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# Efficacy of biocontrol agents and natural compounds against powdery mildew of zucchini

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**Abstract** The activity of different types of natural compounds and of two biofungicides based on *Bacillus subtilis* and *Ampelomyces quisqualis* alone and in combination with fungicides was tested against powdery mildew of zucchini. The efficacy was compared to the activity of fungicides used alone in four experimental trials carried out in the open field and under greenhouse conditions. The *Podosphaera xanthii* population used throughout the work was partially resistant to azoxystrobin, whereas it was susceptible to mychlobutanil. Sulphur plus terpenes and mustard oil consistently controlled powdery mildew, followed by mychlobutanil alone or in combination with *A. quisqualis*. *B. subtilis* and *A. quisqualis* when tested alone were partially effective. The combination of azoxystrobin and *B. subtilis* only delayed the spread of the pathogen.

**Keywords** *Ampelomyces quisqualis* · *Bacillus subtilis* · Biological control · *Cucurbita pepo* · Integrated disease management · *Podosphaera xanthii*

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## Introduction

Powdery mildew, incited by *Podosphaera xanthii*, previously known as *Sphaerotheca fuliginea* and *S. fusca* (Braun and Takamatsu 2000), is a severe disease of cucurbits and one of two species of powdery mildew of cucurbits worldwide (Sitterly 1978; Zitter *et al.* 1996). The disease is particularly important in the Mediterranean countries, where it causes severe losses to crops grown in the open field as well as in greenhouses. Powdery mildew in Italy is particularly serious on crops such as melon and zucchini.

The most common strategy to control powdery mildew of zucchini includes the use of resistant cultivars and the application of fungicides. Actually, chemical control has a key role and it is the principal tool to manage cucurbit powdery mildew (McGrath 2001). However, in spite of this, powdery mildew continues to cause serious losses worldwide (Zitter *et al.* 1996). The intensive use of chemicals against *P. xanthii* often resulted in the development of resistance; this has happened in the case of most of the groups of chemicals applied (McGrath 2001, 2007). During the past few years, resistance became widespread also in the case of Quinone outside Inhibitors (QoIs) fungicides (Ishii 2010; McGrath 2007).

Biological control agents as well as natural compounds are possible alternatives to the use of chemicals, which have been proposed and evaluated in numerous pathosystems, with different degrees of success. Among biocontrol agents, *Ampelomyces quisqualis* and *Bacillus subtilis* have been widely tested and are registered for use in several countries (Copping 2004). In many cases, their application within integrated disease management strategies offered interesting results (Gilardi *et al.* 2008; Paulitz and Bélanger 2001). Moreover, a synergistic effect between *B. subtilis* and QoI fungicides was observed in the control of powdery mildew of zucchini (Gilardi *et al.* 2008).

Different types of so-called natural compounds, ranging from salts such as sodium bicarbonate to plant extracts and oils, have been widely exploited against several agents of powdery mildews on a number of crops (Hagiladi and Ziv 1986; Horst *et al.* 1992; Pasini *et al.* 1997), providing in many cases very interesting results. Moreover, in some cases a positive effect of mineral fertilizers has been shown (Reuveni and Reuveni 1998).

The main objective of this study was to evaluate the activity of different types of natural compounds, mineral fertilizers, and of two biofungicides based on *B. subtilis* and *A. quisqualis* alone and in combination with fungicides, in comparison with fungicides (including sulphur) used alone against *P. xanthii* on zucchini (*Cucurbita pepo* L.) under open field and greenhouse conditions.

## Materials and methods

*Field trials* Two trials were carried out in the open field at Boves, in the Cuneo province of northern Italy. Zucchini plants (cv. Xsara), 18 days old, were transplanted into soil covered with black plastic mulch by following a randomized block design, with three replicates and eight plants per replicate.

*Greenhouse trials* Two trials were carried out in a greenhouse at Grugliasco, in the Turin province of northern Italy. Zucchini plants (cv. Genovese) were grown in pots (14×14 cm, 2*l* volume of soil) in a peat: clay: perlite substrate (65:30:5 v/v). Two plants were planted in each pot. They were maintained at temperatures ranging between 24°C and 27°C, at 60–70% R.H.. Fifteen-day-old plants with their second true expanded leaf were used, and laid out in a randomized block design with four replicates.

*Sensitivity of the pathogen to the fungicides used* The strain AG1 of *P. xanthii* was collected in Piedmont (northern Italy) from infected zucchini. The sensitivity of *P. xanthii* AG1 strain towards azoxystrobin and mychlobutanil was evaluated by treating zucchini seedlings at the cotyledon stage with increasing rates of the two fungicides up to twice their field dosages, corresponding respectively to 0.186 ml$l^{-1}$ for azoxystrobin and 0.056 ml$l^{-1}$ for mychlobutanil. The treated seedlings were placed in a greenhouse at a temperature of 22–25°C. The artificial inoculation was carried out 24 h after the fungicide treatment using a paint-brush, with $1\times10^5$ conidia cm$^{-2}$. Inoculated and non-treated plants were used as control. Seven to 14 days after the last treatment, the percentage of zucchini leaves affected by *P. xanthii* (disease incidence) was evaluated on a scale from 0 to 5 (0=no infection, 1=0 to 0.99% infected leaf area; 2=1–4.99% infected leaf area; 3=5–19.9% infected leaf area; 4=20–40% infected leaf area; 5=>40% infected leaf area). The minimal inhibitory concentration (MIC) and the concentrations able to inhibit 50% ($ED_{50}$) of the development of *P. xanthii* in comparison with the inoculated and non-treated control were evaluated.

*Treatments* Two or three sprays were carried out in the different trials (Table 1). *Bacillus subtilis* QST 713 (Serenade WP, 10% a.i.; AgraQuest Inc., Davis, CA, USA) and *Ampelomyces quisqualis* (AQ 1058% a.i.; Intrachem Bio Italia S.p.A., Bergamo, Italy) were used as commercial formulations and applied at the suggested dosages, as foliar sprays, as reported in Tables 2, 3, 4, 5, 6, 7 and 8. AQ 10 was applied in combination with Nu-Film P, as recommended by the company.

Azoxystrobin (Ortiva, 23.2% a.i.; Syngenta Crop Protection S.p.A., Milano, Italy), mychlobutanil (Thiocur forte, 4.5% a.i.; DowAgrosciences), sulphur plus terpenes (Heliosoufre S, 51.1% a.i.; Intrachem Bio Italia), mustard oil (Duolif, soluble organic nitrogen 3%, soluble sulphur 15%, organic matter 80%; Cerealtoscana S.p.A., Livorno, Italy), organic-mineral fertilizer N:K (Kendal, soluble organic nitrogen 3.5%, soluble potassium oxide 15.5%, organic carbon 3–4%; Valagro, Atessa, Chieti, Italy), mineral fertilizer N:K+B, and Mo (Silvest, soluble organic nitrogen 8%, soluble potassium oxide 8%, soluble boron 0.1%, soluble molybdenum 0.01%; Green Has Italia S.p.A., Canale d'Alba, Cuneo, Italy) were

**Table 1** Time table for the four trials in the powdery mildew experiments

| Operation | Trial No. | | | |
|---|---|---|---|---|
| | Field trials[z] | | Greenhouse trials | |
| | 1 | 2 | 3 | 4 |
| First treatment | 6[y] | 7 | 4 | 5 |
| Artificial inoculation with *Podosphaera xanthii* | 7 | 24 | 6 | 6 |
| Second treatment | 15 | 15 | 11 | 12 |
| Third treatment | - | 31 | 19 | 20 |
| First evaluation | 35 | 37 | 11 | 19 |
| Second evaluation | 49 | 44 | 19 | 25 |
| Third evaluation | - | - | 26 | 32 |
| Fourth evaluation | - | - | 33 | - |
| Biomass evaluation | - | - | 33 | 32 |

[z]Dates of transplant for the four trials: August 7 (Trial 1, 2008); July 13 (Trial 2, 2010); February 10 (Trial 3, 2011); February 25 (Trial 4, 2011)

[y]Numbers indicate days after transplanting

applied at the dosages reported in Tables 2, 3, 4, 5, 6, 7 and 8.

When applied together, chemicals and biofungicides were mixed before spraying. Treatments were carried out, at 6–8-day intervals, using 800 $l\,ha^{-1}$ with an Efco atomizer (Norwalk Power Equipment Company, UK). Treatments were applied 24 h before the artificial inoculation with the pathogen and, as reported in Table 1, two or three sprays were carried out in the different trials.

*Data collection* Typical symptoms of powdery mildew started to be visible 7–20 days after artificial inoculation. Plants were checked every 7 days after the last treatment for disease development and the percentage of zucchini leaves affected by *P. xanthii* (disease incidence) was determined. The evaluations were carried out by assessing the upper surfaces of 50 (first and second evaluation, Trial 1) and 100 leaves. Disease severity was evaluated by using a disease index ranging from 0 to 5 (EPPO 2004). The final disease rating was recorded

**Table 2** Effect of different treatments, expressed as disease incidence and disease severity, against *Podosphaera xanthii* on zucchini (cv. Xsara) at 35 and 49 days after transplanting (DAT) (Trial 1, Boves)

| Treatment | Dosage (a.i. g or ml $l^{-1}$) | Disease incidence[z] at | | Disease severity[y] at | |
|---|---|---|---|---|---|
| | | DAT 35 | DAT 49 | DAT 35 | DAT 49 |
| *Bacillus subtilis* | 0.4 | 40.8 bc[x] | 52.0 bcd | 8.8 a | 15.4 ab |
| *Ampelomyces quisqualis* | 0.029 | 51.8 cd | 45.3 abc | 12.3 ab | 15.0 ab |
| Azoxystrobin | 0.186 | 54.7 cd | 63.3 cd | 11.5 ab | 17.8 ab |
| Azoxystrobin+*B. subtilis* | 0.186+0.4 | 45.0 bcd | 48.0 abc | 8.9 a | 10.3 ab |
| Mychlobutanil+*A. quisqualis* | 0.056+0.029 | 34.9 ab | 48.0 abc | 6.6 a | 14.0 ab |
| Sulphur | 1.53 | 21.3 a | 32.7 ab | 2.5 a | 5.8 a |
| Kendal (N:K, organic C) | 3.0[w] | 46.7 bcd | 44.0 abc | 11.5 ab | 10.4 ab |
| Duolif (mustard oil) | 10.0[w] | 44.7 bcd | 27.3 a | 8.9 a | 5.4 a |
| Inoculated control | - | 57.5 d | 70.7 d | 26.0 b | 22.5 b |

[z] Expressed in percent of infected leaves

[y] Expressed in percent of infected leaf area

[x] Within columns, means followed by a common letter do not differ significantly according to Tukey's test ($P < 0.05$)

[w] Dosage (ml $l^{-1}$) of the commercial formulation

**Table 3** Effect of different treatments, expressed as disease incidence and severity, against *Podosphaera xanthii* on zucchini (cv. Xsara) at 37 and 44 days after transplanting (DAT) (Trial 2, Boves)

| Treatment | Dosage (a.i. g or ml $l^{-1}$) | Disease incidence[z] at | | Disease severity[y] at | |
|---|---|---|---|---|---|
| | | DAT 37 | DAT 44 | DAT 37 | DAT 44 |
| *Bacillus subtilis* | 0.4 | 62.8 cd[x] | 80.7 cd | 22.4 bcd | 28.4 bc |
| Azoxystrobin | 0.186 | 59.4 cd | 66.7 bc | 22.1 bcd | 23.6 abc |
| Azoxystrobin+*B. subtilis* | 0.186+0.4 | 65.0 cd | 63.3 bc | 17.6 bcd | 15.1 ab |
| Mychlobutanil | 0.056 | 11.0 a | 40.6 a | 2.3 a | 9.8 a |
| Sulphur | 1.53 | 34.0 ab | 58.0 ab | 8.2 ab | 12.8 a |
| Silvest (N:K+B, Mo) | 3.5[w] | 64.5 cd | 74.0 bcd | 23.1 cd | 20.4 ab |
| Duolif (mustard oil) | 10.0[w] | 44.2 bc | 60.7 b | 12.1 abc | 19.4 ab |
| Inoculated control | - | 79.9 d | 85.3 d | 32.3 d | 36.0 c |

[z] Expressed in percent of infected leaves

[y] Expressed in percent of infected leaf area

[x] Within columns, means followed by a common letter do not differ significantly according to Tukey's test ($P<0.05$)

[w] Dosage (ml $l^{-1}$) of the commercial formulation

30–37 days after inoculation. Biomass, expressed as fresh weight of zucchini plants at the beginning of flowering, was evaluated at the end of trials 3 and 4.

*Statistical analysis* The data from all the experiments were analyzed using ANOVA (SPSS software 18) and means were spread according to Tukey's test ($P=0.05$; Winer 1962). Disease index data were transformed to the respective arcsin values prior to statistical analysis.

## Results

*Sensitivity of P. xanthii AG1 strain towards azoxystrobin and mychlobutanil* The population of *P. xanthii* AG1 used throughout the work for artificial inoculation was able to cause slight infections on zucchini plants treated with the field dosages of 186 mg $l^{-1}$ of azoxystrobin. In the case of azoxystrobin, $ED_{50}$ of the *P. xanthii* population 7 days after the last treatment ranged between 23.2 and 46.4 mg $l^{-1}$,

**Table 4** Effect of different treatments, expressed as disease incidence, against *Podosphaera xanthii* on zucchini (cv. Genovese) at 11, 19, 26 and 33 days after transplanting (DAT) (Trial 3, Grugliasco)

| Treatment | Dosage (a.i. g or ml $l^{-1}$) | Disease incidence[z] at | | | |
|---|---|---|---|---|---|
| | | DAT 11 | DAT 19 | DAT 26 | DAT 33 |
| *Bacillus subtilis* | 0.4 | 5.0 a[y] | 40.0 b | 48.5 abc | 87.0 c |
| Azoxystrobin | 0.186 | 30.5 b | 44.3 b | 51.0 bc | 71.0 abc |
| Azoxystrobin+*B. subtilis* | 0.186+0.4 | 5.5 a | 41.5 b | 56.7 c | 83.0 bc |
| Mychlobutanil | 0.056 | 1.5 a | 10.9 a | 31.8 ab | 59.5 ab |
| Sulphur | 1.53 | 0.5 a | 9.5 a | 29.3 a | 46.5 a |
| Duolif (mustard oil) | 10.0[x] | 0.5 a | 9.5 a | 33.3 ab | 57.0 ab |
| Silvest (N:K+B, Mo) | 3.5[x] | 41.5 c | 47.3 b | 54.5 c | 70.0 abc |
| Inoculated and not treated control | - | 43.8 c | 63.0 c | 79.0 d | 95.5 c |

[z] Expressed in percent of infected leaves

[y] Within columns, means followed by a common letter do not differ significantly according to Tukey's test ($P<0.05$)

[x] Dosage (ml $l^{-1}$) of the commercial formulation

**Table 5** Effect of different treatments, expressed as disease severity, against *Podosphaera xanthii* on zucchini (cv. Genovese) at 11, 19, 26 and 33 days after transplanting (DAT) (Trial 3, Grugliasco)

| Treatment | Dosage (a.i. g or ml $l^{-1}$) | Disease severity[z] at | | | |
|---|---|---|---|---|---|
| | | DAT 11 | DAT 19 | DAT 26 | DAT 33 |
| *Bacillus subtilis* | 0.4 | 0.3 a[y] | 5.6 b | 13.8 bc | 44.8 de |
| Azoxystrobin | 0.186 | 5.1 c | 13.6 d | 18.5 c | 37.0 cd |
| Azoxystrobin+*B. subtilis* | 0.186+0.4 | 0.6 a | 6.7 bc | 20.7 c | 41.6 de |
| Mychlobutanil | 0.056 | 0.1 a | 0.8 a | 3.7 ab | 18.3 abc |
| Sulphur | 1.53 | 0.1 a | 1.0 a | 3.0 a | 11.3 a |
| Duolif (mustard oil) | 10.0[x] | 0.0 a | 1.0 a | 3.2 a | 17.1 ab |
| Silvest (N:K+B, Mo) | 3.5[x] | 3.6 b | 11.2 cd | 14.3 c | 31.5 bcd |
| Inoculated and not treated control | - | 5.6 c | 27.6 e | 44.5 d | 57.0 e |

[z] Expressed in percent of infected leaf area

[y] Within columns, means followed by a common letter do not differ significantly according to Tukey's test ($P<0.05$)

[x] Dosage (ml $l^{-1}$) of the commercial formulation

while MIC was higher than 372 mg $l^{-1}$. The $ED_{50}$ of mychlobutanil, was 14–28 mg $l^{-1}$, while the MIC was 56 mg $l^{-1}$.

The decreased sensitivity of the population of *P. xanthii* to QoI was confirmed by the low to poor efficacy shown by azoxystrobin in all trials (Tables 2, 3, 4, 5, 6, 7 and 8).

*Efficacy of biocontrol agents and natural compounds against powdery mildew* The artificial inoculation with *P. xanthii* resulted in high infection levels in all trials (Tables 2, 3, 4, 5, 6 and 7), with disease incidence ranging, at the end of the trials in the inoculated untreated controls, from 61% to 96% and disease severity ranging from 20% to 57%.

**Table 6** Effect of different treatments, expressed as disease incidence, against *Podosphaera xanthii* on zucchini (cv. Genovese) at 19, 25 and 32 days after transplanting (DAT) (Trial 4, Grugliasco)

| Treatment | Dosage (a.i. g or ml $l^{-1}$) | Disease incidence[z] at | | |
|---|---|---|---|---|
| | | DAT 19 | DAT 25 | DAT 32 |
| *Bacillus subtilis* | 0.4 | 44.7 c[y] | 48.0 de | 71.5 de |
| Azoxystrobin | 0.186 | 17.9 b | 41.0 cde | 56.7 abcd |
| Azoxystrobin+*B. subtilis* | 0.186+0.4 | 19.4 b | 33.7 cd | 56.0 abcd |
| *Ampelomyces quisqualis* | 0.029 | 39.1 c | 56.0 e | 56.7 abcd |
| Mychlobutanil+*A. quisqualis* | 0.056+0.029 | 13.4 ab | 27.0 bc | 50.5 abc |
| Mychlobutanil | 0.056 | 13.3 ab | 26.5 bc | 49.8 ab |
| Sulphur | 1.53 | 4.5 a | 10.5 ab | 41.5 a |
| Duolif (mustard oil) | 10.0[x] | 4.0 a | 9.0 a | 44.0 ab |
| Kendal (N:K, organic C) | 3.0[x] | 48.8 c | 53.5 e | 62.5 bcde |
| Silvest (N:K+B, Mo) | 3.5[x] | 39.5 c | 46.4 de | 69.5 cde |
| Inoculated and not treated control | - | 63.5 d | 73.5 f | 77.6 e |

[z] Expressed in percent of infected leaves

[y] Within columns, means followed by a common letter do not differ significantly according to Tukey's test ($P<0.05$)

[x] Dosage (ml $l^{-1}$) of the commercial formulation

**Table 7** Effect of different treatments, expressed as disease severity, against *Podosphaera xanthii* on zucchini (cv. Genovese) at 19, 25 and 32 days after transplanting (DAT) (Trial 4, Grugliasco)

| Treatment | Dosage (a.i. g or ml $l^{-1}$) | Disease severity[z] at | | |
|---|---|---|---|---|
| | | DAT 19 | DAT 25 | DAT 32 |
| *Bacillus subtilis* | 0.4 | 7.8 b[y] | 13.8 de | 30.1 cd |
| Azoxystrobin | 0.186 | 1.8 a | 7.7 bc | 22.1 abc |
| Azoxystrobin+*B. subtilis* | 0.186+0.4 | 1.3 a | 8.3 cd | 19.8 abc |
| *Ampelomyces quisqualis* | 0.029 | 9.6 b | 20.7 f | 22.8 abc |
| Mychlobutanil+*A. quisqualis* | 0.056+0.029 | 0.8 a | 2.0 ab | 17.3 abc |
| Mychlobutanil | 0.056 | 0.8 a | 3.9 abc | 13.1 ab |
| Sulphur | 1.53 | 0.3 a | 1.3 a | 9.6 a |
| Duolif (mustard oil) | 10.0[x] | 0.2 a | 1.1 a | 9.9 a |
| Kendal (N:K, organic C) | 3.0[x] | 10.8 b | 18.2 ef | 24.9 bc |
| Silvest (N:K+B, Mo) | 3.5[x] | 8.3 b | 14.6 e | 28.9 cd |
| Inoculated and not treated control | - | 21.9 c | 35.9 g | 40.0 d |

[z]Expressed in percent of infected leaf area

[y]Within columns, means followed by a common letter do not differ significantly according to Tukey's test ($P<0.05$)

[x]Dosage (ml $l^{-1}$) of the commercial formulation

In trial 1, carried out in an open field, the best results, in terms of reduction of disease incidence and disease severity, were achieved, at the end of the trial, by mustard oil and sulphur, followed by the organic-mineral fertilizer N:K 3.5-15.5 (Kendal), *A. quisqualis* alone and in mixture with mychlobutanil, and by the mixture of *B. subtilis* with azoxystrobin. The two biocontrol agents, *B. subtilis* and *A. quisqualis*, when applied alone, only partially controlled the disease. Azoxystrobin and the mineral fertilizer Silvest did not satisfactorily control powdery mildew (Table 2). In particular, at the last reading, in the presence of 70.7% disease incidence in the control plots, mustard oil reduced disease incidence to 27.3%, sulphur to 32.7%, Kendal to 44%, *A. quisqualis* to 45.3%, when applied alone and to 48% when applied in mixture with mychlobutanil (Table 2). Disease severity was reduced from 22.5% in the untreated control to 5.4% and 5.8%, respectively, by mustard oil and terpenic sulphur. The mixture of *B. subtilis*+azoxystrobin reduced disease severity to 10.3% and mychlobutanil+*A. quisqualis* to 14%. *A. quisqualis* and *B. subtilis* alone reduced disease severity, respectively, to 15% and 15.4% (Table 2).

In trial 2, in the open field, in the presence of 85.3% disease incidence and 36.0% disease severity in the untreated control at the end of the trial, mychlobutanil provided the best control of powdery mildew (reducing disease incidence to 40.6% and disease severity to 9.8%), followed by sulphur plus

**Table 8** Effect of different treatments against *Podosphaera xanthii* on biomass of zucchini (cv. Genovese) (Trials 3 and 4, Grugliasco)

| Treatment | Dosage (a.i. g or ml $l^{-1}$) | Biomass (g) | |
|---|---|---|---|
| | | Trial 3 | Trial 4 |
| *Bacillus subtilis* | 0.4 | 118.1 abcd[z] | 120.0 cde |
| Azoxystrobin | 0.186 | 82.1 cd | 141.7 abc |
| Azoxystrobin+*B. subtilis* | 0.186+0.4 | 106.3 bcd | 150.4 ab |
| *Ampelomyces quisqualis* | 0.029 | n.t.[k] | 110.5 de |
| Mychlobutanil+*A. quisqualis* | 0.056+0.029 | n.t.[k] | 93.5 e |
| Mychlobutanil | 0.056 | 138.3 abc | 146.4 abc |
| Sulphur | 1.53 | 169.8 a | 203.5 a |
| Duolif (mustard oil) | 10.0[y] | 158.1 ab | 165.1 b |
| Kendal (N:K, organic C) | 3.0[y] | n.t.[k] | 126.9 cde |
| Silvest (N:K+B, Mo) | 3.5[y] | 102.3 bcd | 152.6 ab |
| Inoculated and not treated control | - | 71.9 d | 126.3 cde |

[z]Within columns, means followed by a common letter do not differ significantly according to Tukey's test ($P<0.05$)

[y]Dosage (ml $l^{-1}$) of the commercial formulation

[k]not tested

terpenes, which reduced disease incidence to 58.0% and disease severity to 12.8%. Mustard oil provided partial control of the disease. The other tested compounds were only partially effective. In particular, azoxystrobin alone and in mixture with *B. subtilis* provided limited disease control. The same poor disease control was observed by applying the mineral fertilizer N:K+Mo and B (Silvest) (Table 3).

In trial 3, under greenhouse conditions, the best disease control was offered by sulphur plus terpenes, followed by mustard oil and mychlobutanil (Tables 4 and 5). Disease incidence, which was 95.5% in the untreated plots, was reduced to 46.5% by terpenic sulphur, 57.0% by mustard oil and 59.5% by mychlobutanil (Table 4). Disease severity, which was 57.0% in the untreated control, was reduced to 11.3% by sulphur, to 17.1% by mustard oil and to 18.3% by mychlobutanil (Table 5). Azoxystrobin, alone and in mixture with *B. subtilis.* provided partial control of powdery mildew as did the mineral fertilizer N:K+Mo and B (Silvest), whereas *B. subtilis* alone was not effective (Tables 4 and 5).

In trial 4, under greenhouse conditions, sulphur plus terpenes and mustard oil confirmed their good activity, followed by mychlobutanil alone and in mixture with *A. quisqualis* (Tables 6 and 7). Disease incidence was reduced from 77.6% in the control plots to 41.5% by sulphur, 44.0% by mustard oil, 49.8% by mychlobutanil and 50.5% by the mixture mychlobutanil+*A. quisqualis* (Table 6). Disease severity was 39.9% in the control plots and was reduced to 9.9% by sulphur plus terpenes and mustard oil, 13.1% by mychlobutanil and 17.2% by the mixture mychlobutanil+A. *quisqualis* (Table 7). Azoxystrobin and the mineral fertilizer Silvest were less effective.

In trials 3 and 4, where also biomass at the end of the trials was considered, sulphur plus terpenes provided the best results, followed by mustard oil (Table 8).

## Discussion

The cucurbit powdery mildew fungus *P. xanthii* has a high potential for developing fungicide resistance, thus complicating disease management. Actually, resistance developed to benzimidazoles, DMIs, organophosphates, hydroxypyrimidines, QoIs, and quinozalines (McGrath 2001) and in some cases, such as DMIs and QoIs, it developed quickly. Following resistance development towards DMIs, it was shown that control with this class of fungicides could be improved by decreasing spray intervals, increasing water volumes, and increasing fungicide dosages (Huggenberger *et al*. 1984). In 1999, after only 2 years of commercial use, strains of *P. xanthii* resistant to QoIs were found in field and greenhouse crops of melon and cucumber in Japan, Taiwan, Spain and France (Heaney *et al.* 2000)

In Italy, resistance to demethylation inhibitors and QoI fungicides has been reported (Gilardi *et al*. 2008). The widespread presence of populations of the pathogen resistant to several of the most commonly used fungicides leads to great interest in the exploitation of control strategies, also based on non-chemical measures (McGrath 2007).

In this study, sulphur consistently provided good disease control both in the open field and under greenhouse conditions. The same good results were provided by mustard oil. Vegetable oil-based fungicides could represent a good alternative to chemical fungicides: they are effective in controlling a number of plant pathogens at low dosages and induce little or no resistance in target fungi (Martin *et al.* 2005); they have very good spreading and leaf surface adhesion characteristics; and, due to their rapid biodegradation, have a low toxicity for human beings and a limited environmental impact.

Serenade biofungicide is based on a naturally occurring strain of *B. subtilis* QST-713 and is registered and used in several countries (Copping 2004; Paulitz and Bélanger 2001). It works through complex modes of action that entail biological action of the bacteria and also lipopeptide compounds (iturins, agrastatin/plipastatins and surfactins) produced by it, which are well known for their antimicrobial properties (Manker 2005; Marrone 2002). The complex mode of action of *B. subtilis* (Jacobsen *et al*. 2004; Romero *et al.* 2007) is well suited for its use in integrated control.

AQ 10, based on the strain AQ 10 of *A. quisqualis* and commercialized in several countries, parasitizes powdery mildew colonies and is active against several powdery mildews on different hosts (Copping 2004; Hofstein *et al.* 1996; Paulitz and Bélanger 2001). Also AQ 10 is intended for use as part of an integrated disease management programme and is compatible with a wide range of chemicals (McGrath and Shishkoff 1999; Shishkoff and McGrath 2002). Previous works carried out on cucurbits showed that the same formulation of *B. subtilis* gave inconsistent results (from ineffective to very effective) against powdery mildews when applied alone. In alternation with QoIs, *B. subtilis* was significantly more effective (Keinath and DuBose 2004). *B. subtilis* QST 713 alternated with sulphur, mychlobutanil and trifloxystrobin provided good control of powdery mildew of lettuce (Matheron and Porchas 2000). A synergistic effect among *B. subtilis* and QoI fungicides when applied against *P. xanthii* on zucchini was reported by Gilardi *et al.* (2008).

In this work, in the presence of high disease pressure, it was possible to manage powdery mildew of zucchini effectively with both sulphur plus terpenes and mustard oil. Mychlobutanil alone and in combination with *A. quisqualis* provided interesting results. The good activity shown by the formulation containing sulphur and terpenes as well as mychlobutanil, and the possibility of introduction of a natural product such as mustard oil, and biocontrol agents in integrated disease management strategies provides choices for the extension service and growers. Azoxystrobin, due to the presence of resistance, did not provide satisfactory control of the pathogen.

This study provides further information to previous work, showing the possibility of introducing natural compounds such as mustard oil within management strategies. It shows that an old fungicide such as sulphur plus terpenes can perform well, if applied properly.

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## References

Braun, U., & Takamatsu, S. (2000). Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (Erysipheae) and *Cystotheca*, *Podosphaera*, *Sphaerotheca* (Cystotheceae) inferred from rDNA ITS sequences – some taxonomic consequences. *Schlechtendalia, 4*, 1–33.

Copping, L. G. (Ed.). (2004). *The manual of biocontrol agents*. Alton, Hampshire, UK: British Crop Protection Council.

EPPO. (2004). *EPPO Standards PP1* (2nd ed., Vol. 2). Paris, France: European and Mediterranean Plant Protection Organization.

Gilardi, G., Manker, D. C., Garibaldi, A., & Gullino, M. L. (2008). Efficacy of the biocontrol agents *Bacillus subtilis* and *Ampelomyces quisqualis* applied in combination with fungicides against powdery mildew of zucchini. *Journal of Plant Diseases and Protection, 115*, 208–213.

Hagiladi, A., & Ziv, O. (1986). The use of antitranspirants for the control of powdery mildew of roses in the field. *Journal of Environmental Horticulture, 4*, 69–71.

Heaney, S. P., Hall, A. A., Davies, S. A., & Olaya, G. (2000). Resistance to fungicides in the QoI-STAR cross-resistance group: current perspectives. *Proc. British Crop Protection Conference on Pests and Diseases, 2*, 755–762.

Hofstein, R., Daoust, R. A., & Aeschlimann, J. P. (1996). Constraints to the development of biofungicides: the example of AQ 10, a new product for controlling powdery mildews. *Entomophaga, 41*, 455–460.

Horst, R. K., Kawamoto, S. O., & Porter, L. L. (1992). Effect of sodium bicarbonate and oils on the control of powdery mildew and black spot of roses. *Plant Disease, 76*, 247–251.

Huggenberger, F., Collins, M. A., & Skylakakis, G. (1984). Decreased sensitivity of *Sphaerotheca fuliginea* to fenarimol and other ergosterol-biosynthesis inhibitors. *Crop Protection, 3*, 137–149.

Ishii, H. (2010). QoI fungicide resistance: current status and the problems associated with DNA-based monitoring. In U. Gisi, I. Chet, & M. L. Gullino (Eds.), *Recent developments in management of plant diseases* (pp. 37–45). Dordrecht, the Netherlands: Springer.

Jacobsen, B. J., Zidack, N. K., & Larson, B. J. (2004). The role of *Bacillus*-based biological control agents in integrated pest management systems: plant diseases. *Phytopathology, 94*, 1272–1275.

Keinath, A. P., & DuBose, V. B. (2004). Evaluation of fungicides for prevention and management of powdery mildew on watermelon. *Crop Protection, 23*, 35–42.

Manker, D. C. (2005). Natural products as green pesticides. In J. M. Clark & H. Ohkawa (Eds.), *New discoveries in agrochemicals* (pp. 283–294). Washington, DC: American Chemical Society.

Marrone, P. G. (2002). An effective biofungicide with novel modes of action. *Pesticide Outlook, 13*, 193–194.

Martin, B., Hernandez, S., Silvarrey, C., Jacas, J. A., & Cabaleion, C. (2005). Vegetable, fish and mineral oils control grapevine powdery mildew. *Phytopathologia Mediterranea, 44*, 169–179.

Matheron, M. E., & Porchas, M. (2000). Evaluation of fungicide performance for control of powdery mildew on lettuce in 2000. Online publication no. *AZ1177 in: Vegetable:* College of Agriculture Report 2000. College of Agriculture, University of Arizona, Tucson, AZ, USA.

McGrath, M. T. (2001). Fungicide resistance in cucurbit powdery mildew, experiences and challenges. *Plant Disease, 85*, 236–245.

McGrath, M. T. (2007). Managing cucurbit powdery mildew and fungicide resistance. *Acta Horticulturae, 731*, 211–216.

McGrath, M. T., & Shishkoff, N. (1999). Evaluation of biocompatible fungicides for managing cucurbit powdery mildew. *Crop Protection, 18*, 471–478.

Pasini, C., D'Aquila, F., Curir, P., & Gullino, M. L. (1997). Effectiveness of antifungal compounds against rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*) in glasshouses. *Crop Protection, 16*, 251–256.

Paulitz, T. C., & Bélanger, R. B. (2001). Biological control in greenhouse systems. *Annual Review of Phytopathology, 39*, 103–133.

Reuveni, R., & Reuveni, M. (1998). Foliar-fertilizer therapy – a concept in integrated pest management. *Crop Protection, 17*, 111–118.

Romero, D., Devicente, A., Rakotoaly, R. H., Dufour, S. E., Veening, J. W., Arrebola, E., *et al*. (2007). The iturin and fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* toward *Podosphaera fusca*. *Molecular Plant-Microbe Interactions, 20*, 430–440.

Shishkoff, N., & McGrath, M. T. (2002). AQ10 biofungicide combined with chemical fungicides or AddQ spray adjuvant for control of cucurbit powdery mildew in detached leaf culture. *Plant Disease, 86*, 915–918.

Sitterly, W. R. (1978). Powdery mildew of cucurbits. In D. M. Spencer (Ed.), *The powdery mildews* (pp. 359–379). London, UK: Academic Press.

Winer, B. J. (1962). *Statistical principles in experimental design* (2nd ed.). New York, NY: McGraw-Hill.

Zitter, T. A., Hopkins, D. L., & Thomas, C. E. (1996). *Compendium of cucurbit diseases*. St. Paul, MN, USA: APS Press.