Salvia officinalis Extract Can Protect Grapevine Against *Plasmopara viticola*

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ABSTRACT

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The ability of sage (*Salvia officinalis*) extract to control grapevine downy mildew under greenhouse and field conditions was tested. The persistence and rainfastness of sage extract were also investigated. Sage extract provided a high level of sustained disease control in artificially inoculated, potted grapevine under greenhouse conditions. However, even small amounts of simulated rainfall (10 mm) significantly reduced efficacy of sage extract. In a field experiment in 2006, sage extract provided 94% reduction in disease incidence and 63% reduction in area under the disease progress curve for disease severity on berries and leaves, respectively, reaching a level of disease control not significantly different from that provided by copper hydroxide. In 2007, the sage extract provided only a partial reduction (less than 30%) of downy mildew on leaves, probably as a result of a long rainy period between two of the consecutive treatments. Overall, sage extract effectively controlled grapevine downy mildew and could be a promising alternative to copper in organic viticulture. However, the low rainfastness of this treatment adversely affected its efficacy.

Plasmopara viticola (Berk. & M.A. Curt.) Berl. & De Toni is a grapevine pathogen found worldwide that can cause significant losses, especially in areas characterized by warm and wet conditions during the growing season. The pathogen attacks leaves and bunches, affecting both the size and quality of crop yields. *P. viticola* overwinters in soil as thick-walled oospores, which can germinate and release zoospores throughout the growing season (15). Secondary infections lead to explosive increases in disease development that continue until leaf fall. When susceptible plant tissues are moist, the pathogen can develop across a wide range of temperatures (10 to 30°C) (23). The most popular grape cultivars are highly susceptible to downy mildew. Fungicides are generally applied throughout the season. Contact fungicides are usually applied at weekly intervals in order to protect the newly grown vegetation.

Metallic copper compounds have been used against downy mildew since 1885 (25). Today, copper is the most important fungicide in organic agriculture because it is a compound originating from nature with a wide spectrum of activity. Recently,

copper has come under increased toxicological scrutiny, due to its ability to persist and accumulate in the soil and to harm soil microorganisms (12). For this reason, there is great interest in reducing copper applications or identifying alternative treatments; several alternatives to copper have also been studied in other plant-pathogen systems such *Malus* × *domestica*–*Venturia inaequalis* (2), *Solanum* spp.–*Phytophthora infestans* (6,14), and *Citrus* spp.–*Mycosphaerella citri* (42).

Aromatic plants and herbs have been extensively investigated as potential sources of natural compounds with medicinal and antimicrobial activity. The chemical composition and activities of members of the family *Lamiaceae* have been widely studied, including sage (*Salvia fruticosa*, *S. officinalis*, and *S. lavandulifolia*), oregano (*Origanum vulgare*), savory (*Satureja hortensis*), thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), lavender (*Lavandula latifolia* and *L. officinalis*), basil (*Ocimum gratissimum*), lemon grass (*Cymbopogon citratus*), peppermint (*Mentha* × *piperita*), and melissa (*Melissa officinalis*) (8–10,17,33,34,38). The antioxidant activity of members of *Lamiaceae* is mainly due to carnosic acid, carnosol, rosmarinic acid, and other phenolic compounds (38,45), whereas the fungicidal activity of these species seems to be due to molecules such as carvacrol, thymol, and *p*-cymene (36).

The genus *Salvia* (sage) includes nearly 900 species worldwide. These species are known for their pharmacological proper-

ties and have been used as food, flavoring agents, perfumes, and drugs since ancient times. The compositions of essential oils and extracts of *Salvia fruticosa*, *S. canariensis*, *S. argentea*, *S. spinosa*, African species (i.e., *S. verbenaca*, *S. radula*, and *S. dolomitica*), and *S. officinalis* have been reported (1,4,5,13,20,24,43). Each *Salvia* sp. has marked morphological and genetic variations that reflect their geographical origins, and these variations provide each extract with a specific fingerprint composition with distinct organoleptic properties (17). Moreover, the compositions of essential oils and extracts obtained from the same plant depend on the time of harvest and the specific plant organ (35). Essential oils and organic extracts of *S. officinalis* have been widely tested against human pathogens. Sage has been found to be effective against yeasts, dermatophytes, and bacteria in vitro (26,27,37). The effects of *S. officinalis* on phytopathogens such as *Botrytis*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Fusarium*, and *Phytophthora* spp. have been demonstrated in vitro (37,46,47). Disease control properties of sage extract against *Pseudoperonospora cubensis* (cucumber downy mildew), *Peronospora destructor* (onion downy mildew), *Bremia lactucae* (lettuce downy mildew), and *Phytophthora infestans* (tomato late blight) were demonstrated with preliminary in planta studies (6,30,40).

Our objectives were to evaluate (i) the efficacy of *S. officinalis* extract against grapevine downy mildew under greenhouse and field conditions compared with the control provided by copper hydroxide, (ii) the persistence of its disease control, and (iii) the rainfastness of the extract on plants, in relation to its effect on downy mildew.

MATERIALS AND METHODS

Preparation of the sage extract. Dried leaves of *S. officinalis* (200 g) from Dalmatia (Biokyma, Arezzo, Italy) were extracted with 99.8% ethanol (1 liter) in a Soxhlet extractor (Microglass Heim, Naples, Italy). After 8 h of extraction, the extract (800 ml) was concentrated to dryness under reduced pressure with a rotavapor (Laborota 4000/G3; Heidolph Instruments GmbH, Schwabach, Germany), and the residue was dissolved in the ethanol (180 ml) collected from the concentration process to minimize the loss of puta-

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tive active ingredients. Addition of distilled water (20 ml) was necessary in order to dissolve the water-soluble compounds. A fresh extraction was prepared from the same plant material before each treatment and stored at 4°C in the dark until use. Sage extract was applied in greenhouse and field trials at a final concentration of 50 ml/liter of water. This sage extract concentration was deduced from the effective concentration values of the sage extract against *Pseudoperonospora cubensis* obtained on cucumber potted plants under greenhouse conditions (*data not shown*).

Evaluations of sage extract activity, persistence of activity, and rainfastness under greenhouse conditions. In all of the greenhouse experiments, we used 2-year-old grapevine plants (*Vitis vinifera* cv. Pinot gris grafted onto Kober 5BB rootstock) in individual 2.5-liter pots of peat-rich prefertilized (NPK: 14-16-18) soil (Topfsubstrat D450; Stender, Schermbeck, Germany), which were grown under natural light at temperatures ranging from 18 to 28°C.

Copper hydroxide (Kocide 2000, 1.42 g/liter; Du Pont De Nemours, Wilmington DE) and water were used as the standard treatment and control, respectively. Sage extract, water, and the standard treatment were applied to plants with an air compressor system (Advance; Fini S.p.A., Bologna, Italy) equipped with a spray gun working at 400 kPa of pressure at the nozzle. Each plant received approximately 40 ml of treatment solution applied on all leaves to both adaxial and abaxial leaf surfaces.

In the initial experiments to evaluate the activity of the sage extract, the treatments (sage extract, copper hydroxide, and water) were applied 6 h before inoculation with the suspension of *P. viticola* sporangia. The solvent solo (ethanol) was included as control in the initial experiments. In further experiments to evaluate the persistence of activity over time, the treatments were applied once at 6, 3, 2, or 1 days or 6 h before pathogen inoculation. To evaluate rainfastness, plants were sprayed with sage extract, copper hydroxide, or water and then, 4 h after treatment, subjected to 0, 10, or 30 mm of simulated rain (30 mm/h) applied using 14 sprinklers positioned 2.2 m above the soil, protected from wind, and regulated to produce drops similar in size to raindrops (0.3 to 2.5 mm). After receiving the simulated rainfall, plants were left to dry (4 h) and then inoculated with the pathogen as described below.

All the treatments were applied to plants that had 10 to 15 fully developed leaves on one to three shoots. Three to five plants (replicates) were used for each treatment, so that there were at least 45 leaves per treatment. Each experiment (activity, persistence, and rain-fastness) was carried out twice.

Pathogen inoculation and disease assessment in greenhouse experiment. *P.*

viticola sporangia from infected leaves collected from untreated vineyards on various cultivars (Chardonnay, Pinot gris, Merlot, and Cabernet) in the Trentino region (northern Italy) were used as inoculum. Sporangia were washed from freshly sporulating lesions with cold (4°C) distilled water. An aqueous suspension of sporangia was diluted to a concentration of 10^5 to 10^6 sporangia/ml. For trials on potted plants, grape plants were inoculated by spraying the abaxial surface of each fully expanded leaf with the sporangia suspension (approximately 40 ml/plant), using the air compressor system working at 200 kPa of pressure at the nozzle. Inoculated plants were transferred to a growth chamber and kept in the dark for 12 h at $20 \pm 1^\circ\text{C}$ and relative humidity (RH) of 80 to 99%. The plants were then transferred to the greenhouse (temperature of 18 to 25°C, RH of 60 to 80%, and natural light regime), where they were kept for 10 days (incubation period). After the incubation period, plants were placed overnight (12 h) in a growth chamber at $20 \pm 0.5^\circ\text{C}$ and 99% RH to promote *P. viticola* sporulation. The percentage of leaf surface covered by sporulation was visually estimated on all leaves. Disease severity was evaluated for each treatment as the mean percentage of total leaf area covered by sporulating lesions per plant.

Evaluations of sage extract activity under field conditions. Field trials to evaluate the efficacy of the sage extract for controlling grapevine downy mildew were carried out in northern Italy (Rovereto; 45°53' N, 11°02' E; 204 m above sea level) in 2006 and 2007. The experimental vineyard was cultivated according to European organic production practices (Council Regulation No. 2092/91) and cv. Cabernet Sauvignon on Kober 5 BB rootstock was used. The vineyard was planted in 1999. A pergola trentina (2.80 by 0.91 m) trellis system was used. There were four replicates of each treatment. Each plot (replicate) had a surface area of approximately 18 m² and included six to eight plants.

The treatments were sage extract (applied at a rate of 60 liters/ha), copper hydroxide (1.2 kg/ha), and the water control. Products were dissolved in water and sprayed with an experimental atomizer (Waibl Diethart, Merano, Italy) using a spray volume of 1,200 liters/ha. Products were applied weekly, starting when each plant had three unfolded leaves (stage 13 of BBCH identification keys for phenological growth of *V. vinifera*). This growth stage was reached on 3 May in both years. In all, 18 treatments were applied in 2006 and 17 treatments were applied in 2007.

Activity against downy mildew was assessed based on the presence of disease symptoms or signs on leaves (oil spots or sporulation) and bunches (sporulation or necrosis). Disease severity was calculated as the mean percentage of leaf or bunch

area showing signs of infection and disease incidence indicated the number, expressed as percentage, of leaves or bunches showing signs of infection. For each replicate, 50 leaves and 50 bunches were evaluated from among the four to six most-central plants in each plot. The first assessment was carried out immediately before the second treatment was applied. Further assessments were made once a week before each treatment. Phytotoxicity was assessed throughout the season by checking for modification of leaf or berry color and necrosis on leaves and bunches.

Assessments of grape yield and juice quality were made on grape berries hand harvested on 17 October 2006 and 25 September 2007. Numbers of bunches and the total weights of bunches were recorded, and average yield per plant and average bunch weight were calculated for each treatment. The berries were then crushed and juice filtered. The percentage of sugars in the juice (grams per milliliter, expressed as °Brix) was measured using a digital refractometer (MTD-033; Three-In-One Co., Taipei, Taiwan). To determine total titratable acidity, water (40 ml) was added to the juice (7.5 ml) and, after the addition of the chemical indicator (bromothymol blue; Sigma-Aldrich, St. Louis), the juice was titrated with 0.1 N NaOH (Sigma-Aldrich). Acidity was expressed as grams per liter of tartaric acid. The pH of the juice was measured using a pH meter (pH 538; WTW GmbH, Weilheim, Germany).

Statistical analysis. Randomized complete block designs were used in all of the experiments. In the experiments performed under greenhouse conditions, the data for disease severity on leaves were analyzed. Repeated experiments showing nonsignificant treatment-experiment interactions (*F* test; *P* > 0.05) were pooled and compared. Reduction in disease (disease control) was calculated using the equation disease control = $100 (y_U - y_T) y_U^{-1}$, in which y_U and y_T are the levels of disease severity in the water control plots and the treated plots, respectively. Sage extract and copper hydroxide disease control values, assessed in the rainfastness and persistence experiments, were plotted against rainfall amount and days, respectively. Differences in disease severity in the sage extract and copper treatments for each volume of simulated rain were compared using Student's *t* test.

Area under the disease progress curve (AUDPC) values for disease severity on leaves over time and disease incidence on bunches at harvest time were considered in the analysis of the field data. AUDPC values were calculated according the equation

$$\sum_{i=1}^{n-1} (y_i + y_{i+1})(t_{i+1} - t_i) / 2$$

In this equation, *y* is disease severity at time *t*, *i* is the number of assessments, and *n* is the total number of assessments. The

interval between two subsequent assessments was 7 days.

Yield per plant (grams), average bunch weight (grams), sugar content (°Brix), pH, and titratable acidity (tartaric acid grams per liter) were used to evaluate crop quality in the different treatments.

Field data, including those for the water control and standard treatments, were normalized (log-transformed) and subjected to analysis of variance and Tukey's test ($\alpha = 0.05$).

All statistical analyses were performed using SPSS 15.0 (Chicago).

RESULTS

Evaluations of sage extract activity, persistence of activity, and rainfastness under greenhouse conditions. In each experiment conducted under greenhouse conditions, sage extract exhibited a high level of preventive activity, with an efficacy ranging from 79.9 to 95.8%, which was similar to that of copper hydroxide (80.5 to 96.0%). For both treatments, disease severities were significantly different from the water control. The solvent solo did not show any effect (no difference with water control) and, therefore, was not included in the following experiments.

In the persistence study, the disease severity of untreated controls was $53.6 \pm 1.8\%$ (standard error [SE]). Regression analysis did not indicate that the significant effect of the sage extract and copper on grapevine downy mildew decreased for up to 6 days after product application (Fig. 1). In the rainfastness study, sage extract showed a high level of disease control (about 80%) without rain, with disease severity in the untreated control being $56.4 \pm 4.5\%$ (SE). Regression analysis indicated that the level of disease control provided by the sage extract decreased proportionally to the rain amount following a logarithmic curve ($Y = -4.82\text{Ln}(X) + 57.90$; $R^2 = 0.99$; $P = 0.003$) (Fig. 2). Linear regression analyses did not indicate that disease control obtained with copper hydroxide decreased significantly with the increased amount of simulated rain ($Y = -0.3008X + 80.834$; $R^2 = 0.7879$; $P = 0.112$) (Fig. 2). Significant differences (t test, $P = 0.008$) were observed between the sage extract treatment and copper hydroxide treatment when the plants received 10 mm of simulated rain (29.76 ± 4.84 and $11.65 \pm 2.15\%$ disease severity, respectively). However, there were no significant differences between the sage extract and copper treatments at the other levels of simulated rain.

Evaluation of the activity of sage extract against downy mildew under field conditions. In 2006, the weather conditions were highly conducive for infection, starting from late July (Fig. 3A). The first disease symptoms were recorded on water control plants on 18 July. Disease severity on leaves of the water control developed rapidly, reaching 97% within 1 month. On

the sage-extract-treated plants, disease symptoms first appeared on leaves on 18 July but disease severity on leaves was low until 24 August. Between 8 and 20 August, there was a long period of rain and the treatment scheduled for 15 August was delayed until 19 August, resulting in 80% disease severity on leaves on 31 August. Copper hydroxide effectively controlled downy mildew, reducing the AUDPC of disease severity on leaves by 73% and reducing the incidence of disease on bunches of grape berries by 95% (Table 1). The results obtained for sage extract were not significantly different from those obtained for copper hydroxide. Applications

of sage extract caused a 63% reduction in AUDPC for disease severity on leaves and a 94% reduction in the incidence of disease on bunches (Table 1).

In 2007, weather conditions were highly conducive to infection throughout the growing season, particularly during August (Fig. 3B). The first symptoms appeared on leaves on 11 July. A moderate increase in disease was recorded during July. From August through the beginning of September, the severity of grapevine downy mildew on the leaves of the water control increased from 37 to 80%. By harvest time, the water control was defoliated. Copper hydroxide provided the most effective

Fig. 1. Persistence of the effect of *Salvia officinalis* extract on downy mildew severity on leaves of potted grapevine (cv. Pinot Gris) under greenhouse conditions. Sage extract and copper hydroxide were applied 6, 3, 2, or 1 days (1, 2, 3, and 6) or 6 h (0) before inoculation with *Plasmopara viticola* sporangia suspended in water (10^5 to 10^6 sporangia/ml). For each treatment, the average disease control value (based on disease severity reduction in the treated plots compared with the water control plots) for eight replicates (two pooled experiments with experimental factor not significant) and the standard error of the mean are represented. Solid line represents the regression line for copper hydroxide and the dashed line represents the regression line for sage extract.

Fig. 2. Effect of simulated rain on the effects of *Salvia officinalis* extract (sage) and copper hydroxide on downy mildew severity on leaves of potted grapevine (cv. Pinot Gris) under greenhouse conditions. Plants were treated with sage extract or copper hydroxide and were exposed to 0, 10, 30, or 50 mm of simulated rain before they were inoculated with *Plasmopara viticola* (10^5 to 10^6 sporangia/ml). Average disease control value (based on disease severity reduction in the treated plots compared with the water control plots) for eight replicates (two pooled experiments with experimental factor not significant) and the standard error of the mean are represented. Solid line represents the regression line for copper hydroxide and the dashed line represents the regression line for sage extract.

tive disease control. This treatment reduced downy mildew severity (AUDPC) by 79% and the incidence of disease on bunches by 74% (Table 1). Sage extract controlled the disease on leaves but not on bunches. On the sage-extract-treated leaves, the first symptoms occurred during the rainy period that lasted from 3 through 13 July. Until then, the sage extract provided 58% disease control compared with the water control (Fig. 3B). The level of control provided by the sage extract de-

creased to 21% as the disease spread more rapidly due to rain events in late August. Similarly, the sage extract provided 56% disease control on bunches (*data not shown*) until the end of the season, when the level of control fell to 37%. No symptoms of phytotoxicity were observed on leaves or bunches of any of the plants treated with sage extract or copper hydroxide in 2006 or 2007.

The assessment of grape yield and juice quality showed that treatments with sage

extract protected the crop and, in both 2006 and 2007, the yields of the extract-treated plants were as high or significantly higher than those from plants treated with copper hydroxide (Table 2). In 2006, the total acidity levels for the sage extract treatments were significantly lower than for the control and similar to those observed for the copper treatments. In 2007, there were no significant differences among treatments and control. In 2007, the sugar content of berries from the sage-treated plots was similar to that of the berries from the copper-treated and control plots. However, in 2006, the sugar contents of the berries from the sage-treated plots and the water treated plots were only 13.4 and 12.2°Brix, respectively, significantly lower than that of the copper-treated berries. These sugar contents were lower than requested for vinification and, if such berries should be used for wine production, the addition of supplemental sugar (i.e., concentrated must) would be necessary. However, our data indicate that, in terms of the parameters that were considered, the grape berries from the sage-treated plots were otherwise quite suitable for wine production.

DISCUSSION

In planta, the ability of *S. officinalis* to control diseases has been previously tested for late blight of potato (6) and downy mildew of cucumber, onion, and lettuce (30,40). This is the first report on the activity of *S. officinalis* extract against *P. viticola* on grapevine. The results obtained under greenhouse conditions were confirmed in trials conducted under field conditions. These results demonstrate that sage extract is a promising natural product for *P. viticola* control in organic viticulture. Its efficacy was almost comparable with that of a commercial copper fungicide. These results confirm the antimicrobial activity of sage that has been demonstrated in in vitro studies involving human pathogens (dermatophytes) and phytopathogenic fungi (33,37).

In experiments carried out under greenhouse conditions, the sage extract showed good persistence of action on grapevine leaves. Nevertheless, the performance of sage extract was strongly affected by simulated rainfall. Its low rainfastness can be attributed to its high solubility in water. This problem should be addressed when this crude extract has to be formulated. However, the level of activity did not decrease linearly as the amount of simulated rainfall increased, and most of the loss of activity was observed with low quantities of rain, whereas the effectiveness of the extract remained almost stable when rainfall was further increased. This typical tendency, expressed as extract residual efficacy, was described by a logarithmic function. Similar results have been noted for chemicals such as fenvalerate, mancozeb, and captan as they are washed off

Fig. 3. Trend of infection on leaves of the untreated plants (solid line), sage-treated plants (dotted line), and copper-treated plants (dashed line) and daily rainfall amounts (vertical bars). Treatments were applied at regular intervals of 7 days. Data were collected from June through September in **A**, 2006 and **B**, 2007 at the field site in Rovereto, Italy.

Table 1. Effects of sage extract, copper hydroxide, and the water control treatment on grapevine downy mildew in an organic vineyard in northern Italy^x

Treatment	2006		2007	
	AUDPC $\pm$ SE ^y	Incidence $\pm$ SE ^z	AUDPC $\pm$ SE	Incidence $\pm$ SE
Sage extract	619 $\pm$ 73 a	5.5 $\pm$ 2.6 a	1118 $\pm$ 110 b	62.5 $\pm$ 10.5 b
Copper hydroxide	448 $\pm$ 47 a	5.0 $\pm$ 1.7 a	380 $\pm$ 44 a	26.0 $\pm$ 5.4 a
Water control	1659 $\pm$ 96 b	99.0 $\pm$ 0.6 b	1825 $\pm$ 154 c	100.0 $\pm$ 0.0 b

^x Values followed by different letters are significantly different from one another (analysis of variance and Tukey's test, $\alpha = 0.05$).

^y Area under the disease progress curve (AUDPC) on leaves (percent $\times$ days) based on disease severity (percentage of leaf area with downy mildew symptoms); SE = standard error of the mean, $n = 4$.

^z Incidence on bunches (%) calculated as percentage of bunches with downy mildew symptoms.

from cotton plants, apple seedlings, and apple trees, respectively (18,28,41). Similar to what was reported by Fife and Nokes (11), the remaining active portion of extract is probably held in the leaf matrix and not easily dislodged by additional rain. Wash-off and efficacy losses are affected not only by the active ingredient and its formulation but also by drying time of the fungicide before the rain event (3,29). However, contradictory results have been reported for contact fungicides. Drying time is reported to affect the washing-off of chlorothalonil and mancozeb but not that of fluazinam (3,18,39). Moreover, the rain intensity and the mechanical impact of the rain droplets play important roles in the rainfastness of contact fungicides. A short-duration/high-intensity rain washes off significantly more fungicide than a long-duration/low-intensity rain (11,22). In our greenhouse experiments, we selected a short drying time (4 h) and a rain intensity similar to the average rain intensity observed in 2006, to better estimate the possible effect of rain on fungicide removal under field conditions.

Nevertheless, the results obtained under greenhouse conditions cannot always completely mimic the behavior of products under field conditions because of the different sensitivity of preparations to greenhouse and field conditions, such as light (photolabile compounds) and temperature (volatile and thermolabile compounds), or the rapid biodegradation of active molecules. Moreover, canopy density, redeposition of fungicide, dilution by new foliage growth, crop cultivar (leaf surface characteristics may affect leaf wettability), and retention of spray droplets should be considered in attempts to predict rainfastness (3,19,22).

Under field conditions, sage extract provided better downy mildew control compared with water control in 2006 than in 2007, both on leaves and on bunches. The same experimental vineyard was used in both years, with no change in the canopy density and similar meteorological conditions. The difference in the downy mildew control provided by sage extract in 2006 compared with 2007 might reflect not only the differences in rainfall patterns in the 2 years but also the different amounts of time between the applications and rain events. In 2006, the sage extract was not washed from the vine canopy until the first days of August, and this resulted in good

control of downy mildew. However, later heavy rains washed off the sage extract, allowing infection to occur. Thus, the first symptoms of disease on plants treated with sage extract appeared in the middle of August. The subsequent heavy rains caused a rapid spread of the disease on leaves but not on bunches. The very low incidence of disease on bunches at the end of the season could be explained as the consequence of the late development of the pathogen, which coincided with the acquisition of ontogenic resistance by the berries. It has been suggested that, during the growing season, the berries become less susceptible to pathogens as they age and stomata convert into lenticels, which then cannot serve anymore as an infection court (21). Nevertheless, *P. viticola* seems to be able to enter the otherwise resistant berry through the still-susceptible pedicel and proliferate internally, but are unable to sporulate through the stomata.

In contrast, in 2007, the early appearance of disease symptoms on the sage-treated plants suggested that the rain events during early fruit ripening in mid-June were sufficient to dislodge a large fraction of the sage extract so that infection could take place. Consequently, during the next rain period in the first days of July, infection on leaves as well as on the susceptible bunches occurred. The smaller amount of rainfall in the later part of the season allowed the sage extract to control infection spreading on leaves, even if it could not do so on bunches.

A formulation of sage extract with a high level of rainfastness should be developed for improving retention, enhancing spray adhesion, avoiding run-off, and encouraging an even spreading of active ingredients on the leaf tissue (44). Chemical adjuvants such as silicone surfactants, alkyl alkoxylate, and acryl polymers are well-known rainfastness enhancers. However, few adjuvants are permitted in organic crop protection and those that are permitted are generally less effective than conventional adjuvants (16).

As determined in preliminary experiments, a high concentration of sage extract is necessary to obtain good antifungal activity. This confirms the high concentration necessary for the practical antimicrobial activity of sage leaf extract (37,43,47). The large amount of starting material necessary to obtain a sufficient quantity of

extract for pathogen control in a commercial vineyard is one of the main economic limitations on the use of *S. officinalis* as an organic fungicide. To solve this problem, it might be useful to change the conventional extraction method. For example, Soxhlet, distillation, and maceration, which require large amounts of starting plant material and organic solvents, might be replaced with new techniques, such as pressurized hot water extraction. This method requires smaller amounts of starting material and, therefore, could be more convenient than the conventional method that we used (31).

Essential oil of Dalmatian sage is known to contain α - $\pm$ -thujone, camphor, 1,8-cineole, and *p*-cymene as its most dominant components (33). In general, ethanol extracts of *S. officinalis* contain the specific components that define the chromatograph profile of the essential oil (5). The ethanol content of the solution has the most pronounced effect on the recovery of sage bioactive compounds. At high ethanol contents (99.6%), there was a high recovery of the carnosolic compounds (lipophilic) and good recovery of essential oil but a low recovery of total phenols and rosmarinic acid (hydrophilic) (7). In particular, the ethanol extract obtained from sage leaves contains camphene, limonene, α - $\pm$ -thujone, camphor, bornyl acetate, and α -humulene while the flower extract contains high level of α -pinene and 1,8-cineole (43). Nevertheless, a range of lower molecular weight hydrocarbons were found to be absent in the Soxhlet extracts of *Origanum onites*; these compounds were probably lost during the extraction or in the solvent reduction steps (32). The observed in vitro fungicidal activity seems to be related to these components but contrasting results were obtained with thujone. In a study conducted by Pinto et al. (37), the highest level of antifungal activity corresponded to the lowest *cis*-thujone content, demonstrating that thujone content may not be related to the inhibition of fungal development. On the other hand, Papeijnjak et al. (33) confirmed that an oil sample with more α - $\pm$ -thujone than 1,8-cineole had a stronger effect against dermatophytes.

S. officinalis is considered to be a plant strengthener in Germany. However, further studies should be carried out to identify the chemical structure of the active ingredient or blend of ingredients with respect to its

Table 2. Mean grape yield per plant, bunch weight per cluster, sugars, pH, and total acidity for grape plants treated with sage extract, copper hydroxide, or water in an organic vineyard in northern Italy^y

Treatment	2006					2007				
	Yield (kg)	Weight (g)	Sugars (°Brix)	pH	Acidity ^z	Yield (kg)	Weight (g)	Sugars (°Brix)	pH	Acidity ^z
Sage extract	1.8 b	68.1 a	13.4 a	3.14 a	6.2 a	2.0 b	66.4 a	20.3 ab	3.78 b	5.9 a
Copper hydroxide	2.3 b	85.4 a	19.7 b	3.16 a	8.9 a	1.0 a	62.4 a	21.2 b	3.43 a	5.5 a
Water control	0.2 a	34.4 b	12.2 a	3.22 a	11.3 b	1.1 a	48.3 a	19.6 a	3.60 ab	6.2 a

^y Values followed by different letters are significantly different from one another (analysis of variance and Tukey's test, $\alpha = 0.05$).

^z Total acidity measured as grams of tartaric acid per liter.

effect on grapevine and *P. viticola*. Moreover, the identification of the active ingredients will allow the toxicological and ecotoxicological studies necessary for the registration of a sage-extract-based product in the European Union.

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