Phytoparasitica (2013) 41:59–72
DOI 10.1007/s12600-012-0264-y

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

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Received: 6 April 2012 / Accepted: 26 August 2012 / Published online: 13 September 2012

Abstract To control downy mildew of sweet basil (*Ocimum basilicum* L.), incited by *Peronospora belbahrii* Thines, a number of compounds were tested in 2011 and 2012 under glasshouse conditions. These included copper-based fungicides, biocontrol agents, and compounds previously reported to induce resistance in plants to pathogens. Results were compared with those provided by fungicides registered for downy mildew control in Italy. The tested compounds were used alone or applied in rotation in spray programs. In all trials, the greatest reduction in disease incidence and severity was found with treatments that included metalaxyl-M+copper hydroxide, a mineral fertilizer 'Alexin', mandipropanid, and azoxystrobin. The glucohumates activator complex and acibenzolar-S-methyl also provided significant disease control ($P<0.05$). The mineral fertilizer Alexin, the glucohumates activator complex and acibenzolar-S significantly reduced disease incidence and severity 20 days after the last treatment compared with the untreated control ($P<0.05$). Among the copper-based products, the greatest reductions in disease incidence and severity were provided by copper hydroxide with terpenic alcohols and copper oxychloride+copper hydroxide. The mineral fertilizer Kendal and prohexadione-Ca, as well as mustard oil, partially reduced disease incidence and severity compared with the untreated control ($P<0.05$), whereas the biocontrol agent *Bacillus subtilis* QST 713 and thyme oil extract were not effective in two out of three trials. When different combinations of various products used in rotation were tested, effective control was found either using a rotation of fungicides with compounds that can induce resistance, as well as by using rotation with different resistance-inducing compounds on their own.

Keywords Acibenzolar-S-methyl · *Bacillus subtilis* · Integrated control · Organic and mineral fertilizers

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Introduction

Sweet basil (*Ocimum basilicum* L.) is an economically important herb crop in Mediterranean regions, the USA and 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 in the Riviera Ligure, and 'Genovese Gigante' is the most appreciated variety for fresh consumption (ISTAT 2011). This has been the case in the past (Montalti 1995) as well as in the current days—where it is the only cultivar used for industrial production of pesto sauce and covers 90% of the total growing area (personal communication). Basil production in Italy is 4,500–4,900 tons/year or more if minor producers are considered. It is an important crop for pesto production in the Piedmont region, with an average production of 392 tons/year (ISTAT 2011). In this area, it is grown primarily outdoors. Basil is grown mostly in glasshouses; in this environment average night temperatures and dew depositions caused by high relative humidity favor the development of diseases such as gray mold (*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, 2011).

A particularly severe problem, downy mildew of basil, incited by *P. belbahrii* (Belbahri *et al.* 2005; Thines *et al.* 2009), was observed in northern Italy in 2003 (Garibaldi *et al.* 2004b) and quickly spread to other Italian regions in central and southern Italy (Minuto *et al.* 2004) as well as France (Garibaldi *et al.* 2005). This pathogen was first reported in Uganda, identified as *Peronospora* sp. (Hansford 1932), and much later in Switzerland (Lefort *et al.* 2003). After the report in Switzerland, the pathogen spread to many basil-growing areas. The disease was recently reported also in Belgium (Coosemans 2004), the USA (Roberts *et al.* 2009), Cuba (Martinez de la Parte *et al.* 2010) and Hungary (Nagy & Horvath 2011). Its spread probably has been favored by the fact that it is seed-transmitted (Garibaldi *et al.* 2004a, b).

Basil plants affected by *P. belbahrii* showed an extensive leaf chlorosis, especially near the central vein, and under favorable environmental conditions (high relative humidity for 6–12 h at 20 °C), a characteristic gray sporulation quickly develops on the lower surface of the infected leaves. The pathogen can then readily spread, as shown for many other downy mildews (Garibaldi *et al.* 2007; Spencer 1981).

The management of downy mildew of basil is complicated by the very limited availability of chemicals for the crop, due to the risk of the presence of residues at harvest as well as the difficulty to obtain fungicide registration for minor crops (Leadbeater & Gisi 2010) such as basil. The search for basil varieties resistant to the pathogen is still in its early stages (Wyenandt *et al.* 2010). The few fungicides registered in Italy on basil [azoxystrobin, belonging to the Quinone outside inhibitor QoI of the strobilurin class; metalaxyl-M, belonging to the phenylamide; and mandipropamid, belonging to the carboxylic acid amides] must be used in a very limited number of sprays to avoid selection of resistant strains of the pathogen. Fungicide applications need to begin before the pathogen is present to obtain effective control, and products belonging to different FRAC code groups must be used in alternation or in mixture (Brent & Hollomon 2007; Choen *et al.* 2008; Gisi & Sierotzki 2008).

Among resistance-inducing products known for the efficacy against oomycetes, phosphite-based products showed the ability to activate plant defence by inducing the synthesis and translocation of phytoalexins (Guest & Grant 1991; Smille *et al.* 1989) and their efficacy has been reported in several pathosystems (Bock *et al.* 2012; Jackson *et al.* 2000; Silva *et al.* 2011). Acibenzolar-S-methyl, as well as rhizobacteria and prohexadione–Ca have been shown to induce resistance in different crops (Bazzi *et al.* 2003; Oostendorp *et al.* 2001; Van Loon *et al.* 1998), while thyme oil extract and mustard oil have been poorly investigated against downy mildew agents.

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

Materials and methods

Basil growth and experimental conditions Five experimental trials (Table 1) were carried out in 2011 and 2012 at Grugliasco (Torino) in a glasshouse belonging to Agroinnova. Main information about the trials is 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 1.5 *l* plastic pots (12×12 cm) containing a white peat : perlite (80:20 v/v) mix (Turco Silvestro, Albenga, Savona) that had been steamed (90°C for 30 min). Fertilization was carried out once prior to sowing, by mixing OSMOFORM 2, Scotts (18%N, 5% P_2O_5, 13% K_2O) at 2 kg $m^{-3}$of soil.

For each treatment, four replicates (two pots each) in a completely randomized design were used. The basil pots were maintained on the benches and covered with a transparent polyethylene film, 50 μ thick, 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. During the trials the environmental condition was maintained, with high relative

Table 1 Main operations carried out in the trials

Operations	Trial no.				
	1	2	3	4	5
Date of sowing	18/08/2011	21/09/2011	22/09/2011	27/12/2011	1/03/2012
Dates of treatments with Plant inducers, salt, fertilizers, plant extract, copper-based products, BCA	9/09; 15/09/2011	7/10; 13/10/2011	14/10; 20/10/2011	11/01/2012; 18/01/2012; 25/01/2012	21/03/2012; 28/03/2012; 4/04/2012
Dates of systemic fungicides applications	9/09/2011	7/10/2011	14/10/2011	11/01/2012; 18/01/2012; 25/01/2012	21/03/2012; 28/03/2012; 4/04/2012
Date of artificial inoculation with a spore suspension of *Peronospora belbarhii* at 1×10^5 CFU ml^{-1}	16/09/2011	14/10/2011	21/10/2011	12/01/2012; 26/01/2012	22/03/2012; 29/03/2012
Dates of downy mildew incidence and severity assessment	23/09; 30/09; 5/10/2011	26/10; 2/11; 7/11/2011	31/10; 7/11; 14/11/2011	1/02/2012; 8/02/2012; 17/02/2012	5/04/2012; 13/04/2012; 20/04/2012
Date of biomass evaluation as plants fresh weight. End of the trial	–	–	–	17/02/2012	20/04/2012

humidity, close to 85–95%, and temperatures ranging between 19°C and 25°C by using a program (software Magricomp Multilab, Agricontrol S.n.c., SV, Italy) to obtain the environmental conditions set in the greenhouse. This system constantly measures the humidity and manages the number of mistings during the experimental trials. The dates of the main operations carried out during the trials are reported in Table 1.

Artificial inoculation The populations of *Peronospora belbahrii*, collected in Piedmont (northern Italy) from diseased plants, were maintained on basil plants. Infected leaves were shaken in 100 ml of sterile water containing 2 μl of Tween 20 and the obtained conidial suspension was adjusted with the aid of a haemocytometer to 1×10^5 sporangia ml^{-1}. The artificial inoculation was carried out immediately through nebulisation with a laboratory spray bottle (10 ml capacity). One ml of suspension/repetition per treatment (corresponding to 1 ml per two pots), was used. The artificial inoculation was carried out 24 h after the second treatment in trials 1–3, while in trials 4 and 5 two artificial inoculations were carried out (Table 1).

Treatments-application and products Several compounds known for their capability to induce resistance in the host, salts, fertilizers, copper-based fungicides as well as fungicides registered for use on basil, were tested. In trials 1–3 the different compounds were tested alone, while in trials 4 and 5 selected products were used alone and in rotation.

All treatments were applied as foliar spray on basil plants 16–21 days after sowing, using the dosage in accordance with manufacturer's instructions, on the dates reported in Table 1. The water suspension (800 *l* ha^{-1}of volume) of each product was sprayed on the plants at the third true leaf stage, using a handheld 1-*l*-capacity sprayer.

In trials 1–3, two treatments were applied at 6-day intervals except for systemic chemical fungicides that were applied once on the dates reported in Table 1. One artificial inoculation with the pathogen was done 24 h after the last treatment applications.

In trials 4 and 5, three treatments were carried out at 6-day intervals, using the tested products alone and in rotation as described in Table 1. For evaluating the persistence of the protection offered by the different treatments tested, two artificial inoculations with the pathogen were carried out 24 h after the first and at the last treatment, respectively (Table 1).

Plant inducers, salts and fertilizers Acibenzolar-S-methyl (Bion 50WG, 50% a.i., Syngenta, Italy), prohexadione-Ca (Regalis, 10% i.e., BASF, Italy), organic-mineral fertilizer based on glucohumate complex (Glucoinductor+GlucoActivator, N 4%, P_2O_5 18%, International patent PCT, IB2004\001905, Fertirev, Torino, Italy); organic-mineral fertilizer (Kendal, soluble organic N 3.5%, soluble K_2O 15.5%, organic carbon 3–4%, Valagro, Atessa, Chieti, Italy); mineral fertilizers (Kendal TE, Cu 23%, Mn 0.5%, Zn 0.5%, Valagro); and mineral fertilizer based on phosphate salts (Alexin 95PS, P_2O_5 52%, K_2O 42%, Massò, Milano, Italy) were tested. Dosages are given in Tables 2, 3, 4, 5 and 6 and the timing of application is indicated in Table 1.

Plant extracts and biocontrol agents (BCAs) Thyme oil plant extract (*Thymus vulgaris* 100 % a. i., 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.

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); copper sulfate+copper gluconate, tackifying compound and natural inducers of systemic induced resistance (SAR) (Labimethyl, 3%+ 2% a.i., Macasa, Spain) were tested at the dosages given in Tables 2–6.

Systemic chemical fungicides Azoxystrobin (Ortiva, Syngenta Crop Protection S.p.A., Milano, Italy, 23.2% a. i.), mandipropamid (Pergado SC, 23.4% a.i., Syngenta), metalaxyl-M+copper oxychloride (Ridomil R WG 2.5%+40% a. i., Syngenta) were tested. These fungicides are registered for use on basil in Italy as reported on the ministerial label of these products.

Data collection and analysis Trials were monitored daily and starting at the appearance of the first symptoms, the percent of infected leaves (disease incidence) and of diseased leaf area affected by the pathogen (disease severity) were evaluated on a disease rating scale (EPPO 2004). Disease severity was calculated using the formula: DS=[∑(n° leaves× x 0–5) / (total of leaves recorded)] with x 0–5 corresponding to: 1=from 1% to 10% (midpoint 5%) infected leaf area; 2=from 11% to 25% (midpoint 18%) infected leaf area; 3=from 26% to 50% (midpoint 38%) infected leaf area; 4=from 51% to 75% (midpoint 63%) infected leaf area; 5=from 76% to 100% (midpoint 85%) infected leaf area.

Disease incidence and severity were estimated on 100 leaves per treatment. Forty to 50 leaves of basil chosen randomly from each pot per repetition were visually examined.

At the end of trials 4 and 5, plant biomass, as fresh weight of plants corresponding to two pots per repetition, was measured using a technical balance (Orma SNC).

All data collected were statistically analyzed by univariate ANOVA with SPSS software 18 and means were spread according to Tukey's test ($P<0.05$).

Results

In all the five trials the artificial inoculation with *P. belbahrii* led to a high level of disease in the untreated inoculated control; at the final assessment, carried out 20–25 days after the last treatment, the disease incidence ranged from 66.8% to 88.8% of infected leaves and disease severity from 40.7% to 66.6% of the leaf area affected by the pathogen. The level of infection reached with the artificial inoculation was high in the different trials, however differences were observed in the onset of the infections. Signs of downy mildew were first observed 6 days after the artificial inoculation in trial 1, 10 days were needed in trials 2 and 3, while 21 and 14 days after the first inoculation were required in trials 4 and 5, respectively (Tables 2–6). 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 contamination of the seeds used (Garibaldi *et al.* 2004a) as well as to the spread of the pathogen among plants (Table 2). However, it is possible that the pathogen was present in the greenhouse before the start of the treatments or that it was already present in the greenhouse before the start of the first trial.

In trial 1, downy mildew incidence and severity were significantly reduced by the mixture of metalaxyl-M+ copper hydroxide, by the mineral fertilizer Alexin, by

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', Trial 1

Active ingredient	Commercial product[z]	Dosage	DI at day after last treatment						DS at day after last treatment					
		g a.i.100 l^{-1}	8		15		20		8		15		20	
–	Inoculated control	–	40.4	gh[y]	66.8	e	88.8	f	22.4	f-i[y]	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-Ca	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	Thyme 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
Glucohumate activator complex	Glucoinductor	400[x]	7.8	ab	16.5	ab	30.8	bc	5.5	a-c	11.5	ab	22.2	ab
Copper sulfate+copper gluconate	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
–	Non-inoculated control	–	37.5	f-h	48.2	d	73.0	d-f	25.8	hi	33.6	cd	54.4	d-f

[z] Two treatments were carried out, on 9/09/2011 and 15/09/2011, with the exception of metalaxyl-M+copper hydroxide, mandipropamid and azoxystrobin applied once on 9/09/2011. One artificial inoculation was carried out on 16/09/2011

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

[x] 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 [z]	Dosage	DI at day after last treatment						DS at day after last treatment					
		g a.i. $100 l^{-1}$	13		20		25		13		20		25	
–	Inoculated control	–	53.5	e[y]	81.8	f	84.5	f	32.9	e[y]	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-Ca	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	Thyme oil	100	36.2	de	58.5	de	61.3	ef	22.9	de	41.3	e	44.7	de
Mustard oil [x]	Duolif	1000[x]	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
Glucohumate activator complex[x]	Glucoinductor	400[x]	0.0	a	4.3	ab	3.8	ab	0.0	a	1.6	ab	2.1	a
Copper sulfate+copper gluconate	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

[z] Two treatments were carried out, on 7/10 and 13/10, with the exception of metalaxyl-M+copper hydroxide, mandipropamid and azoxystrobin applied once on 7/10/2011. One artificial inoculation was carried out on 14/10

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

[x] 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 [z]	Dosage	DI at day after last treatment						DS at day after last treatment					
		g a.i.100 l^{-1}	11		18		25		11		18		25	
–	Inoculated control	–	38.3	e[y]	47.5	h	83.8	h	26.8	f[y]	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-Ca	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	Thyme oil	100	21.8	d	39.8	gh	58.8	fg	14.8	e	24.9	g	44.1	fg
Mustard oil[x]	Duolif	1000[x]	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
Glucohumate activator complex [x]	Glucoinductor	400[x]	0.0	a	5.5	ab	10.8	ab	0.0	a	3.7	ab	8.1	ab
Copper sulphate+copper gluconate	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

[z] Two treatments were carried out, on 14/10 and 20/10, with the exception of metalaxyl-M+copper hydroxide, mandipropamid and azoxystrobin applied once on14/10/2011. One artificial inoculation was carried out on 21/11

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

[x] 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[z]	Dosage	DI at days after the last treatment						DS at days after the last treatment						Biomass (g)	
		g a.i.100 l^{-1}	8		13		22		8		13		22			
–	Inoculated control	–	22.5	fg[y]	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	Thyme oil	100	24.5	g	36.0	e	58.8	f	11.4	f	23.6	c	37.8	f	68.0	a-d
Mustard oil[x]	Duolif	1000[x]	21.8	fg	28.8	e	37.8	e	7.8	de	19.3	c	24.6	e	60.8	d
Glucohumate activator complex [x] (F)	Glucoinductor	400[x]	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 [w]	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	cd
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	63.8	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

Table 5 (continued)

Active ingredient (code)	Commercial product[z]	Dosage	DI at days after the last treatment			DS at days after the last treatment			Biomass (g)
		g a.i.100 l^{-1}	8	13	22	8	13	22	
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
–	Non-inoculated control	–	0.0 a	1.5 ab	8.3 ab	0.0 a	0.5 a	3.1 a	93.8 a-c

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

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

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

[w] For the combined spray programs A corresponds to Airone (Copper oxychloride+copper hydroxide); H to Alexine (Mineral fertilizer P_2O_5 52%, K_2O 42%), I to Ridomil Gold R (Metalaxyl-M+copper hydroxide); L to Pergado (Mandipropamid); M to Ortiva (azoxystrobin); B to Bion (Acibenzolar-S-methyl); F to Glucoinductor (Glucohumates activator complex); G to Heliocuivre (Copper hydroxide and terpenic alcohols)

mandipropamid, by azoxystrobin, and by the glucohumate activator complex; these products provided a significantly better level of disease control than other tested compounds at the end of the trial as well as at the earlier rating times (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% of infected leaves 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% of the affected leaf area, respectively (Table 2). Copper hydroxide mixed with terpenic alcohols and copper sulphate+copper gluconate, probexadone-Ca, as well as acibenzolar-S-methyl, significantly differ in disease incidence and severity from the untreated control in all the assessments carried out. *B. subtilis* QST 713, the mineral fertilizer Kendal, alone or combined with copper (Kendal TE) treatments, determined a significantly lower disease incidence and severity than the inoculated control at the first and second rating points after treatments, while 20 days after treatments no differences from the untreated control were observed. Thyme oil extract did not provide any control of downy mildew, with results statistically similar to the untreated control (Table 2).

In trial 2, the first signs of downy mildew in the untreated control were observed 10 days after artificial inoculation of the pathogen. At the first assessment, 53.5% of basil leaves were infected with 32.9% of the leaf area affected, whereas no disease was observed on the non-inoculated basil plants. In the presence of 84.5% infected leaves with 61.8% of the affected leaf area in the untreated control at the last assessment, metalaxyl+copper hydroxide, mandipropanid and azoxystrobin significantly reduced to 0.8%, 1.3%, 1.3% the disease incidence and to 0.2%, 0.8%, 0.6%, the severity of the disease, respectively. The potassium phosphite-based products (Alexin and the glucohumate activator complex) and acibenzolar-S-methyl showed a statistically similar downy mildew control in terms of disease incidence and severity compared with the chemical fungicides tested. Among the different copper-based products tested, copper oxychloride+copper hydroxide and copper hydroxide with terpenic alcohols provided statistically similar results in disease incidence reduction compared with the phosphite-based products and acibenzolar-S-methyl. This trend was clear considering the severity of the pathogenic attacks 25 days after the last application of the products. At the last assessment,

Table 6 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 5

Active ingredient (code)	Commercial product[z]	Dosage	DI at days after the last treatment			DS at days after the last treatment			Biomass (g)
		g a.i.100l^{-1}	8	16	23	8	16	23	
–	Inoculated control	–	13.5 h[y]	29.8 d	60.3 e	7.1 e	22.3 d	45.2 e	57.5 h
Copper oxychloride+ copper hydroxide (A)	Airone	40+40	5.8 e	5.5 ab	24.8 bc	3.9 d	4.1 ab	18.6 bc	98.0 a-d
Acibenzolar-S-methyl (B)	Bion	1	0.0 a	1.5 a	11.0 a-c	0.0 a	1.1 a	8.25 a-c	77.2 e-h
Mineral fertilizer Cu+Mn+Zn	Kendal TE	46+1.5+1.5	4.3 de	16.0 c	26.0 cd	2.8 cd	12.0 c	19.5 cd	67.3 f-h
Thyme oil	Thyme oil	100	9.3 f	30.5 d	58.8 e	6.6 e	22.9 d	44.1 e	58.2 h
Mustard oi l[x]	Duolif	1000[x]	11.8 g	29.3 d	43.9 de	8.3 e	21.9 d	33.0 de	65.3 gh
Glucohumates activator complex [x](F)	Glucoinductor	400[x]	0.0 a	1.3 a	3.8 a	0.0 a	0.7 a	2.8 a	80.0 c-g
Copper hydroxide and terpenic alcohols (G)	Heliocuivre	60	3.3 b-e	11.0 bc	17.5 a-c	1.1 a-c	8.3 bc	13.1 a-c	80.3 c-g
Mineral fertilizer P_2O_5 52%, K_2O 42% (H)	Alexin	130+105	0.5 ab	0.5 a	4.5 a	0.4 ab	0.4 a	3.4 a	102.8 a-c
Metalaxyl-M+copper hydroxide (I)	Ridomil Gold R	7.5+ 120	0.0 a	0.8 a	6.0 ab	0.0 a	0.6 a	4.5 a	94.5 a-e
Mandipropamid (L)	Pergado	11.7	0.0 a	0.0 a	5.0 a	0.0 a	0.0 a	3.8 a	89.3 b-e
Azoxystrobin (M)	Ortiva	18.6	0.0 a	0.0 a	5.3 a	0.0 a	0.0 a	3.9 a	94.5 a-e
A-H-A[w]	Airone; Alexin; Airone	40+40; 130+105; 40+40	0.8 a-c	0.5 a	9.5 a-c	0.5 ab	0.4 a	7.1 a-c	84.5 c-g
A-I-A	Airone; Ridomil Gold R; Airone	40+40; 7.5+ 120; 40+40	0.0 a	0.0 a	11.3 a-c	0.0 a	0.0 a	8.4 a-c	76.3 e-h
A-L-A	Airone; Pergado; Airone	40+40;11.7;40+40	0.0 a	0.0 a	8.5 a-c	0.0 a	0.0 a	6.4 a-c	76.4 e-h
A-M-A	Airone; Ortiva; Airone	40+40;18.6;40+40	1.5 a-d	1.5 a	5.5 a	0.9 a-c	1.1 a	4.1 a	93.7 a-e
A-B-A	Airone; Bion; Airone	40+40; 1; 40+40	1.3 a-d	1.5 a	14.5 a-c	0.3 ab	1.1 a	10.9 a-c	112.2 a
A-F-A	Airone; Glucoinductor; Airone	40+40; 400; 40+40	0.0 a	0.0 a	13.3 a-c	0.0 a	0.0 a	9.9 a-c	86.2 c-f
B-H-B	Bion ; Alexin; Bion	1; 130+105; 1	0.0 a	0.0 a	7.8 a-c	0.0 a	0.0 a	5.8 ab	93.7 a-e
B-I-B	Bion ; Ridomil Gold R; Bion	1; 7.5+120; 1	0.0 a	1.0 a	9.5 a-c	0.0 a	0.8 a	7.1 a-c	82.7 c-g
B-L-B	Bion; Pergado; Bion	1; 11.7; 1	0.0 a	0.0 a	9.5 a-c	0.0 a	0.0 a	7.1 a-c	75.0 e-h
B-M-B	Bion; Ortiva; Bion	1; 18.6; 1	0.0 a	1.3 a	10.3 a-c	0.0 a	0.9 a	7.7 a-c	77.7 d-h
B-F-B	Bion; Glucoinductor; Bion	1; 400; 1	0.0 a	1.0 a	13.3 a-c	0.0 a	0.8 a	6.2 a-c	87.7 b-f
F-H-F	Glucoinductor; Alexin; Glucoinductor	400; 130+105;400	0.3 ab	1.3 a	3.5 a	0.2 ab	0.9 a	2.6 a	107.2 ab
F-I-F	Glucoinductor ; Ridomil Gold R; Glucoinductor	400; 7.5+120;400	0.0 a	0.0 a	6.5 ab	0.0 a	0.0 a	4.9 a	110.4 a
F-L-F	Glucoinductor; Pergado; Glucoinductor	400; 11.7;400	0.0 a	0.8 a	2.8 a	0.0 a	0.6 a	2.1 a	86.1 c-f
F-M-F	Glucoinductor; Ortiva; Glucoinductor	400; 18.6;400	0.0 a	1.3 a	3.3 a	0.0 a	0.9 a	2.4 a	85.9 c-g
G-H-G	Heliocuivre; Alexin; Heliocuivre	60; 130+105;60	0.0 a	0.5 a	4.0 a	0.0 a	0.4 a	3.0 a	86.9 b-f

Table 6 (continued)

Active ingredient (code)	Commercial product[z]	Dosage	DI at days after the last treatment			DS at days after the last treatment			Biomass (g)
		g a.i.100 l^{-1}	8	16	23	8	16	23	
G-I-G	Heliocuivre ; Ridomil Gold R; Heliocuivre	60; 7.5+120;60	0.0 a	0.0 a	6.3 ab	0.0 a	0.0 a	4.7 a	85.9 c-g
G-L-G	Heliocuivre; Pergado; Heliocuivre	60; 11.7;60	0.0 a	0.0 a	11.0 a-c	0.0 a	0.0 a	8.3 a-c	89.0 b-e
G-M-G	Heliocuivre; Ortiva; Heliocuivre	60; 18.6; 60	0.0 a	0.0 a	5.3 a	0.0 a	0.0 a	3.9 a	94.7 a-e
–	Non-inoculated control	–	0.0 a	0.0 a	2.3 a	0.0 a	0.0 a	1.5 a	94.6 a-e

[z] Three treatments were carried out, on21/3, 28/3, 4/04. Two artificial inoculations were carried out, on 22/03 and 29/03

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

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

[w] For the combined spray programs A corresponds to Airone (Copper oxychloride+copper hydroxide); H to Alexine (Mineral fertilizer P_2O_5 52%, K_2O 42%), I to Ridomil Gold R (Metalaxyl-M+copper hydroxide); L to Pergado (Mandipropamid); M to Ortiva (azoxystrobin); B to Bion (Acibenzolar-S-methyl); F to Glucoinductor (Glucohumates activator complex); G to Heliocuivre (Copper hydroxide and terpenic alcohols)

there were no significant differences between plants treated with the copper sulphate compounds, the mineral fertilizer Kendal TE, prohexadione-Ca and with mustard oil, which showed a disease incidence ranging from 18.3% to 27.8% and a disease severity from 10.3% to 18.% (Table 3). The effectiveness of copper oxychloride, of the mineral fertilizer Kendal, has been limited but significantly different from the untreated control (Table 3). Two applications of *Bacillus subtilis* and thyme oil extract were not enough to achieve a suitable reduction of the pathogenic attacks and, at the last assessment, no significant differences from the untreated control were observed.

In trial 3, the lowest absolute downy mildew incidence and severity were observed until 18 days after the last treatment by using chemical fungicides. However, acibenzolar-S-methyl, Alexin, Glucoinductor give a significantly similar disease reduction as the fungicides tested. The efficacy of these products was maintained for 25 days after the last application. All the copper-based products are shown to have a significantly lower disease incidence and severity than the inoculated control until the end of the trial, whereas copper hydroxide showed this trend only at the first and second rating points after treatment. The mineral fertilizer Kendal TE gave significantly better downy mildew control than Kendal, mustard oil, and prohexadione-Ca. *B. subtilis* and thyme oil were confirmed to be slightly effective, but their applications gave a significant reduction compared to the untreated control for 18 days after the last treatment (Table 4).

In trials 4 and 5, when used alone in three treatments, metalaxyl-M+copper hydroxide, mandipropamid, azoxystrobin, the mineral fertilizer Alexin, and glucohumate activator complex showed the highest reduction of downy mildew incidence and severity (Tables 5 and 6). When different combinations of various products were tested in rotation, it was possible to reduce the fungicidal application to obtain a significantly similar reduction of downy mildew incidence and severity. Trials 4 and 5 showed the same trend in terms of disease control. When the copper-based products have been applied in rotation with Alexin there was a significant improvement in their effectiveness (Tables 5 and 6). When the phosphate-based products Alexin and Glucoinductor were used in rotation, they provided a statistically similar downy mildew control as the systemic fungicides tested (Tables 5 and 6).

The biomass as fresh weight of basil often reflected the degree of disease incidence (Tables 5 and 6); this trend was much more clear in trial 5 compared with trial 4, where thyme oil extract, Kendal TE, the mustard oil, copper-based product Airone in rotation with metalaxyl-M or with mandipropamide were statistically similar to the inoculated and non-treated control. Among the plant resistance inducers tested, the phosphate-based product Alexin significantly improved the fresh weight in both the trials, whereas acibenzolar-S-methyl, applied at 1 g 100 l^{-1}, was not significantly different from the inoculated control.

Discussion

All trials were carried out under conditions favorable for infection by *P. belbahrii*. The downy mildew signs observed in some trials in the non-inoculated control were probably caused by natural infection of seeds (Garibaldi *et al.* 2004a) as well as by the spread of the pathogen among plants with consequent cross contamination. In trial 1, it is possible that *P. belbahrii* was present in the greenhouse prior to the application of the first treatment, 22 days after sowing. This hypothesis is supported by the lower efficacy of acibenzolar-S-methyl (44% of disease incidence reduction compared with the untreated control at the last assessment), compared with the results obtained in trials 2–4 that showed a 91%, 83% and 81% reduction of downy mildew at the last assessment, respectively. Results were consistent with prior observations for this compound in other foliar diseases, such as gray mold (*Botrytis cinerea*) on tomato (Malolepsza 2006), anthracnose (*Colletotrichum lagenarium*) on cucumber (Ishii *et al.* 1999), and blue mold (*Peronospora tabacina)* on tobacco (LaMondia 2009). The copper-based products showed the same trend of acibenzolar-S-methyl and provided a better disease control of basil downy mildew in trials 2–5 compared with trial 1.

Among the tested fungicides, metalaxyl-M+copper hydroxide and azoxystrobin confirmed their efficacy (Gullino *et al.* 2009). Also mandipropamid was quite effective in this study. This fungicide is registered for use on basil in Italy but, like other fungicides with a specific mode of action, it is labelled for use in rotation with fungicides having other modes of action to prevent fungicide resistance (Brent & Hollomon 2007). Copper oxychloride+copper hydrochloride, copper sulphate and copper hydroxide and terpenic alcohols showed results statistically similar to acibenzolar-S-methyl and phosphite-based products in trials 2 and 3. These copper products when applied in rotation with resistance inducers such as the mineral fertilizer Alexin and with the glucohumate activator product in trials 4 and 5 improved their efficacy.

The results provided by Alexin and by the glucohumate activator complex, used alone or in alternation with other products, 20–25 days after the last treatment, were statistically similar to those offered by the most active systemic fungicides.

The mineral fertilizer Kendal TE gave a better result than Kendal in trials 2 and 3, whereas in trial 1 at the last evaluation no differences from the untreated control were observed for either product.

The biocontrol agent *B. subtilis* and thyme oil extract gave results significantly different from the untreated control at the first evaluation in trial 1, and for two of the three evaluations carried out in trial 2. Downy mildew incidence in plants treated with mustard oil and prohexadione-Ca was significantly different from that of the inoculated control in all the trials and showed an efficacy at the last evaluation between 43% and 69% and from 33% to 70%, respectively.

Resistance inducers are legal to use under the rules for fertilizers in Italy under current regulations. 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 to health maintenance and product quality (Kappert *et al.* 2011; Mersha *et al.* 2012).

Plant resistance inducers or improvers are reported to provide disease reduction of oomycetes under different conditions, for instance against *Peronospora destructor*, *P. parasitica*, *Bremia lactucae* and *Pseudoperonospora cubensis* under greenhouse conditions (Kofoet & Fischer 2007), and in the open field against *Peronospora manshurica* on soybean and *P. hyoscyami* f.sp. *tabacina* on tobacco (Perez *et al.* 2003; Silva *et al.* 2011). The efficacy of several improvers is influenced by different factors, such as host genotype, inoculum density, nutrition and climatic conditions: high levels of disease control are reported under controlled conditions while their effectiveness in the field is variable (Walters & Fountaine 2009). In the tobacco–blue mold and barley–powdery mildew pathosystem, it has been observed that plant resistance genes can influence the efficacy of the resistance inducers products (Martinelli *et al.* 1993; Perez *et al.* 2003). In the case of the downy mildews of vegetables, only the efficacy of phosphonates was confirmed also under field conditions (Kofoet & Fischer 2007) and the positive effects of their post-infection application have been reported (Wicks *et al.* 1991). In the case of *Peronospora parasitica* on cauliflower, acibenzolar-S-methyl was effective by inducing the production of pathogenesis-related proteins (Ziadi *et al.* 2001). The same compound proved effective against Phytophthora blight, caused by *P. capsici*, on squash (*Cucurbita pepo*) (Ji *et al.* 2011), while, Mersha *et al.* (2012) have shown the effect of rate, type of application and pre- or post-inoculation application of acibenzolar-S-methyl treatment against downy mildew of basil.

The aim of the present work was to evaluate the possibility of adopting disease management programs focused on a more flexible use of systemic chemical fungicides in order to reduce the risk of the presence of residues at the end of each production cycle. Spray programs to control downy mildew of basil must have short pre-harvest interval residues. In the case of basil for pesto production, where harvest is carried out every 20 days, this aspect severely restricts the choice of products that should be applied on the basis of their effectiveness as well as in respect to the Maximum Residue Limits in food, and their compatibility in an integrated pest management program (IPM). As shown in trials 4 and 5, several combinations of products, applying two products in alternation in three treatments at 6-day intervals, enable us to reduce strongly downy mildew incidence and severity compared with the untreated control. These spray programs provide a significantly similar disease control compared with systemic chemicals and do not reduce the marketable yield. As demonstrated for the blue mold–tobacco pathosystem, the rotation of effective fungicides with resistance inducers has improved the disease control as well as marketable yield (LaMondia 2008). Morever, Molina *et al.* (1998) reported that the synergistic effect between fungicides and induced resistance enabled reduction of total fungicide application. In the meantime, the rotation between fungicides - especially those with a specific mode of action - and resistance inducers will reduce the selection pressure by the fungicides, thus reducing the risk of resistance development towards the few registered fungicides (Skylakakis 1981; Vallad & Goodman 2004). The results of this work provided evidence that some of the resistance inducers and phosphorus-based fertilizers tested provided significant control of downy mildew on basil, when applied alone as well as in several combinations.

Acknowledgments Work carried out in the framework of the project SAFEFOODCONTROL, funded by Regione Piemonte “Sviluppo di sistemi e tecnologie innovative per la produzione, conservazione, trasformazione e valorizzazione dell’orto-frutticoltura piemontese di qualità”. The authors thank Dr. Piter Mazzoglio for language revision.

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