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1 **Management of downy mildew of sweet basil (*Ocimum basilicum* L.) caused by *Peronospora***  
2 ***belbahrii* by means of resistance inducers, fungicides and alternative control measures**

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11

12 **Abstract** In the present study a number of compounds (acibenzolar-S-methyl, mineral and organic  
13 fertilizers, plant extracts), known for their capability of inducing resistance in plants to several  
14 pathogens, were tested in 2011 and 2012, in comparison with registered fungicides, copper-based  
15 fungicides and biocontrol agents, to control downy mildew of sweet basil (*Ocimum basilicum* L.),  
16 incited by *Peronospora belbahrii*. Four experimental trials were carried out under glasshouse  
17 conditions in the presence of a high disease incidence. The tested compounds were used alone or  
18 applied in rotation in spray programmes. In all trials the best results, in terms of reduction of disease  
19 incidence and disease severity were offered by metalaxyl-M + copper hydroxide, by the mineral  
20 fertilizer Alexin, by mandipropanid and by azoxostybin, followed by the glucohumates activator  
21 complex and acibenzolar-S-methyl. Such products did significantly reduce disease incidence and  
22 severity also 20 days after the last treatment. Among the copper-based products, the best results  
23 were provided by copper hydroxide with terpenic alcohols and copper oxychloride + copper  
24 hydroxide. The mineral fertilizer Kendal and prohexadione calcium, the biocontrol agent *B. subtilis*  
25 QST 713, thyme oil extract and mustard oil on the contrary, were not effective. When different  
26 combination of various products used in rotation were tested, it was possible to reduce disease  
27 incidence and severity with different strategies, based on the rotation of fungicides and resistance  
28 inducers as well as with the rotation of resistance inducers.

29

30 **Keywords** organic and mineral fertilizers; fungicides; acibenzolar-S-methyl; *Bacillus subtilis*;  
31 integrated control

32

33 **Introduction**

34

35 Sweet basil (*Ocimum basilicum* L.) is an economically important herb crop in several  
36 Mediterranean regions, in the USA and in many other parts of the world. This popular herb is used  
37 both as a fresh and dried food spice, and in traditional medicine (Csizinsky, 1993; Lucier, 1993). In  
38 Italy, most of the basil production takes place on the Riviera Ligure, and ‘Genovese Gigante’ is the  
39 most appreciated variety for fresh consumption. It is the only cultivar used for industrial production  
40 of the pesto sauce and covers 90% of the total growing area (Montalti, 1995). Also in Piedmont  
41 basil is an important crop, grown in particular outdoor, especially for the production of pesto sauce,  
42 with an average production of 4,000-4,500 tons/year, not considering the small producers. In  
43 general, basil is mostly grown under greenhouses; in this environment average night temperatures  
44 and dew depositions caused by high RH favour the development of diseases such as grey mould  
45 (*Botrytis cinerea*), downy mildew (*Peronospora belbahrii*) and foliar spots (*Alternaria alternata*,  
46 *Colletotrichum* sp.) especially after cool nights following warm, humid days (Garibaldi *et al.*, 1997;  
47 Garibaldi *et al.*, 2011).

48 In particular, downy mildew, incited by *P. belbahrii*, was observed in northern Italy in 2003  
49 (Garibaldi *et al.*, 2004) and quickly spread to other Italian regions in Central and Southern Italy  
50 (Minuto *et al.*, 2004) as well as in France (Garibaldi *et al.*, 2005). This pathogen was first reported  
51 in Uganda, identified as *Peronospora* sp. (Hansford, 1934) and much later in Switzerland (Lefort *et al.*,  
52 2003). After this report in Switzerland, the pathogen spread to many basil growing areas. Its  
53 spread has been probably favoured by the fact that it is seed-transmitted (Garibaldi *et al.*, 2004).  
54 The causal agent of basil downy mildew has been recently identified as *Peronospora belbahrii*  
55 (Belbahri *et al.*, 2005; Thines *et al.*, 2009).

56 The disease was recently reported also in Belgium (Coosemans, 2004), in USA (Roberts *et al.*,  
57 2009), in Cuba (Martinez de La Parte *et al.*, 2010) and in Hungary (Nagy and Horvath, 2011).

58 One of the hypothesis of its sudden, almost simultaneous spread in so many countries, is linked to  
59 the production of seeds in developing countries where the pathogen is endemic.

60 The management of downy mildew of basil is complicated by the very limited availability of  
61 chemicals on the crop, due to the risk of the presence of residues at harvest as well as on the  
62 difficulty to have registered fungicides on minor crops such as basil (Leadbeater and Gisi, 2010).  
63 The few fungicides registered in Italy on basil (azoxystrobin, belonging to QoI, metalaxyl-M,  
64 belonging to phenylamides, and mandipropamid belonging to carboxylic acid amides) must be used  
65 in a very limited number of sprays for avoiding the selection of resistant strains of the pathogen.  
66 The search for varieties of basil resistant to the pathogen are still at early stages (Wyenandt *et al.*,  
67 2010).

68 In the present study a number of compounds, known for their capability of inducing resistance  
69 in plants to several pathogens, was tested, in comparison with registered fungicides and biocontrol  
70 agents to control basil downy mildew.

71

## 72 **Materials and methods**

73

74 *Basil growth and experimental conditions* Four experimental trials (Table 1) were carried in 2011  
75 and 2012 out at Grugliasco (Torino) in a glasshouse belonging to Agroinnova. Details about the  
76 trials are reported in table 1. Basil seeds (cv. Genovese selection 'Italiano classico', Pagano  
77 Sementi) were sown (0.2 g/pot, corresponding to 70-80 plants/pot) in plastic pots (1.5 L vol., 12 x  
78 12 cm) containing a peat:perlite (80:20 v/v) mix (Turco Silvestro, Albenga, Savona), steamed (90°C  
79 for 30 minutes).

80 For each treatment, four replicates (2 pots each) with a randomized design were used. All replicates  
81 of the same treatment were managed similarly in terms of fertilization.

82 During the trials temperatures ranged between 19 and 25 °C. The basil pots were maintained over  
83 the benches and covered with a plastic sheet placed over all the plants by using iron supports (1.0  
84 m high, 3.0 m wide and 6.0 m long). The plastic sheets were placed on the iron support immediately  
85 after each artificial inoculation and maintained until the last assessment. The relative humidity was  
86 maintained close to 100% by misting during the experimental trials. The dates of the different  
87 operations carried out are reported under table 1.

88

89 *Artificial inoculation* The populations of *Peronospora belbahrii*, collected in Piedmont (Northern  
90 Italy) from diseased plants, were maintained on basil plants. The artificial inoculation was carried  
91 out throughout nebulisation with a laboratory spray bottle (10 ml of capacity) of a suspension of  
92  $1 \times 10^5$  sporangia/ml. One ml of suspensions/treatment (corresponding to 8 pots), was used. The  
93 artificial inoculation was carried out 24 h after the second treatment in trials 1-3, while in trial 4 two  
94 artificial inoculations were carried out (Table 1).

95

96 *Treatments* Several compounds, known for their capability to induce resistance in the host, salts,  
97 fertilizers, copper-based fungicides as well as fungicides registered on basil were tested. In trial 1-3  
98 the different compounds were tested separately, while in trial 4 several spray programmes were  
99 tested.

100

101 *Plant inducers, salts and fertilizers* Acibenzolar-S-methyl (Bion 50WG, 50% a.i., Syngenta, Italy),  
102 prohexadione calcium (Regalis, 10% a.i, BASF, Italy), organic-mineral fertilizer based on  
103 glucohumates complex N:P (Glucoinductor + GlucoActivator, N 4%, P<sub>2</sub>O<sub>5</sub> 18%, International  
104 patent PCT, IB2004\001905, Fertirev, Torino, Italy), organic-mineral fertilizer N:K (Kendal,  
105 soluble organic nitrogen 3.5%, soluble potassium oxide 15.5%, organic carbon 3-4% Valagro,  
106 Atessa, Chieti, Italy), mineral fertilizer (Kendal TE, Cu 23%, Mn 0,5%, Zn 0,5%, Valagro, Atessa,  
107 Chieti, Italy) and (Alexin 95PS, P<sub>2</sub>O<sub>5</sub> 52%, K<sub>2</sub>O 42%, Massò, Milano, Italy), were tested at the  
108 dosages reported under tables 2- 5.

109

110 *Plant extracts and BCAs* Thyme oil (*Thymus vulgaris* 100% a.i. plant extract, Soave & C, Italy),  
111 mustard oil (Duolif, soluble organic nitrogen 3%, soluble sulphur 15%, organic matter 80%,  
112 Cerealtoscana S.p.A., Livorno, Italy), *Bacillus subtilis* QST 713 (Serenade MAX, 15,67% a.i.,  
113 BASF, Italy) were tested alone or in alternation.

114

115 *Copper-based products* Copper oxychloride (Cupravit Flow, 20% a.i., Bayer, Italy), copper  
116 oxychloride and copper hydroxide (Airone, 10%+10% a.i. Isagro, Italy), copper sulfate (Cuproxat  
117 SDI, 15.2% a.i., Nufarm GnbH & CoKG, Austria), copper hydroxide and terpenic alcohols  
118 (Heliocuvire, 26.7% a.i., Intrachem Bio Italia, Italy), peptide complex copper sulfate, tackifying  
119 compound and natural inducers of systemic induced resistance (SAR) (Labimethyl, 3%+ 2% a.i.,  
120 Macasa, Spain), were tested at the dosages reported under tables 2-5.

121

122 *Chemical fungicides* Azoxystrobin (Ortiva, Syngenta Crop Protection S.p.A., Milano, Italy, 23.2%  
123 a.i.), mandipropamid (Pergado SC, 23.4% a.i., Syngenta Crop Protection S.p.A., Italy), metalaxyl-  
124 M + copper oxychloride (Ridomil R WG 2.5% + 40% a. i., Syngenta Crop Protection S.p.A., Italy)  
125 were tested. Such fungicides are registered for use on basil in Italy and routinely applied in the  
126 commercial production.

127 All treatments were carried out on basil plants 16-21 days after sowing at the dates reported under  
128 table 1 by using 800 L ha<sup>-1</sup>.

129 In the trials 1-3, two treatments at 6 day interval were carried out, with the exception of chemical  
130 fungicides tested that were applied once at the dates reported under table 1. One artificial  
131 inoculation with the pathogen was made 24 hours after the last treatment applications.

132 In trial 4, three treatments were carried out at 6 days interval, by using the tested products alone and  
133 in alternation. Two artificial inoculations with the pathogen were carried out respectively, 24 hours  
134 after the first and third treatment (Table 1).

135

136 *Data collection and analysis* Starting at the appearance of the first symptoms, the evaluation of the  
137 percent of infected leaves (disease incidence) and of diseased leaf tissue (disease severity) was  
138 made by using a disease rating scale with: 1 = up to 5 % infected leaf area; 2 = up to 10% infected  
139 leaf area; 3 = up to 25% infected leaf area; 4 = up to 50% infected leaf area; 5= up to 75 %  
140 infected leaf area. Disease incidence and severity was estimated on 100 leaves/treatment.

141 In trials 4, plant biomass, as fresh weight of plants at the end of the trial, was also measured.

142 All data collected were statistically analysed by univariate Anova with Tukey's test using SPSS  
143 software 18.

144

## 145 **Results**

146

147 In all the four trials the artificial inoculation with *P. belbahrii* led to a high level of disease in the  
148 untreated inoculated control; at the last estimating the development of pathogen attacks, disease  
149 incidence ranged respectively from 66.8 to 88.8 and disease severity from 40.7 to 66.6. The level of  
150 infection reached with the artificial inoculation was consistent in the different trials (Tables 2-5). In  
151 the different trials and especially in the first one, infection was present also in the non inoculated  
152 control plants; this is probably due to a contamination of the used seeds as well as with the spread  
153 of the pathogen among plants (Table 2).

154 In trial 1, the best results, in terms of reduction of disease incidence and disease severity were  
155 offered by the mixture metaxyl-M + copper hydroxide, by the mineral fertilizer Alexin, by  
156 mandipropanid and by azoxostybin, followed by the glucohumates activator complex. Such  
157 products did significantly reduce disease incidence and severity also 20 days after the last treatment  
158 (Table 2). Twenty days after the last treatment, disease incidence was reduced from 88.8 in the  
159 inoculated control to 5.5, 13.5, 16.3, 18.0 and 30.8 in the plants treated with the above mentioned  
160 products. The same treatments reduced disease severity from 66.6 to 3.8, 9.1, 9.8, 11.9 and 22.0,  
161 respectively (Table 2). The copper-based treatments offered a partial control of the disease as well  
162 as acibenzolar-S-methyl. Among the copper-based products, the best results were provided by  
163 copper hydroxide mixed with terpenic alcohols and copper sulphate + copper gluconate.  
164 Probexadone calcium and mineral fertilizer Kendal, alone or combined with copper (Kendal TE)  
165 provided only a very partial activity. *Bacillus subtilis* QST 713 and thyme oil extract did not  
166 control downy mildew (Table 2).

167 Similar results were obtained in trial 2, in the presence of a similar level of infection. Metalaxyl-  
168 M + copper hydroxide, mandipropanid, azoxystrobin, the mineral fertilizer Alexin, followed by

169 glucohumates activator complex were very effective in reducing downy mildew incidence and  
170 severity. In this trial acibenzolar-S-methyl offered very good results. Also prohexadione calcium,  
171 and the mustard oil provided interesting results in terms of reducing disease incidence and disease  
172 severity. Among the different copper-based products tested, copper oxychloride + copper  
173 hydroxide and copper hydroxide with terpenic alcohols provided interesting results followed by  
174 copper sulphate compounds and by the mineral fertilizer Kendal TE (Table 3). *Bacillus subtilis* and  
175 thyme oil extract were not effective (Table 3).

176 In trial 3, a similar trend was observed, with the same products providing the best control, in  
177 terms of reduction of disease incidence and severity. Among the copper based products, with the  
178 exception of copper oxychloride + copper hydroxide, copper hydroxide with terpenic alcohols, and  
179 copper sulphate, copper oxychloride and the mineral fertilizer Kendal TE were partially effective,  
180 as well as mustard oil and prohexadione calcium. *B. subtilis* and thyme oil confirmed to be not  
181 effective (Table 4).

182 In trial 4, when used alone in 3 treatments, the fungicides metalaxyl-M + copper hydroxide,  
183 mandipropanid, azoxystrobin, the mineral fertilizer Alexin and glucohumates activator complex  
184 confirmed their very good activity (Table 5). Very interestingly, when different combination of  
185 various products used in rotation were tested, it was possible to reduce disease incidence and  
186 severity with different strategies, based on the rotation of fungicides and resistance inducers as well  
187 as with the rotation of resistance inducers.

188 When also the effect on biomass was evaluated, in trial 4, the mineral fertilizer Alexin provided the  
189 best results, while the glucohumate activator complex caused some reduction (Table 5).

190

## 191 **Discussion**

192

193 Downy mildew incidence was consistent and relevant in all trials as a consequence of the artificial  
194 inoculation and permitted to evaluate the different products and strategies of disease control under  
195 severe disease pressure. The infection observed in some trials in the not inoculated control was  
196 probably caused by natural infection of seeds as well as by the spread of the pathogen among plants  
197 with consequent cross contamination. The results obtained in the different trials were quite  
198 consistent in terms of response of the different tested products. Among the tested fungicides,  
199 metalaxyl-M + copper hydroxide and azoxystyrobin confirmed its efficacy (Gullino *et al.*, 2009).  
200 Also mandipropamid, admitted for use on basil in Italy with a maximum of 50% of the total number  
201 of applications or in alternation with fungicides having other modes of action, was quite effective.  
202 The copper-based fungicides provided a partial control of the disease, except copper oxychloride

203 which was not effective. Copper-based products were mostly effective when applied in rotation  
204 with resistance inducers such as the mineral fertilizer Alexin and with the glucohumates activator  
205 product.

206 The most interesting results were consistently provided by the mineral fertilizer Alexin and by  
207 the glucohumates activator complex, which provided, used alone or in rotation with other products,  
208 also 20 days after the last treatment, results similar to those offered by the most active fungicides.  
209 Also acibenzolar-S-methyl provided a good disease control of basil downy mildew with pre-  
210 infection treatment, thus confirming what already observed for this compound when applied against  
211 other foliar diseases, such as grey mould (*Botrytis cinerea*) on tomato (Malolepsza, 2006),  
212 anthracnose (*Colletotrichum lagenarium*) on cucumber (Ishii *et al.*, 1999) and blue mould  
213 (*Peronospora tabacina*) on tobacco (LaMondia, 2009).

214 The mineral fertilizer Kendal, the biocontrol agent *B. subtilis*, thyme oil extract and mustard oil, on  
215 the contrary, were not effective.

216 Resistance inducers, also frequently referred to as plant strengthening agents, are legal under  
217 current understanding and are treated under the fertilizer regulation (Kappert *et al.*, 2011). In  
218 horticulture, particularly on vegetables and ornamentals, they are increasingly used as a means of  
219 production and disease control. Their application is particularly interesting in the case of minor  
220 crops, because of the lack of registered fungicides, as well as in organic farming, where they can  
221 contribute in health maintenance and production quality (Kappert *et al.*, 2011).

222 Plant resistance inducers or improvers provided good results under different conditions, for  
223 instance against *Peronospora destructor*, *P. parasitica*, *Bremia lactucae* and *Pseudoperonospora*  
224 *cubensis* under greenhouse conditions (Kofot and Fisher, 2007): in general they are more effective  
225 in the presence of moderate infection pressure and are generally influenced by different factors,  
226 such as genotype, inoculum density, climatic conditions. In the case of the previously mentioned  
227 pathogens, only phosphonates confirmed their efficacy also under field conditions (Kofot and  
228 Fischer, 2007). Also a positive effects of their post-infection application have been reported (Wicks  
229 *et al.*, 1999). In the case of *Peronospora parasitica* of cauliflower, acibenzolar-S-methyl was  
230 effective by inducing the production of pathogenesis-related proteins (Ziadi *et al.*, 2001). The same  
231 compound proved effective against Phytophthora blight, caused by *P. capsici*, on squash (*Cucurbita*  
232 *pepo*) (Ji *et al.*, 2011).

233 In our study, some of the resistance inducers and fertilizers provided very interesting results,  
234 when applied alone as well as in rotation with fungicides. As shown in trial 4, several combination  
235 of products, applied in rotation in three treatments, permit to strongly reduce downy mildew  
236 incidence and severity. Of particular interest are the strategies that, besides providing a good disease

237 control, do not cause strong reduction in the biomass. Moreover, it must be pointed out that the  
238 rotation of effective fungicides with resistance inducers strongly reduces the risk of presence of  
239 fungicide residues in a crop such as basil characterized by a high number of harvests. In the case of  
240 basil crops for pesto production, harvest is carried out every 20 days. In the mean time, the rotation  
241 between fungicides with resistance inducers will reduce the selection pressure by the fungicides,  
242 thus reducing the risk of development of resistance towards the few registered fungicides.

243

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245

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249

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324

325 **Table 1** Operations carried out in the four trials

| Operation                      | Trial number          |                      |                      |                                       |
|--------------------------------|-----------------------|----------------------|----------------------|---------------------------------------|
|                                | 1                     | 2                    | 3                    | 4                                     |
| Date of sowing                 | 18/08/2011            | 21/09/2011           | 22/09/2011           | 27/12/2011                            |
| Dates of treatments            | 9/09; 15/09           | 7/10; 13/10          | 14/10; 20/10         | 11/01/2012; 18/01/2012;<br>25/01/2012 |
| Date of artificial inoculation | 16/09                 | 14/10                | 21/10                | 12/01/2012; 26/01/2012                |
| Dates of disease assessment    | 23/09; 30/09;<br>5/10 | 26/10; 2/11;<br>7/11 | 31/10;<br>7/11;14/11 | 1/02/2012;<br>8/02/2012;17/02/2012    |
| Date of biomass evaluation     | -                     | -                    | -                    | 17/02/2012                            |

**Table 2** Efficacy of different treatments, expressed as disease incidence (DI, % of infected leaves) and disease severity (DS, % of infected leaf area) against downy mildew, incited by *Peronospora belbahii*, on basil cv. Genovese, selection ‘Italiano Classico’, Trial

| Active ingredient                                                          | Commercial product <sup>x</sup> | Dosage<br>a.i.100 L | DI at day after last treatment <sup>x</sup> |                 |      | DS at day after last treatment <sup>x</sup> |      |     |      |                  |      |    |      |     |
|----------------------------------------------------------------------------|---------------------------------|---------------------|---------------------------------------------|-----------------|------|---------------------------------------------|------|-----|------|------------------|------|----|------|-----|
|                                                                            |                                 |                     | 8                                           | 15              | 20   | 8                                           | 15   | 20  |      |                  |      |    |      |     |
| -                                                                          | Inoculated control              | -                   | 40.4                                        | gh <sup>k</sup> | 66.8 | e                                           | 88.8 | f   | 22.4 | f-i <sup>k</sup> | 46.7 | e  | 66.6 | f   |
| Copper oxychloride                                                         | Cupravit flow                   | 50                  | 33.3                                        | d-h             | 44.9 | d                                           | 64.3 | de  | 23.3 | g-i              | 28.7 | cd | 46.8 | c-e |
| Copper oxychloride + copper hydroxide                                      | Airone                          | 40+40               | 27.5                                        | c-g             | 39.0 | d                                           | 56.5 | de  | 18.8 | e-h              | 25.8 | cd | 40.1 | b-e |
| Acibenzolar-S-methyl                                                       | Bion                            | 1                   | 5.5                                         | ab              | 21.5 | bc                                          | 49.3 | cd  | 1.0  | a                | 12.4 | ab | 32.6 | bc  |
| Organic mineral fertilizer N:K                                             | Kendal                          | 10.5+45             | 35.6                                        | e-h             | 46.3 | d                                           | 72.5 | d-f | 23.3 | g-i              | 33.2 | cd | 54.4 | d-f |
| Mineral fertilizer Cu+Mn+Zn                                                | Kendal TE                       | 46+1.5+1.5          | 26.8                                        | c-f             | 39.1 | d                                           | 65.0 | d-f | 16.4 | d-g              | 25.7 | cd | 47.3 | c-f |
| Prohexadione calcium                                                       | Regalis                         | 5                   | 22.0                                        | cd              | 42.0 | d                                           | 58.8 | de  | 14.1 | c-f              | 29.3 | cd | 41.9 | b-e |
| Thyme oil extract                                                          | Tyme oil                        | 100                 | 44.0                                        | h               | 50.5 | de                                          | 77.5 | ef  | 30.4 | i                | 36.5 | de | 57.5 | ef  |
| <i>Bacillus subtilis</i> QST713                                            | Serenade                        | 58.4                | 23.5                                        | c-e             | 38.0 | cd                                          | 70.0 | d-f | 16.1 | d-g              | 24.8 | cd | 52.5 | d-f |
| Glucosaminates activator complex <sup>z</sup>                              | Glucosinductor                  | 400                 | 7.8                                         | ab              | 16.5 | ab                                          | 30.8 | bc  | 5.5  | a-c              | 11.5 | ab | 22.2 | ab  |
| Peptide complex copper sulfate                                             | Labimethyl                      | 9+6                 | 22.3                                        | c-e             | 40.9 | d                                           | 54.9 | c-e | 14.8 | d-g              | 25.7 | cd | 39.9 | b-e |
| Copper hydroxide and terpenic alcohols                                     | Heliocouivre                    | 60                  | 15.5                                        | bc              | 35.0 | cd                                          | 51.5 | cd  | 10.0 | b-d              | 21.9 | bc | 36.1 | b-d |
| Copper sulfate                                                             | Cuproxtat                       | 53.2                | 16.6                                        | bc              | 40.3 | d                                           | 56.3 | de  | 11.8 | b-e              | 26.3 | cd | 39.5 | b-e |
| Mineral fertilizer P <sub>2</sub> O <sub>5</sub> 52%, K <sub>2</sub> O 42% | Alexin                          | 130+105             | 0.0                                         | a               | 6.3  | ab                                          | 13.5 | ab  | 0.0  | a                | 3.7  | a  | 9.1  | a   |
| Metalaxyl-M + copper hydroxide                                             | Ridomil Gold R                  | 7.5+ 120            | 0.0                                         | a               | 1.3  | a                                           | 5.5  | a   | 0.0  | a                | 0.7  | a  | 3.8  | a   |
| Mandipropamid                                                              | Pergado                         | 11.7                | 4.5                                         | ab              | 9.5  | ab                                          | 16.3 | ab  | 3.4  | ab               | 5.4  | a  | 9.8  | a   |
| Azoxystrobin                                                               | Ortiva                          | 18.6                | 1.0                                         | a               | 6.0  | ab                                          | 18.0 | ab  | 0.8  | a                | 2.7  | a  | 11.6 | a   |
| -                                                                          | Not inoculated control          | -                   | 37.5                                        | f-h             | 48.2 | d                                           | 73.0 | d-f | 25.8 | hi               | 33.6 | cd | 54.4 | d-f |

<sup>x</sup> Two treatments were carried out on: 9/09/2011; 15/09/2011. One artificial inoculation was carried out on: 16/09/2011

<sup>k</sup> Means of the same column, followed by the same letter do not differ according to Tukey’s test (P<0.05)

<sup>z</sup> Dosage ( ml 100 L<sup>-1</sup>) of the commercial formulation

**Table 3** Efficacy of different treatments, expressed as disease incidence (DI, % of infected leaves) and as disease severity (DS, % of infected leaf area) against downy mildew, incited by *Peronospora belbahii* on basil cv. Genovese, selection ‘Italiano Classico’, Trial 2

| Active ingredient                                                          | Commercial product <sup>x</sup> | Dosage<br>g a.i.100 L <sup>-1</sup> | DI at at day after last treatment |                |      |                 |      |     | DS at day after last treatment <sup>x</sup> |                |      |     |      |     |
|----------------------------------------------------------------------------|---------------------------------|-------------------------------------|-----------------------------------|----------------|------|-----------------|------|-----|---------------------------------------------|----------------|------|-----|------|-----|
|                                                                            |                                 |                                     | 13 <sup>x</sup>                   | 20             | 25   | 13 <sup>x</sup> | 20   | 25  |                                             |                |      |     |      |     |
| -                                                                          | Inoculated control              | -                                   | 53.5                              | e <sup>k</sup> | 81.8 | f               | 84.5 | f   | 32.9                                        | e <sup>k</sup> | 58.6 | f   | 61.8 | e   |
| Copper oxychloride                                                         | Cupravit flow                   | 50                                  | 19.0                              | b-d            | 36.3 | cd              | 43.8 | c-e | 7.1                                         | a-c            | 25.0 | d   | 28.9 | b-d |
| Copper oxychloride + copper hydroxide                                      | Airone                          | 40+40                               | 11.0                              | ab             | 10.8 | ab              | 13.0 | ab  | 3.6                                         | a              | 6.8  | a-c | 7.3  | a   |
| Acibenzolar-S-methyl                                                       | Bion                            | 1                                   | 2.0                               | ab             | 6.0  | ab              | 7.5  | ab  | 0.8                                         | a              | 3.9  | a-c | 4.7  | a   |
| Organic mineral fertilizer N:K                                             | Kendal                          | 10.5+45                             | 31.3                              | cd             | 26.3 | bc              | 50.5 | de  | 17.9                                        | b-d            | 18.4 | cd  | 36.1 | cd  |
| Mineral fertilizer Cu+Mn+Zn                                                | Kendal TE                       | 46+1.5+1.5                          | 9.3                               | ab             | 25.3 | bc              | 27.8 | b-d | 3.1                                         | a              | 16.3 | b-d | 18.1 | a-c |
| Prohexadione calcium                                                       | Regalis                         | 5                                   | 13.3                              | a-c            | 26.0 | bc              | 25.3 | a-e | 5.3                                         | ab             | 17.6 | b-d | 18.4 | a-c |
| Thyme oil extract                                                          | Time oil                        | 100                                 | 36.2                              | de             | 58.5 | de              | 61.3 | ef  | 22.9                                        | de             | 41.3 | e   | 44.7 | de  |
| Mustard oil <sup>z</sup>                                                   | Duolif                          | 1000                                | 12.5                              | ab             | 16.5 | a-c             | 25.5 | a-e | 5.1                                         | ab             | 10.5 | a-d | 15.4 | a-c |
| <i>Bacillus subtilis</i> QST713                                            | Serenade                        | 58.4                                | 35.5                              | de             | 74.5 | ef              | 83.8 | f   | 20.0                                        | c-e            | 55.8 | ef  | 60.3 | e   |
| Glucohumates activator complex <sup>z</sup>                                | Glucoinductor                   | 400                                 | 0.0                               | a              | 4.3  | ab              | 3.8  | ab  | 0.0                                         | a              | 1.6  | ab  | 2.1  | a   |
| Peptide complex copper sulfate                                             | Labimethyl                      | 9+6                                 | 6.0                               | ab             | 15.5 | a-c             | 24.8 | a-e | 2.7                                         | a              | 10.9 | a-d | 15.4 | a-c |
| Copper hydroxide and terpenic alcohols                                     | Heliocuvire                     | 60                                  | 1.3                               | ab             | 7.3  | ab              | 13.3 | ab  | 0.4                                         | a              | 4.5  | a-c | 7.4  | a   |
| Copper sulfate                                                             | Cuproxat                        | 53.2                                | 8.5                               | ab             | 15.0 | a-c             | 18.3 | a-c | 2.8                                         | a              | 9.9  | a-d | 10.3 | ab  |
| Mineral fertilizer P <sub>2</sub> O <sub>5</sub> 52%, K <sub>2</sub> O 42% | Alexin                          | 130+105                             | 0.0                               | a              | 0.0  | a               | 2.5  | ab  | 0.0                                         | a              | 0.0  | a   | 1.8  | a   |
| Metalaxyl-M + copper hydroxide                                             | Ridomil Gold R                  | 7.5+ 120                            | 0.0                               | a              | 0.0  | a               | 0.8  | a   | 0.0                                         | a              | 0.0  | a   | 0.2  | a   |
| Mandipropamid                                                              | Pergado                         | 11.7                                | 0.0                               | a              | 0.3  | a               | 1.3  | ab  | 0.0                                         | a              | 0.1  | a   | 0.8  | a   |
| Azoxystrobin                                                               | Ortiva                          | 18.6                                | 0.0                               | a              | 0.0  | a               | 1.3  | ab  | 0.0                                         | a              | 0.0  | a   | 0.6  | a   |
| -                                                                          | Non inoculated control          | -                                   | 0.0                               | a              | 0.3  | a               | 6.8  | ab  | 0.0                                         | a              | 0.1  | a   | 4.6  | a   |

<sup>x</sup> Two treatments were carried out on:7/10; 13/10. One artificial inoculation was carried out on: 14/10

<sup>k</sup> Means of the same column, followed by the same letter do not differ according to Tukey’s test (P<0.05)

<sup>z</sup> Dosage ( ml 100 L<sup>-1</sup>) of the commercial formulation

**Table 4** Efficacy of different treatments, expressed as disease incidence (DI, % of infected leaves) and as disease severity (DS, % of infected leaf area) against downy mildew, incited by *Peronospora belbahii* on basil cv. Genovese, selection ‘Italiano Classico’, Trial 3

| Active ingredient                                                          | Commercial product <sup>x</sup> | Dosage<br>g a.i.100 L <sup>-1</sup> | DI at day after last treatment <sup>x</sup> |                |      |     |      |     | DS at day after last treatment <sup>x</sup> |                |      |     |      |     |
|----------------------------------------------------------------------------|---------------------------------|-------------------------------------|---------------------------------------------|----------------|------|-----|------|-----|---------------------------------------------|----------------|------|-----|------|-----|
|                                                                            |                                 |                                     | 11                                          | 18             | 25   | 11  | 18   | 25  | 11                                          | 18             | 25   |     |      |     |
| -                                                                          | Inoculated control              | -                                   | 38.3                                        | e <sup>k</sup> | 47.5 | h   | 83.8 | h   | 26.8                                        | f <sup>k</sup> | 35.6 | h   | 62.8 | h   |
| Copper oxychloride                                                         | Cupravit flow                   | 50                                  | 22.0                                        | d              | 27.5 | e-f | 61.3 | f-h | 15.8                                        | e              | 20.6 | fg  | 45.9 | f-h |
| Copper oxychloride + copper hydroxide                                      | Airone                          | 40+40                               | 8.5                                         | a-c            | 11.7 | a-c | 21.3 | a-d | 6.0                                         | a-d            | 8.1  | a-d | 15.9 | a-d |
| Acibenzolar-S-methyl                                                       | Bion                            | 1                                   | 3.3                                         | a              | 6.5  | ab  | 14.0 | a-c | 2.1                                         | a              | 3.6  | ab  | 10.5 | a-c |
| Organic mineral fertilizer N:K                                             | Kendal                          | 10.5+45                             | 17.0                                        | cd             | 25.0 | d-f | 53.8 | e-g | 12.0                                        | de             | 18.8 | e-g | 40.3 | e-g |
| Mineral fertilizer Cu+Mn+Zn                                                | Kendal TE                       | 46+1.5+1.5                          | 7.0                                         | ab             | 13.8 | b-d | 30.0 | b-e | 4.6                                         | a-c            | 10.3 | b-e | 22.5 | b-e |
| Prohexadione calcium                                                       | Regalis                         | 5                                   | 16.0                                        | b-d            | 16.3 | b-e | 37.5 | c-f | 11.3                                        | c-e            | 12.2 | b-f | 28.1 | c-f |
| Thyme oil extract                                                          | Tyme oil                        | 100                                 | 21.8                                        | d              | 39.8 | gh  | 58.8 | fg  | 14.8                                        | e              | 24.9 | g   | 44.1 | fg  |
| Mustard oil <sup>z</sup>                                                   | Duolif                          | 1000                                | 14.5                                        | b-d            | 23.3 | c-f | 42.5 | d-g | 10.1                                        | b-e            | 17.4 | e-g | 31.9 | d-g |
| <i>Bacillus subtilis</i> QST713                                            | Serenade                        | 58.4                                | 14.8                                        | b-d            | 26.3 | e-f | 62.5 | gh  | 10.6                                        | b-e            | 19.7 | fg  | 46.9 | gh  |
| Glucohumates activator complex <sup>z</sup>                                | Glucoinductor                   | 400                                 | 0.0                                         | a              | 5.5  | ab  | 10.8 | ab  | 0.0                                         | a              | 3.7  | ab  | 8.1  | ab  |
| Peptide complex copper sulfate                                             | Labimethyl                      | 9+6                                 | 21.0                                        | d              | 27.5 | e-f | 53.8 | e-g | 14.4                                        | e              | 20.6 | fg  | 40.3 | e-g |
| Copper hydroxide and terpenic alcohols                                     | Heliocuire                      | 60                                  | 4.8                                         | a              | 9.0  | ab  | 22.5 | a-d | 3.6                                         | ab             | 6.8  | a-c | 16.9 | a-d |
| Copper sulfate                                                             | Cuproxtat                       | 53.2                                | 6.8                                         | ab             | 29.5 | fg  | 20.0 | a-d | 4.6                                         | a-c            | 14.4 | c-f | 15.0 | a-d |
| Mineral fertilizer P <sub>2</sub> O <sub>5</sub> 52%, K <sub>2</sub> O 42% | Alexin                          | 130+105                             | 2.8                                         | a              | 6.3  | ab  | 11.3 | ab  | 2.1                                         | a              | 4.6  | ab  | 8.4  | ab  |
| Metalaxyl-M + copper hydroxide                                             | Ridomil Gold R                  | 7.5+ 120                            | 0.0                                         | a              | 0.0  | a   | 5.0  | a   | 0.0                                         | a              | 0.0  | a   | 3.8  | a   |
| Mandipropamid                                                              | Pergado                         | 11.7                                | 0.0                                         | a              | 0.0  | a   | 10.0 | ab  | 0.0                                         | a              | 0.0  | a   | 7.5  | ab  |
| Azoxystrobin                                                               | Ortiva                          | 18.6                                | 0.0                                         | a              | 0.0  | a   | 9.3  | ab  | 0.0                                         | a              | 0.0  | a   | 6.9  | ab  |
| -                                                                          | Non inoculated control          | -                                   | 15.5                                        | b-d            | 22.5 | c-f | 56.3 | fg  | 10.4                                        | b-e            | 16.9 | d-g | 42.2 | fg  |

<sup>x</sup> Two treatments were carried out on:14/10; 20/10. One artificial inoculation was carried out on: 21/11

<sup>k</sup> Means of the same column, followed by the same letter do not differ according to Tukey’s test (P<0.05)

<sup>z</sup> Dosage ( ml 100 L<sup>-1</sup>) of the commercial formulation

**Table 5** Effect of different treatments against downy mildew, incited by *Peronospora belbaharii*, expressed as disease incidence (DI, % of infected leaves) as disease severity (DS, % of infected leaf area) and on the yield of basil cv. Genovese, selection 'Italiano Classico', Trial 4.

| Active ingredient (code)                                                       | Commercial product <sup>x</sup>                 | Dosage<br>g a.i.100 L <sup>-1</sup> | DI at days after the last treatment <sup>x</sup> |                 |      |     |      |     | DS at days after the last treatment |     |      |    |      |     | Biomass (g) |     |
|--------------------------------------------------------------------------------|-------------------------------------------------|-------------------------------------|--------------------------------------------------|-----------------|------|-----|------|-----|-------------------------------------|-----|------|----|------|-----|-------------|-----|
|                                                                                |                                                 |                                     | 8                                                | 13              | 22   | 8   | 13   | 22  | 8                                   | 13  | 22   |    |      |     |             |     |
| -                                                                              | Inoculated control                              | -                                   | 22.5                                             | fg <sup>k</sup> | 36.8 | e   | 66.8 | f   | 10.7                                | ef  | 25.8 | c  | 40.7 | f   | 64.0        | cd  |
| Copper oxychloride + copper hydroxide (A)                                      | Airone                                          | 40+40                               | 9.3                                              | c-e             | 17.5 | cd  | 24.5 | d   | 2.6                                 | a-c | 9.8  | b  | 14.9 | d   | 90.7        | b-d |
| Acibenzolar-S-methyl (B)                                                       | Bion                                            | 1                                   | 2.8                                              | a-c             | 5.5  | ab  | 12.8 | a-d | 0.8                                 | ab  | 1.4  | a  | 8.1  | a-d | 80.4        | a-d |
| Mineral fertilizer Cu+Mn+Zn                                                    | Kendal TE                                       | 46+1.5+1.5                          | 10.5                                             | de              | 10.8 | a-d | 24.5 | d   | 3.4                                 | bc  | 5.3  | ab | 14.0 | cd  | 69.5        | a-d |
| Thyme oil                                                                      | Tyme oil                                        | 100                                 | 24.5                                             | g               | 36.0 | e   | 58.8 | f   | 11.4                                | f   | 23.6 | c  | 37.8 | f   | 68.0        | d   |
| Mustard oil <sup>z</sup>                                                       | Duolif                                          | 1000                                | 21.8                                             | fg              | 28.8 | e   | 37.8 | e   | 7.8                                 | de  | 19.3 | c  | 24.6 | e   | 60.8        | cd  |
| Glucosaminates activator complex <sup>z</sup> (F)                              | Glucosinductor                                  | 400                                 | 0.5                                              | a               | 0.5  | a   | 6.3  | ab  | 0.1                                 | a   | 0.0  | a  | 3.4  | a   | 75.4        | b-d |
| Copper hydroxide and terpenic alcohols (G)                                     | Heliocuvivre                                    | 60                                  | 15.8                                             | ef              | 17.8 | d   | 40.3 | e   | 5.0                                 | cd  | 10.1 | b  | 24.7 | e   | 86.2        | a-d |
| Mineral fertilizer P <sub>2</sub> O <sub>5</sub> 52%, K <sub>2</sub> O 42% (H) | Alexin                                          | 130+105                             | 0.0                                              | a               | 0.0  | a   | 6.0  | ab  | 0.0                                 | a   | 0.0  | a  | 1.9  | a   | 124.8       | a   |
| Metalaxyl-M + copper hydroxide (I)                                             | Ridomil Gold R                                  | 7.5+ 120                            | 0.0                                              | a               | 7.0  | a-d | 9.8  | ab  | 0.0                                 | a   | 2.3  | a  | 4.6  | ab  | 89.4        | a-c |
| Mandipropamid (L)                                                              | Pergado                                         | 11.7                                | 2.3                                              | a-c             | 3.0  | ab  | 8.3  | ab  | 0.6                                 | ab  | 0.4  | a  | 3.1  | a   | 78.0        | a-c |
| Azoxystrobin (M)                                                               | Ortiva                                          | 18.6                                | 0.0                                              | a               | 0.0  | a   | 8.0  | ab  | 0.0                                 | a   | 0.0  | a  | 3.1  | a   | 76.6        | a-d |
| A-H-A <sup>y</sup>                                                             | Airone; Alexin; Airone                          | 40+40; 130+105; 40+40               | 1.5                                              | a               | 3.0  | ab  | 11.8 | a-c | 0.4                                 | ab  | 0.9  | a  | 4.6  | ab  | 78.2        | a-c |
| A-I-A                                                                          | Airone; Ridomil Gold R; Airone                  | 40+40; 7.5+ 120; 40+40              | 0.0                                              | a               | 4.5  | ab  | 10.5 | a-c | 0.0                                 | a   | 1.3  | a  | 4.9  | ab  | 64.0        | a-d |
| A-L-A                                                                          | Airone; Pergado; Airone                         | 40+40;11.7;40+40                    | 5.5                                              | a-d             | 9.5  | a-d | 16.8 | b-d | 1.8                                 | ab  | 2.8  | ab | 8.3  | a-d | 73.6        | cd  |
| A-M-A                                                                          | Airone; Ortiva;Airone                           | 40+40;18.6;40+40                    | 0.0                                              | a               | 8.3  | a-d | 10.3 | ab  | 0.0                                 | a   | 2.2  | a  | 4.0  | ab  | 95.2        | a-c |
| A-B-A                                                                          | Airone; Bion; Airone                            | 40+40; 1; 40+40                     | 8.8                                              | b-e             | 11.8 | b-d | 22.8 | cd  | 1.2                                 | ab  | 1.6  | a  | 5.9  | a-c | 100.4       | a-d |
| A-F-A                                                                          | Airone; Glucosinductor; Airone                  | 40+40; 400; 40+40                   | 4.3                                              | a-d             | 4.3  | ab  | 10.5 | a-c | 1.3                                 | ab  | 2.5  | ab | 3.2  | a   | 84.4        | a-c |
| B-H-B                                                                          | Bion ;Alexin; Bion                              | 1; 130+105; 1                       | 0.5                                              | a               | 0.8  | a   | 4.0  | a   | 0.1                                 | a   | 0.2  | a  | 1.6  | a   | 93.4        | a-c |
| B-I-B                                                                          | Bion ;Ridomil Gold R; Bion                      | 1; 7.5+120; 1                       | 0.0                                              | a               | 5.3  | ab  | 7.0  | ab  | 0.0                                 | a   | 1.6  | a  | 2.8  | a   | 83.2        | a-c |
| B-L-B                                                                          | Bion; Pergado; Bion                             | 1; 11.7; 1                          | 2.0                                              | ab              | 9.5  | a-d | 17.5 | b-d | 0.6                                 | ab  | 2.8  | ab | 7.6  | a-d | 66.4        | a-d |
| B-M-B                                                                          | Bion; Ortiva; Bion                              | 1; 18.6; 1                          | 0.0                                              | a               | 2.8  | ab  | 5.8  | ab  | 0.0                                 | a   | 0.7  | a  | 2.0  | a   | 85.0        | a-d |
| B-F-B                                                                          | Bion; Glucosinductor; Bion                      | 1; 400; 1                           | 5.0                                              | a-d             | 6.8  | a-c | 8.3  | ab  | 1.1                                 | ab  | 0.6  | a  | 2.8  | a   | 94.4        | a-c |
| F-H-F                                                                          | Glucosinductor; Alexin; Glucosinductor          | 400; 130+105;400                    | 4.0                                              | a-d             | 2.0  | ab  | 5.5  | ab  | 0.2                                 | a   | 0.0  | a  | 2.4  | a   | 36.8        | cd  |
| F-I-F                                                                          | Glucosinductor ; Ridomil Gold R; Glucosinductor | 400; 7.5+120;400                    | 0.8                                              | a               | 0.0  | a   | 6.8  | ab  | 0.0                                 | a   | 0.4  | a  | 4.7  | ab  | 79.0        | a-d |
| F-L-F                                                                          | Glucosinductor; Pergado; Glucosinductor         | 400; 11.7;400                       | 0.0                                              | a               | 1.8  | ab  | 12.3 | a-d | 0.1                                 | a   | 0.0  | a  | 2.2  | a   | 100.6       | a-d |
| F-M-F                                                                          | Glucosinductor; Ortiva; Glucosinductor          | 400; 18.6;400                       | 0.5                                              | a               | 0.0  | a   | 4.0  | a   | 0.5                                 | ab  | 0.3  | a  | 4.4  | ab  | 111.6       | ab  |
| G-H-G                                                                          | Heliocuvivre; Alexin; Heliocuvivre              | 60; 130+105;60                      | 2.0                                              | ab              | 1.3  | ab  | 7.5  | ab  | 1.1                                 | ab  | 1.1  | a  | 3.9  | ab  | 104.4       | a-c |
| G-I-G                                                                          | Heliocuvivre ; Ridomil Gold R; Heliocuvivre     | 60; 7.5+120;60                      | 4.0                                              | a-d             | 3.8  | ab  | 8.5  | ab  | 1.6                                 | ab  | 4.5  | ab | 9.0  | a-d | 106.2       | a-c |
| G-L-G                                                                          | Heliocuvivre; Pergado; Heliocuvivre             | 60; 11.7;60                         | 5.0                                              | a-d             | 8.8  | a-d | 17.3 | b-d | 0.8                                 | ab  | 1.6  | a  | 6.1  | a-d | 88.6        | a-d |
| G-M-G                                                                          | Heliocuvivre; Ortiva; Heliocuvivre              | 60; 18.6; 60                        | 3.0                                              | a-c             | 4.0  | ab  | 12.3 | a-d | 2.6                                 | abc | 5.3  | ab | 12.4 | b-d | 97.6        | a-c |
| -                                                                              | Not inoculated control                          | -                                   | 0.0                                              | a               | 1.5  | ab  | 8.3  | ab  | 0.0                                 | a   | 0.5  | a  | 3.1  | a   | 93.8        | a-c |

<sup>x</sup> Three treatments were carried out on: 11/1; 18/1; 25/1. Two artificial inoculations were carried out on: 12/01 and 26/01

<sup>k</sup> Means of the same column, followed by the same letter do not differ according to Tukey's test ( $P < 0.05$ )

<sup>z</sup> Dosage ( ml 100 L<sup>-1</sup>) of the commercial formulation

<sup>y</sup> Spray program adopted where letter corresponding to A, Copper oxychloride + copper hydroxide (Airone); H, Mineral fertilizer P<sub>2</sub>O<sub>5</sub> 52%, K<sub>2</sub>O 42% (Alexine); I, Metalaxyl-M + copper hydroxide (Ridomil Gold R); L, Mandipropamid (Pergado); M, azoxystrobin (Ortiva); B, Acibenzolar-S-methyl (Bion); F, Glucohumates activator complex (Glucoinductor); G, Copper hydroxide and terpenic alcohols (Heliocuire)