



Evaluation of the short term effect of nursery treatments with phosphite-based products, acibenzolar-S-methyl, pelleted *Brassica carinata* and biocontrol agents, against lettuce and cultivated rocket fusarium wilt under artificial inoculation and greenhouse conditions

Giovanna Gilardi ^{a, b}, Stefano Demarchi ^a, Maria Lodovica Gullino ^{a, b, *}, Angelo Garibaldi ^a

^a Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, Largo P.Braccini 2, 10095 Grugliasco, TO, Italy

^b Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo P.Braccini 2, 10095 Grugliasco, TO, Italy

ARTICLE INFO

Article history:

Received 3 January 2015

Received in revised form

12 February 2016

Accepted 11 March 2016

Keywords:

Lactuca sativa

Eruca vesicaria

Fusarium oxysporum f. sp. *lactucae*

Fusarium oxysporum f. sp. *raphani*

Integrated control

ABSTRACT

Experimental trials have been carried out in order to evaluate the efficacy of preventative treatments based on plant defense activator products, biocontrol agents, a microbial complex with arbuscular mycorrhizal fungi, and *Brassica carinata* pellets against *Fusarium oxysporum* f. sp. *lactucae* race 1 on lettuce and *Fusarium oxysporum* f. sp. *raphani* on cultivated rocket under greenhouse conditions. These products were compared with fungicides known for their ability to induce host resistance (phosethyl-Al and acibenzolar-S-methyl), and with azoxystrobin. Three and four applications of the tested products were carried out on lettuce and rocket seedlings grown in nursery conditions. Treated and untreated plants were transplanted into soil infested with *Fusarium* wilt agents to obtain an average disease severity (DS) of 65.6–69.2 and of 56.9–62.1 on the untreated lettuce and rocket plants, respectively. The best *Fusarium* wilt biocontrol was obtained after four applications of *Bacillus subtilis* Qst713 and with the Glomas microbial complex (42 and 46.7% efficacy, respectively). *B. carinata* pellets provided a consistent control when applied 14 days before the rocket and lettuce were transplanted into the infested soil. Acibenzolar-S-methyl, applied at 0.025 g/Liter, showed a DS reduction in *F. oxysporum* f. sp. *lactucae* from 36 to 61% and of *F. oxysporum* f. sp. *raphani* from 54 to 73%, thus showing statistically similar results to those of azoxystrobin, which was used as a reference (DS reduction from 59 to 65%). Although the *Fusarium* wilt control provided by such products was not complete in the present experimental conditions, these products can be considered interesting components for an integrated pest management of the *Fusarium* wilt of leafy vegetables, starting from nursery applications. Moreover, the tested BCAs could become potentially useful, especially for plant monocultures. This study has been produced new information on the effects of potassium phosphite, applied at the nursery level, on reducing lettuce and rocket fusarium wilt. An average efficacy of 69.5% was observed for lettuce, while an average efficacy of 65.2% was observed for cultivated rocket. The good fungicidal activity of the phosphite-based product, coupled with the positive effect on plant biomass, is of special interest.

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1. Introduction

In recent years, the economic relevance of lettuce (*Lactuca sativa* L.) and cultivated rocket [*Eruca vesicaria* (L.) Cav.] has increased, since many farms produce fresh cut and ready-to-eat salads. In

these intensive systems, where leafy vegetables are continuously grown in the same soil, the phytopathological situation is constantly in evolution, as a consequence of the dynamism and specialization of such crops (Garibaldi and Gullino, 2010; Garibaldi et al., 2014). *Fusarium* wilt incited by *Fusarium oxysporum* f. sp. *lactucae* on lettuce and by *Fusarium oxysporum* ff. spp. *raphani* and *conglutinans* on cultivated rocket, can lead to serious losses (Matheson and Gullino, 2012). The *Fusarium* wilt of lettuce was detected for the first time in Europe, in northern Italy, in 2002 (Garibaldi and Gullino, 2010). The two *formae speciales* that affect

* Corresponding author. Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, Largo P.Braccini 2, 10095 Grugliasco, TO, Italy.
E-mail address: marialodovica.gullino@unito.it (M.L. Gullino).

cultivated rocket, that is, *Fusarium oxysporum* ff.spp. *raphani* and *conglutinans*, the first of which is more frequently detected (Garibaldi et al., 2006; Srinivasan et al., 2012), also affect other genera belonging to Brassicaceae, such as cabbage, brussels sprouts, broccoli, turnip, radish and stock (Garibaldi et al., 2006). The *Fusarium* wilt of lettuce and rocket are easily and frequently seed-transmitted (Gullino et al., 2014), thus suggesting the importance of preventative disease management strategies.

Lettuce varieties that are resistant or at least tolerant to *Fusarium* wilt are available (Scott et al., 2010a, b; Matheron and Gullino, 2012; Gilardi et al., 2014b), but their effective use is complicated by the presence of three races of the pathogen (Fujinaga, 2005). In the case of rocket, the use of resistant varieties is still very limited (Gilardi et al., 2007).

In general, the management of soil-borne pathogens is complicated by the limited number of registered chemicals and by the restrictions in the use of pre-plant fumigants, including metam sodium and dazomet (Colla et al., 2012). Several approaches to soil-borne disease management have been investigated intensively in an attempt to find an answer to the many practical problems associated with the loss or limitation of use of effective fumigants encountered by growers. Moreover, more emphasis is now given to crop and soil health instead of disease control (Barrière et al., 2014). Among the exploited strategies for disease management, systemic acquired resistance (SAR) and induced systemic resistance (ISR), which are mainly triggered by microorganisms, such as plant growth-promoting rhizobacteria, by the metabolic products of affected plants, or by chemicals (Sticher et al., 1997; Oostendorp et al., 2001; Vallad and Goodman, 2004; Shores et al., 2005) are at present attracting a great deal of interest. Induced systemic and localized resistance to soil-borne pathogens, by means of *Trichoderma* treatments, has been well documented (Shores et al., 2005; Vinale et al., 2008). Kloepper et al. (2004) proved the ability of *Bacillus* spp. to induce systemic resistance, and in most cases, to also elicit plant growth promotion. Moreover, arbuscular mycorrhizal fungi have been reported to be implicated in protecting plants against soil-borne pathogens, through different mechanisms, including an improvement in plant nutrition, damage compensation, competition, changes in the root system and activation of plant defense signalling (Whipps, 2004; Pozo and Azcón-Aguilar, 2007). Moreover, among the chemical resistance inducers, the phosphite-based fertilizers and acibenzolar-S-methyl have been shown to lead to a reduction in disease against soil-borne pathogens, on vegetable and ornamental crops (Eikemo et al., 2003; Elmer, 2004, 2006; Hyeon et al., 2009; Bubici et al., 2006; Walters, 2012; Gilardi et al., 2014a), but no data are available concerning their efficacy against the *Fusarium* wilts of lettuce and rocket.

This study has been carried out in order to evaluate the efficacy of preventative treatments, including SAR and IRS activator products, biocontrol agents, a microbial complex based on arbuscular mycorrhizal fungi, and *Brassica carinata* pellets against *Fusarium oxysporum* f. sp. *lactucae* race 1 on lettuce and *Fusarium oxysporum* f. sp. *raphani* on cultivated rocket under simulated nursery conditions, in a greenhouse.

2. Material and methods

2.1. Plant material and experimental layout

Experimental trials (Table 1) were carried out in 2012 and 2013 under greenhouse conditions in order to test the efficacy of different products against the *Fusarium* wilts of lettuce and cultivated rocket. Lettuce (cv. Crispilla) and cultivated rocket (cv. Coltivata) seeds, which are very susceptible to *Fusarium* wilt (Garibaldi

et al., 2004; Gilardi et al., 2014a, b), were sown in 100-plug trays (2.5 cm Ø per pot, 4-L of soil capacity) filled with a steamed (90 °C for 30 min) peat mix substrate (blond peat:black peat 15:85, pH 5.5–6.0, 1100 g m⁻³ of N:P:K and traces of molybdenum, Brill Type 5, Georgsdorf, Germany).

Fifteen-day-old lettuce and cultivated rocket seedlings were transplanted into the same substrate in 12-L plastic pots. Ten plants/pot of each tested crop were kept in a greenhouse on benches with air temperatures ranging from 26 to 34 °C during the day and from 20 to 25 °C during the night (Table 1). The substrate was artificially infested with the *Fusarium* wilt agents, as described hereafter. Forty plants per treatment were arranged in a complete randomized block design in each trial, which represented the experimental unit, with three (Protocol 1) and four replicated trials (Protocol 2) as reported in Table 1.

2.2. Fungal strains and artificial inoculation

A highly virulent strain of *F. oxysporum* f. sp. *lactucae*, isolated in 2002 from infected lettuce plants in north-western Italy, ATCC-MYA3040, belonging to Race 1 of the pathogen (Garibaldi et al., 2002), and the FusRuc 13/03 strain, isolated from rocket grown in a commercial plastic greenhouse in northern-Italy in 2003 and identified as *F. oxysporum* f. sp. *raphani* (Garibaldi et al., 2006), were used throughout the experiments. The single-spore culture of each isolate was stored in glycerol at –80 °C.

These strains were grown in potato dextrose broth (Sigma–Aldrich, St. Luis, USA) and kept in a rotatory shaker working at 90–100 rpm for 10 days at 25 °C. The biomass produced after centrifugation (9600 g at 4 °C) was prepared as a dry talc-based powder (biomass: talc 2:1 w/w), as described by Locke and Colhoun (1974). After 20 days at 22–25 °C, the number of chlamydospores per gram of talc was assessed by serial plating on potato dextrose agar, PDA (Merck, Darmstadt, Germany), which contained 25 mg L⁻¹ streptomycin sulphate. The talc formulations of *F. oxysporum* f. sp. *lactucae* (strain MYA3040) and of *F. oxysporum* f. sp. *raphani* (strain FusRuc13/03), at 2 and 5 × 10⁷ chlamydospores/g, respectively, were used. These formulated pathogens (2 and 1 g liter/respectively) were mixed into the steamed substrate as chlamydospores dispersed in talc to achieve a final concentration of 5 × 10⁴ chlamydospores ml⁻¹ of substrate. A non-infested substrate was used as a control (Table 1).

2.3. Products used in the test

Different compounds known for their capability to induce resistance in the host, that is, phosphite-based fertilizers, organic amendments, biocontrol agents (BCAs) and fungicides were tested (Table 2).

Among the considered phosphite-based products and organic amendments, a phosphite-based glucohumate complex (Glucoinductor + GlucoActivator, N 4%, P₂O₅ 18%, International patent PCT, IB2004/001905, Fertirev, Torino, Italy), a mineral fertilizer based on potassium phosphite (Alexin 95PS, P₂O₅ 52%, K₂O 42%, Massò, Spain), and a patented formulation of *B. carinata* defatted seed meal (Biofence, N organic 3%, P 2.2%, K 2%, organic C 52%, Triumph, Spain) were tested.

Among the BCAs, *Bacillus subtilis* QST 713 (Serenade, 14.6% a.i., BayerCropScience, Italy), *Bacillus velezensis* (Cilus Plus IT45, 95%, Massò, Spain), *Trichoderma asperellum* + *Trichoderma gamsii* (Remedier WP, Isagro Ricerca, Milano, Italy), a product based on arbuscular mycorrhizal fungi combined with a microbial complex of *Trichoderma* and *Bacillus* (Rizocore, *Glomus* spp. 5% + *Bacillus megaterium* 10⁴ UFCg⁻¹ + *Trichoderma* 10¹⁰ UFCg⁻¹, Biogard, division of CBCEurope, Italy) were tested.

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