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Control of powdery mildew of grape in Greece using Sporodex® L and Milsana®

Wirkung von Sporodex® und Milsana® gegenüber dem Echten Mehltau der Rebe

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Summary

Six field trials were conducted in Greece to study the efficacy of two formulations of *Reynoutria sachalinensis* extract (Milsana® VP 1999 & 2001) and a formulation of *Pseudozyma flocculosa* (Sporodex® L) against powdery mildew (*Uncinula necator*) of grapes. In three trials where Milsana® was tested alone it was found that a) both formulations significantly reduced disease severity on berries, b) its efficacy was moderate to low but within the range of sulphur treatment alone and c) significant increase in yield was obtained in one out of two trials where yield was measured. In two field trials where Sporodex® L was tested it was found that a) it was effective on moderate to high disease pressure on bunches, but its efficacy declined when disease severity was extremely high and b) its efficacy was similar or inferior to that of sulphur alone. Alternated applications of Milsana® and Sporodex® L tested in one trial improved the efficacy of Milsana®, but not that of Sporodex® L. Alternation of Milsana® and Sporodex® L with sulphur did not result in significantly better efficacy than the stand alone applications of the control agents (one trial). The potential use of Milsana® or Sporodex® L in low input systems in grapes is discussed.

Key words: biological control, plant extract, *Pseudozyma flocculosa*, *Reynoutria sachalinensis*, *Uncinula necator*

Zusammenfassung

Sechs Feldversuche wurden in Griechenland zur Untersuchung der Wirksamkeit zweier Formulierungen von *Reynoutria sachalinensis*-Extrakten (Milsana® VP 1999 & 2001) und einer Formulierung von *Pseudozyma flocculosa* (Sporodex® L) gegenüber dem Echten Mehltau der Rebe (*Uncinula necator*) durchgeführt. In drei Versuchen mit Milsana® als alleinigem Präparat wurde gefunden, dass a) beide Formulierungen die Befallsstärke des Mehltaus an den Trauben signifikant verminderten, b) die Wirksamkeit gering bis moderat, aber innerhalb des Bereichs von Schwefelbehandlungen war, und c) eine signifikante Ertragssteigerung in einem von zwei Versuchen mit Ertragsmessungen erzielt wurde. In zwei Versuchen mit Sporodex® L wurde festgestellt, dass das Präparat a) bei moderaten bis hohen Befallsstärken an Trauben wirkte, während die Wirksamkeit bei sehr hohen Befallsstärken abnahm, und b) seine Wirksamkeit mit der einer alleinigen Schwefelbehandlung vergleichbar oder geringer war. Abwechselnde Behandlungen mit Milsana® und Sporodex® L erhöhten die Wirksamkeit von Milsana®, nicht aber von Sporodex® L in einem Versuch. Abwechselnde Behandlungen mit Milsana®, Sporodex® L und Schwefel in einem weiteren Versuch erhöhten die Wirksamkeit der entsprechenden Einzelbehandlungen nicht signifikant. Das Potenzial von Milsana® und Sporodex® L im Weinbau mit geringer Bewirtschaftungsintensität wird diskutiert.

Stichwörter: biologische Kontrolle, Pflanzenextrakt, *Pseudozyma flocculosa*, *Reynoutria sachalinensis*, *Uncinula necator*

1 Introduction

Powdery mildew caused by the biotrophic ascomycete fungus *Uncinula necator* (Schwein) Burrill is a widely distributed destructive disease of grape (*Vitis vinifera* L.). It can affect all stages of plant growth, without necessarily causing obvious symptoms, and may have a negative effect on the vine production in terms of quantity and quality (Halleen and Holz 2001). Cluster and blossom infection with *U. necator* before or shortly after bloom may result in poor fruit set and considerable yield loss (Evans et al. 1996) and a decrease in wine quality (Gump et al. 1996; Sawyer Ostrom et al. 1996). The pathogen follows a specific pattern in each part of the world to create an epidemic, which is determined by biological characteristics of the fungus, climatic factors, cultivation practices and cultivar choices.

Chemical control of the disease includes elemental sulphur and frequent applications of fungicides. Resistance of *U. necator* to selective fungicides like DMIs (Brent 1995) along with increased world-wide emphasis on the production of grapes with minimal fungicide input provides a strong and sound reason for exploring more consumer friendly, environmentally safe disease management strategies.

Pseudozyma flocculosa (former *Sporothrix flocculosa*), a basidiomycetous yeast related to anomorphs of Ustilaginales, is one of the most recent and efficient natural antagonists of powdery mildews to be identified (Bélanger and Labbé 2002). There are several reports on its efficacy against powdery mildews in cucumbers (Jarvis et al. 1989; Boekhout 1995), roses (Hajlaoui and Bélanger 1991) and wheat (Hajlaoui and Bélanger 1993). Cytological and macroscopic studies in cucumbers (Hajlaoui et al. 1992) indicated that *P. flocculosa* destroyed the integrity of host cell membranes, causing cell leakage, but did not appear to colonize host hyphae by penetrating its host. It was thus speculated that the antagonist acted by antibiosis. Chemical analysis of culture filtrates revealed the presence of at least four molecules with antifungal activity, three of which were closely related to fatty acids (Benyagoub et al. 1996; Choudhury et al. 1994). In two large scale studies, it was shown that Sporodex® L was more effective than AQ-10 (the commercial product based on *Ampelomyces quisqualis*) and *Verticillium lecanii* in controlling powdery mildew in cucumbers (Dik et al. 1998). Finally, the aforementioned formulation controlled powdery mildew on roses as well as chemicals and yielded flowers of better quality in

commercial greenhouses in Colombia and the Netherlands (Bélanger and Labbé 2002). The technical grade active ingredient *P. floculossa* strain PF-A22 UL and the associated end use pesticide BCA product Sporodex® L (Plant Products Co. Ltd., Brampton, OT, Canada) is now registered in Canada for the control of powdery mildew on roses and cucumbers and currently under registration in EU.

Research with plant extracts from *Reynoutria sachalinensis* (formulated extract under the name Milsana®) showed positive control of powdery mildew diseases in vegetables and ornamentals (Herger and Klingauf 1990; Daayf et al. 1995; Petsikos-Panayotarou et al. 2002; Von Amsberg and Watanabe 2002). The mechanisms by which *R. sachalinensis* extracts protect plants from powdery mildew were investigated in cucumbers and could be attributed to induced resistance. The defence against the pathogen was shown to be, among other processes, based on the involvement of reactive oxygen species directly upon priming with the inducing extract (Müller 2004) as well as on the build-up of phytoalexins in induced and infected plants with effects on conidial germination (Daayf et al. 1995, 1997a and 1997b). In addition, disorganization of the pathogen (extracellular and endocellular) including inhibition of haustoria formation or haustoria collapse was reported after localized rapid production of phenolic compounds in cucumber plants following Milsana® treatment (Wurms et al. 1999). Two liquid formulations of the extract, namely, Milsana® VP 1999 and Milsana® VP 2000, VP 2001 (lots of the same formulation) were studied in Greece and Germany and were found effective against cucumber powdery mildew (*Podosphaera xanthii* former *Sphaerotheca fuliginea*) (Petsikos-Panayotarou et al. 2002). All the above-mentioned formulations were tested against powdery mildew of grapes for a period of 3 years in organic farms in Germany and moderate disease control of *U. necator* was achieved equal to that of sulphur (Schmitt et al. 2002).

In preliminary small scale trials in Greece, it was shown that a) Milsana® (1% v/v) was highly effective (> 98.1%) against powdery mildew on leaves of potted vines, independently of the susceptibility of the cvs to the pathogen (cvs "Soultanina", "Black Corinth" and "Cabernet Sauvignon"-percentage of infected leaf area ranged from 45 to 62%) and the spray interval (7, 10 and 14 days), b) *R. sachalinensis* water extract had no effect on conidial germination of *P. xanthii* (tests on leaf discs) and on the length of primary or secondary hyphae, and c) Milsana® reduced conidial germination (about 80% at concentrations > 0.1%) of the pathogen on vine leaves over a period of 24 hours.

Based on these findings, the current study focussed on investigations of the efficacy of Milsana® and Sporodex® L against powdery mildew in grapes in the field. Experiments took place in commercial vineyards in Greece where environmental conditions are extremely favourable for the development of powdery mildew. Specifically, this study tested the efficacy of Milsana® and Sporodex® L applied as stand alone treatments, on leaves and bunches infection, in relation to a) rate, spray interval and timing of application, b) their combined efficacy and c) their efficacy when alternating treatments with sulphur.

2 Materials and methods

2.1 Efficacy of Milsana® against powdery mildew in the field

Three trials in two different grape cultivars were carried out over two years in 1999 and 2001 in the area of Peloponnese, aimed to test the efficacy of Milsana® in relation to:

a) Application rate (Peloponnese-1999). One trial was established in a commercial organic vineyard in Peloponnese (cv. "Black Corinth"). Plants were sprayed with a) water, b) Milsana® VP 1999 (0.5% and 1% v/v) and c) wettable sulphur (2 g l^{-1}, Bayer 80 WP) using a knapsack sprayer with medium volume spray. Each treatment was applied to four plots of 12 plants each (four plants x three rows). The first application of treatments was carried out when plants had reached the stage of the 5th–6th leaf. Milsana® and water were sprayed at 7 day intervals (12 applications in total), while sulphur was applied according to the recommendation of the Local Advisory Service (six applications in total). Plants (with no foliage support wires) were arranged in a complete randomised block design.

Percentage infected bunch area was assessed in a sample of 12 bunches/plot, which were cut into small clusters of 25–30 berries. Assessments were carried out with the use of a scale (1 = 0%; 2 = 0–5%; 3 = 5–25%; 4 > 25% infected area) according to BBA Guideline (Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln, 1988) at the beginning of ripening (BBCH 81). The number of berries/cluster in each class of the scale was recorded and mean infected bunch area per vine was hence calculated using the formula of Townsend-Heuberger (Townsend and Heuberger 1943). Additionally, the weight of grapes per vine was recorded at the time of harvesting.

b) Application interval (Peloponnese-1999). The efficacy of Milsana® VP 1999 (1% v/v) applied at 7, 10 and 15 day intervals was tested in one large scale trial carried out in a vineyard (cv. "Roditis") in the Plant Protection Institute of Patras (PPIP). Each treatment was applied to three plots of nine plants each (three plants with no foliage-support wires x three rows). Plots were arranged in a complete randomised block design. Milsana® applications started when plants had reached the stage of the 3rd–4th leaf. Plants were sprayed with medium volume spray with a knapsack sprayer (totally 16, 12 and 8 applications in 7, 10 and 15 days intervals, respectively). Disease assessment (the number of infected berries per bunch) was carried out in a sample of 21 bunches randomly selected from each plot (growth stage BBCH 81-beginning of ripening).

c) Formulation (bridge study, Peloponnese-2001). This trial was established in a commercial vineyard (cv. "Roditis") in Peloponnese. Plants were sprayed with a) water, b) wettable sulphur (2 g l^{-1}), c) Milsana® VP 1999 (1% v/v) and d) Milsana® VP 2001 (2% v/v) using a knapsack sprayer with medium volume spray. Applications of treatments started when plants were at the stage of the 5th–6th leaf. Both formulations of Milsana® were applied at 7 day and sulphur at 12 day intervals (15 and 10 applications in total, respectively). Plots (18 plants) were arranged in a complete randomised block design with four replicates per treatment.

Disease severity (% infected bunch area) was assessed on a sample of 32 bunches/plot (eight bunches/vine from four vines in the central row). Three assessments were carried out at the growth stages BBCH 73, 79 and 83. Average disease severity was calculated per plot. The influence of the treatments on yield [weight (kg) of grapes per vine] was also assessed at the time of harvesting.

2.2 Efficacy of Sporodex® L against powdery mildew

a) Under controlled conditions (growth chamber). Two experiments were carried out to test the efficacy of Sporodex® L against *U. necator* in young grapevines of the cv. "Soultanina" (susceptible to the pathogen). In the first experiment, plants of 20 cm height were used. Before treatment applications, all leaves were removed except for three younger leaves per plant. All thirty-two vines were artificially inoculated by a conidial suspension of the pathogen (1 x 10^6 conidia ml^{-1}), obtained from infected grapevine plants. Vines were divided into two groups of 16 plants each (four treatments x four plants). One group of 16 vines was sprayed with Sporodex® L (1.25, 2.50 or 5.00 ml l^{-1}) or water two hours after artificial inoculation, while the remaining 16 plants were sprayed with the antagonist and water, 5 days after inoculation. Hence, plants of the second group (+ 5 days) were sprayed with water twice, both on the day of inoculation and 5 days later. Inoculated plants were placed in a growth chamber set at 75 (± 5)

% relative humidity (RH) for the first 24 hours and 55 (± 5) % RH thereafter. Temperature was set at 22 (± 2)°C and light intensity was 20,000 lux on a 12/12 h light/dark photoperiod. In the second experiment, the same procedure was followed with a modification of the inoculum pressure. Potted vines were inoculated with a less concentrated conidial suspension (1 x 10^4 conidia ml^{-1}). Disease severity on all leaves was assessed in terms of percentage infected leaf area, 20 days after inoculation. Plots in both experiments were arranged in a complete randomised design.

b) In the field (Crete-1999). One experiment was conducted in a vineyard (cv. "Soultanina") in the farm of the Technological Education Institute of Crete (TEI). Plants (with no foliage support wires) were treated with a) water, b) Sporodex® L (2.5 and 5.0 ml l^{-1}) and c) wettable sulphur (Bayer 80 WP) at the recommended lower rate (1.5 g l^{-1}) using a knapsack sprayer with medium volume spray. Sulphur was sprayed according to the recommendation of the Local Advisory Service (four applications in total), while water and Sporodex® L at 7 day intervals (12 applications in total). Plots were arranged in a complete randomised block design with four replicates (15 plants/plot).

Assessments (in terms of % infected bunch area) were carried out, in a sample of 10 bunches/plot, at the growth stages BBCH 79 (berry touch completed) and 81 (beginning of ripening).

2.3 *Compatibility studies of Sporodex® L with Milsana®* in vivo *and* in vitro

The compatibility of Sporodex® L with Milsana® VP 2000 was tested on a) leaf tissues and b) in the tank mix. Leaf disks of the cv. "Soultanina" (12 disks per treatment x three replications) were sprayed on the upper side with a) Sporodex® L (5.0 ml l^{-1}) and b) Sporodex® L (5.0 ml l^{-1}) + Milsana® at different concentrations (0.1%, 0.3%, 0.5% and 1% v/v). One day after spraying, the leaf disks were homogenized with 10 ml of sterile distilled water and plated on triplicate Petri dishes (dilutions up to 10^{-3}) containing potato dextrose agar with 0.2% water soluble chloramphenicol (Sigma C3175). When distinct white colonies of *P. flocculosa* were observed, the number of colony forming units (cfu's) cm^{-2} of leaf surface was estimated. In addition, the compatibility of the BCAs in the tank, 30 and 60 minutes after mixing, was tested in all cases using the same spread plate technique.

2.4 *Efficacy of combined or alternated applications of Sporodex® L with Milsana® in the field (small scale trial)*

The trial was conducted in a vineyard (cv. "Roditis") located in the PPIP aimed to test the combined efficacy of Sporodex® L and Milsana® VP 1999 applied as stand alone treatments or in combination/alternation. The treatments applied were as follows: a) water, b) Milsana® (0.5% v/v), c) Sporodex® L (5 ml l^{-1}), d) Milsana® + Sporodex®, e) Milsana® alternated with Sporodex®. Treatments were applied using a knapsack sprayer with medium volume spray at 7 day intervals (12 applications in total). Powdery mildew incidence (number of infected berries/bunch) on a sample of 12 bunches, randomly selected and marked, from three plants per treatment was assessed twice (according to EPPO Guidelines) at the stage of fruit setting (BBCH 71) and at the beginning of ripening (BBCH 81). Plants were arranged in a complete randomised block design and each treatment was applied to five single-plant replicates.

2.5 *Efficacy of combined applications of Milsana® or Sporodex® L with sulphur in the field (Crete-2001)*

This experiment was conducted in the vineyard (cv. "Soultanina") of TEI Crete. Plants were arranged in a complete randomised block design with four replicates. Each plot consisted of eighteen plants. Treatments applied were as follows: a) water, b) Sporodex® L (5 ml l^{-1}), c) Milsana® VP 2001 (2% v/v), d) wettable sulphur (1.5 g l^{-1}, Bayer 80 WP) e) Sporodex® L + wettable sulphur and f) Milsana® + wettable sulphur. Milsana®, Sporodex® L and control plots were sprayed on a weekly basis (12 applications in total). Sulphur was applied four times during the experimental period, according to the recommendations of the Local Advisory Service. Applications of the biocontrol agents were initiated early in the growing season, prophylactically. In the plots where the alternated treatments were applied the procedure followed is described below.

Applications were halted when there was a recommendation for sulphur. In the mid-time between two recommendations and at least one week after sulphur application (residual action of sulphur), treatments with the BCAs were initiated and continued at 7 d intervals.

Disease severity on bunches was assessed on a sample of 20 bunches/plot (5 bunches/vine from the middle four vines). Two assessments were carried out at the following growth stages: BBCH 71 (fruit set, young fruits begin to swell, remaining of flower lost) and BBCH 81 (beginning of ripening, berries begin to brighten in colour). Average disease severity was calculated per plot.

2.6 *Environmental data*

Temperature and relative humidity data were obtained from nearby standard weather stations. At Patras (Peloponnese), data were collected within 100 m of the PPIP and approximately 10 km from the commercial vineyards where trials were conducted. In Heraklion (Crete) data were collected approximately 5 km from the vineyard site and about 50 m lower, at the airport.

2.7 *Statistical analysis*

Data on disease severity (mean percentage infected leaf or bunch area) were arcsine transformed when needed and were subjected to Analysis of Variance (ANOVA), (GENSTAT, 8.0 for Windows) at $P \leq 0.05$. The comparison of treatment means were tested using Duncan's multiple range test.

3 Results

3.1 *Efficacy of Milsana® against powdery mildew in the field*

a) In relation to the application rate (Peloponnese-1999). Both Milsana® VP 1999 rates (0.5% and 1% v/v) significantly reduced disease severity on bunches of the cv. "Black Corinth". When the higher rate was used, a higher disease reduction on grapes was achieved compared to the lower one and similar to that of sulphur (Table 1). Also yield was significantly increased compared to the control by Milsana at the rate of 1% v/v and sulphur. (Table 1).

b) In relation to the application interval (Peloponnese-1999). Milsana® VP 1999 significantly reduced the number of infected berries/bunch of the cv. "Roditis" regardless of the application intervals. The highest disease reduction was achieved when Milsana® was applied at 7 day intervals (Table 2).

c) In relation to the formulation (bridge studies, Peloponnese-2001). Both Milsana® formulations and sulphur were effective against powdery mildew on bunches of the cv. "Roditis". Milsana® formulations were equally effective with sulphur. No differences in yield were obtained (Table 3). The 'absolute infection rate' [difference in % infected area/no of days between two successive assessments] in Milsana plots ranged from 1.9 to 2.1 (1^{st}–2^{nd} assessment) and 0.85 to 1% day^{-1} (2^{nd}

Table 1: Powdery mildew severity on grape bunches (% infected area) and yield (kg of grapes vine^{-1}) in the cv. "Black Corinth", after treatment with Milsana® (VP1999) and wettable sulphur (Peloponnese, Greece, Field Trial-1999)

Treatment	Mean % infected bunch area (BBCH-81)[a]	Yield (kg vine^{-1})
Control	82.1 a*	24.8 a
Milsana® (VP1999) 0.5%	71.8 b	30.9 ab
Milsana® (VP1999) 1%	63.1 c	34.0 b
Wettable sulphur	68.4 bc	37.0 b
F prob.	< 0.001	0.05

[a] BBCH scale (growth stage identification keys for mono- and dicotyledonous plants): 81 – beggining of ripening, berries begin to brighten in colour (BBA, BSA, IVA, AgrEvo, BASF, Bayer, Novartis).
* Means with the same letter, within the same column, are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Table 2: Powdery mildew incidence on grape bunches (% infected berries bunch^{-1}) in the cv. "Roditis" treated with Milsana® (VP1999) at 7, 10 and 15 day intervals (Peloponnese, Greece, Field Trial-1999)

Treatment	(%) infected berries/bunch (BBCH-81)[a]
Control	98.4 a*
Milsana® at 7 d. intervals	45.5 d
Milsana® at 10 d. intervals	60.3 c
Milsana® at 15 d. intervals	81.5 b
F prob.	< 0.001

[a] BBCH scale (growth stage identification keys for mono- and dicotyledonous plants): 81 – begginnig of ripening, berries begin to brighten in colour (BBA, BSA, IVA, AgrEvo, BASF, Bayer, Novartis).
* Means with the same letter, within the same column, are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

Table 3: Severity of powdery mildew on grape bunches and yield in the cv. "Roditis" treated with two formulations of Milsana® and wettable sulphur applied as stand alone treatments (Peloponnese, Greece, Field Trial-2001)

Treatments	Mean % infected bunch area			
Treatments	BBCH[a]-73	BBCH-79	BBCH-83	Yield (kg vine^{-1})
Control	5.6 a*	80.1 a	85.8 a	8.24
Milsana® (VP 1999) 1%	0.9 bc	45.7 b	68.6 b	10.58
Milsana® (VP 2001) 2%	0.6 c	39.8 b	65.6 b	9.64
Wettable sulphur**	1.7 bc	53.4 b	74.8 b	10.45
F-prob	< 0.001	< 0.001	0.004	0.25

[a] BBCH scale (growth stage identification keys for mono and dico-tyledonous plants): 73 – berries groat sized, bunches begin to hang; 79 – berries touch completed; 83 – berries begin to brighten in colour (BBA, BSA, IVA, AgrEvo, BASF, Bayer, Novartis)
* Means with the same letter, within the same column, are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)
** Formulation: Bayer 80 WP (sulphur 80% w/w)
No of days between successive assessments 21 (1st–2nd) and 27 days (2nd–3rd)

to 3rd assessment) while the respective values of the control were 3.5 and 0.2% day^{-1}. Parameters of this equation are presented in Table 3.

3.2 *Efficacy of Sporodex® L against powdery mildew*

a) Under controlled conditions. In the first experiment (high inoculum pressure), timing of application was shown to be important since Sporodex® L at all rates was more effective when applied on the day than 5 days after artificial inoculation (Fig. 1 A). Under conditions of high inoculum pressure no rate effect was observed in any case. On the contrary, in the second experiment (low inoculum pressure), Sporodex® L was effective regardless of the time of application and there was a rate effect, with Sporodex® L 5 ml l^{-1} being the most effective treatment (Fig. 1B) of all. Mean efficacy of Sporodex® L calculated from all experiments was as follows: 66.5% (median 73.6), 55.7% (median 59.6) and 43.5% (median 45) for the rates of 5, 2.5 and 1.25 ml l^{-1}, respectively. Analysis of all available data (two experiments) also showed that there were marginally no significant differences in efficacy among rates (F-prob. 0.061). Differences in infection levels between controls of the group 0 and that of + 5 days were observed in both experiments, due to the additional water application, which coincided with the day of inoculation, in the second group of plants (Fig. 1).

b) In the field (Crete-1999 and 2001). It was shown that Sporodex® L applied at 5 ml l^{-1} was significantly different from the control, in both trials and equally effective to sulphur against the pathogen under conditions of low disease severity (Trial 2001). However, Sporodex® L at the rate of 2.5 ml l^{-1} (trial of 1999) did not differ from the controls (Table 4).

3.3 *Compatibility studies of Sporodex® L with Milsana®* in vivo *and* in vitro

The number of viable cfu's cm^{-2} of *P. flocculosa* on leaf discs of the grapevine cv. "Soultanina " *in vivo* was significantly affected by Milsana® VP 2000 at concentrations ≥ 0.5% v/v (48.8 cfu's cm^{-2}) in comparison to the controls (80.22 cfu's cm^{-2}) (P= 0.004, data not shown). This toxic effect was also evident when tank mix suspensions were plated out *ca.* 60 min after the preparation [decline of cfu's ml^{-1} from 2.53 x 10^5 (control) to 7.7 x 10^4 (Milsana® VP 2000, 1% v/v)].

3.4 *Efficacy of combined or alternated applications of Sporodex® L and Milsana® in the field (small scale trial)*

Infected bunch area in the controls of the cv. "Roditis" was extremely high reaching the level of 84.7% (Fig. 2). Mean disease reduction achieved by Milsana® VP 1999 (0.5% v/v), and Sporodex® L (5 ml l^{-1}) applied as stand-alone treatments was 20% and 38.7%, respectively. The combination or alternation of Milsana® with Sporodex® L increased the efficacy of Milsana® but not that of Sporodex®.

3.5 *Efficacy of alternated applications of Milsana® or Sporodex® L with sulphur in the field (Crete-2001)*

There were no significant differences in efficacy (P= 0.64) among treatments on the bunches of the cv. "Soultanina under extremely low disease pressure (4% infected bunch area). In the 2nd assessment, under moderate disease pressure,

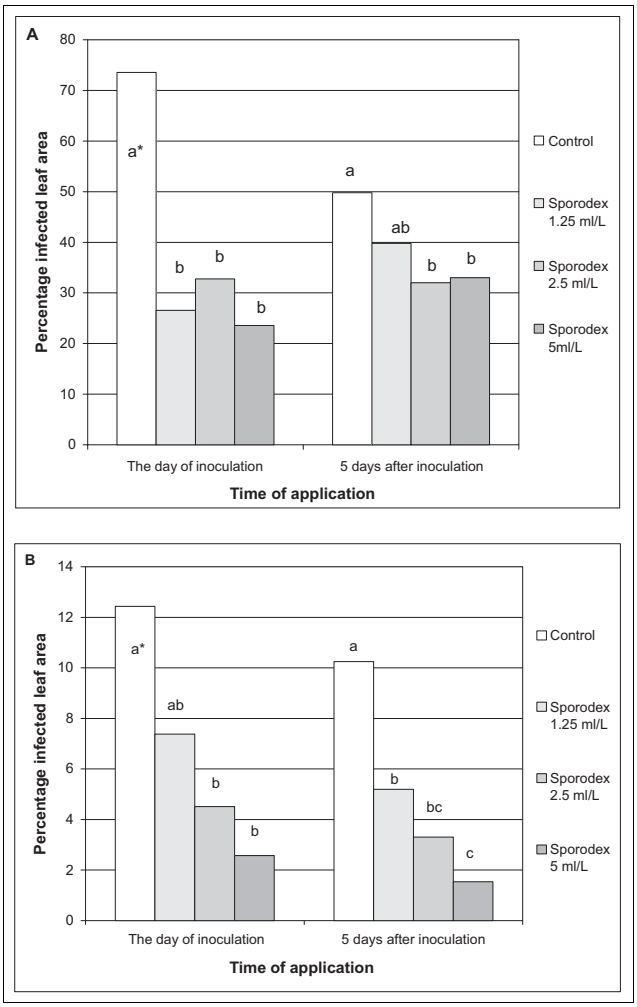

Fig. 1: Powdery mildew severity on leaves of the grapevine cv. "Soultanina" (% infected leaf area) treated with different rates of Sporodex® L on the day (0 d.) and 5 days (+ 5 d.) after inoculation of potted vines with A: 1 x 10^6 and B: 1 x 10^4 conidia ml^{-1}.
A: F-prob. < 0.01 for 0 d.; F-prob. < 0.01 for + 5 d. B: F-prob. 0.016 for 0 d.; F-prob. < 0.001 for + 5 d
* Means with the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Sporodex® L (5 ml l^{-1}) was equally effective to sulphur and Milsana® while the plant extract was not different to the control (Table 4). Their alternation with sulphur did not result in significant changes in efficacy.

3.6 Environmental data

Environmental data (mean monthly temperature and relative humidity) in Crete and Peloponnese where field trials were conducted in the years 1999, 2000 and 2001 are presented in Table 5.

4 Discussion

Reports on the efficacy of Milsana® and Sporodex®, against *U. necator* are very limited. In several trials conducted in commercial vineyards in Germany (Schmitt et al. 2002) it was found that the efficacy of Milsana® against powdery mildew of grapes was similar to that of sulphur. In the field trials conducted in Peloponnese, in the frame of the current study,

Table 4: Severity of powdery mildew on grape bunches (cv. "Soultanina") treated with Sporodex® L and Milsana® applied as stand alone treatments or alternated with wettable sulphur (Crete, Greece, Field Trials-1999 and 2001)

	Crete-1999		Crete-2001	
Treatment	BBCH[a]-79	BBCH-81	BBCH-71	BBCH-81
Control	71.7 a*	85.4 a	4.3	23.7 a
Sporodex® L 2.5 ml l^{-1}	55.9 a	76.5 a	–	–
Sporodex® L 5 ml l^{-1}	23.9 b	39.8 ab	1.3	5.7 b
Milsana® (VP 2001) 2%	–	–	3.6	14.6 ab
Milsana® 2% alt. sulphur	–	–	3.3	10.5 b
Sporodex® L 5 ml l^{-1} alt. sulphur	–	–	2.0	8.6 b
Wettable sulphur**	2.9 c	3.1 c	0.9	7.5 b
F-prob.	0.043	0.02	0.64	0.049

[a] BBCH scale (growth stage identification keys for mono- and dicotyledonous plants): 71 – fruit set, young fruits begin to swell, remaining of flower lost; 79 – berrie touch completed; 81 – begging of ripening, (BBA, BSA, IVA, AgrEvo, BASF, Bayer, Novartis)
* Means with the same letter, within the same column, are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Milsana® was nearly as effective as sulphur (applied according to label recommendations) in controlling grape mildew infections regardless of the application rates and different formulations and lots. Nonetheless, Milsana® tended to be more effective when applied at higher rates and at shorter application intervals. On the contrary, in Crete, its efficacy (one trial) was lower than that of sulphur. Differences in efficacy between locations might be attributed to the different prevailing environmental conditions and their possible effect on the pathogen and the response of the host (highly susceptible cv.). The overall efficacy of Milsana® (in both areas), was calculated to be ca 25% (by regressing % infected bunch area in Milsana® on the controls). Its efficacy ranged from 13 to 83% in all assessments in a total of 5 trials in three different years and two locations.

Moreover in this study, it was found that Milsana® a) was most effective at the early stages of the disease development (efficacy approx. 89%) – when berries were groat sized and bunches began to hang (BBCH 73, Table 3) – while later on, efficacy started to decline with the 'absolute infection rate' close to ripening, being higher than that of the control b) had a positive effect on the weight of harvested grapes despite of infection level, but the observed increase of yield was not always statistically significant (based on F-values).

The induced resistance properties of this extract have been extensively studied on cucumbers (annual plants) and its efficacy against tomato and cucumber powdery mildews has been proven (see introduction). The extent to which a known inducer of resistance could be used for the control of powdery mildew in grapevine which is a perennial crop was considered to be of scientific importance. Stimulation of a plant's defence mechanism(s) against disease is a relatively new concept in plant protection of grapes. Today, several reports have been published on the activation of defence responses in grapevine following the application of an elicitor and their potential use for the control of fungal foliar diseases (screening and testing compounds in potted vines), but little research has been carried out in large scale where the host, the environment and the pathogen interact in a different way. In this field, it had been shown that an oligosaccharide (OS) induced resistance in grapevine (by acting rather like a priming agent than a true elicitor) against *Plasmopara viticola* but its efficacy was signif-

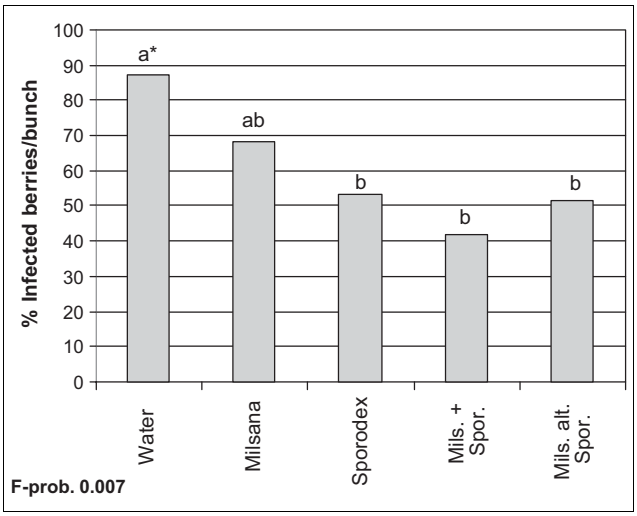

Fig. 2: Powdery mildew incidence on grape bunches (% infected berries bunch^{-1}) in the cv. "Roditis" after single, combined or alternated applications of Sporodex® L (5 ml l^{-1}) and Milsana® (VP1999) 0.5% in small scale (Peloponnese, Greece, Field Trial-2000).
* Means with the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Table 5: July to August mean monthly temperatures and relative humidities at each site in each year

		Temperature (°C)		Relative humidity (rh-%)	
Site	Year	July	August	July	August
Crete	1999	26.4	27.3	60.6	56.5
	2000	26.8	27.8	44.3	37.2
	2001	26.7	28.0	57.8	55.0
Peloponnese	1999	26.1	27.1	63.0	57.0
	2000	26.4	25.7	53.0	59.0
	2001	27.0	26.6	57.0	61.0

icantly influenced by leaf age (Trouvelot et al. 2006). Researchers in Israel also reported the control of downy mildew in grapevine via induced resistance in experimental systems (Cohen et al. 1999; Aziz et al. 2003) and in the field (Reuveni et al. 2001). Riches and Holmes (2005) showed that a plant nutrient and chitosan controlled *P. viticola* on leaves under field conditions (artificial inoculations), at flowering. In the case of the plant nutrient, there was evidence of systemic activity consistent with an induced resistance response while chitosan was found to act via local acquired resistance (LAR). In addition, both agents were shown to have contact fungicide activity. In Greece, there is only one report where the registered elicitor laminarin was shown to reduce the percent powdery mildew infected leaf area (by 66%) and the percent of infected berries (by 90%) when berries began to brighten in colour (Lefranc and Joubert 2004).

Data for the induction of defence responses associated with Milsana® in grape vine are not yet available. However, induced resistance was proven as mode of action of Milsana® in cucumber before (Müller 2004; Daayf et al. 1997a,b; Wurms et al. 1999; McNally et al. 2003; Schmitt 2002).

Efficacy of Sporodex® L in the growth chamber depended on inoculum pressure. In the field Sporodex® L was moderately effective on bunches. Its overall efficacy was ca 59% (range from 53–75%). The highest efficacy was achieved under conditions of low disease pressure. The activity of *P. flocculosa* depends on relative humidity (RH). It requires > 70% RH in order to survive and act on the phylloplane (Bélanger and Labbé 2002). In the trials presented, mean daily RH ranged from 37.2 to 61% during July and August when grapes were close to ripening. Detailed environmental data showed that RH was above the threshold of 70% only for certain hours per day (mainly during nights). Sporodex® L was applied in the field late in the afternoon to enhance its chance of survival on the phylloplane, but in Greece, environmental conditions were rather unfavourable for its optimal performance. At a molecular level it has been shown that *Pseudozyma* acts via its secondary metabolites (mainly three unsaturated fatty acids) which due to their unusual structure can rapidly partition into fungal membranes. Kates (1986) showed that lipids are generally labile towards peroxidation and hydrolysis. This indicates that under Mediterranean natural conditions especially with the relatively high temperature and humidity unsaturated fatty acids will variably oxidise. In literature, there are several reports on environmental conditions, which regulate the survival and action of microbial fungicides *in situ* targeted to the control of powdery mildews (Paulitz and Bélanger 2001).

The first series of experiments (in 1999) and the fact that disease control was declining toward ripening of fruit indicated that it would not be advisable to rely on the stand alone applications of an inducer of resistance (Milsana®) to control powdery mildew in organic grape vines under Mediterranean conditions. At the same time, the application of Sporodex® L as stand-alone treatment appears to be not sufficiently reliable under the given conditions. Combination or alternation of Milsana® with the biological agent Sporodex® L or a chemical agent (sulphur/alternation) showed, in Peloponnese-2000 and Crete-2001 respectively, that the efficacy of Milsana® was improved. Similar findings were reported for the combination of Milsana® with another non-commercialised BCA, *Brevibacillus brevis* (Schmitt and Seddon 2005).

In conclusion, Milsana® or Sporodex® L could be potentially used in organic farming and/or in IPM systems in grapes, under certain circumstances in the Mediterranean region (e.g epidemiology of the disease, cost-efficiency of these novel compounds compared to standard sulphur and/or conventional preventive fungicides, impact on the environment, public health etc). Since their efficacy is expected to be lower than that of fungicides and highly dependent on disease pressure and prevailing environmental conditions, they could be applied either before or at the onset of the disease and also in suitable spray programmes possibly close to harvest where residues is of critical importance. As a general approach it could be mentioned that these alternatives could be used as a tool to minimise the use of conventional fungicides to the absolutely necessary applications, in line with the principles of food safety as required by the EU, stakeholders and consumers.

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