathrm{WD}_h\mathrm{TWD}_h \geq 60,\ \ \mathrm{INF}_h = 1,\ \ \iota = h,\ \ \mathrm{ZIN}_\iota = \mathrm{ZDI}_\delta$$
$$\text{otherwise } \mathrm{INF}_h = 0,\ \ \mathrm{ZDI}_h = 0\ \text{(i.e. zoospores die)}$$

where WD is the number of wet hours from δ to the hour when the condition is satisfied for the first time; TWD is the average T of this wet period; 60 is the minimum favourable combination of WD and TWD (Blaeser and Weltzien, 1979); ι is the time of infection.

The last state variable is represented by the oil spots on leaves (OSL). Oils spots appear after an incubation period, whose length depends mainly on T (Goidanich et al., 1957). The model calculates the hourly progress of incubation (INC) and particularly its confidence interval, INC′ to INC″ (Rossi et al., 2002):

$$\mathrm{INC}'_h = \frac{1}{(24\cdot(45.1 - 3.45\cdot T_h + 0.073\cdot T_h^2))} \tag{8}$$

$$\mathrm{INC}''_h = \frac{1}{(24\cdot(59.9 - 4.55\cdot T_h + 0.095\cdot T_h^2))} \tag{9}$$

so that incubation finishes in the time interval:

$$\sum_{h=\iota}^{\eta}\mathrm{INC}'_h \leq 1 : \sum_{h=\iota}^{\eta}\mathrm{INC}''_h \leq 1,\ \ \tau = h$$

where ι and η have the previously cited means (hour of infection occurrence, and current hour, respectively), while τ is the time of disease onset.

2.4. *Model evaluation*

The model was evaluated using data from 43 natural epidemics recorded in the grape-growing regions of Emilia-Romagna, Northern Italy, representing a 12-year period (1995–2006) with 2–5 vineyards each year (Table 2). Vineyards were selected to be representative of different vine-growing areas, for soil type, varieties, training systems and cropping regimes. Vineyards were supposed to have a representative dose of overwintering oospore populations because a regular fungicide schedule was applied the previous season to control downy mildew.

A plot including several rows of vines (at least 500 m^2 wide) was set apart in each vineyard, and no fungicides against *P. viticola* were applied until the time of first disease onset. These unsprayed plots were carefully inspected at least once per week starting from bud burst, to determine the time of appearance of the first disease symptoms, such as “oil spots” on leaves or as rotted bunches.

Hourly meteorological data of T, RH, R and W were supplied by the agro-meteorological regional network; until the year 2000 the service supplied, for each vineyard, meteorological data from automatic stations (SIAP, Bologna, Italy) not farer than 15 km, while from the year 2001 it supplied inter-

Table 2 – Number of vineyards used for model validation in 1995–2006 and total number of simulations per year, separated in accurate and wrong

Year	Vineyards	Simulations				
		Total[a]	Accurate		Wrong	
			Successful[b]	Aborted[c]	Successful[d]	Aborted[e]
1995	3	18	6	11	1	0
1996	4	19	3	16	0	0
1997	4	70	5	61	4	0
1998	4	40	4	32	4	0
1999	2	16	2	13	1	0
2000	3	24	4	17	3	0
2001	5	42	8	33	1	0
2002	5	34	7	25	2	0
2003	3	29	4	25	0	0
2004	5	73	4	59	10	0
2005	3	26	5	18	3	0
2006	2	20	5	14	1	0
Total	43	411	57	324	30	0

[a] Several simulations were performed in each vineyard according to the number of oospore cohorts formed between the beginning of the primary inoculum season and the time of first disease onset.
[b] The simulated appearance of the disease corresponded to the actual appearance in the vineyard.
[c] The simulation was interrupted and no disease appeared in the vineyard during the time period when the correspondent symptoms should appear in the case that simulation proceeded successfully, or disease appeared but it was explained by another accurate successful simulation.
[d] The model simulated a successful infection but the disease did not appear.
[e] The model did not simulate an infection that actually occurred and was not explained by an accurate successful simulation.

polated data for the grids (5 km × 5 km wide) which comprised the vineyards (Bottarelli and Zinoni, 2002).

The model was used to simulate, for each vineyard, the progress of its infection cycle for each oospore cohort between the beginning of PIS and the time of first disease onset in the vineyard.

Total simulations were firstly distinguished in aborted and successful. A simulation was considered aborted when the process was interrupted during any stage prior to infection, while it was considered successful when all stages progressed until onset of disease symptoms. Simulations were distinguished in: (i) accurate successful, when a predicted appearance of the disease corresponded to the actual appearance in the vineyard; (ii) accurate aborted, when the simulation was interrupted and no disease appeared in the vineyard during the time period when the correspondent symptoms should appear in case that simulation should proceed successfully, or disease appeared but it was explained by another accurate successful simulation; (iii) wrong successful, when the model predicted a successful infection but the disease did not appear; (iv) wrong aborted, when the model did not predict an infection that actually occurred and was not explained by an accurate successful simulation.

All the possible combinations of observed (O) versus predicted (P) infections were organized in a 2 × 2 contingency table, where the two groups $O-$,$P-$ (no observed and no predicted infection) and $O+$,$P+$ (yes observed and yes predicted infection) were the right estimates, while the two groups $O-$,$P+$ and $O+$,$P-$ were the wrong ones. Sensitivity, specificity and accuracy of the model predictions were evaluated by means of the Bayesian analysis (Yuen and Hughes, 2002). To asses the advantages rising from the model in practice, the probabilities that an oospore cohort of a particular vineyard result or not in a downy mildew infection were determined as $P(O+,P+)$ and $P(O-,P-)$ following Madden (2006), and compared with the correspondent prior probabilities, $P(O+)$ and $P(O-)$, respectively.

3. Results

The date of the first seasonal onset of downy mildew symptoms in the 43 vineyards ranged between 11th May and 29th June (Fig. 4). In 50% of cases, the first disease symptoms appeared between mid May and early June, with a median on 25 May (Fig. 4). In two vineyards (in 2003 and 2004) the disease did not appear all season long.

Sixteen to 73 simulation runs were performed for year in the time window between the beginning of the primary inoculum season and the first seasonal onset of downy mildew

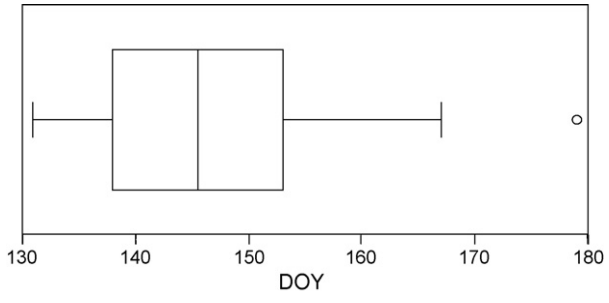

Fig. 4 – Box plot showing the distribution over time (expressed as day of the year, DOY) of the first seasonal appearance of downy mildew symptoms in the 43 vineyards considered for model validation.

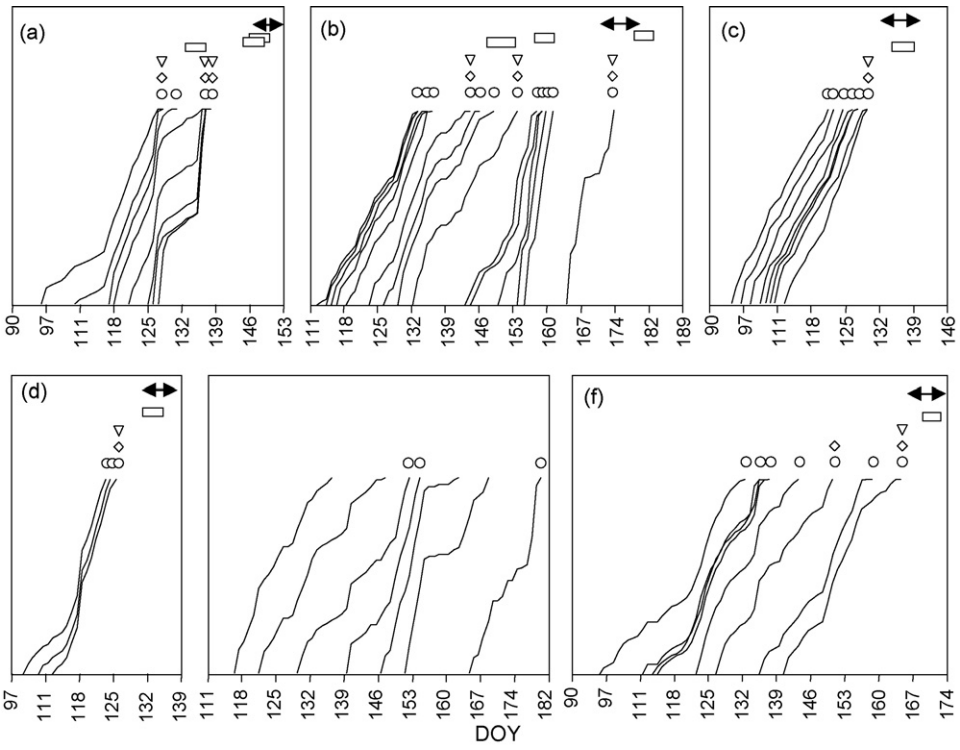

Fig. 5 – Examples showing model simulations and validation in the vineyards of Castelfranco Emilia 1995 (a), Cavriago 1997 (b), Lavezzola 2001 (c), Castelfranco Emilia 1996 (d), Piacenza 2003 (e), and Gaggio 2006 (f). DOY is the day of the year, (—), shows the germination course of an oospore cohort; (○), zoospore release; (◊), zoospore dispersal; (▽), infection; (□), and (↔), estimated and actual periods of downy mildew onset, respectively.

(Table 2), depending on the number of oospore cohorts formed in such a window, for a total of 411 simulations. Seventy-nine percent of these simulations were aborted because environmental conditions were not favourable for the progress of the infection cycle. Some representative examples of these simulations are shown in Fig. 5.

For instance, at Lavezzola 2001 (Fig. 5c), the model calculated that the PIS began on 5th April. At this date, rainfall triggered the germination of the first oospore cohort and seven further cohorts began germination in the following 2 weeks. The simulations of the first six cohorts were aborted because no rainfall dispersed the zoospores released from sporangia during the survival period of these zoospores. The rain events on 14th and 16th April triggered germination of two oospore cohorts; zoospores were released and dispersed on grape leaves on 3rd May and caused infection on the same day. The model simulated a probable onset of symptoms between 8th and 13th May, and the downy mildew symptoms appeared on 13th May. Similar situations occurred at Castelfranco Emilia 1996 (Fig. 5d) and Gaggio 2006 (Fig. 5f).

At Cavriago 1997 (Fig. 5b), the model performed 15 simulations after the beginning of PIS (23rd April). The model aborted 12 simulations because zoospores released from sporangia did not survive. The sixth and the ninth cohort completed the infection on 26th May and 5th June, respectively, but disease symptoms did not appear in the vineyard within the expected period. The rainfall of 15th June triggered germination of the 15th cohort, which produced sporangia on 25th June; on the same day zoospores were dispersed and caused infection. The model estimated the onset of symptoms between 29th June and 3rd July, and first downy mildew spots were observed on 29th June. In Castelfranco Emilia in 1995, there was also an estimated infection that did not correspond to an actual disease onset in the vineyard (Fig. 5a).

At Piacenza 2003 (Fig. 5e), the model performed only seven simulations: four and three of them were aborted because sporangia or zoospores did not survive, respectively. Actually, the disease never appeared in the vineyard.

All the actual infections were correctly predicted, giving a true positive proportion TPP = 1, (Table 3). Also, 324 out of 354 aborted infections were correctly predicted, giving a true negative proportion of TNP = 0.92. An analysis of these simulations (Fig. 6) showed that in few cases the infection cycle was interrupted because sporangia did not survive till zoospore release (5.6% of the aborted cases), or because the zoospores that dispersed to leaves did not cause infection (10.8%). In most cases (83.6%), the infection cycle stopped because zoospores released from sporangia did not survive until dispersal.

In no cases, there were wrong aborted infections, because infections never occurred in the vineyard without a successful simulation by the model, giving a false positive proportion of FNP = 0 (Table 3). In 30 cases, simulations were wrong successful, because the model predicted an infection that did not result in actual disease onset, giving a false negative proportion of FPP = 0.08. These cases were more frequent in the early season: 85% of them occurred within the first week of May (as

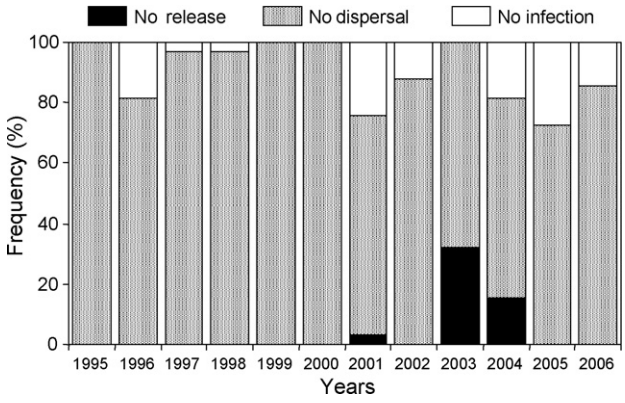

Fig. 6 – Percent distribution of 324 simulations in which the model did not complete the infection cycle because the environmental conditions were unfavourable for zoospore release, zoospore dispersal or infection.

in Fig. 5a), and only 15% after mid May (as in Fig. 5b). In all these latter cases, wrong simulations started with a rain of only 0.2 mm occurred after some dry days (not shown).

Overall accuracy was 0.93 considering accurate versus total simulations, while it was $J = 1$ using the Youden's index (J = TPP-FPP), which is a perfect simulation. The likelihood ratio (LR) of a successful infection was LR(O+) = TPP/FPP = ∞, or 1000 when FPP was cautiously set = 0.001 (i.e. 0.1% of actual infections not correctly predicted by the model), and the likelihood ratio for an aborted simulation was LR(O−) = FNP/TNP = 0.093.

The probability that there was infection when the infection was predicted was $P(O+,P+) = 1$ (or 0.993 under the cautious hypothesis that the false positive proportion was FPP = 0.001 instead of the observed value of 0), and the probability that there was no infection when the infection was aborted by the model was $P(O-,P-) = 0.999$, while the prior probabilities for infection and no infection were $P(O+) = 57/411 = 0.14$ and $P(O-) = 354/411 = 0.86$, respectively (Table 3). The posterior probability that there was no infection when the model predicted an infection was $P(O-,P+) = 0.006$, and the posterior probability that there was infection when infection was not predicted by the model was $P(O+,P-) = 0$ (or 0.00016 under the cautious hypothesis) (Table 3).

4. Discussion

In this work, a new model for *P. viticola* primary infections was elaborated, following a mechanistic approach. Mechanistic modelling uses fundamental knowledge of the interaction between variables involved in the system to be modelled to define the model structure, and requires the precise definition of functional relationships between these variables (Tham, 2000). Mechanistic models offer possibilities for increasing understanding of the behaviour of a pathosystem and can lead to prediction of disease under a range of circumstances (Campbell and Madden, 1990). To elaborate this model, development of the sexual stage of *P. viticola* was split into different state variables, and changes from one state to the following one were determined by rate variables or switches depending on environmental conditions by means of mathematical equations taken from the literature or elaborated from previously published data; in the absence of precise information, assumptions were made based on the available knowledge.

From a conceptual point of view, the model defines a primary inoculum season and a seasonal oospore dose, divides this dose into many cohorts and defines their relative density. This concept is similar to PAD, the potential ascospore dose used for the apple scab pathogen *Venturia inaequalis* (Rossi, 2005), that is the total amount of ascospores available inside pseudothecia during the primary season. PAD can be calculated for each apple orchard based on the number of pseudothecia formed in the leaf litter (Gadoury and MacHardy, 1986), or can be estimated based on apple scab incidence on leaves in autumn (MacHardy et al., 1999). Quantification of PAD makes simulations performed with weather-driven models able to estimate the severity of each ascosporic infection event throughout the season (Rossi et al., 2000b). At the moment, there are no methods for quantifying the oospore dose in a vineyard. When such as method is provided, the structure of this model will make quantitative simulation possible.

Table 3 – Comparison between *Plasmopara viticola* infections predicted by the model and observed in the vineyard, and correspondent properties of the model

	Predicted infection		Total (T)	Youden's index[e] (J)	Likelyhood ratio (LR)	Prior probability (P)	Posterior probability (P)	
	Yes ($P+$)	No ($P-$)						
Observed infection								
Yes ($O+$)	57 TPP[a] = 1.00	0 FNP[b] = 0.00	57 ($T+$)	1	LR($O+$) = 1000	$P(O+)$ = 0.14	$P(O+,P+)$ = 0.994	$P(O+,P-)$ = 0.00016
No ($O-$)	30 FPP[c] = 0.08	324 TNP[d] = 0.92	354 ($T-$)		LR($O-$) = 0.093	$P(O-)$ = 0.86	$P(O-,P+)$ = 0.006	$P(O-,P-)$ = 0.999
Total								
	87	324	411					

[a] True positive proportion (sensitivity).
[b] False negative proportion (set to 0.001 for calculation of LR and P).
[c] False positive proportion.
[d] True negative proportion (specificity).
[e] J = TPP − FPP (accuracy).

In the model, oospores go through different development stages: from morphologically mature, to physiologically mature, to germinated. The development rate is regulated by a physiological time, with rainfall triggering germination. This conceptual construction produced consistent simulations compared to the literature information. Some works showed a close relationship between rainfall and oospore development (Zachos, 1959; Tran Manh Sung et al., 1990; Kast et al., 2004), with long dry periods blocking development (Rouzet and Jacquin, 2003) and delaying occurrence of primary infections (Rossi et al., 2002). Burruano et al. (1987, 1992) postulated a key role of soil moisture on oospore maturation and germination, and Serra and Borgo (1995) stated that rainfall is important to maintain soil humidity. Rossi and Caffi (2007) demonstrated the direct effect of the available water in the leaf residues on oospore development, and observed that under field conditions the water content in the leaf litter is frequently too low when there is no rainfall.

The model was evaluated over a 12-year period in Emilia-Romagna (Northern Italy) by comparing aborted and successful simulations with actual observations of the first seasonal symptoms of downy mildew in 43 vineyards. For this purpose, aborted simulations were considered accurate when the model stopped the infection cycle and no symptoms appeared when they should appear (in case that simulation should proceed). Actually, there is no evidence that an aborted simulation really occurred in the vineyard; anyhow, the model correctly predicted that there was not risk for infection.

The model showed very high sensitivity (TPP = 1) and specificity (TNP = 0.92) (Madden, 2006). Also overall accuracy was high (0.93) with a Youden's index showing perfect simulations. Also the likelihood ratios of successful (LR($O+$) = ∞) and aborted (LR($O-$) = 0.093) infections showed good accuracy, because an accurate model has, in general, large LR($O+$) (above 1) and small LR($O-$) (close to 0) (Madden, 2006). Considering that (i) the data set used for validation was not used in model building, (ii) it is representative of different epidemiological conditions, with both early and late *P. viticola* infections, and (iii) neither calibration nor empirical adjustment of model parameters were necessary to obtain accurate simulation, it can be stated that the mechanistic construction of this model produces a reasonable approximation of the primary infection cycle of *P. viticola*.

Nevertheless, a small false positive proportion (FPP = 0.08) existed. Most of these errors occurred in April and early May. In this period, grapevine plants were between bud break and 5–6 leaves unfolded (about 10 cm shoot length) and the extent of the host surface susceptible to infection was very low. Based on a long term experience, grapevines are not susceptible to *P. viticola* before they reach the stage of 5–6 leaves unfolded (Baldacci, 1947; Park et al., 1997), even if a recent work (Kennely et al., 2007) showed that artificially inoculated leaves became infected also in earlier growth stages. Furthermore, the oospore cohorts that germinated during this period had a low density (Rossi et al., 2007), so that the inoculum available for infection is low. The probability that a few inoculum meet a few susceptible host tissue may be very low, so that a predicted infection may result in no disease.

Some wrong simulations were triggered by isolated weak rain events. Likely these rains were not sufficient for providing the leaf litter with sufficient moisture for oospore germination to occur (Rossi and Caffi, 2007). To avoid these errors, the minimum rainfall required for triggering oospore germination should be increased when the rainfall is preceded by a dry period, but this modification needs further investigations.

In conclusion, the new model represents a considerable enhancement in modelling each key stage of the sexual stage of *P. viticola* that leads to primary infections on leaves compared to the existing empirical models. The model is clearly of practical value because the achieved probability that an oospore cohort produce infection (0.994) was much more higher than 0.5 (Madden, 2006) and more than seven times higher than the prior probability. Furthermore, the achieved probability that an oospore cohort does not produce infection was 0.999, while the prior probability was 0.86. Because of the properties of the model and the prior probability of an infection, one would have more confidence in prediction of non-infections than in prediction of infections, because there was still a small probability (<0.01) that a simulated infection is not a true infection, while there was practically no probability that an oospore cohort produce infection when infection was not predicted.

The new model could then contribute to practical control of grapevine downy mildew. Fungicide sprays could be avoided when the model does not predict an infection, and initiated only when the model simulates a successful infection. In this case, a small probability of unjustified sprays persists, but there is anyhow an improvement compared to the current situation characterized by many unjustified treatments. Considering that oosporic infections contribute to the disease epidemic for long time during the season (Gobbin et al., 2005; Kennely et al., 2007), the model could be applied also for timing further sprayings taking the advantage due to the fact that requirements for oosporic infections might be met more frequently than those for secondary infections (Kennely et al., 2007).

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