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## Research Papers

# Biocontrol agents and resistance inducers reduce *Phytophthora* crown rot (*Phytophthora capsici*) of sweet pepper in closed soilless culture

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**Summary.** Twelve trials, in closed soilless culture under controlled conditions, were carried out to evaluate the efficacy of resistance inducers (based on K-phosphite and K-silicate used alone or in combination), and of experimental biocontrol agents (*Trichoderma* sp. TW2, a mixture of *Pseudomonas* FC 7B, FC 8B, and FC 9B, *Fusarium solani* FUS25, *Pseudomonas* sp. PB26), and a commercial formulation of *Trichoderma gamsii* + *T. asperellum*, against diseases caused by *Phytophthora capsici* of sweet pepper. The products were applied using three different protocols, and effects on incidence of *Phytophthora* crown, stem and root rots (% dead plants), disease development (area under the disease progress curve; AUDPC), and plant fresh weights were evaluated. Potassium phosphite, applied directly at standard  $P_2O_5:K_2O$ , 1.30 + 1.05 g L<sup>-1</sup>) and at half standard rates, onto growing media, or via nutrient solution, and before infestation of peat plant growing medium with *P. capsici*, provided the best disease management in a dose-dependent manner, with an 80% reduction of *Phytophthora* crown, stem and root rots for the standard dosage and for both types of application. These treatments also reduced proportions of dead plants by 47% from the standard rate and by 62% at the half standard rate, when applied via the nutrient solution or directly to the substrate. K-silicate alone partially reduced the percentage of dead plants, with efficacy of 20–23%. No improvement in disease control was observed when K-silicate was applied in combination with phosphite, while K-silicate alone or combined with K-phosphite reduced disease development, compared to untreated controls. Biocontrol agents (BCAs), applied preventively, reduced *Phytophthora* crown, stem and root rots, with similar or better results than those from the commercial mixture of *Trichoderma asperellum* + *T. gamsii*. Among the tested BCAs, *Fusarium solani* FUS25 provided the most consistent disease reduction (60–65%) and gave increased plant fresh weights. All the tested BCAs reduced disease development, with a similar trend for different disease pressures. The least AUDPC values, compared to the non-treated controls, were from *Fusarium solani* FUS25, followed by the tested *Pseudomonas* strains and *Trichoderma* sp. TW2. These results indicate the potential for potassium phosphite and biocontrol agents in management of *Phytophthora* crown, stem and root rots of pepper grown in soilless systems.

**Keywords.** Hydroponics, *Phytophthora* control, *Capsicum annuum*, salts, microorganisms.

## INTRODUCTION

Sweet pepper (*Capsicum annuum*) is a popular vegetable crop, which is greatly appreciated for its quality and taste for fresh consumption and for processing, and is increasingly grown in greenhouse environments. The commercial greenhouse production of this vegetable is extensive in European countries, including Spain (12,420 ha), Italy (2,370 ha), Poland (1,830 ha) and The Netherlands (1,320 ha) (2017 data; FAOSTAT 2019).

However, sweet pepper is frequently affected by pathogens, with soil-borne pathogens being important and causing severe crop losses (Owen-Going *et al.*, 2003; Pernezny *et al.*, 2003; Messelink *et al.*, 2020). Among these *Phytophthora capsici*, which causes various diseases such as root, stem, and crown rots, resulted in economically important losses in most production areas (Matta and Garibaldi, 1981; Ristaino and Johnston, 1999; Hausbeck and Lamour, 2004; Granke *et al.*, 2012). This pathogen is highly variable (Ristaino and Johnston, 1999; Matta and Garibaldi, 1981; Lee *et al.*, 2001; Tamietti and Valentino, 2001; Foster and Hausbeck, 2010), and causes severe losses when grown either in soil or in soilless systems (Nielsen *et al.*, 2006b), because it is difficult to manage (Messelink *et al.*, 2020).

These diseases have been known in Italy since 1949 (Sibilia, 1952), and are still widespread, as a result of a lack of effective fumigants, no commercially acceptable resistant cultivars, and limited availability of resistant rootstocks which often only have partial resistance to the pathogen (Gilardi *et al.*, 2014). Although several sweet pepper cultivars show full or partial resistance to *P. capsici*, stem blight, root rot and foliar blight of these cultivars are probably reduced by separate genetic systems, so that cultivars resistant to crown rot may not be resistant to foliar blight (Foster and Hausbeck, 2010). This complicates the use of genetic disease resistance in sweet pepper, increasing the need to improve marketable yield potential and good fruit quality (Barchenger *et al.*, 2018; Acquadro *et al.*, 2020). Development of pathogen resistance to fungicides, including the commonly used mefenoxam and metalaxyl (Bower and Coffey, 1985; Lamour and Hausbeck 2001; Parra and Ristaino, 2001; Tamietti and Valentino, 2001), and alternatives to phenylamides, such as cyazofamid, dimethomorph, flumorph and oxathiapoprolin (Bower and Coffey 1985; Kousik *et al.*, 2008; Bi *et al.*, 2014; Miao *et al.*, 2016), has complicated management of these diseases.

Soilless cultivation is increasingly being adopted for many vegetable crops (Lee and Lee, 2015. Sambo *et al.*, 2019), as this is a good option when crop rotation is not feasible and resistant cultivars are not available, and

because of increasing limitations in the use of fumigants and fungicides (Garibaldi *et al.*, 2014). Host species, plant growth substrate type and the target pathogen all play important roles in management of diseases in soilless cultivation (Zheng *et al.*, 2000; van der Gaag and Wever, 2005; Khalil *et al.*, 2009; Lee and Lee, 2015), and specific evaluations are required for new crops. The effects of soilless cultivation on sweet pepper have not been fully exploited, particularly in terms of effects on the reduction of soil-borne diseases, such as *Phytophthora crown rot*.

Apart from their intrinsic capability of reducing problems caused by soil-borne pathogens, soilless cultivation systems offer good opportunities for exploiting new disease management strategies, including the use of resistance inducers and biocontrol agents, where increasing limitations apply to use of traditional fungicides.

Several biocontrol agents (Rankin and Paulitz, 1994; Paulitz, 1997; Chatterton *et al.*, 2004; Mercier and Manker, 2005; Calvo-Bado *et al.*, 2006; Liu *et al.*, 2007; Segarra *et al.*, 2010; Köhl *et al.*, 2011; Vallance *et al.*, 2011; Gilardi *et al.*, 2020), and other biocide products such as salts of phosphites and silicates, and nonionic surfactants (Stanghellini and Tomlinson, 1987; Cherif *et al.*, 1994; Stanghellini and Rasmussen, 1994; Stanghellini *et al.*, 1996; Stanghellini and Miller, 1997; Förster *et al.*, 1998; Nielsen *et al.*, 2006a; French-Monar *et al.*, 2010; Song *et al.*, 2016; Gilardi *et al.*, 2020) have been tested against a number of soil-borne pathogens, including *P. capsici*, in soilless systems. However, most of the applications of these disease management tools require fine-tuning for type and application timing, particularly in soilless systems, before they can be practically implemented (Vallance *et al.*, 2011).

The present study was carried out in an experimental closed soilless system, under controlled conditions, to evaluate the efficacy of host resistance inducers, based on K-phosphite and K-silicate, used alone or combined, and of experimental biocontrol agents, against *P. capsici* on sweet pepper. These treatments were compared with a commercial formulation of *Trichoderma gamsii* + *T. asperellum*. Different treatments and types and timing of application were tested, aiming to develop appropriate strategies for their application in practical soilless production systems.

## MATERIALS AND METHODS

### *Soilless growing system and experimental layout*

Twelve trials were carried out in a glasshouse at the AGROINNOVA Centre of Competence of the University

of Torino, in Grugliasco, Torino, Italy, at temperatures ranging from 22 to 30°C, using fully automated closed soilless system. This is a small-scale hydroponic system, with recirculating nutrient solution. Each hydroponic unit consisted of one channel (6 m long and 25 cm wide) connected to a storage tank (300 L capacity) filled with nutrient solution, which was automatically delivered to the plants using an electronic control unit (Idromat2, Calpeda S.p.a.). Nutrient solution (at 1.5–1.6 mS cm<sup>-1</sup>) was pumped by emitters (one per pot) at a flow rate of 6 L h<sup>-1</sup> from the storage tank, and fed to plants through drip emitters, and was left to drain back into the storage tank by gravity. The plants were fertigated with 5:3:8 N:P:K fertilizer (nutrient solution containing 120 mg L<sup>-1</sup> total N, 30 mg L<sup>-1</sup> P and 150 mg L<sup>-1</sup>; conductivity 1.5–1.6 mS cm<sup>-1</sup>). Nutrient solutions contained: 11.24 mM NO<sub>3</sub>, 4.8 mM NH<sub>4</sub>, 0.75 mM KH<sub>2</sub>PO<sub>4</sub>, 0.75 mM K<sub>2</sub>SO<sub>4</sub>, 0.012 mM Iron chelate EDTA, 2 mM MgO, 2 mM SO<sub>3</sub>, 0.2 mM B, 0.001 mM Mo, 0.15 mM Zn, 3.1 mM CaO, 0.05 mM Cu<sup>++</sup>, 0.25 mM Mn, and 12.2 mM K. The pH and conductivity of the nutrient solutions were checked regularly by using a pH meter and a SevenGo DUO TM SG23 conductivity meter (Tettler). The irrigation programme (three to six times per day) was revised according to the environmental conditions, particularly temperature. Each experimental unit consisted of six pots replicated five times (n = 30 pots). Two plants were planted in each pot, and six pots were each sub-replicate of 12 plants each. Five replicates were used per treatment (60 plants per treatment).

#### *Plant material, and tested products and protocols*

Fifteen-day-old plants of sweet pepper ‘Corno di Toro’ (Furia Sementi), which is susceptible to *Phytophthora* crown rot, were transplanted into 3 L capacity plastic pots filled with a growing medium containing: black peat soil (Brill Type 5: 15% of blond peat, 85% of black peat; pH 5.5–6.0, 1,100 g m<sup>-3</sup> of N:P:K and traces of molybdenum, Georgsdorf) in all the trials.

Each set of trials included one untreated and inoculated experimental control, and different treatments with salt products or biocontrol agents tested alone or in combinations, which were applied according to three different protocols (Table 1). These were:

**Protocols I and II.** The fertilizer-based phosphite (Alexine 95PS: 52% P<sub>2</sub>O<sub>5</sub> + 42% K<sub>2</sub>O, Massò), which is labelled as a phosphorus supplement for soilless application, and potassium silicate (K<sub>2</sub>SiO<sub>3</sub>, 33.7 to 34.7%, Andrea Gallo S.r.l.) were tested (Trials 1 to 6) in 2018 and 2019, to select the optimal rates and types of application for potassium phosphite and potassium silicate,

used alone or in combinations. K<sub>2</sub>SiO<sub>3</sub> at 200 mg L<sup>-1</sup> and K-phosphite were added directly to the nutrient solutions (NS) at the standard rate of 1.30 g L<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> + 1.05 g L<sup>-1</sup> K<sub>2</sub>O, or at half this rate (0.65 P<sub>2</sub>O<sub>5</sub> + 0.52 g L<sup>-1</sup> K<sub>2</sub>O + 0.525 g L<sup>-1</sup>) in experimental Protocol I. The same products, alone or in combinations, were applied to each pot around the crown of the seedlings using 100 mL per pot of the suspension prepared according to Protocol II. The treatments were carried out three times every 7 d (Table 1).

**Protocol III.** The following BCAs, isolated from suppressive composts, were tested: *Pseudomonas* sp. PB26 (Pugliese *et al.*, 2008), *Fusarium solani* FUS25 (Gullino and Pugliese, 2011), *Trichoderma* sp. TW2 (Cucu *et al.*, 2020), and a 1:1:1 mixture of three *Pseudomonas* spp. strains: *Pseudomonas* sp. FC 7B (EU836174), *Pseudomonas putida* FC 8B (EU836171), and *Pseudomonas* sp. FC 9B (EU836172). These strains were previously isolated from a suppressive rockwool substrate in a soilless system (Clematis *et al.*, 2009; Srinivasan *et al.*, 2009) (Table 1). The bacterium strains were maintained at 4°C in Luria Bertani (LB) slants throughout the study. The fresh bacterium suspensions were each prepared by inoculating a loop-full of cells into 30 mL of LB medium in 100 mL capacity Erlenmeyer flasks, and then incubating the suspension on a rotary shaker at 600 rpm. The cell suspension was centrifuged and resulting pellets were re-suspended in sterile deionized water. The optical densities (OD<sub>600</sub>) of cultures incubated for 48 h at 23°C were checked immediately before application, and was adjusted with sterile deionized water to 1 × 10<sup>8</sup> cells mL<sup>-1</sup> before application. *Trichoderma* sp. TW2 was grown in a 1000 mL capacity flask containing 200 mL of potato dextrose broth (Sigma-Aldrich) and maintained under static conditions at 23°C. After 13 to 15 d, the produced conidia and mycelium were transferred to 200 mL of sterile distilled water and homogenized using a hand-held rotary mixer. The conidium suspension obtained for the *Trichoderma* sp. TW2 isolate was standardized to 1 × 10<sup>7</sup> cfu mL<sup>-1</sup>.

The antagonist *Fusarium solani* FUS25 was propagated in 1000 mL capacity-flasks each containing 250 mL of potato dextrose broth maintained on a rotary shaker for 8 to 10 d at 160 rpm. The cultures were centrifuged at 8,000 g for 20 min at 4°C. Conidia and mycelium pellets were each transferred into 200 mL of sterile distilled water and homogenized using a rotary mixer. The inoculum suspensions were adjusted with sterile deionized water to 1 × 10<sup>7</sup> conidia mL<sup>-1</sup> before application.

Each BCA suspension was applied at a final concentration of 1 × 10<sup>7</sup> cfu mL<sup>-1</sup> to each pot and around the base of 15-d-old seedlings immediately after planting.

The BCAs were applied to the growing medium five times at 7 d intervals using 100 mL per pot of the suspension, following experimental Protocol III (Table 1). The experimental BCAs were compared with a commercial formulation of *Trichoderma asperellum* + *T. gamsii* (10% a.i.; 'Remedier', Isagro), applied at the label rate of 0.25 g per liter of peat substrate (Table 1).

#### Phytophthora capsici strain and inoculation

A highly virulent strain of *Phytophthora capsici* (coded PHC 6/16; AGROINNOVA culture collection), originally isolated from sweet pepper, was cultured on selective oomycete medium (Masago *et al.*, 1977) at 20°C for 1 week. Zoospores were produced according to a modified protocol of that described by Kim *et al.*, (1997). One mycelium/agar plug (5 mm in diam.), taken from an actively growing colony, was transferred to a 1000 mL capacity flask containing a wheat-hempseed medium (200 g wheat kernels, 100 g hempseeds, 320 mL water, sterilized at 121°C for 30 min), and then incubated at 22°C in a growth chamber under continuous light for 7 d, followed by 3 d at 15°C in darkness. Zoospore suspension was prepared by mixing 80 g of the oomycete biomass in 1 L of distilled water, mixing the suspension for

10 min, then removing the aqueous extract from solid sediment by filtering through two layers of cheesecloth and vigorously mixing it. Zoospores were released by chilling the liquid cultures at 4°C for 1 h, followed by 1 h at room temperature (25°C). The zoospore concentration was adjusted to  $1 \times 10^5$  zoospores mL<sup>-1</sup> using a haemocytometer. A 10 mL aliquot of the zoospore suspension was then pipetted onto the peat media around the base of each plant.

In trials 1 to 6, the first treatment with salts was carried out 48 h before infestation of the peat substrate, while in trials 7 to 12, the first treatment with BCAs was carried out 48–72 h before infestation of the peat substrate (Table 1).

#### Disease assessments, and statistical analyses

The sweet pepper plants were assessed starting from the first appearance of wilt caused by *P. capsici* root and crown infections. Disease incidence (DI) was evaluated at intervals of 3 to 7, d by counting and removing the dead plants with symptoms of Phytophthora root, crown and stem rot. At the final assessment, the remaining plants were removed by each pot and the total number of dead plants including those wilted, due to severe root

**Table 1.** Protocols adopted for different trials.

Experimental Protocol (Trial number, year)	Artificial inoculation	Tested treatment and concentration	Standard reference (dosage)	Application of the treatments	Number and interval between treatments
I (1, 2 and 3; 2018)	48 h after the first treatment, with $1 \times 10^5$ zoospores mL <sup>-1</sup>	Potassium phosphite (P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O 0.65+0.525 g L <sup>-1</sup> ) and K-silicate (200 mg L <sup>-1</sup> ), alone or combined	Potassium phosphite (P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O 1.30 + 1.05 g L <sup>-1</sup> )	In nutrient solution (NS)	Three applications, at 7 d intervals
II (4, 5 and 6; 2019)	48 h after the first treatment, with $1 \times 10^5$ zoospores mL <sup>-1</sup>	Potassium phosphite (P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O 0.65+0.525 g L <sup>-1</sup> ) and K-silicate (200 mg mL <sup>-1</sup> ), alone or combined	Potassium phosphite (P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O 1.30 + 1.05 g L <sup>-1</sup> )	To soil substrate	Three applications, at 7 d intervals
III (7, 8, 9, 10, 11 and 12; 2019-2020)	48-72 h after the first treatment, with $1 \times 10^5$ zoospores mL <sup>-1</sup>	<i>Fusarium solani</i> FUS25 ( $1 \times 10^7$ cfu mL <sup>-1</sup> ) <i>Pseudomonas</i> (FC 7B, FC 8B, FC 9B) ( $1 \times 10^7$ cfu mL <sup>-1</sup> ) <i>Pseudomonas</i> sp. PB26 ( $1 \times 10^7$ cfu mL <sup>-1</sup> ) <i>Trichoderma</i> TW2 ( $1 \times 10^7$ cfu mL <sup>-1</sup> )	<i>Trichoderma gamsii</i> + <i>T. asperellum</i> (0.25 g L <sup>-1</sup> )	To soil substrate	Five applications, at 7 d intervals

infection by the pathogen, were counted. Final disease incidence (DI) was then calculated, expressed as percent, of dead and wilted plants over the total plants examined. Areas under the disease-progress curves (AUDPCs) were calculated using the formula of Shaner and Finney (1977), for a total number of observations per trial of three to five disease incidence assessments. The biomass of healthy plants (fresh weight from 1–2 cm above soil surface) in each experimental unit (six pots), was measured at the end of trials 7 to 12 in Protocol III.

The experimental units each consisted of a 3 L capacity pot with two plants (six pots), and sub-replicates contained 12 plants/treatment. Trials carried out using each of the three protocols were repeated at least three times (Tables 1 and 1S), and they were combined when the 'trial' factor was not statistically significant ( $P > 0.05$ ).

Analysis of variance (ANOVA) with SPSS software (Version 26) was used to determine the effects of trial, treatments and their interactions, on disease incidence (DI) calculated at the end of each trial, AUDPC and fresh weight data. Prior to ANOVA, homogeneity of variances was evaluated and arcsin transformation of the percentage data was applied when necessary to normalize variances. When a statistically significant F test was obtained for treatments ( $P \leq 0.05$ ), the data were subjected to mean separation using Tukey's test at  $P \leq 0.05$ .

## RESULTS

### *Efficacy of resistance inducers (dosage, type of application) against Phytophthora crown rot*

First symptoms of *Phytophthora* root and crown rot and sometimes black lesion on stems started to be visible between 4 to 7 d after inoculation during the trials carried out using Protocols I and II. These symptoms then developed quickly (average air temperature ranging from 24 to 28°C), with the final assessments carried out 27 to 33 d after planting (Protocol I), or 17 to 28 d after planting (Protocol II).

Both K-phosphite (df = 2, F = 261.324;  $P \leq 0.0001$ ) and K-silicate, (df = 1, F = 5,220;  $P \leq 0.024$ ), and K-phosphite + K-silicate (df = 2, F = 13.685;  $P \leq 0.0001$ ) affected the percentage of dead plants (DI) and the development of *Phytophthora* crown rot (AUDPC) (K-phosphite: df = 2, F = 161,784;  $P \leq 0.0001$ . K-silicate: df = 1; F = 5,747;  $P \leq 0.018$ . K-phosphite + K-silicate: df = 2; F = 13.823;  $P \leq 0.0001$ ) (Table 2). Neither the type of application of K-phosphite (df = 2, F = 0.431;  $P = 0.263$ ) or K-silicate (df = 1; F = 0.501;  $P = 0.480$ ), nor the interaction of the K-phosphite + K-silicate treatment with the type of application (df = 2; F = 1.089;  $P = 0.339$ ), affect-

**Table 2.** Analyses of variance for disease incidence (DI) as percentage of dead and wilted plants and areas under disease progress curve (AUDPCs) for pepper 'Corno di toro' plants inoculated with *P. capsici* and treated with salts treatments (K-phosphite, K-silicate or K-phosphite + K-silicate), the type of application (in nutrient solution or to the soil substrate), and their interactions, calculated at the end of trials 1 to 6, according to F tests.

Considered factors and their interaction	df <sup>a</sup>	F values <sup>b</sup>	
		DI	AUDPC
K-phosphite treatment	2	261.324***	161.784***
K-silicate treatment	1	5.220*	5.747**
K-phosphite + K-silicate combined treatment	1	461.312***	90.003***
K-phosphite × K-silicate interaction	2	13.685***	13.823***
K-phosphite × type of application	1	0.431	0.679
K-silicate × type of application	1	0.501	0.021
K-phosphite × K-silicate × type of application	2	1.089	0.288

<sup>a</sup> Degrees of freedom between groups.

<sup>b</sup> Symbols \*, \*\*, \*\*\* indicate significance at, respectively,  $P \leq 0.05$ ,  $P \leq 0.001$  or  $P \leq 0.0001$ , according to F tests.

ed disease incidence. Similarly, these treatments did not affect crown rot development (K-phosphite: df = 2, F = 0.679;  $P = 0.359$ . K-silicate: df = 1, F = 0.021;  $P = 0.885$ . K-phosphite + K-silicate: df = 2, F = 0.288;  $P = 0.750$ ) (Table 2).

The DI and AUDPC data from each set of trials carried out according to Protocol I (Trials 1 to 3) or Protocol II (trials 4 to 6) were analyzed together for each experimental run (Tables 3 and 4), because statistically significant differences were not observed among trials ( $P > 0.05$ ). The average incidence of *Phytophthora* root and crown rots was 46% in the untreated controls in trials 1, 2 and 3, and was greater (DI = 93%) in trials 4, 5 and 6 (Table 3).

The most efficacious control of the pathogen was observed from K-phosphite at the standard dosage (80% efficacy) in all the experiments for both types of application (Table 3), while efficacy of the half rate treatments also reduced percentage of dead plants (compared to the untreated controls) by 47%, when applied via the nutrient solution, or by 62% when applied directly to the soil substrate.

K-silicate alone reduced the percentage of dead plants with 20–23% efficacy. No improvement in disease control was observed when K-silicate was applied in combination with K-phosphite for either of the tested doses. Slightly less disease control was observed for K-phosphite, at the standard dosage, combined with K-silicate, when applied via nutrient solution (68% efficacy) or to

**Table 3.** Mean disease incidences from K-silicate or K-phosphite salt treatments, alone or in combinations, when applied against *Phytophthora* crown rot caused by *Phytophthora capsici* on soilless grown sweet pepper 'Corno di Toro' plants. Data are average percentages of wilted and dead plants (disease incidence, DI) at the end of trials 1, 2 and 3, where Protocol I was used (see text), and trials 4, 5 and 6, where Protocol II was used.

Treatment (dosages: a.i.) <sup>a</sup>	Mean Disease Incidence (DI)					
	Treatments applied to the NS (Protocol I)			Treatments applied to the substrate (Protocol II)		
Untreated control	45.7 ± 3.2	d <sup>c</sup>	E % <sup>d</sup>	93.3 ± 2.0	d	E %
K-silicate (200 mg L <sup>-1</sup> )	35.0 ± 3.4	cd	23.4	73.3 ± 5.0	c	20.4
K-phosphite (P <sub>2</sub> O <sub>5</sub> :K <sub>2</sub> O 0.65, + 0.525 g L <sup>-1</sup> )	24.4 ± 3.4	bc	46.5	35.6 ± 3.2	b	61.1
K-phosphite (P <sub>2</sub> O <sub>5</sub> :K <sub>2</sub> O, 0.65 + 0.525 g L <sup>-1</sup> ) + K-silicate (200 mg L <sup>-1</sup> )	21.7 ± 2.5	b	52.6	34.4 ± 1.8	b	63.1
K-phosphite (P <sub>2</sub> O <sub>5</sub> :K <sub>2</sub> O, 1.30 + 1.05 g L <sup>-1</sup> )	8.9 ± 2.1	a	80.5	18.3 ± 2.3	a	80.4
K-phosphite (P <sub>2</sub> O <sub>5</sub> :K <sub>2</sub> O, 1.30 + 1.05 g L <sup>-1</sup> ) + K-silicate (200 mg L <sup>-1</sup> )	14.4 ± 1.3	ab	68.4	24.4 ± 2.6	ab	73.8
Trial <sup>b</sup>	df = 2; F = 2.888; P = 0.057			df = 2; F = 0.9555; P = 0.389		
K-phosphite	df = 1; F = 50.910; P < 0.0001			df = 1; F = 186.005; P < 0.0001		
K-silicate	df = 1; F = 7.704; P = 0.007			df = 1; F = 9.469; P = 0.003		
K-phosphite + K-silicate combined treatment	df = 1; F = 6.429; P = 0.017			df = 1; F = 9,870; P = 0.004		
K-phosphite × K-silicate interaction	df = 1; F = 8.432; P = 0.005			df = 1; F = 2.918; P = 0.05		
Treatments	df = 5; F = 24.040; P < 0.0001			df = 5; F = 97.006; P < 0.0001		

<sup>a</sup> K<sub>2</sub>SiO<sub>3</sub> at 200 mg L<sup>-1</sup> and K-phosphite (standard dosage of P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O, 1.30 + 1.05 g L<sup>-1</sup> or half dosage of P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O, 0.65 + 0.525 g L<sup>-1</sup>) or their combinations, were added directly to the nutrient solution NS (Protocol I), or to the soil substrate (Protocol II) in each pot using 100 mL/pot of suspension. The treatments were carried out three times, at 7 d intervals.

<sup>b</sup> Values from trials 1, 2 and 3 or trials 4, 5 or 6, each with five replicates per treatment, were combined when statistically significant differences were not observed (Trial P > 0.05). Significant according to the F tests and degrees of freedom (df) used in its calculation. Each mean is associated with its standard error (± SE).

<sup>c</sup> Means in the same column, followed by the same letter, do not differ according to Tukey's Test (P ≤ 0.05). Each mean is associated with its standard error (± SE).

<sup>d</sup> E%: percentage reduction of disease incidence of wilted and dead plants, compared to the untreated controls, at the end of trials 1, 2 or 3, corresponding to, respectively, 30, 25 or 24 d after inoculation (Protocol I), and at the end of trials 4, 5 or 6, corresponding to, respectively, 35, 21 or 17 d after inoculation (Protocol II).

the substrate (74% efficacy) (Table 3). Similar effects were observed for K-phosphite, at a reduced dosage, combined with K-silicate, when applied via nutrient solution (53% efficacy) or to the substrate (63% efficacy) (Table 3).

For AUDPC values, K-silicate reduced disease development, compared to the untreated control, for both types of application. K-phosphite, at both tested dosages, applied alone or combined with K-silicate, affected AUDPC compared to the untreated control, and differences among these treatments (Table 4).

K-phosphite and K-silicate either alone or combined, applied via nutrient solution or when distributed to the growing medium, did not reduce the development of sweet pepper plants (data not shown).

#### Effects of biocontrol agents

First symptoms of *Phytophthora* root and crown rots started to be visible at 5 to 13 d after inoculations with *P. capsici* carried out 48–72 h after planting during the tri-

als carried out using Protocol III. These symptoms rapidly under the experimental conditions used (average air temperature 24 to 28°C), with the final assessment carried out between 34 to 37 d after planting trials 7–9, and 23 to 26 d in trials 10–12 carried out with Protocol III.

The data from trials 7, 8 and 9 (DI, df = 2, F = 0.369, P = 0.545; AUDPC, df = 2, F = 0.073, P = 0.930), and from trials 10, 11 and 12 (DI, df = 2, F = 2.252, P = 0.111; AUDPC, df = 2, F = 0.781, P = 0.461), were combined when there was heterogeneity between the trial runs (Tables 5 and 6). Inoculation with the pathogen led to high disease incidence in the untreated controls (78% in trials 7, 8 and 9 and 63% in trials 10, 11 and 12), permitting evaluation of the different BCAs under study (Table 5). All the BCAs reduced DI in the trials 7, 8 and 9 (df = 5, F = 18.021, P < 0.0001) and in trials 10, 11 and 12 (df = 5, F = 15.538, P < 0.0001), compared to the untreated controls. The experimental biocontrol agents generally gave more disease control than the commercial formulation of *Trichoderma asperellum* + *T. gamsii*

**Table 4.** Mean areas under disease progress (AUDPCs) after treatments with K-silicate and K-phosphite salts, alone or in combinations, applied against *Phytophthora* crown rot caused by *Phytophthora capsici* to soilless grown plants of sweet pepper 'Corno di Toro'. These data were obtained at the end of trials 1, 2 and 3, using Protocol I, and trials 4, 5 and 6 using Protocol II (see text).

Treatment (Dosage a.i.) <sup>a</sup>	AUDPC <sup>b</sup>			
	Treatment applied to the NS (Protocol I)		Treatment applied to the substrate (Protocol II)	
Untreated control	846.4 ± 76.01	c <sup>d</sup>	1,376.9 ± 60.8	c
K-silicate (200 mg L <sup>-1</sup> )	533.6 ± 47.7	b	1,045.0 ± 105.9	b
K-phosphite (P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O 0.65 + 0.525 g L <sup>-1</sup> )	413.3 ± 59.7	ab	396.9 ± 46.2	a
K-phosphite (P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O 0.65 + 0.525 g L <sup>-1</sup> ) + K-silicate (200 mg L <sup>-1</sup> )	452.8 ± 54.3	ab	404.4 ± 33.4	a
K-phosphite (P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O 1.30 + 1.05 g L <sup>-1</sup> )	236.9 ± 45.2	a	219.2 ± 35.7	a
K-phosphite (P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O 1.30 + 1.05 g L <sup>-1</sup> ) + K-silicate (200 mg L <sup>-1</sup> )	259.4 ± 31.9	a	321.4 ± 43.2	a
Trial <sup>d</sup>	df = 2; F = 2.442; P = 0.093		df = 2; F = 0.388 P = 0.244	
K-phosphite	df = 1; F = 28.861; P < 0.0001		df = 1; F = 26.036; P < 0.0001	
K-silicate	df = 1; F = 9.202; P = 0.003		df = 1; F = 4.951; P = 0.029	
K-phosphite + K-silicate combined treatment	df = 1; F = 9.423; P = 0.005		df = 1; F = 5.311; P = 0.014	
K-phosphite × K-silicate interaction	df = 1; F = 9.485; P = 0.003		df = 1; F = 12.976; P = 0.001	
Treatment	df = 5; F = 16.800; P < 0.0001		df = 5; F = 61.923; P < 0.0001	

<sup>a</sup> K<sub>2</sub>SiO<sub>3</sub> at 200 mg L<sup>-1</sup> and K-phosphite (standard dosage of P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O 1.30 + 1.05 g L<sup>-1</sup> or half dosage of P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O 0.65 + 0.525 g L<sup>-1</sup>) or their combinations, added directly to the nutrient solution NS (Protocol I), or to the substrate in each pot using 100 mL per pot of the suspension prepared (Protocol II). The treatments were carried out three times every 7 days.

<sup>b</sup> AUDPC values were calculated for three to four assessments at 6-7 d intervals during trials 1, 2 and 3 (Protocol I), and four or five assessments at 3-6 d intervals during trials 4, 5 and 6 (Protocol II).

<sup>c</sup> Values from trials 1, 2 and 3 or trials 4, 5 and 6, each with five replicates per treatment, are combined when statistically significant differences (trial  $P > 0.05$ ; F tests) were not observed.

<sup>d</sup> Means in each column followed by the same letter are not different ( $P \leq 0.05$ ; Tukey's Test). Each mean is associated with its standard error ( $\pm$  SE).

**Table 5.** Mean *Phytophthora* crown rot incidences after applications of experimental BCA treatments, applied according to Protocol III, on for soilless grown sweet pepper 'Corno di Toro'. The data are average disease incidence (DI) at the end of trials 7, 8 and 9 and 10, 11 and 12.

Treatment <sup>a</sup>	Disease incidence (DI)					
	Trials 7, 8, and 9			Trials 10, 11 and 12		
Untreated control	77.8 ± 3.2	d <sup>c</sup>	% E <sup>d</sup>	62.5 ± 4.2	c	% E
<i>Fusarium solani</i> FUS 25	30.6 ± 4.0	a	60.7	22.2 ± 3.3	a	64.4
<i>Pseudomonas</i> sp. FC 7, FC 8, FC 9	42.8 ± 4.1	ab	45.0	30.4 ± 3.0	ab	51.4
<i>Pseudomonas</i> sp. PB 26	40.6 ± 3.5	ab	47.9	34.4 ± 3.2	ab	44.9
<i>Trichoderma</i> sp. TW 2	46.7 ± 4.7	b	40.0	36.9 ± 3.0	b	40.9
<i>Trichoderma asperellum</i> + <i>T. gamsii</i>	61.1 ± 4.2	c	21.4	36.4 ± 3.8	b	41.8
Trial <sup>b</sup>	df = 2; F = 0.369; P = 0.545			df = 2; F = 0.781; P = 0.461		
Treatment	df = 5; F = 18.021; P < 0.0001			df = 5; F = 15.538; P < 0.0001		

<sup>a</sup> The BCA suspensions were applied (at  $1 \times 10^7$  cfu mL<sup>-1</sup>) to each pot and around the base of 15 d-old seedlings after planting, six times at 5 d intervals using 100 mL per pot of the suspension. The experimental BCAs were compared with a commercial formulation of *Trichoderma asperellum* + *T. gamsii* (10% a.i., 'Remedier', Isagro), applied at the label rate of 0.25 g L<sup>-1</sup> of peat substrate.

<sup>b</sup> Values from trials 7, 8 and 9 or trials 10, 11 and 12, each with five replicates per treatment, are combined when statistically significant differences ( $P > 0.05$ ; F tests) were not observed.

<sup>c</sup> Means in each column followed by the same letter are not different ( $P \leq 0.05$ ; Tukey's Test). Each mean is associated with its standard error ( $\pm$  SE).

<sup>d</sup> E%: percent disease incidence reduction, calculated as dead and wilted plants, compared to the untreated controls, at the end of the trials corresponding to 31, 31 or 34 d after inoculation for, respectively, trials 7, 8 and 9, and 23, 21 or 22 days after inoculation for, respectively, trials 10, 11 and 12.

**Table 6.** Mean areas under disease progress curves after applications of different experimental BCA treatments, applied according to Protocol III, for *Phytophthora crown rot* caused by *P. capsici* on soilless grown sweet peppers ‘Corno di Toro’. The data are expressed as average area under disease progress (AUDPC) at the end of trials 7–9 and 10–12.

Treatment <sup>a</sup>	AUDPC <sup>b</sup>			
	Trials 7, 8 and 9		Trials 10, 11 and 12	
Untreated control	1,814.4 ± 75.7	d <sup>d</sup>	1,457.2 ± 62.9	c
<i>Fusarium solani</i> FUS 25	584.7 ± 72.2	a	442.6 ± 57.8	a
<i>Pseudomonas</i> sp. FC 7, FC 8, FC 9	852.8 ± 103.7	a-c	574.6 ± 55.5	ab
<i>Pseudomonas</i> sp. PB 26	806.1 ± 76.3	ab	673.3 ± 72.1	ab
<i>Trichoderma</i> sp. TW 2	947.2 ± 80.7	bc	727.5 ± 57.5	b
<i>Trichoderma asperellum</i> + <i>T. gamsii</i>	1,170.0 ± 77.2	c	660.3 ± 61.1	ab
Trial <sup>c</sup>	df = 2; F = 0.073; P = 0.930		df = 2; F = 2.252; P = 0.111	
Treatment	df = 5; F = 27.655; P < 0.0001		df = 5; F = 33.733; P < 0.0001	

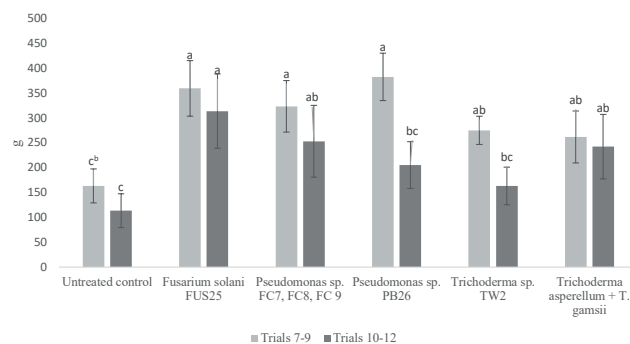
<sup>a</sup> The BCA suspensions ( $1 \times 10^7$  cfu mL<sup>-1</sup>) were applied five times after planting at 7 d intervals to individual pots around the base of 15 d-old seedlings, using 100 ml of suspension per pot. The experimental BCAs were compared with a commercial formulation of *Trichoderma asperellum* + *T. gamsii* (10% a.i.; ‘Remedier’, Isagro), applied at the label rate of 0.25 g L<sup>-1</sup> of peat substrate.

<sup>b</sup> AUDPC values were calculated considering four to five assessments at 4 to 9 d intervals during trials 7, 8 and 9, and four assessments at 4 to 8 d intervals during trials 10, 11 and 12.

<sup>c</sup> Values from trials 7, 8 and 9 or trials 10, 11 and 12, each with five replicates per treatment, are combined when statistically significant differences ( $P > 0.05$ ; F tests with and degrees of freedom (df) indicated).

<sup>d</sup> Means each column accompanied by the same letter are not different ( $P \leq 0.05$ ; Tukey’s Test). Each mean is accompanied by its standard error ( $\pm$  SE).

(22 to 42% efficacy). The greatest and most consistent *P. capsici* control was from *Fusarium solani* FUS25, which reduced proportions of dead plants by 61 to 65%. Both of the tested experimental *Pseudomonas* treatments (PB 26 and the mixture of FC 7B, FC 8B and FC 9B) showed



**Figure 1.** Mean fresh weight biomass (g)<sup>a</sup> of healthy sweet peppers ‘Corno di Toro’ plants treated according to Protocol III at the end of trials 7, 8 and 9 (31 to 34 d after inoculation), and trials 10, 11 and 12 (21 to 23 d after inoculation). <sup>a</sup> Mean fresh weight of plants per treatment from trials 7, 8 and 9 (df = 5; F = 30.446;  $P < 0.0001$ ) or trials 10, 11 and 12 (df = 5; F = 34.384;  $P < 0.0001$ ), each with five replicates per treatment, are combined because significant differences were not observed for trials 7, 8 and 9 (df = 2; F = 2.684  $P = 0.055$ ) or trials 10, 11 and 12 (df = 2; F = 1.701  $P = 0.190$ ), as indicated by F tests. <sup>b</sup>Means accompanied by the same letter do not differ ( $P \leq 0.05$ ; Tukey’s Test). Each mean is accompanied by associated with its standard error ( $\pm$  SE).

similar disease control trends, with 45 to 51% disease reduction, compared to the untreated controls. *Trichoderma* sp. TW2, applied to the growing media every 7 d, provided a consistent disease reduction of 40–41%.

Disease reductions were observed for all the tested BCAs, and a similar trend was observed for the different disease amounts in trials 7, 8 and 9 (df = 5, F = 27.655,  $P < 0.0001$ ) and trials 10, 11 and 12 (df = 5, F = 33.733,  $P < 0.0001$ ). The least AUDPC values, compared to the non-treated controls, were from *Fusarium solani* FUS25, followed by the tested *Pseudomonas* strains and *Trichoderma* TW2 (Table 6).

Applications of the BCAs to the growing media generally increased fresh plant biomass at the end of both sets of trials, compared to the untreated controls, although inconsistent results were observed for *Pseudomonas* PB26 and *Trichoderma* TW2 (Figure 1).

## DISCUSSION

*Phytophthora capsici* is a broad host range pathogen (Granke *et al.*, 2012), important in most sweet pepper growing areas. There are few chemical options for management of the diseases caused by this pathogen, due to the lack of effective soil fumigants, its resistance to some effective fungicides (Bower and Coffey, 1985; Hwang *et al.*, 1996; Lamour and Hausbeck, 2001; Parra and



Ristaino, 2001; Tamietti and Valentino, 2001; Kousik *et al.*, 2008; Miao *et al.*, 2016; Barchenger *et al.*, 2018; Hunter *et al.*, 2018), and the complexity for host breeding to select cultivars resistant to local pathogen isolates (Acquadro *et al.*, 2020).

Soilless crop cultivation is expanding throughout the world, but by itself this approach cannot solve the problems of managing soil-borne pathogens (Jenkins and Averre, 1983), since many of them, including *P. capsici*, may be introduced through infected planting material (Granke *et al.*, 2012) or irrigation water (Ristaino *et al.*, 1993; Hong and Moorman, 2005; Gevens *et al.*, 2007). Growth and spread of zoospore pathogens are particularly severe problems in soilless systems (Stanghellini and Rasmussen, 1994). Management strategies that minimize inoculum dispersal have, therefore, considerable potential for disease reduction. Increased understanding of the impacts of crop management measures in soilless systems is needed for the sweet pepper-*P. capsici* pathosystem.

The present study examined the efficacy of resistance inducers, based on K-phosphite and K-silicate, used alone or in combinations, and of experimental biocontrol agents under conditions of high disease pressure, in a closed soilless system. K-silicate only partially reduced the incidence of *P. capsici* (20–23% efficacy) and its development, also when combined with K-phosphite, at the tested standard or reduced dosages. However, phosphite combinations could possibly reduce the risks of selection of *Phytophthora* isolates resistant to phosphite (Hunter *et al.*, 2018), and address control of more than one pathogen in integrated pest management. Addition of 75 or 100 mg L<sup>-1</sup> of silicon to the hydroponic nutrient solution reduced the severity of anthracnose caused by *Colletotrichum capsici* or *C. gloeosporioides* in *Capsicum annum* (Jayawardana *et al.*, 2015).

The mode of action of silicates and their functioning in several pathosystems are not yet fully understood. French-Monar *et al.*, (2010) reported that bell pepper plants treated with calcium silicate had reduced root lesions and crown and stem necrosis caused by *P. capsici*, although at lower levels of reduction than found in other hosts. Together with possible formation of physical barriers to pathogens, from silicon accumulation in plants with differences depending on species, this element can influence plant defense responses and interact with key components of plant stress signalling systems, thus leading to induced resistance to pathogens (Liang *et al.*, 2006; Wang *et al.*, 2017).

Of the products tested in the present study, potassium phosphite provided the best control of *P. capsici*, when applied to pepper plants by adding soluble forms

to nutrient solution of the closed hydroponic system, and when added to the growing medium. No effects on pepper development were detected after addition of this compound. However, the possible phytotoxic effects of phosphite, applied in soilless systems via a nutrient solution, should be considered. These compounds may precipitate and accumulate, as has been described for several fertilizers (Sambo *et al.*, 2019). For example, tomato and pepper plants treated with either commercial or technical formulations of phosphite in a soilless system exhibited reduced growth (Forster *et al.*, 1998).

The results obtained in the present study are similar to those of Förster *et al.* (1998), who observed reduced symptoms caused by *P. capsici* on tomato and pepper plants grown in a greenhouse hydroponic system treated with phosphite. Several factors are associated with the inhibition of pathogen growth as a result of phosphite treatments. These include phosphite concentration, the nature of the salts, the acidification of the plant growth medium and the pathogen life cycle (Guest and Bompeix, 1990; Smillie *et al.*, 1989; Khalil *et al.*, 2009; Khalil and Alsanius, 2011; Sambo *et al.*, 2019). The results obtained in the present study show that the concentration of applied K-phosphite affected control of *Phytophthora* root and crown rot of sweet pepper, with greatest control provided by the standard dosages here tested.

Phosphite may act directly on pathogens, by inhibiting their growth (Fenn and Coffey, 1984, Smillie *et al.*, 1989; Grant *et al.*, 1990; Smillie *et al.*, 1989), and possibly by priming the host defense in several pathosystems during pre-infection or post-inoculation stages of the pathogens. Liu *et al.* (2016) reported that phosphite, at >5 µg mL<sup>-1</sup>, had a direct effect on mycelium growth and zoospore production in the sweet pepper-*P. capsici* pathosystem. Moreover, this compound increased transcription of antioxidant enzyme genes, and those involved in ethylene and abscisic acid biosynthesis, which mediated control of the pathogen at a higher phosphite concentration (1 g L<sup>-1</sup>) (Liu *et al.*, 2016).

Phosphites are receiving a growing interest in horticulture (Gomez-Merino and Trejo-Téllez, 2015). Their application to soilless cultivation should be tested on different host-pathogen combinations, because some host plants are more responsive to phosphite than others (Guest and Bompeix, 1990; Shearer and Crane, 2012). Potassium phosphite reduced the *Phytophthora* crown rot of soilless grown zucchini grown by 62–94%, when applied directly to growing media, or via a nutrient solution, when the pathogen was inoculated 5–7 d before planting (Gilardi *et al.*, 2020). Phosphites are particularly active against oomycetes and have long been used for management of *Phytophthora* diseases in several

crops (Fenn and Coffey 1984; Smillie *et al.*, 1989; Guest and Bompeix, 1990; Förster *et al.*, 1998, Dobrowolski *et al.*, 2008), and in specific situations, based on greenhouse and field studies of soil-borne pathogens in several pathosystems. These include tomato/*Phytophthora nicotianae* (Gilardi *et al.*, 2014); zucchini/*P. capsici* (Gilardi *et al.*, 2015); lettuce/*Fusarium oxysporum* f. sp. *lactucae*; rocket/*Fusarium oxysporum* f. sp. *raphani* (Gilardi *et al.*, 2016); potato/*Rhizoctonia solani* (Lobato *et al.*, 2010), and common bean/*Sclerotinia sclerotiorum* (Fagundes-Nacarrath *et al.*, 2008).

Biocontrol agents may be worthwhile for disease management in soilless production systems (Paulitz, 1997; Postma, 2009; Vallance *et al.*, 2011; Lee and Lee, 2015). In the past 20 years, several studies have demonstrated reduced disease and practical implementation (Lamichhane *et al.*, 2017; Villeneuve, 2017; Barratt *et al.*, 2018; Raymaekers *et al.*, 2020). In the present study, the *Pseudomonas putida* isolate mixture (FC 7B, FC 8B, FC 9B), *Pseudomonas* sp. PB26, *F. solani* FUS25 and *Trichoderma* sp. TW2, introduced into the soilless system 48-72 h before the pathogen inoculation, followed by four applications at 7 d intervals, reduced *P. capsici* on pepper by 45 to 64%. This result indicates further research is warranted. The most consistent results in control of Phytophthora crown rot were provided by *F. solani* FUS25 (60-64% efficacy). The same biocontrol strain sometimes provided good, but variable results (8-54% efficacy) on zucchini, when applied to growing medium immediately at inoculation with *P. capsici* and 5-6 d before planting (Gilardi *et al.*, 2020). Although the mechanism of action of *F. solani* FUS25 is not known, the most likely strategy for its use should be as a protectant or in preventative treatments. Establishment of BCAs in host root systems can also vary according to the host. The root systems of sweet pepper have greater surface areas than root systems of zucchini, which instead develops roots with few branches.

The good results obtained in the present study add evidence for applying BCAs in soilless cultivation systems against diseases caused by oomycetes. *Pseudomonas* sp. is known to be effective in reducing cucumber root colonization by *Pythium aphanidermatum* (Moulin *et al.*, 1994; Chatterton *et al.*, 2004), and Pythium disease on cucumber grown in a closed rockwool system (Postma *et al.*, 2000). *Streptomyces griseoviride* ('Mycostop') is effective against *Pythium ultimum* on cucumber (Wolfhechel and Funck Jensen, 1991), while *Trichoderma virens* ('Soilgard') and *Gliocladium catenulatum* ('Prestop') are active against *Pythium aphanidermatum*, the causes of the damping-off of cucumbers grown in rockwool (Punja and Yip, 2003).

Increased understanding of BCA modes of action is needed to achieve a widespread application of these agents. Disease suppression could be related to different mechanisms: including production of antibiotics, secondary metabolites, lytic enzymes, phytohormones, siderophores, volatiles, and induction of host resistance (Köhl *et al.*, 2019). For example, pepper plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* developed local and systemic resistance against *P. capsici* (Silvar *et al.*, 2009), and the foliar pathogen *Botrytis cinerea* (Díaz *et al.*, 2005). Endophytic *Trichoderma* isolates induced resistance in hot pepper to *P. capsici* (Bae *et al.*, 2011). *Pseudomonas* induced motility inhibition of *P. capsici* zoospores (Zohara *et al.*, 2016), which are the only mobile propagules found in recirculating nutrient solutions (Stanghellini *et al.*, 1996). Iron competition was important in the antagonistic activity of *Trichoderma asperellum* against *F. oxysporum* f. sp. *lycopersici* of tomato grown in a soilless medium based on perlite (Segarra *et al.*, 2010).

Little is known about the capability of biocontrol agents to suppress Phytophthora blight on peppers grown in soilless systems.

Selected strains of the *Pseudomonas*, *Bacillus*, and *Trichoderma* have long been known for ability to improve plant growth and induce host systemic resistance against diseases and pests in different ecosystems, including soilless systems (Paulitz, 1997; Domenech *et al.*, 2006; Gravel *et al.*, 2007; Berg, 2009; Lee and Lee, 2015.; Sambo *et al.*, 2019). Several biocontrol agents introduced into hydroponically grown fruit and vegetables have provided positive effects on yields and quality of horticultural products. In the present study, significant reduction in Phytophthora crown rot observed after treatment with *Fusarium solani* FUS25 was confirmed, by increased biomass fresh weight, with similar or greater increases than those from the commercial mixture of *Trichoderma asperellum* + *T. gamsii*.

In general, if a biocontrol agent is given time to become well-established in plant growth media, before pathogens are introduced (often through planting material), it can prevent infections or can interfere with pathogen inoculum production that may spread throughout the systems (Fry, 1982). *Pseudomonas chlororaphis*, *Bacillus cereus*, and *B. gladioli* strains, applied in small-scale hydroponic units, suppressed root colonization of chrysanthemum by *Pythium aphanidermatum* when applied 14-7 d before pathogen inoculation, rather than at the same time as inoculation (Liu *et al.*, 2007). Selected antagonistic *Pseudomonas*, *Fusarium* or *Trichoderma* strains, previously tested in pot trials in peat medium against Fusarium wilt agents of lettuce and wild rocket

(Gilardi *et al.*, 2019; Srinivasan *et al.*, 2009), or in naturally infested soil in a zucchini-*P. capsici* pathosystem (Cucu *et al.*, 2020), were here used in preventative treatments in a closed soilless system, before inoculation of peat substrate with *P. capsici*. Optimizing the conditions in the soilless environment to which biocontrol agents were introduced, resulted in improved disease management consistency.

The results obtained in this study provide evidence for using phosphite-based products and biocontrol agents against *Phytophthora* crown and root rot of pepper, grown in soilless systems. They also show that there is not one solution for the management of these diseases. Different options should be considered and adapted to the different situations, relying on good extension services. Soilless cultivation provides good opportunities for exploitation and practical application of new disease management tools, such as resistance inducers and biocontrol agents. These have been intensively studied in recent years, expanding the integrated disease management options for intensive vegetable production (Paulitz, 1997; Lamichhane *et al.*, 2017; Messelink *et al.*, 2020).

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