A MODEL PREDICTING PRIMARY INFECTIONS OF *PLASMOPARA VITICOLA* IN DIFFERENT GRAPEVINE-GROWING AREAS OF ITALY

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SUMMARY

A dynamic model for *Plasmopara viticola* primary infections was evaluated by comparing model predictions with disease onset in: (i) 100 vineyards of northern, southern and insular Italy (1995 to 2007); (ii) 42 groups of potted grapevine plants exposed to inoculum (2006 to 2008). The model simulates the development of any oospore cohort during the primary inoculum season, including oospore germination, production and survival of sporangia, release, survival and dispersal of zoospores, and infection and incubation. The model showed high sensitivity, specificity, and accuracy both in vineyards and in potted plants. The true positive and negative proportions were TPP=0.99 and TNP=0.87, respectively. Because of a certain proportion of false positive predictions (FPP=0.13), confidence in prediction of non-infections was higher than in prediction of infections. These wrong predictions occurred early in the season or when oospore inoculum was low, or were triggered by isolated weak rain events. In only one case (a group of potted plants) there was infection when infection was not predicted (FNP=0.005). The model can be considered an accurate and robust predictor of *P. viticola* oospore infections and could be used to reduce or improve the timing of fungicide sprays.

Key words: primary infections, dynamic modelling, grapevine, downy mildew.

INTRODUCTION

Plasmopara viticola (Berk *et* Curt.) Berlese *et* de Toni is the causal agent of grapevine downy mildew, a potentially destructive disease in all grape-growing areas characterized by frequent rain (Lafon and Clerjeau, 1988). *P. viticola* epidemics involve primary (sexual) and secondary (asexual) infection cycles. Oospores, which represent the sexual stage of the pathogen (Berlese, 1898), are the sole relevant source of inoculum for primary infections in the following season (Galbiati and Longhin, 1984). Oospores overwinter in leaf debris above ground or buried in soil; in spring they germinate to form a macrosporangium that releases zoospores which are responsible for primary infections on grape leaves and clusters. After 5 to 18 days, depending on the temperature, the pathogen produces sporangia containing asexually produced zoospores (Agrios, 1988). First infections are therefore followed by successive asexual cycles (Blaeser and Weltzien, 1979). Primary and secondary infections overlap for part of the season. Recent studies showed that oospores not only trigger the epidemics but play a key role in epidemic development during a prolonged period, from May to August (Rumbou and Gessler, 2004; Gobbin *et al.*, 2005; Kennely *et al.*, 2007).

A properly timed spray programme is essential for managing downy mildew in the vineyard. Since the disease spreads very fast during the secondary infection cycles, successful control depends on controlling the primary infections (Schwinn, 1981). In Northern Italy, 6 to 9 fungicide sprays are used to control the disease (Borgo *et al.*, 2004), with estimated costs of 30 Euros ha^{-1} $spray^{-1}$ (Monchiero *et al.*, 2005); only for the Piedmont region, annual costs for downy mildew control typically range 8 to 16 million Euros depending on the weather conditions (Salinari *et al.*, 2006). Some of these sprays are usually unnecessary given the sporadic occurrence of the disease.

In order to identify high risk periods for primary infections and to time fungicide sprays, weather-driven models have been proposed (Strizyk, 1983; Tran Manh Sung *et al.*, 1990; Hill, 2000; Park *et al.*, 1997). These models have been evaluated under several environmental conditions in Italy, but none of them was accurate and robust enough to be used for scheduling fungicide applications (Egger *et al.*, 1994; Vercesi *et al.*, 1999; 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.*, 2000), although it has often indicated risk of infections that did not occur (Vercesi, 1995; Serra and Borgo, 1995; Vercesi *et al.*,

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1999). 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).

A new dynamic model for *P. viticola* primary infections was recently developed according to a mechanistic approach (Rossi *et al.*, 2008). The model is based on the definition of a primary inoculum season, a seasonal oospore (inoculum) dose, and its division into many coeval oospore cohorts, which develop simultaneously. First model evaluations showed promising results (Rossi *et al.*, 2008).

The aim of this work was to compare model predictions with (i) first seasonal appearance of downy mildew in several commercial vineyards of different grapevine-growing areas of Italy, and (ii) first seasonal and further oosporic infections in potted grapevine plants placed on an 'artificial leaf litter'.

MATERIALS AND METHODS

Model description. The model has been described in detail (Rossi *et al.*, 2008). It is a dynamic mechanistic model which simulates, with a time step of 1 h, the infection process from oospore germination to the onset of disease symptoms, including the germination progress of the oospores, the survival of sporangia, zoospore release and survival, zoospore dispersal, infection and incubation (Fig. 1).

The model calculates the length of the primary inoculum season, as the time from the first to the last oospore able to germinate in the current year. Within this time period, the model performs several simulation runs. A simulation starts when a measurable rainfall (i.e., ≥ 0.2 mm of rain per hour) wets the leaf litter containing the oospores; the oospores which have broken dormancy at the time of rainfall form a cohort which develops in a similar way. The model calculates the germination course of the oospore cohort until the production of sporangia; germination progress depends on temperature when oospores are sufficiently wet. In the presence of a film of water, sporangia release zoospores; otherwise they can survive for a few days and then die, depending on temperature and relative humidity. These zoospores reach the grape leaves by splashes and aerosols triggered by rainfall, but if the litter surface dries up before rainfall they do not survive. Zoospores deposited on the leaves can cause infection if wetness duration and the corresponding temperature are favourable; if the leaf surface dries the zoospores dry out. At the end of incubation, the infection sites become visible as disease symptoms; incubation progress is influenced by temperature and relative humidity. When the weather conditions are favourable, the simulation continues until disease appearance; otherwise, the simulation stops at any stage of pathogen development.

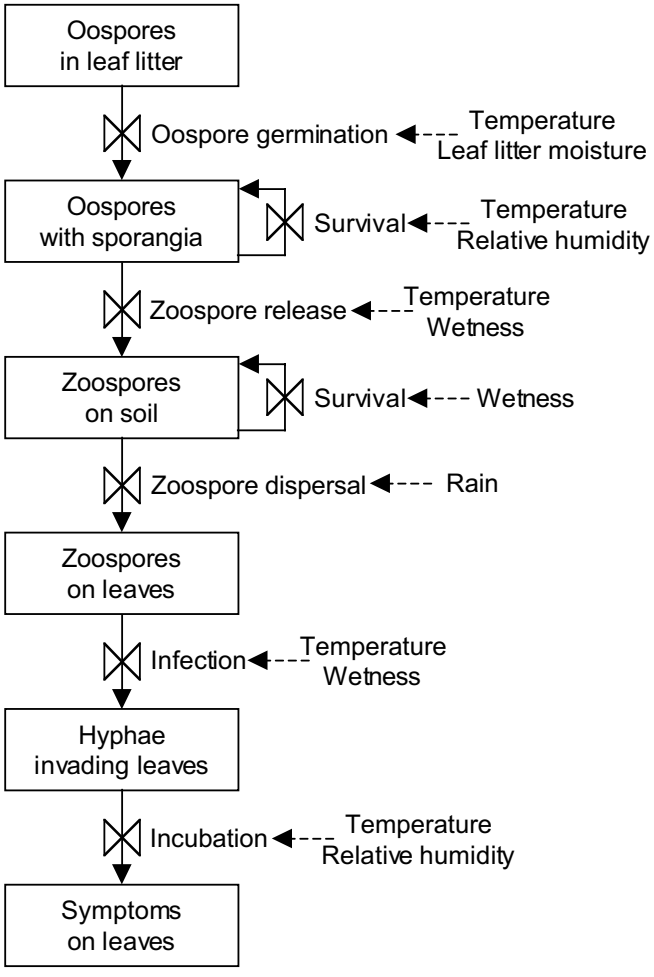

Fig. 1. Flow diagram of the model simulating the development of the *Plasmopara viticola* oospores, from germination to disease appearance (redrawn from Rossi *et al.*, 2008). The model considers the different stages of the primary infection cycle. Oospores form within the affected leaf tissue and overwinter in the leaf litter or buried in the soil. During the winter, the oospores break dormancy progressively, and germinate under favourable conditions. Germinating oospores form sporangia that survive for a few hours to a few days and release zoospores in the presence of water. Survival of the zoospores is strictly dependent on the presence of a film of 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".

The model provides both Tables showing the hourly progress of each simulation run and graphs showing the stage of pathogen development in each day of the primary inoculum season (Fig. 2).

Field observations. The model was evaluated using data from 100 natural epidemics recorded in grapevine-

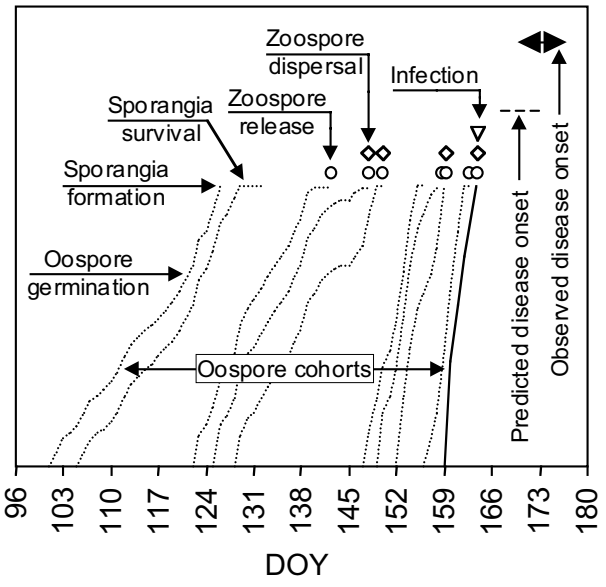

Fig. 2. Representation of the outputs of the model simulating the development of *Plasmopara viticola* oospores. DOY is the day of the year.

growing areas of Piedmont, Lombardy (Oltrepo Pavese), Emilia-Romagna, Marche, Basilicata, and Sardinia, representing a 13-year period (1995 to 2007) with 1 to 6 vineyards each year (Table 1). Vineyards were selected to be representative of the different areas, for soil type, varieties, training systems, and cropping regimes. Vineyards were assumed to have a representative dose of overwintering oospores 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 seasonal 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 temperature (in °C), relative humidity (in %), rainfall (in mm), and presence of wetness (yes or no) were collected in different ways. In Piedmont and Lombardy, data were measured by automatic and mechanical weather stations, respectively, installed in the vineyards. In the other regions data were supplied by the regional networks for the nearest automatic station (not farer than 15 km). In Emilia-Romagna, from the year 2001 the regional network supplied interpolated data for the grids (5×5 km wide) which comprised the vineyards (Bottarelli and Zinoni, 2002).

Experiments with potted plants. Grape leaves were collected in autumn 2004 to 2007 from the leaf litter of a cv. Barbera vineyard located in Canneto Pavese (Oltrepo Pavese) which had been affected by downy mildew at the end of the season. Leaves were checked for the presence of oospores by microscopic observation: oospores were always abundant except in 2005, because of the low downy mildew severity in that year. Further leaves were then collected from other vineyards, but the oospore density was sporadic everywhere. Leaves were overwintered outside in plastic net cages placed above the soil at the University campus in Piacenza (North Italy); leaves were arranged in a square 3×3 m wide, about 600 g of leaves per m^2 of soil.

Starting from the normal time of bud break (mid April), groups of three 3-year-old potted plants of cv. Barbera were placed at the edge of the 'artificial leaf litter', and remained there until the end of the wet period triggered by the first rainfall; afterwards, pots were placed under a cover far from any additional source of *P. viticola* inoculum. Forty-two groups were exposed in aggregate: 11 in 2005, 11 in 2006, 7 in 2007, and 13 in 2008. Plants were observed daily in order to determine the time of oil spot appearance and their number. Before exposure, the plants were grown in a greenhouse at about 10°C in darkness and 20°C in daylight, and managed in such a way that they had 3 to 4 shoots of 20 to 30 cm in length at the time of exposure. Hourly meteorological data were recorded by an automatic station (Davis Instruments, USA) located 5 m from the potted plants.

Running the model. The model was operated for each vineyard and for the potted plants starting from 1 January using the weather data collected. For the vineyards, the model was used to calculate the development of any oospore cohort in the time window between the beginning of the primary inoculum season and the first seasonal onset of downy mildew. In the vineyards where the disease did not appear for the entire season, simulations were performed over the whole primary inoculum season. For the potted plants, the model was operated until the last group of plants was removed from the artificial leaf litter.

Model evaluation. Total model simulations were first distinguished as successful (i.e., resulting in infection) or aborted (i.e., resulting in non-infection). A simulation was considered aborted when the process was interrupted at any stage prior to infection, while it was considered successful when all stages progressed until onset of disease symptoms. Simulations were distinguished as: (i) accurate positives, when a predicted appearance of the disease corresponded to the actual appearance in the vineyard; (ii) accurate negatives, when the simulation was interrupted and no disease appeared in the vineyard during the period when the corresponding symptoms should appear in case that simulation

should proceed successfully, or disease appeared but it was explained by another accurate successful simulation; (iii) wrong positives, when the model predicted a successful infection but the disease did not appear; (iv) wrong negatives, when the model did not predict an infection that actually occurred and was not explained by an accurate successful simulation. To make these comparisons, it was considered that the downy mildew symptoms should appear on any day between the last negative (no disease observed) and the first positive (symptoms observed) disease assessment; the period between two successive disease assessments was maximum one week.

All the possible combinations of observed (O) versus predicted (P) infections were organized in a 2x2 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 using Bayesian analysis (Yuen and Hughes, 2002). To asses the advantages of the model in practice, the probabilities that an oospore cohort of a particular vineyard did or did not result 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. Accuracy of the model is given by the overall accuracy index (correct/total cases) and by the Youden's index (TPP-FPP) which both are equal to one in case of perfect model prediction.

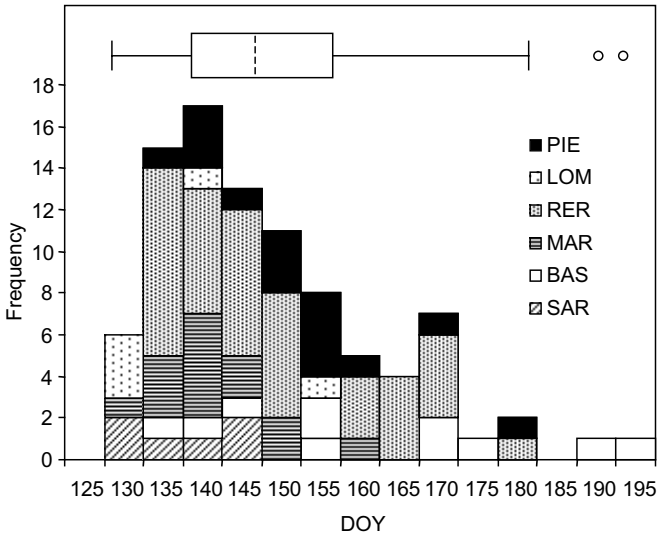

Fig. 3. Distribution over time of the first seasonal appearance of downy mildew symptoms in the 100 vineyards considered for model evaluation, in Piedmont (PIE), Lombardy (LOM), Emilia-Romagna (RER), Marche (MAR), Basilicata (BAS), and Sardinia (SAR). DOY is the day of the year, bars show the number of vineyards for each 5-day interval, the box plot shows data distribution: the box contain 50% of the data, the broken line is the median, whiskers extend to the extremes, while are outliers.

RESULTS

Model evaluation in the vineyards. The date of the first seasonal onset of downy mildew symptoms in the 100 vineyards ranged between 7 May (DOY=126, Marche 2007) and 11 July (DOY=191, Basilicata 2005) (Fig. 3). In 50% of cases, the first disease symptoms appeared between mid May and early June (DOY=136 to 154) (Fig. 3). The average data of first seasonal disease appearance was 26 May (DOY=146). In nine vineyards (six in 2003, one in 2004, 2005, and 2007) the disease did not appear all season long.

One to 35 simulation runs were performed for vineyards in the time window between the beginning of the primary inoculum season and the first seasonal onset of downy mildew, depending on the number of oospore cohorts formed in such a window, for a total of 922 simulations (Table 1). In vineyards where the disease did not appear for the entire season, simulations were performed over the whole primary inoculum season. Twenty-nine percent of the total simulations resulted in infection, while 71% were aborted because environmental conditions were not favourable for the completion of the process (Table 1). Nineteen percent of the predicted infections were accurate because they resulted in actual downy mildew appearance in the vineyard, while 10% were wrong because the disease did not appear during the time period when the correspondent symptoms should appear in the case that simulation proceeded successfully (Table 1). All the simulations that predicted no infection were right because no downy mildew symptoms appeared in the vineyard (Table 1). An analysis of these 657 simulations showed that in some cases the infection cycle was interrupted because sporangia did not survive till zoospore release (13% of cases), or because the zoospores that dispersed to leaves did not cause infection (12% of cases); in most cases (75%), the infection cycle stopped because zoospores released from sporangia did not survive until dispersal (data not shown).

Some representative examples of the model simulations are shown in Fig. 2. For instance, in Oltrepo Pavese, Lombardy 1998 (Fig. 4a), the model calculated that the primary inoculum season should begin in early April. Between 9 April and 3 May (DOY=99 to 122), rainfall triggered the germination of seven oospore cohorts. The simulations of these cohorts were interrupted because no rainfall dispersed the zoospores released from sporangia during the survival period of these zoospores. A rain event on 6 May (DOY=126) triggered germination of an oospore cohort; zoospores were released and dispersed on grape leaves on 24 May

Table 1. Number of vineyards used for model evaluation in each region, from 1995 to 2007, and total number of simulations, distinguished as accurate or false.

Region	Years	Vineyards	Number of simulations				
			Total[a]	Accurate		False	
				Infection[b] (P+,O+)	Non-infection[c] (P-,O-)	Infection[d] (P+,O-)	Non-infection[e] (P-,O+)
Piedmont	1999 - 2004	19	153	38	109	6	0
Lombardy	1998 - 2002	5	26	12	13	1	0
Emilia-Romagna	1995 - 2007	45	449	59	357	33	0
Marche	2004 - 2007	15	113	34	61	18	0
Basilicata	2004 - 2007	9	112	23	75	14	0
Sardinia	1996 - 2004	7	69	8	42	19	0
Total		100	922	174 (19%)	657 (71%)	91 (10%)	0 (0%)

[a]Several simulations were performed per vineyard according to the number of oospore cohorts formed between the beginning of the primary inoculum season and the time of first disease onset, or during the whole primary inoculum season for the vineyards where the disease did not appear; the number of oospore cohorts is equal to the number of rain events above 0.2 mm.
[b]The predicted appearance of the disease (P+) corresponded to the actual appearance in the vineyard (O+).
[c]The model predicted non-infection (P-) and no disease was observed in the vineyard (O-) during the time period when the correspondent symptoms should appear in the case that simulation proceeded.
[d]The model predicted an infection (P+) but the disease did not appear (O-).
[e]The model did not predict an infection (P-) that actually occurred (O+).

Table 2. Comparison between *Plasmopara viticola* infections predicted by the model and observed in the vineyards indicated in Table 1, and corresponding properties of the model.

Regions	Proportions				Likelihood ratio		Accuracy		Prior probability		Posterior probability			
	TPP[a]	FNP[b]	FPP[c]	TNP[d]	LR(+)	LR(-)	J[e]	Overall[f]	*P*(O+)[g]	*P*(O-)	*P*(P+,O+)	*P*(P-,O-)	*P*(P+,O-)	*P*(P-,O+)
Piedmont	1.00	0.00	0.05	0.95	19.2	0.0	0.95	0.96	0.25	0.75	0.8636	0.9997	0.1364	0.0003
Lombardy	1.00	0.00	0.07	0.93	14.0	0.0	0.93	0.96	0.46	0.54	0.9231	0.9991	0.0769	0.0009
Emilia-Romagna	1.00	0.00	0.08	0.92	11.8	0.0	0.92	0.93	0.13	0.87	0.6413	0.9998	0.3587	0.0002
Marche	1.00	0.00	0.23	0.77	4.4	0.0	0.77	0.84	0.30	0.70	0.6538	0.9996	0.3462	0.0004
Basilicata	1.00	0.00	0.16	0.84	6.4	0.0	0.84	0.88	0.21	0.79	0.6216	0.9997	0.3784	0.0003
Sardinia	1.00	0.00	0.31	0.69	3.2	0.0	0.69	0.72	0.12	0.88	0.2963	0.9999	0.7037	0.0001
Total	1.00	0.00	0.12	0.88	8.2	0.0	0.88	0.90	0.19	0.81	0.6566	0.9998	0.3434	0.0002

[a]True Positive Proportion (sensitivity).
[b]False Negative Proportion (set to 0.001 for calculation of *P*(P-,O-)).
[c]False Positive Proportion.
[d]True Negative Proportion (specificity).
[e]Youden's index J=TPP-FPP.
[f]Overall accuracy = ((P+,O+) + (P-,O-))/total simulations (see Table 1).
[g]Actual infections were defined based on the appearance of downy mildew symptoms in the vineyard.

(DOY=144) and caused infection on the same day. The model simulated a probable onset of symptoms between 29 May and 2 June (DOY=149 to 153), and the downy mildew symptoms appeared in the week 29 May to 4 June (DOY=149 to 155). Similar situations occurred at Siniscola, Sardinia 2000 (Fig. 4b), Oltrepo Pavese, Lombardy 2001 (Fig. 4c), and Montefano, Marche 2006 (Fig. 4h): first predicted infections indeed corresponded

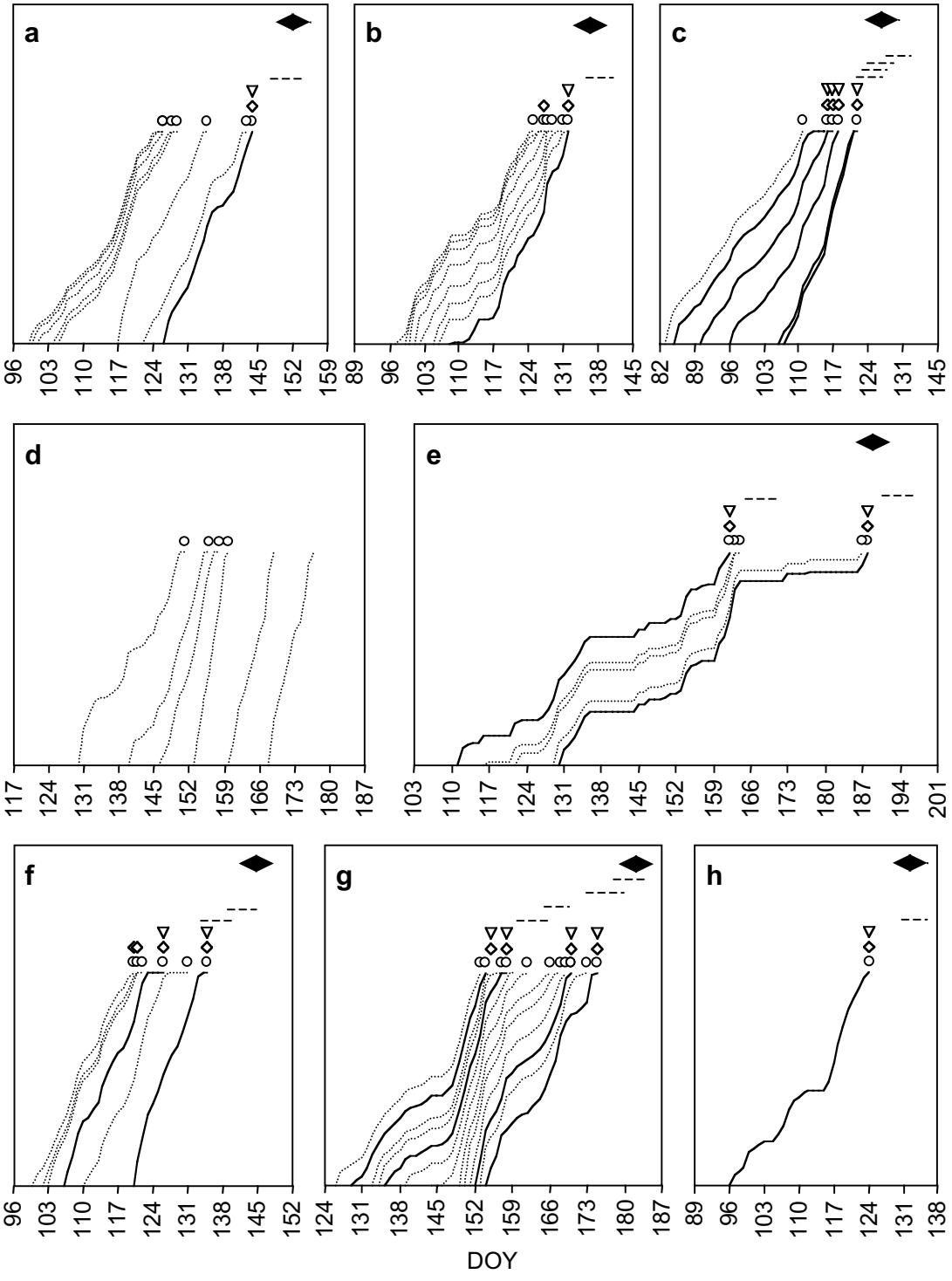

Fig. 4. Examples showing model simulations in the vineyards of Oltrepo Pavese (Lombardy) 1998 (a), Siniscola (Sardinia) 2000 (b), Oltrepo Pavese (Lombardy) 2001 (c), Alba (Piedmont) 2003 (d), Venosa (Basilicata) 2005 (e), Serralunga (Piedmont) 2004 (f), Boscogaldo (Basilicata) 2005 (g), and Montefano (Marche) 2006 (h). DOY is the day of the year; unbroken and dotted lines show the germination course of oospore cohorts which cause or do not cause infection, respectively; circle, zoospore release; diamond, zoospore dispersal; triangle, infection; broken line and left-right arrow, estimated and actual periods of downy mildew onset, respectively.

to actual infections. In Oltrepo Pavese (Fig. 4c), the appearance of first downy mildew spots (10 May, DOY=130) was predicted by four infection events (26, 27 and 28 April, 2 May, DOY=116 to 122), which involved five oospore cohorts.

At Alba, Piedmont 2003 (Fig. 4d), the model performed only six simulations between 10 May (DOY= 130) and 17 June (DOY=168) because only six rainfalls occurred in this time period (data not shown). The model interrupted all these simulations because zoospores released from sporangia did not survive (1st to 4th cohort) or sporangia did not release zoospores (5th and 6th cohort). The disease never in fact appeared in the vineyard.

At Venosa, Basilicata 2005 (Fig. 4e), according to the model, the first seasonal oospore cohort began germination on 21 April (DOY=111) and produced sporangia 51 days later because of both low temperatures and dry periods slowed down the germination process; on 11 June (DOY=162), the model predicted that these sporangia released zoospores which caused infection. However, the disease did not appear in the vineyard within the period predicted by the model. The rainfall of 10 May (DOY=130) triggered germination of the 5th cohort, which produced sporangia on 7 July; on the same day zoospores were dispersed and caused infection. The model estimated the onset of symptoms between 11 and 17 July (DOY=192 to 195), and first downy mildew spots were observed on 11 July. Also at Serralunga, Piedmont 2004 (Fig. 4f), and Bosco Galdo, Basilicata 2005 (Fig. 4g), the model wrongly predicted one or two infections, respectively, before the simulation that correctly predicted the actual disease onset in the vineyard.

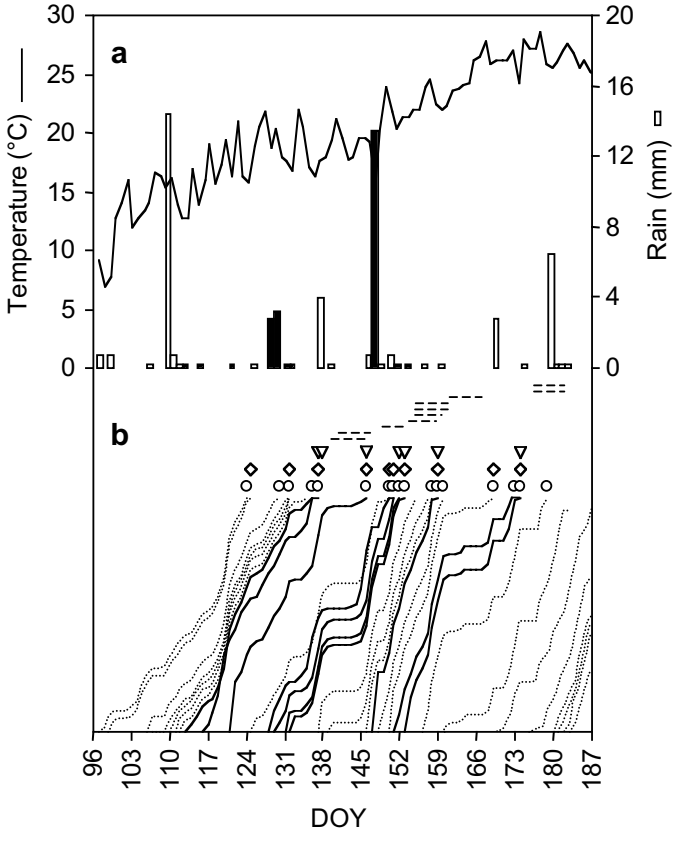

Fig. 5. Data of air temperature and rainfall measured at Siniscola (Sardinia) 2003 (a) and corresponding model simulations (b). In (a): black bars show rains which triggered germination of infection-causing oospores. In (b): unbroken and dotted lines show the germination course of oospore cohorts which do or do not cause infection, respectively; circle, zoospore release; diamond, zoospore dispersal; triangle, infection; broken line, estimated period of downy mildew onset; the disease did not appear all season long. DOY is the day of the year.

All the actual infections were correctly predicted by the model, giving a sensitivity of TPP=1 (Table 2). Also, 657 out of 748 simulations that predicted no infection were correct because the disease did not appear, giving a specificity of TNP=0.88. Infections never occurred in the vineyard without a successful simulation by the model, giving a false positive proportion of FNP=0 (Table 2). Finally, 91 out of 748 simulations predicted an infection that did not result in actual disease onset, giving a false negative proportion of FPP=0.12. Model accuracy was high because both overall accuracy (=0.90), which considers accurate versus total simulations, and the Youden's index (J= 0.88) were close to one. The likelihood ratio (*LR*) of a successful infection was *LR*(O+)=8.2, and the likelihood ratio for an aborted simulation was *LR*(O-)=0.

The probability that there was infection when infection was predicted was *P*(O+,P+)=0.6566, and the probability that there was no infection when infection was aborted by the model was *P*(O-,P-)=0.9998, while the prior probabilities for infection and no infection were *P*(O+)=174/922=0.19 and *P*(O-)=748/922=0.81, respectively (Table 2). The posterior probability that there was no infection when the model predicted an infection was *P*(O-,P+)=0.3434, and the posterior probability that there was infection when infection was not predicted by the model was *P*(O+,P-)=0 (or 0.0002 when FPP was cautiously set = 0.001, i.e. 0.1% of actual infections not correctly predicted by the model) (Table 2).

The model performances showed little change when the predictions were analysed for the different regions separately (Table 2). Sensitivity was TPP=1.00 for all regions, while specificity ranged between TNP=0.69 (for Sardinia) and 0.95 (for Piedmont). This means that the frequency of false positive predictions was higher in some regions than in others. In particular, these wrong predictions were higher for the vineyards of Sardinia (FPP=0.31), Marche (0.23), and Basilicata (0.16) than for the other regions (0.05 to 0.08) (Table 2). An analysis of these errors showed that the false positive predictions were caused i) by oospore cohorts which began to develop when a rain episode of only 0.2 mm occurred after some dry days, or ii) by infections which occurred in the early season, i.e., between April and early May,

when grapevine plants were in early growth stages (data not shown).

For instance, in the vineyard of Siniscola, Sardinia 2003, there were 31 rain events (total of 54.8 mm of rainfall) between 6 April and 6 July (DOY=96 to 187), which triggered the germination of as many oospore cohorts (Fig. 5a). The model predicted that 10 of these cohorts should produce infection, on 5 periods: 17-18 May (DOY=137-138, 2 cohorts), 26 May (DOY=146, 1 cohort), 1–2 June (DOY=152-153, 4 cohorts), 8 June (DOY=159, 1 cohort), and 23 June (DOY=174, 2 cohorts) (Fig. 5b). The germination of 7 of these oospore cohorts was triggered by rainfall of only 0.2 mm. Since downy mildew did not appear in this vineyard all season long, these simulations produced false positives.

In a similar way, in the vineyard of Agugliano, Marche 2007, 316 mm of rain fell in 35 days between 16 April and 15 June (DOY=75 to 166) (Fig. 6a). Eleven of the 35 oospore cohorts that began germination produced infection, on 3 periods: 21-23 April (DOY=111-113, 8 cohorts), 4 May (DOY=124, 1 cohort), 6-7 June (DOY=157-158, 2 cohorts) (Fig. 6b). The germination of the first and last groups of oospores was triggered by heavy rainfall, while that of the second group began germination with 0.2 mm of rain (Fig. 6a). All these simulations were wrong, because downy mildew symptoms did not appear.

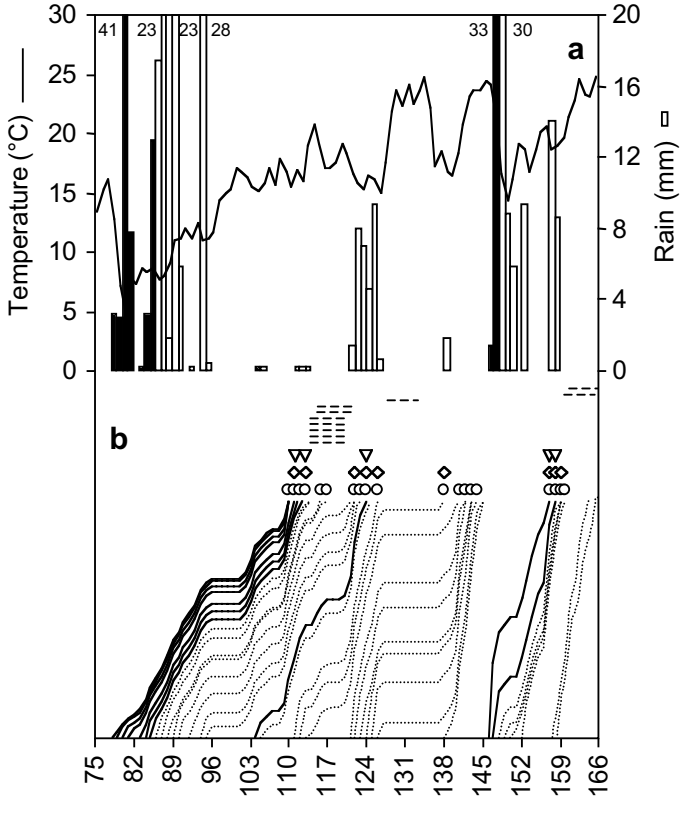

Fig. 6. Data of air temperature and rainfall measured at Agugliano (Marche) 2007 (a) and corresponding model simulations (b). In (a): black bars show rains which triggered germination of infection-causing oospores. In (b): unbroken and dotted lines show the germination course of oospore cohorts which do or do not cause infection, respectively; circle, zoospore release; diamond, zoospore dispersal; triangle, infection; broken line, estimated period of downy mildew onset; the disease did not appear all season long. DOY is the day of the year.

Model evaluation with potted plants. Forty-two groups of potted plants were exposed to the single rain events that occurred between late April and early to late June each year (Table 3). Rainfall ranged between 0.2 and 62.4 mm per exposure period. Disease symptoms appeared on 20 plant groups: 4 groups in 2005, 5 in 2007, and 11 in 2008; numbers of oil spots ranged between 1 and 24 per group. Nineteen of the 20 infections which caused these symptoms were correctly predicted by the model, giving a true positive proportion TPP=0.95 (Tab. 4).

The infection observed in the plants exposed to the rainfall of 22 and 23 May 2008 (group VII), resulting in two oil spots, was not correctly predicted, giving a false negative proportion FNP=0.05. The oospore cohort which began germination on 5 May (1.8 mm of rain) produced sporangia on 22 May, 02.00 h, and immediately released zoospores because of the presence of wetness and suitable temperature; these zoospores survived until 06.00 h when the wet period finished (Fig. 7). Since no rain fell between 02.00 and 06.00, the oospores did not disperse and the simulation was interrupted. A rain occurred between 14.00 and 18.00 (26.6 mm of rain), with favourable conditions for infection to occur (18 hours of wetness at 15.3°C); therefore, the 6-hour long dry period (07.00 and 13.00 h) may not have occurred in reality.

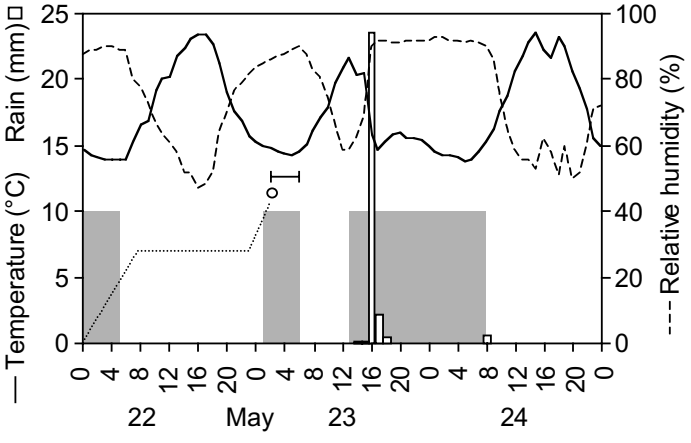

Fig. 7. Hourly weather conditions registered at Piacenza, 22 to 24 May 2008, and corresponding model simulations: dotted line shows the final part of the germination course of the oospore cohort which began germination on 5 May; circle, zoospore release; bar, zoospore survival; shaded area, presence of wetness.

Table 3. Rain periods during which potted grapevine plants were exposed over a leaf litter containing *Plasmopara viticola* oospores and corresponding number of downy mildew spots on leaves, compared with model predictions.

Year	Plant group[a]	Exposure period[b]	Rain mm	N. of spots[c]	Predicted infection
	I	24-26 April	23.6	-	no
	II	3-5 May	4.6	-	no
	III	10-11 May	2.4	-	no
	IV	14 May	9.2	-	no
	V	17-18 May	10.4	1	17-18 May
2005	VI	20 May	0.2	-	no
	VII	23 may	5.2	3	23 May
	VIII	31 May	0.2	4	31 May
	IX	6 June	2.4	12	6 June
	X	14-15 June	11.6	-	14 June
	XI	25 June	17.2	-	no
	I	3-4 May	1.0	-	no
	II	5 May	3.6	-	5 May
	III	10 May	1.6	-	no
	IV	11 May	0.8	-	no
	V	12 May	0.2	-	no
2006	VI	14 May	9.2	-	14 May
	VII	17-18 May	10.4	-	18 May
	VIII	20 May	0.2	-	no
	IX	23 May	5.2	-	no
	X	6 June	2.4	-	no
	XI	14 June	11.4	-	14 June
	I	2-7 May	62.0	-	5 May
	II	14-15 May	0.8	-	no
	III	25-28 May	13.2	1	28 May
2007	IV	31 May-1 June	17.0	2	31 May
	V	4-7 June	62.4	2	5 June
	VI	10 June	14.0	8	10 June
	VII	12-15 June	21.8	7	12-13 June
	I	28 April	4.4	-	no
	II	30 April	0.2	-	no
	III	16-17 May	6.6	10	17 May
	IV	18 May	8.2	15	18 May
	V	19 May	7.6	1	19 May
	VI	20 May	19.6	11	20 May
2008	VII	22-23 May	27.2	2	no
	VIII	24 May	1.2	10	24 May
	IX	25 May	1.2	18	25 May
	X	28-29 May	6.8	24	29 May
	XI	30 May	22.2	6	30 May
	XII	2 June	1.2	2	2 June
	XIII	4 June	11.0	1	4 June

[a]Each group was composed of three 3-year-old potted grapevine plants with 3 to 4 shoots of 20 to 30 cm in length.
[b]Plants were exposed over an 'artificial leaf litter' composed by leaves collected in autumn of the previous year from a vineyard which had been affected by downy mildew, and remained there until the end of the wet period triggered by the first rainfall.
[c]After exposure plants were placed under a cover and observed daily for the presence of oil spots; data are numbers of spots on the three plants.

Table 4. Comparison between *Plasmopara viticola* infections predicted by the model and observed in the potted grapevine plants of Table 3, and corresponding properties of the model.

	Proportions				Likelihood ratio		Accuracy		Prior probability		Posterior probability			
	TPP[a]	FNP[b]	FPP[c]	TNP[d]	LR(+)	LR(-)	J[e]	Overall[f]	*P*(O+)[g]	*P*(O-)	*P*(P+,O+)	*P*(P-,O-)	*P*(P+,O-)	*P*(P-,O+)
Potted plants	0.95	0.05	0.27	0.73	3.5	0.07	0.68	0.83	0.48	0.52	0.7600	0.9565	0.2400	0.0435

[a]True Positive Proportion (sensitivity).

[b]False Negative Proportion (set to 0.001 for calculation of *P*(P-,O-)).

[c]False Positive Proportion.

[d]True Negative Proportion (specificity).

[e]Youden's index J=TPP-FPP.

[f]Overall accuracy = ((P+,O+) + (P-,O-))/total simulations (see Table 3).

[g]Actual infections were defined based on the appearance of downy mildew symptoms in the potted plants.

The model predicted the occurrence of infection for six plant groups in which the disease did not appear, giving a false positive proportion FPP=0.27. Four of these wrong predictions occurred in the plants exposed in 2006. In 2006, the disease did not appear for the entire experiment, probably because of the sporadic presence of oospores in the artificial leaf litter. In 16 groups the disease did not appear and the model correctly predicted non-infection, giving a true negative proportion TNP=0.73.

The properties of the model for the potted plants (Table 4) were similar to those obtained for the vineyard observations (Table 2). Considering both vineyards and potted plants, model sensitivity was 0.99, specificity was 0.87, overall accuracy was 0.90, with a Jouden's index of 0.87. The likelihood ratios were 7.9 and 0.01 for predicted infections and non-infections, respectively. Posterior probabilities were 0.666 for infection when infection is predicted, 0.999 for non-infection when infection is not predicted, 0.334 for non-infection when infection is predicted, and 0.001 for infection when infection is not predicted.

DISCUSSION

The model was evaluated by comparing model simulations with observations of: (i) the first seasonal symptoms of downy mildew in 100 vineyards, from northern to southern and insular Italy, over a 13-year period; (ii) both first seasonal and further infections in 42 groups of potted grapevine plants exposed to inoculum over a 4-year period. The data set used in this work can be considered representative enough for a robust evaluation of the model, for the three reasons listed in the following paragraph.

First, the data set was not used in model building (Teng, 1981). Second, the grape-growing areas considered represent the different climatic zones of grape cultivation in Italy. Based on the Köppen-Geiger climate classification, four different types of temperate climate were represented: (i) Cfb (fully humid with warm summer); (ii) Cfc (fully humid with cold summer); (iii) Csa (dry and hot summer), Csb (dry and warm summer) (Kottek *et al*., 2006). These climate types also cover all the grape-growing areas of Europe and of the Mediterranean Basin (Kottek *et al*., 2006). Third, the date of the primary *P. viticola* infections ranged between early May and mid July, which is the time period when oospore-derived infections usually contribute to the epidemics (Rumbou and Gessler, 2004; Gobbin *et al*., 2005; Kennely *et al*., 2007); in nine vineyards and in the potted plants exposed in 2006 the disease did not appear all season long.

For evaluation purposes, actual downy mildew onset was compared with model simulations. Simulations

were considered accurate when the predicted appearance of the disease corresponded to the actual appearance in the vineyard, or when the model stopped the oospore development and no symptoms appeared when they would have appeared in the case that simulation should proceed until infection establishment. There is no proof that an interrupted simulation actually corresponded to an aborted infection event in the vineyard; however from a practical point of view, the model produces accurate information in any case, because it predicted no risk of infection.

The model showed very high sensitivity (TPP>0.99) and specificity (TNP=0.87) (Madden, 2006). Overall accuracy was 0.90, and the likelihood ratios of predicted infections (*LR*(O+)=7.9) and non-infections (*LR*(O-)=0.20) also showed good accuracy, because an accurate model has, in general, large *LR*(O+) (above 1) and small *LR*(O-) (close to 0) (Madden 2006). Nevertheless, a false positive proportion (FPP=0.13) existed.

Comparison between model performances in the different grape-growing areas showed that sensitivity did not change, being always TPP=1, while specificity was higher in Northern Italy (TNP>0.9) than in the other areas, with a minimum for Sardinia (TNP=0.69). This difference was due to a different incidence of false positive predictions, i.e., the model predicted an infection but the disease did not appear. As a general statement, false positive predictions can be due to either errors in model development or lack of relevant biological phenomena in the model. Most of the false positive predictions observed in this work occurred between 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. Long experience suggests that grapevines are not susceptible to *P. viticola* before they reach the stage of 5-6 leaves unfolded (Baldacci *et al*., 1947; Park *et al*., 1997), even if a recent study (Kennely *et al*., 2007) showed that artificially inoculated leaves became infected even at 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 was low. The probability that a few spores encounter small amount of susceptible host tissue may be very low, so that a predicted infection may prove false. This phenomenon should have a greater impact for the vineyards of southern and insular Italy than for those of northern Italy. In fact, the incidence of downy mildew is usually lower in the former areas than in the latter (Various Authors, 1989), and consequently the inoculum (oospore) density may be lower. The results obtained with potted grapevines supported this consideration, because no downy mildew appeared in 2006, when the presence of oospores in the leaf litter was sporadic due to a very low disease level in autumn 2005.

Some incorrect model predictions which occur in early season may be avoided accounting for the phenological stage of the plant at the time when infection is predicted, or linking the downy mildew model to a grape phenological model (Ortega *et al*., 2002; Mariani *et al*., 2007). On the contrary, there are no possibilities to improve model predictions by accounting for the oospore dose in a vineyard because no methods for quantifying oospore density are available to date. When such as method is provided, the structure of this model will make quantitative simulation possible.

Some other wrong simulations were triggered by isolated weak rain events. Probably these rains were not sufficient to provide the leaf litter with sufficient moisture for oospore germination (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 investigation.

In conclusion, considering that this evaluation was carried out using independent data which represents many different epidemiological conditions and that neither calibration nor empirical adjustment of model parameters were necessary to obtain accurate simulation, it can be stated that the model developed by Rossi *et al.* (2008) produced accurate and robust predictions. The model is clearly of practical value because the achieved probability that an oospore cohort would produce infection (*P*(P+/O+)=0.67) was higher than 0.5 (Madden, 2006) and more than 3 times higher than the prior probability (*P*(O+)=0.19). Furthermore, the achieved probability that an oospore cohort does not produce infection was >0.99, while the prior probability was 0.80. 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 probability (*P*(P+/O-)=0.34) that a simulated infection is not a true infection, while there was practically no probability that an oospore cohort would produce infection when infection was not predicted. This event occurred in only one case (group VII in 2008, where two unpredicted oil spots appeared) over the 142 observations in the total of the vineyards and potted plants.

The new model can then contribute to practical control of grapevine downy mildew, particularly in the critical control period, which is early in the season when active vegetative growth occurs (Magarey *et al.,* 1994) and requirements for oosporic infections might be met more frequently than those for secondary infections (Kennely *et al*., 2007). Fungicide sprays could be avoided when the model does not predict an infection, and applied only when it does. Considering that oosporic infections contribute to disease epidemics over a large part of the season (Gobbin *et al*., 2005; Kennely *et al.,* 2007), the model could also be applied for improved timing of

sprays. In this case, a certain probability of unjustified sprays persists, but there is anyhow an improvement compared to the current situation characterized by many unjustified treatments.

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