

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/89319> since 2016-11-12T13:11:05Z

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

Gilardi G.; Baudino M.; Garibaldi A.; Gullino M.L.. Efficacy of biocontrol agents and natural compounds against powdery mildew of zucchini.. PHYTOPARASITICA. 40 pp: 147-155.

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/89319>

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

2
3 Giovanna Gilardi, Michele Baudino, Angelo Garibaldi and Maria Lodovica Gullino

4
5 ¹Centre of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA), University of
6 Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy

7 ² Consorzio di Ricerca e Sviluppo per l'Ortofrutticoltura Piemontese (CReSO)-
8 Via Albertasse 16 - 12012 Boves (CN), Italy.

9
10 Corresponding author. Tel.:+ 39 0116708540; Fax: +39 0116709307.

11 E-mail address: marialodovica.gullino@unito.it

12 13 Abstract

14
15 The activity of different types of natural compounds and of two biofungicides based on *Bacillus subtilis* and
16 *Ampelomyces quisqualis* alone and in combination with fungicides against *Podosphaera xanthii* on zucchini was
17 tested and compared to the activity of fungicides used alone in four experimental trials carried out in open field
18 and under greenhouse conditions. The population of *P. xanthii* used throughout the work for artificial
19 inoculation was able to cause infections on zucchini plants treated with the field dosages of azoxystrobin, while
20 was susceptible to mychlobutanil. Sulphur plus terpenes and mustard oil consistently controlled powdery
21 mildew, followed by mychlobutanil alone or combined with *A. quisqualis*. *B. subtilis* and *A. quisqualis* when
22 tested alone were partially effective. Azoxystrobin in all the four trials only partially controlled powdery
23 mildew. The combination of azoxystrobin and *B. subtilis* was only delaying the spread of the pathogen.

24
25 **Key words:** *Podosphaera xanthii*; natural compounds; biological control; integrated disease management

26 27 INTRODUCTION

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

33 Fungicide application and the use of resistant cultivars are the main means of disease control. However, in spite
34 of this, powdery mildew continues to cause serious losses worldwide (Zitter et al. 1996). In practice, fungicide
35 treatments are the main disease management strategy for cucurbit powdery mildew control (McGrath 2001).
36 Unfortunately, the intensive use of chemicals against *P. xanthii* often resulted in the development of resistance:
37 this has happened in the case of most of the groups of chemicals applied (McGrath 2001 and 2007). During
38 the past few years, resistance became widespread also in the case of QoIs fungicides (McGrath, 2007; Ishii,
39 2010)

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

47 Different types of so called natural compounds, ranging from salts such as sodium bicarbonate to plant extracts
48 and oils have been largely exploited against several agents of powdery mildews on a number of crops (Horst et
49 al., 1992; Pasini et al., 1997; Hagiladi and Ziv, 1986; Martin et al., 2005; Stephan et al., 2005; Rongai et al.,
50 2009), providing in many cases very interesting results. Moreover, in some cases a positive effective of mineral
51 fertilisers has been shown (Reuveni and Reuveni, 1998).

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

56

57 MATERIALS AND METHODS

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

60

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

63

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

68

69 **Sensitivity of the pathogen to the fungicides used during the trials.** The strain AG 1 of *P. xanthii* was
70 collected in Piedmont (Northern Italy) from infected zucchini. The sensitivity of *P. xanthii* AG1 strain towards
71 azoxystrobin and myclobutanil was evaluated by treating zucchini seedlings at the cotyledon stage with
72 increasing rates of the two fungicides up to twice their field dosages, corresponding respectively to 0.186 ml L⁻¹
73 for azoxystrobin and 0.056 ml L⁻¹ for myclobutanil. The seedlings treated were placed in a greenhouse at a
74 temperature of 22-25°C. The artificial inoculation was carried out 24 h after the fungicide treatment by using a
75 paint-brush, with 1x10⁵ conidia cm⁻². Inoculated and not treated plants were used as control. After 7-14 days
76 from the last treatment, the percentage of zucchini leaves affected by *P. xanthii* (disease incidence) was
77 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 %
78 infected leaf area; 3 = 5-19.9 % infected leaf area; 4 = 20-40% infected leaf area; 5 = > 40%). The minimal

79 inhibitory concentration (MIC) and the concentrations able to inhibit 50% (ED₅₀) of the development of *P.*
80 *xanthii* in comparison with the inoculated and non-treated control were evaluated.

81

82 **Treatments.** *Bacillus subtilis* QST 713 (Serenade WP, AgraQuest Inc, USA, 10% a.i.) and *Ampelomyces*
83 *quisqualis* (AQ 10, Intrachem Bio Italia S.p.A., Bergamo, Italy, 58% a.i.) were used as commercial
84 formulations and applied, as foliar sprays, at the suggested dosages, as reported under Tables 2-8. AQ 10 was
85 applied in combination with Nu-Film P, as recommended by the company.

86 Azoxystrobin (Ortiva, Syngenta Crop Protection S.p.A., Milano, Italy, 23.2% a.i.), myclobutanil (Thiocur
87 forte, DowAgrosciences, 4.5 % a.i.), sulphur plus terpenes (Heliosoufre S, Intrachem Bio Italia S.p.A.,
88 Bergamo, Italy, 51,1% a.i.), mustard oil (Duolif, Cerealtoscana S.p.A., Livorno, Italy, soluble organic nitrogen
89 3%, soluble sulphur 15%, organic matter 80%), organic-mineral fertiliser N:K (Kendal, soluble organic nitrogen
90 3.5%, soluble potassium oxide 15.5%, organic carbon 3-4% Valagro, Atessa, Chieti, Italy), mineral fertiliser
91 N:K+ B, and Mo (Silvest, soluble organic nitrogen 8%, soluble potassium oxide 8%, soluble boron 0.1%,
92 soluble molybdenum 0.01%, Green Has Italia S.p.A., Canale d'Alba, Cuneo, Italy) were applied at the dosages
93 reported under Tables 2 - 8.

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

97

98 **Data collection.** Typical symptoms of powdery mildew started to be visible 7-20 days after artificial
99 inoculation. Plants were checked every 7 days after the last treatment for disease development and the
100 percentage of zucchini leaves affected by *P. xanthii* (disease incidence) was evaluated. The evaluations were
101 carried out by assessing the upper surfaces of 50 (first and second evaluation, Trial 1) and 100 leaves. Disease
102 severity was evaluated by using a disease index ranging from 0 to 5 (EPPO 2004). The disease index used
103 throughout the experiments ranged from 0 to 100 (0 = healthy plant; 1 = 0-0.99 % of infected leaf area; 2 = 1-
104 4.99 % infected leaf area; 3 = 5-19.99 % infected leaf area; 4 = 20-40% infected leaf area; 5 = > 40%). The
105 final disease rating took place 30-37 days after inoculation. Biomass, expressed as fresh weight of zucchini
106 plants at beginning of flowering, was also evaluated at the end of trials 3 and 4.

107

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

111

112 **RESULTS**

113 **Sensitivity of *P. xanthii* AG1 strain towards azoxystrobin and myclobutanil.** The population of *P. xanthii*
114 AG1 used throughout the work for artificial inoculation was able to cause slight infections on zucchini plants
115 treated with the field dosages of 186 mg L⁻¹ of azoxystrobin. In the case of azoxystrobin, ED₅₀ of *P. xanthii*
116 population after 7 days from the last treatment ranged between 23.2 and 46.4 mg L⁻¹, while MIC was higher
117 than 372 mg L⁻¹. In the case of myclobutanil, its ED₅₀ was 14-28 mg L⁻¹, while the MIC was 56 mg L⁻¹. MIC.

118 The decreased sensitivity of the population of *P. xanthii* to QoI was confirmed by the low to poor efficacy
119 shown by azoxystrobin in all trials (Tables 2-8).

120

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

124 In trial 1, carried out in open field, the best results, in terms of reduction of disease incidence and disease
125 severity were provided, at the end of the trial, by mustard oil and sulphur, followed by the organic-mineral
126 fertiliser N:K 3.5-15.5 (Kendal), *A. quisqualis* alone and in mixture with mychlobutanil and by the mixture of
127 *B. subtilis* with azoxystrobin. The two biocontrol agents, *B. subtilis* and *A. quisqualis*, when applied alone, only
128 partially controlled the disease. Azoxystrobin and the mineral fertilizer Silvest did not satisfactorily control
129 powdery mildew (Table 2). In particular, at the last reading, in the presence of 70.7% disease incidence in the
130 control plots, mustard oil reduced disease incidence to 27.3%, sulphur to 32.7%, Kendal to 44%, *A. quisqualis*
131 to 45.3%, when applied alone and to 48% when applied in mixture with mychlobutanil (Table 2). Disease
132 severity was reduced from 22.5 % in the untreated control to 5.4 and 5.8% respectively by mustard oil and
133 terpenic sulphur. The mixture of *B. subtilis* + azoxystrobin reduced disease severity to 10.3% and mychlobutanil
134 + *A. quisqualis* to 14%. *A. quisqualis* and *B. subtilis* alone reduced disease severity respectively to 15 and
135 15.4% (Table 2).

136 In trial 2, in the open field, in the presence of 85.3 % disease incidence and 36.0% disease severity in the
137 untreated control at the end of the trial, mychlobutanil provided the best control of powdery mildew (reducing
138 disease incidence to 40.6 and disease severity to 9.8%), followed by sulphur plus terpenes, which reduced
139 disease incidence to 58.0 and disease severity to 12.8%. Mustard oil provided a partial control of the disease.
140 The other tested compounds were only partially effective. In particular, azoxystrobin alone and in mixture with
141 *B. subtilis* provided a limited disease control. The same poor disease control was observed by applying the
142 mineral fertilizer N:K+Mo and B (Silvest) (Table 3).

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

150 In trial 4, under greenhouse conditions, sulphur plus terpenes and mustard oil confirmed their good activity,
151 followed by mychlobutanil alone and in mixture with *A. quisqualis* (Tables 6 and 7). Disease incidence was
152 reduced from 77.6% in the control plots to 41.5% by sulphur, 44.0 % by mustard oil, 49.8 % by mychlobutanil
153 and 50.5% by the mixture mychlobutanil + *A. quisqualis* (Table 6). Disease severity was 39.9 % in the control
154 plots and was reduced to 9.9 % by sulphur plus terpenes and mustard oil, 13,1 % by mychlobutanil and 17.2%
155 by the mixture mychlobutanil + *A. quisqualis* (Table 7). Azoxystrobin and the mineral fertilizer Silvest were
156 less effective.

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

159

160 **DISCUSSION**

161

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

170 In Italy, resistance to demethylation inhibitors and QoI fungicides has been reported (Gilardi et al., 2008). The
171 widespread presence of populations of the pathogen resistant to several of the most commonly used fungicides
172 makes very interesting the exploitation of control strategies, also based on non-chemical measures (McGrath,
173 2007).

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

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

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

196 In this work, in the presence of high disease pressure, it was possible to manage effectively powdery mildew of
197 zucchini with both sulphur plus terpenes and mustard oil. Mychlobutanil alone and in combination with *A.*
198 *quisqualis* provided interesting results.
199 The good activity shown by the formulation containing sulphur and terpenes as well as mychlobutanil, and the
200 possibility of introduction of natural product such as mustard oil, and biocontrol agents in integrated disease
201 management strategies provides choices for extension services and growers.
202 Azoxystrobin, due to the presence of resistance, did not provide a satisfactory control of the pathogen.
203 This study offers further development to the previous ones, showing the possibility of introducing natural
204 compounds such as mustard oil within management strategies. In the mean time, it shows that an old fungicide
205 such as sulphur plus terpenes can perform well, if applied properly.
206

207 **Acknowledgements**

208
209 This work was carried out with a grant from of “Valorizzazione dell’orticoltura transfrontaliera VALORT”
210 supported by the ALCOTRA programme of the European Union.
211

212 **REFERENCES**

- 213
214 Braun, U., & Takamatsu S. (2000) Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (Erysipheae) and
215 *Cystotheca*, *Podosphaera*, *Sphaerotheca* (Cystothecaceae) inferred from rDNA ITS sequences – some taxonomic
216 consequences. *Schlechtendalia* 4, 1-33.
217 Copping, L.G. (ed.), (2004) The Manual of Biocontrol Agents. British Crop Protection Council, Alton,
218 Hampshire, UK.
219 EPPO, (2004) EPPO Standards PP1, 2nd Edition, Vol. 2. European and Mediterranean Plant Protection
220 Organization, Paris.
221 Gilardi, G., Manker, D.C., Garibaldi, A., & Gullino, M.L. (2008) Efficacy of the biocontrol agents *Bacillus*
222 *subtilis* and *Ampelomyces quisqualis* applied in combination with fungicides against powdery mildew of
223 zucchini. *Journal of Plant Diseases and Protection*, 115, 208-213.
224 Hagiladi, A., & Ziv, O. (1986) The use of antitranspirants for the control of powdery mildew of roses in the
225 field. *Journal Environmental Horticulture* 4, 69-71.
226 Heaney, S.P., Hall, A.A., Davies, S.A., & Olaya, G. (2000) Resistance to fungicides in the QoI-STAR cross-
227 resistance group: current perspectives. *Proc. BCPC Conf. Pests Dis.* 2, 755-762.
228 Hofstein, R., Daoust, R.A., & Aeschlimann, J.P. (1996) Constraints to the development of biofungicides: the
229 example of AQ 10, a new product for controlling powdery mildews. *Entomophaga* 41, 455-460.
230 Horst, R.K., Kawamoto, S.O. & Porter, L.L. (1992) Effect of sodium bicarbonate and oils on the control of
231 powdery mildew and black spot of roses. *Plant Disease* 76, 247-251.
232 Huguenberger, F., Collins, M.A., & Skylakakis, G. (1984) Decreased sensitivity of *Sphaerotheca fuliginea* to
233 fenarimol and other ergosterol-biosynthesis inhibitors. *Crop Prot.* 3, 137-149.
234 Ishii, H., (2010). QoI fungicide resistance: current status and the problems associated with DNA-based
235 monitoring. In: Recent developments in management of plant diseases (Gisi U., Chet I., Gullino M.L. ds.),
236 Springer, Dordrecht, The Netherlands, 37-45.

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

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

241 Manker, D.C. (2005) Natural products as green pesticides. In: J.M. Clark, H. Ohkawa (eds.): *New Discoveries*
242 *in Agrochemicals*, pp. 283-294. American Chemical Society, Washington, DC and Columbus, OH, USA.

243 Marrone, P.G., (2002) An effective biofungicide with novel modes of action. *Pestic. Outl.* 13, 193-194.

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

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

249 McGrath, M.T. (2001) Fungicide resistance in cucurbit powdery mildew, experiences and challenges. *Plant Dis.*
250 85, 236-245.

251 McGrath M.T. (2007) Managing cucurbit powdery mildew and fungicide resistance. *Acta Horticulturae*, 731:
252 211-216.

253

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

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

258 Paulitz, T.C., & Bélanger, R.B. (2001) Biological control in greenhouses systems. *Annu. Rev. Phytopathol.* 39,
259 103-133.

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

262 Romero, D., Devicente, A., Rakotoaly, R.H., Dufour, S.E., Veening, J.W. , Arrebola, E., Cazorta, F.M.,
263 Kuipers, O.P., Paquot, M., & Perez-Garcia, A. (2007) The iturin and fengycin families of lipopeptides are key
264 factors in antagonism of *Bacillus subtilis* toward *Podosphaera fusca*. *Mol. Plant-Microbe Interact.* 20, 430-440.

265 Rongai, D., Cerato, C., & Lazzeri, L. (2009) A natural fungicide for the control of *Erysiphe betae* and *Erysiphe*
266 *cichoracearum*. *European Journal of Plant Pathology*, 124, 613-619.

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

269 Sitterly, W.R., (1978) Powdery mildew of cucurbits. In: D.M. Spencer (ed.): *The Powdery Mildews*, pp. 359-
270 379. Academic Press, London.

271 Stephan, D., Schmitt, A., Martins Carvalho, S., Seddon, B., & Koch, E. (2005) Evaluation of biocontrol
272 preparations and plant extracts for the control of *Phytophthora infestans* on potato leaves. *Eur. J. Plant Pathol.*
273 112, 235-246.

274 Winer, B.J., 1962: *Statistical Principles in Experimental Design*, 2nd Edition. McGraw-Hill, New York.

275 Zitter, T.A., Hopkins, D.L., & Thomas, C.E. (1996) *Compendium of Cucurbit Diseases*. APS Press, St. Paul,
276 MN, USA.

277

278 **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 <i>Podosphaera xanthii</i>	14/08/2008	06/08/2010	16/02/2011	09/03/2011
First treatment	13/08/2008	20/07/2010	15/02/2011	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	23/03/2011
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

279

280

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

Treatment	Dosage	Disease incidence at ^x		Disease severity at ^y	
	a.i. g or ml L ⁻¹	11/09/2008	25/09/2008	11/09/2008	25/09/2008
<i>Bacillus subtilis</i>	0.4	40.8 bc ^w	52.0 bcd	8.8 a	15.4 ab
<i>Ampelomyces quisqualis</i>	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 + <i>B. subtilis</i>	0.186+0.4	45.0 bcd	48.0 abc	8.9 a	10.3 ab
Mychlobutanil + <i>A. quisqualis</i>	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

283 ^xExpressing the percent of infected leaves

284 ^y Expressing the percent of infected leaf area.

285 ^wMeans within a column, followed by the same letter do not significantly differ following
 286 Tukey's Test $P < 0.05$.

287 ^z Dosage (ml L⁻¹) of the commercial formulation.

288

289

290

291

292 **Table 3** Effect of different treatments, expressed as disease incidence and severity, against
 293 *Podosphaera xanthii* on zucchini (cv. Xsara) (Trial 2, Boves).

Treatment	Dosage	Disease incidence at ^x		Disease severity at ^y	
	a.i. g or ml L ⁻¹	19/08/2010	26/08/2010	19/08/2010	26/08/2010
<i>Bacillus subtilis</i>	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 + <i>B. subtilis</i>	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

294 ^x Expressing the percent of infected leaves

295 ^y Expressing the percent of infected leaf area.

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

298 ^z Dosage (ml L⁻¹) of the commercial formulation.

299

300

301 **Table 4** Effect of different treatments, expressed as disease severity, against *Podosphaera*
 302 *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
<i>Bacillus subtilis</i>	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 + <i>B. subtilis</i>	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

303 ^x Expressing the percent of infected leaves

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

306 ^z Dosage (ml L⁻¹) of the commercial formulation.

307

308 **Table 5** Effect of different treatments, expressed as disease severity, against *Podosphaera*
 309 *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
<i>Bacillus subtilis</i>	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 + <i>B. subtilis</i>	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

310 ^y Expressing the percent of infected leaf area.

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

313 ^z Dosage (ml L⁻¹) of the commercial formulation.

314

315 **Table 6** Effect of different treatments, expressed as disease severity, against *Podosphaera*
 316 *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
<i>Bacillus subtilis</i>	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 + <i>B. subtilis</i>	0.186+0.4	19.4 b	33.7 cd	56.0 abcd
<i>Ampelomyces quisqualis</i>	0.029	39.1 c	56.0 e	56.7 abcd
Mychlobutamil + <i>A. quisqualis</i>	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

317 ^x Expressing the percent of infected leaves

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

320 ^z Dosage (ml L⁻¹) of the commercial formulation.

321

322

323 **Table 7** Effect of different treatments, expressed as disease severity, against *Podosphaera*
 324 *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
<i>Bacillus subtilis</i>	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 + <i>B. subtilis</i>	0.186+0.4	1.3 a	8.3 cd	19.8 abc
<i>Ampelomyces quisqualis</i>	0.029	9.6 b	20.7 f	22.8 abc
Mychlobutamyl + <i>A. quisqualis</i>	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

325 ^y Expressing the percent of infected leaf area.

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

328 ^z Dosage (ml L⁻¹) of the commercial formulation.

329

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

Treatment	Dosage a.i. g or ml L ⁻¹	Biomass (g)	
		Trial 3	Trial 4
<i>Bacillus subtilis</i>	0.4	118.1 abcd ^w	120.0 cde
Azoxystrobin	0.186	82.1 cd	141.7 abc
Azoxystrobin + <i>B. subtilis</i>	0.186+0.4	106.3 bcd	150.4 ab
<i>Ampelomyces quisqualis</i>	0.029	n.t.	110.5 de
Mychlobutamil + <i>A. quisqualis</i>	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

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

334 ^z Dosage (ml L⁻¹) of the commercial formulation.

335
 336