

Multivariate forecasting model to optimize management of grape downy mildew control

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Summary

Aim of this study was to develop a forecasting model for *Plasmopara viticola* to achieve rational disease management and to reduce the use of copper treatments in organic farming. Starting from meteo-climatic, agronomic and phytopathological data a partial least squares discriminant analysis was developed. Three different strategies were compared: treatments according to the established organic agricultural practice (standard); treatments according to the predictive model and untreated control where no fungicides against downy mildew were applied. The modelling approach was divided into three phases: 1) model calibration; 2) field testing and 3) *a posteriori* model performance evaluation. The prediction was separately considered and modelled for: i) disease onset and ii) disease progress. The results for phase 1 show a percentage of correct classification equal to 91.8 % for the disease onset with 3 days elapsed between the prediction of first potential attack and disease onset and to 91.23 % for disease progress. In field testing phase the percentage of correct classification was equal to about 81 % for both the analysed years (2009 and 2010). In the phase 3 the percentages were quietly higher for the 2009. The number of fungicide applications on the partial least squares discriminant analysis model was almost half compared with standard schedule both in 2009 and 2010. Finally this approach showed the possibility to reduce fungicidal treatments and to avoid applying copper not essential for disease control representing a first step in the model validation.

Key words: Partial least squares discriminant analysis, copper, forecasting model, host-pathogen interaction, organic farming, *Plasmopara viticola*.

Introduction

The choice and timing of pesticide applications are essential to achieve a correct plant protection. Directive 2009/128/EC on sustainable use of pesticides encourage the development of forecasting diagnosis systems to determine whether and when to apply plant protection measures. Forecasting models could give a decisive support to the farmers, especially to the organic growers because organic production method implies that only few plant protection products could be used and they have preventive activity.

The models simulate epidemic processes and provide the onset and development of harmful organisms. Epidemiological models can be either empirical or mechanistic (BRUNELLI *et al.* 2002). Empirical models are elaborated starting from data collected under specific field conditions and not necessarily contain cause-effect relationship between variables. On the contrary, mechanistic models are based on an *a priori* analysis of the factor influencing epidemics (CAFFI *et al.* 2007). When the warning system indicates a critical situation based on a risk index, the alarm is given for carrying out the treatments. Optimizing the placement of applications it is possible to reduce the number of treatments and so the negative impacts due to the pesticides on the environment and health of farmers and consumers. Starting from Goidanich model (GOIDANICH *et al.* 1957) and the "three ten rule" (BALDACCIO 1947) several predictive models have been developed in order to determine the optimal time for applying fungicides against *Plasmopara viticola* (BERK. et CURT.) BERL. et DE TONI (MAGAREY *et al.* 1991, HILL 2000, VIRET and BLOESCH, 2002, ROSSI *et al.* 2008) but generally these approaches trend to overestimate the risk of infections and induce to treat the vineyard even if not necessary with an excessive application of pesticides. Some forecasting models, otherwise, underestimate the risk of infection leaving the pathogen to breakout.

The aim of this study was to develop a multivariate statistical model based on the partial least squares discriminant analysis (PLSDA) for prediction of *P. viticola* infections in order to reduce the use of copper in organic farming starting from meteo-climatic, agronomic and phytopathological data. This approach was carried out considering two different kinds of prediction: the disease onset and the disease progress (infection cycle development during the remaining season).

Material and Methods

Field trials: Two years (2009 and 2010) of field trials were carried out in an organic vineyard near Rome (central Italy) (lat. 41.4°N, long. 12.3°E, 180 m a.s.l.). The grape variety tested was 'Malvasia di Candia', and the rootstock (44 years old) employed was Kober 5BB (*Vitis berlandieri* × *V. riparia*). The training system was "tendone", consisting of a continuous overhead canopy under which the bunches are disposed (RANA *et al.* 2004). Plots were prepared, each containing 12 plants and repeated four times in randomized blocks.

Distances between vines were 2.50 m × 2.50 m, with a buffer row between treatments. The test organism was *P. viticola*. The trials were carried out according to European and Mediterranean Plant Protection Organization Standard PP1/31(3) (EPPO, 2004).

Three different application strategies were compared: i) treatments according to the established organic agricultural practice of the area (Standard); ii) treatments according to the predictive model PLSDA; iii) untreated control where no fungicides against downy mildew were applied. The treatments, established by the modelling PLSDA application, were different from the standard treatments only on the base of the time of anti-downy mildew application and not for the type of product used (Tab. 1). The products were sprayed until near run-off at a pressure of 1.5 bar, with sufficient coverage of the lower and upper surfaces of the leaves. For all the products the equipment used to spray was a pulled sprayer (Martignani K.W.H. electrostatic sprayer system - Martignani s.r.l., S. Agata sul Santerno, Ravenna - Italy).

Meteorological data: A weather station (Wireless Vantage Pro 2™ - Davis Instruments, Hayward, California-USA) was placed into the trial site to record meteo-climatical data. Data were acquired through a GSM modem integrated with the weather station for remote transmission to a data management software (WeatherLink 5.9.0). Data from Remote Transmit Units (RTUs) on the field are collected every 15 minutes.

Disease assessment: The vineyard was carefully inspected twice a week to provide a description of *P. viticola* development and to verify the efficacy of treatments. The time of first appearance of downy mildew lesions was detected. Grapevine leaves and bunches were visually assessed, 100 leaves and 100 bunches were picked randomly from the central 10 vines of each plot. The disease incidence was calculated as the percentage of leaves and bunches diseased out of a total number assessed. The area of leaves and bunches showing symptoms of disease

(disease severity), were also estimated to compute disease severity (infection degree, ID) that was calculated using a scale of nine classes (0-8) using the Townsend-Heuberger formula (TOWNSEND-HEUBERGER 1943).

Predictive model description: A PLS-DA was considered in order to predict the optimal time for applying copper compounds against grape downy mildew and it was built by examining the interactions between pathogen, host and environment. This consists of a classical partial least squares (PLS) regression analysis where the response variable is categorical (Y-block; replaced by a set of dummy variables describing the categories, see below), thus expressing the class membership of the statistical units (SABATIER *et al.* 2003, COSTA *et al.* 2010). The modelling approach was divided into three different phases: 1) model calibration; 2) field testing and 3) *a posteriori* model performance evaluation. The datasets were constructed considering the following variables: Y: disease incidence and severity on the untreated control; X: 18 daily variables: ordinal date (starting from January 1st of the same year), air temperature (maximum, minimum and mean; °C), air Relative Humidity (RH; maximum, minimum and mean; %), mean soil temperature at 20 cm depth (°C), rain rate (mm), mean soil RH at 20 cm depth, mean soil RH at 40 cm depth, mean wind speed (km·h⁻¹), mean solar radiation (Watt·m⁻²), total solar radiation (Watt m⁻²), upper foliar page wet (h), lower foliar page wet (h), GOIDANICH T (°C), grapevine phenological stages according to the BBCH-identification grapevine keys (LORENZ *et al.* 1994) and epidemiological characteristic of *P. viticola* in order to establish the infection risk.

Being PLSDA a classification method, quantitative response of Y was converted into binary response variable of daily disease presence (1) or absence (0). The binary response variable was based on the daily differential increase of pathogen attack and calculated as the first derivative of the infection percentage showing a value greater than a specific threshold (PathogenThresh). The PathogenThresh

Table 1

Commercial products and active ingredients used against *P. viticola*. The parameters reported are: Treatments (ST = Standard where vines were sprayed according to the grower's schedule; PLSDA = Partial Least Squares Discriminant Analysis where vines were sprayed according to the forecasting model); Commercial name of the products used; Composition of the products used; the percentage of copper (% Cu⁺⁺) and the years of activity

Treatments	Commercial name	Composition	% Cu ⁺⁺	Years of activity
ST	Cuprobenton blu	Copper oxychloride	14	2009
	+	+	+	
PLSDA	Cuproxat SDI	Tribasic copper sulphate	15.2	
	+	+	+	
	Cuproxat SDI	Tribasic copper sulphate	15.2	
ST	Cuproxat SDI	Tribasic copper sulphate	15.2	2010
	+	+	+	
	Bentoram	Copper hydroxide	10	
PLSDA	Cuproxat SDI	Tribasic copper sulphate	15.2	
	+	+	+	
	Bentoram	Copper hydroxide	10	

was empirically calculated as the minimum daily value (0.4 %) that allowed a statistically significant difference between two the disease incidence assessments at two times. Similarly, for severity index, a PathogenThresh daily value of 0.02 % was empirically calculated.

The prediction was separately considered and modelled for: 1) the disease onset and 2) the infection cycle development during the remaining season (disease progress) because secondary infections required different environmental conditions than the primary infections. For the disease onset was considered the presence/absence of the pathogen. Therefore, two different provisional models were adopted for the disease onset (MDO) and disease progress (MDP). The models were developed using a procedure written in MATLAB 7.1 R14.

The statistical models with respect to the deterministic ones, consider that the pathogen expression, measured on a certain date, is depending on the parameters acquired the days before: normally is accepted a difference in time (TimeLag) of 3 d among the climatic change (event) and the pathogen appearance. TimeLag represents the elapse between the pathogen level and the daily-meteo-climatic data (X-block) shifted i days before. Furthermore, it was considered the possibility that the event could be related to the variable of specific adjacent (n) days (TimeSeries, X-block - 1, X-block - 2, X-block - n). Consequently, the possibility to combine the TimeSeries variables was considered in order to account variables weights different from the "X-block - 0" condition.

The daily prediction value was expressed as probability of significant daily increment or absence one of the disease. For the whole model performance evaluation a confusion matrix has been used returning indication of the correctly predicted values together with the false positives (when the model predicted an infection but the disease did not appear) and false negatives (when the model did not predict an infection that actually occurred). The best model relative to the disease onset was chosen following two parameters: i) the percentage of correct classification of the observed values with respect to the predicted ones (CorrClass); ii) days elapsed between the prediction of the first potential attack and the disease onset (DeltaDayFirstAttack).

Based on the three different phase analysis, the following dataset compositions were used:

Model calibration phase: PLSDA model calibration on 2006 and 2007 yearly data of disease incidence, validation on yearly data 2008 disease incidence; extraction of best models in terms of i) days elapsed between the prediction of the first potential attack and the disease onset (DeltaDayFirstAttack) for model disease onset (MDO), ii) the percentage of correct classification of the observed values with respect to the predicted (CorrClass) for model disease progress (MDP).

Field testing phase: application of best models MDO and MDP, extracted in phase 1, to predict disease onset and disease progress on data 2009 and 2010, respectively and fungicide applications positioning.

A posteriori model performance evaluation: a: model calibration on 2006, 2007, 2008,

2009 yearly data of disease incidence and severity, validation on yearly data 2010 disease incidence and severity; b: model calibration on 2006, 2007, 2008, 2010 yearly data of disease incidence and severity, validation on yearly data 2009 disease incidence and severity; c: extraction of best models in terms of MDO and MDP for disease incidence and severity.

The MDP predictions were performed considering a progressive dataset being built by model subset plus the days of the last analysis year (2009 or 2010 respectively) prior the testing ones.

Statistical inferential analysis: Statistics were performed with GraphPad InStat version 3.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). Data on disease incidence and disease severity were arcsine transformed. Data obtained were subjected to statistical analysis using an analysis of variance (ANOVA) and TUKEY's test ($P < 0.05$) for quantitative variables (disease incidence). For ordinal variables (disease severity) individual antifungal activity differences of the products were compared by a KRUSKAL-WALLIS test (a nonparametric test) with DUNN's post hoc test ($P < 0.05$).

Results

Model calibration phase: The best model chosen for the model calibration phase for the disease onset (MDO) was identified selecting a CorrClass value as high as possible (91.8 %), paired with the lowest associable DeltaDayFirstAttack (-3 d) (Tab. 2). The best model relative to the disease progress phase (MDP) was chosen following the CorrClass parameter. After disease onset, during disease progress phase (MDP), other parameters as the false positive and the false negative were used to optimize the models. Therefore, for field testing phase both for 2009 and 2010, a combining prediction approach using MDO for the disease onset and MDP for disease progress phase, was used respectively.

Field testing phase: Figs 1 and 2 show the relationship among meteorological data (A), considering the importance of climatic conditions for the epidemiology of grape downy mildew, phenological growth stage of host (B), considering that susceptibility of grape depends on its phenological status, and epidemiological situation (B) for 2009 and 2010. Epidemiological situation in 2009 (Fig. 1B) indicates that starting from the "three ten rule" (BALDACCIO 1947) the infective rain causing disease onset, at the end of incubation, was the rain of 117th DOY (May, 28). This rain permitted zoospores to reach the stomata and to penetrate into the plant through the stomata. The appearance of oil spots was on 153rd DOY (June, 3). Thereafter the further development of the epidemic is reported in Fig. 1B. Epidemiological situation in 2010 (Fig. 2B) indicates that disease symptoms occurred on 144th DOY (May, 24). Primary and secondary infections are reported (Fig. 2B). The incubation period was calculated on the basis of the method proposed by GOIDANICH *et al.* (1957). PLSDA model was able to forecast the alarms (Figs 1B and 2B), considering environmental factors, host factors, epidemiological

Table 2

Results relative to the best model chosen for the model calibration, field testing (for both 2009 and 2010) and a posteriori (for both incidence and severity and for the two years of analysis 2009 and 2010) phases reporting: model (MDO = model for the disease onset; MDP = model for the disease progress); number of LV (Latent Vector); number of observed values, false positives and negatives; percentage of correct classification and DeltaDayFirstAttack (days elapsed between the prediction of the first potential attack and the disease onset). For the a posteriori model to predict the disease incidence data of the year 2009 was added to the historical dataset (2006, 2007 and 2008) and tested with data of the year 2010 and data of the year 2010 was added to the historical dataset (2006, 2007 and 2008) and tested with data of the year 2009

Phase	Model	Pre-processing	N° LV	N° Observed values	N° False positives	N° False negatives	% Correct classification	DeltaDayFirst Attack
Model calibration	MDO	autoscale	2	171	1	13	91.80	-3
	MDP	autoscale	2	171	8	7	91.23	2
Field testing	MDO + MDP (2009)	autoscale	2	128	17	7	81.30	-3
	MDO + MDP (2010)	autoscale	2	190	24	11	81.60	+2
A posteriori (incidence)	MDO + MDP (2009)	baseline	13	121	3	3	89.19	-3
	MDO + MDP (2010)	normalize	4	97	10	8	78.04	-3
A posteriori (severity)	MDO + MDP (2009)	median center	7	121	4	1	96.22	-5
	MDO + MDP (2010)	baseline	13	96	14	2	81.11	-4

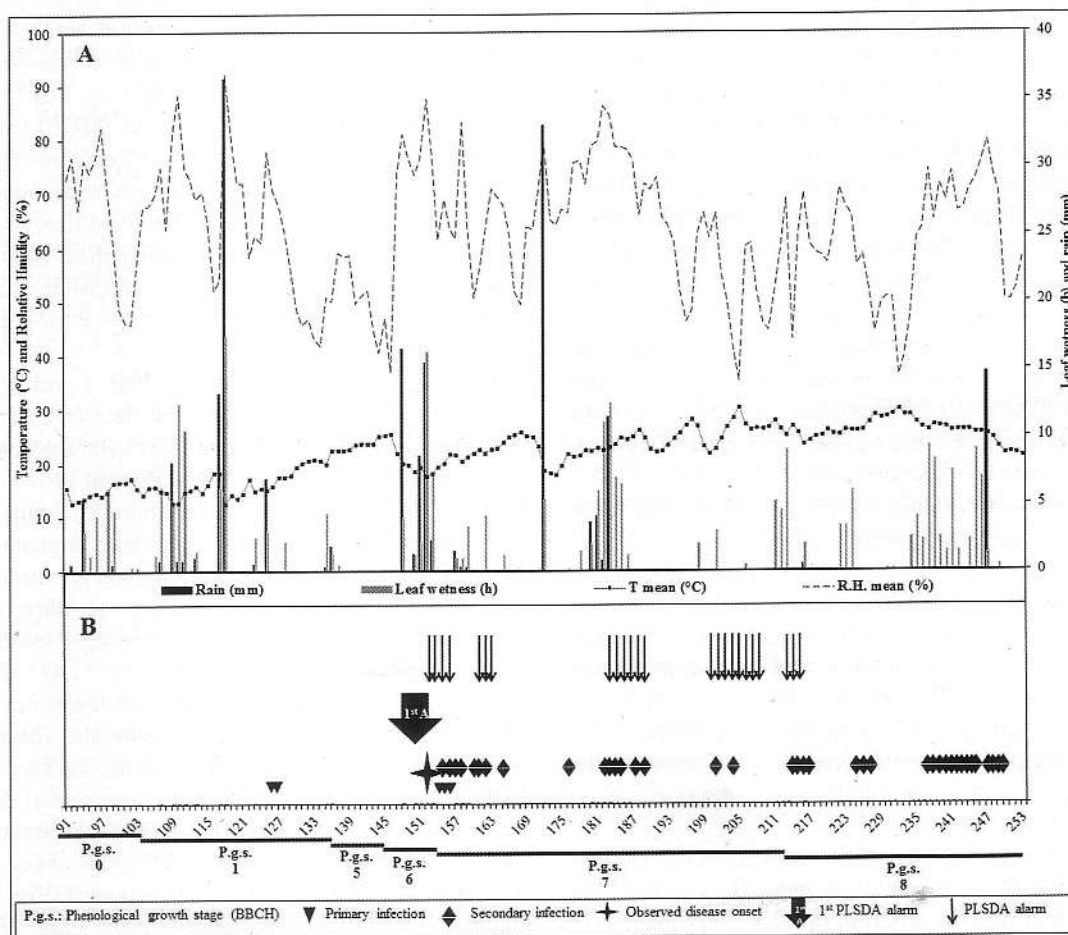


Fig. 1: Relationship among meteorological data (A), pathogen epidemiological parameters (B), host phenological growth stage (B) and partial least squares discriminant analysis (PLSDA) model alarm (B) for the first year of analysis (2009).

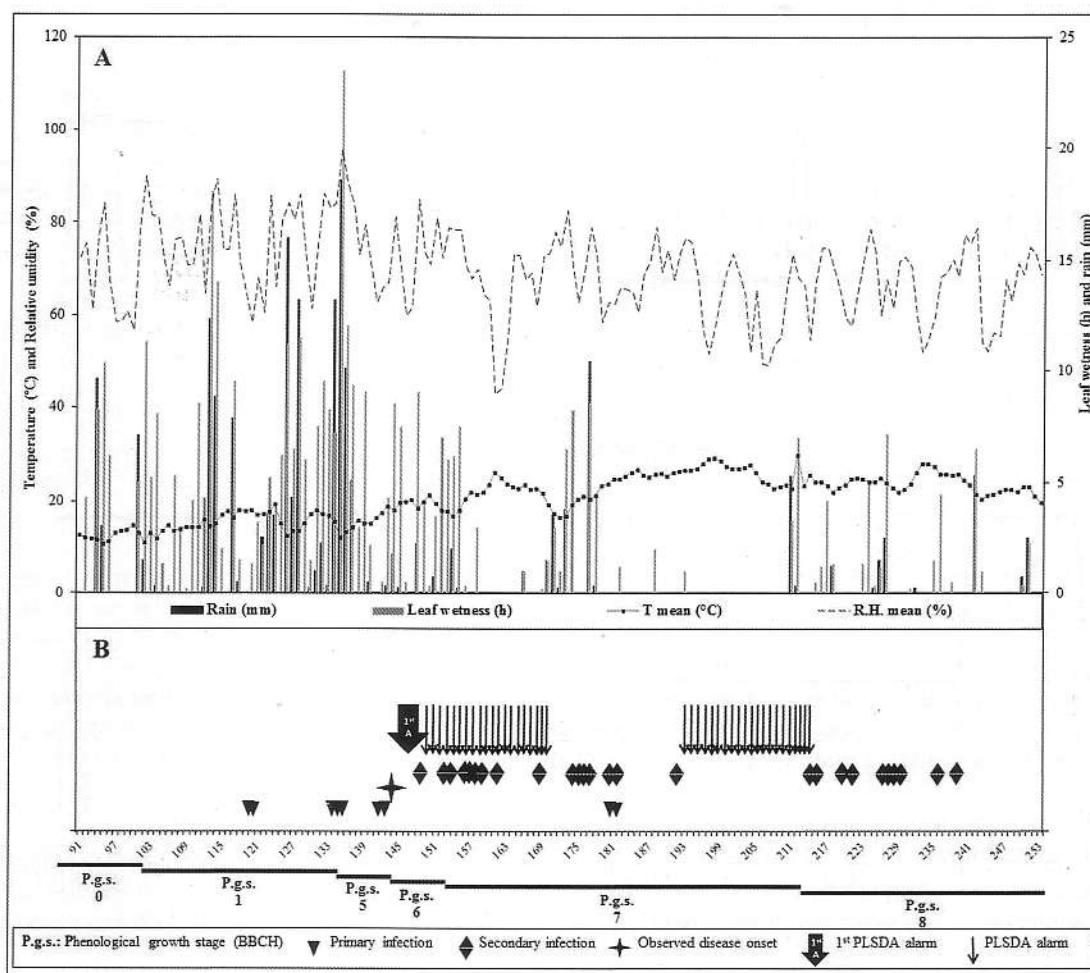


Fig. 2: Relationship among meteorological data (A), pathogen epidemiological parameters (B), host phenological growth stage (B) and partial least squares discriminant analysis (PLSDA) model alarm (B) for the second year of analysis (2010).

conditions and the interactions among them. Tab. 2 shows the whole prediction results found in the field testing phase by applying the two best models for the MDO and the MDP both for 2009 and 2010. For 2009, the DeltaDay-FirstAttack resulted to be of -3 d: on May, 31 [(Day Of Year (DOY) = 151] the first alarm was signalled, indeed, on June 3 (DOY = 154) when the first downy mildew symptoms appeared on the leaves of the untreated control (Fig. 3A). The alarm on May 31 was ignored because between May, 31 and June, 2 26.4 mm of rainfall occurred and it was impossible to spray with a tractor-operated sprayer. Based on forecasting model there were 25 alarms between May, 31 (DOY = 151) and August, 5 (DOY = 217) but only five treatments were carried out. The alarms where the fungicide application already protected plants (the period of protectant activity of fungicide application was estimated between 7 and 10 d based on grapevine phenological stages) were disregarded. Generally the predictive model provided 24 wrong predictions on a total of 128 with 17 false positives and 7 false negatives (Tab. 2; Fig. 3A). For the second year of analysis (2010) the first alarm occurred on May, 26 (DOY = 146), 2 d later the first downy mildew symptoms were observed in the field, but it was notified in time to perform the first treatment on May, 24 (DOY = 144), thanks to the TimeLag of the model of 3 d (Tab. 2; Fig. 3B). The second alarm was notified on May, 30 (DOY

= 150) but the second treatment was carried out on June, 1 (DOY = 152) because the first treatment already protected vines from this predicted infection. The alarms continued until June, 18 (DOY = 169) with two treatments on June, 8 and 15 (DOY = 159 and DOY = 166). After the treatment made on July, 13 (DOY = 194) other 2 treatments were made on July, 23 (DOY = 204) and on August, 2 (DOY = 214). Generally the predictive model provided 35 wrong predictions on a total of 190 with 24 false positives and 11 false negatives (Tab. 2; Fig. 3B).

Disease control: Tabs 3 and 4 report the results of the assessments on leaves and bunches for 2009 and 2010 respectively. At harvest (BBCH-89), both standard and PLSDA showed significant differences in disease incidence in comparison with the untreated control, although 2010 PLSDA was not as effective as the reference product (Standard). Disease severity on plants treated according to the grower's schedule was not statistically different in comparison with plants treated using PLSDA model.

A posteriori model performance evaluation: To predict the disease incidence, data of the year 2009 were added to historical datasets (2006, 2007 and 2008) and tested with data of the year 2010. Data of the year 2010 were added to historical datasets (2006, 2007 and 2008) and tested with data of the year 2009 for the MDO and MDP (Tab. 2). The test average correct classification

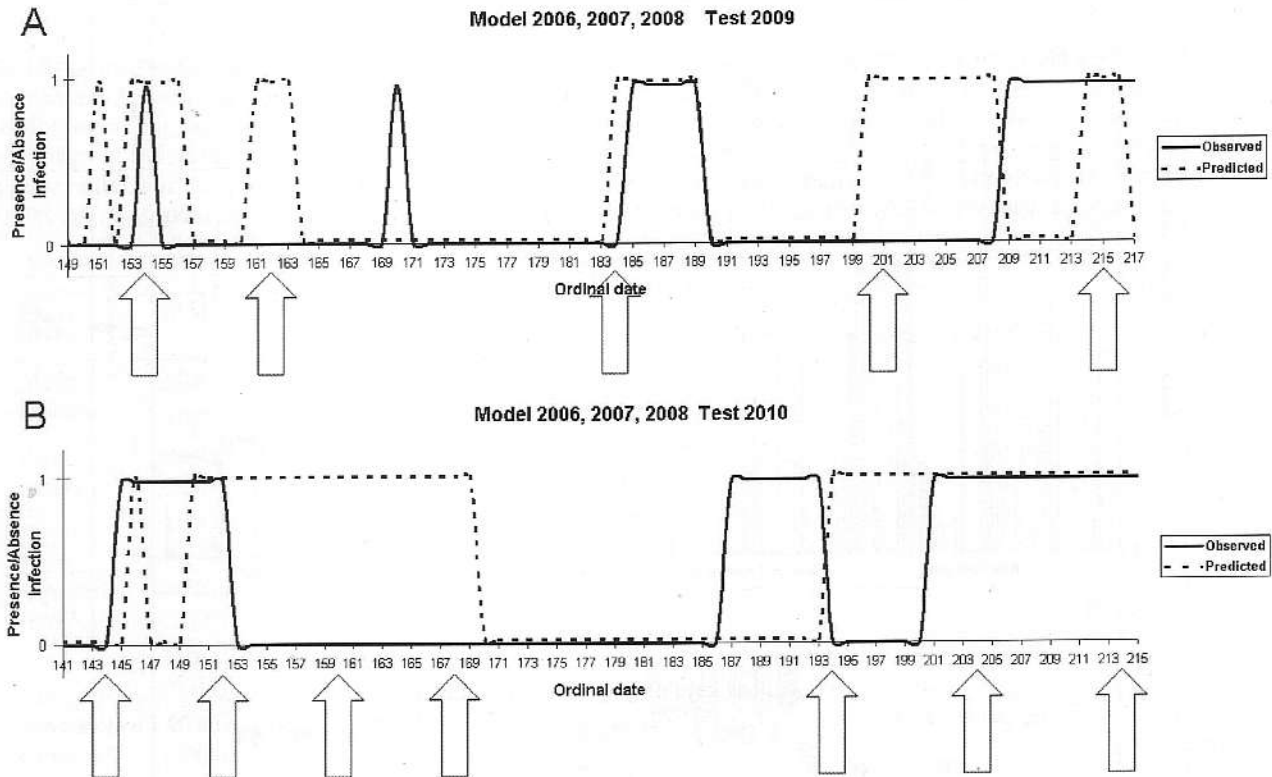


Fig. 3: Plot of the presence/absence (1/0) of the observed (continuous line) and predicted (dotted line) infections for A) Model on 2006, 2007 and 2008 and Test on 2009 and B) Model on 2006, 2007, 2008, 2009 and Test on 2010. Only the central infection periods (68 d for tested year 2009 and 74 for tested year 2010) are reported. The arrows indicate the treatments carried out during the experiment.

Table 3

Effect of different treatments on leaves and bunches, expressed as disease incidence and disease severity against *Plasmopara viticola* (2009)

	Disease incidence*									
	03/06/09 (BBCH ^a -71)		19/06/09 (BBCH-75)		20/07/09 (BBCH-79)		18/08/09 (BBCH-85)		08/09/09 (BBCH-89)	
	leaves	bunches	leaves	bunches	leaves	bunches	leaves	bunches	leaves	bunches
Untreated control	1.33 a	6.77 a	14.00 a	0.67 a	23.67 a	3.00 a	34.33 a	4.67 a		
Standard	0.33 a	2.33 b	3.00 b	0 a	5.67 b	0 a	9.00 b	0 b		
PLSDA	0.33 a	2.67 b	3.33 b	0 a	6.00 b	0 a	9.33 b	0.33 b		
	Disease severity**									
Untreated control	0.25 a	1.46 a	3.63 a	0.21 a	7.46 a	0.54 a	11.46 a	0.88 a		
Standard	0.08 a	0.33 b	0.63 a	0 a	1.13 a	0 a	1.79 b	0 b		
PLSDA	0.04 a	0.50 ab	0.75 a	0 a	1.42 a	0 a	2.08 ab	0.04 ab		

* Expressing the percent of leaves and bunches diseased out of a total number assessed. Means in the same row followed by same letter are not significantly different according to the TUKEY test at $P < 0.05$.

** Expressing the percent area of leaves and bunches showing symptoms of disease. Means in the same row followed by same letter are not significantly different according to the KRUSKAL-WALLIS test followed by DUNN's multiple comparison test at $P < 0.05$.

^a Phenological growth stages and BBCH-identifications keys of grapevine: 71 – fruit set; young fruits begin to swell, remains of flowers lost; 75 – berries pea-sized, bunches hang; 79 – majority of berries touching; 85 – softening of berries; 89 – berries ripe for harvest.

is equal to 89.19 % for the tested year 2009 and to 78.04 % for the tested year 2010. In both cases it is possible to observe that DeltaDayFirstAttack is equal to -3 d. For the prediction of disease severity a PathogenThresh daily value of 0.02 % was empirically calculated. Also in this case data of

the year 2009 were added to historical datasets (2006, 2007 and 2008) and tested with data of the year 2010. Data of the year 2010 were added to historical datasets (2006, 2007 and 2008) and tested with data of the year 2009 for MDO and MDP. The test average correct classification is equal

Table 4

Effect of different treatments on leaves and bunches, expressed as disease incidence and disease severity, against *Plasmopara viticola* (2010)

	Disease incidence*									
	26/05/10 (BBCH ^a -57)		28/06/10 (BBCH-75)		19/07/10 (BBCH-77)		02/08/10 (BBCH-81)		09/09/10 (BBCH-89)	
	leaves	bunches	leaves	bunches	leaves	bunches	leaves	bunches	leaves	bunches
Untreated control	2.00 a	1.25 a	6.75 a	1.25 a	23.75 a	15.50 a	29.00 a	20.25 a	36.50 a	31.00 a
Standard	1.00 a	0 b	4.00 a	0 b	9.75 b	3.00 b	11.50 b	4.25 b	16.00 b	10.00 c
PLSDA	1.25 a	0.25 b	5.00 a	0.25 b	14.25 b	4.75 b	17.25 b	8.75 b	22.00 b	16.50 b
	Disease severity**									
Untreated control	0.31 a	0.25 a	1.22 a	0.25 a	6.23 a	3.31 a	7.34 a	4.47 a	9.19 a	7.63 a
Standard	0.13 b	0 b	0.56 a	0 b	1.63 b	0.41 b	1.94 b	0.56 b	2.75 b	1.59 b
PLSDA	0.19 ab	0.03 ab	0.81 a	0.03 ab	2.72 ab	0.88 ab	3.34 ab	1.66 ab	4.41 ab	3.34 ab

* Expressing the percent of leaves and bunches diseased out of a total number assessed. Means in the same row followed by same letter are not significantly different according to the TUKEY test at $P < 0.05$.

** Expressing the percent area of leaves and bunches showing symptoms of disease. Means in the same row followed by same letter are not significantly different according to the KRUSKAL-WALLIS test followed by DUNN's multiple comparison test at $P < 0.05$.

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to 96.22 % for the tested year 2009 and to 81.11 % for the tested year 2010. The DeltaDayFirstAttack is equal to -5 d for the first case and to -4 for the latter.

Discussion

The most encouraging results emerged from the use of the forecasting model, concerning the prediction of first treatment (MDO) which is essential in order to avoid the establishment of the disease in the vineyard. Indeed, the early prevention is essential for good control of grape downy mildew disease particularly in organic farming where no curative pesticides are available.

In this study, the PLSDA forecasting model allows a reduction of treatments and avoids unnecessary anti-mildew treatments of organic farmers. The field testing phase was carried out on a very limited number of temporal observations for model calibration (2006, 2007 and 2008). This allowed to improve the model robustness (WISE and RICKER 1991) being one of the few cases in literature of two years of applications in field based on only 3 years as historical data. Nevertheless, the model provided a good level of prediction (81 % of correct classification in both tested years). In 2009, during the monitored period, 9 treatments were carried out on standard treatment, while, in the same period the PLSDA model provides the need of just 5 treatments, underlining the superfluous remaining 4. In 2010 the first downy mildew symptom observed in the field, occurred 2 d after the first alert, but it was notified in time to perform the first treatment that is May, 24, thanks to the TimeLag of the model of 3 d. In this case 13 treatments were carried out by the farmer, while, in the same period the PLSDA treatment provides the need of just 7 ones. The *a posteriori* model performance evaluation both for the tested year

2009 and 2010 predicting both the disease incidence and severity. This resulted in better performance with respect to the in field one by considering: the DeltaDayFirstAttack (incidence: -3 d vs. -3 and +2 for the year 2009 and 2010 respectively; severity: -5/-4 vs. -3 and +2 for the year 2009 and 2010 respectively). The total number of false positives and negatives (tested year 2009: 24 for the field phase, 6 and 5 for incidence and severity prediction for the *a posteriori* phase respectively; tested year 2010: 35 for the field phase, 18 and 16 for incidence and severity prediction for the *a posteriori* phase respectively) and finally for the percentage of correct classification (tested year 2009: 81.3 % for the field phase, 89.19 % and 96.22 % for incidence and severity prediction for the *a posteriori* phase respectively; tested year 2010: 81.6 % for the field phase and only for the severity prediction for the *a posteriori* phase respectively with a value equal to 81.11 %).

Conclusions

This work shows the possibility to reduce fungicidal treatments and to avoid copper treatments not essential for disease control with economics and environmental advantages. For all these reasons, this study represents a first step in the model validation in order to try to overcome the typical limitations of the actual forecasting models for infections with the aim of rationalizing treatments and make a more punctual pest control.

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