Pythium Root Rot and Growth Responses of Organically Grown Geranium Plants to Beneficial Microorganisms

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Abstract. *Pythium* root rot, caused by *Pythium ultimum*, is responsible for important losses in geranium plant production, mainly as a result of the decrease in the plant overall quality. An organic production system for geranium plants based on fertilization using a filtered suspension of dehydrated hen manure was compared with a conventional fertilization system to evaluate their tolerance to root disease. Under typical greenhouse conditions, geranium plants were inoculated with a suspension of *Pseudomonas putida*, *Trichoderma atroviride*, a mixture of both or with *Trichoderma harzianum*, and a commercially available product, Rootshield[®], 1 and 4 weeks after planting. Four weeks after the first inoculation, *Pseudomonas putida* and *Trichoderma atroviride* stimulated plant growth (shoot and root dry weight) compared with the control regardless of the fertilization. The results also showed that the colonization of geranium roots by *Pythium* spp. was significantly lower for organically grown plants for all treatments compared with the inoculated control under conventional fertilization. Inoculation with *T. atroviride* under conventional fertilization was the only treatment that did not significantly reduce root colonization by *Pythium* spp. compared with the conventional control. For both organically and conventionally grown plants, the coinoculation with both *P. putida* and *T. atroviride* resulted in the weakest colonization of roots by the pathogen. The inoculation of *P. putida*, *T. atroviride*, and the mixture of the bacterium and the fungus also significantly increased the fresh and dry weight of roots regardless of the fertilization used. All microorganism treatments in conventionally grown plants significantly increased the fresh and dry weight of the shoot compared with the control.

Growing concerns over the protection of the environment is now widespread, especially in the field of agriculture. Consumers are progressively seeking quality produce grown under systems respecting the increasingly restrictive environmental guidelines. In this context, ornamental plant growers should also aspire to achieve a more sustainable production system. Organic amendments of diverse origin such as composts or manure are considered a good source of nutrients and are often used as the main source of fertilization in container-grown organic crops (Raviv et al., 2005). The use of extracts from such organic materials was therefore thought to be an easier way to substitute a conventional production system with an organic ornamental crop such as geranium.

In addition to the potential environmental benefits attached to the use of organic amendments, organic fertilization could also be viewed as a preventive method to control soilborne plant pathogens (Hoitink and Fahy,

1986; Raviv, 2008; Raviv et al., 2005). The effectiveness of the use of organic amendments such as composts of diverse origin in the suppression of plant diseases has widely been described in the literature (Chen et al., 1987; Cronin et al., 1996; Hadar and Mandelbaum, 1986; Lumsden et al., 1983; Zhang et al., 1996). Considering that geranium plants are susceptible to root diseases caused by a multitude of pathogens, including numerous *Pythium* species such as *P. splendens* (Griffin, 1972), *P. ultimum* (Desilets et al., 1994), and *P. irregulare* (Moorman et al., 2002), the use of extracts from organic materials may prevent losses resulting from such diseases. Moreover, the use of organic amendments for fertilization and to prevent root diseases is also compatible with the inoculation of beneficial rhizosphere microorganisms such as biological control agents or plant growth-promoting microorganisms and would likely lead to an improved repressive effect (Avis et al., 2008).

The objectives of this study were therefore 1) to compare, in terms of plant growth, an organic production system based on dehydrated hen manure extracts for geranium plants to a conventional growing system; and 2) to evaluate the effect of an organic fertilization on the development of *Pythium*

root rot of geranium plants inoculated or not with beneficial microorganisms.

Materials and Methods

Plant material. Seeds of *Pelargonium* hybrid cultivar “Moulin Rouge” were sown on 15 Mar. 2007 to obtain stock plants. Geranium plants for the experiment were propagated by cuttings on 1 Aug. 2007. Cuttings were placed in vermiculite in a mist chamber to stimulate root development. On 22 Aug. 2007, uniform cuttings (shoot and root development) were planted in 15-cm plastic pots containing PRO-MIX BX Professional (Premier Tech, Rivière-du-Loup, Québec, Canada), a peat-based growing medium. Pots were then placed in the greenhouse for 1 week before the start of the experiment to assure that the cuttings were developing normally. Growing conditions remained constant during the course of the experiment: temperatures of 22 °C (day) and 17 °C (night), 75% relative humidity, natural daylight supplemented with high-pressure sodium lamps (100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for a 12-h photoperiod, and no CO₂ enrichment.

Plant fertilization. Geranium plants received either a conventional or an organic fertilization. Plants were fertilized three times a week with 100 mL of their respective nutrient solution. The conventional fertilization consisted of a nutrient solution containing 200 ppm of nitrogen (electrical conductivity of 1.5 $\text{mS}\cdot\text{cm}^{-1}$) and made with 2 $\text{g}\cdot\text{L}^{-1}$ of 10–52–10 (N–P₂O₅–K₂O; Plant-Prod, Laval, Québec, Canada) for the first week and 1 $\text{g}\cdot\text{L}^{-1}$ of 20–8–20 (N–P₂O₅–K₂O; Plant-Prod) for the rest of the experiment. The organic fertilization was done using a nutrient solution consisting of a filtered extract of dehydrated and pelleted hen manure. To make the suspension, 0.5 kg of 8–2–2 (N–P₂O₅–K₂O) and 0.5 kg of 7–3–9 (N–P₂O₅–K₂O) (Acti-sol Inc., St-Wenceslas, Québec, Canada) were placed in 12 L of water for 3 h. The solution was mixed every hour and was then filtered through a cotton material. A fresh extract was made every time plants needed to be fertilized. The solution was diluted (1:10) to obtain an electric conductivity of 2.1 $\text{mS}\cdot\text{cm}$. According to preliminary results, the final organic solution contained 200 ppm of nitrogen (NO₃ and NH₄) (Table 1). Plants were watered as needed. The experimental design was a split-plot design with six replicates. Main plots (two fertilization treatments) and subplots (five microbial treatments) were randomized within each block. Each experimental unit consisted of three geranium plants except for one of the control plants that consisted of four geranium plants (one plant to be used as an uninoculated control in the final evaluation).

Pathogen. A virulent strain of *Pythium ultimum* was provided by the laboratory of Dr. Russell Tweddell (Université Laval, Québec, Québec, Canada). The organism was grown on potato dextrose agar (PDA; Difco Laboratories, Becton Dickinson, Sparks, MD) at 24 °C. PDA disks covered with *P. ultimum*

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Table 1. Composition of the filtered and diluted extract of dehydrated and pelleted hen manure used as the organic nutrient solution and theoretical composition of the conventional nutrient solution.

	Nutrient solution content (ppm)	
	Organic solution ^z	Conventional solution ^y
NO ₃	193.2	200 ^x
NH ₄	6.51	
Phosphorus	7.31	34.88
Potassium	22.80	166.02
Calcium	3.95	
Magnesium	1.65	1.5
Chlorine	84.32	
SO ₄	87.6	
Na	15.5	
Zinc	0.25	0.5
Manganese	<0.02	0.5
Copper	0.22	0.5
Iron	0.25	1.0

^zThe organic nutrient solution was sampled and analyzed right after the filtration and the dilution of the hen manure extract.

^yThe conventional nutrient solution was prepared with 1 g·L⁻¹ of 20–8–20.

^xTotal nitrogen.

mycelium conserved in sterile distilled water at 24 °C served as stock cultures. Considering that, at room temperature, zoospores are not produced by *P. ultimum* (van der Plaats-Niterink, 1981), the propagule suspension of *P. ultimum* consisted of hyphae and oospores. The propagule suspension was prepared from liquid cultures (250-mL flasks) containing 100 mL of potato dextrose broth (Difco Laboratories) incubated on a rotary shaker (150 rev/min) at 24 °C for 1 week. PDA disks covered with actively growing mycelium of *P. ultimum* were used to inoculate the flasks.

Beneficial microorganisms. The bacterium and fungus tested in this study were isolated from the rhizosphere of tomato plants (Gravel et al., 2005). The bacterium tested was *Pseudomonas putida* (Trevisan) Migula Subgroup B Strain 1 and the fungus tested was *Trichoderma atroviride* Karsten. The bacterium was grown on tryptic soy agar (Sigma-Aldrich, Mississauga, Ontario, Canada) and stock cultures were kept in 80% glycerol at -80 °C. Bacterial suspensions were prepared in a 500-mL flask containing 250 mL of tryptic soy broth (TSB; Sigma-Aldrich). Flasks were inoculated with 1 mL of an overnight culture of the bacterium grown in TSB. Flasks were incubated on a rotary shaker (150 rev/min) at 24 °C for 24 h. Bacterial cells were removed by centrifugation (4000 g, 10 min), resuspended in a phosphate buffer (0.1 M), and diluted to 4 × 10⁷ bacteria/mL. The fungus was grown on PDA (Difco Laboratories) and stock cultures were freeze-dried and kept at -20 °C. Spore suspensions were prepared by scraping the surface of a 2-week-old mycelium grown on PDA using a glass rod. The spore suspension was adjusted by dilution with a phosphate buffer (0.1 M) to 4 × 10⁷ spores/mL based on hemacytometer counts. Rootshield® (*Trichoderma harzianum* Rifai strain KRL-AG2; BioWorks Inc., Geneva, NY) suspension

was prepared according to the recommended application rate of the product (0.75 g·L⁻¹ of phosphate buffer).

Plant inoculation. Each geranium plant received by drenching 50 mL of one of the following treatments: a suspension of Rootshield®, of *P. putida*, of *T. atroviride*, or a mixture of *P. putida* and *T. atroviride*. Control plants received 50 mL of the phosphate buffer (0.1 M). Plants were inoculated 1 week after planting and 4 weeks later.

Evaluation of plant growth and development. Plant growth was evaluated 4 and 9 weeks after planting on one plant per experimental unit. The number of leaves per plant was counted and the total leaf area was measured using a LI-3100 area meter (LI-COR Inc., Lincoln, NE). The fresh and dry weight of both the shoot and the roots were then measured. Flower production (number of flowers) was determined 8 and 12 weeks after planting.

CO₂ emission from the growing medium. A 35-g sample of the growing medium was collected from each pot after the evaluation of biomass was completed and placed in a petri dish. Fifteen milliliters of a glucose solution (33.3 g·L⁻¹) was added and mixed thoroughly with the sample to activate the microorganisms present in the growing medium. Samples were incubated at 23 °C for 24 h after which the CO₂ efflux emitting from the sample was measured using a CO₂ flux chamber (Li-6400-09; LI-COR Inc.). The results were expressed in μmol CO₂/m²·s⁻¹.

Evaluation of *Pythium* infection on plant growth and root colonization by the pathogen. Eight weeks after planting, one plant per experimental unit was inoculated with 50 mL of a propagule suspension of *P. ultimum* (1 × 10⁶ propagules/mL). The 12 remaining control plants (one plant per main plot) received 50 mL of water. Four weeks later (12 weeks after planting), biomass, as an indicator of plant growth and development, was evaluated for three blocks as described previously. The other three blocks were used to evaluate root colonization by *P. ultimum* according to the method described by Chérif et al. (1997). Briefly, 3 g of geranium roots were rinsed thoroughly and blended in 100 mL of distilled water. Successive dilutions 1, 1/10, 1/100, and 1/1000 were made and 200 μL of each dilution were plated on a corn meal agar, a *Pythium*-selective medium (Jeffers and Martin, 1986). Three replicates of three plates were made for each dilution. *Pythium* colonies were counted after 72 h of incubation in the dark at 24 °C. Results were expressed as number of propagules per gram of fresh roots.

Statistical analyses. The effects of fertilization, microbial inoculation, and the interaction of these fixed effects on plant growth, CO₂ effluxes, and *Pythium* colonization were analyzed using the SAS Mixed Models procedure (SAS Institute, Cary, NC) with replicates as random effects. When significant ($P \leq 0.05$), means were compared by the Tukey's multiple range test.

Results

Evaluation of plant growth and development. After 4 weeks after plant inoculation with beneficial microorganisms, regardless of the fertilization used, total leaf area was not significantly different for plants inoculated with *P. putida*, *T. atroviride*, or the mixture of both compared with the control but 16% higher than plants treated with *T. harzianum* (Table 2). The mean area per leaf was significantly higher (9.3%) for organically grown plants (Table 2). Regardless of the fertilization treatment, the fresh and dry weight of the shoot was on average 9.9% and 10.4% higher ($P \leq 0.05$), respectively, for plants treated with *P. putida*, *T. atroviride*, or the mixture of both compared with the control (Table 2). The fresh and dry weights of the roots were significantly higher (4.5% and 32% to 70%, respectively) for plants treated with *P. putida*, *T. atroviride*, or the mixture of both compared with the control (Table 2). Results for the plants treated with *T. harzianum* showed root weights similar to the control (Table 2).

After 8 weeks after the initial inoculation or 4 weeks after the second inoculation, no significant difference was observed between treatments except for the mean area per leaf, which was 12% higher ($P \leq 0.05$) for plants grown under an organic fertilization. Overall, the average value for the mean total leaf area was 1271.4 ± 64.7 cm². Weights of the shoot were similar between treatments with average values of 113.9 ± 5.4 g (fresh) and 13.6 ± 0.7 g (dry). Similar results were observed for the roots with average values of 12.1 ± 1.5 g (fresh) and 1.7 ± 0.2 g (dry).

CO₂ efflux from the growing medium. CO₂ emission 4 weeks after the initial inoculation with the beneficial microorganisms was four times higher ($P \leq 0.05$) for organically grown plants compared with the conventionally grown ones (Table 3). The same tendency was also observed after 8 and 12 weeks after the inoculation of plants. After 8 weeks, conventionally grown plants treated with beneficial microorganisms showed lower CO₂ emission compared with the conventional control (Table 3). Organically grown plants treated with the mixture of *P. putida* and *T. atroviride* had significantly lower CO₂ emission compared with other organically grown plants (Table 3). After 12 weeks, CO₂ emission was generally lower for organically grown plants compared with the measurement taken 4 weeks prior, but was still significantly higher than in the conventional treatment (Table 3).

Evaluation of the effect of *Pythium* infection on plant growth and root colonization by the pathogen. After 4 weeks after inoculation with *P. ultimum* (12 weeks after the first inoculation with the beneficial microorganisms), the total leaf area was significantly lower for the conventional *P. ultimum*-inoculated control compared with all other treatments (Table 4), whereas the organic *P. ultimum*-inoculated control was not significantly different from the organic nor the conventional

Table 2. Plant biomass and leaf area of geranium plants grown under an organic or a conventional fertilization system (4 weeks after the first inoculation with beneficial microorganisms).

	Shoot					Roots	
	Total leaf area (cm ²)	Mean area/leaf (cm ²)	Number of leaves	Fresh wt (g)	Dry wt (g)	Fresh wt (g)	Dry wt (g)
Organic ^z		21.2 a [*]					
Conventional ^y		19.4 b					
Control	750.8 ab			54.55 b	6.05 b	17.63 bc	1.04 c
<i>Pseudomonas putida</i>	811.5 a			60.53 a	6.87 a	18.47 a	1.69 a
<i>Trichoderma atroviride</i>	820.9 a			59.55 a	6.48 a	18.08 ab	1.54 a
<i>P. putida</i> / <i>T. atroviride</i>	809.6 a			59.99 a	6.80 a	18.40 a	1.37 b
<i>T. harzianum</i>	699.1 b			51.79 b	5.69 b	17.30 c	1.16 bc
Organic							
Control	790.4	20.6	39.3	56.66	6.37	17.97	1.02
<i>Pseudomonas putida</i>	885.6	21.7	41.0	63.39	7.26	18.85	1.48
<i>Trichoderma atroviride</i>	834.0	22.0	38.2	60.16	6.53	18.13	1.47
<i>P. putida</i> / <i>T. atroviride</i>	841.7	22.0	38.3	61.05	6.94	18.55	1.23
<i>T. harzianum</i>	659.1	19.5	34.0	49.29	5.47	17.07	1.04
Conventional							
Control	711.3	18.9	37.5	52.45	5.71	17.30	1.06
<i>Pseudomonas putida</i>	737.5	18.0	40.7	57.66	6.47	18.08	1.90
<i>Trichoderma atroviride</i>	808.0	19.6	38.2	58.92	6.42	18.02	1.61
<i>P. putida</i> / <i>T. atroviride</i>	777.6	18.9	40.3	59.00	6.66	18.26	1.52
<i>T. harzianum</i>	739.1	21.4	39.2	54.29	5.91	17.52	1.29
Probability level							
Fertilization	NS	0.0407	NS	NS	NS	NS	NS
Microbial treatment	0.0207	NS	NS	0.0016	0.0072	0.0022	0.0134
Fertilization × microbial treatment	NS	NS	NS	NS	NS	NS	NS

^zThe organic fertilization consisted of a filtered extract of pelleted dry hen manure (200 ppm of nitrogen).

^yThe conventional fertilization consisted of a nutritive solution containing 1 g·L⁻¹ of 20–8–20.

Data are means of six replicates (n = 6).

^{*}Within a column, values followed by a similar letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$).

NS = Nonsignificant at $\alpha = 0.05$.

Table 3. CO₂ efflux from the substrate medium of geranium plants grown under an organic or a conventional fertilization system.

	CO ₂ efflux (μmol CO ₂ /m ² /s)		
	4 wk	8 wk	12 wk
Organic ^z	14.4 a ^x		
Conventional ^y	3.6 b		
Organic			
Control	14.8	16.2 a	9.0 a
<i>Pseudomonas putida</i>	16.6	16.2 a	8.3 ab
<i>Trichoderma atroviride</i>	13.1	16.1 a	9.1 a
<i>P. putida</i> / <i>T. atroviride</i>	14.2	13.2 b	8.4 ab
<i>T. harzianum</i>	13.4	15.9 a	7.2 b
Conventional			
Control	3.0	6.6 c	2.5 cd
<i>Pseudomonas putida</i>	3.5	1.3 d	3.5 c
<i>Trichoderma atroviride</i>	3.2	3.6 d	2.5 cd
<i>P. putida</i> / <i>T. atroviride</i>	3.2	2.3 d	1.3 d
<i>T. harzianum</i>	4.9	1.6 d	3.0 cd
Probability level			
Fertilization	0.0002	0.0001	0.0001
Microbial treatment	NS	0.0025	NS
Fertilization × microbial treatment	NS	0.0327	0.0269

^zThe organic fertilization consisted of a filtered extract of pelleted dry hen manure (200 ppm of nitrogen).

^yThe conventional fertilization consisted of a nutritive solution containing 1 g·L⁻¹ of 20–8–20.

Data are means of six replicates (n = 6).

^xWithin a column, values followed by a similar letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$).

NS = Nonsignificant at $\alpha = 0.05$.

uninoculated control (Table 4). Similar results were also observed for the fresh weight of the shoot. No significant difference was observed in the dry weight of the shoot among the four controls. Regardless of the microbial treatment, the number of leaves per plant was significantly lower (8.5%) for the organically grown plants than conventional ones. In addition, it was also significantly lower for the uninoculated control compared with all other microbial treatments regardless

of the fertilization used. For the root fresh weight, values of all treatments were not significantly different from the uninoculated control but were significantly higher from the *P. ultimum*-inoculated control. Root dry weight of *P. ultimum*-inoculated plants with *P. putida*, *T. atroviride*, or a mix of both was 37% higher than the *P. ultimum*-inoculated control. However, no significant difference between the *P. ultimum*-inoculated control and noninoculated control was ob-

served (Table 4). No significant difference was observed between treatments for flower production (data not shown). Consequently, no quality index was determined nor evaluated.

Conventional uninoculated control plants were not colonized with *Pythium* spp., whereas the conventional inoculated control showed the highest colonization (388.8 propagules/g of fresh roots) (Table 5). Inoculation with *T. atroviride* under conventional fertilization was the only treatment that did not significantly reduce root colonization by *Pythium* spp. (Table 5). Colonization of geranium roots by *Pythium* spp. was significantly lower for organically grown plants for all treatments compared with the inoculated control under conventional fertilization (Table 5). For both organic and conventional fertilization, the coinoculation with *T. atroviride* and *P. putida* resulted in the lowest root colonization by *Pythium* spp. (Table 5).

Discussion

The first objective of this study was to evaluate the potential of an organic fertilization in the production of geranium plants. In the first 8 weeks of the experiment, growth of organically and conventionally grown plants were very similar. The main difference observed between the two growing systems was in the mean area per leaf. This can affect the visual quality of plants produced, although in this case, the difference between the two was relatively small, being at most 2.6 cm² after 8 weeks of treatment. Flower production and the plant overall quality were unaffected by the fertilization used. This study therefore

Table 4. Plant biomass and leaf area of geranium plants grown under an organic or a conventional fertilization system and inoculated with *Pythium ultimum* 4 weeks before evaluation (12 weeks after inoculation with beneficial microorganisms).

	Shoot					Roots	
	Total leaf area (cm ²)	Mean area/leaf (cm ²)	Number of leaves	Fresh wt (g)	Dry wt (g)	Fresh wt (g)	Dry wt (g)
Organic ^z			61.6 z [†]				
Conventional ^y			67.3 y				
Control ^x			53.6 c			13.36 ab	1.81 ab
Control + ^w			63.3 b			8.80 c	1.70 b
<i>Pseudomonas putida</i>			66.9 ac			15.58 b	2.38 a
<i>Trichoderma atroviride</i>			67.5 ac			15.08 a	2.42 a
<i>P. putida</i> / <i>T. atroviride</i>			70.4 a			15.44 a	2.35 a
<i>T. harzianum</i>			65.2 ab			12.94 a	1.89 ab
Organic							
Control	1,386.8 abcd	21.6 bc	53.1	112.02 cd	15.81 bc	14.03	1.51
Control +	1,258.5 cd	20.9 bc	60.4	113.18 cd	15.16 bc	10.82	1.35
<i>Pseudomonas putida</i>	1,649.1 a	22.9 bc	67.2	134.58 ab	17.53 ab	15.14	2.20
<i>Trichoderma atroviride</i>	1,537.6 a	22.3 abc	63.5	132.54 ab	17.24 ab	14.76	2.18
<i>P. putida</i> / <i>T. atroviride</i>	1,621.8 a	24.7 a	65.5	143.93 a	18.57 a	15.08	2.17
<i>T. harzianum</i>	1,292.1 b	21.6 bc	60.1	110.41 d	14.45 c	12.78	1.68
Conventional							
Control	1,233.7 d	22.8 ab	54.1	114.01 cd	16.04 bc	12.69	2.11
Control +	966.1 e	17.3 d	66.3	90.90 e	10.03 cd	6.77	2.04
<i>Pseudomonas putida</i>	1,427.4 ab	21.5 bc	66.6	126.54 bcd	17.15 ab	16.03	2.55
<i>Trichoderma atroviride</i>	1,478.9 ab	20.8 bc	71.5	119.50 bcd	16.81 ab	15.40	2.65
<i>P. putida</i> / <i>T. atroviride</i>	1,501.1 a	19.9 cd	75.3	122.38 bcd	16.98 ab	15.81	2.53
<i>T. harzianum</i>	1,535.7 a	21.8 a	70.3	128.94 abc	18.19 a	13.10	2.10
Probability level							
Fertilization	NS	NS	0.0407	NS	NS	NS	NS
Microbial treatment	0.0001	0.0398	0.0001	0.0001	0.0001	0.0001	0.0478
Fertilization × microbial treatment	0.0062	0.0478	NS	0.0062	0.0001	NS	NS

^zThe organic fertilization consisted of a filtered extract of pelleted dry hen manure (200 ppm of nitrogen).

^yThe conventional fertilization consisted of a nutritive solution containing 1 g·L⁻¹ of 20–8–20.

^xUninoculated control plants.

^w*P. ultimum*-inoculated control plants.

Data are means of three replicates (n = 3).

[†]Within a column, values followed by a similar letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$).

NS = Nonsignificant at $\alpha = 0.05$.

Table 5. Root colonization by *Pythium* spp. of *Pelargonium* plants grown under an organic or a conventional fertilization system and inoculated with *Pythium ultimum* 4 weeks before evaluation.

	Root colonization by <i>Pythium</i> spp. (no. of propagules/g of root)
Organic ^z	
Control ^x	148.1 cd ^y
Control + ^w	222.2 bc
<i>Pseudomonas putida</i>	166.6 cd
<i>Trichoderma atroviride</i>	166.6 cd
<i>P. putida</i> / <i>T. atroviride</i>	74.0 de
<i>T. harzianum</i>	111.1 d
Conventional ^y	
Control	0 e
Control +	388.8 a
<i>Pseudomonas putida</i>	240.7 bc
<i>Trichoderma atroviride</i>	296.3 ab
<i>P. putida</i> / <i>T. atroviride</i>	92.5 de
<i>T. harzianum</i>	129.6 d
Probability level	
Fertilization	0.0481
Microbial treatment	0.0001
Fertilization × microbial treatment	0.0090

^zThe organic fertilization consisted of a filtered extract of pelleted dry hen manure (200 ppm of nitrogen).

^yThe conventional fertilization consisted of a nutritive solution containing 1 g·L⁻¹ of 20–8–20.

^xUninoculated control plants.

^w*P. ultimum*-inoculated control plants.

Data are means of three replicates (n = 3).

[†]Within a column, values followed by a similar letter are not significantly different according to Tukey's multiple range test ($P \leq 0.05$).

shows that it is possible to produce high-quality organically grown geranium plants by following a similar fertilization schedule as for conventionally grown plants.

Organic fertilization is not as precise as conventional chemical fertilizers. However, the use of compost or manure extract allows

for better management of the nutrients readily available for the plant because it does not rely mainly on a turnover of nutrient as is the case with solid organic amendments for soil crop production. It should nonetheless be noted that the use of organic fertilizers is optimized when plants are also inoculated

with beneficial microorganisms such as arbuscular mycorrhizal fungi (Perner et al., 2007). In the case of this experiment, results show that inoculation with *P. putida* and *T. atroviride*, two beneficial microorganisms, improved the growth of geranium plants, especially when organic fertilization was used. Considering that the sole source of fertilizer was the manure extract, which was adjusted according to the plant need for available nitrogen, it is most likely that the microorganisms had rather a limited effect on nutrient availability. It is also likely that every time plants were fertilized with the filtered solution, microbial activity in the growing medium was stimulated, which could explain the higher CO₂ efflux in the organic treatment. Nevertheless, the use of organic residues, including composted material, should be viewed with caution. Adverse effects on the development (reduced growth index and dry weight) and marketability (lower quality index based on plant height, flower counts, and leaf necrosis) of geranium plants grown using such materials as fertilizers or as part of a growing medium have been reported (Gauthier et al., 1998).

Gladstone and Moorman (1990) demonstrated that losses resulting from *Pythium* root rot affecting geranium plants are greater when high nitrogen fertilization rates were used. In the case of this experiment, similar nitrogen fertilization rates were used for the conventional and the organic plants to test the effect of the fertilization system itself while

eliminating the effect of fertilization rates. In the case of this experiment, nitrogen fertilization rates could therefore not explain the effect on *Pythium* spp. development. Fertilization rates should be sufficient in regard to plant nutrient needs but should not exceed them to avoid overfertilization and the resulting losses of nutrients in the environment.

In addition to their use as a source of nutrients, organic amendments can also play a role in the repression of certain diseases. The use of compost and green manure against pathogens such as *Pythium* spp. has been described in the literature (Erhart et al., 1999; Hibar et al., 2006; Hoitink and Fahy, 1986; Manici et al., 2004). Suppressiveness of composts has been associated with the reduction of plant pathogens during the composting stages themselves, the presence of antagonist microorganisms in the composts as well as to its chemical and physical factors (Hoitink and Fahy, 1986). Compost has in the past been used as a source of antagonists against *P. ultimum* (Carisse et al., 2003). Craft and Nelson (1996) also concluded that the suppression of *Pythium* diseases in bentgrass was dependent on microbial properties of compost and of the soil in which it is amended. McKellar and Nelson (2003) showed that the compost-induced suppression of *Pythium* damping-off of cottonseeds was the result of the increased presence of fatty acid-metabolizing bacteria. Compost-amended substrate has been shown to induce a systemic acquired resistance in cucumber against *Pythium* root rot, perhaps through the increased activity of the microflora (Zhang et al., 1996). The use of compost tea as a treatment against numerous pathogens has also been tested over the years with sometimes mixed results (Scheuerell and Mahaffee, 2006; Sturz et al., 2006). The source and composition of composts used therefore play an important role in the efficacy of the treatments. Our study showed for the first time that the use of hen manure tea as organic fertilizer significantly reduced the root *Pythium* population (43% reduction of the number of propagules per gram of root) of organic *P. ultimum*-inoculated control compared with conventional *P. ultimum*-inoculated plants, suggesting that this organic source of fertilizer may constitute a preventive means against *Pythium* infection.

On the other hand, the inoculation with specific beneficial microorganisms has been intensely studied to help control infections by pathogenic *Pythium* species (Carisse et al., 2003; Rankin and Paulitz, 1994). Such biological control is also relevant in organic production in which preventive and curative treatments are limited. In this study, microorganisms already known to reduce *Pythium* diseases of tomato (Gravel et al., 2006, 2007) were tested to evaluate their effect on the development of *Pythium* root rot in geranium plants in organic and conventional systems. Our results are a clear example of the pathogen repression benefits that can be exploited from the use of beneficial microorganisms as described by Avis et al. (2008).

Beneficial microorganisms (*P. putida* and *T. atroviride*) used in this study, alone or in coinoculation, were first shown to have a stimulating effect on geranium plant growth in the early development of the plant (first 4 weeks of the experiment). Such a stimulating effect of those two microorganisms used alone on tomato plants had already been reported (Gravel et al., 2007). In this experiment, these two microorganisms were also shown to reduce the damage caused by *P. ultimum* on geranium shoot and root development. The beneficial effect on plant growth was, however, not observed with *T. harzianum* (Rootshield®), a commercial biofungicide well known to be effective against soilborne pathogens such as *Pythium* spp. It should, however, be noted that this strain of *T. harzianum* is not known to stimulate the growth of geranium plants, which is in accordance with results observed in this experiment (Harman, 2000). Nevertheless, this commercial product had a repressive effect on the development of *P. ultimum* on the roots of the plants.

Soil CO₂ efflux is a good indicator of the microbial activity. In this experiment, the CO₂ emissions from the substrate samples were 4.9, 2.45, and 3.6 times higher than conventional CO₂ efflux control, for a similar root fresh weight ($P > 0.05$), suggesting a higher microbial activity. The filtered hen manure extract used as the source of fertilization in the organic treatment represented a constant source of microorganisms in the growing medium. The microflora was constantly renewed, every 3 d, when plants were fertilized. This probably benefited the development of the plant, especially once *P. ultimum* had been inoculated in the substrate. This is shown in this experiment by the decrease in the root colonization by *Pythium* spp. under organic fertilization compared with the conventional fertilization. The inoculation with beneficial microorganisms, however, had either a little or no effect on the CO₂ effluxes in the organic treatment at each measuring time. The already high microbial activity resulting from the constant inoculation through the dehydrated manure extract and the resulting high microbial competition within the growing medium could explain the observed results. This could also explain, at least partially, the lower root colonization by *Pythium* spp. in the inoculated organic control compared with the inoculated conventional control. Root colonization by *Pythium* spp. was, however, observed in the uninoculated organic control indicating that *Pythium* spp. was naturally present in the organic treatment. Considering that no root colonization was observed in the uninoculated conventional control, the results indicate that the growing medium was not a source of *Pythium* spp. but that the organic fertilization introduced *Pythium* propagules. Considering that the selective medium is not specific to pathogenic species of *Pythium*, but rather to the genus in general, it is possible that nonpathogenic species of *Pythium* were also detected in the process.

Although fertilization in organic production systems for ornamental potted plants such as geranium needs to be optimized, this study shows that benefits can be obtained from the synergic effect of beneficial microorganisms and the use of organic sources of fertilizers for such a production.

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