Management of downy mildew of sweet basil (Ocimum basilicum) caused by Peronospora belbahrii by means of resistance inducers, fungicides, biocontrol agents and natural products.

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

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

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

Introduction

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

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

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

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

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

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

Materials and methods

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

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

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

Artificial inoculation The populations of *Peronospora belbahrii*, collected in Piedmont (Northern Italy) from diseased plants, were maintained on basil plants. The artificial inoculation was carried out throughout nebulisation with a laboratory spray bottle (10 ml of capacity) of a suspension of $1x10^5$ sporangia/ml. One ml of suspensions/treatment (corresponding to 8 pots), was used. The artificial inoculation was carried out 24 h after the second treatment in trials 1-3, while in trial 4 two artificial inoculations were carried out (Table 1).

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

Plant inducers, salts and fertilizers Acibenzolar-S-methyl (Bion 50WG, 50% a.i., Syngenta, Italy), prohexadione calcium (Regalis, 10% a.i, BASF, Italy), organic-mineral fertilizer based on glucohumates complex N:P (Glucoinductor + GlucoActivator, N 4%, P_2O_5 18%, International patent PCT, IB2004\001905, Fertirev, Torino, Italy), organic-mineral fertilizer N:K (Kendal, soluble organic nitrogen 3.5%, soluble potassium oxide 15.5%, organic carbon 3-4% Valagro, Atessa, Chieti, Italy), mineral fertilizer (Kendal TE, Cu 23%, Mn 0,5%, Zn 0,5%, Valagro, Atessa, Chieti, Italy) and (Alexin 95PS, P_2O_5 52%, K_2O 42%, Massò, Milano, Italy), were tested at the dosages reported under tables 2- 5.

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

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

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

All treatments were carried out on basil plants 16-21 days after sowing at the dates reported under table 1 by using 800 L ha^{-1}.

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

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

Data collection and analysis Starting at the appearance of the first symptoms, the evaluation of the percent of infected leaves (disease incidence) and of diseased leaf tissue (disease severity) was made by using a disease rating scale with: 1 = up to 5 % infected leaf area; 2 = up to 10% infected leaf area; 3 = up to 25% infected leaf area; 4 = up to 50% infected leaf area; 5= up to 75 % infected leaf area. Disease incidence and severity was estimated on 100 leaves/treatment.
In trials 4, plant biomass, as fresh weight of plants at the end of the trial, was also measured.
All data collected were statistically analysed by univariate Anova with Tukey's test using SPSS software 18.

Results

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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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; 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; 5/10	26/10; 2/11; 7/11	31/10; 7/11;14/11	1/02/2012; 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 belbarhii*, on basil cv. Genovese, selection 'Italiano Classico', Tria1

Active ingredient	Commercial product [x]	Dosage a.i.100 L	DI at day after last treatment [x]						DS at day after last treatment [x]					
			8		15		20		8		15		20	
-	Inoculated control	-	40.4	gh[k]	66.8	e	88.8	f	22.4	f-i[k]	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
Bacillus subtilis 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
Glucohumates activator complex [z]	Glucoinductor	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	Heliocuivre	60	15.5	bc	35.0	cd	51.5	cd	10.0	b-d	21.9	bc	36.1	b-d
Copper sulfate	Cuproxat	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_2O_5 52%, K_2O 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

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

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

[z] Dosage (ml 100 L^{-1}) 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 belbarhii* on basil cv. Genovese, selection 'Italiano Classico', Trial 2

Active ingredient	Commercial product [x]	Dosage g a.i.100 L $^{-1}$	DI at at day after last treatment						DS at day after last treatment [x]					
			13 [x]		20		25		13 [x]		20		25	
-	Inoculated control	-	53.5	e[k]	81.8	f	84.5	f	32.9	e[k]	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[z]	Duolif	1000	12.5	ab	16.5	a-c	25.5	a-e	5.1	ab	10.5	a-d	15.4	a-c
Bacillus subtilis 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 [z]	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	Heliocuivre	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_2O_5 52%, K_2O 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

[x] Two treatments were carried out on:7/10; 13/10. One artificial inoculation was carried out on: 14/10

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

[z] Dosage (ml 100 L^{-1}) 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 belbarhii* on basil cv. Genovese, selection 'Italiano Classico', Trial 3

Active ingredient	Commercial product [x]	Dosage g a.i.100 L $^{-1}$	DI at day after last treatment [x]						DS at day after last treatment [x]					
			11		18		25		11		18		25	
-	Inoculated control	-	38.3	e[k]	47.5	h	83.8	h	26.8	f[k]	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[z]	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
Bacillus subtilis 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 [z]	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	Heliocuivre	60	4.8	a	9.0	ab	22.5	a-d	3.6	ab	6.8	a-c	16.9	a-d
Copper sulfate	Cuproxat	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_2O_5 52%, K_2O 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

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

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

[z] Dosage (ml 100 L^{-1}) of the commercial formulation

Table 5 Effect of different treatments against downy mildew, incited by *Peronospora belbarhii*, 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[x]	Dosage g a.i.100 L $^{-1}$	DI at days after the last treatment [x]						DS at days after the last treatment						Biomass (g)	
			8		13		22		8		13		22			
-	Inoculated control	-	22.5	fg[k]	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[z]	Duolif	1000	21.8	fg	28.8	e	37.8	e	7.8	de	19.3	c	24.6	e	60.8	cd
Glucohumates activator complex [z] (F)	Glucoinductor	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)	Heliocuivre	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_2O_5 52%, K_2O 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[y]	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; Glucoinductor; 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; Glucoinductor; 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	Glucoinductor; Alexin; Glucoinductor	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	Glucoinductor ; Ridomil Gold R; Glucoinductor	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	Glucoinductor; Pergado; Glucoinductor	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	Glucoinductor; Ortiva; Glucoinductor	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	Heliocuivre; Alexin; Heliocuivre	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	Heliocuivre ; Ridomil Gold R; Heliocuivre	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	Heliocuivre; Pergado; Heliocuivre	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	Heliocuivre; Ortiva; Heliocuivre	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

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

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

[z] Dosage (ml 100 L^{-1}) of the commercial formulation

[y] Spray program adopted where letter corresponding to A, Copper oxychloride + copper hydroxide (Airone); H, Mineral fertilizer P_2O_5 52%, K_2O 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 (Heliocuivre)