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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 against *Podosphaera xanthii* on zucchini was tested and compared to the activity of fungicides used alone in four experimental trials carried out in open field and under greenhouse conditions. The population of *P. xanthii* used throughout the work for artificial inoculation was able to cause infections on zucchini plants treated with the field dosages of azoxystrobin, while was susceptible to mychlobutanil. Sulphur plus terpenes and mustard oil consistently controlled powdery mildew, followed by mychlobutanil alone or combined with *A. quisqualis*. *B. subtilis* and *A. quisqualis* when tested alone were partially effective. Azoxystrobin in all the four trials only partially controlled powdery mildew. The combination of azoxystrobin and *B. subtilis* was only delaying the spread of the pathogen.

Key words: *Podosphaera xanthii*; natural compounds; biological control; integrated disease management

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 on crops grown in open field as well as under greenhouse. Powdery mildew in Italy is particularly serious on crops such as melon and zucchini.

Fungicide application and the use of resistant cultivars are the main means of disease control. However, in spite of this, powdery mildew continues to cause serious losses worldwide (Zitter et al. 1996). In practice, fungicide treatments are the main disease management strategy for cucurbit powdery mildew control (McGrath 2001). Unfortunately, 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 and 2007). During the past few years, resistance became widespread also in the case of QoIs fungicides (McGrath, 2007; Ishii, 2010)

Biological control agents as well as natural compounds are possible alternatives to the use of chemicals, that 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 (Paulitz and Bélanger 2001; Gilardi et al., 2008). 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 largely exploited against several agents of powdery mildews on a number of crops (Horst et al., 1992; Pasini et al., 1997; Hagiladi and Ziv, 1986; Martin et al., 2005; Stephan et al., 2005; Rongai et al., 2009), providing in many cases very interesting results. Moreover, in some cases a positive effective of mineral fertilisers 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 fertilisers, and of two biofungicides based on *B. subtilis* and *A. quisqualis* alone and in combination with fungicides, in comparison with fungicides (included sulphur) used alone against *P. xanthii* on zucchini (*Cucurbita pepo* L.) under open field and greenhouse conditions.

MATERIALS AND METHODS

Experimental trials. Two experimental trials were carried out in open field at Boves (Cuneo) and two under glasshouse at Grugliasco (TO).

Field trials. Zucchini plants (cv. Xsara) 18 day-old, were transplanted into soil covered with black plastic mulch by following a randomized block design, with three replicates and 8 plants/replicate.

Greenhouse trials. Zucchini plants (cv. Genovese) were grown in pots (14x14 cm, 2 L volume of soil) in a peat: clay: perlite substrate (65:30:5 v/v). Two plants/pot were planted. Plants were maintained at temperatures ranging between 24 and 27 °C, at 60-70% RH. Fifteen-day old plants with their second true expanded leaf were used. A randomised block design with four replicates was used.

Sensitivity of the pathogen to the fungicides used during the trials. The strain AG 1 of *P. xanthii* was collected in Piedmont (Northern Italy) from infected zucchini. The sensitivity of *P. xanthii* AG1 strain towards azoxystrobin and myclobutanil 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 myclobutanil. The seedlings treated were placed in a greenhouse at a temperature of 22-25°C. The artificial inoculation was carried out 24 h after the fungicide treatment by using a paint-brush, with $1x10^5$ conidia cm^{-2}. Inoculated and not treated plants were used as control. After 7-14 days from the last treatment, the percentage of zucchini leaves affected by *P. xanthii* (disease incidence) was evaluated by using a scale from 0 to 5 (0: No infection, 1= 0 to 0.99 % of 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%). 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. *Bacillus subtilis* QST 713 (Serenade WP, AgraQuest Inc, USA, 10% a.i.) and *Ampelomyces quisqualis* (AQ 10, Intrachem Bio Italia S.p.A., Bergamo, Italy, 58% a.i.) were used as commercial formulations and applied, as foliar sprays, at the suggested dosages, as reported under Tables 2-8. AQ 10 was applied in combination with Nu-Film P, as recommended by the company.

Azoxystrobin (Ortiva, Syngenta Crop Protection S.p.A., Milano, Italy, 23.2% a.i.), myclobutanil (Thiocur forte, DowAgrosciences, 4.5 % a.i.), sulphur plus terpenes (Heliosoufre S, Intrachem Bio Italia S.p.A., Bergamo, Italy, 51,1% a.i.), mustard oil (Duolif, Cerealtoscana S.p.A., Livorno, Italy, soluble organic nitrogen 3%, soluble sulphur 15%, organic matter 80%), organic-mineral fertiliser N:K (Kendal, soluble organic nitrogen 3.5%, soluble potassium oxide 15.5%, organic carbon 3-4% Valagro, Atessa, Chieti, Italy), mineral fertiliser 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 applied at the dosages reported under Tables 2 - 8.

When applied together, chemicals and biofungicides were mixed before spraying. Treatments were carried out, at 6-8 day intervals, by using 800 l ha^{-1} with a EFCO atomizer. Treatments were carried out 24 h before the artificial inoculation with the pathogen. Two to three sprays were carried out in the different trials (Table 1).

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 evaluated. 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 disease index used throughout the experiments ranged from 0 to 100 (0 = healthy plant; 1 = 0-0.99 % of infected leaf area; 2 = 1-4.99 % infected leaf area; 3 = 5-19.99 % infected leaf area; 4 = 20-40% infected leaf area; 5 = > 40%). The final disease rating took place 30-37 days after inoculation. Biomass, expressed as fresh weight of zucchini plants at beginning of flowering, was also evaluated at the end of trials 3 and 4.

Statistical analysis. The data from all the experiments were analysed 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 myclobutanil. 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 *P. xanthii* population after 7 days from the last treatment ranged between 23.2 and 46.4 mg L^{-1}, while MIC was higher than 372 mg L^{-1}. In the case of mychlobutanil, its ED_{50} was 14-28 mg L^{-1}, while the MIC was 56 mg L^{-1}. MIC.

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 2-8).

Efficacy of biocontrol agents and natural compounds against powdery. The artificial inoculation with *P. xanthii* resulted in high infection levels in all trials (Tables 2-7), 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 %.

In trial 1, carried out in open field, the best results, in terms of reduction of disease incidence and disease severity were provided, at the end of the trial, by mustard oil and sulphur, followed by the organic-mineral fertiliser 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 terpenes, which reduced disease incidence to 58.0 and disease severity to 12.8%. Mustard oil provided a partial control of the disease. The other tested compounds were only partially effective. In particular, azoxystrobin alone and in mixture with *B. subtilis* provided a 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 a only partial control of powdery mildew as well as the mineral fertilizer N:K+Mo and B (Silvest), while *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). Resistance did develop quickly in some cases, such as DMIs and QoIs. 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 two 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 demethtylation 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 makes very interesting the exploitation of control strategies, also based on non-chemical measures (McGrath, 2007).

In this study, sulphur consistently provided a 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 quick biodegradation rate, they have a low toxicity for human beings and cause 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 (Paulitz and Bélanger 2001; Copping 2004). 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, well known for their antimicrobial properties (Marrone 2002; Manker, 2005). The complex mode of action of *B. subtilis* (Jacobsen et al., 2004; Romero et al, 2007) is well suited for its use under integrated control strategies.

AQ 10, based on 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 (Hofstein et al. 1996; Paulitz and Bélanger 2001; Copping 2004). 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* showed 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, myclobutanil 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 effectively powdery mildew of zucchini 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 natural product such as mustard oil, and biocontrol agents in integrated disease management strategies provides choices for extension services and growers.

Azoxystrobin, due to the presence of resistance, did not provide a satisfactory control of the pathogen.

This study offers further development to the previous ones, showing the possibility of introducing natural compounds such as mustard oil within management strategies. In the mean time, it shows that an old fungicide such as sulphur plus terpenes can perform well, if applied properly.

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Table 1 Dates of the operations carried out and calendar of treatments in the different trials.

Operation	Trial			
	1	2	3	4
Transplant	7/08/2008	13/07/2010	10/02/2011	25/02/2011
Artificial inoculation with *Podosphaera xanthii*	14/08/2008	06/08/2010	16/02/2011	09/03/2011
First treatment	13/08/2008	20/07/2010	15/02011	08/03/2011
Second treatment	22/08/2008	28/07/2010	22/02/2011	15/03/2011
Third treatment	-	13/08/2010	01/03/2011	23/0372011
First evaluation	11/09/2008	19/08/2010	22/02/2011	22/03/2011
Second evaluation	25/09/2008	26/08/2010	08/03/2011	28/03/2011
Third evaluation	-	-	15/03/2011	04/04/2011
Biomass evaluation	-	-	15/03/2011	04/04/2011

Table 2 Effect of different treatments, expressed as disease incidence and disease severity, against *Podosphaera xanthii* on zucchini (cv. Xsara) (Trial 1, Boves)

Treatment	Dosage a.i. g or ml L^{-1}	Disease incidence at [x]		Disease severity at [y]	
		11/09/2008	25/09/2008	11/09/2008	25/09/2008
Bacillus subtilis	0.4	40.8 bc[w]	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[z]	46.7 bcd	44.0 abc	11.5 ab	10.4 ab
Duolif (mustard oil)	10.0 [z]	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

[x]Expressing the percent of infected leaves

[y] Expressing the percent of infected leaf area.

[w]Means within a column, followed by the same letter do not significantly differ following Tukey's Test $P < 0.05$.

[z] 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) (Trial 2, Boves).

Treatment	Dosage a.i. g or ml L^{-1}	Disease incidence at [x]		Disease severity at [y]	
		19/08/2010	26/08/2010	19/08/2010	26/08/2010
Bacillus subtilis	0.4	62.8 cd[w]	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 [z]	64.5 cd	74.0 bcd	23.1 cd	20.4 ab
Duolif (mustard oil)	10.0[z]	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

[x] Expressing the percent of infected leaves

[y] Expressing the percent of infected leaf area.

[w] Means within a column, followed by the same letter do not significantly differ following Tukey's Test $P < 0.05$.

[z] Dosage (ml L^{-1}) of the commercial formulation.

Table 4 Effect of different treatments, expressed as disease severity, against *Podosphaera xanthii* on zucchini (cv. Genovese) (Trial 3, Grugliasco)

Treatment	Dosage a.i. g or ml L^{-1}	Disease incidence[x] at			
		22/02/2011	01/03/2011	08/03/2011	15/03/2011
Bacillus subtilis	0.4	5.0 a[w]	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 [z]	0.5 a	9.5 a	33.3 ab	57.0 ab
Silvest (N:K+B, Mo)	3.5[z]	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

[x] Expressing the percent of infected leaves

[w] Means within a column, followed by the same letter do not significantly differ following Tukey's Test *$P < 0.05$.*

[z] 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) (Trial 3, Grugliasco)

Treatment	Dosage a.i. g or ml L^{-1}	Disease severity[y] at			
		22/02/2011	01/03/2011	08/03/2011	15/03/2011
Bacillus subtilis	0.4	0.3 a[w]	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 [z]	0.0 a	1.0 a	3.2 a	17.1 ab
Silvest (N:K+B, Mo)	3.5[z]	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

[y] Expressing the percent of infected leaf area.

[w] Means within a column, followed by the same letter do not significantly differ following Tukey's Test *$P < 0.05$.*

[z] Dosage (ml L^{-1}) of the commercial formulation.

Table 6 Effect of different treatments, expressed as disease severity, against *Podosphaera xanthii* on zucchini (cv. Genovese) (Trial 4, Grugliasco)

Treatment	Dosage a.i. g or ml L^{-1}	Disease incidence [x] at		
		23/03/2011	28/03/2011	04/04/2011
Bacillus subtilis	0.4	44.7 c[w]	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
Mychlobutamil + *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[z]	4.0 a	9.0 a	44.0 ab
Kendal (N:K, organic C)	3.0[z]	48.8 c	53.5 e	62.5 bcde
Silvest (N:K+B, Mo)	3.5[z]	39.5 c	46.4 de	69.5 cde
Inoculated and not treated control	-	63.5 d	73.5 f	77.6 e

[x] Expressing the percent of infected leaves

[w] Means within a column, followed by the same letter do not significantly differ following Tukey's Test $P < 0.05$.

[z] 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) (Trial 4, Grugliasco)

Treatment	Dosage a.i. g or ml L^{-1}	Disease severity [y] at		
		23/03/2011	28/03/2011	04/04/2011
Bacillus subtilis	0.4	7.8 b[w]	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
Mychlobutamil + *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[z]	0.2 a	1.1 a	9.9 a
Kendal (N:K, organic C)	3.0[z]	10.8 b	18.2 ef	24.9 bc
Silvest (N:K+B, Mo)	3.5[z]	8.3 b	14.6 e	28.9 cd
Inoculated and not treated control	-	21.9 c	35.9 g	40.0 d

[y] Expressing the percent of infected leaf area.

[w] Means within a column, followed by the same letter do not significantly differ following Tukey's Test $P < 0.05$.

[z] Dosage (ml L^{-1}) of the commercial formulation.

Table 8 Effect of different treatments, against *Podosphaera xanthii* on zucchini (cv. Genovese) on biomass (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[w]	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.	110.5 de
Mychlobutamil + *A. quisqualis*	0.056+0.029	n.t.	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[z]	158.1 ab	165.1 b
Kendal (N:K, organic C)	3.0[z]	n.t.	126.9 cde
Silvest (N:K+B, Mo)	3.5[z]	102.3 bcd	152.6 ab
Inoculated and not treated control	-	71.9 d	126.3 cde

[w] Means within a column, followed by the same letter do not significantly differ following Tukey's Test $P < 0.05$.

[z] Dosage (ml L^{-1}) of the commercial formulation.