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, biocontrol agents and natural products

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Abstract 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 programmes. In all trials, the greatest reduction in disease incidence and severity were 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), while the biocontrol agent *B. 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 rotation of fungicides with compounds that can induce resistance as well as by using rotation with different resistance-inducing compounds on their own.

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 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 the 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 mostly grown in glasshouses; in this environment average night temperatures and dew depositions caused by high relative humidity 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).

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*., 2004) 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 this report in Switzerland, the pathogen spread to many basil growing areas. The disease was recently reported also in Belgium (Coosemans, 2004), in the USA (Roberts *et al*., 2009), in Cuba (Martinez de La Parte *et al*., 2010) and in Hungary (Nagy and Horvath, 2011). Its spread probably has been favoured by the fact that it is seed-transmitted (Garibaldi *et al*., 2004).

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

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 the difficulty to obtain fungicide registration for minor crops (Leadbeater and Gisi, 2010), such as basil. The search for basil varieties resistant to the pathogen is still at 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 and Hollomon, 2007).

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 and Grant 1991; Smillie et al., 1989) and their efficacy has been reported in several Molinapathosystems (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 were 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, 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 out in 2011 and 2012 at Grugliasco (Torino) in a glasshouse belonging to Agroinnova. Main information 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 white peat : perlite (80:20 v/v) mix (Turco Silvestro, Albenga, Savona), that had been steamed (90°C for 30 minutes). Fertilization was carried out once before 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 (2 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 microns 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 humidity, close to 85-95%, and temperatures ranging between 19 and 25 °C by using a program (software Magricomp Multilab, Agricontrol S.n.c., SV, Italy)

allowing to obtain the environmental conditions set in the greenhouse. This system constantly measures the humidity and manages the number of misting 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 1x10^5 sporangia/ml. 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 2 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 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, by using a dosage in according to manufacturer's instructions at the dates reported under table 1. The water suspension (800L ha^{-1}of volume) of each product was sprayed on the plants at stage of development of three true leaf, by using a handheld of 1L capacity.

In the trials 1-3, two treatments were applied at 6 days interval except of systemic chemical fungicides that were applied once on the dates reported in table 1. One artificial inoculation with the pathogen was made 24 hours after the last treatment applications.

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

Plant inducers, salts and fertilizers Acibenzolar-S-methyl (Bion 50WG, 50% a.i., Syngenta, Italy), prohexadione-Ca (Regalis, 10% a.i, 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 ~~N:K~~ (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, Atessa, Chieti, Italy) 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 2-6 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 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. These fungicides are registered for use on basil in Italy as reported in Ministerial label of these products.

Data collection and analysis Trials were monitored daily and starting at the appearance of the first symptoms, the evaluation of the percent of infected leaves (disease incidence) and of diseased leaf area affected by the pathogen (disease severity) was made by using a disease rating scale /EPPO, 2004). Disease severity was calculated by using the formula: DS= [∑(n° leaves × x 0-5) / (total of leaves recorded)] with x 0-5 corresponding to: 1 = from1 to 10% (midpoint 5 %) infected leaf area; 2 = from11 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 was estimated on 100 leaves/treatment. Ten-fifteen leaves of basil randomly chosen from each pot were visually examined.

At the end of the trials 4 and 5, plant biomass, as fresh weight of plants, corresponded to two pots per repetition, was measured by 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 6, respectively (Tables 2-6). In the different trials and especially in the first one, infection was present also in the not inoculated control plants; this is probably due to a contamination of the seeds used (Garibaldi *et al*., 2004) 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 starting treatments. It is not excluded that the pathogen was already present in the greenhouse before starting the first trial.

In trial 1, downy mildew incidence and severity was significantly reduced by the mixture of metalaxyl-M + copper hydroxide, by the mineral fertilizer "Alexin", by 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 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 day after treatments no differences from the untreated control were observed. Thyme oil extract did not provide any control of downy mildew, resulting 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 the artificial inoculation of the pathogen. At the first assessment, 53.5% of basil leaves were infected with the 32.9% of the leaf area affected, while, no disease was observed on the not inoculated basil plants. In the presence of 84.5% of infected leaves with a 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”, 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 clearly shown considering the severity of the pathogenic attacks 25 days after the last application of the products. At the last assessment, there were not significant differences between plants treated with the copper sulphate compounds, the mineral fertilizer “Kendal TE”, prohexadione-Ca and with mustard oil, that 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 as having a significantly lower disease incidence and severity than the inoculated control until the end of the trial, while copper hydroxide showed this trend only at the first and second rating points after treatment. The mineral fertilizer “Kendal TE” showed a significantly better downy mildew control than “Kendal”, mustard oil, and prohexadione-Ca. *B. subtilis* and thyme oil confirmed to be slightly effective, however, 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 3 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 (Table 5). 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 of their effectiveness (Tables 5 and 6). When the phosphate-based products “Alexin” and “Glucoinductor” were used in rotation, provided a statistically similar downy mildew control as 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 not treated control. Among the plant resistance inducerstested, the phosphate-based product “Alexin” significantly improve the fresh weight in both the trials, while, Acibenzolar-S-methyl, applied at 1 g 100 L^{-1}, was not significant different from the inoculated control.

Discussion

All trials were carried out under conditions favourable to the infection by *P. belbahrii*. The downy mildew signs observed in some trials in the not inoculated control were probably caused by natural infection of seeds (Garibaldi *et al*., 2004) 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 the trials 2-4 that showed a 91%, 83% and 81% of downy mildew reduction at the last assessment, respectively. Results were consistent with prior observations for this compound in 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 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 actions, it is labelled for use in rotation with fungicides having other modes of action to prevent fungicide resistance (Brent and Hollomon, 2007; Cohen *et al*. 2008). 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 the mineral fertilizer "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 trial 2 and 3, while in trial 1 at last evaluation no differences from the untreated control were observed for both products.

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 trails 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 production quality (Mersha *et al*., 2012; Kappert *et al*., 2011).

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 and Fisher, 2007), and in the open field against *Peronospora manshurica* of soybean and *P. hyoscyami* f.sp. *tabacina* on tobacco (Silva *et al*., 2011; Perez *et al*., 2003). 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 and 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 (Perez *et al*., 2003; Martinelli *et al*., 1993). In the case of the downy mildews of vegetables, only phosphonates confirmed their efficacy also under field conditions (Kofoet and Fischer, 2007) and the positive effects of their post-infection application have been reported (Wicks *et al*., 1991). 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 Phytophthora blight, caused by *P. capsici*, on squash (*Cucurbita pepo*) (Ji *et al*., 2011). 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 programmes focused on a more flexible use of systemic chemicals fungicides in order to reduce the risk of the presence of residues at the end of each production cycle. Spray programmes 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 their respect of the Maximum Residue Limits in food, and their compatibility in a integrated pest management programmes (IPM). As shown in trials 4 and 5, several combinations of products, applying two products in alternation in three treatments at 6 days interval, permit to reduce strongly downy mildew incidence and severity compared with the untreated control. These spray programmes, 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 the synergistic effect between fungicides and induced resistance able to allow the 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 (Vallad and Goodman, 2004 Skylakakis, 1981). The result of this work put in evidence that some of the resistance inducers and phosphorous-based fertilizers tested provided a significant control of downy mildew on basil, when applied alone as well as in several combinations.

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Table 1 Main operations carried out in the five trials

Operations	Trial number				
	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	7/10; 13/10	14/10; 20/10	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;	7/10;	14/10;	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 $1x10^5$ CFU/ml	16/09	14/10	21/10	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	26/10; 2/11; 7/11	31/10; 7/11;14/11	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.

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[x]	Dosage g a.i.100 L	DI at day after last treatment						DS at day after last treatment					
			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-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	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
Glucohumate activator complex [z]	Glucoinductor	400	7.8	ab	16.5	ab	30.8	bc	5.5	a-c	11.5	ab	22.2	ab
Copper sulphate +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
-	Not inoculated control	-	37.5	f-h	48.2	d	73.0	d-f	25.8	hi	33.6	cd	54.4	d-f

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

[x] 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 at 9/09/2011. One artificial inoculation was carried out on 16/09/2011

[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 day after last treatment						DS at day after last treatment					
			13 [x]		20		25		13		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-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	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
Glucohumate activator complex [z]	Glucoinductor	400	0.0	a	4.3	ab	3.8	ab	0.0	a	1.6	ab	2.1	a
Copper sulphate +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

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

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

[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						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-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	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
Glucohumate activator complex [z]	Glucoinductor	400	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

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

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

[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						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	a-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	d
Glucohumate 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	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
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

[k] Means of the same column, followed by the same letter do not differ according to Tukey's test (*P*<0.05)
[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
[y] 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)
[z] Dosage (ml 100 L^{-1}) of the commercial formulation

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[x]	Dosage g a.i.100 L^{-1}	DI at days after the last treatment						DS at days after the last treatment						Biomass (g)	
			8		16		23		8		16		23			
-	Inoculated control	-	13.5	h	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	Tyme oil	100	9.3	f	30.5	d	58.8	e	6.6	e	22.9	d	44.1	e	58.2	h
Mustard oil[z]	Duolif	1000	11.8	g	29.3	d	43.9	de	8.3	e	21.9	d	33.0	de	65.3	gh
Glucohumates activator complex[z] (F)	Glucoinductor	400	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[y]	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
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
-	Not inoculated control	-	0.0	a	0.0	a	2.3	a	0.0	a	0.0	a	1.5	a	94.6	a-e

[k] Means of the same column, followed by the same letter do not differ according to Tukey's test ($P<0.05$)
[x] Three treatments were carried out on: 21/3, 28/3, 4/04. Two artificial inoculations were carried out on: 22/03 and 29/03
[y] 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)
[z] Dosage (ml 100 L^{-1}) of the commercial formulation