

Efficacy of Milsana[®], a formulated plant extract from *Reynoutria sachalinensis*, against powdery mildew of tomato (*Leveillula taurica*)

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Abstract. The efficacy of Milsana[®] VP 1999 and 2000 (a formulated plant extract of *Reynoutria sachalinensis*), known to induce resistance to powdery mildew on cucumbers, was tested against *Leveillula taurica* (Lév.) Arn. on greenhouse tomato. In four out of five trials, Milsana[®] achieved a disease reduction ranging from 42.2 to 64.6%. In one trial only, its efficacy was exceptionally low (23%). Application rates and disease pressure proved to be the main factors affecting the level of control. Milsana[®] was significantly less effective than fungicides (alternated DMIs and penconazole) *in situ*. In contrast, Milsana[®] was equally effective to wettable sulphur indicating that its effect was rather preventive than curative. The level of efficacy achieved by either Milsana[®] or fungicides did not result in a significant increase of yield. Laboratory tests showed that Milsana[®] (VP 1999) had a direct effect on conidial germination. Whether this effect significantly contributes to its field efficacy, remains to be elucidated. Overall, results indicate that Milsana[®] could play an important role in disease management of powdery mildew in organic and low input tomato production.

Key words: biological control, disease severity, *Leveillula taurica*, plant extracts, yield

Introduction

Several species of powdery mildew fungi have been reported to occur in tomato (Mielserová and Lebeda, 1999). One of these species, *Leveillula taurica* (Lév.) Arn, is endemic in the Mediterranean region and Central Africa but is gradually spreading to new regions as supported by publications on the fungus' first appearance in tomato in Germany (Amelung, 1990) and Mexico (Garcia-Estrada et al., 1993).

Leveillula taurica is an endoparasite forming both endophytic and epiphytic mycelium with branched conidiophores growing through stomata (Mielserová and Lebeda, 1999). In tomato, the main symptoms are yellow lesions on the upper leaf surface with a powdery sporulation appearing on the lower surface. Severe infections may result in considerable damages. In Canada, *L. taurica* infections resulted in serious yield losses in greenhouse tomatoes (Cerkauskas et al., 2000), while in field tomato losses up to 40% as well as sunburns due to severe defoliation, have been reported in the US (Jones and Thomson, 1987). On the other hand, Correll et al. (1988) did not notice any yield reduction or any other adverse side effect, such as sunscalds or downgrade of the quality in fresh market tomatoes, in California. However, they did accept that in canning tomatoes, which are harvested much later in the cropping season, disease severity on leaves might have a substantial impact on fruit quality. Koren (1978) in Israel and Moens et al. (1984) in Tunisia, as cited by Palti (1988), found no yield increase despite the high level of disease control on leaves. In Greece, unpublished data and local experience indicate that losses vary depending on the type of crop, growing period and cultivar.

Tomato cultivars vary in their susceptibility to *L. taurica* (Malathrakis, 1997), but there are no resistant cultivars available in the market, and fungicides remain the main method of control. Conventional fungicides such as dinocap were moderately effective, and the systemic ones often failed to control powdery mildew, probably due to the predominance of less susceptible strains of the pathogen (Jones and Thomson, 1987). Other inorganic products, as alternatives to fungicides, include the fertiliser monopotassium phosphate that was tested on pepper. Reuveni et al. (1998) claimed that it induced local and systemic resistance against *L. taurica* and was as effective as a sterol-inhibiting fungicide (DMI). Diop-Bruckler and Molot (1987) tested hyperparasites, with yeast as a nutrient, against *L. taurica* on tomato, but results obtained showed no differences between the fungi and the yeast (control). Overall, there is a need for further testing of biological means (antagonists, plant extracts, etc.) against *L. taurica*, which could be used as alternatives to fungicides when resistance occurs, or as a valid control measure in low input/organic tomato production systems.

There are several reports on the positive results of aqueous and ethanolic extracts of the giant knotweed *Reynoutria sachalinensis* (F. Schmidt) Nakai. When applied as preventive treatments, they

gave satisfactory results against powdery mildews on greenhouse cucumber, tomato, begonia and potted apple plants (Herger et al., 1988; Herger and Klingauf, 1990; Neuhaus and Pallut, 1992; Konstantinidou-Doltsinis and Schmitt, 1998; Konstantinidou-Doltsinis et al., 2001). Daayf et al. (1995, 1997) provided conclusive proof of phenolics being produced in powdery mildew infected and uninoculated Milsana® treated cucumber leaves, confirming former indications for resistance induction. Specifically, after application of Milsana® phenolics produced in cucumbers included flavonoids with strong antifungal activity. Down regulating chalcone synthase, a key enzyme of the flavonoid pathway, resulted in the nearly complete suppression of induced resistance by Milsana® (development of healthy haustoria) (Fofana et al., 2005).

In Germany, Milsana® is registered as a plant strengthener. So far, experiments with liquid formulations have been conducted mainly in the pathosystem cucumber/powdery mildew with good results (Daayf et al., 1995; Dik and van der Staay, 1995; Wurms et al., 1999; Petsikos-Panayotarou et al., 2002).

The companies Dr. Schaette AG (Germany) and KHH Bioscience (North America) purchased the patent, trademark and technology rights for Milsana® from BASF, for Europe and the Americas; and Japan and South Africa, respectively. Both companies developed new improved liquid formulations from *R. sachalinensis* extracts, from which the following two formulations have resulted: (a) Milsana® (VP 1999) at KHH Bioscience and (b) Milsana® (VP 2001) at Dr. Schaette AG.

The objectives of this study were: (a) to test whether there is a direct effect of *R. sachalinensis* extract and Milsana® on conidial germination of *L. taurica*, (b) to evaluate the efficacy of both Milsana® formulations against the pathogen in the greenhouse and (c) to examine their effect on yield, compared to conventional fungicides and sulphur, registered in Greece for use against tomato powdery mildew.

Materials and methods

The tomato cultivar 'Manthos' GC 785, F1 (S&G, Holland), highly susceptible to powdery mildew, was used in all of the experiments. The two different formulations of Milsana® VP 1999 and 2001 were applied at different rates as recommended by the company according to results of bridging studies on potted tomatoes and cucumbers (1 field trial), in Germany and Greece.

*Laboratory tests**Effect of Milsana® on conidial germination of L. taurica*

In vitro bioassays. In two *in vitro* bioassays, different concentrations of Milsana® (VP 1999): 0.032, 0.160, 0.800, 4.000 mg l⁻¹ (first) and 0.030, 0.060, 0.120, 0.240, 0.480, 0.960 mg l⁻¹ (second) were incorporated into an agar (7.5 g l⁻¹), dextrose (10 mg l⁻¹), mannitol (20 g l⁻¹) medium (ADM). Ten millilitres of the suspension of each concentration were poured in Petri dishes. Conidia of *L. taurica*, obtained from colonies on cucumber cotyledons maintained on ADM, were suspended in distilled sterile water. Suspensions (1x10⁴ conidia ml⁻¹; 0.5 ml/dish) were spread plated onto the medium and incubated at 21 °C. Germination was assessed after 24 h.

In vivo bioassays. Leaflets of the 5th and 6th leaves, from disease-free plants, were placed onto moistened sterile filter paper in Petri dishes (5 leaflets dish⁻¹) on the adaxial or abaxial side. They were sprayed with (a) an aqueous extract of *R. sachalinensis* or (b) Milsana® (VP 1999), both at the concentrations: 10, 50, 100, 500 and 1000 mg l⁻¹ (final concentration in water) and were left to dry for half an hour. They were inoculated with *L. taurica* conidia from naturally infected tomato leaves (greenhouse) by blowing, in a settling tower and were incubated for 24 h at 20 ± 1 °C and 16 h L/8 h D photoperiod. Imprints were obtained with the use of strips of an adhesive tape. The percentage of germinated conidia (in a sample of 100 conidia replicate⁻¹) and the length of the germ tubes were assessed. In a second bioassay, leaflets were sprayed on their adaxial side with (a) *R. sachalinensis* extract (1% v/v) or (b) water. After 24 h, they were rinsed with water for 30 min and were inoculated. Percentage of germinated conidia was assessed after 24 h as described above.

Growth chamber experiment (GC-exp)

Milsana® (VP 1999) was tested in relation to: (a) application rate (1.0, 0.5, 0.25 and 0.12% v/v) and (b) time of application (one day before and on the day of artificial inoculation). Tomato plants (4th leaf stage) in pots were arranged in a complete randomized design with 5 replicates per treatment and were sprayed with a Humbrol spray gun until run-off. They were inoculated by blowing conidia from infected tomato leaves. Plants were incubated at 23 ± 1 °C, 70 ± 5% RH for 24 h and 60 ± 5% RH for the rest of the time; 12 h L/12 h D

photoperiod and light intensity of 10,000 lux. Disease severity (% infected leaf area) was assessed, 14 days after artificial inoculation.

Greenhouse trials

Greenhouse trials (GTs) in Crete (CR) (south) and Peloponnesus (PS) (central-west) Greece

Trials in Crete were carried out in a greenhouse (300 m²) at the farm of TEI Heraklion. Trials in Peloponnesus were carried out in a glasshouse (350 m²) located in the Plant Protection Institute of Patras. Crops in the greenhouse trials were grown according to the system adopted locally. All treatments were sprayed until run-off.

Plants were arranged in a complete randomized block design with 4 and 5 replicates in Crete and Peloponnesus, respectively. Plants were planted, in double rows, in each plot. Treatments were randomly assigned between plots of 12 plants (Crete)/14 plants (Peloponnesus), in each block. In all experiments, disease severity (% infected area) on individual leaves was assessed visually, at 7 day intervals and the mean infected leaf area per plant was calculated.

Preliminary trial – Crete (CRPT-1999). The trial was carried out from May (transplanting date) until June (last assessment), 1999. The following treatments were applied: Milsana® (VP 1999) at the rate of 0.5%, the fungicide pyrazophos (Afugan EC, 29.4% a.i. w/v, AgrEvo) at the rate of 0.3 ml l⁻¹ and water. Milsana® was applied at 7 day intervals and pyrazophos at 10 day intervals, with a 16 l knapsack sprayer (OVAC PE-HD).

1st trial – Crete (CRGT-1999). The trial was carried out from September (transplanting date) until December 1999 (last assessment). The following treatments were applied: Milsana® (VP 1999) at the rates of 0.3, 0.5, 0.7 and 1.0% v/v, penconazole (Topas 20 EW, 20% w/v, Novartis AG) at the rate of 0.1 ml l⁻¹ and water. Application started 5 days after transplanting, before any disease symptoms appeared. Milsana® and water were applied with a 16 l knapsack sprayer (OVAC PE-HD) at 7 day intervals and penconazole at 10 day intervals.

2nd trial – Crete (CRGT-2001). The trial was carried out from April (transplanting date) until August 2003 (last assessment). The following treatments were applied: penconazole (Topas 20 EW, 20% a.i. w/v, Novartis AG) at the rate of 0.1 ml l⁻¹, Milsana® (VP 2001) at

the rate of 2%, defoliation (1–10th leaf), Milsana® + penconazole (at full rates), Milsana® + defoliation, penconazole + defoliation and water.

Demonstration trial in Crete (CRGT-2003). The trial was carried out from October 2003 until February 2004. There were three separate plots of 80 plants each. Plants were planted in rows, 1 m apart (6 plants/row). The following treatments were applied at 7 day intervals: Milsana® VP 2001 (2%), Milsana® VP 2001 + defoliation (1–10th leaf) and water. Besides the percentage infected leaf area, weight of harvested fruits was also recorded.

1st trial – Peloponnesus (PSGT-1999). The trial was carried out from July 1999 (transplanting date) until February 2000 (last assessment). The following treatments were applied: Milsana® (VP 1999) at the rates of 0.2 and 0.5%, fungicides (alternated): myclobutanil (Systhane 12 E, EC, 12.5% a.i. w/v, AgrEvo) at the rate of 0.4 ml l⁻¹, penconazole (Topas 10 EC, 10% a.i. w/v, Novartis AG) at 0.15 ml l⁻¹, triforine (Saprol 16 EC, 16% a.i. w/v, Bayer) at 1.5 ml l⁻¹ and water. Treatment applications started 5 days after transplanting, before any disease symptoms appeared. All treatments were applied at medium volume spray (500–1000 l ha⁻¹) with a 20 l knapsack sprayer (Berthoud Vermorel 2000 HP, 3 bars). Besides the percentage infected leaf area, weight (kg) of fruits per plant was also recorded at 2–5 day intervals.

2nd trial – Peloponnesus (PSGT-2001). The trial was carried out from September 2001 (transplanting date) until February 2002 (last assessment). The following treatments were applied: water, Milsana® VP 2001 at the rate of 2%, sulphur (Bayer 80 WP, 80% a.i.w/v) at the rate of 3 g l⁻¹ and Milsana® alternated with sulphur at full rates (1st appl: Milsana, 2nd appl: sulphur, 3rd and 4th appl.: Milsana, 5th appl.: sulphur, 6th and 7th appl: Milsana). Treatment applications started after transplanting. Besides the percentage infected leaf area, weight of harvested fruits was also recorded.

Statistical analysis

Laboratory tests

Data obtained on conidial germination were subjected to Probit Analysis using SPSS 11 for Windows (SPSS Inc., Chicago, US). EC₅₀

(concentration causing 50% reduction in germination of conidia) values were estimated.

Greenhouse trials and growth chamber experiment

Data of the percentage infected leaf area were used to calculate the Area Under Disease Progress Curve (AUDPC) per plant (Campbell and Madden, 1990).

Data of: (a) the percentage infected leaf area per plant (growth chamber experiment), (b) the AUDPC values (all GTs 1999 and 2001) and (c) the weight of harvested fruits per plant (GTs-1999 and 2001, Peloponnesus) were subjected to Analysis of Variance (ANOVA) and mean differences among treatments were tested using Duncan's multiple range test at $p \leq 0.05$. For the demonstration trial in Crete (CRGT-2003) only means (disease severity and weight of tomatoes) and standard deviations were calculated due to lack of replications (blocks) of treatments.

Lastly, the AUDPC values of Milsana® (from all GTs 1999 and 2001) were regressed on the AUDPCs of the controls (SPSS 11, SPSS Inc., Chicago, US).

Results

Laboratory tests

Effect of Milsana® on conidial germination of L. taurica

Results from *in vitro* and *in vivo* bioassays showed that both *R. sachalinensis* aqueous extract and Milsana® (VP 1999) inhibited conidial germination of *L. taurica*.

In vitro bioassays. The EC_{50} values of Milsana® were 0.149 ml l^{-1} (lower limit: 0.135 , upper limit: 0.167 ml l^{-1}) and 0.145 ml l^{-1} (lower limit: 0.118 , upper limit: 0.177 ml l^{-1}) in the first and second bioassay, respectively.

In vivo bioassays. On detached tomato leaves the EC_{50} values of Milsana® and the aqueous *R. sachalinensis* extract were 17.46 and $< 1 \text{ ml l}^{-1}$, respectively. Both Milsana® and *R. sachalinensis* extract at the concentration of 1000 mg l^{-1} reduced the length of the germ tubes by 29.7 and 85.5% , respectively (mean length of germ tubes of the controls: 18.76μ , data not shown). Conidial germination on rinsed leaflets, 48 h after treatment with Milsana® or *R. sachalinensis*, was inhibited by 33.3 ± 7.2 (SD) and $98.3 \pm 1.3\%$, (SD), respectively.

Growth chamber experiment (GC-exp.)

The infection level was generally low in this trial. Results (Table 1) showed that Milsana® was more effective when applied on the day of inoculation (0 day) than 1 day before (-1 day). At both application times, the rates of 0.5 and 1.0% were more effective than the lower ones (0.25 and 0.125%). Disease reduction achieved by Milsana® at the rate of 1% was 96.2% (0 day) and 73.5% (-1 day).

Greenhouse trials

Greenhouse trials (GTs) in Crete (CR) (south) and Peloponnesus (PS) (central-west) Greece

Preliminary trial – Crete (CRPT-1999). Results are presented in Figure 1. Neither Milsana® nor pyrazophos were highly effective against *L. taurica* although disease severity in treated plots was significantly lower ($p \leq 0.05$) compared to that of the control. At low levels of infection (< 15% in the control plots) Milsana® was equally effective to the fungicide. Pyrazophos was significantly better than Milsana® as the infection level increased.

1st trial – Crete (CRGT-1999). Disease progress is shown in Figure 2a. Penconazole and Milsana® (VP 1999) at rates > 0.5% significantly reduced disease severity compared to the control ($p \leq 0.05$) as indicated

Table 1. Disease severity (% infected leaf area) caused by *L. taurica* on tomato treated with different rates of Milsana® (VP 1999), applied on the day (0 day) or one day before (-1 day) artificial inoculation (assessment was carried out 14 days after artificial inoculation, GC-exp.)

Treatments	Means of % infected leaf area	
	0 day	-1 day
Control (water)	21.0 a*	31.0 a
Milsana® 1% v/v	0.8 c	8.2 c
Milsana® 0.5% v/v	2.2 bc	7.8 c
Milsana® 0.25% v/v	5.0 b	15.0 bc
Milsana® 0.125% v/v	6.0 b	23.0 ab

*Means followed by the same letter, within the same column, are not statistically different by Duncan's multiple range test ($p \leq 0.05$).

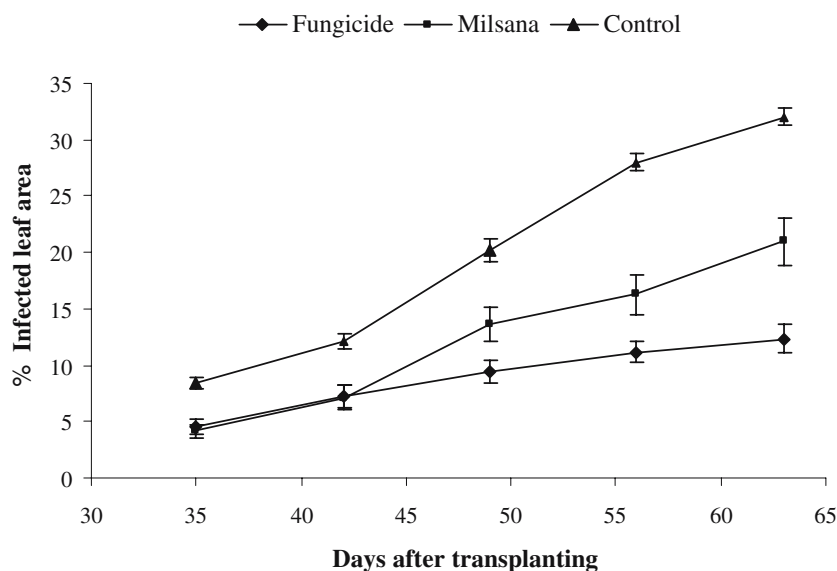


Figure 1. Mean disease severity (% infected leaf area, ± 1 SEM) caused by *L. taurica* on tomato treated with Milsana® (VP 1999) 0.5% and pyrazophos, in a preliminary greenhouse trial (CRPT-1999).

by the AUDPC values (Table 2). Milsana® at the rates of 0.7 and 1.0% was as effective as the reference fungicide. Applications reduced disease severity by 27.4% (penconazole) and 23.2% (Milsana® 0.7%).

2nd trial – Crete (CRGT-2001). Disease progress is shown in Figure 2b. Milsana® VP 2001 and penconazole significantly reduced disease severity by 58.4 and 82%, respectively, while their combinations with the technique of defoliation did not increase their efficacy. Penconazole was significantly better than Milsana® VP 2001 (Table 2).

Demonstration trial in Crete (CRGT-2003). Powdery mildew progress on leaves is presented in Figure 3a. The mean value of AUDPC for the untreated plots was 194.2 while that of Milsana® 87.2%-days (approx. 55% reduction, data not shown). Defoliation did not increase the efficacy of Milsana®. The total mean weight (g) of harvested fruits plant⁻¹ was 1726.7 [± 586.1 (SD)] and 2181.6 [± 871.5 (SD)] in the controls and Milsana® treated plots, respectively (Figure 3b).

1st trial – Peloponnesus (PSGT-1999). Disease progress is shown in Figure 4a. AUDPC values (Table 2) showed that both Milsana® and fungicides significantly reduced disease severity ($p \leq 0.05$). Fungicides

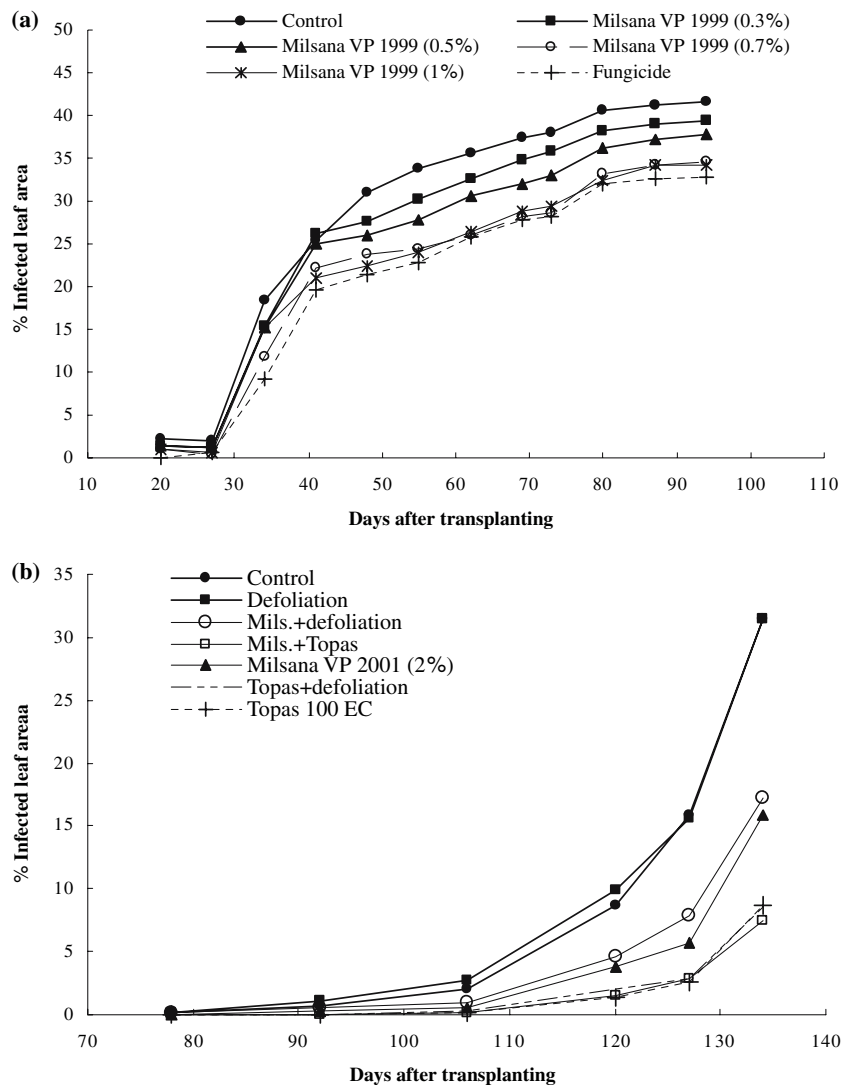


Figure 2. Mean disease severity (% infected leaf area) caused by *L. taurica* on tomato treated with: (a) different rates of Milsana® (VP 1999) and penconazole (CRGT-1999) and (b) Milsana® (VP 2001) and penconazole combined with defoliation in the greenhouse (CRGT-2001).

were significantly better than both Milsana® rates. The rate of 0.5% was more effective than the rate of 0.2%. Treatments reduced disease severity by: 92% (fungicides), 64.8% (Milsana® 0.5%) and 39.7% (Milsana 0.2%). No significant differences in yield were found (weight of fruits plant⁻¹) among treatments (Table 2).

EFFICACY OF MILSANA®, FROM REYNOUTRIA SACHALINENSIS 385

Table 2. Values of Areas Under Disease Progress Curves (AUDPC – %-days) of tomatoes treated with different formulations and rates of Milsana®, fungicide(s) and water; and yield in four greenhouse trials carried out in Crete and Peloponnesus in 1999 and 2001

Treatments	Disease severity on leaves AUDPC (%-days)				Yield (kg plant ⁻¹)	
	Crete		Peloponnesus		Peloponnesus	
	(FT-1999)	(FT-2001)	(FT-1999)	(FT-2001)	(FT-1999)	(FT-2001)
Control (water)	2290 a*	358 a	3920 a	6050 a	3.55 a	2.90 a
Milsana® (VP-1999) 0.2%	–	–	2365 b	–	3.41 a	–
Milsana® (VP-1999) 0.3%	2116 ab	–	–	–	–	–
Milsana® (VP-1999) 0.5%	1995 bc	–	1389 c	–	3.15 a	–
Milsana® (VP-1999) 0.7%	1759 d	–	–	–	–	–
Milsana® (VP-1999) 1%	1779 cd	–	–	–	–	–
Milsana® (VP-2001) 2% ^a	–	149 b	–	3496 b	–	3.0 a
Milsana® (VP-2001) 2%	–	–	–	3421 b	–	3.0 a
alt. sulphur						
Milsana® (VP-2001) 2% + defoliation	–	190 b	–	–	–	–
Defoliation (1–10th leaf)	–	360 a	–	–	–	–
Fungicide (penconazole)– + defoliation	–	77 c	–	–	–	–
Fungicide(s) ^b	1662 d	65 c	311 d	–	3.67 a	–
Sulphur	–	–	–	3642 b	–	3.2 a

*Means followed by the same letter, within the same column, are not statistically different by Duncan's multiple range test ($p \leq 0.05$).

^a2% (Milsana® VP 2001) equivalent to 0.5% (Milsana® VP 1999).

^bMyclobutanil (Systhane 12 E, EC, 12.5% a.i. w/v, AgrEvo at the rate of 0.4 ml l⁻¹), penconazole (Topas 10 EC, 10% a.i. w/v, Novartis AG, 0.15 ml l⁻¹) and triforine (Saprol 16 EC, 16% a.i. w/v, Bayer, 1.5 ml l⁻¹) in PSGT-1999; penconazole (Topas 10 EC, 10% a.i. w/v, Novartis AG, 0.20 ml l⁻¹) in CRGT-1999.

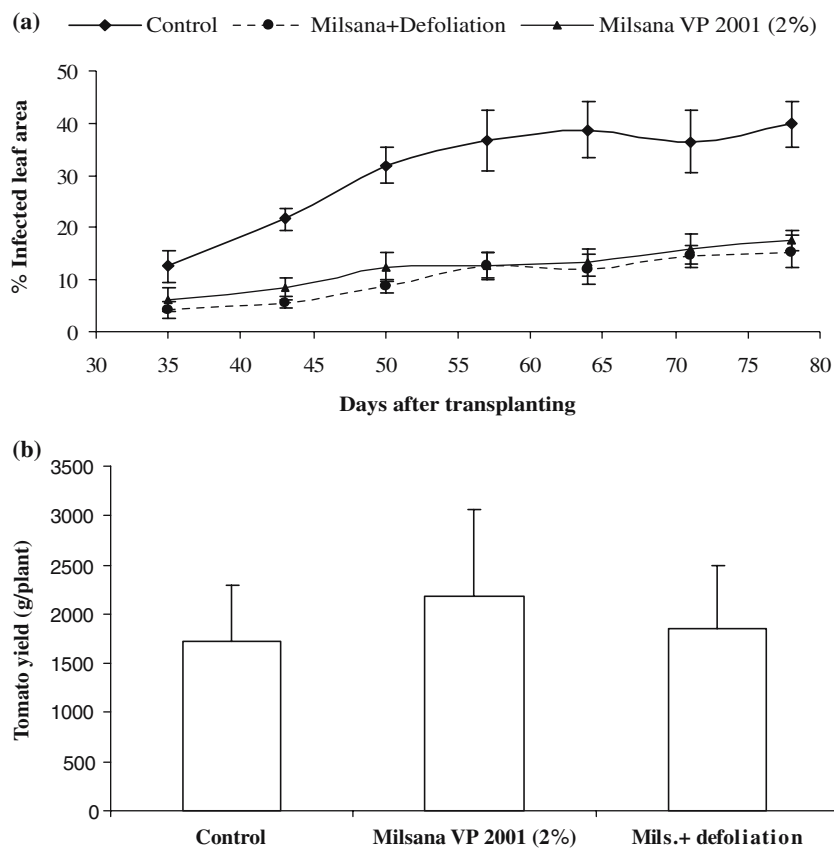


Figure 3. Mean disease severity (% infected leaf area, ± 1 SD) caused by *L. taurica* on tomato treated with: (a) Milsana® VP 2001 (CRGT-2003) and (b) mean weight ($+1$ SD) of harvested fruits (kg^{-1}) per plant in the greenhouse (CRGT-2003).

2nd trial – Peloponnesus (PSGT-2001). Disease progress is shown in Figure 4b. Milsana®, sulphur and their alternation were equally effective in controlling *L. taurica* (Table 2). Treatments reduced disease severity on leaves by: 58% (Milsana®), 60% (sulphur) and 57% (Milsana® and sulphur alternated). No significant differences in yield (weight of fruits plant^{-1}) were found among treatments.

Overall efficacy of Milsana®. A plot of AUDPCs of Milsana® and fungicides vs. control are illustrated in Figure 5. It was found that the efficacy of Milsana® (VP 1999 or 2001) was correlated to the level of infection in the controls in a linear way. The mean efficacy

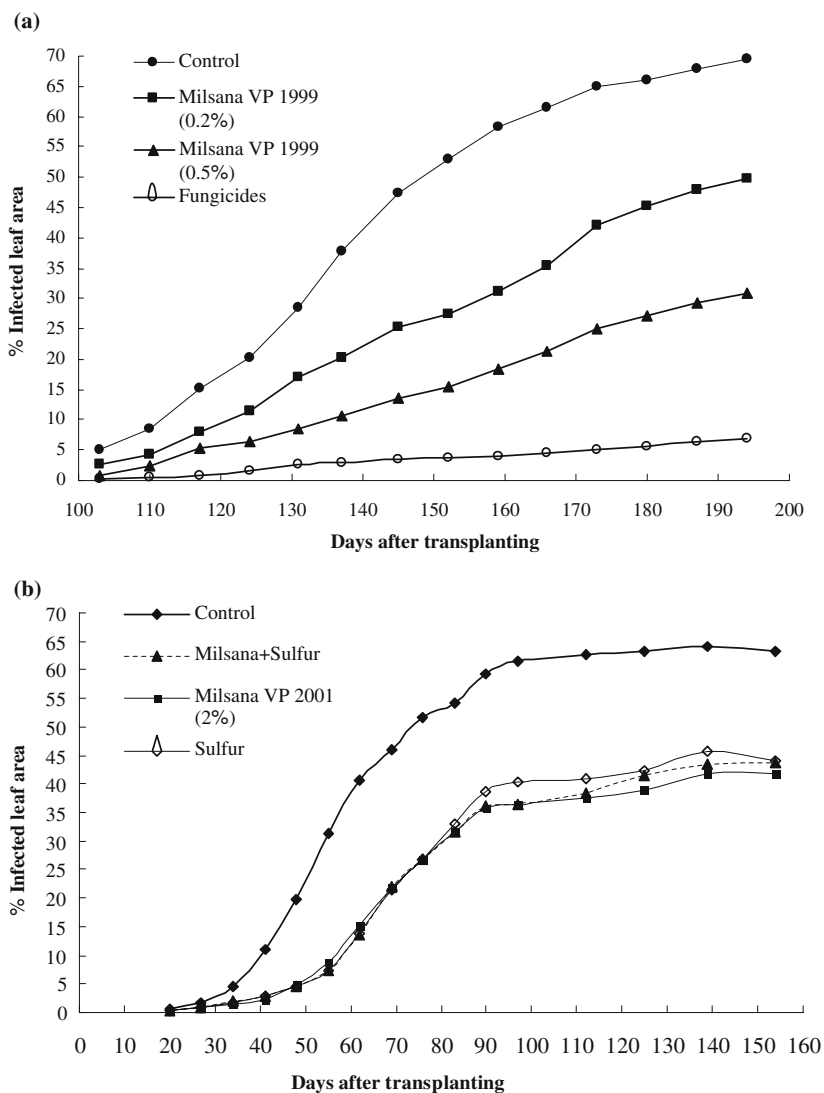


Figure 4. Mean disease severity (% infected leaf area) caused by *L. taurica* on tomato treated with: (a) two rates of Milsana® (VP 1999) and DMI fungicides (PSGT-1999) and (b) Milsana® (VP 2001) and sulphur applied alone and alternated in the greenhouse (PSGT-2001).

achieved by Milsana® over the three years (1999, 2001 and 2003) was 46% as calculated by subtracting the coefficient of x (0.54) from 1 (representing 100% disease reduction).

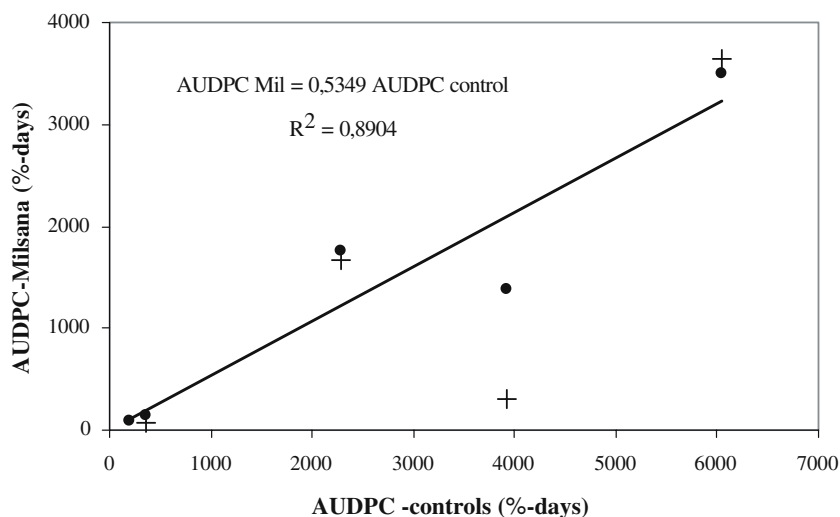


Figure 5. Powdery mildew reduction achieved by two Milsana® formulations (●) and fungicides (+) in multi-year trials carried out on greenhouse tomato in Crete and Peloponnesus.

Discussion

The results from the growth chamber experiment (85.5% disease reduction) and the preliminary greenhouse trial provided evidence that Milsana® could be an effective control agent against *L. taurica* on tomato as it was found for other powdery mildews (Herger et al., 1988; Herger and Klingauf, 1990; Neuhaus and Pallut, 1992; Konstantinidou-Doltsinis et al., 2001). The rate of application proved significant and higher rates were more effective against *L. taurica* than lower ones regardless of the time of application.

In four out of five greenhouse trials (GTs 1999--2003), Milsana® had an efficacy ranging from 42.2 to 64.6%. Only in the CRGT-1999, Milsana® failed to control powdery mildew (low efficacy: 23%). Disease in that trial appeared much sooner (20 days after transplanting) than in the CRGT-2001 (80 days after transplanting) and PSGT-1999 (100 days after transplanting) (Figures 2 and 4), resulting in a considerable build up of inoculum in the crop, at the early stages of the epidemic. Although in the PSGT-2001 (Figure 4b) disease also appeared very early in the cropping season (ca. 20 days after transplanting), the curve of its absolute rate of infection ($dy(\text{severity})/dt$) vs. time was significantly different (data not shown). Specifically the calculated maximum absolute rate of infection in PSGT-2001 was 0.017 (1.7%

increase day⁻¹, 48–55 days after transplanting) while in CRGT-1999 was 0.023 (2.3% increase day⁻¹, 26–33 days after transplanting), indicating a much more aggressive epidemic in Crete (1999) than in Peloponnesus (2001). It is also speculated that leaves, in CRGT-1999, were infected by *L. taurica* very close to transplanting without showing symptoms of the disease. This assumption might explain the fact that disease severity increased from 2 to 18.4% in a period of 7 days, between the second and third assessment (Figure 2a). It is possible that in this case, applications of Milsana® did not serve as a preventive treatment thus failed to control disease at the onset of the epidemic.

Sterol demethylation inhibitors (DMIs) were significantly better than Milsana® as expected, since they are systemic with residual action achieving good efficacy under conditions of high disease pressure. In contrast, Belanger and Labbe (2002) stated that high disease pressure is limiting for the effectiveness of non-chemical agents (e.g. microbial agents) and inducers of resistance. The same holds for preventive fungicides (e.g. sulphur). Milsana® was shown to be equally effective to sulphur, the only 'natural' fungicide permitted for organic crops. Thus, Milsana® could be a potential alternative, since sulphur can be damaging to beneficials and toxic to crops at high temperatures. Milsana® proved to be harmless to beneficial insects and mites (Hafez et al., 1999; Schuld et al., 2002).

Lastly defoliation, a common cultural and sanitation technique among local growers, did not increase the efficacy of Milsana® or penconazole (CRGT-2001) and was not effective when applied alone. This does not necessarily mean that repeated defoliation is ineffective. The removal of a limited number of leaves (1–10th true leaves) in this study did not substantially contribute to the reduction of the total inoculum in the environment of the greenhouse.

There are limited published data on the impact of powdery mildew severity on leaves on tomato yield (see Introduction). Data from the greenhouse experiments in Peloponnesus (GTs 1999 and 2001) showed that despite the high levels of disease reduction achieved by Milsana® or fungicide(s), yield was not significantly increased. In the GT-1999 in Peloponnesus, infection started late in the growing season (100 days after transplanting) when a lot of flowers were already set and the time left for the disease to affect yield was probably short while in GT-2001 this was not the case as disease symptoms appeared soon after transplanting. The data reported here suggest very little effect of disease on yield (in absolute values) and this may be related to the

relatively more luxuriant growth of the green house tomatoes, in which leaf area is often not limiting (undetermined type of cultivars). Our results are similar to those reported by Correll et al. (1988) and Koren (1978) and Moens et al. (1984) (as cited by Palti, 1988) and were verified in the demonstration trial carried out in Crete (2003). However, direct effect on yield is not the only reason why tomato powdery mildew should be controlled. In areas where tomatoes are grown during winter, such as in the Mediterranean basin, powdery mildew infection sites might facilitate the colonization of senescent leaves by destructive necrotrophic pathogens such as *Botrytis cinerea* and/or *Didymella lycopersici*.

In vitro, it was found that the EC₅₀ value of Milsana® (0.15 mg l⁻¹), was lower than that of penconazole (3.33 mg l⁻¹) (Malathrakis unpublished data). Although, EC₅₀ values of Milsana® *in vitro* and *in vivo* (17.46 mg l⁻¹) cannot be directly related to its efficacy in the field, they provide evidence that Milsana® has a direct effect on the conidia of *L. taurica* over a period of 48 h. Further studies are needed to elucidate the duration of this direct effect and its contribution to the efficacy of Milsana® in the field. Alternatively/additively, Milsana® could be acting as an inducer of resistance on tomato, as it was shown for the cucumber/powdery mildew pathosystem (Mueller et al., 1998; Fofana et al., 2005).

In practice, Milsana® could be used preventively in spraying programmes alternated with sulphur (organic farming) or systemic fungicides depending on the risk of infection and/or disease progress (conventional farming). It appears that Milsana® is the first plant extract suitable for the control of *L. taurica* in commercially grown greenhouse tomatoes.

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