DEVELOPMENT AND EVALUATION OF A WARNING MODEL FOR THE OPTIMAL USE OF COPPER IN ORGANIC VITICULTURE

A. Pellegrini[1], D. Prodorutti[1], A. Frizzi[1], C. Gessler[2] and I. Pertot[1]

[1] *Fondazione Edmund-Mach, Via Mach 1, 38010 S. Michele all'Adige (TN), Italy*
[2] *Department of Plant Pathology, Institute of Integrative Biology ETH, Zürich, Switzerland*

SUMMARY

Downy mildew caused by *Plasmopara viticola* is one of the most serious diseases affecting grapevine worldwide, and can significantly affect crop quality and quantity. In organic viticulture, downy mildew control is mainly based on copper compounds, which are toxic to organisms in the soil. For this reason, restrictions concerning the maximum amount of copper that organic growers can apply per hectare have been enacted in several countries. We have developed a warning model and a decision-making procedure to help organic grapevine growers optimize their use of copper against downy mildew. This system is based on treating vines with a variable rate of copper when there is an immediate risk of infection and the vegetation is not sufficiently protected by previous treatments. In 2007, the warning model and the decision-making procedure were tested in two vineyards in northern Italy and compared with the local common practice and an untreated control. The treatment timings and copper application rates recommended by the system resulted in control of downy mildew equivalent to that associated with the common practice, but with a considerable reduction in the total amount of copper applied.

Key words: downy mildew, *Plasmopara viticola, Vitis vinifera,* control, fungicides.

INTRODUCTION

Organically grown food is produced without the use of synthetic fertilizers or pesticides (USDA, http://www.ams.usda.gov). The prevention of damage caused by pests, diseases and weeds relies primarily on protection by natural enemies, the choice of crop species and cultivar, crop rotation, cultivation techniques and other physical processes, and, in the case of an established threat to a crop, the application of plant protection products derived from natural sources [see for example, for the USA, the National Organic Program (USDA); for the European Union, the Council Regulation (EC) No. 834/2007 (Council of the European Union, 2007); and for Canada, the Organic Products Regulations (CFIA, 2006)].

Downy mildew is an important disease of grapevine that can cause heavy yield losses (Agrios, 1997; Dalla Marta *et al.*, 2005). It is caused by *Plasmopara viticola* (Berk. et Curt.) Berl. et De Toni and is present worldwide. Small, pale yellow circular lesions develop on the upper part of leaf surfaces (oil spots). These spots later become necrotic and white-to-grey pathogen growth (sporangiophores and sporangia) is observed on the undersides of the infected leaves. Infected young clusters turn light green-yellow and become covered with fungal growth. If clusters are attacked later in the season, the lesions are restricted to cluster stems and all or part of the cluster will shrivel, but there will be no visible sporulation. The older-infected berries turn red or yellow and then harden and shrivel instead of ripening.

P. viticola overwinters on the ground as oospores, which germinate in the spring and throughout the growing season and produce macrosporangia that contain zoospores. Splashing rain (10 mm, but even less can be sufficient) and wind can spread zoospores to aerial parts of the vine (Gobbin *et al.*, 2003, 2005). When water is present on the plant surface and temperatures are between 6 and 26°C, zoospores reach the stomata, and the pathogen then starts to penetrate the plant through these. After a latent period, whose length depends on temperature and which requires at least 3 h of darkness and relative humidity (RH) higher than 93%, the pathogen emerges from the stomata to produce secondary sporangia. These are spread by splashing rain and if they land on wet, green grapevine tissue, they will release zoospores. Under low RH conditions and in the presence of sunlight, sporangia rapidly die. The conditions necessary for secondary infection and the mechanisms involved are similar to those of primary infection (Agrios, 1997; Caffi *et al.*, 2007; Rosa *et al.*, 1993).

Leaf tissues are susceptible to infection once stomata have developed (when shoots are approximately 10 cm long or leaves are approximately 10 cm^2 in area). Leaves

Corresponding author: A. Pellegrini
Fax: +39.0461.615500
E-mail: alberto.pellegrini@iasma.it

remain susceptible throughout the growing season, even if to a reduced level. Infections on older leaves develop as small lesions (1-2 mm in diameter), which have less sporulation than lesions that form on younger leaves. Clusters are susceptible from the prebloom period; as the season proceeds, they become less susceptible and 5-6 weeks after bloom they become resistant (Kennelly *et al.*, 2005). Therefore, infections occurring before and after bloom and until veraison cause the greatest damage, either by directly destroying clusters or by killing leaf tissues and reducing photosynthesis. Infections occurring after veraison do not directly kill fruit or leaf tissue, but do affect photosynthesis. Therefore, they are less of a threat to the crop.

In most vineyards, control of grapevine downy mildew is based on application of synthetic fungicides. In organic cropping systems, naturally derived products, such as rock dust and clays, biocontrol agents, chitosan, plant extracts, and copper can be used. Aside from copper, these products often give inconsistent and unsatisfactory results under field conditions. Therefore, copper, which is also commonly used in conventional farming, remains for organic systems the only active ingredient effective against grapevine downy mildew (Dagostin *et al.*, 2006). Copper can be applied as a hydroxide, oxychloride, oxide or sulfate. Its activity depends on the amount of free Cu^{2+} in the aqueous solution and there do not seem to be any significant differences in the downy mildew control provided by different copper formulations under field conditions (Goidanich, 1964).

Copper is not cytotropic or systemic; it works as a prophylactic measure and is not eradicant. It has to be present at a sufficient concentration on susceptible leaf tissue to prevent infection, so, in organic viticulture, treatments are often applied weekly throughout the season, to provide continuous protection.

Copper has other positive effects on grapevine. For example, it promotes the lignification of shoots in late summer and provides some protection against other grapevine diseases (Agrios, 1997; Nita *et al.*, 2006). It is also relatively inexpensive. However, copper is a heavy metal and accumulates in the soil, where it has the negative effect of decreasing microbial and earthworm activity (Van-Zwieten *et al.*, 2004).

For this reason, the European Union has set a limit on the amount of copper that can be applied in organic cropping systems (The Commission of the European Communities, 2002). Other limitations on the use of copper in organic agriculture are under discussion worldwide (Wightwick *et al.*, 2008).

Several approaches have been proposed for reducing the use of copper in organic vineyards: the use of resistant/tolerant cultivars; the use of more effective, improved formulations and the use of reduced application rates combined with treatments precisely timed in relation to the time of infection. The use of resistant or less susceptible cultivars is rarely accepted by the market because of the poor quality of wines produced from these cultivars and none of the new copper formulations examined to date have provided any significant improvements in efficacy (Pontiroli *et al.*, 2006). Several research projects have evaluated the effects of reduced application rates (single copper applications), but none of these studies examined the optimal timing of treatments (Cravero *et al.*, 2004; Egger *et al.*, 1998; Ferrari *et al.*, 2000; Morando *et al.*, 2005; Pertot *et al.*, 2002).

Many forecasting/epidemiological models have been developed in order to help determine the optimal time for applying fungicides against downy mildew. One of the first empirical models to be developed was the "3-10 rule" (Baldacci, 1947), which states that primary infections occur in the simultaneous presence of vine shoots longer than 10 cm, air temperatures of at least 10°C, and at least 10 mm of rain within a 24-48 h period. The EPI (Etat Potential d'Infection) is a more complex model developed in France (Strizyk, 1983) that is based on the potential impact of the pathogen and its ability to cause infection. Other tools include PRO (Plasmopara Risk Oppenheim), which helps growers decide when downy mildew has reached a stage at which it will begin to damage the crop (Hill, 1990); DMODEL, a downy mildew simulator developed in Australia (Wachtel and Magarey, 1997); PLASMO, which simulates the limited survival of sporangia, the start of all possible infections and the duration of incubation periods (Rosa *et al.*, 1993); DMCast (Downy Mildew Forecast), a weather-driven model that provides estimations of both primary and secondary *P. viticola* infections (Park *et al.*, 1997); and a model simulating *P. viticola* infections based on fuzzy logic (Orlandini *et al.*, 2003). The UCSC (Università Cattolica del Sacro Cuore, Piacenza, Italy) has developed a mechanistic model which simulates the entire downy mildew disease cycle from oospore maturation through the onset of symptoms (Caffi *et al.*, 2007). Other models include Vinemild, a quantitative model based on the biology of the pathogen and its reproductive capacity (Blaise *et al.*, 1999); and the Vitimeteo Plasmopara, a qualitative model based on climatic conditions fostering infection and the readiness of oospores to germinate (Siegfried *et al.*, 2004). In their practical use, these models often rely on a wide range of fungicides and/or synthetic fungicides with high efficacies and curative/eradicative properties (Madden *et al.*, 2000).

Several forecasting models are not implemented in the field because of the unavailability of technical equipment on small farms (organic vineyards tend to be small) or imprecise meteorological measurements, which yield incorrect predictions (Dalla Marta *et al.*, 2005). Organic growers rely mostly on the preventive use of copper and cannot intervene after infections with synthetic curative products. Moreover, low or very low doses of copper can be easily washed away by rain, exposing the vegetation to downy mildew infections. Therefore, to support the optimal use of copper in organic agriculture, the use of a specific warning model and a decision-making procedure that envisage the sole use of copper is desirable.

A warning model to guide preventive treatment before disease onset is the most appropriate (Shtienberg, 2000). A warning model and a decision-making procedure that integrate data and expert opinion can then be implemented in a computerized decision-support system to enable growers to independently access information at any time. From the grape growers' point of view, a good warning model will be characterized by the need to input few and simple-to-measure parameters, a low risk of failure, high reliability, and a level of plant protection comparable to that of common practice. Not advising a treatment when an infection event will occur cannot be tolerated, but a treatment not followed by an infection is acceptable.

Our objective was to develop and validate such a model for use of copper in organic viticulture. We focused on developing a model and validating it in two organic vineyards in northern Italy, comparing our system with the local common practice. We discuss potential advantages and limitations of our approach with particular regard to the threshold of 6 kg Cu^{2+} ha^{-1} $year^{-1}$, which is the current EU limit, possibly to be extended to other grape-growing areas outside Europe (Ministero della Salute, 2004).

MATERIALS AND METHODS

Rainfastness and efficacy of different copper treatments. An experiment was conducted in a greenhouse under controlled conditions (temperature 22±4°C and RH 60±20%), using plants of a susceptible cultivar (Pinot Gris) that had two shoots with four fully expanded leaves. Three copper formulations were used: copper hydroxide (Kocide® 2000, DuPont De Nemours Italiana, Italy), copper oxychloride (Cuproxat, Isagro Italia, Italy), and copper sulphate, as Bordeaux mixture (Poltiglia Bordolese Disperss Blu, Cerexagri, Italy). For each product, four different concentrations of Cu^{2+} were tested (1.0, 0.7, 0.5 and 0.3 g l^{-1}). Considering a volume of solution applied per hectare of 1200 litres, these four concentrations correspond to 1200, 840, 600, and 360 g ha^{-1} of Cu^{2+} respectively. Forty ml of solution were sprayed onto each plant. Upper and lower leaf surfaces were sprayed using an air compressor and spray gun working at a pressure of 400 kPa. Water was applied as a control treatment. Rain (15 mm h^{-1}) was simulated with overhead irrigation placed 3 m above the plants. The quantities of artificial rain applied to the plants were 0, 10, 30, 40, and 60 mm and these applications were made once the copper treatment had dried (approximately 1 h after application). Each formulation, concentration, quantity of rain treatment was applied to four replicates of five plants each. The treatments were arranged in a completely randomized block design. Fresh *P. viticola* sporangia were used as inoculum. A sporangia-water suspension (10^6 sporangia ml^{-1}) was sprayed on the underside of each fully expanded leaf once plants were dry (approximately 1 h after the artificial rain). Inoculated plants were incubated for 12 h in darkness at approximately 90% RH and a temperature of 20°C. Seven to ten days after inoculation, the plants were again incubated in darkness, overnight at 80% RH and 20°C, to promote sporulation.

Disease severity (DS) was evaluated as the percentage of symptomatic leaf area (EPPO, 2004). The efficacy of each treatment (T) was calculated according the formula 100 (DS_{untrT} – DS_T) DS_{untrT}^{-1}, in which untrT is the untreated control for each quantity of artificial rain. This experiment was carried out twice. Factorial ANOVA was used to compare the results. If the effect of the "experiment" variable was not significant ($P>0.05$), results were pooled and means separated using Tukey's test ($p = 0.05$). Throughout this study, the Statistica 7.1 software package (Statsoft, USA) was used.

Warning model development and implementation. The warning model is based on preventive applications of copper when the risk of infection is high and on adjusting copper application rates based on rain forecasts, plant susceptibility (risk of losses in case of infection), and the residues predicted to be present on the leaves after the last treatment. Infection is considered likely (high risk) if rain is predicted for the following days (Fig. 2).

Agronomic, environmental and host factors were taken into account in developing the model, which is based on a simple epidemiological model and data from our own experiments, as well as the expert opinions of growers and technicians.

Agronomic factors. Copper residues left on the leaves from the last treatment and the number of new leaves emerged since the last copper treatment were considered among the agronomic factors. From past experience, vineyards were subjectively divided into two groups, representing two different levels of expected damage from first downy mildew infections. Generally, in the first group, few oil spots develop and they do not cause damage (low risk vineyard). Therefore, primary infection can be tolerated in these vineyards. In the second group, primary infections will usually cause severe losses in yield quality and quantity or complicate the prevention of secondary infection due to the high presence of inoculum (high risk vineyard); growers will aim

Table 1. Recommended actions by phenological stage when there are fewer than three new unprotected leaves: "Do not treat, and check the system again the following day" (No) and "Treat as soon as possible with the specified copper concentration" (0.3, 0.5, 0.7 and 1.0 g Cu^{2+} l^{-1}), based on the copper concentration of the last treatment (CC), forecast chance of rain the next day (No, Low, Medium, High) and rainfall (SR_d) on the days since the last treatment.

		Plant phenological stage												
		Before bloom Risk of rain				Bloom Risk of rain	Between bloom and veraison Risk of rain				After veraison and before harvest Risk of rain			
CC (g Cu^{2+} l^{-1})	ΣR_d (mm)	No	Low	Medium	High	Any	No	Low	Medium	High	No	Low	Medium	High
0.3	≤10	No	No	No	0.7	No	No	No	No	0.5	No	No	No	No
	>10 and ≤30	No	0.3	0.5	0.7	No	No	0.3	0.5	0.7	No	No	No	0.3
	>30 and ≤40	No	0.3	0.5	0.7	No	No	0.3	0.5	0.7	No	No	0.3	0.3
	>40	0.3	0.5	0.5	1.0	No	0.3	0.3	0.5	0.7	No	No	0.3	0.3
0.5	≤10	No	No	No	0.7	No	No	No	No	0.3	No	No	No	No
	>10 and ≤30	No	0.3	0.5	0.7	No	No	No	0.3	0.5	No	No	No	0.3
	>30 and ≤40	No	0.3	0.5	0.7	No	No	No	0.3	0.5	No	No	No	0.3
	>40	0.3	0.5	0.5	1.0	No	No	0.3	0.5	0.7	No	No	No	0.3
0.7	≤10	No	No	No	0.3	No	No	No	No	No	No	No	No	No
	>10 and ≤30	No	No	0.3	0.5	No	No	No	No	0.3	No	No	No	No
	>30 and ≤40	No	No	0.3	0.5	No	No	No	0.3	0.5	No	No	No	No
	>40	No	0.3	0.5	0.7	No	No	No	0.3	0.7	No	No	No	0.3
1.0	≤10	No	No	No	No	No	No	No	No	No	No	No	No	No
	>10 and ≤30	No	No	No	0.3	No	No	No	No	No	No	No	No	No
	>30 and ≤40	No	No	0.3	0.5	No	No	No	No	0.3	No	No	No	No
	>40	No	No	0.5	0.7	No	No	No	No	0.3	No	No	No	No

to prevent primary infections in these vineyards. The difference between the two groups is due to a complex interaction among cultivar, trellis system, the presence of overwintering inoculum, and environmental conditions. For the grower the choice between the two levels is related to the willingness to tolerate different levels of risk (to treat or not to treat the first primary infections).

Environmental factors. Temperature, rain, and forecasted rain were the factors considered. In constructing our model, we did not consider RH and leaf wetness duration, because these factors are difficult to measure. Their measurement requires the use of expensive sensors, which are not always available on small organic farms.

Host factors. The model considers the target varieties to be highly/extremely highly susceptible to downy mildew; therefore, tolerant hybrids were not considered. Considering the age-related susceptibility of grapevines, the growing season was divided into four stages: prebloom, bloom, between bloom and veraison, and after veraison until harvest. Prebloom was considered the most susceptible stage, during which only the lowest infection risk can be tolerated. Treatments are stopped during bloom for one week to avoid damaging flowers (because of copper toxicity) and disturbing pollination (Pimentel *et al.*, 1992). After bloom, a higher level of risk can be tolerated.

First treatment. The system allows a choice between two epidemic situations based on the history of early disease in the specific vineyard. This choice determines the starting time for the first treatment. In both cases, the treatment is applied before a forecasted rain. In a high risk vineyard, the first copper treatment (0.70 g Cu^{2+} l^{-1}) is prescribed as soon as the plant is susceptible to infection in the spring (when shoot length reaches approximately 10 cm) and the forecasted weather is cloudy with any risk of rain. To determine the optimal timing for the first treatment in a low risk vineyard, the system first considers whether the following conditions have been concurrently fulfilled: the shoot length is ≥ 10 cm, the average temperature over the last 24 h has been ≥ 10°C, the quantity of rainfall in the last 24 h has been ≥ 10 mm and 70% of the latent period has been completed. The progression of the latent period is calculated based on the average daily temperature in the vineyard (Goidanich, 1964). Once all the above conditions have been met, the system considers the weather forecast and recommends "no action" if sunny to partly cloudy weather with no risk of rain is predicted. Weather conditions should be checked again the next day. The system recommends treatment (0.70 g Cu^{2+} l^{-1}) if the weather is predicted to be cloudy with any risk of rain. Weather forecasts were acquired from Meteotrentino regional weather forecasting service (http://www.meteotrentino.it).

Subsequent treatments. In the model, the conditions for further infections are extremely simplified. The model assumes that infection can occur if the average temperature is between 6 and 26°C with any amount of rain. Therefore, if the weather forecast indicates any risk of rain the following day, there is a risk of infection. The decision (treatment/no action) is based on a combination of the following factors: plant growth (number of leaves unprotected by the previous treatment), wash-off of copper (amount of rain between the last treatment and the time of the decision), and the risk of infection the following day (whether rain is forecast). New leaves that have grown since the last treatment are considered to be unprotected. The number of new leaves can be evaluated by marking 10 shoots in the vineyard and counting the number of new susceptible leaves (larger than 10 cm^2) that have developed since the previous treatment. If the average number of unprotected leaves is three or more and if there is any risk of rain the following day, the system recommends a treatment (0.7 g Cu^{2+} l^{-1}) independent of any existing residues on older leaves.

If the number of unprotected leaves is less than three, the action and the recommended rate of copper to be applied are based on the rainfastness of the copper product and the grower's experience and tolerance for disease risk. The rain prediction on the coming day is rated in four risk categories (Table 1): none (sunshine predicted); low (partly cloudy with scattered rain); medium (cloudy and rainy, with less than 40 mm of rain predicted for the next two days); and high (cloudy with heavy rain, i.e. more than 40 mm predicted for the next two days). Basically, the concept is to guarantee a high level of protection before bloom, a medium level between bloom and veraison, and a basic level after veraison. The system recommends that no action be taken during full bloom (for a maximum of seven days) and that the last prebloom application be made when 10% of the clusters are already blooming. A low dose treatment (0.3 g Cu^{2+} l^{-1}) is allowed after one week of flowering only if the risk of rain is high. Before bloom, if the last treatment was made with 0.3 or 0.5 g Cu^{2+} l^{-1} and the risk of rain is high, the recommended rate is always 0.7 g Cu^{2+} l^{-1}, unless the amount of rain between the last treatment and the decision is more than 40 mm. In that case, the system suggests the highest rate of copper (1.0 g Cu^{2+} l^{-1}) to be applied as a precaution, even though our experimental results indicated no difference between the efficacies of 0.7 and 1 g Cu^{2+} l^{-1} (Table 1, Fig. 2). If the rate of the last treatment was 0.5 g Cu^{2+} l^{-1} or more, the recommended rate is always calculated based on copper rainfastness and the risk of rain. Between bloom and veraison, a lower level of efficacy can be tolerated, as compared to the situation before bloom.

Therefore, a general reduction in application rate is suggested; recommended actions for the last treatment with 0.5 and 0.7 g Cu^{2+} l^{-1} between bloom and veraison are the same as for the last treatment with 0.7 and 1.0 g Cu^{2+} l^{-1} before bloom. No action is prescribed if the last treatment was made with 1.0 g Cu^{2+} l^{-1}. After veraison, only treatments with a rate of 0.3 g Cu^{2+} l^{-1} in the most risky situations are suggested. The system's recommendations end four weeks before the estimated harvest time. The volume of spray solution should be adapted to the canopy size. In our study it was 1200 litres ha^{-1}.

Simulation. In order to verify the system's ability to predict infections and estimate the potential reduction in the amount of copper applied, we simulated its use from 2004 through 2006 in an organic vineyard in Rovereto (northern Italy). The cultivar was Chardonnay, the vineyard was nine-year-old at the beginning of the simulation and the trellis system was "pergola trentina". The simulation started when the shoots reached 10 cm and ended four weeks before the estimated harvest time. During the growing season, plants were routinely checked for the appearance of new leaf spots. One hundred leaves were randomly checked every week and any increase in the appearance of symptoms on the leaves was considered to be the result of an infection. The potential moment of infection was estimated by calculating the latent period, based on Goidanich (1964). Although Kennelly *et al.* (2007) suggest that the latent periods can be much longer than expected, we decided to use Goidanich's model because, based on preliminary field observation, it accurately predicts the latent period under Trentino conditions (data not shown). Meteorological data (temperature and rain) were acquired from an automated weather station located in the experimental vineyard (http://meteo.iasma.it/meteo/) and weather forecasts were acquired from Meteotrentino regional weather forecasting service (www.meteotrentino.it).

In this vineyard, treatments against downy mildew were applied according to the common local practice for downy mildew control, based on weekly treatments of 0.5 - 0.7 g Cu^{2+} l^{-1}. Treatments were first applied when shoots are 10 cm long and discontinued four weeks before harvest. A plot (one fifth of the vineyard) was left untreated to monitor the development of natural infections.

Any day on which the system prescribed a treatment because of a risk, the following day was considered to be an estimated infection. Any (simulated) treatment that was followed by a real infection assessed in the vineyard was considered to be correctly estimated and any (simulated) treatment followed by a second (simulated) treatment before a new real infection was considered incorrectly estimated. Accuracy was calculated as the percentage of treatments correctly estimated relative to the total number of simulated treatments. The simulation considered a high and low level of *P. viticola* risk in the vineyard and the number and timing of treatments in the simulation were compared with the common practice applications.

Validation in organic vineyards. In 2007, the system was evaluated in two organic vineyards in northern Italy, 45 km apart, in the Adige Valley. The first vineyard (cv. Schiava, 13-year-old, pergola trentina) was located in S. Michele all'Adige at 300 m above sea level (a.s.l.) on the side of a hill. It was considered to be at low risk because, in previous years, the first primary infections did not threaten the crop. The second vineyard (cv. Cabernet Sauvignon, nine-year-old), was located in Rovereto, in a flat area at 200 m a.s.l. In this vineyard, downy mildew infections usually cause damage in the early part of the season, so it was considered to be at high risk.

A randomized block design was used and each treatment (untreated control, treated according to the system's recommendations and treated according to the common practice) was replicated four times (16 vines per treatment per block). The "common practice" treatment consisted of weekly applications of copper at variable rates. We always used a volume of 1200 litres ha^{-1} of treatment solution per application. The total amount of copper applied per hectare per season was calculated by multiplying the sprayed volume by the Cu^{2+} concentration in the solution by the number of treatments.

Treatments were made using a backpack atomizer (SOLO450, Fiaba, Italy). We always used copper hydroxide (Kocide® 2000). Copper was applied at different concentrations according to the system's recommendations (0.3, 0.5, 0.7, and 1.0 g Cu^{2+} l^{-1}) or the common practice of the area (between 0.4 and 0.7 g Cu^{2+} l^{-1}). Sulphur (3 g l^{-1} of Thiovit® 80; Syngenta Crop Protection, Italy) was applied weekly in all plots to control powdery mildew. The treatments were sprayed in the morning, in the absence of wind and when the vegetation was completely dry.

Assessments of the incidence (percentage of leaves and clusters showing symptoms) and severity (percentage of leaf area or cluster area showing symptoms) of downy mildew infections were carried out weekly on 50 leaves or clusters per replicate. Results were compared using the Kruskal-Wallis nonparametric test. Meteorological data were acquired from automated weather stations located in the experimental vineyard in Rovereto and 300 m from the vineyard in S. Michele (http://meteo.iasma.it/meteo/) and weather forecasts were acquired from Meteotrentino regional weather forecasting service.

Assessment of must quality and copper residues. Five kg of ripe grapes were randomly collected for each replication (20 kg per treatment) on the 6th of September 2007 in Rovereto and on the 13th of September

2007 in S. Michele. The samples were pressed and the must was filtered. Sugar contents (°Brix) were measured using a digital refractometer (IMF Digitale ATC, Inderst, Italy) and total acidity (expressed as g l^{-1} tartaric acid) was determined by titrating with NaOH (0.1 N). Results were compared using the Kruskal-Wallis non-parametric test.

Copper residues (ppm) in each vineyard were determined on the 27th of August in a randomly collected sample of 50 leaves and 4 kg of grapes for each treatment. The copper residues on these samples were collected in acid solution. The leaves or filtered ground grapes were put in a water solution of 1% nitric acid (1 g leaves or grape in 10 ml acid solution) and manually shaken for 3 min. Copper was measured using plasma spectrophotometry (Perkin Elmer OPTIMA 3300 PV, USA).

RESULTS

Rainfastness and efficacy of different copper treatments. Results were pooled because no effect of the variable "experiment" was observed (P>0.05). No significant differences were present among copper formulations applied at the same concentration and after the same amount of artificial rain (P>0.05). The four concentrations of copper gave the same level of efficacy ± standard error (92.59% ± 1.38) when no artificial rain was applied. When the quantity of artificial rain was increased, the efficacy of copper applications decreased (Fig. 1). For each copper concentration, significant efficacy differences (Tukey's test, P≤0.05) were observed between two subsequent quantities of artificial rain, except for 0.7 and 1.0 g Cu^{2+} l^{-1} between 0 and 10 mm of rain and 0.3 and 5.0 g Cu^{2+} l^{-1} between 10 and 30 mm of rain. The lowest concentration (0.3 g Cu^{2+} l^{-1}) was least effective with all of the amounts of artificial rain (Fig. 1).

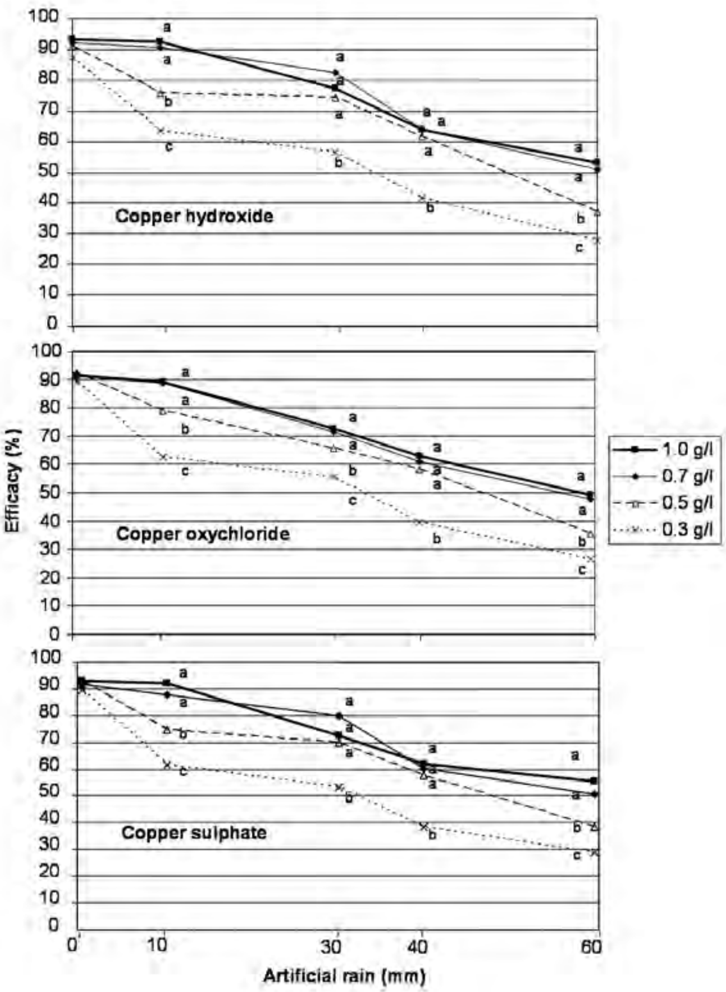

Fig. 1. Efficacies of different concentrations of copper formulations used against *Plasmopara viticola* on potted grapevines following artificial rain and artificial inoculation under controlled conditions. Different letters within the same rain quantity and the same copper formulation indicate significant differences according to Tukey's test (P≤0.05).

Table 2. Simulation of the decision support system in an organic vineyard in northern Italy (Rovereto) from 2004 through 2006. The recommended treatments were determined by simulating the use of the system (SR) according the high level (HR) and the low level of downy mildew risk (LR) of vineyard, as compared with the treatments that were actually applied during these seasons, in accordance with the common practice (CP). New infections were recorded weekly. Accuracy is the percentage of prescribed (simulated) treatments followed by an infection.

	Year								
	2004 Treatment			2005 Treatment			2006 Treatment		
	SR-HR	SR-LR	CP	SR-HR	SR-LR	CP	SR-HR	SR-LR	CP
Copper* (kg/ha)	4.96	2.80	7.46	5.40	4.56	8.57	4.68	3.12	7.97
Accuracy (%)	85.7	80.0	53.8	50.0	71.4	34.0	57.1	80.0	43.0
No. of Treatments	7	5	13	8	7	14	8	6	13

* The total amount of copper per hectare was calculated for a volume of 1200 l ha^{-1} per each spray

System simulation. At the beginning of the 2004 growing season, the system estimated two primary infection events. However, only the second predicted infection corresponded to a confirmed infection in the vineyard. During the season, the system identified 12 days as risky for secondary infections, and infections developed on 75% of these days. If treatments had been applied according to the system's recommendations, reductions of 33.5 and 62.5% in the quantity of copper applied and of 42.5 and 61.5% in the number of treatments could have been achieved for the high and low risk scenarios, respectively (Table 2).

At the beginning of the 2005 growing season, the system estimated four primary infection events. Of these, only the first predicted infection was not confirmed by real infections in the vineyard. During the season, the system identified 11 days as risky for secondary infections and confirmed infections developed on 63.4% of those days. If treatments had been applied according to our decision support system, reductions of 37.0 and 46.8% in the quantity of copper applied and of 42.9 and 50.0% in the number of treatments could have been achieved for the high and low risk scenarios, respectively.

At the beginning of the 2006 growing season, the system predicted two primary infection events. The first of these predicted infections was not confirmed by events in the vineyard. During the season, the system identified 10 days as risky for secondary infections and infections developed on 50% of these days. If treatments had been

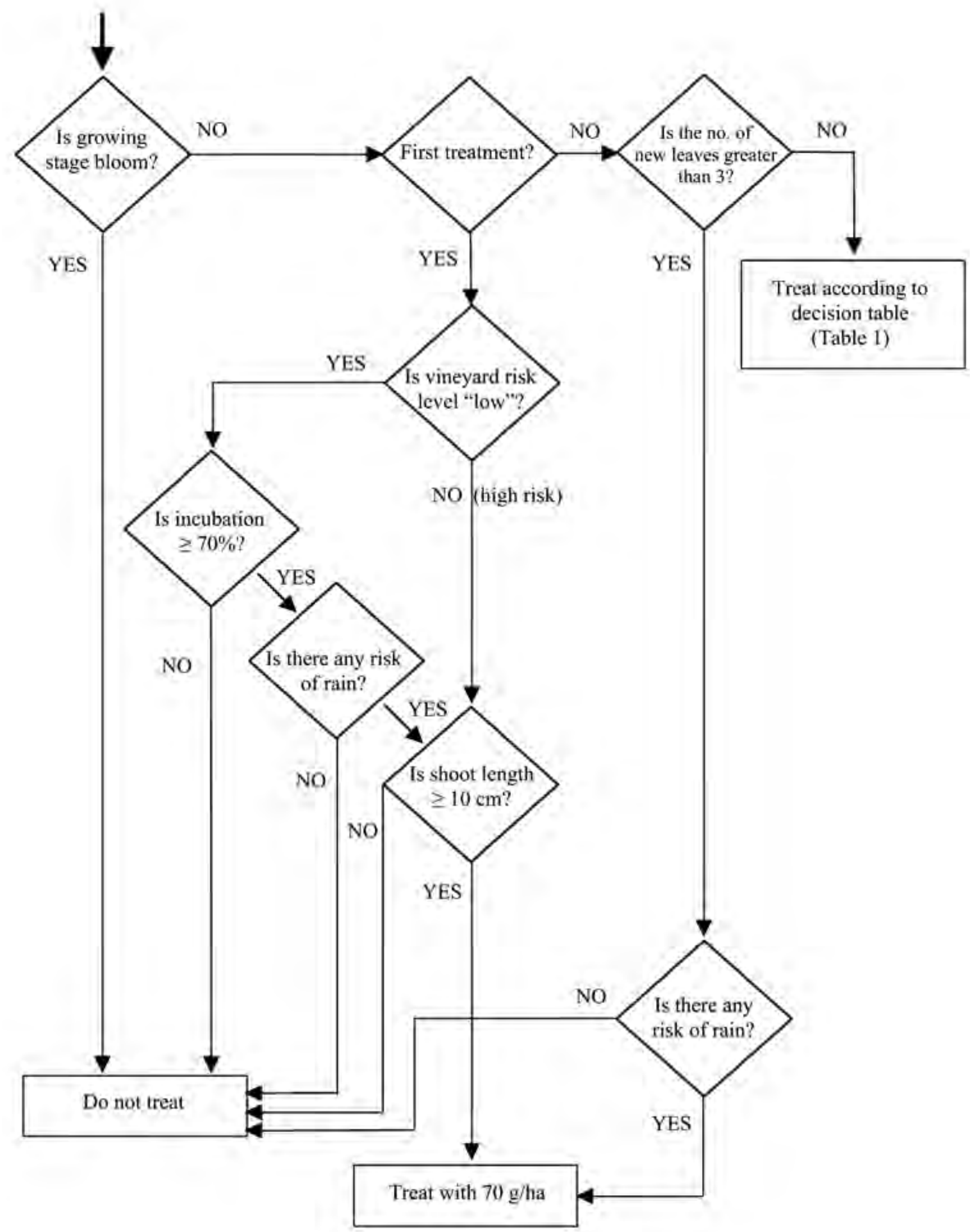

Fig. 2. Flow chart showing decision-making within the system.

applied according to the recommendations of our decision support system, reductions of 41.3 and 60.9% in the quantity of copper applied and of 38.5 and 53.8% in the number of treatments could have been achieved for the high and low risk scenarios, respectively.

The system was quite accurate (Table 2) in the sense that in both the simulations, with low and high levels of risk tolerance, no infections occurred without a preventive copper treatment having been recommended. Thus the system never recommended "no treatment" when a treatment was necessary. But in a few cases, much less often than for the common practice, a recommendation "to treat" was issued that was later determined to have been unnecessary.

System validation in organic vineyards. In 2007, in both Rovereto and San Michele all'Adige, the first downy mildew symptoms appeared on the leaves of the untreated control at the end of June and newly infected leaves emerged throughout the season. Symptoms appeared on clusters, especially in the summer. On developed berries, infection was observed as brown rot. In untreated plots in both vineyards, downy mildew damaged the crop to an unacceptable extent; almost all clusters were considered to be damaged when evaluated at harvest (incidence greater than 90%; Figs. 3 and 4). The common practice treatment program reduced the losses, but the amount of copper used exceeded the EU limit by 75% (Table 3). Using the application timings

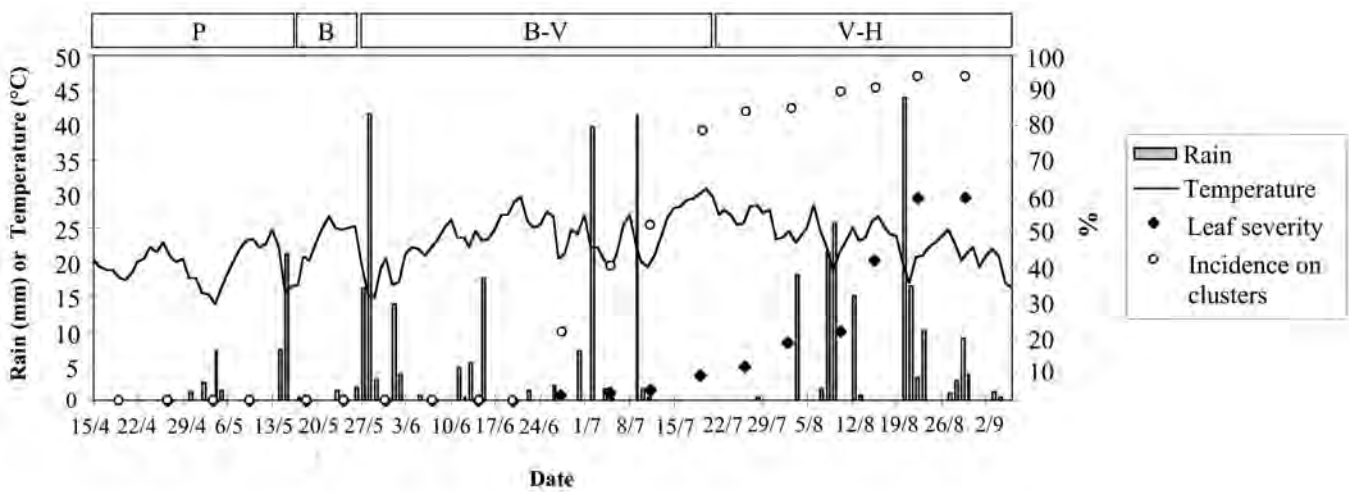

Fig. 3. Average daily rainfall, average daily temperature, severity of *Plasmopara viticola* infections on leaves and incidence of infections on clusters in S. Michele (2007) in the plots without any control measures. P = prebloom; B = bloom; B-V = between bloom and veraison; V-H = between veraison and harvest.

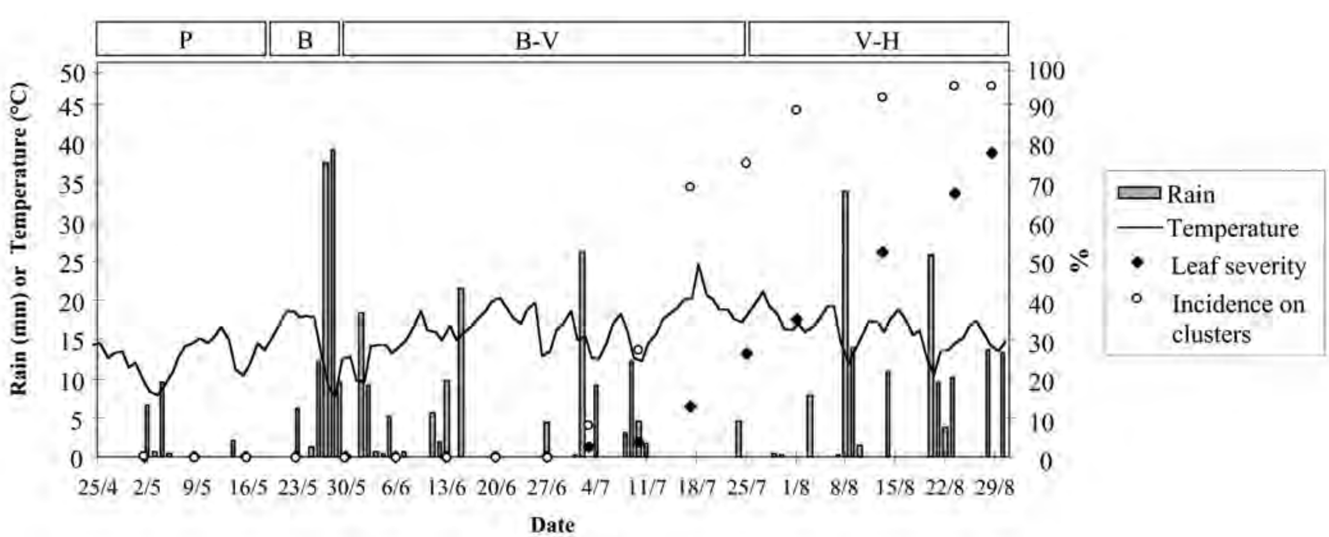

Fig. 4. Average daily rainfall, average daily temperature, severity of *Plasmopara viticola* infections on leaves and incidence of infections on clusters in Navicello (Rovereto, 2007) in the plots without any control measure. P = prebloom; B = bloom; B-V = between bloom and veraison; V-H = between veraison and harvest.

Table 3. Application rates of copper in the common practice (CP) treatment and in the treatment recommended by the system (SP), chance (risk) of rain the following day, accumulated rainfall since the last treatment (rainfall) and the average number of new leaves that had emerged since the last treatment in the two vineyards in which the system was tested in northern Italy in 2007.

Location	Date (day month^{-1})	Copper concentration (g ha l^{-1})		Risk of rain	Rainfall (mm)	New leaves
		CP	SP			
	03/05	0.7	0.7	high	0	0
	10/05	0.7	-			
	11/05	-	0.3	medium	11.4	2.4
	16/05	-	0.3	low	28.6	2.5
	17/05	0.7	-			
	24/05	0.5	-			
	25/05	-	0.3	high	1.8	2.0
	31/05	0.7	0.5	medium	62.4	1.8
S. Michele	07/06	0.7	-			
	09/06	-	0.3	medium	18.6	1.5
	14/06	0.5	0.3	low	10.4	1.3
	21/06	0.7	-			
	28/06	0.7	-			
	29/06	-	0.3	low	22.2	1.8
	03/07	-	0.5	medium	46.6	0.4
	05/07	0.7	-			
	12/07	0.7	-			
	19/07	0.5	0.3	low	45	0.4
	26/07	0.5	-			
Total copper (g/ha)*		9960	4560			
No. of applications		13	10			
	03/05	0.6	0.7	high	0	0
	09/05	0.6	-			
	16/05	0.6	-			
	18/05	-	0.7	low	12.4	5.3
	23/05	0.4	-			
	25/05	-	0.3	high	6.2	2.8
	30/05	0.6	0.7	high	113	2.0
	06/06	0.6	-			
	08/06	-	0.3	medium	34.4	1.9
Rovereto	13/06	0.6	-			
	14/06	-	0.3	low	17	1.8
	20/06	0.6	-			
	25/06	-	0.5	medium	21.4	1.8
	27/06	0.6	-			
	03/07	-	0.3	medium	30.8	1.3
	04/07	0.6	-			
	11/07	0.6	0.3	low	28.8	0.5
	18/07	0.6	-			
	26/07	0.6	-			
	01/08	0.6				
Total copper (g/ha)*		9840	4920			
No. of applications		14	9			

* The total amount of copper per hectare was calculated for a volume of 1200 l ha^{-1} per each spray

and rates prescribed by our decision support system resulted in fewer applications, as well as reductions in the total amount of copper applied. Instead of the 13 and 14 treatments in the common practice plots in the two vineyards, 10 and 9 applications were made in the decision support system plots, respectively. The first treat-

Table 4. Severity and incidence of downy mildew infections on leaves and clusters at harvest time in the two vineyards.

Vineyard	Treatment	Leaves		Clusters	
		Incidence (%)*	Severity (%)	Incidence (%)	Severity (%)
S. Michele	Common practice	16.5 a	5.0 a	22.0 a	5.2 a
	System recommendations	22.5 a	6.5 a	22.5 a	5.1 a
	Untreated	98.0 b	59.5 b	94.0 b	49.1 b
Rovereto	Common practice	24.5 a	8.0 a	28.5 a	7.3 a
	System recommendations	33.0 a	10.8 a	35.0 a	8.9 a
	Untreated	99.5 b	67.1 b	96.0 b	49.8 b

* Different lower-case letters within the same column and the same vineyard indicate significant differences according to Kruskal-Wallis test ($P \leq 0.05$).

ment was applied in both vineyards on the 3rd of May. In Rovereto (high risk), plants reached 10 cm of shoot length on the 18th of April and the first rain was forecast and happened on the 4th of May. In S. Michele all'Adige (low risk) all conditions for primary infection (3-10 rule and 70% of latent period) were fulfilled on the 15th of April and the next rain was forecast and happened with high risk on the 4-5th of May. No significant differences (Kruskal-Wallis, $P>0.05$) in disease incidence and severity between the common practice treatment plots and the decision support system plots were noted (Table 4). However, a slight increase in disease level was noted on the leaves for both treatments. At harvest time, disease incidences of about 20-30% on leaves and 25-35% on clusters were observed in both vineyards and for both treatments. The incidence of disease in untreated plots was significantly higher (Kruskal-Wallis, $P\leq0.05$) and reached, in both vineyards, more than 90% on clusters before harvest. Assessments of disease severity on leaves and clusters confirmed the results observed for disease incidence.

Assessment of must quality and copper residues. A decrease in grape quality can be detected in untreated plots through measurements of sugar content and acidity, although statistical analysis did not show significant differences between treatments (Kruskal-Wallis, $P>0.05$) (Table 5). In the untreated plots, a slight reduction in sugar content and increase in acidity, as compared to the common practice treatment plots and the system's recommendation treatment plots, could indicate a reduction in net photosynthesis. In any case, the values of sugar and acidity of grapes collected in treated and untreated plots were acceptable for vinification.

The legal maximum copper residue level (20 ppm for wine grapes in Italy) was never reached for grapes in any of the treatments. Copper residue levels were 1.8 and 1.4 ppm for the grapes treated according to the system's recommendations in S. Michele all'Adige and Rovereto, respectively (Table 6).

DISCUSSION

The treatments prescribed by the decision support system provided good control of downy mildew while significantly reducing the amount of copper applied per hectare, which was always below the European legal

Table 5. Quality values of musts (sugar and acidity) obtained at harvest from plots treated according to common practice, or according to the system's recommendations, and untreated plots in S. Michele (cv. Schiava) and Rovereto (cv. Cabernet Sauvignon) in 2007.

Vineyard	Treatment	Sugar (°Brix)*	Acidity (g l^{-1} tartaric acid)*
S. Michele	Common practice	18.1	6.6
	System recommendations	17.6	6.4
	Untreated	17.0	7.6
Rovereto	Common practice	21.7	8.6
	System recommendations	20.6	9.5
	Untreated	19.3	10.0

*No significant differences were present within the same vineyard according Kruskal-Wallis test ($P \leq 0.05$).

Table 6. Copper residues on leaves and grapes at harvest time in 2007 in plots at S. Michele (cv. Schiava) and Rovereto (cv. Cabernet Sauvignon) that were treated according the common practice and the system's recommendations.

Vineyard	Sample	Treatment	Copper residues (ppm)
S. Michele	leaf	Common practice	146.0
		System	81.0
	grape	Common practice	3.1
		System	1.8
Rovereto	leaf	Common practice	390.0
		System	85.0
	grape	Common practice	2.9
		System	1.4

limit. As the summer of 2007 was relatively wet, in drier years the advantages of timing the applications according to the system's recommendations could give even greater reductions in the amount of copper applied.

The proposed system is extremely easy to use. It requires simple observations of the vegetation (phenological stage and number of new leaves that have emerged since the last treatment), simple rain and temperature measuring equipment and daily logging of this data, and daily early-in-the-day checking of the weather forecast for the next two days. With this information, the grower can decide, on a daily basis, whether a copper application is advisable. The recommended treatments are customized for each individual vineyard and are based on the characteristics of the specific vineyard and treatments previously applied during the current season. By changing the values in the decision Table, the system could also be adapted to different situations, including new copper products and different levels of risk tolerance.

Recent trends in decision-support systems include a movement from growers making decisions for a single site toward consultants or managers compiling and integrating information to make decisions for multiple sites across diverse regions. There has also been a movement toward more complex, technologically based agriculture, with governments and processors regulating the use of pesticides and requiring that the use of these products be reported (Magarey *et al*., 2002). These trends often do not match the needs of small organic farms, which often are not included in broader pest management programs. In contrast to other more sophisticated decision-making systems, our system requests very little input data from the grower, so it can be easily adopted by small organic farms that are not under the supervision of a consultant.

Some limitations of the system should be noted. It is based only on copper applications, so if new alternatives to copper appear on the market, the system will need to be adapted accordingly. If a very low limit on copper applications is fixed in areas where downy mildew pressure is high, then the copper concentrations in the decision-making process will have to be reduced and the risk tolerance increased. Alternatively, the grower might choose to switch to disease-tolerant hybrids. The system is also not suitable for use on farms where the grower can only spray on certain days. In order to follow the system's recommendations, the grower must be ready to spray on any day.

The next step in our work is the development of a web-based version of this decision-support system to facilitate its wider use. In fact, only after several years of use will it be possible to evaluate growers' satisfaction with the system and its true efficacy under commercial conditions.

ACKNOWLEDGEMENTS

We thank C. Sicher for technical support and M. Ramponi for the chemical analysis. This research was funded by SafeCrop Centre, Fondo per la Ricerca, Autonomous Province of Trento, Italy (system development) and by the Ministero per le Politiche Agricole e Forestali, Italy (system validation).

REFERENCES

Agrios G.N., 1997. The downy mildews. In: Plant Pathology*,* pp. 278-283. 4th Ed. Academic Press, San Diego, CA, USA.

Baldacci E., 1947. Epifitie di *Plasmopara viticola* (1941-46) nell'Oltrepò Pavese ed adozione del calendario di incubazione come strumento di lotta. *Atti Istituto Botanico, Laboratorio Crittogamico* **7**: 45-85.

Blaise P., Dietrich R., Gessler C., 1999. Vinemild: An application-oriented model of *Plasmopara viticola* epidemics on *Vitis vinifera*. *Acta Horticulturae* **499**: 187-192.

Caffi T., Rossi V., Cossu A., Fronteddu F., 2007. Empirical vs. mechanistic models for primary infections of *Plasmopara viticola*. *Bulletin OEPP/EPPO Bulletin* **37**: 261-271.

CFIA, Canadian Food Inspection Agency, 2006. Organic Products Regulations. *Canada Gazette* **140 (35)**: 2502-2514.

Council of the European Union, 2007. Council Regulation (EC) No. 834/2007. *Official Journal of the European Union* **L 189**: 1-23.

Cravero S., Ferrari D., Crovella P., Bassignana E., 2004. Confronto tra diversi fungicidi rameici impiegati a basso dosaggio contro *Plasmopara viticola* con lo scopo di ridurre l' apporto di rame in viticoltura biologica. *Atti Giornate Fitopatologiche, Montesilvano 2004* **2**: 171-176.

Dagostin S., Ferrari A., Pertot I., 2006. Efficacy evaluation of biocontrol agents against downy mildew for copper replacement in organic grapevine production in Europe. *Bulletin OILB/SROP* **29 (11)**: 15-21.

Dalla Marta A., Magarey R.D., Orlandini S., 2005. Modeling leaf wetness duration and downy mildew simulation on grapevine in Italy. *Agricultural and Forest Meteorology* **132**: 84-95.

Egger E., D'Arcangelo M.E.M., Petrucci I., Lugugnani U., Valentini P., 1998. Efficacia del rame sotto forme e a dosi diverse nella difesa antiperonosporica della vite in Toscana. *Atti Giornate Fitopatologiche, Scicli e Ragusa 1998*: 531-536.

EPPO, European and Mediterranean Plant Protection Organization, 2004. EPPO standards PP 1/31(3). *Efficacy Evaluation of Fungicides & Bactericides* **2**: 47-49.

Ferrari D., Bassignana E., Pensabene G., 2000. Efficacy evaluation of different low-rate copper formulations and acupric compounds against grapevine downy mildew (*Plasmopara viticola*) in Piedmont (north-western Italy) during the period 1994-1999. IFOAM 2000-*The World Grows Organic. Proceedings 6th International Congress on Organic Viticulture, Basel, 2000*: 163-165.

Gobbin D., Jermini M., Loskill B., Pertot I., Raynal M., Gessler C., 2005. Importance of secondary inoculum of

Plasmopara viticola to epidemics of grapevine downy mildew. *Plant Pathology* **54**: 522-534.

Gobbin D., Pertot I., Gessler C., 2003. Genetic structure of a *Plasmopara viticola* population in an isolated Italian mountain vineyard. *Journal of Phytopathology* **151**: 636-646.

Goidanich G., 1964. Manuale di Patologia Vegetale, Vol. 2, Edizioni Agricole, Bologna, Italy.

Hill G.K., 1990. Plasmopara Risk Oppenheim - A deterministic computer model for the viticultural extension. *Convegno su Modelli Euristici ed Operativi in Agricoltura. Caserta, 1990*: 182-194.

Kennelly M.M., Gadoury D.M., Wilcox W.F., Magarey P.A., Seem R.C., 2005. Seasonal development of ontogenic resistance to downy mildew in grape berries and rachises. *Phytopathology* **95**: 1445-1452.

Kennelly M.M., Gadoury D.M., Wilcox W.F., Magarey P.A., Seem R.C., 2007. Primary infection, lesion productivity, and survival of sporangia in the grapevine downy mildew pathogen *Plasmopara viticola. Phytopathology* **97**: 512-522.

Madden L.V., Ellis M.A., Lalancette N., Hughes G., Wilson L.L., 2000. Evaluation of a disease warning system for downy mildew of grapes. *Plant Disease* **84**: 549-554.

Magarey R.D., Travis J.W., Russo J.M., Seem R.C., Magarey P.A., 2002. Decision support systems: Quenching the thirst. *Plant Disease* **86**: 4-14.

Ministero della Salute, 2004. D.M. 27 August 2004, Prodotti fitosanitari: limiti massimi di residui delle sostanze attive nei prodotti destinati all'alimentazione. *Gazzetta Ufficiale della Repubblica Italiana 292. Supplemento Ordinario* **179**: 3-238.

Morando A., Sozzani F., Moiraghi G., 2005. Rameici a dosaggio ridotto contro la peronospora della vite. *Informatore Agrario* **61(13)**: 67-69.

Nita M., Ellis M.A., Wilson L.L., Madden L.V., 2006. Effects of application of fungicide during the dormant period on Phomopsis cane and leaf spot of grape disease intensity and inoculum production. *Plant Disease* **90**: 1195-1200.

Orlandini S., Dalla Marta A., D'Angelo I., Genesio R., 2003. Application of fuzzy logic for the simulation of *Plasmopara viticola* using agrometeorological variables. *Bulletin OEPP/EPPO Bulletin* **33**: 415-420.

Park E.W., Seem R.C., Gadoury D.M., Pearson R.C., 1997. DMCAST: A prediction model for grape downy mildew development. *Viticultural and Enological Science* **52**: 182-189.

Pertot I., Delaiti M., Mescalchin E., Zini A., Forti D., 2002. Attivita antiperonosporica di nuove formulazioni di composti rameici utilizzati a dosi ridotte e prodotti alternativi al rame impiegabili in viticoltura biologica. *Atti Giornate Fitopatologiche, Baselga di Pine, Trento 2002* **2**: 297-302.

Pimentel D., Acquay H., Biltonen M., Rice P., Silva M., Nelson J., Lipner V., Giordano S., Horowitz A., D'Amore M., 1992. Environmental and economic costs of pesticide use *BioScience* **42**: 750-760.

Pontiroli R., Bergaglio S., Barbieri E., Delpero M., 2006. Riduzione dei dosaggi di rame nella difesa antiperonosporica della vite in Oltrepò Pavese. *Atti Convegno Internazionale Impiego del Rame in Viticoltura. E.R.S.A.F, Torrazza Coste 2006*: 17-26.

Rosa M., Genesio R., Gozzini B., Maracchi G., Orlandini S., 1993. PLASMO: A computer program for grapevine downy mildew development forecasting. *Computers and Electronics in Agriculture* **9**: 205-215.

Shtienberg D., 2000. Modelling: The basis for rational disease management. *Crop Protection* **19**: 747-752.

Siegfried W., Viret O., Bloesch B., Bleyer G. Kassemeyer H.-H., 2004. VitiMeteo Plasmopara ein neues Prognosemodell für den Echten Mehltau. *Schweizerische Zeitschrift für Obst- und Weinbau* **140 (23):** 10-13.

Strizyk S., 1983. Modèle d'état potentiel d'infection: Application à *Plasmopara viticola*. Association de Coordination Technique Agricole, Maison Nationale des Eleveurs: 1-46.

The Commission of the European Communities 2002. Commission Regulation EC 473/2002. *Official Journal of the European Union* **L 075**: 21-24.

Van-Zwieten L., Merrington G., Van-Zwieten M., 2004. Review of impacts on soil biota caused by copper residues from fungicide application. *Proceedings of the SuperSoil: 3rd Australian New Zealand Soils Conference. University of Sydney, Australia.* CD-ROM.

Wachtel M.F., Magarey P.A., 1997. The field use of a simulator for *Plasmopara viticola* to improve disease management for Australian grape growers. *Proceedings International Workshop on Grapevine Downy and Powdery Mildew Modelling. Freiburg 2004*: 193-194.

Wightwick A.M., Mollah M.R., Partington D.L., Allinson G., 2008. Copper fungicide residues in Australian vineyard soils. *Journal of Agricultural and Food Chemistry* **56**: 2457-2464.

Received January 21, 2009
Accepted September 2, 2009