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Biological control of Fusarium wilt of tomato with *Fusarium equiseti* GF191 in both rock wool and soil systems

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Abstract The plant growth-promoting fungus (PGPF) *Fusarium equiseti* GF191 was tested for its ability to control Fusarium wilt of tomato (FWT) caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in both a hydroponic rock wool and soil system. *F. equiseti* effectively controlled FWT, with protective effects based on disease severity of 66.7–88.6% in four experiments. The numbers of colony-forming units of FOL per gram fresh weight of stems were significantly reduced ($P < 0.05$) in plants treated with *F. equiseti*. Stem extracts from *F. equiseti*-treated and pathogen-challenged plants significantly inhibited the germination and germ-tube length of FOL microconidia and the production of FOL budding-cells. Tomatine content in tomato stems treated with *F. equiseti* was significantly increased compared with the non-treated control.

Keywords Biological control · *Fusarium oxysporum* f. sp. *lycopersici* · *Lycopersicon esculentum* · PGPF *Fusarium equiseti*

Abbreviations

FOL	*Fusarium oxysporum* f. sp. *lycopersici*
FWT	Fusarium wilt of tomato
PGPF	Plant growth-promoting fungus

Introduction

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), a soil-inhabiting fungus that invades plant roots and vascular tissue, is one of the most economically important and destructive diseases of tomato. It causes severe damage to both field- and greenhouse-grown tomato plants, resulting in stunted seedlings and drooping and yellowing leaves. Infected plants frequently wilt and die (Jones et al. 1991). Although the use of Fusarium-resistant tomato cultivars can provide some degree of protection against this disease, the emergence of new races of the pathogen that overcome resistance to the cultivar is a continuing problem. The most effective method of controlling Fusarium wilt of tomato (FWT) to date

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has been soil disinfection using methyl bromide. However, the use of methyl bromide was outlawed in 2005 since it caused severe environmental problems. In recent years, the production of greenhouse tomatoes has begun to shift from soil cultivation to various hydroponic culture systems, including the rock wool system (Mihuta-Grimm et al. 1990). However, there is no fungicide registered for use in controlling FWT in such hydroponic systems. Thus, alternative control methods need to be made available as soon as possible.

Biological control methods have been reported in the management of FWT, with several antagonists already known to reduce the incidence of FWT, such as *Penicillium oxalicum* (De Cal et al. 1999), non-pathogenic *Fusarium* (Lemanceau and Alabouvette 1993; Larkin and Fravel 1998, 2002) and *Pseudomonas fluorescens* (Lemanceau and Alabouvette 1993). However, their biological control efficacy was found to differ depending on the culture conditions of tomato plants.

The saprophytic plant growth-promoting fungus (PGPF) *Fusarium equiseti* isolated from turfgrass rhizospheres has been found to promote significant plant growth and suppress several soil-borne diseases, such as *Pythium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Gaeumannomyces graminis* var. *tritici* and *Cochliobolus sativus* (Hyakumachi 1994; Hyakumachi and Kubota 2004). The underlying mechanisms of the disease suppression engendered by PGPF have also been intensively studied, and induced systemic resistance is thought to be involved in one of those mechanisms (Koike et al. 2001; Meera et al. 1995; Shivanna et al. 1996). We have previously shown that *F. equiseti* is able to control Fusarium crown and root rot of tomato in both a hydroponic rock wool system and a soil system (Horinouchi et al. 2007, 2008) and may also prevent Fusarium wilt of spinach in transplanting systems using paper pots (Horinouchi et al. 2010). Based on these results, we suspect that *F. equiseti* may effectively control Fusarium diseases under various culture conditions.

The involvement of tomatine, an antifungal compound, in disease resistance has been studied with plant pathogenic fungi, especially *F. oxysporum* f. sp. *lycopersici* (Kimmerchlag and Mace 1975; Lairini et al. 1997; Langcake et al. 1972; MacCane and Drysdale 1975). MacCane and Drysdale (1975) reported that tomatine inhibited FOL spore germination at an ED_{50} (effective dose in 50% of plants) of about 50 μg ml^{-1} and completely inhibited it at 100 μg ml^{-1}. In our previous studies, pre-inoculation of tomato plants treated with *F. equiseti* reduced the *F. oxysporum* f. sp. *radics–lycopersic* population and inhibited the germination and proliferation of the pathogen (Horinouchi et al. 2007, 2008). From these results, we hypothesized that *F. equiseti* may induce the production of antifungal compound in tomato plants.

The objective of this study was to investigate the effect of *F. equiseti* in controlling FWT in both rock wool hydroponic and soil systems. The effects of stem extracts from tomatoes treated with *F. equiseti* on the germination and proliferation of the pathogen and on the tomatine content in the stems were also determined.

Materials and methods

Organisms

The PGPF isolate of *F. equiseti* GF191 was used as a putative biocontrol agent. The FOL isolate Ku11 (race 2), obtained from a tomato plant with FWT, was used as a pathogen.

Plant material

The tomato cv. House Momotaro (Takii Seed Co, Kyoto, Japan), which is a popular cultivar used mainly in greenhouses in Japan but which is susceptible to FWT, was used in all experiments. All seeds were surface-disinfected with 1% sodium hypochlorite solution for 5 min, followed by rinsing three times in sterile distilled water prior to sowing.

Inoculum preparation

The PGPF isolate GF191 and FOL isolate Ku11 were separately cultured on potato dextrose agar in 9-cm petri dishes for five days in the dark at 25°C. Five mycelial disks (diameter 5 mm) taken from the edges of the cultures were transferred into 100 ml of potato dextrose broth in 300-ml flasks and incubated for seven days at 25°C in a rotary shaker (model NR-150; Taitec Co, Koshigaya, Japan) at 120 rpm.

To obtain budding-cells from both isolates, the fungal cultures were filtered through three layers of sterile gauze. The fungal suspensions of GF191 and Ku11 were then diluted with sterile distilled water to 10^7 budding-cells and 10^5 budding-cells ml^{-1}, respectively, and each suspension was used as an inoculum source.

Assay for control of FWT in a hydroponic rock wool system

Two sizes of cube and rockwool substrate were used in the experiments: mini-cubes (3.6 × 3.6 × 4 cm) and large cubes (7.5 × 7.5 × 6.5 cm) (Grodan; Grodania A/S, Hedehusene, Denmark). Tomato seeds were individually sown in rock wool mini-cubes, and a 20-ml suspension of GF191 was then poured over each cube. The seedlings were grown for about 20 days in a greenhouse maintained at 20–30°C, under a 14/10-h L:D photoperiod. Each mini-cube with its tomato seedling was then inserted into a large cube over which was poured a 200-ml suspension of GF191 immediately after insertion. Using this system, experiments 1 and 2 were conducted from 1999 to 2002 in the greenhouse for 60 and 90 days after pathogen inoculation, respectively, at 25–35°C. In both experiments, four large-cube transplants were placed on rock wool slabs (15 × 45 × 7.5 cm) (Grodan) in polypropylene boxes (50 × 50 × 50 cm). Each plant was grown in a subirrigation culture system. Plastic baskets (38 × 29 × 13 cm) were placed on the bottom of the boxes to create a space underneath the slabs. Tap water was added to the boxes up to 3 cm below the surface of the slabs. The plant fertilizers Nutricote 12-0-0-23 (NPKCa) and Nutricote 13-11-13 (NPK) (Asahikasei Co, Tokyo, Japan) were used at 4.3 and 19.2 g per plant, respectively. Each box contained two rockwool slabs into which two tomato plants were inserted about 20 cm apart. After seven days, a 100-ml suspension of FOL was inoculated into each rockwool cube. Rockwool cubes untreated with GF191 and either challenged or unchallenged with FOL were prepared as controls. Disease severity based on foliar symptoms of wilting was monitored throughout the experiments, and the discoloration severity of the vascular tissue/cortex/xylem was assayed at the end of the experiments on a scale of 0–4 where 0 = healthy, 1 = yellowing, 2 = slight wilting, 3 = severe wilting and 4 = dead plant. The disease severity in each replication within each treatment was calculated using the formula $(4A + 3B + 2C + D)/4N \times 100$, where A is the number of plants assessed at scale 4, B is the number of plants at scale 3, C is the number of plants at scale 2, and D is the number of plants on scale 1; N is the total number of plants. The discoloration severity of vascular tissue/cortex/xylem was assessed subjectively on a scale of 0–3 after cutting the basal stem or crown of each plant, where 0 = healthy (no vascular discoloration), 1 = ≤33% discoloration, 2 = >33–67% and 3 = >67–100%.

Assay for control of FWT in a soil system

A plug tray (28.0 × 54.5 cm) containing 128 pots [4.5 cm (depth) × 3.0 cm^2 per pot) was filled with Star Bed potting soil (Zen-noh, Tokyo, Japan) containing clay, peat, zeolite and composted plant material. The respective nitrogen, phosphorus and potassium contents of the potting soil were 200:1500:200 mg l^{-1}, and the pH ranged from 6.0 to 7.0. A 10-ml suspension of GF191 was poured into each pot, and one tomato seed was sown. The 19-day-old seedlings were transferred into 9-cm diameter pots filled with potting soil. A 100-ml suspension of GF191 was then poured into the pot. Eighteen days later, the seedlings were transplanted into artificially pathogen-infested soil (FOL concentration 1.0 × 10^4 cfu g^{-1} soil) at 15-cm intervals in plastic boxes [64 × 22 × 18 cm (length × width × depth)]. Seedlings untreated with GF191 were used as controls. All treatments included four replicates, with three plants per replicate. Experiments 3 and 4 were conducted in the greenhouse for 90 and 75 days at 25–37°C between 11 July and 11 October 2000 and between 29 June and 4 October 2007, respectively.

In experiments 3 and 4, disease severity was monitored for 90 and 72 days, respectively, after the seedlings had been transplanted into pathogen-infested soil. The levels of disease and discoloration severity were assessed as previously described.

Monitoring of FOL in stems

Populations of FOL in the stems were estimated at the end of experiments 1 and 4. Based on the discoloration severity within each treatment, the 20-cm long stems of all plants in each disease score category were combined. Following washing of each

stem separately in tap water to remove adhering rockwool or soil, all stems in each score category were homogenized in sterile distilled water (1:10 w/v) using a blender (model AM, Ace Homogenizer; Nihonseiki Kaisha, Tokyo, Japan) at 8,000 rpm for 10 min. The homogenized stems were filtered through two layers of gauze, diluted 10- to 1000-fold and plated on Komada's selective medium (Komada 1975) with six plates per replication. After seven days of incubation at 25°C in the dark, number of colony-forming units of FOL per gram fresh weight of the stems was recorded for each score category based on the discoloration severity within each treatment. The average populations of FOL in each replication within each treatment were calculated using the formula $(P_0A + P_1B + P_2C + P_3D)/N \times 100$, where $P_{0,1,2, \text{ and } 3}$ = the population of pathogens in scale categories 0, 1, 2 and 3, respectively, A = fresh weight of stems in scale category 0, B = fresh weight in scale category 1, C = fresh weight in scale category 2, D = fresh weight in scale category 3 and N = total weight of stems. The experiment included four replicates.

Effect of stem extracts on germination and proliferation of FOL

Tomato plants either treated or non-treated with GF191 and challenged with FOL, and showing scale 0 discoloration, were used in the experiments. The stems were collected at the end of experiments 1 and 4 for the germination and proliferation of FOL, respectively. The filtrates of homogenized stems were centrifuged at 3,000 rpm for 10 min and the supernatant solutions collected and filtered through a 0.45-μm Millipore filter (Millex-HV; Millipore, Bedford, MA, USA). Stem extracts (2 ml) were placed in small petri dishes (40 × 20 mm). Cellophane was then positioned on the surface of the extract, and 40 μl of spore suspension FOL (5×10^5 budding-cells ml^{-1}) was immediately placed on the cellophane. The petri dishes were maintained at 25°C under the dark condition. After a 12-h incubation, the germination percentages were determined by randomly counting 400 spores per petri dish. Germ-tube lengths for 40 spores per petri dish were also measured under a microscope. The effect of stem extracts on pathogen proliferation was evaluated by counting newly formed budding-cells in the stem extracts. Nine milliliters of stem extracts and 1 ml of budding-cells of FOL (1.0×10^6 budding-cells ml^{-1}) were mixed in a 100-ml Erlenmeyer flask which was then incubated at 25°C on a reciprocal shaker at 120 rpm for seven days. The numbers of budding-cells formed were determined with a hemocytometer 1, 3, 4 and 7 days after incubation. Each petri dish and Erlenmeyer flask constituted one replicate, and each experiment was conducted four times.

Tomatine content in stems treated with *F. equiseti*

The tomato plants treated or non-treated with GF191 and unchallenged with FOL were used in experiments to determine the content of tomatine in the tomato stems. The tomato plants were grown in the same way as described for the assay on the control of FWT in a soil system at 25–35°C between September and December 2002. The 20-cm-long stems extending above the soil surface from two plants were collected 90 days after seeding and homogenized in a blender (model AM, Ace Homogenizer; Nihonseiki Kaisha, Japan) at 8,000 rpm for 5 min. The homogenized stems were frozen and then lyophilized for two days. The freeze-dried material was reduced to a fine powder and stored at −20°C. The tomatine content was measured by an absorptiometric method (Furui et al. 1998). Powdered tomato stem (0.5 g) was used for this experiment, which was conducted in triplicate.

Data analysis

The experiments were arranged in a randomized complete block design of four replications, with four plants per replication in experiments 1 and 2 and three plants per replication in experiments 3 and 4. Data from the experiments were first tested for the homogeneity of variances and then subjected to analysis of variance (ANOVA) for disease severity, discoloration severity and pathogen populations. When the F test results were significant, means were compared using Fisher's protected least significant difference, with a $P < 0.05$. Data on both disease and discoloration severity and spore germination of FOL were analyzed after the data had been subjected to arcsine-square-root transformation.

Results

Assay for control of FWT in a rockwool system

Tomato plants treated with GF191 showed less disease than pathogen-control plants throughout experiments 1 and 2 (Fig. 1). At the end of experiments 1 and 2, the disease severity of GF191-treated plants was significantly reduced by 88.6% and 72.7%, respectively, relative to the pathogen-control plants. GF191 also significantly reduced the discoloration severity by 85.0% and 64.1% (experiments 1 and 2, respectively) compared to the pathogen-control plants (Table 1).

Assay for control of FWT in a soil system

Tomato plants treated with GF191 also exhibited less disease than pathogen-control plants in the soil system experiments (Fig. 2). At the end of experiments 3 and 4, disease severity was significantly reduced by 66.7% and 82.7%, respectively, relative to the pathogen-control plants. GF191 also significantly reduced the discoloration severity by 53.7% and 67.7% (in experiments 3 and 4, respectively) as compared to the pathogen-control (Table 2).

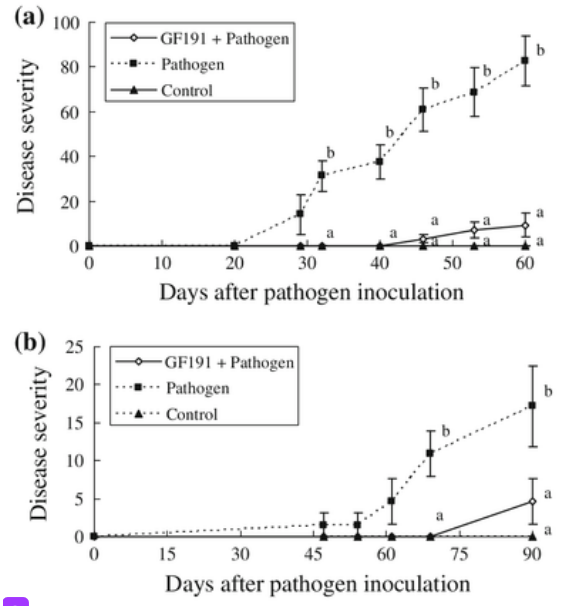

Fig. 1 Progress of disease severity of foliar symptoms caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) on tomato plants treated with *F. equiseti* GF191 in experiments 1 (**a**) and 2 (**b**) in a greenhouse rockwool system. Values with the same letter at each time point are not significantly different ($P < 0.05$) according to Fisher's protected least significant difference test ($F_{2,9} = 19.51$, 21.68, 23.15, 11.81 and 21.90 and $P = 0.0005$, 0.0004, 0.0003, 0.0030 and 0.0003 at 32, 40, 46, 53 and 60 days, respectively, in experiment 1; $F_{2,9} = 13.24$ and 6.24, and $P = 0.0021$ and 0.0199 at 69 and 90 days, respectively, in experiment 2). Bars represent SE

Table 1 Effect of *Fusarium equiseti* GF191 on the discoloration severity of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) in a greenhouse rockwool system (experiments 1 and 2)

Treatment	Discoloration severity	
	Experiment 1	Experiment 2
GF191 + pathogen	12.5 ± 5.4 a	6.9 ± 4.2 a
Pathogen	83.3 ± 11.3 b	19.2 ± 8.0 b
Control	0.0 ± 0.0 a	0.0 ± 0.0 a

Discoloration severity was assessed at the end of experiments 1 and 2

Values presented as the mean ± SE of four replications. Values followed by the same letter in each column are not significantly different at $P < 0.05$ according to Fisher's protected least significant difference test ($F_{2,9} = 22.02$, $P = 0.0003$ for experiment 1; $F_{2,9} = 8.48$, $P = 0.0085$ for experiment 2)

Monitoring of FOL in tomato stems

In a hydroponic rookwool system (experiment 1), FOL population densities in stems treated with GF191 at scales 2 and 3 of discoloration severity were reduced by 95.9% and 100%, respectively, compared to the pathogen-infested control (Table 3). The average FOL population in stems treated with GF191 was also significantly ($P < 0.05$) reduced by 99.6% relative to the pathogen-infested control.

In a soil system (experiment 4), the average FOL population in stems treated with GF191 was also significantly reduced (90.7%; $P < 0.05$) compared to the pathogen-infested control (Table 3). FOL population densities in stems treated with GF191 that were assessed with a discoloration severity of 3 were reduced by 89.1% compared to the pathogen-infested control.

Effects of stem extracts on FOL germination and proliferation

Stem extracts from tomato plants treated with GF191 and challenged with pathogen significantly

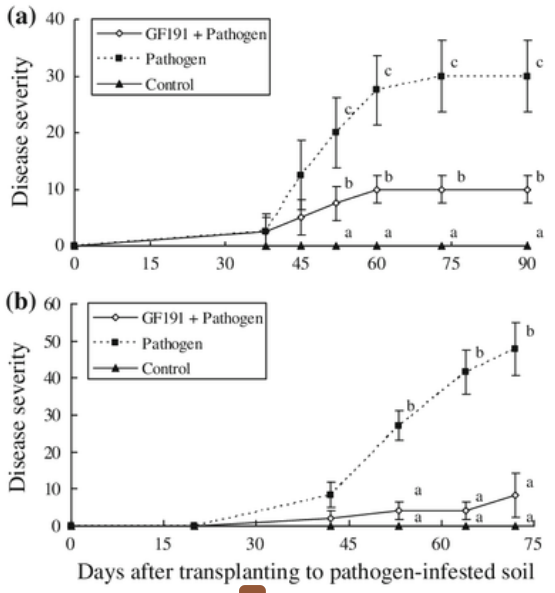

Fig. 2 Progress of disease severity of foliar symptoms caused by FOL on tomato plants treated with *F. equiseti* GF191 in experiment 3 (**a**) and experiment 4 (**b**) in the greenhouse soil system. Values with the same letter at each time point are not significantly different ($P < 0.05$) according to Fisher's protected least significant difference test ($F_{2,9} = 16.28$, 20.18, 22.00 and 22.00, and $P = 0.0004$, 0.0001, <0.0001 and <0.0001 at 52, 60, 72 and 90 days, respectively, in experiment 3; $F_{2,9} = 28.81$, 36.64 and 20.93, and $P = 0.0001$, <0.0001 and 0.0004 at 53, 64 and 72 days, respectively, in experiment 4). Bars represent SE

($P < 0.05$) inhibited FOL spore germination and germ-tube length after a 12-h incubation of the pathogen (Table 4).

Table 2 Effects of *F. equiseti* GF191 on the discoloration severity of Fusarium wilt of tomato caused by FOL in the soil system (experiments 3 and 4)

Treatment	Discoloration severity	
	Experiment 3	Experiment 4
GF191 + pathogen	27.8 ± 8.5 a	27.8 ± 14.7 a
Pathogen	60.0 ± 4.1 b	86.1 ± 2.8 b
Control	0.0 ± 0.0 a	0.0 ± 0.0 a

Discoloration severity was assessed at the end of experiments 3 and 4

Values are presented as the mean ± SE of four replications. Values followed by the same letter in each column are not significantly different at $P < 0.05$ according to Fisher's protected least significant difference test ($F_{2,9} = 30.69$, $P < 0.0001$ for experiment 3; $F_{2,9} = 30.40$, $P = 0.0001$ for experiment 4)

The stem extracts from tomato plants treated with GF191 and challenged with pathogen significantly ($P < 0.05$) inhibited the production of new budding-cells of FOL compared with those of both untreated and unchallenged and untreated and challenged plants 1, 3, 4 and 7 days after inoculation (Fig. 3). Consequently, the rates of budding-cell formation were reduced by 62.5, 62.0, 58.3 and 49.5%, respectively, relative to the untreated and unchallenged control and by 65.5, 48.6, 45.5 and 33.1%, respectively, relative to the untreated and challenged plants.

Tomatine content in stems treated with *F. equiseti*

The tomatine content in 20-cm-long stems from tomato plants treated with GF191 (118.5 ± 15.4 μg g^{-1} fresh weight) was significantly ($P < 0.05$) increased (156%) compared that in non-treated controls (75.8 ± 0.3 μg g^{-1} fresh weight).

Discussion

In this study, the PGPF *Fusarium equiseti* GF191 effectively inhibited the development of FWT under four different experimental conditions in both hydroponic rockwool and soil systems. GF191 was also able to control FWT in long-term experiments (90 days after the inoculation of pathogen and 90 days after the plant had been transplanted in pathogen-infested soil). Fairly high protective effects (66.7–88.6%) based on the disease severity of foliar symptoms against FWT by GF191 were obtained in the four experiments. These effects were almost identical to those with non-pathogenic *F. oxysporum* (Larkin and Fravel 1998), although the experimental periods differed. In the latter case, non-pathogenic *F. oxysporum* provided a 50–80% reduction of disease incidence during a shorter period of 4–6 weeks. De Cal et al. (1999) also reported the longer protective effect (60–100 days) of *Penicillium oxalicum* against FWT, but those effects were comparatively low at 24–69%. In general, fumigation is considered to be necessary to control FWT in highly pathogen-infested fields (Sivan et al. 1987). However, the results obtained from our experiments 3 and 4 showed that, even without using soil fumigation, GF191 could significantly control

Table 3 Effect of *F. equiseti* GF191 treatment on the population density of FOL in tomato stems in the rockwool system (experiment 1) and soil system (experiment 4)

Treatment	Population of FOL in tomato stems of different discoloration score categories ($\times 10^2$ cfu g^{-1} fresh weight)				Average population of FOL ($\times 10^2$ cfu g^{-1} fresh weight)
	0	1	2	3	
Experiment 1					
GF191 + pathogen	6.7 ± 0.7	0.0 ± 0.0	1.3 ± 0.9	1.0 ± 0.7	5.6 ± 0.3 b
Pathogen	3.2 ± 0.5	n.s.	32.0 ± 2.5	2176.7 ± 181.9	1278.3 ± 53.2 c
Control	0.0 ± 0.0	n.s.	n.s.	n.s.	0.0 ± 0.0 a
Experiment 4					
GF191 + pathogen	0.7 ± 0.7	1.3 ± 0.7	2.7 ± 1.8	4.0 ± 1.2	2.1 ± 0.9 y
Pathogen	n.s.	2.0 ± 0.0	2.7 ± 1.3	36.7 ± 4.7	22.6 ± 3.1 z
Control	0.0 ± 0.0	n.s.	n.s.	n.s.	0.0 ± 0.0 y

n.s., No sample

Values are presented as the mean ± SE of six replications. Values followed by the same letter in each column are not significantly different at $P < 0.05$) according to Fisher's protected least significant difference test ($F_{2,9} = 30.69$, $P < 0.0001$ for experiment 1; $F_{2,9} = 30.40$, $P = 0.0001$ for experiment 4)

Table 4 Effect of stem extracts from plants treated with *F. equiseti* GF191 and the pathogen on spore germination and germ-tube length of FOL

Treatment	Germination (%)	Germ-tube length (μm)
GF191 + pathogen	50.5 ± 5.2 a	6.0 ± 0.4 a
Pathogen	68.7 ± 5.6 b	10.7 ± 1.1 b
Control	65.8 ± 2.2 b	11.0 ± 1.2 b

Values are presented the mean ± SE of four replications. Stem samples were obtained at the end of experiment 1. Values followed by the same letter in each column are not significantly different at $P < 0.05$ according to Fisher's protected least significant difference test ($F_{2,9} = 4.62$, $P = 0.0417$ for germination; $F_{2,9} = 8.35$, $P = 0.0185$ for germ-tube length)

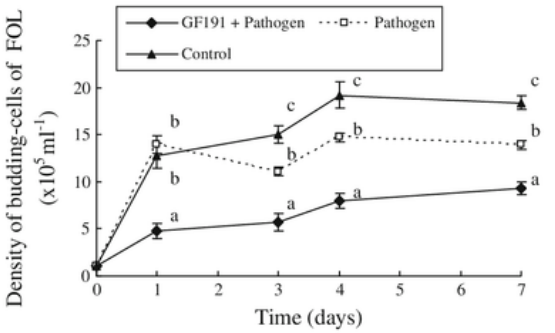

Fig. 3 Suppression of production of new budding-cells of FOL in extracts of tomato stems showing discoloration scale 0 treated with *F. equiseti* GF191 and pathogen. Stem samples were obtained at the end of experiment 4. Values with the same letter at each time point are not significantly different ($P < 0.05$) according to Fisher's protected least significant difference test. ($F_{2,9} = 21.79$, 33.50, 36.70 and 55.62, and $P = 0.0003$, <0.0001, <0.0001 and <0.0001 at 1, 3, 4 and 7 days, respectively). Bars represent SE

tomato FWT even in a heavily pathogen-infested field (FOL concentration 10^4cfu g^{-1} soil).

There are only a few published reports on the utilization of *F. equiseti* as a biocontrol agent. However, in our previous studies, GF191 was able to control Fusarium crown and root rot of tomatoes by 63–100% and 53–62%, respectively, in both hydroponic rockwool and soil systems (Horinouchi et al. 2007, 2008). A different strain, GF183 of *F. equiseti*, was also able to effectively control Fusarium wilt of spinach (Horinouchi et al. 2010). Based on these results, PGPF *F. equiseti* may be a potential candidate as a biocontrol agent. Moreover, *F. equiseti* has been characterized as a natural root endophyte able to colonize plant roots and endowed with properties that could make it a promising candidate for the biological control of root pathogens and nematodes (Maciá-Vicente et al. 2009; Nitao et al. 2001).

GF191 was frequently re-isolated from tomato roots throughout their growing periods (Horinouchi et al. 2007, 2008). This result suggests that the high ability of *F. equiseti* to colonize plant roots may be related to its mechanism of disease suppression. This

finding is similar to that reported for non-pathogenic *F. oxysporum* (Olivain et al. 2006; Yamaguchi et al. 1992), which showed that the magnitude of the protection effect corresponded to the frequency of isolation of non-pathogenic *Fusarium*. PGPF isolates have been found to colonize the epidermal and outer cortical cell layers of roots (Hyakumachi 1994; Shivanna et al. 1996). The continuous presence of PGPF isolates on/in roots may trigger the plants to produce protective signaling processes (Meera et al. 1995).

Our results demonstrate that stem extracts from *F. equiseti*-pathogen-treated plants had an inhibitory effect on pathogen germination and proliferation (Table 4, Fig. 3). In previous studies, we also observed such inhibitory effects of stem or root extracts from both *F. equiseti*-treated plants and *F. equiseti*–pathogen-treated plants on the pathogen germination and proliferation of *F. oxysporum* f. sp. *radicis-lycopersici* and f. sp. *spinaciae* (Horinouchi et al. 2007, 2010). These results suggest that *F. equiseti* may induce physiological changes in the composition of plant extracts. In support of this theory, the amount of tomatine in tomato stems treated with GF191 (118.5 ± 15.4 μg g^{-1} fresh weight) was significantly increased compared with non-treated plants (75.8 ± 0.3 μg g^{-1} fresh weight). The antifungal compound tomatine has been reported to provide a chemical barrier in tomato plants against plant pathogenic fungi, including *F. oxysporum* (Lairini et al. 1997). In our studies, the pre-inoculation of tomato seedlings with *F. equiseti* GF191 not only suppressed the disease but also reduced the number of pathogen populations in their stems. This result is similar to those of previous studies in which the inhibition of pathogens in the roots and stems of resistant tomato cultivars after inoculation with Fusarium wilt pathogen was related to the accumulation of tomatine and rishitin (Hammerchlag and Mace 1975; Hutson and Smith, 1980; Langcake et al. 1972; MacCane and Drysdale 1975). These changes enabled reduced inoculum levels of pathogen in the plants. Furthermore, *F. equiseti* GF191 was re-isolated from tomato roots—but not from their stems—indicating that the hyphae of *F. equiseti* do not migrate between the two sites (Horinouchi et al. 2007, 2008). In other words, there is no direct interaction (parasitism and antibiosis) in the stems between GF191 and FOL, suggesting that, in this system, the observed disease reduction may be attributed to the indirect antagonism mediated through the host plant in response to root colonization by GF191 and that the induced resistance may be one of the mechanisms involved in the biocontrol of FWT.

We have shown here that *F. equiseti* GF191 is an effective biocontrol agent against FWT in both hydroponic rockwool and soil systems. To the best of our knowledge, this is the first report of the biocontrol of FWT by *F. equiseti*. Further research will be needed to determine the complete mechanisms of disease suppression involved in the *F. equiseti* system and to develop methods for its practical use as a biocontrol agent.

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References

De Cal A, Garcia-Lepe R, Pascual S, Melgarejo P (1999) Effects of timing and method of application of *Penicillium oxalicum* on efficacy and duration of control of Fusarium wilt of tomato. Plant Pathol 48:260–266

Furui H, Inakuma T, Ishiguro Y, Kiso M (1998) Tomatine content in host and transgenic tomatoes by absorptiometric measurement. Biosci Biotechnol Biochem 62:556–557

Hammerchlag F, Mace ME (1975) Antifungal activity of extracts from fusarium wilt-susceptible and -resistant tomato plants. Phytopathology 65:93–94

Horinouchi H, Muslim A, Suzuki T, Hyakumachi M (2007) *Fusarium equiseti* GF191 as an effective biocontrol agent against Fusarium crown and root rot of tomato in rock wool systems. Crop Prot 26:1514–1523

Horinouchi H, Katsuyama N, Taguchi Y, Hyakumachi M (2008) Control of Fusarium crown and root rot of tomato in a soil system by combination of a plant growth-promoting fungus, *Fusarium equiseti*, and biodegradable pots. Crop Prot 27:859–864

Horinouchi H, Muslim A, Hyakumachi M (2010) Biocontrol of Fusarium wilt of spinach by the plant growth promoting fungus *Fusarium equiseti* GF183. J Plant Pathol 92: 251–256

Hutson RA, Smith IM (1980) Phytoalexins and tyloses in tomato cultivars infected with *Fusarium oxysporum* f. sp. *lycopersici* or *Verticillium albo-atrum*. Physiol Plant Pathol 17:245–257

Hyakumachi M (1994) Plant growth-promoting fungi from turfgrass rhizosphere with potential for disease suppression. Soil Microorg 44:53–68

Hyakumachi M, Kubota M (2004) Fungi as plant growth promoter and disease suppressor. In: Arora DK (ed) Mycology series. Fungal biotechnology in agricultural, food, and environmental applications, vol 21. Marcel Dekker, New York, pp 101–110

Jones JB, Jones JP, Stall RE, Zitter TA (1991) Compendium of tomato diseases. American Phytopathological Society, St. Paul

Koike N, Hyakumachi M, Kageyama K, Tsuyumu S, Doke N (2001) Induction of systemic resistance in cucumber against several diseases by plant growth-promoting fungi: lignification and superoxide generation. Eur J Plant Pathol 107:523–533

Komada H (1975) Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev Plant Prot Res (Tokyo) 8:114–125

Lairini K, Perez-Espinosa A, Ruiz-Rubio M (1997) Tomatinase induction in formae speciales of *Fusarium oxysporum* non-pathogenic of tomato plants. Physiol Mol Plant Pathol 50:37–52

Langcake P, Drysdale RG, Smith H (1972) Post-infectional production of an inhibitor of *Fusarium oxysporum* f. sp. *lycopersici* by tomato plants. Physiol Plant Pathol 2:17–25

Larkin RP, Fravel DR (1998) Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. Plant Dis 82:1022–1028

Larkin RP, Fravel DR (2002) Effects of varying environmental conditions on biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 92:1160–1166

Lemanceau P, Alabouvette C (1993) Suppression of fusarium wilts by fluorescent pseudomonads: mechanisms and applications. Biocontrol Sci Technol 3:219–234

MacCane DJ, Drysdale RB (1975) Production of tomatine and rishitin in tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici*. Physiol Plant Pathol 7:221–230

Maciá-Vicente JG, Jansson H-B, Talbot NJ, Lopez-Llorca LV (2009) Real-time PCR quantification and live-cell imaging of endophytic colonization of barley (*Hordeum vulgare*) roots by *Fusarium equiseti* and *Pochonia chlamydosporia*. New Phytol 182:213–228

Meera MS, Shivanna MB, Kageyama K, Hyakumachi M (1995) Persistence of induced systemic resistance in cucumber in relation to root colonization by plant growth promoting fungal isolates. Crop Prot 14:123–130

Mihuta-Grimm L, Erb WA, Rowe RC (1990) Fusarium crown and root rot of tomato in greenhouse rock wool systems: sources of inoculum and disease management with benomyl. Plant Dis 74:996–1002

Nitao JK, Meyer SLF, Schmidt WF, Fettinger JC, Chitwood DJ (2001) Nematoda-antagonistic trichothecenes from *Fusarium equiseti*. J Chem Ecol 27:859–868

Olivain C, Humbert C, Nahalkova J, Fatehi J, L'Haridon F, Alabouvette C (2006) Colonization of tomato root by pathogenic and nonpathogenic *Fusarium oxysporum* strains inoculated together and separately into the soil. Appl Environ Microbiol 72:1523–1531

Shivanna MB, Meera MS, Hyakumachi M (1996) Role of root colonization ability of plant growth promoting fungi in the suppression of take-all and common root rot of wheat. Crop Prot 15:497–504

Sivan A, Ucko O, Chet I (1987) Biological control of fusarium crown rot of tomato by *Trichoderma harzianum* under field condition. Plant Dis 71:587–592

Yamaguchi K, Sano T, Arita M, Takahashi M (1992) Biocontrol of Fusarium wilt of tomato and Verticillium wilt of eggplant by non-pathogenic *Fusarium oxysporum* MT0062. Ann Phytopathol Soc Jpn 58:188–194

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Biological control of Fusarium wilt of tomato with Fusarium equiseti GF191 in both rock wool and soil systems

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