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RESEARCH PAPERS

# Managing Phytophthora crown and root rot on tomato by pre-plant treatments with biocontrol agents, resistance inducers, organic and mineral fertilizers under nursery conditions

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**Summary.** Five trials were carried out under greenhouse conditions to test the efficacy of spray programmes based on biocontrol agents, phosphite-based fertilizers and a chemical inducer of resistance (acibenzolar-S-methyl, phosethyl-Al) to control crown and root rot of tomato incited by *Phytophthora nicotianae*. The best disease control, under high disease pressure resulting from artificial inoculation, was obtained with three pre-plant leaf sprays at 7 d intervals with acibenzolar-S-methyl and with two mineral phosphite-based fertilizers. The disease reduction achieved was similar to that obtained with a single application of azoxystrobin and metalaxyl-M. Phosetyl-Al and the biocontrol agents *Glomus* spp. + *Bacillus megaterium* + *Trichoderma*, *B. subtilis* QST713, *B. velezensis* IT45 and the mixture *T. asperellum* ICC012 + *T. gamsii* ICC080 provided a partial disease control. *Brassica carinata* pellets did not control the disease.

**Key words:** *Solanum lycopersicum*, *Phytophthora nicotianae*, chemical control.

## Introduction

Among the solanaceous crops grown in Italy, tomato (*Solanum lycopersicum* L.) covers over 70,000 ha. With 7 million ton of tomato production, Italy ranks first in Europe and fifth in the world (FAO, 2008). *Phytophthora nicotianae* is a common and destructive pathogen of tomato. It spreads through asexual reproduction, by massive release of zoospores. This pathogen causes seedling damping off, stem canker, buckeye rot of fruits, and causes important yield losses (Jones *et al*., 1991; Erwin and Ribeiro, 1996; Bruna and Tobar, 2004). In Italy, *P. nicotianae* is reported on tomato hybrids in protected crops (Pane *et al*., 2000) and is an emerging problem on tomato grafted on *Solanum lycopersicum* x *S. hirsutum* rootstocks (Garibaldi and Gullino, 2010).

The management of the diseases caused by *P. nicotianae* using grafted tomato onto resistant rootstocks is complicated by pathogenic variation in different strains of the pathogen (Gilardi *et al*., 2011). Furthermore, chemical control is complicated by increasing limitations in the availability of registered fungicides, and the risk of development of resistance towards some classes of chemicals (phenylamides and Quinone outside inhibitors) at present registered for use on tomato (Cohen and Coffey, 1986; Leadbeater and Gisi, 2010).

European Regulation No. 1107/2009, concerning the placing of plant protection products on the market, and European Directive No. 2009/128/EC, establishing a framework for Community action to achieve the sustainable use of pesticides, requires, by 2014, that all professional users implement the gen-

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eral IPM principles (Martin, 2003; Colla *et al*., 2012). Therefore, alternative strategies for disease control are needed. The study outlined in this paper was carried out to test preventive treatments based on biocontrol agents, compounds known for capability to induce resistance to several pathogens in plants, phosphite-based fertilizers, organic amendments based on *Brassica carinata* and fungicides, for control of *P. nicotianae* on tomato under nursery conditions.

## Material and methods

### Plant material and experimental layout

Five trials were carried out in 2012 under greenhouse conditions at Grugliasco (Torino, Italy), at 20–27°C and 65–75% RH. Seeds of tomato cv. Cuore di Bue (Furia sementi, Monticelli Terme) were sown in 60-plug trays (4.05 cm diam. per pot, 2 L soil capacity) filled with 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). The same substrate and fertilization were used for the 3.5 L plastic pots used for transplanting the 20- to 30-d-old tomato seedlings (Table 1). Five tomato plants were used per pot, in pots filled with the described substrate and artificially infested with the pathogen. Two pots per replicate (ten plants per replicate represented the experimental unit) with four replicates were arranged in complete randomized block designs in all trials.

### Pathogen cultures and artificial inoculation

Isolate PHT29 of *P. nicotianae*, obtained from infected tomato plants and maintained on a *Phytophthora*-selective medium (Masago *et al*., 1977) at 12°C, was inoculated in a sterile mixture of wheat-hemp kernels (2:1 v/v) in a 1 L flask and kept at room temperature. Seven d before tomato transplanting, the 20-d-old culture of the pathogen was mixed with peat mix substrate see above) at a rate of 1 g $L^{-1}$ (Brill Type 5) (Table 1). The 3.5 L pots containing the artificially infested substrate were maintained in the greenhouse under the same conditions as the 60-plug trays and watered daily.

### Tested products

Several biocontrol agents and compounds known for capability to induce host resistance, phosphite-based fertilizers, organic amendments, and fungicides were tested (Table 2).

Five biocontrol agents were tested: *Bacillus subtilis* QST 713 (Serenade Max, 14.6% a.i., BASF, Italy), *Bacillus velezensis* (Cilus Plus IT45, 95%, Massò, Italy), *Trichoderma asperellum* ICC012 + *T. gamsii* ICC080 (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* $10^4$ CFU $g^{-1}$ + *Trichoderma* $10^{10}$ CFU $g^{-1}$, Intrachem Bio Italia, Italy) and a microbial complex combined with arbuscular mycorrhizal fungi (Micosat, 14%

**Table 1.** Calendar of the main operations carried out in five trials.

| Operation | Trial number | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Sowing | 26/03/2012 | 06/04/2012 | 05/06/2012 | 01/08/2012 | 24/09/2012 |
| First treatment (T0) | 11/04/2012 | 17/04/2012 | 25/06/2012 | 21/08/2012 | 8/10/2012 |
| Second treatment (T 7) | 17/04/2012 | 24/04/2012 | 02/07/2012 | 28/08/2012 | 15/10/2012 |
| Third treatment (T 14) | 24/04/2012 | 02/05/2012 | 06/07/2012 | 04/09/2012 | 19/10/2012 |
| Artificial infestation of the substrate in pot conditions (T7) | 17/04/2012 | 24/04/2012 | 2/07/2012 | 28/08/2012 | 15/10/2012 |
| Transplanting | 26/04/2012 | 3/05/2012 | 07/07/2012 | 05/09/2012 | 19/10/2012 |
| End of the trial | 22/05/2012 | 3/06/2012 | 26/07/2012 | 24/09/2012 | 21/11/2012 |

**Table 2.** List of the products tested and experimental protocol used for treatments applied to tomato plants.

| BCA or active ingredient | Commercial formulation | Dosage (g a.i. $L^{-1}$) | Time (days) of application in tray conditions, and type of application | Time (days) of application in plastic pots (3.5 L) and type of application |
|---|---|---|---|---|
| *Bacillus subtilis* QST713 | Serenade Max | 0.58 | T0[a],T7, T14, leaf spray | - |
| *Glomus* spp. + *Bacillus velezensis* | Cilus Plus | 0.4[b] | T0,T7, T14, leaf spray | - |
| *T. asperellum* + *T. gamsii* | Remedier | 0.04 | T0, T7, T14, leaf spray | - |
| Acibenzolar-S-methyl | Bion 50 WG | 0.025; 0.0125 | T0, T7, T14, leaf spray | - |
| Phosetyl-Al | Aliette | 1.6 | T0, T7, T14, leaf spray | - |
| *Glomus* spp. + *Bacillus megaterium* + *Trichoderma* | Rizocore | 0.08[b] | T0, T7, T14, leaf spray | - |
| *Glomus* spp. + microbial complex | Micosat | 1.5[b] | T0, soil mixing | - |
| Mineral fertilizer P:K 52:42 | Alexin | 1.3 + 1.06 | T0, T7, T14, leaf spray | - |
| Organic Mineral fertilizer N:P 4:18 | Glucohumate complex | 1.6 + 0.72 | T0, T7, T14, leaf spray | - |
| Azoxystrobin | Ortiva | 0.19 | T14, leaf spray | - |
| Metalaxyl-M | Ridomil gold | 0.48 | T14, leaf spray | - |
| *Brassica carinata* pellet N:P:K: C organic | Biofence | 0.15 + 0.055 + 0.05 + 1.13 | - | T7, soil mixing |

[a] T0 corresponding to the development stage of 3–4 true leaves.
[b] Corresponding to the dosage (g $L^{-1}$) of the commercial formulation.

a.i., CCS Aosta, Italy). The fertilizers and organic amendments tested were: the fertilizer based on the glucohumate complex (Glucoinductor + GlucoActivator, N 4%, $P_2O_5$ 18%, International patent PCT, IB2004\001905, Fertirev, Torino, Italy), a mineral fertilizer based on potassium phosphite (Alexin 95PS, $P_2O_5$ 52%, $K_2O$ 42%, Massò, Italy) and a patented formulation of *Brassica carinata* defatted seed meals (Biofence, N organic 3%, P 2.2%, K 2%, organic C 52%, Triumph, Italy). The resistance inducing chemicals tested were: acibenzolar-S-methyl (Bion 50WG, 50% a.i., Syngenta Crop Protection, Italy) and phosetyl-Al (Aliette, 80% a.i, Bayer Crop Science, Italy), both known for capabilities to induce resistance mechanisms in plants. The efficacy of different treatments was compared with chemical fungicides registered for use on tomato in Italy, including azoxystrobin (Ortiva, 23.2% a. i., Syngenta Crop Protection, Italy) and metalaxyl-M (Ridomil gold, 480 g $L^{-1}$, Syngenta Crop Protection, Italy). The timing of applications is reported in Tables 1 and 2, while the dosages of applications are outlined in Tables 3 to 7.

### Timing and treatment applications

The biocontrol agents, acibenzolar-S-methyl, phosetyl-Al, the fertilizers based on phosphite salts, as well as the fungicides tested, were applied as leaf sprays at high volume of water (1,500 L $ha^{-1}$) using a hand sprayer. The product based on arbuscular mycorrhizal fungi and microbial complex (Micosat) tested in trials 3 to 5 was mixed with 2 L substrate used per plug tray (Tables 5 to 7), while the patented formulation of *B. carinata* defatted seed meals was mixed with the substrate used to fill the plastic 3.5 L pots. Both of these treatments were carried out 1 week before transplanting at the same time as the inoculation with *P. nicotianae* was carried out (Tables 1 and 2).

**Table 3.** Trial 1. Mean proportions of dead plants, mean disease indices (0–100) and mean total plant fresh weights, for tomato plants receiving different treatments.

| BCA or active ingredient | Commercial formulation | Dosage (g a.i. L-1) | Percentage of dead plants on 14/05/2012 | Disease index[b] on 22/05/2012 |
|---|---|---|---|---|
| Inoculated non-treated control | - | - | 27.5 bc[c] | 62.5 bc |
| *Bacillus subtilis* QST713 | Serenade Max | 0.58 | 25.0 bc | 37.5 ab |
| *Glomus* spp. + *Bacillus velezensis* | Cilus Plus | 0.4[d] | 12.5 ab | 30.0 ab |
| *T. asperellum* + *T. gamsii* | Remedier | 0.04 | 15.0 ab | 37.5 ab |
| Acibenzolar-S-methyl | Bion | 0.025 | 0.0 a | 0.0 a |
| Phosetyl-Al | Aliette | 1.6 | 17.5 a-c | 35.0 ab |
| *Glomus spp. +Bacillus megaterium +Trichoderma* | Rizocore | 0.08 | 17.5 a-c | 37.5 ab |
| Mineral fertilizer P:K 52:42 | Alexin | 1.3 + 1.06 | 0.0 a | 7.5 a |
| Organic mineral fertilizer N:P 4:18 | Glucohumate complex | 1.6 + 0.72 | 0.0 a | 15.0 a |
| Metalaxyl-M | Ridomil gold | 0.48 | 0.0 a | 2.5 a |
| *Brassica carinata* pellet N:P:K: C organic | Biofence [a] | 0.15 + 0.055 + 0.05 +1.13 | 37.5 c | 82.5 c |
| Non-inoculated and non-treated control | - | | 0.0 a | 0.0 a |

[a] Applied by mixing with the substrate at T7.
[b] Disease Index 0–95 [0, no symptoms, healthy plants; 1, 1 to 30% wilting (midpoint 15%); 2, 31 to 50% wilting (midpoint 40%); 3, 51 to 70% wilting (midpoint 60%); 4, 71 to 90% wilting (midpoint 80%); 5, over 90% wilting or dead plant (midpoint 95%)].
[c] The mean values of the same column followed by the same letter do not differ significantly according to Tukey's test ($P = 0.05$).
[d] Corresponding to the dosage (g $L^{-1}$) of the commercial formulation.

The tomato seedlings grown in each tray were treated by leaf spray applications at 6–7 d intervals, at least three times, with the exception of *B. carinata* pellets, Micosat and fungicides that were all applied once (Tables 1 and 2). The first treatment was carried out on tomato plants still in the plug trays, at the 3–4 true leaf stage, corresponding to 14–20 d after sowing. Product dosage was performed according to the manufacturer's instructions at the dates reported in Tables 2 to 7.

### Data collection and analysis

After transplanting, the plants were monitored weekly and Phytophthora crown rot infection was recorded starting from the appearance of the first symptoms of yellow leaves. The number of infected plants showing wilting and stem necrosis was counted to assess disease incidence. Disease severity was evaluated at the end of each trial as disease index (DI) ranging from 0 to 5 according to Quesada-Ocampo and Hausbeck (2010). Disease index data also included dead plants observed in previous assessments. Disease index was calculated using the formula [$\sum$(n° plants × $x_{0\text{-}5}$) / (total of plants recorded)] with $x_{0\text{-}5}$ corresponding to the midpoint value reported: 0 = no symptoms, healthy plants; 1 = 1 to 30% wilting (midpoint 15%); 2 = 31 to 50% wilting (midpoint 40%); 3 = 51 to 70% wilting (midpoint 60%); 4 = 71 to 90% wilting (midpoint 80%): 5 = more than 90% wilting or dead plant (midpoint 95%) (Tables 3 to 7). Data were examined to evaluate the correlation (positive or negative) between the effect of each product on disease severity compared with the inoculated and non-treated control plants, and the disease severity on the inoculated and non-treated control plants.

**Table 4.** Trial 2. Mean proportions of dead plants, mean disease indices (0-100) and mean total plant fresh weights, for tomato plants receiving different treatments.

| BCA or active ingredient | Commercial formulation | Dosage (g a.i. $L^{-1}$) | Percentage of dead plants on 18/05/2012 | Disease index [b] on 3/06/2012 | Total plants fresh weight (g) on 3/06/2012 |
|---|---|---|---|---|---|
| Inoculated non-treated control | - | - | 35.0 cd[c] | 70.0 de | 75.8 ef |
| *Bacillus subtilis* QST713 | Serenade Max | 0.58 | 22.5 b-d | 59.5 cd | 99.8 c-e |
| *Glomus* spp. + *Bacillus velezensis* | Cilus Plus | 0.4[d] | 17.5 a-c | 56.5 cd | 92.0 de |
| *T. asperellum* + *T. gamsii* | Remedier | 0.04 | 20.0 bc | 58.0 cd | 90.0 de |
| Acibenzolar-S-methyl | Bion | 0.025 | 0.0 a | 5.5 a | 149.0 a-c |
| Phosetyl Al | Aliette | 1.6 | 12.5 ab | 56.5 cd | 105.0 c-e |
| *Glomus spp. + Bacillus megaterium + Trichoderma* | Rizocore | 0.08[d] | 20.0 bc | 44.5 bcd | 105.8 c-e |
| Mineral fertilizer P:K 52:42 | Alexin | 1.3 + 1.06 | 0.0 a | 35.5 bc | 117.5 b-e |
| Organic mineral fertilizer N:P 4:18 | Glucohumate complex | 1.6 + 0.72 | 0.0 a | 38.0 bc | 106.0 c-e |
| Azoxystrobin | Ortiva | 0.19 | 0.0 a | 15.0 ab | 164.8 ab |
| *Brassica carinata* pellet N:P:K: C organic | Biofence [a] | 0.15 + 0.055 + 0.05 + 1.13 | 40.0 d | 91.5 e | 24.3 f |
| Non-inoculated and non-treated control | - | - | 0.0 a | 0.0 a | 199.8 a |

[a, b, c, d] See Table 3.

At the end of trials 2 to 5, the fresh weight of tomato plant was measured to evaluate any effects of the treatments on plant development.

The data obtained from counts of diseased plants at different assessments and the disease severity data (DI) were arcsine transformed to normalize their distributions. All data were then analysed by univariate ANOVA in SPSS 18.0, and means were separated using Tukey's test.

## Results

The method for inoculation with *P. nicotianae* gave mean disease severities ranging from 56.0 to 75.8 for inoculated non-treated control plots in the five trials (Tables 3 to 7). a. This allowed evaluation of the efficacy of the different products tested under severe disease conditions. There were negative correlations between the severity of *P. nicotianae* in the control pots and the efficacy of *B. subtilis* (R = - 0.65), the microbial complex combined with arbuscular mycorrhizal fungi, Micosat (R = - 0.98), acibenzolar-S-methyl used at 0.025 mg $L^{-1}$ (R = - 0.89), the phosphite-based fertilizers, Alexin (R = - 0.43) and phosetyl-Al (R = -0.03). Positive correlations with disease severity in the inoculated and non-treated control were determined for of *B. velezensis* (R = 0.09), the mixture of *T. harzianum* ICC012 + *T. viride* ICC080 (R = 0.32), the *B. carinata* defatted seed meals (R = 0.3), the phosphite-based glucohumate complex (R = 0.2), acibenzolar-S-methyl used at 0.0125 mg $L^{-1}$ (R = 0.6), metalaxyl-M (R = 0.53), and azoxystrobin (R = 0.02).

In Trial 1, the greatest reductions in disease severity, compared to the non-treated control, were provided by acibenzolar-S-methyl (100% efficacy), the phosphite-based fertilizer, Alexin (88% disease reduction) and by the product based on glucohumate complex (76% disease reduction). These treatments

**Table 5.** Trial 3. Mean proportions of dead plants, mean disease indices (0-100) and mean total plant fresh weights, for tomato plants receiving different treatments.

| BCA or active ingredient | Product | Dosage (g a.i. $L^{-1}$) | Percentage of dead plants on 17/07/2012 | Disease Index [b] on 26/07/2012 | Total plants fresh weight (g) on 26/07/2012 |
|---|---|---|---|---|---|
| Inoculated non- treated control | - | - | 57.5 d[c] | 67.5 f | 107.3 a-d |
| *Bacillus subtilis* QST713 | Serenade Max | 0.58 | 37.5 cd | 45.0 c-f | 127.0 a-c |
| *Glomus* spp. + *Bacillus velezensis* | Cilus Plus | 0.4[d] | 42.5 cd | 57.5 d-f | 65.1 d |
| *T. asperellum* + *T. gamsii* | Remedier | 0.04 | 42.5 cd | 60.0 ef | 73.5 cd |
| Acibenzolar S-methyl | Bion | 0.025 | 0.0 a | 0.0 a | 137.1 ab |
| Acibenzolar S-methyl | Bion | 0.0125 | 0.0 a | 2.5 a | 155.9 ab |
| Phosetyl-Al | Aliette | 1.6 | 0.0 a | 40.0 b-e | 116.6 a-d |
| *Glomus* spp. + *Bacillus megaterium* + *Trichoderma* | Rizocore | 0.08[d] | 30.0 c | 32.5 bc | 126.8 abc |
| *Glomus* spp. + microbial complex | Micosat[d] | 1.5[d] | 35.0 c | 45.0 c-f | 112.3 a-d |
| Mineral fertilizer P:K 52:42 | Alexin | 1.3 + 1.06 | 0.0 a | 17.5 ab | 138.0 ab |
| Organic mineral fertilizer N:P 4:18 | Glucohumate complex | 1.6 + 0.72 | 5.0 ab | 17.5 ab | 144.1 ab |
| Metalaxyl-M | Ridomil Gold | 0.48 | 2.5 a | 5.0 a | 130.0 a-c |
| *Brassica carinata* pellet N:P:K: C organic | Biofence [a] | 0.15 + 0.055 + 0.05 + 1.13 | 37.5 cd | 52.5 c-f | 96.8 b-d |
| Non-inoculated and non-treated control | - | | 0.0 a | 0.0 a | 164.3 a |

[a, b, c, d] See Table 3.

were as effective as metalaxyl-M (Table 3). The biocontrol agents (*B. velezensis*, *Trichoderma harzianum* + *T. viride* and *B. subtilis*), the mixture *Glomus* spp. + *B. megaterium* + *Trichoderma* (Rizocore) and phosetyl-Al provided disease reductions ranging from 40 to 52%, but these reductions not statistically different from the inoculated and non-treated control (Table 3). *Brassica carinata* pellets were not effective and caused an increase in disease incidence and severity in comparison with the inoculated and non-treated control plots (Table 3).

In Trial 2, even in the presence of a high disease pressure (DI = 70; Table 4), disease severity was least on plants treated with acibenzolar-S-methyl and azoxystrobin with disease reductions of 92% and 79%, respectively. Disease control provided at the end of the trial by the phosphite-based fertilizers (Alexin and Glucoinductor complex) was still considerable, with 50% and 46% reductions, respectively, compared with the inoculated and non-treated control, respectively. Phosetyl-Al reduced the percentage of dead plants on 18th May 2012, giving a 64% reduction of disease compared with the non-treated control. Disease reduction provided by this product was not significant at the last evaluation on 3rd June, however. Disease severity on plants treated with the biocontrol agents was not significantly different from the inoculated non-treated control. In this trial the *B.carinata* pellets also caused increased disease incidence and severity compared with the untreated control plots. In terms of fresh biomass production, azoxystrobin and acibenzolar-S-methyl gave results

**Table 6.** Trial 4. Mean proportions of dead plants, mean disease indices (0–100) and mean total plant fresh weights, for tomato plants receiving different treatments.

| BCA or active ingredient | Commercial formulation | Dosage (g a.i. $L^{-1}$) | Percentage of dead plants on 19/09/2012 | Disease index [b] on 24/09/2012 | Total plants fresh weight (g) on 24/09/2012 |
|---|---|---|---|---|---|
| Inoculated non- treated control | - | - | 48.0 c-e[c] | 56.0 c-f | 61.4 a-d |
| *Bacillus subtilis* QST713 | Serenade Max | 0.58 | 28.0 a-d | 38.4 b-f | 58.8 a-d |
| *Glomus* spp. + *Bacillus velezensis* | Cilus Plus | 0.4[d] | 40.0 b-e | 57.6 d-f | 33.7 cd |
| *T. asperellum* + *T. gamsii* | Remedier | 0.04 | 52.0 de | 63.2 ef | 28.6 d |
| Acibenzolar-S-methyl | Bion | 0.025 | 0.0 a | 12.8 ab | 47.7 a-d |
| Acibenzolar-S-methyl | Bion | 0.0125 | 0.0 a | 1.6 a | 71.7 a-d |
| Phosetyl Al | Aliette | 1.6 | 4.0 a | 18.4 ab | 79.5 ab |
| *Bacillus megaterium* + *Trichoderma* | Rizocore | 0.08[d] | 64.0 e | 71.2 f | 38.8 b-d |
| *Glomus spp.* + microbial complex | Micosat | 1.5[d] | 0.0 a | 24.8 a-d | 54.8 a-d |
| Mineral fertilizer P:K 52:42 | Alexin | 1.3 + 1.06 | 0.0 a | 10.4 ab | 79.6 ab |
| Organic mineral fertilizer N:P 4:18 | Glucohumate complex | 1.6 + 0.72 | 8.0 ab | 22.4 a-c | 83.2 a |
| Metalaxyl-M | Ridomil Gold | 0.48 | 0.0 a | 8.0 ab | 76.7 a-c |
| Azoxystrobin | Ortiva | 0.19 | 0.0 a | 6.4 ab | 88.0 a |
| *Brassica carinata* pellet N:P:K: C organic | Biofence [a] | 0.15 + 0.055 + 0.05 + 1.13 | 44.0 c-e | 58.4 d-f | 54.2 a-d |
| Non-inoculated and non-treated control | - | | 20.0 a-d | 20.8 ab | 89.4 a |

[a, b, c, d] See Table 3.

similar to the non-treated and non-inoculated control (Table 4).

In Trial 3, on 26th July 2012, the non-treated control showed a mean disease severity of 67.5: acibenzolar-S-methyl, at both tested dosages, and metalaxyl-M reduced *P. nicotianae* symptoms by 100–96.3% and 92.6%, respectively. Also the phosphite-based fertilizers and phosetyl-Al provided significant disease control, with average disease severity reductions of 74.1% and 40.7%, respectively (Table 5). Among the biocontrol agents, only *Glomus* spp. + *B. megaterium* + *Trichoderma* (Rizocore) gave partial disease control with a significant disease severity reduction of 52%. The products based on *Glomus* spp. with the microbial complex (Micosat), *B. subtilis*, *B. velezensis,* the mixture *T. harzianum* + *T. viride*, and *B. carinata* pellets did not control the disease (Table 5). Measurements of the fresh weights of tomato plants at the end of this trial showed that acibenzolar-S-methyl, at both tested dosages, and the phosphite-based fertilizers and phosetyl-Al did not negatively affect plant development compared with metalaxyl-M and the non-treated and non-inoculated control (Table 5).

In Trial 4, disease reductions were: acibenzolar-S-methyl at both dosages, 77%; metalaxyl-M, 97%; azoxystrobin, 86%; phosetyl-Al, 89%; and the phosphite-based fertilizer Alexin, 69% (Table 6). There were no significant differences in the survival of tomato plants

**Table 7.** Trial 5. Mean proportions of dead plants, mean disease indices (0–100) and mean total plant fresh weights, for tomato plants receiving different treatments.

| BCA or active ingredient | Product | Dosage (g a.i. $L^{-1}$) | Percentage of dead plants on 2/11/2012 | Disease index[b] on 9/11/2012 | Total plants fresh weight (g) on 9/11/2012 |
|---|---|---|---|---|---|
| Inoculated non-treated control | - | - | 35.0 b-d[c] | 71.5 b | 63.3 b-d |
| *Bacillus subtilis QST713* | Serenade Max | 0.58 | 40.0 d | 58.0 b | 68.3 a-d |
| *Glomus* spp. + *Bacillus velezensis* | Cilus Plus | 0.4[d] | 55.0 d | 64.0 b | 63.6 b-d |
| *T. asperellum* + *T. gamsii* | Remedier | 0.04 | 55.0 d | 65.0 b | 52.3 cd |
| Acibenzolar-S-methyl | Bion | 0.025 | 0.0 a | 4.5 a | 92.4 a-d |
| Acibenzolar-S-methyl | Bion | 0.0125 | 0.0 a | 4.0 a | 73.5 a-d |
| Phosetyl-Al | Aliette | 1.6 | 5.0 a-c | 3.0 a | 119.5 a-d |
| *Bacillus megaterium* + *Trichoderma* | Rizocore | 0.08[d] | 37.5 cd | 58.0 b | 84.0 a-d |
| *Glomus spp.* + microbial complex | Micosat | 1.5[d] | 42.5 d | 49.5 b | 99.8 a-d |
| Mineral fertilizer P:K 52:42 | Alexin | 1.3 + 1.06 | 0.0 a | 12.0 a | 103.3 a-d |
| Organic mineral fertilizer N:P 4:18 | Glucohumate complex | 1.6 + 0.72 | 0.0 a | 8.0 a | 145.4 a |
| Metalaxyl-M | Ridomil Gold | 0.48 | 0.0 a | 7.0 a | 114.1 a-d |
| Azoxystrobin | Ortiva | 0.19 | 2.5 ab | 2.5 a | 131.0 ab |
| *Brassica carinata* pellet N:P:K:C organic | Biofence [a] | 0.15 + 0.055 + 0.05 + 1.13 | 42.5 d | 56.0 b | 91.3 a-d |
| Non-inoculated and non-treated control | - | | 0.0 a | 0.0 a | 127.8 a-c |

[a, b, c, d] See Table 3.

grown in soil infested with *P. nicotianae* and treated with the products based on the glucohumate complex, *Glomus* spp. + microbial complex (Micosat) and the other biocontrol agents, or with the *B. carinata* pellets compared with the inoculated and non-treated control (Table 6). The assessment of total fresh weight of plants at the end of this trial showed that there were no significant differences between treatments and non-inoculated and non-treated control plants, with the exception of the results from the Remedier, Rizocore and Cilus Plus treatments (Table 6).

Similar trends in disease control were observed in Trial 5 (Table 7): in the presence of severe disease (mean severity = 71.5), metalaxyl-M, azoxystrobin, acibenzolar-S-methyl, the phosphite-based fertilizer and phosetyl-Al all significantly reduced disease severity, with reductions in disease of from 96.5% to 83.2%. There were no significant effects of the biocontrol agents tested or *B. carinata* pellets on disease severity. Plants treated with the product based on glucohumate complex showed an increase in fresh weight compared with the non-treated inoculated control (Table 7).

## Discussion

Several products, including microbial extracts, chemicals, phosphite-based fertilizers, plant growth

promoting rhizobacteria and arbuscular mycorrhiza, have been shown to induce systemic acquired resistance in a number of pathosystems (Kessmann *et al*., 1994; Pieterse *et al*., 1996; Eshraghi *et al*., 2011; Sukhada *et al*., 2011; Vos *et al*., 2012). Many studies have investigated the practical application of induced resistance products to control plant diseases (Walters and Fountaine, 2009; Walters *et al*., 2013). However the effects of pre-plant treatments based on resistance inducing products and *Brassica carinata* amendments in controlling Phytophthora crown and root rot on tomato have not been previously reported.

Among the resistance inducing chemicals, phosphites have been used previously in the management of several *Phytophthora* spp., including *P. cinnamomi* on *Eucalyptus marginata* (Jackson *et al*., 2000), against *P. cactorum* on strawberry (Eikemo *et al*., 2003), *P. cinnamomi* on *Arabidopsis thaliana* (Eshraghi *et al*., 2011) and *P. capsici* on squash (Ji *et al*., 2011). Soil drench with phosphite was effective in reducing stem necrosis caused by *P. cinnamomi* on lupin, *P. nicotianae* on tabacco and *P. palmivora* on *Carica papaya* (Smillie *et al*., 1989). The effects of phosphite on plant development and susceptibility of tomato and pepper to Phytophthora root and crown rot has also been investigated in hydroponic conditions (Föster *et al*., 1998).

Among the chemical resistance inducers tested in the present study, effective control of *P. nicotianae* on tomato was achieved from three treatments of 12.5 mg $mL^{-1}$ of acibenzolar-S-methyl. However, Koné *et al.* (2009) indicated that several systemic acquired resistance inducers tested (including acibenzolar-S-methyl, DL-3-aminobutyric acid, and 2,6-dichlor-oisonicolinic acid) applied as leaf sprays or soil drenches at 25 or 50 mg $mL^{-1}$ reduced leaf necrosis caused by *P. capsici* on squash. Eikemo *et al.* (2003) reported some significant differences in reducing *P. cactorum* and *P. fragariae* on strawberry using acibenzolar-S-methyl at dosages from 10 to 1000 mg per plant. On pepper, Matheron and Porchas (2002) obtained the best pepper stem canker reduction, caused by *P. capsici*, using four treatments with acibenzolar-S-methyl at 75 mg $L^{-1}$ in comparison with one treatment with mefenoxam. Ji *et al.* (2011) suggested the integration of acibenzolar-S-methyl with standard fungicides (copper, mandipropamide and mefenoxam) improved *P. capsici* control on squash under field conditions compared with the results provided by acibenzolar-S-methyl applied alone. In our study, Phytophthora crown and root rot control provided by three treatments with acibenzolar-S-methyl was comparable to that obtained with single treatment of azoxystrobin or metalaxyl-M. Our study also showed that both of these fungicides, applied as pre-planting treatments, were effective for control of Phytophthora crown root rot of tomato, with efficacy continuing for 20–30 d after spray application to leaves.

Among the resistance inducing chemicals tested, the present work showed that phosphite-based fertilizers reduced Phytophthora crown root rot on tomato. Three treatments with the phosphite-based fertilizers Alexin and Glucoinductor complex reduced disease severity by between 66% and 88% compared with the inoculated and non-treated experimental controls. Pepper plants grown in a recirculating hydroponic system, fertilized with a commercial phosphite, and with a phosphite formulation at 1mM and 0.1 mM, have also been shown to be protected against *P. capsici* infections (Förster *et al*., 1998).

Among the biocontrol agents tested here, *B. velezensis*, *Glomus* spp. + *Trichoderma* gave partial disease reductions, but these were not significantly different from the inoculated and non-treated control. Beneficial effects of arbuscular fungi was reported against *Phytophthora parasitica* var. *nicotianae* on papaya (Sukhada *et al*., 2011) and against *Meloidogyne incognita* in tomato (Vos *et al*., 2012). The only partial effects obtained in our study with the application of biological control agents confirmed that in most cases, biocontrol agents can only play a role when applied within integrated control programmes. Previous studies have demonstrated the effectiveness of plant growth-promoting rhizobacteria applied with acibenzolar-S-methyl against *Xanthomonas campestris* pv. *vesicatoria* on tomato (Obradovic *et al*., 2005; Abo-Elyousr and El-Hendawy, 2008). Pre-planting application of acibenzolar-S-methyl on tomato seedlings has been shown to control *Pseudomonas syringae* pv. *syringae* (Gilardi *et al*., 2010) and fungal diseases of tomato, including grey mould (Malolepsza, 2006). The possibility of using acibenzolar-S-methyl against fungal and bacterial pathogens is a positive aspect to be considered for the practical application of disease management strategies. Resistance inducers such as the phosphite-based products can be applied legally, under current regulations in Italy, as fertilizers. Their application is of great interest in all cases when few chemicals are registered and/or in organic farming and in IPM programmes.

The lack of effectiveness of *B. carinata* pellets in all trials, and the occasional increase in disease severity, could be explained by the fact that such amendments can act as nutrients for the pathogen. A possible negative effect due to an increase in the initial *Pythium* species composition or changes in microbial community structure, as well as conflicting data on the efficacy of biofumigation in field trials, have been reported in several pathosystems (Manici *et al*., 2004; Cohen, *et al*., 2005; Motisi *et al*., 2010; Mazzola *et al*. 2012).

Tomato yields were not evaluated in the present study, while no toxicity effects were observed on the development of tomato plants cv. Cuore di Bue treated with the tested products. This study showed no effects of phosphite-based fertilizer treatments on tomato plant fresh weight compared with the non-inoculated and non-treated controls. This is in agreement with results reported by Föster *et al.* (1998), with tomato plants fertilized with phosphite at 0.1 or 1 mM, where leaf area and dry weights, of leaves, stems and roots were improved by using phosphate as the only phosphorus source. Silva *et al.* (2011) reported no differences in yield between soybeans treated with phosphite and non-treated crops under high downy mildew pressure. The present study also reports no negative effects on tomato development from three application of acibenzolar-S-methyl at two dose rates. Phytotoxicity symptoms have been observed, however, by Koné *et al.*, (2009) on *Cucurbita pepo* treated with acibenzolar-S-methyl at 50 mg $mL^{-1}$.

In our study, the efficacy of acibenzolar-S-methyl and mineral based-phosphite fertilizers as possible preventative products, as additional products or alternative to systemic fungicides, against *Phytophthora* crown root rot of tomato, has been demonstrated in trials carried out under high disease pressure in nursery conditions. Their application will also be particularly valuable for organic farming because of the lack of effective fumigants in that sector of horticulture. In addition, rotation of the materials with fungicides, especially those with a specific modes of action, and with resistance inducers, will reduce the selection pressure by the fungicides, thus reducing the risk of development of pathogen resistance towards the few registered fungicides for control of crown and root rot of tomato (Vallad and Goodman, 2004).

The availability of these compounds might foster the implementation of integrated disease management strategies for the control of *P. nicotianae* of tomato.

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