European Journal of Plant Pathology **109:** 243–251, 2003.

Activity of the new fungicide benthiavalicarb against *Plasmopara viticola* and its efficacy in controlling downy mildew in grapevines

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Accepted 22 October 2002

Key words: disease control, resistance, systemic fungicides

Abstract

Benthiavalicarb is a new fungicide active against Oomycetes fungal plant pathogens. The present study shows that benthiavalicarb is effective for controlling the Oomycete fungal pathogen *Plasmopara viticola,* which causes downy mildew in grapevines. The fungicide did not affect zoospore discharge from sporangia of *P. viticola*, but strongly inhibited zoospore encystment, cystospore germination *in vitro* and mycelial growth, together with sporangial production *in vivo*. Benthiavalicarb showed strong prophylactic and local activity in intact plants or detached leaves and low translaminar activity. The compound was not translocated from leaf to leaf in either a acropetal or basipetal direction. Benthiavalicarb applied at 1, 3 and 6 days post-inoculation protected grapevine plants against downy mildew and inhibited sporulation of the pathogen. Similar results were obtained on leaf disks if benthiavalicarb was applied up to 96 h post-inoculation. Benthiavalicarb diminished the sporulation of *P. viticola* when applied to established disease in the tissue. Benthiavalicarb remained active on leaves for a period up to 28 days. Two foliar applications of benthiavalicarb, 2 weeks apart, to field-grown grapevines inhibited downy mildew development and were as effective as the standard metalaxyl-Cu treatment in controlling the disease. A formulated mixture of benthiavalicarb + Folpet was similar or superior in performance to metalaxyl-Cu and the new strobilurin trifloxystrobin in controlling downy mildew. The effectiveness of benthiavalicarb makes it well suited for integration into a control programme against downy mildew disease in vineyards, and as a component to delay resistance buildup.

Introduction

Downy mildew is a widely distributed and destructive disease of field-grown grapevines. Cluster and blossom infection with *Plasmopara viticola* before, during or after bloom may result in poor fruit set and quality, and considerable crop loss (Pearson and Goheen, 1988). When commercially acceptable resistant plants are not available, disease control is generally achieved with fungicides. However, fungicide-resistant strains of the pathogen have developed (Leroux and Clerjeau, 1985; Pearson and Goheen, 1988). Once resistant strains appear, they can survive for several years, and the risk of re-enforcing the resistant subpopulation through further applications of fungicides is very high (Cohen and Coffey, 1986; Dekker, 1987; Staub, 1991). This risk intensifies the need for new compounds, with different modes of actions for disease control, and for their appropriate use in fungicide spray programmes. The strobilurins, as a new class of fungicidal compounds, are an example for such compounds, which have been recently introduced. Among them, azoxystrobin (Baldwin et al., 1996) and trifloxystrobin (Flint) (Margot et al., 1998) are active against grapevine diseases in Israel (Reuveni, 2001), USA, and several other countries. Strobilurin fungicides inhibit mitochondrial respiration at the Qo site in the citochrome bc_1 complex, and although resistant strains of *P. viticola* have been isolated from crops in Southern Europe (Bartlett et al., 2002), this new class

of broad-spectrum fungicides is valuable for inclusion in strategies to minimize the development of fungicide resistance.

Benthiavalicarb is a novel amino acid carbamate fungicide with protectant, curative, and antisporulant activities against plant pathogens of the Oomycetes (Kumiai Chemical Ind., Japan). This compound is still in development and has not yet been registered for use (Makhteshim-Agan, Israel – personal communication). However, very little information on the primary mode of action of benthiavalicarb against downy mildew in grapevines, is known. The present paper provides data on biological activity of benthiavalicarb against *P. viticola* in grapevines, and its efficacy in controlling downy mildew in field-grown grapevines.

Materials and methods

Experimental fungicides

Benthiavalicarb (KIF 230, Kumiai Chemical Industry Ltd., Tokyo, Japan) is a new fungicide (Ag Chem new compounds review, 2001). The chemical name (IUPAC) is: Isopropyl[(S)-1-[(R)-1-(6-flourobenzothiazolel-2-yl)ethylcarbamoyl]-2-methyl-propyl] carbamate. The 10 WP (Wettable Powder) formulation of benthiavalicarb was used. The concentrations of the laboratory experiments are presented as active ingredients (a.i.). A ready for-use formulated mixture of 1.75% benthiavalicarb + 70% Folpet (provided by Makhteshim, Israel) was tested in the field in 1999–2001.

Metalaxyl-copper (Metalaxyl-Cu, 45 WP, Syngenta, Basel, Switzerland, containing 5% metalaxyl plus 40% copper oxychloride) was used as a standard treatment in field experiments and compared to the efficacy of benthiavalicarb or benthiavalicarb + Folpet in controlling downy mildew. In addition, the strobilurins azoxystrobin (Abound, Amistar, 250 SC, Syngenta, United Kingdom) and trifloxystrobin (Flint, 50 WG Bayer), were included in field experiments.

Growth chambers experiments

Plants

Grapevines of *Vitis vinifera* cv. 'Emerald Riesling' were used to study the effect of benthiavalicarb on downy mildew. Plants were grown from seeds (one plant per 0.1-l pot) and grown in the growth room (23 °C, 100–120 μE m^{-2} s^{-1}, 16 h light per day). Seedlings were fertilized twice weekly with a 1 g of 20–20–20 (N–P–K) fertilizer l^{-1} solution. Eight-week-old seedlings with at least four fully developed leaves were used.

Pathogen

An isolate of *P. viticola,* obtained from infected plants in a vineyard in the Golan region, Israel, was maintained on reinfected plants grown in a growth chamber (24 °C, 100–120 μE m^{-2} s^{-1}, 16 h light per day). Inoculum was obtained from freshly sporulating leaves 8–12 days after inoculation. Sporangia were suspended in distilled water and adjusted with the aid of a haemocytometer to a concentration of 4×10^4 sporangia ml^{-1} of water.

Leaf disk assay and inoculation with P. viticola

Ten leaf disks (each 12 mm in diameter) were cut with a cork borer from healthy leaves (second from the tip) of different plants and placed, abaxial side up, in 10-cm diameter plastic Petri dishes containing filter paper, moistened with 5 ml of water. Disks were inoculated by placing one drop (5 μl) of inoculum (4×10^4 sporangia ml^{-1}) mixed with various concentrations of benthiavalicarb (0.0–2.0 μg ml^{-1}) on the middle of the lower surface of each disk. After inoculation, leaf disks and the inner surfaces of the Petri dish lids were sprayed with distilled water. The Petri dishes containing 10 leaf disks were incubated at 19 °C for 24 h in darkness and were then kept in a growth chamber (24 °C, 100–120 μ m^{-2} s^{-1}, 16 h light per day) for disease development. Downy mildew lesions accompanied by whitish sporangia were rated according to a 0–4 scale, as previously described (Reuveni, 1998): 0, no lesions; 1, 1–10% of the leaf disk area infected and sporulating; 2, 11–25%; 3, 26–50%; and 4, >50%. At the end of the experiment, the Petri dishes were kept at 19 °C for 24 h in darkness, to induce sporulation, and the numbers of sporangia produced on leaf disks were estimated. Sporangia were washed from 10 leaf disks in a known volume of ethanol–formaldehyde–acetic acid solution (90 : 5 : 5, v/v/v), and counted with the aid of a haemocytometer (six counts per 10 leaf disks). The number of sporangia produced per square centimeter of leaf disk area was calculated. The experiment was conducted twice, with three Petri dishes for each treatment.

Effect of prophylactic treatment on attached leaves

Grape plants were sprayed with 0, 0.1, 0.5 and 1.0 μg benthiavalicarb ml^{-1} on both surfaces, and 24 h later inoculated on the lower surface with a sporangial suspension of *P. viticola.* The lower surface of each of six to eight attached leaves on each of six plants was uniformly sprayed with 2 ml of a sporangial suspension of 4×10^4 sporangia ml^{-1}, with the aid of a glass chromatography sprayer. After inoculation, plants were covered with plastic bags lightly sprayed on the inside with water, and incubated at 19 °C for 20 h in darkness. The plants were then uncovered and kept in a growth chamber, as described for leaf disks. Nine days after inoculation, the plants were lightly sprayed with water, covered with plastic bags and incubated at 19 °C for 24 h in darkness, to induce sporulation. Leaves were detached and the percentage of leaf area covered with sporangiophores and sporangia of *P. viticola* were estimated visually and recorded. The number of sporangia produced per square centimeter of leaf tissue was calculated as described above for leaf disks.

Spore germination

Sporangial suspensions were mixed with 0, 0.01, 0.1, 0.5, 1.0 and 2.0 μg benthiavalicarb ml^{-1}, and 0.1 ml droplets were transferred to depression glass slides (four slides for each concentration). Slides were incubated in moist Petri dishes at 20 °C in darkness for 8 h. The percentages of sporangia releasing zoospores and of cystospores producing germ tubes, were counted under the microscope. The same sporangial suspensions mixed with benthiavalicarb were used to inoculate leaf disks floating on water.

Translaminar activity of benthiavalicarb

Benthiavalicarb was applied at 10 μg ml^{-1} to either the adaxial or the abaxial leaf surfaces of 6-leaf-old plants. Plants were allowed to dry and were inoculated with *P. viticola*. Results were obtained 9 days after inoculation.

Systemic activity of benthiavalicarb

Benthiavalicarb was sprayed on both leaf surfaces of either the lower or upper four leaves of 8-leaf-old plants. One day later, plants were inoculated with sporangia of *P. viticola* on all leaves. Results were obtained 9 days after inoculation.

Longevity of benthiavalicarb

Benthiavalicarb was sprayed at 10 μg ml^{-1} on lower surfaces of 6-leaf-old plants, and at 1, 14, 21 and 28 days after application plants were inoculated with sporangia of *P. viticola*. Nine days after inoculation plants were incubated at 19 °C for 24 h in darkness to induce sporulation. The number of sporangia produced on leaf tissue was estimated and calculated.

Curative activity against downy mildew

In the first experiment, 6-leaf-old plants were inoculated with sporangia of *P. viticola*. One, three or six days after inoculation, benthiavalicarb was sprayed at 10 μg ml^{-1} on lower surfaces of each leaf of each of six plants per treatment. Results were obtained 9 days after inoculation. In another experiment, leaf disks were placed in Petri dishes on filter paper moistened with water and inoculated with *P. viticola*. One, 24, 48, 72 and 96 h after inoculation, leaf disks were transferred to new Petri dishes containing filter papers moistened with 5 μg benthiavalicarb ml^{-1}. Untreated leaf disks remained on filter papers containing water as controls. Downy mildew lesions were rated 8 days after inoculation and sporangia produced on leaf disks were estimated as described above. The experiment was conducted twice, with three Petri dishes for each treatment.

The antisporulant effect of benthiavalicarb

Six-leaf-old plants were inoculated with sporangia of *P. viticola* and kept in a growth chamber. Infected leaves bearing chlorotic lesions were sprayed on the lower surface with suspensions containing 0, 1, 5 and 10 μg benthiavalicarb ml^{-1}. Leaves of each of six plants per treatment were then detached and placed, abaxial side up, in a dew chamber (20 °C, dark) for 24 h to allow fungal sporulation. The number of sporangia produced per cm^2 leaf tissue for each treatment was estimated.

Field experiments

Field experiments were conducted in 1998–2001 using *V. vinifera*, 'Cabernet Sauvignon' on Richter 110

rootstock (9, 10, 13 and 14 years old, respectively) in commercial vineyards in the Golan region of Israel. Methods of fertilization, irrigation, and other cultural practices for this crop were as recommended to commercial growers by the Extension Service of the Ministry of Agriculture, Israel. It was sunny most of the summer (950 W m^{-2} s^{-1}). Occasionally, however, during some nights from June to September, the temperatures were 14–20 °C and dew accumulated on the leaf surfaces. Drip irrigation was used from end of April, with 0.5 mm day^{-1} and increased gradually to 3 mm day^{-1} at 'veraison' stage. The rainfall in this region during the winter period is 750–850 mm, and the average midday relative humidity (RH) and temperature in summer are 35–40% and 28 °C, respectively.

Experimental design

Treatments were arranged in a randomized complete block design. Plots consisting of six adjacent vines, were replicated four times. Fungicides were sprayed twice to run-off (2500 l ha^{-1}) with a 100-l gun-sprayer (1400 kPa) at 14-day intervals. Six treatments consisting of either untreated control, benthiavalicarb (25 and 50 g 100 l^{-1}), 30 and 40 g azoxystrobin 100 l^{-1}, and 400 g of metalaxyl-Cu 100 l^{-1} of water, as a standard, were evaluated in 1998. Fungicides were sprayed on 3 August 1998, when symptoms of downy mildew were evident on leaves, and 14 days later.

Three experiments were conducted in 1999–2001 using a ready for-use formulation of 1.75% benthiavalicarb + 70% Folpet. The experiment in 1999 included the following five treatments: untreated control, 200 and 300 g of 1.75% benthiavalicarb + 70% Folpet 100 l^{-1}, 20 g trifloxystrobin 100 l^{-1}, and 400 g metalaxyl-Cu 100 l^{-1} of water, as a standard. The experiments in 2000 and 2001 included untreated control, 200 and 400 g of the 1.75% benthiavalicarb + 70% Folpet 100 l^{-1}, and 400 g metalaxyl-Cu 100 l^{-1} of water, as a standard. Fungicides were sprayed twice at 14-day intervals, starting on 12 August 1999, 10 August 2000 and 19 August 2001 when symptoms of downy mildew were evident on leaves.

Assessment of downy mildew on leaves in the field

Grapevine leaves, which were naturally infected with the downy mildew pathogen, were assessed visually for the percentage of leaf area covered with sporangiophores and sporangia of *P. viticola* and presented as percentage of leaf area infected. Five shoots of the central four vines of each replicate plot were randomly selected and each of the top 10 leaves (with midvein longer than 5 cm in length) of each shoot were evaluated for the percentage of leaf area infected. The percentage of leaves with downy mildew sporulation in each treatment was calculated.

Data analysis

The laboratory and growth chamber experiments were conducted at least twice, with four to six replicate plants per treatment. Results of representative experiments are reported. An arc-sin transformation was performed on percentage data. Analysis of variance (ANOVA) using the SAS GLM (SAS Institute, Inc., Cary, NC) procedure was applied to the transformed data. Duncan's Multiple Range Test was used to determine significant differences between treatments.

Results

Fungicidal effects on spore germination and sporangial production

Benthiavalicarb had a weak effect (42.3–49.1%) on zoospore discharge from sporangia of *P. viticola* at concentrations of up to 1.0 μg ml^{-1}. However, concentrations of 0.01, 0.1, 1.0 and 2.0 μg ml^{-1}, strongly inhibited germ tube formation of cystospores by 30.5%, 50.5%, 96.0% and 100.0%, respectively (Table 1). High levels of inhibition of sporangial production were found on leaf disks floating on water and inoculated with sporangial suspensions mixed with benthiavalicarb to give the concentrations mentioned above and a concentration of 0.5 μg ml^{-1} provided total inhibition (Table 1).

Effects of prophylactic treatment on downy mildew development

There was a reduction in the percentage of infected leaf area at concentrations of 0.1 and 0.5 μg benthiavalicarb ml^{-1}, and total inhibition of disease development on plants treated with benthiavalicarb at 1 and 5 μg ml^{-1}. Concentrations of 0.1 and 0.5 μg benthiavalicarb ml^{-1} inhibited sporangial

Table 1. Effects of benthiavalicarb on germination of zoospores of *P. viticola* on depression slides and sporangial production on leaf disks

Concentration ($\mu g\ ml^{-1}$, a.i.)[1]	Germinated zoospores (%)	% inhibition as to control	Sporangia production cm^{-2}	% protection as to control
0	67.4 a[2]	—	45354.0 a	—
0.01	46.8 b	30.5	9601.8 b	78.8
0.1	33.3 c	50.5	177.0 b	99.6
0.5	22.3 d	67.00	0.0 b	100
1.0	2.8 e	96.0	0.0 b	100
2.0	0.0 e	100.0	0.0 b	100

[1]The given concentrations were used to determine germination of zoospores on depression slides and sporangial production on leaf disks after inoculation with sporangial suspensions.
[2]Mean numbers within columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.

Table 2. Efficacy of benthiavalicarb applied prophylactically on downy mildew development induced by *P. viticola* on inoculated potted grapevines[1]

Concentration ($\mu g\ ml^{-1}$, a.i.)	Infected leaf area (%)	% inhibition of infected leaf area[2]	Sporangia cm^{-2} leaf area ($\times 1000$)	% inhibition of sporulation[2]
0	91.6 a[3]	—	240.7 a	—
0.1	37.7 b	59.0	27.3 b	88.7
0.5	0.8 b	99.0	0.22 b	99.9
1.0	0.0 b	100	0.0 b	100
5.0	0.0 b	100	0.0 b	100

[1]Plants were sprayed with benthiavalicarb and inoculated with *P. viticola* after 24 h.
[2]Relative to infected leaf area and sporulation of untreated control plants.
[3]Mean numbers within columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.

production almost completely, at 1.0 $\mu g\ ml^{-1}$ there was no sporulation at all (Table 2).

Translaminar activity and systemic protection against downy mildew

Benthiavalicarb at a concentration of 10 $\mu g\ ml^{-1}$ completely inhibited disease development on leaves treated on the abaxial surfaces, but no significant protection was observed when leaves were treated on the adaxial surfaces, showing that benthiavalicarb was not transported through the leaf blade (results not shown).

Nine days after inoculation, all the leaves treated with 10 μg benthiavalicarb ml^{-1} were fully protected against the mildew and showed no visible symptoms. However, the untreated leaves were as mildewed as those in inoculated control plants, showing that benthiavalicarb failed to translocate from a treated to untreated leaf in either acropetal or basipetal direction (data not shown).

Longevity of benthiavalicarb

Downy mildew symptoms developed on leaves of untreated control plants at each of the tested intervals (1–28 days). An average of 20.9×10^4 sporangia cm^{-2} of leaf tissue were produced on these leaves following wet and dark conditions. However, leaves sprayed with benthiavalicarb at 10 $\mu g\ ml^{-1}$ were fully protected against *P. viticola* at all tested intervals, showing that benthiavalicarb was active for a period of up to 28 days.

Curative activity against downy mildew

Benthiavalicarb at concentration of 10 $\mu g\ ml^{-1}$ applied to 6-leaf-old plants at one, three or six days after inoculation significantly ($p < 0.05$) inhibited downy mildew development. Application of benthiavalicarb at one or three days post-inoculation was more effective than at 6 days in decreasing the percentage of infected leaf area (Table 3). Sporangial production on plants treated with benthiavalicarb within this period

Table 3. Curative activity of benthiavalicarb against downy mildew as applied to attached leaves at various intervals after inoculation

Application days after inoculation[1]	Infected leaf area (%)	Sporangia cm^{-2} leaf area ($\times 1000$)	% protection as relative to control
1	2.1 c[2]	0.7 b	97.9
3	5.2 c	2.1 b	93.3
6	15.7 b	1.3 b	95.9
Control	57.3 a	32.6 a	—

[1]Plants were inoculated with *P. viticola* and sprayed with benthiavalicarb at concentration of 10 μg ml^{-1} 1, 3 and 6 days after inoculation. Control plants were sprayed with water.
[2]Mean numbers within columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.

decreased by 93–98%, compared to untreated control plants (Table 3).

Similar results were obtained in another experiment using leaf disks. Results showed that benthiavalicarb significantly reduced development of downy mildew symptoms and sporangial production, even if applied up to 96 h post-inoculation (Figure 1). The inhibitory effect against sporulation of *P. viticola* was significantly ($p < 0.05$) increased from 78.7% to 99.3%, as time of application decreased from 96 to 1 h after inoculation, respectively, as relative to control untreated disks (Figure 1).

The antisporulant effect of benthiavalicarb

The number of sporangia per cm^2 leaf area produced on leaves treated with 0.0, 1.0, 5.0 and 10.0 μg benthiavalicarb ml^{-1} was 18.4×10^3, 6.1×10^3, 2.2×10^3 and 0.0, respectively. Thus, concentrations of 1.0, 5.0 and 10 μg benthiavalicarb ml^{-1} provided 66.8%, 88.0% and 100% inhibition of sporulation, respectively, as relative to the control.

Field experiments

Two foliar applications of benthiavalicarb or azoxystrobin or metalaxyl-Cu inhibited downy mildew development by a significant reduction in the percentage of infected leaf area on the mildewed leaves (Table 4). Benthiavalicarb and metalaxyl-Cu applications accelerated necrosis of oilspots, which dried out and died quickly. One day before the first application, the infected leaf area on nontreated vines

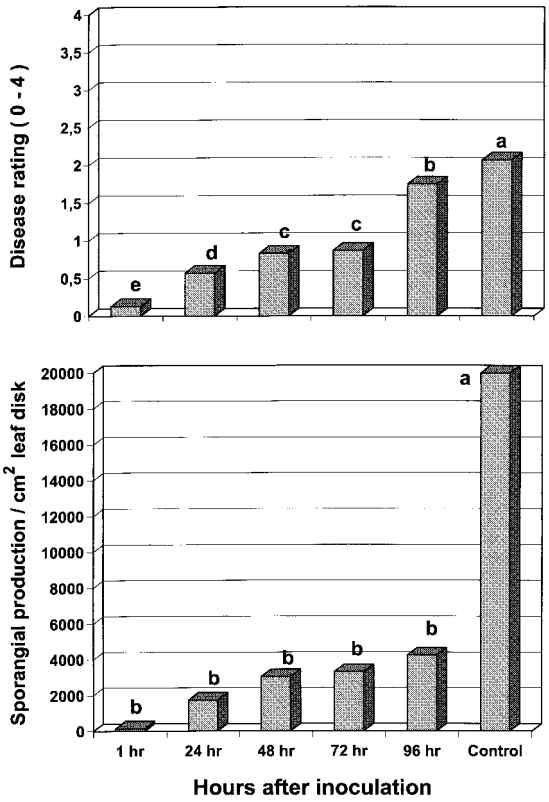

Figure 1. Curative activity of benthiavalicarb against *P. viticola* as applied to leaf disks at various intervals after inoculation. Leaf disks were inoculated with *P. viticola* and transferred to new Petri dishes containing filter papers moistened with 5 μg benthiavalicarb ml^{-1} 1, 24, 48, 72 and 96 h after inoculation. Untreated control leaf disks remained on filter paper containing water. Disease and sporangia production were evaluated as described in Material and methods. Results represent one of two experiments. Three Petri dishes were used for each treatment. Bars with different letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.

was 1.5%. Nine days after the second application, downy mildew was significantly suppressed on vines treated with 25 g benthiavalicarb $100\,l^{-1}$, with the infected leaf area being 0.4% while it increased up to 25.1% on nontreated vines (Table 4). Applications of 25 g benthiavalicarb $100\,l^{-1}$ were similarly effective as metalaxyl-Cu and slightly more effective than azoxystobin in controlling downy mildew (Table 4).

Results of the 1999 experiment showed that applications of 200 g $100\,l^{-1}$ of a formulated mixture containing 1.75% benthiavalicarb + 70% Folpet, provided almost full control of downy mildew (Table 5). One day before the first application, the infected leaf area on

nontreated vines was 1.0%. Eleven days after the second application, downy mildew was significantly suppressed on vines treated with 200 g 100 l^{-1} of a mixture containing 1.75% benthiavalicarb + 70% Folpet, with the infected leaf area being 0.1% while it increased up to 25.1% on nontreated vines, providing 99.6% protection compared to controls (Table 5). This formulated mixture was significantly more effective than metalaxyl-Cu or trifloxystrobin in controlling downy mildew (Table 5). Results in 2000 and 2001 showed that applications of 200 g 100 l^{-1} of the formulated mixture containing 1.75% benthiavalicarb + 70% Folpet provided excellent control and was as effective as metalaxyl-Cu in controlling downy mildew (Table 5).

Table 4. Efficacy of benthiavalicarb, azoxystrobin and metalaxyl-copper on control of downy mildew in field-grown grapevine

Treatment and conc. per 100 l water[1]	Sporulating leaves (%)	Infected leaf area (%)	Protection relative to control (%)
Control	100.0 a[2]	25.1 a[2]	—
Metalaxyl-Cu (400 g)	14.5 d	0.2 b	99.4
Azoxystrobin (30 g)	40.5 b	1.7 b	93.3
Azoxystrobin (40 g)	31.0 c	1.0 b	95.9
Benthiavalicarb (25 g)	14.0 d	0.4 b	98.4
Benthiavalicarb (50 g)	10.0 d	0.05 b	99.8

[1]Fungicides were sprayed twice with a 14-day separation, starting on 2 August 1998, when symptoms of downy mildew were evident in leaves. Nine days after the second application, leaves were evaluated for the percentage of leaf area infected.
[2]Mean values within columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.

No phytotoxicity to the foliage was observed as a result of foliar applications of benthiavalicarb.

Discussion

Benthiavalicarb is a new amino acid carbamate fungicide active as a foliar spray against fungal plant pathogens of the Oomycetes under both greenhouse and field conditions. The present paper reports on the activity of benthiavalicarb in controlling downy mildew in grapevines. Special emphasis was given to the biological mode of action of benthiavalicarb.

Benthiavalicarb had excellent prophylactic activity against downy mildew in grapevines. The mechanism by which the compound acts against the fungus is not fully understood. Judging from the presented findings, it is assumed that benthiavalicarb acts on cell-wall biosynthesis because it had no effect on zoospore discharge but prevented zoospores from producing cell walls and consequently germ tube. Also, lesion formation and sporangia production were inhibited *in vivo*. Inhibition of sporangia formation was also observed when benthiavalicarb was applied to normally developed lesions just before sporangiophores were induced, by high humidity, to emerge from stomata. Sporangia production was highly sensitive to applications of benthiavalicarb.

Data provided here show that benthiavalicarb protected grape leaves from the mildew when applied post-infectionally. Even when applied 6 days after

Table 5. Efficacy of a formulated mixture of benthiavalicarb + Folpet, trifloxystrobin and metalaxyl-Cu on control of downy mildew in field-grown grapevine

Treatment and conc. per 100 l water[1]	1999		2000		2001	
	Sporulating leaves (%)	Infected leaf area (%)	Sporulating leaves (%)	Infected leaf area (%)	Sporulating leaves (%)	Infected leaf area (%)
Control	87.5 a[2]	26.0 a[2]	31.1 a[2]	7.1 a[2]	76.3 a[2]	18.1 a[2]
Metalaxyl-Cu (400 g)	30.0 c	2.2 b	1.0 b	0.1 b	0.0 b	0.0 b
Trifloxystrobin (20 g)	51.5 b	3.7 b	Not tested	Not tested	Not tested	Not tested
Benthiavalicarb (1.75%)+ Folpet (70%) (200 g)	4.0 d	0.1 c	0.0 b	0.0 b	2.9 b	0.1 b
Benthiavalicarb (1.75%)+ Folpet (70%) (300 g)	11.0 d	0.3 c	Not tested	Not tested	Not tested	Not tested
Benthiavalicarb (1.75%)+ Folpet (70%) (400 g)	Not tested	Not tested	1.0 b	0.1 b	0.0 b	0.0 b

[1]Fungicides were sprayed twice with a 14-day separation, starting on 12 August 1999 and 10 August 2000, when symptoms of downy mildew were evident in leaves. Eleven days after the second application, leaves were evaluated for the percentage of leaf area infected.
[2]Mean values within columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's Multiple Range Test.

inoculation to leaves or to leaf disks, protection achieved was 96.0%. It may be speculated that benthiavalicarb damages penetrated cells, so that translocation of nutrients from the affected host cells into the haustoria is blocked, thus prohibiting sporangial production. Histochemical data are required to prove such a hypothesis.

Similar to the new strobilurin trifloxystrobin, benthiavalicarb failed to translocate from leaf to leaf in either the acropetal or the basipetal direction, which shows that there was no transport in the vascular system (Herman et al., 1998; Reuveni, 2001). Unlike trifloxystrobin, benthiavalicarb had no translaminar activity, as could be judged from its ineffectiveness, when applied to the leaf surface opposite to the inoculated surface. The activity on leaf disks, however, could be a result of diffusion of the compound through the wounded tissue.

Benthiavalicarb has been found to protect potato against *Phytophthora infestans* (Y. Cohen, personal communication, 2000), and cucumber and melon against *Pseudoperonospora cubensis* (J. Henen, personal communication, 2000). It thus appears that benthiavalicarb has good activity against several fungal pathogens belonging to Oomycetes. The compound is reported to be active against *P. infestans*, regardless of their sensitivity to phenylamide fungicides (Y. Cohen, personal communication, 2000), thus making it a possible alternative to phenylamide fungicides which have since the early 1980s suffered from the appearance of resistant sub-populations (Leroux and Clerjeau, 1985). Resistant strains to phenylamide fungicides of *P. viticola* have, as yet, not been detected in Israel. The mode of action of benthiavalicarb is likely to differ from that of metalaxyl and other phenylamide fungicides (Cohen and Coffey, 1986). Alternating benthiavalicarb in spray programmes or the use of its mixtures with other fungicides, such as cymoxanil or Folpet should reduce the development of populations of *P. viticola* resistant to either fungicide (Samoucha and Gisi, 1987). This practice could also enhance the performance of benthiavalicarb, provide greater timing flexibility and delay or reduce the selection process of resistant strains (Gisi, 1996; Samoucha and Gisi, 1987).

The field data demonstrate that biweekly post-infection foliar applications of benthiavalicarb in the vineyard inhibited development of downy mildew in grapevines and provided 98.4% protection compared with the control. The inhibitory effect was expressed as a reduction in sporulation, and accelerated necrosis of oilspots, which dried out and died quickly, thus eradicating the disease more effectively. Benthiavalicarb was as effective as the standard metalaxyl-Cu and more effective than the new stobilurin fungicide azoxystrobin (Baldwin et al., 1996) in reducing the percentage of sporulating leaves (Table 4). Applications of a formulated mixture of benthiavalicarb with Folpet, at reduced rates, effectively controlled downy mildew and was more effective than both the standard metalaxyl-Cu and the new strobilurin trifloxystrobin in particular on high disease pressure conditions as in 1999 season (Table 5). Results obtained in 2000 and 2001 showed that applications of this formulated mixture provided excellent control and was as effective as metalaxyl-Cu in controlling downy mildew (Table 5). These field data support the findings on potted plants regarding the properties of benthiavalicarb and its prophylactic and therapeutic activities against *P. viticola* (Tables 2 and 3).

The presented data, on the biological mode of action and control of grape downy mildew, as observed on potted and field-grown grapevines, makes benthiavalicarb an attractive compound for practical agronomic use against *P. viticola* in grapevine. A programme, including alternating applications of benthiavalicarb with those of phenylamide fungicides and phosphonates (Reuveni, 1997; Wicks et al., 1991), could reduce the intensive use of site-specific fungicides against downy mildew.

Acknowledgements

This study was supported by 'Makhteshim' Ltd., Israel. The author thanks Mr. D. Scheglov for his technical assistance.

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