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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 18.7 mL/100 L 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.

1. Introduction

Wild rocket [*Diplotaxis tenuifolia* (L.) DC], an interspecific hybrid of *Diplotaxis viminea* × *Diplotaxis 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 3600 Hectares (Anonymous, 2012).

The observation of a new leaf spot caused by *Plectosphaerella cucumerina* (Garibaldi et al., 2012) 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

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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 × 18 cm) containing a white peat: perlite (80:20 v/v) mix (Turco Silvestro, Albenga, Savona), which had been steamed (90 °C for 30 min). 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 1×10^5 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.

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

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 95 PS, 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 $[\sum(n^\circ \text{ leaves} \times x_{0-5})/(\text{total leaves recorded})]$ with x_{0-5} corresponding to the midpoint value reported: 0 = no symptoms, healthy plants; 1 = 1–30% affected leaf area (midpoint 15%); 2 = 31–50% affected leaf area (midpoint 40%); 3 = 51–70% affected leaf area (midpoint 60%); 4 = 71–90% 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).