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Management of leaf spot of wild rocket using fungicides, resistance inducers and a biocontrol agent, under greenhouse conditions

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ABSTRACT

A new leaf spot of wild rocket (*Diplotaxis tenuifolia*), caused by the seed transmitted pathogen *Plectosphaerella cucumerina,* is causing severe losses on this crop. Disease management is complicated, as few chemicals are registered for use on rocket. Four experimental trials using fungicides, resistance inducers, and a biocontrol agent against *P. cucumerina* were carried out on two cultivars of wild rocket, under greenhouse conditions, in order to evaluate the efficacy of the different treatments. In the presence of a disease severity ranging from 5.8 to 26.6 in the control plots of wild rocket (cvs Grazia and Rucola selvatica), one foliar application of azoxystrobin, boscalid alone or combined with pyraclostrobin provided a significant reduction in disease severity, compared with the untreated control, at values ranging between 48.5% and 84.8%. The phosphites-based products and acibenzolar-S-methyl provided the most constant results when applied in three treatments, with a disease severity reduction ranging between 34%-82% and 45%-70.3%, respectively. The highest disease reduction in disease severity was observed by using two sprays of mandipropamid at 18.7mL/100L with an efficacy ranging between 64% and 92%. Among the copper-based products, the most effective treatments compared with the untreated control plants were the copper hydroxide and terpenic alcohols. Thiram provided a significant reduction in disease severity compared with the untreated control plants at values ranging from 53% to 78%; however, the plants generally did not improve in biomass compared with the untreated control. The most effective treatment in terms of disease severity reduction did not always provide a better response in plant biomass. *Streptomyces griseoviridis* provided only a partial, but consistent, reduction of leaf spot on wild rocket when applied in three treatments at seven day-intervals, with results statistically different from those observed in the untreated control plants. Considering the difficulty in reaching a complete disease management level against *P. cucumerina*, an integrated approach should be considered.

Keywords: *Diplotaxis tenuifolia*; *Plectosphaerella cucumerina*; phosphite; acibenzolar-S-methyl; *Streptomyces griseoviridis*; disease control

1. Introduction

Wild rocket [*Diplotaxis tenuifolia* (L.) DC], an interspecific hybrid of *D. viminea* x *D. muralis*, is a crop increasingly grown in many countries because of its broad use in Italian and International cuisine. The crop, native to central and southern Europe and Asia, has become highly popular in the Mediterranean region as a constituent of ready-to-eat fresh cut salad mix (Pignone, 1997; Bianco, 2009). During recent years, several diseases caused by soil-borne and foliar pathogens have been observed for the first time on wild and cultivated rocket, grown in intensive cropping systems (Gilardi et al., 2013a). In many cases the new diseases have appeared for the first time in Italy, a country where the crop is very popular and is planted in approximately 3,600 Hectares (Anonymous, 2012).

The observation of a new leaf spot caused by *Plectosphaerella cucumerina* (Garibaldi et al., 2013a) on wild rocket leads to complications in crop management, as few chemicals are registered. *P. cucumerina* (syn. *Plectosporium tabacinum*) (Palm et al., 1995) causes a wide range of disease symptoms, including crown and root rot, and leaf necrosis, on a large number of vegetable hosts such as melon, watermelon, squash, zucchini, cucumber, sunflower, white lupin, tomato, parsley, endive, wild rocket (Matta, 1978; Saad and Black 1981; Zazzerini and Tosi,1987; Youssef et al., 2001; Carlucci et al., 2012; Garibaldi et al., 2012; 2013), and fruit crops such as banana (Kanakala and Singh, 2013). The same pathogen causes a basal rot on basil and tomato grown in soilless systems (Egel et al., 2010; Vallance et al., 2011).

Information regarding the management of this pathogen is very scarce, especially in relation to the very limited availability of registered fungicides on rocket, which is considered a minor crop. The occurrence of *P. cucumerina* on wild rocket seeds is one of the causes of the spread of the pathogen to new cultivation areas (Gilardi et al., 2013b), further complicating disease management. The aim of this study was to evaluate the efficacy of different treatments including fungicides, resistance inducers, and a biocontrol agent against *P. cucumerina*, under greenhouse conditions.

2. Materials and Methods

2.1. Plant material and experimental conditions

Four experimental trials (Table 1) were carried out in 2012 and 2013, in a glasshouse at temperatures ranging from 22 to 28°C. Wild rocket seeds belonging to cvs Grazia (Enza Zaden) and Rucola selvatica (Suba) were sown (0.025 g/pot, corresponding to 35-40 plants/pot) in plastic pots (3.5 L vol., 18 x 18 cm) containing a white peat : perlite (80:20 v/v) mix (Turco Silvestro, Albenga, Savona), which had been steamed (90°C for 30 minutes). Fertilization was carried out before sowing, by mixing OSMOFORM 2, Scotts (18% N, 5% P_2O_5, 13% K_2O) at 2 kg $m^{-3}$of soil.

The rocket seeds of cv Rucola selvatica used during the trials were contaminated by *P. cucumerina* at a level of 0.3%, while in the case of seeds belonging to the cv Grazia no seed contamination was detected by isolation of subsamples represented by 400 seeds, following the method described by Mathur and Kongsdal (2003).

For each treatment, four replicates (2 pots each) in a completely randomized block design were used. Pots were maintained on benches, covered with a transparent polyethylene film (50 microns thick), placed on an iron support immediately after artificial inoculation, and maintained until the last assessment.

2.2. Artificial inoculation

The strain of *P. cucumerina* (code PLC-1, GenBank Accession No. JX185769), obtained from wild rocket plants and maintained on potato dextrose agar media at 8°C, was used for artificial inoculation. The isolate was grown in a Petri dish containing potato dextrose agar (PDA, Merck) for 7 days, at temperatures ranging from 20 to 23 °C, with a 12 h/d fluorescent light regime, for inoculum production. A conidial suspension, adjusted with a haemocytometer to $1x10^{5}$ conidia mL^{1}, was applied to healthy plants of wild rocket with a hand-held canister (10 mL capacity) until run-off was achieved. The artificial inoculation of the pathogen was carried out 24 h after treatment. The number and date of artificial inoculations carried out in the different trials are reported in tables 1-5.

2.3. Tested products

Fungicides, phosphite-based products and organic amendments, known for their capability to induce resistance in several hosts, and the biocontrol agent *Streptomyces griseoviridis* (Mycostop, 30% in dried spores and mycelium of ray fungus, Verdera), reported in Tables 2-5, were tested.

Commercial formulations of azoxystrobin (Ortiva, 23.2% a. i., Syngenta Crop Protection), pyraclostrobin (Cabrio F, 25% a.i., BASF Crop Protection), pyraclostrobin + boscalid (Signum, 6.7% + 26.7% a.i., BASF Crop Protection), belonging to Quinone outside inhibitors Q_OI (FRAC code11), mandipropamid (Pergado SC, 23.4% a. i., Syngenta Crop Protection), belonging to CAA –fungicides (Carboxylic acidamides) (FRAC code 40), prochloraz (Octave, 46.1% a.i., BASF Crop Protection) belonging to DMI group (FRAC code 3), thiram (Tetrasol 50 WG, 47.5% a.i., Taminco Italia) of the ethylene bisdithiocarbamate chemical class (FRAC code M3), iprodione (Rovral, 25% a.i., BASF Crop Protection) of the dicarboximides (FRAC code 2), boscalid (Cantus, 50% a.i., BASF Crop Protection) of pyridine carboxamides (FRAC code 7), represent the different chemicals tested.

Among multi-site fungicides (FRAC code M) the copper-based products, copper oxychloride, copper hydroxide (Airone, 10%+10% a.i. Isagro), copper hydroxide, and terpenic alcohols (Heliocuivre, 26.7% a. i., Biogard) were tested.

Among the resistance inducers, the phosphite-based on the glucohumate complex (Glucoinductor + GlucoActivator, N 4%, P_2O_5 18%, International patent PCT, IB2004\001905, Fertirev) and a mineral fertilizer based on potassium phosphite (Alexin 95PS, P_2O_5 52%, K_2O 42%, Massò), as well as acibenzolar-S-methyl (Bion 50WG, 50% a. i., Syngenta Crop Protection) of the benzo-thiadiazole group (FRAC code P), were used.

The biocontrol agent, acibenzolar-S-methyl, the products based on phosphite salts, as well as the fungicides tested, were applied as leaf spray at a volume of 500 L ha^{-1} using a hand spray (1 L capacity), at dosages according to the manufacturer’s instructions. Two to three treatments were carried out at 7 day intervals, starting 24 h before the artificial inoculation of wild rocket at the age of 10-14 days after sowing (Tables 1-5). The timing of the applications of the tested products is reported in tables 1-5, while the dosages of the applications are given in tables 2-5.

2.4. Data collection and statistical analysis

Plants were checked weekly for disease development and the percentage of infected leaves was evaluated. *P. cucumerina* was consistently reisolated from the lesions. At the end of each trial, plants were harvested to determine the total leaf biomass per replicate, as fresh weight. Disease severity (DS) was estimated on 100 leaves/treatment per replicate by using a disease rating scale calculated as [∑(n° leaves × x_{0-5}) / (total leaves recorded)] with x_{0-5} corresponding to the midpoint value reported: 0=no symptoms, healthy plants; 1=1 to 30% affected leaf area (midpoint 15%); 2=31 to 50% affected leaf area (midpoint 40%); 3=51 to 70% affected leaf area (midpoint 60%); 4=71 to 90% affected leaf area (midpoint 80%): 5=over 90% affected leaf area (midpoint 95%). The data, expressed as a percentage of affected leaf area (disease severity) and fresh weight (per pot) were statistically processed by means of variance analysis (ANOVA) and Tukey's test (P=0.05).

3. Results

The artificial inoculation led to a consistent disease level in the inoculated, not treated, control plants (Tables 2-5). Since seeds of cv Rucola selvatica were contaminated by *P. cucumerina* at 0.3%, some level of disease was also present in the non-inoculated control plants of both tested cultivars (Tables 2-5). The disease observed in the non-inoculated control plants at the end of the trials can be attributed to a possible cross-contamination of the plants probably because treated and untreated rocket plants were maintained under the same environmental conditions under mulching.

In trial 1, all the products tested provided a significant disease reduction compared with the inoculated non-treated control, in the presence of an average disease severity value of 25.0% on cv Grazia and 18.4% on cv Rucola selvatica. The highest level of disease control was provided by prochloraz (83.6% efficacy) and pyraclostrobin (82% efficacy) on cv Grazia (Table 2). In the case of cv Rucola selvatica, boscalid, azoxystrobin and thiram showed an efficacy of 84.8%, 72.8% and 77.7% respectively, compared with the inoculated non-treated control. The other products tested, including the biocontrol agent *S. griseoviridis,* were not significantly different in reducing the level of disease compared with the inoculated and non-treated control plots (Table 2). Generally, the products tested showed a lower impact on plant biomass, with the exception of pyraclostrobin, which improved the plant biomass of cv Grazia, and azoxystrobin and pyraclostrobin + boscalid on cv Rucola selvatica.

In trial 2, in the presence of a disease severity of 21.3% on cv Grazia and 20.0% on cv Rucola selvatica, the best results were obtained with two applications of copper hydroxide and terpenic alcohols (71-74% efficacy). All other products tested, including *S. griseoviridis*, provided a disease reduction ranging from 34% to 64%, not significantly different from the copper hydroxide and terpenic alcohols, and from the untreated control (Table 3). The biomass was not related to the degree of disease severity. The highest fresh weight was observed on cv Grazia treated with azoxystrobin, and on cv Rucola selvatica treated with azoxystrobin and acibenzolar- S-methyl, while the other treatments were not statistically different from the non inoculated control (Table 3).

In trial 3, in the presence of a disease severity of 26.6% on cv Grazia, the best results for disease reduction were provided by mandipropamid at the highest dosage tested (92% efficacy), iprodione (87% efficacy), thiram (88% efficacy), and pyraclostrobin (85% efficacy). Among the resistance inducers, three applications of acibenzolar-S-methyl, at both tested dosages, significantly reduced *P. cucumerina,* with an efficacy from 61% to 70%. Also the phosphite-based products gave a significant level of control of the pathogen, with an average efficacy between 82% and 67%. *S. griseoviridis* provided a reduction in disease severity of 66.2%, significantly similar to that provided by the copper salt Airone (Table 4). On cv Rucola selvatica, with 24.1 disease severity in the untreated control, the best protection was provided by mandipropamid at the highest dosage tested (87% efficacy), followed by thiram and iprodione (83.1% efficacy). Among the resistance inducers, the best results were given by the phosphite-based Alexin, with a disease reduction of 75%. *S. griseoviridis* and the copper based products partially reduced *P. cucumerina* (from 34 to

51% efficacy). The best improvement in biomass as fresh weight, of rocket cvs Grazia and Rucola selvatica, was given by three applications of the mineral phosphite-based products (Table 4).

In trial 4, on both cultivars, the disease severity was relatively low, with an affected leaf area of 5.8 and 10.6% in the inoculated and non-treated control. All treatments carried out on cv Grazia statistically reduced the leaf spot caused by *P. cucumerina*, compared to the non-inoculated and untreated control, with the exception of boscalid, alone and combined with pyraclostrobin. The best level of disease control was provided by two applications of mandipropamid at 12.5 and 18.7 g/100 L, and thiram, which reduced disease severity by 84%, 72% and 71% respectively, and provided a protection level statistically similar to that of three treatments with acibenzolar-S-methyl al the lowest dosage (67% efficacy) (Table 5).

By considering cv Rucola selvatica in trial 4, three treatments with the phosphite-based product, Alexin, provided a 73% disease reduction. The greatest reduction in disease severity was observed with two treatments of thiram (78% efficacy), iprodione (72% efficacy) and mandipropamid (55-64% efficacy) (Table 5). There were no significant differences between the inoculated and untreated control and treatments with products based on copper salts, while the biocontrol agent *S. griseoviridis* gave the worse effect compared to the control. Among the compounds tested, no statistical difference was observed in plant biomass of cv Rucola selvatica compared with both the inoculated and non-inoculated and non-treated control. The greatest increase in fresh weight biomass was given by pyraclostrobin, alone or combined with boscalid, and by mandipropamid. For cv Grazia, only plants treated with mandipropamid were significantly similar in biomass to the control plants (Table 5).

4. Discussion

In this study, the efficacy of fungicides with different modes of action, and belonging to seven different FRAC fungicidal groups, of resistance inducers, and of the biocontrol agent *S. griseoviridis* against *P. cucumeina* was tested on two cultivars of wild rocket.

It is interesting to note that complete control of the disease was very difficult to achieve, even in the trials where disease severity was not very high, such as trial 4. Certainly, the occurrence of the pathogen on seeds (Gilardi et al., 2013b) complicates disease management and favours the spread of the pathogen.

The highest disease reduction was observed by spraying rocket plants with mandipropamid, azoxystrobin, prochloraz, iprodione, the phosphites-based products and acibenzolar-S-methyl. Such treatments significantly reduced leaf spot severity from 14 to 33 days after the last treatment, with an efficacy ranging between 55% and 81%. The most effective products provided the same level of disease control on the two cultivars tested, despite seeds of cv Rucola selvatica having been infected already. Results provided by acibenzolar-S-methyl were consistent with prior observations for this compound against citrus black spot caused by *Guignardia citricarpa* (Miles et al., 2004) and other necrotrophic foliar pathogens of Brassica crops such as *Pyrenopeziza brassicae*, *Cercospora brassicicola* (Kahn et al., 2005; Oxley and Walters, 2012) and *Leptosphaeria maculans* (Liu et al., 2006). On the other hand, it must be considered that the activity of acibenzolar-S-methyl is closely related to the host and cultivar (Oostendorp et al., 2001). Certainly in the presence of even a partial effect of the resistance inducers, a beneficial effect associated with the reduction of the inoculum levels should be considered and further investigated (Walters et al.,2013).

Of some interest is the efficacy against *P. cucumerina* of mandipropamid, a fungicide belonging to the class of amides, as well as that of the phosphite-based products, both of which are mainly effective against Oomycetes (Huggenberger et al., 2005; Smillie et al., 1989). Particularly, our results have shown the highest disease reduction in *Plectosphaerella* leaf spot by using two sprays of mandipropamid at 18.7 mL/100L with an efficacy ranging between 64% and 92%.

Many phosphite products are used as fertilizers with important implications for plant development and, in addition to the Oomycetes, their effect is reported against several plant pathogens of various genera such as *Fusicladium effusum*, *Alternaria alternata*, *Penicillium* *digitatum* and *P. italicum* (McDonald et al., 2001; Agostini et al., 2003; Bock et al., 2012; Cerioni et al., 2013). Our results highlight that phosphite products are effective in reducing the severity of *Plectosphaerella* leaf spot on wild rocket. In addition, as previously reported, the phosphite-based products have shown efficacy also in post-infection application (Wicks et al., 1991).

Our work provides data on the positive effect of one application of azoxystrobin against *Plectosphaerella* leaf spot of wild rocket and indicates an increase in the fresh biomass of plants. In past studies azoxystrobin, a strobilurin fungicide with a wide spectrum of activity against different species belonging to Ascomycota, Basidiomycota, Deuteromycetes, and Oomycetes (Barlett et al., 2002), has been proven to be effective in controlling other brassica pathogens such as *Cercospora brassicicola* on turnip greens (Kahn et al., 2005).

Among the fungicides tested in our trials, thiram provided a significant disease reduction, ranging from 53 to 78%. However, the treated plants did not always show an improved plant biomass, with values close to those observed in the untreated control. In trial 4, two or three spays with the tested products, with the exception of mandipropamid at 18.7 mL/100 L and boscalid + pyraclostrobin, significantly reduced the fresh weight of rocket cv Grazia compared with the untreated control.

It might be useful to check the effect of seed-application of thiram, a practice that is generally done with this product. In the case of iprodione, previous studies indicate one to three foliar sprays at 0.5 kg a.i./ha gave effective control of *Alternaria brassicicola* of *Brassica oleracea* (Humpherson-Jones and Maude, 1982). Our results provide evidence of the significant reduction of Plectosphaerella leaf spot between 43 and 87% with two sprays of iprodione at 50 g/ 100L, compared to the untreated control.

By considering the products admitted in organic farming, such as the copper-based products, the best efficacy was provided by copper hydroxide and terpenic alcohols, which showed 53 and 69% efficacy. We also investigated the effect of *S. griseoviridis* that showed only a partial, but consistent, reduction of leaf spot on wild rocket, with results statistically different from those observed in the untreated control plants. Also Youssef et al., (2001) reported the ability of *Streptomyces* species (S. *cyanoviridis, S. murinus and S. griseoplanus*) to control root rot caused by *Plectosporium tabacinum* on white lupin. A previous study also shows the positive effect of *Trichoderma asperellum* (strain T34) amended to different growth media in hydroponics against both *Plectosphaerella cucumerina* and *Hyaloperonospora parasitica* on cucumber and, on *Arabidopsis* (Segarra et al., 2013).

Regarding the difficulty to reach complete disease management, a more integrated approach, should be considered. In general, considering the losses caused by this pathogen under field conditions and the contribution to its spread to new production areas by its transmission through infected seeds, an integrated approach for disease management, which includes seed dressing, foliar sprays with effective products, and the use of partially resistant cultivars, if available, will be necessary. At present, however, no information concerning the susceptibility of commercial cultivars to the pathogen exists.

This work provides growers useful and practical information concerning the effectiveness of different types of products, including one biocontrol agent, with different modes of action, which can be applied in the field. It will be necessary to evaluate potential phytotoxic effects of such products when applied in a high number of treatments under greenhouse conditions, and under different environmental conditions.

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Table 1

List of the trials carried out and general information.

Trial	Sowing	Artificial inoculation	Number of treatments and date	End of the trial
1	12/07/2012	26/07/2012	2 (25/07/2012; 31/07/2012)	22/08/2012
2	20/08/2012	7/09/2012	2 (30/08/2012; 6/09/2012)	10/10/2012
3	11/03/2013	3/04/2013	3 (28/03/2013; 2/04/2013; 9/04/2013)	24/04/2013
4	6/05/2013	16/05/2013 23/05/2013	3 (8/05/2013 15/05/2013 22/05/2013)	5/06/2013

Table 2

Effect of different treatments against leaf spot, incited by *Plectosphaerella cucumerina,* on wild rocket cvs Grazia and Rucola selvatica, expressed as disease severity (% of affected leaf area) and biomass. In the case of cv Rucola selvatica, seeds were naturally infected (Trial 1).

Active ingredient	Commercial product	Dosage a. i. g or mL / 100L	Number of treatments (days between treatments)	cv Grazia Disease severity [c]		Fresh weight g		cv Rucola selvatica Disease severity		Fresh weight g	
Inoculated and non –treated control	-	-	-	25.0	c[a]	16.2	b	18.4	c	18.9	ef
Azoxystrobin	Ortiva	18.6	1[b]	4.8	ab	36.5	ab	5.0	ab	35.8	a
Boscalid	Cantus	60	1	4.9	ab	30.8	ab	2.8	a	31.4	ab
Acibenzolar –S-methyl	Bion	1.25	2 (7)	12.8	b	19.7	ab	10.0	a-c	20.0	d-f
Acibenzolar –S-methyl	Bion	0.5	2(7)	5.9	ab	26.8	ab	7.2	a-c	25.5	b-e
Phosphite based glucohumate complex	Inductor	16+72	2 (7)	6.1	ab	54.2	ab	12.8	a-c	25.4	b-f
Potassium Phosphite	Alexin	130+105	2 (7)	7.7	ab	21.1	ab	8.8	a-c	28.3	a-d
Prochloraz	Octave	25	1	4.1	a	23.5	ab	16.6	bc	16.3	f
Thiram	Tetrasol	95	1	8.4	ab	29.1	ab	4.1	ab	28.5	a-d
Iprodione	Rovral	50	1	5.0	ab	48.9	ab	9.7	a-c	28.5	a-d
Pyraclostrobin	Cabrio F	7.5	1	4.3	a	59.8	a	9.7	a-c	23.8	b-f
Copper hydroxide and terpenic alcohols	Heliocuivre	60	2(7)	8.4	ab	27.7	ab	7.5	a-c	26.8	a-e
Copper oxychloride + copper hydroxide	Airone	40+40	2(7)	6.3	ab	41.1	ab	12.5	a-c	29.8	a-c
Streptomyces griseoviridis	Mycostop	0.3	2(7)	8.1	ab	27.1	ab	5.8	a-c	20.8	c-f
Non inoculated control	-	-	-	5.9	ab	30.7	ab	8.1	a-c	24.7	b-f

[a] Means within the same column followed by the same letter do not differ according to Tukey's test (P=0.05)

[b] Products were applied once on 31/07/2012 with the exception of acibenzolar-S-methyl, the phosphite-based fertilizer, copper salts and *Streptomyces griseoviridis*, where two treatments were carried out on: 25/07/2012 and 31/07/2012. One artificial inoculation was carried out on 26/07/2012.

[c] Disease severity data were taken 28 days after the first treatment.

Table 3

Effect of different treatments against leaf spot, incited by *Plectosphaerella cucumerina*, on wild rocket cvs Grazia and Rucola selvatica, *expressed* as disease severity (% of affected leaf area) and biomass. In the case of cv Rucola selvatica, seeds were naturally infected (Trial 2).

Active ingredient	Commercial product	Dosage a. i. g or mL / 100L	Number of treatments (days between treatments)	cv Grazia		cv Rucola selvatica	
				Disease severity[c]	Fresh weight g	Disease severity	Fresh weight g
Inoculated and non –treated control	-	-	-	21.3 b[a]	18.2 a-c	20.0 c	12.1 bc
Azoxystrobin	Ortiva	18.6	1 [b]	11.3 ab	29.6 ab	10.3 ab	24.6 a
Boscaid + pyraclostrobin	Signum	40+10	1	11.9 ab	25.8 a-c	10.3 ab	20.2 a-c
Boscalid	Cantus	60	1	14.1 ab	26.5 a-c	10.0 ab	22.4 ab
Acibenzolar –S-methyl	Bion	1.25	2 (7)	7.5 ab	25.4 a-c	10.6 ab	18.0 a-c
Acibenzolar –S-methyl	Bion	0.5	2(7)	13.4 ab	22.3 a-c	10.3 ab	24.6 a
Phosphite based glucohumate complex	Inductor	16+72	2 (7)	12.8 ab	26.7 a-c	14.4 bc	17.9 a-c
Potassium Phosphite	Alexin	130+105	2 (7)	14.1 ab	27.0 a-c	15.3 bc	19.6 a-c
Prochloraz	Octave	25	1	9.4 ab	23.0 a-c	13.4 a-c	14.6 a-c
Thiram	Tetrasol	95	1	12.8 ab	18.6 a-c	11.9 ab	9.2 c
Iprodione	Rovral	50	1	9.3 ab	24.9 a-c	13.1 a-c	14.5 a-c
Pyraclostrobin	Cabrio F	7.5	1	12.5 ab	14.6 a-c	13.8 a-c	13.2 a-c
Copper hydroxide and terpenic alcohols	Heliocuivre	60	2(7)	5.4 a	15.1 a-c	5.9 a	20.5 a-c
Copper oxychloride + copper hydroxide	Airone	40+40	2(7)	11.0 ab	13.8 bc	10.3 ab	20.7 a-c
Streptomyces griseoviridis	Mycostop	0.3	2(7)	14.4 ab	12.2 c	13.8 a-c	11.7 bc
Non inoculated control	-	-	-	4.4 a	31.3 a	9.4 ab	10.5 bc

[a]Means within the same column followed by the same letter do not differ according to Tukey's test (P=0.05)

[b] Products were applied once on 30/08/2012 with the exceptions for acibenzolar-S-methyl, the phosphite-based products, copper salts and *Streptomyces griseoviridis*, where two treatments were carried out on 30/08/2012 and 6/09/2012. One artificial inoculation was carried out on 7/09/2012.

[c] Disease severity data were taken 41 days after the first treatment.

Table 4

Effect of different treatments against leaf spot, incited by *Plectosphaerella cucumerina*, on wild rocket cvs Grazia and Rucola selvatica, expressed as disease severity (% of affected leaf area) and biomass. In the case of cv Rucola selvatica, seeds were naturally infected (Trial 3).

Active ingredient	Commercial product	Dosage a. i. g or mL / 100L	Number of treatments (days between treatments)	cv Grazia Disease severity [c]		cv Grazia Fresh weight g		cv Rucola selvatica Disease severity		cv Rucola selvatica Fresh weight g	
Inoculated and non -treated control	-	-	-	26.6	f[a]	102.8	i	24.1	i	98.0	i
Azoxystrobin	Ortiva	18.6	2 (7)[b]	5.3	bc	149.4	f-h	8.9	d-f	133.0	d-h
Boscaid + pyraclostrobin	Signum	40+10	2 (7)	6.7	cd	197.6	c-e	8.2	c-e	124.1	f-i
Boscalid	Cantus	60	2 (7)	5.0	bc	209.4	b-d	12.6	gh	154.1	b-f
Acibenzolar –S-methyl	Bion	1.25	3 (7)	7.9	de	159.7	e-h	11.7	f-h	195.9	a
Acibenzolar –S-methyl	Bion	0.5	3 (7)	10.3	e	206.9	b-d	13.2	h	121.8	g-i
Phosphite based glucohumate complex	Inductor	16+72	3 (7)	8.7	de	251.6	a	10.5	e-h	124.9	f-i
Potassium Phosphite	Alexin	130+105	3 (7)	4.7	bc	225.7	a-c	6.1	b-d	183.8	ab
Prochloraz	Octave	25	2 (7)	4.7	bc	133.3	hi	9.6	e-g	164.3	a-d
Thiram	Tetrasol	95	2 (7)	3.0	ab	244.2	ab	4.2	ab	126.2	e-i
Iprodione	Rovral	50	2 (7)	3.4	ab	214.7	a-d	4.2	ab	173.8	a-c
Pyraclostrobin	Cabrio F	7.5	2 (7)	4.1	ab	188.7	c-f	9.7	e-g	121.0	g-i
Copper hydroxide and terpenic alcohols	Heliocuivre	60	3 (7)	4.4	a-c	204.5	b-d	8.6	c-e	157.3	b-e
Copper oxychloride + copper hydroxide	Airone	40+40	3 (7)	9.0	de	163.1	e-h	11.8	f-h	144.0	c-g
Streptomyces griseoviridis	Mycostop	0.3	3 (7)	9.0	de	191.1	c-e	12.6	gh	128.6	e-i
Mandipropamid	Pergado	12.5	2 (7)	3.1	ab	177.4	d-g	5.8	a-c	97.9	i
Mandipropamid	Pergado	18.7	2 (7)	2.1	a	207.2	b-d	3.1	a	107.2	hi
Non inoculated non treated control	-	-	-	3.5	ab	146.0	gh	4.6	ab	132.1	e-h

[a] Means within the same column followed by the same letter do not differ according to Tukey's test (P=0.05)

[b] Products were applied twice on 2/04/2013 and 9/4/2013 with the exceptions for acibenzolar-S-methyl, the phosphite-based products, copper salts and *Streptomyces griseoviridis*, where three treatments were carried out on 28/03/2013, 2/04/2013 and 9/04/2013. One artificial inoculation was carried out on 3/04/2013.

[c] Disease severity data were taken 27 days after the first treatment.

Table 5

Effect of different treatments against leaf spot, incited by *Plectosphaerella cucumerina*, on wild rocket cvs Grazia and Rucola selvatica, expressed as disease severity (DS, % of affected leaf area) and biomass. In the case of cv Rucola selvatica, seeds were naturally infected (Trial 4).

Active ingredient	Commercial product	Dosage a. i. g or mL/ 100L	Number of treatments (days between treatments)	cv Grazia				cv Rucola selvatica			
				Disease severity[c]		Fresh weigth g		Disease severity		Fresh weigth g	
Inoculated and non -treated control	-	-	-	5.8	f[a]	101.9	ab	10.6	d-f	19.8	f
Azoxystrobin	Ortiva	18.6	2 (7)[b]	3.3	b-d	31.1	cd	6.7	b-d	80.4	a-d
Boscaid + pyraclostrobin	Signum	40+10	2 (7)	6.0	f	68.3	bc	8.9	c-e	119.8	a
Boscalid	Cantus	60	2 (7)	5.5	ef	48.7	cd	11.7	ef	90.6	ab
Acibenzolar –S-methyl	Bion	1.25	3 (7)	-[d]		34.2	cd	-		75.7	b-d
Acibenzolar –S-methyl	Bion	0.5	3 (7)	1.9	a-c	51.3	cd	8.6	c-e	70.2	b-e
Phosphite based glucohumate complex	Inductor	16+72	3 (7)	3.8	de	29.7	cd	8.2	c-e	56.4	b-f
Potassium Phosphite	Alexin	130+105	3 (7)	3.1	b-d	20.4	d	2.8	ab	49.8	c-f
Prochloraz	Octave	25	2 (7)	3.3	b-d	17.4	d	6.9	b-d	54.4	b-f
Thiram	Tetrasol	95	2 (7)	1.7	ab	18.6	d	2.3	a	20.4	f
Iprodione	Rovral	50	2 (7)	3.3	b-d	32.0	cd	3.0	ab	19.1	f
Pyraclostrobin	Cabrio F	7.5	2 (7)	3.7	c-e	26.7	cd	9.1	d-f	27.8	f
Copper hydroxide and terpenic alcohols	Heliocuivre	60	3 (7)	3.8	de	33.8	cd	13.3	fg	23.5	f
Copper oxychloride + copper hydroxide	Airone	40+40	3 (7)	3.2	b-d	35.4	cd	17.0	gh	34.1	ef
Streptomyces griseoviridis	Mycostop	0.3	3 (7)	3.0	b-d	46.1	cd	18.9	h	25.9	f
Mandipropamid	Pergado	12.5	2 (7)	1.6	ab	39.7	cd	4.7	a-c	55.4	b-f
Mandipropamid	Pergado	18.7	2 (7)	0.9	a	139.4	a	3.8	ab	81.3	a-c
Non inoculated non treated control	-	-	-	2.4	a-d	136.8	a	12.5	ef	31.2	ef

[a] Means within the same column followed by the same letter do not differ according to Tukey's test (P=0.05)

[b]Products were applied twice on 15/05/2013 and 22/5/2013 with the exceptions for acibenzolar-S-methyl, the phosphite-based products, copper salts and *Streptomyces griseoviridis*, where three treatments were carried out on 8/05/2013, 15/05/2013 and 22/05/2013. Two artificial inoculations were carried out on 16/05/2013 and 23/05/2013.

[c] Disease severity data were taken 28 days after the first treatment.

[d]Not tested.