Effects of Using Water Treated by Artificial Wetlands on Root Rot Suppression and Tomato Growth

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SUMMARY. The objectives of this study were to evaluate the risks and benefits of using artificial wetland-treated waters to irrigate tomato plants (*Lycopersicon esculentum*) and the potential for suppression of *Pythium ultimum*. The experiment was conducted in a greenhouse using tap water (control) and treated waters coming from three types of horizontal subsurface flow artificial wetlands filled with pozzolana and implanted with common cattail (*Typha latifolia*). Wetland units contained either a simple [artificial wetland with sucrose (AWS)] or complex [artificial wetland with compost (AWC)] carbon source or no [artificial wetland with no carbon (AW)] additional carbon source. A complete randomized split-block design comparing root sensitivity to root rot (inoculated and uninoculated plants) in main plots and four nutrient solutions [1) control, 2) treated water from AWS, 3) treated water from AWC, and 4) treated water from AW] in subplots was used in six replications. Tomato plants were inoculated with *P. ultimum* twice during the experimental period. The use of treated waters reduced the in vivo root *Pythium* population by 84% and 100% when the treated waters were from AWS and AWC, respectively. In vitro trials showed that sterilization or membrane filtration (0.2 µm) of treated waters significantly reduced the potential for suppression of *P. ultimum*, suggesting that microbial activity played an important role. On the other hand, all AW-treated waters had a negative effect on root development of uninoculated young tomato plants. Root dry weights of plants irrigated with treated waters was 56% lower than in control plants, while their shoot:root ratio was two times higher for plants irrigated with treated waters. The inoculated and AWC-treated water treatments also reduced the Fv:Fm ratio of dark-adapted leaves, representing the maximum quantum efficiency of photosystem II. Organic compounds present in treated waters, expressed as total and dissolved organic compounds, may have affected tomato root development.

An open greenhouse production system growing tomatoes year-round generates as much as

3000 to 4500 m³ of wasted nutrient solution per ha annually, which contains 4 to 10 Mg of environmentally detrimental nutrients such as nitrate (NO₃⁻), sulfate (SO₄²⁻), sodium (Na⁺), and phosphorus (Dorais and Dubé,

2011; Lequillec, 2002). To increase the sustainability of soilless horticulture production systems, nutrient solution recycling, where drained water is collected and returned to the plants, has been used in recent decades. However, specific ions can accumulate to high levels because of plant uptake preferences (NO₃⁻ vs. SO₄²⁻) as well as raw water and fertilizer quality. Consequently, competition between ions may be modified, resulting in reduced crop growth and fruit quality (Dorais et al., 2001; Voogt et al., 2011). However, recirculation of nutrient solutions increases the risk of root diseases because of the presence of water-borne pathogens, such as *Pythium* sp. in the effluent (Ehret et al., 2001; Stanghellini and Rasmussen, 1994). Several approaches for controlling the spread of pathogens are commercially available, but they generally entail large investments and high maintenance and energy costs (Ehret et al., 2001). Active disinfection methods (e.g., ozonation, ultraviolet light, heat) tend to eliminate nonpathogenic microflora (McPherson et al., 1995), which therefore cannot play a role in reducing pathogen dissemination. Passive biological methods, such as slow filters, keep most of the natural microflora alive and showed a similar level of efficiency in terms of reducing plant pathogens (*Fusarium* sp., *Pythium* sp.) in greenhouse effluents (Alsanus et al., 2001; Dénier et al., 2004; Wohanka, 1995).

The use of artificial wetlands (AWs) for greenhouse wastewater treatment could be an alternative way to reduce the environmental burdens associated with nutrient leaching and consequently to increase the sustainability of

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Units			
To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29,574	fl oz	µL	3.3814 × 10 ⁻⁵
29.5735	fl oz	mL	0.0338
0.0283	ft ³	m ³	35.3147
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.0645	inch ²	dm ²	15.5000
1	micron	µm	1
1	mmho/cm	mS·cm ⁻¹	1
28.3495	oz	g	0.0353
7.4892	oz/gal	g·L ⁻¹	0.1335
1	ppm	mg·L ⁻¹	1
0.001	ppm	mg·mL ⁻¹	1000
6.8948	psi	kPa	0.1450
0.9072	ton(s)	Mg	1.1023
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

greenhouse production systems. AWs exploit natural processes involving macrophytes, soils, and associated microbial communities in treating wastewaters (Stottmeister et al., 2003). AWs have been found to reduce population of human pathogens (e.g., *Escherichia coli*, *Salmonella* sp.) with varying but significant degrees of efficiency (Kadlec and Wallace, 2009). For example, Gruyer et al. (2011) showed that AWs were effective in reducing *Pythium ultimum* and *Fusarium oxysporum* populations. Previous studies also showed that AWs reduced NO_3^- and SO_4^{2-} concentrations in agriculture drainage water (Gruyer et al., 2011; Lin et al., 2002; Whitmire and Hamilton, 2005; Xue et al., 1999). When wastewaters are rich in NO_3^- and SO_4^{2-} but poor in organic carbon, as is the case with greenhouse effluents, plant exudates and root decomposition may supply the organic carbon needed by microorganisms and consequently stimulate denitrification and sulfate reduction (Gruyer et al., 2011; Lévesque et al., 2011; Park et al., 2008; Prystay and Lo, 2001). Readily available carbon sources (e.g., glucose, fructose, sucrose) for microorganisms have been used to enhance denitrification and sulfate-reduction capacity in carbon-poor effluent (Lévesque et al., 2011; Park et al., 2008). However, these carbon (C) sources are not sustainable at a commercial scale. On the other hand, it has been shown that a complex source of C, such as compost, provides the required carbon for sulfate reduction (Neculita and Zagury, 2008). Furthermore, compost gives the filter media some interesting properties, including disease suppression (Raviv, 2011). However, the accumulation of organic compounds, such as high concentrations of phenolic compounds, in nutrient solutions can have harmful effects on plant growth (Alsanius et al., 2011; Waechter-Kristensen et al., 1999; Yu and Matsui, 1994). Despite the high potentials of AWs to reduce concentrations of ions, such as NO_3^- and SO_4^{2-} and pathogen populations in wastewaters, beneficial or negative effects may occur when treated water is used to irrigate horticultural crops. To investigate the effect of treated water on root rot disease suppression and tomato plant development, three nutrient solutions made with AW

effluents were compared with a control nutrient solution. The in vivo and in vitro potential of treated waters to suppress the development of *P. ultimum* on tomato root was then studied.

Materials and methods

EXPERIMENTAL SET-UP AND OPERATION. The experiment was conducted using a Venlo-type greenhouse (150 m²) located at Université Laval, Québec City, QC, Canada (lat. 46°46'N, long. 71°16'W). Horizontal subsurface flow artificial wetlands (0.6 × 0.4 × 0.35 m) were filled with pozzolana (10–15 mm diameter) and planted with common cattail 14 months before the beginning of the experiment. The wetland plants and microbial biomass were under steady-state conditions (Gruyer et al., 2011). Treated water from the following three types of AWS were compared: 1) wetland units receiving a simple source of C [AWS (2 g·L⁻¹ of sucrose)]; 2) wetland units receiving a complex source of C [AWC (60% w/w of organic materials, dry weight: 10% of maple wood chips, 20% of sawdust from P.W.I. Industries, St-Hyacinthe, QC, Canada, 10% of FERTILO poultry manure from Fafard, St-Bonaventure, QC, Canada, and 20% of leaf compost from the Botanical Garden, Québec City, QC, Canada)]; and 3) wetland units without any source of C (AW), except the organic carbon coming from the root exudates and the microflora. The complex carbon source selected was based on studies conducted by Neculita and Zagury (2008) to ensure optimal sulfate-reducing bacteria activities. The composition of the reactive mixture is important for the efficiency of biological passive systems (Cocos et al., 2002; Zagury et al., 2006). The porosity of the substrate filter media were 47%, 47%, and 28% for AW, AWS, and AWC, respectively, and the hydraulic retention time was fixed at 5 d for each wetland type. Multiple inlet devices, controlled by timers (13 8-min pulses per day), were used to uniformly distribute the influent to each wetland unit. Influent was made daily with 36 g·L⁻¹ calcium nitrate, 15 g·L⁻¹ potassium nitrate, 12 g·L⁻¹ potassium phosphate, 22 g·L⁻¹ magnesium sulfate, 20 g·L⁻¹ potassium sulfate, and 7 g·L⁻¹ potassium chloride to reach 500 mg·L⁻¹ SO_4^{2-} and 300 mg·L⁻¹ NO_3^- ,

which corresponds to the SO_4^{2-} and NO_3^- concentrations often observed in a soilless tomato greenhouse effluent.

PLANT MATERIAL. 'Trust FI' tomato seeds (De Ruiter Seeds, Columbus, OH) were sown on 15 Dec. 2010 in PROMIX-BX-Professional (Premier Tech, Rivière-du-Loup, QC, Canada), a peat-based growing media. On 3 Jan. 2011, tomato seedlings were transplanted in 15-cm (2.5 L) plastic pots containing the same peat-based growing medium. Pots were then placed in the greenhouse for 1 week before the start of the experiment to ensure that the plants were developing normally. Growing conditions remained constant during the course of the 8-week experiment: temperature of 22 ± 0.8 °C (day) and 17 ± 0.9 °C (night); vapor pressure deficit of 1.56 ± 0.08 kPa. Natural daylight for tomato plant was supplemented using high-pressure sodium lamps (100 μmol·m⁻²·s⁻¹) for a 12-h photoperiod. The experimental design was a randomized split-block design with six replications: *P. ultimum* treatments in main plot and treated water nutrient solution treatments in subplots (2 × 4 × 6). Each experimental unit consisted of one plant.

PLANT FERTILIZATION. Plant nutrient solutions were made every day from AW-, AWS-, and AWC-treated waters (Gruyer et al., 2011). Treated waters were adjusted with mineral elements and tap water to obtain similar balanced nutrient solutions (Table 1). Tomato plants received 150 mL/plant per day of 1) conventional nutrient solution made with tap water (control); 2) nutrient solution made with AW-treated water; 3) nutrient solution made with AWS-treated water; and 4) nutrient solution made with AWC-treated water.

PYTHIUM INOCULATION. A pathogenic strain of *P. ultimum* was provided by the laboratory of R. Tweddell (Université Laval, Québec City, QC, Canada). The organism was grown on potato dextrose agar [PDA (Difco Laboratories, Detroit, MI)] at 24 °C. The propagule suspension was prepared from a liquid culture (250-mL flasks) containing 100 mL of potato dextrose broth [PDB (Difco Laboratories)] incubated on a rotary shaker (150 rpm) at 24 °C for 1 week. PDA disks covered with actively growing

Table 1. Composition of the treated water from three types of wetland [artificial wetland with no carbon source (AW), artificial wetland with sucrose (AWS), artificial wetland with compost (AWC)] and the four nutrient solution treatments made with treated water and adjusted with synthetic fertilizers.

Treated water	pH	EC (mS·cm ⁻¹) ^z	Nitrate- nitrogen ⁻ (mg·L ⁻¹) ^z	Amonium- nitrogen ⁺ (mg·L ⁻¹)	Sulfate (mg·L ⁻¹)	Phosphorus (mg·L ⁻¹)	Potassium (mg·L ⁻¹)	Calcium (mg·L ⁻¹)	Magnesium (mg·L ⁻¹)	Sodium (mg·L ⁻¹)	Total organic carbon (mg·L ⁻¹)	Dissolved organic carbon (mg·L ⁻¹)
Tap water	7.72 a ^y	0.25 b	<1 c	<1 b	<1 c	<1 b	<1 b	26 b	5 b	8 b	<1 d	<1 d
AW	6.24 b	1.55 a	39 a	1 b	268 b	26 a	184 a	99 a	58 a	11 ab	7.20 c	7.00 c
AWS	7.15 a	1.51 a	<1 c	8 a	286 b	25 a	195 a	98 a	64 a	12 a	117.40 a	109.70 a
AWC	7.48 a	1.45 a	8 b	<1 b	447 a	22 a	144 a	118 a	69 a	14 a	34.80 b	41.35 b
Irrigation treatments												
Control	5.9	1.98	134	<1	138	46	235	114	35	6	2.00 c	1.80 c
AW	5.6	2.10	130	<1	130	45	269	126	30	6	3.50 c	3.10 c
AWS	5.7	2.08	130	<1	130	44	274	136	30	6	58.70 a	54.85 a
AWC	5.8	2.10	138	<1	138	46	260	120	25	6	8.70 b	10.33 b

^z1 mS·cm⁻¹ = 1 mmho/cm, 1 mg·L⁻¹ = 1 ppm.

^yAny two means within a column not followed by the same letter are significantly different at $P \leq 0.05$ according to Tukey's multiple range test.

mycelium of *P. ultimum* were used to inoculate the flasks (Gravel et al., 2009). According to the experimental design, half of the plants ($n = 24$) of each treatment were inoculated with 50 mL of a propagule suspension of *P. ultimum* (1×10^6 propagules/mL), while the remaining plants ($n = 24$) received 50 mL of sterile water. Plants were inoculated twice, at 2 weeks and 6 weeks after planting.

MYCELIAL GROWTH EFFECTS. To determine the suppressive effect of wetland effluents on the mycelial growth of *P. ultimum*, treated (AW, AWS, AWC) samples were incorporated into PDA at 25%, 50%, and 75% v/v concentrations. Tap water samples were used as a control. The difference in water volume was taken into account when preparing the agar. Treated waters were added and mixed with PDA when temperatures were below 40 °C and immediately poured into petri dishes. In addition, the effect of sterilized wetland-treated water on mycelium growth was evaluated by incorporating autoclaved (120 °C for 20 min) or membrane-filtered (Acrodisc syringe filters, 0.2 µm pore size; Pall Life Sciences, Port Washington, NY) treated waters into PDA (25%, 50%, and 75% v/v), as described above. After the agar had solidified, mycelial disks (5 mm diameter) of the *P. ultimum* obtained from actively growing colonies were placed in the middle of the agar plates. The petri dishes were then incubated at 24 °C for 2 d. Six replications were used for each sample based on completely randomized design. The percentage of inhibition in the radial colony growth was calculated by the following equation: Inhibition = $C - T/C \times 100$, where C = radial growth (millimeters) in the control sample and T = radial growth (millimeters) in the treated sample.

EVALUATION OF ROOT COLONIZATION BY THE PATHOGEN. Root colonization by *P. ultimum* was assessed according to the method described by Chérif et al. (1997). Three grams of tomato roots were rinsed thoroughly and blended in 100 mL of distilled water; 200 µL was then plated on a *Pythium*-selective medium (Jeffers and Martin, 1986). Half of the plants ($n = 24$) were used to evaluate root colonization by the pathogen, and the rest were used for root growth

measurements. Five laboratory replications of each of 24 plants were made to evaluate root colonization by *Pythium*. *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 (Chérif et al., 1997).

EVALUATION OF PLANT GROWTH AND *Chl a* FLUORESCENCE. Plant growth was evaluated 8 weeks after the beginning of the nutrient solution treatments. The number of leaves per plant was counted, and the total leaf area was measured using a leaf area meter (LI-3100; LI-COR, Lincoln, NE). Roots were carefully washed with water to remove the growing medium. Fresh and dry (5 d at 60 °C) weights of leaves, stem, and roots were measured. To compare the effects of treated water and inoculation treatments on plant development, the leaf area ratio [LAR (i.e., leaf area per unit of the whole plant dry biomass), the specific leaf weight [SLW (i.e., leaf weight per unit of leaf area)], and the shoot:root ratio were calculated. *Chl a* fluorescence was measured on six randomized plants per treatment by using a PEA fluorimeter (Plant Efficiency Analyser, King's Lynn, Norfolk, UK) to determine the Fv:Fm ratio as an indicator of plant stress. The third fully developed leaf from the apex was adapted to darkness during 20 min before the *Chl a* fluorescence measurement (van Kooten and Snel, 1990).

EVALUATION OF MICROBIAL ACTIVITY IN THE TOMATO GROWING MEDIUM. For each plant ($n = 48$), the microbial activity of the tomato growing medium was evaluated. Three laboratory replications were done. Total enzymatic activity was measured using fluorescein diacetate [FDA (Sigma®, St Louis)] according to a protocol adapted from Adam and Duncan (2001). The amount of fluorescein produced by the hydrolysis of FDA is directly proportional to the microbial population. Two grams of growing medium were placed in 50-mL conical flasks with 15 mL of 60 mM potassium phosphate buffer at pH 7.6, and 200 µL of FDA (2 mg·mL⁻¹) was added to activate the reaction. Samples were incubated for 30 min at 30 °C under agitation. The reaction was stopped by adding 15 mL of 2 chloroform:1 methanol (by volume), and the aqueous phase was measured

using a spectrophotometer (490 nm, model U-1100; Hitachi, Tokyo).

STATISTICAL ANALYSES. We analyzed the effects of treated water on plant growth and *P. ultimum* suppression on the studied parameters using the SAS Mixed Model procedures (version 9.0; SAS Institute, Cary, NC). When significant ($P \leq 0.05$), means were compared using Tukey's multiple range test.

Results

PYTHIUM ULTIMUM SUPPRESSION. The in vitro growth of *P. ultimum* was significantly inhibited by treated water compared with the tap water control (Table 2). All studied concentrations (25%, 50%, and 75% v/v) had a similar effect on *P. ultimum* suppression. The inhibition rate was higher (up to 12 times) when crude-treated water was used as opposed to sterile and membrane filtered waters. Sterile and membrane filtered waters had similar effects on *P. ultimum* suppression. In the case of sterile, membrane filtered, or crude-treated waters, the inhibition rate was higher ($P \leq 0.001$) for the AWS-treated water compared with AW and AWC. Effects of treated water were similar (2.75% to 10.13%) when treated waters were sterilized or membrane filtered, and no significant differences between AW and AWC were observed. However, when crude water was added, the inhibition rate was 1.4 times greater for AWC-treated water compared with AW-treated water, while AWS was 3.6 to 5 times more efficient at inhibiting *P. ultimum* development. A strong correlation between percentages of inhibition of *P. ultimum* with the dissolved organic carbon (DOC) content in membrane filtered or crude-treated water ($r = 0.81$, $P < 0.008$ and $r = 0.97$, $P < 0.001$, respectively) was noticed. Plant irrigated with AWS- and AWC-treated waters reduced the root *P. ultimum* colonization by 73% and 100%, respectively, compared with the inoculated control plants (Table 3).

PLANT GROWTH. After 8 weeks of treatment, the total leaf area of plants irrigated with a nutrient solution of treated water from AW was higher than for plants irrigated with tap water and treated water from AWS and AWC [14%, 20%, and 19% higher, respectively (Table 4)]. However, no significant ($P \leq 0.05$) effect of *P. ultimum*

Table 2. Effects of the four nutrient solution treatments made with tap water (control), effluent from artificial wetland with no carbon source (AW), effluent from artificial wetland with sucrose (AWS), and effluent from artificial wetland with compost (AWC) on the percentage inhibition of radial mycelial growth of *Pythium ultimum*. Treated water was sterilized by autoclaving, membrane filtered, or used directly without treatment (crude water). Data from the three concentration treatments (25%, 50%, and 75% v/v) were pooled because no concentration effect was observed at $P > 0.05$.

	<i>P. ultimum</i> inhibition (%)
Sterile water ^z	
Control	0.00 f ^y
AW	4.07 e
AWS	7.56 d
AWC	4.45 e
Filter water ^x	
Control	0.00 f
AW	1.80 e
AWS	10.13 d
AWC	2.75 e
Crude water	
Control	0.00 f
AW	17.99 c
AWS	90.25 a
AWC	25.19 b
Probability	0.001

^zWater was autoclaved at 120 °C (248.0 °F) for 20 min.

^yAny two means within a column not followed by the same letter are significantly different at $P \leq 0.05$ according to Tukey's multiple range test.

^xWater was membrane filtered through 0.2 µm (microns) to remove microorganisms.

inoculation or irrigation water treatments was observed for total leaf dry weight, percentage of leaf and stem dry matter, LAR, or SLW. However, the stem dry weight of inoculated plants irrigated with AW-treated waters was higher than that of the uninoculated control plants. In the case of uninoculated plants, the use of treated water significantly reduced root dry weight compared with control plants, while no significant differences were observed between inoculated plants. Similarly, the percentage of root dry matter was significantly lower for uninoculated plants grown with AW-treated water compared with control plants, while no significant differences were observed between the percentages of root dry matter weight of inoculated plants. The shoot:root

Table 3. Root colonization by *Pythium ultimum* in tomato plants irrigated with four nutrient solution treatments made with tap water (control), effluent from artificial wetland with no carbon source (AW), effluent from artificial wetland with sucrose (AWS), and effluent from artificial wetland with compost (AWC). Data are means of six replications ($n = 6$).

	Root colonization by <i>P. ultimum</i> (log cfu/g root) ^z
Uninoculated	0.00
Inoculated	1.40
Control	1.09
AW	0.91
AWS	0.79
AWC	0.00
Uninoculated	
Control	0.00 c ^y
AW	0.00 c
AWS	0.00 c
AWC	0.00 c
Inoculated	
Control	2.19 a
AW	1.83 ab
AWS	1.59 b
AWC	0.00 c
Probability level	
Irrigation treatments	0.001
Inoculation	0.001
Interaction	0.001

^z1 cfu/g = 28.3495 cfu/oz.

^yAny two means within a column not followed by the same letter are significantly different at $P \leq 0.05$ according to Tukey's multiple range test.

ratio was significantly lower (48%) for uninoculated control plants irrigated with tap water compared with plants irrigated with treated waters. Plants inoculated with *P. ultimum* had similar shoot and root growth development to uninoculated plants irrigated with treated waters.

CHL A FLUORESCENCE. Plant inoculation with *P. ultimum* decreased ($P \leq 0.05$) the value of the Fv:Fm ratio (Table 4). The Fv:Fm ratio for plants irrigated with AWC-treated water was also significantly lower than that of control plants. However, no significant difference was observed between plants irrigated with treated waters (AW, AWS, and AWC).

MICROBIAL ACTIVITY IN THE GROWING MEDIUM. The soil microbial population expressed as enzyme activity was evaluated every week, and no time effects were observed. Plant soil

Table 4. Effects of nutrient solution treatments made with tap water (control), effluent from artificial wetland with no carbon source (AW), effluent from artificial wetland with sucrose (AWS), and effluent from artificial wetland with compost (AWC) on performance of tomato seedling characteristics after 8 weeks of treatment. Plant stress indicator expressed as Fv:Fm ratio (maximum quantum efficiency of photosystem II) of tomato plants. Data are means of six replications ($n = 6$).

	Leaf		Total leaf area (cm ²) ^z	Leaf area ratio	Specific leaf wt	Stem		Root		Plant		Shoot:root ratio	Fv:Fm ratio
	Dry wt (g) ^y	Dry matter (%)				Dry wt (g)	Dry matter (%)	Dry wt (g)	Dry matter (%)	Dry wt (g)	Dry wt (g)		
Uninoculated	7.50	9.18	1906	137.06	341.16	4.50	8.85	1.36	7.01	13.36	13.36	9.58	0.8163 a
Inoculated	7.51	9.21	1934	145.67	243.42	4.67	9.73	1.15	6.73	13.33	13.33	11.10	0.8087 b
Control	7.38	9.48	1905 a ^y	142.43	245.46	4.22	8.97	1.61	7.26	13.21 b	13.21 b	9.11	0.8180 a
AW	8.77	8.99	2214 b	151.61	258.49	4.78	9.22	1.01	5.90	14.56 a	14.56 a	12.52	0.8139 ab
AWS	6.99	9.48	1778 a	129.84	234.18	4.49	9.27	1.29	7.37	12.77 b	12.77 b	10.09	0.8117 ab
AWC	6.85	8.82	1785 a	138.02	430.34	4.83	9.64	1.26	7.01	12.94 b	12.94 b	9.64	0.8051 b
Uninoculated													
Control	7.86	10.25	1823	125.18	235.99	4.04 b	8.61	2.26 a	9.13 a	14.16	14.16	5.59 b	0.8194
AW	8.94	8.80	2255	153.88	253.92	4.39 ab	7.62	0.81 b	5.32 b	14.14	14.14	13.83 b	0.8162
AWS	6.55	9.88	1786	130.79	242.73	4.74 ab	9.22	1.36 b	7.77 ab	12.65	12.65	9.36 a	0.8169
AWC	5.93	7.79	1753	134.44	631.99	4.83 ab	9.75	1.31 b	6.53 ab	12.07	12.07	9.56 a	0.8126
Inoculated													
Control	6.91	8.72	1988	159.68	256.81	4.45 ab	9.39	0.97 b	6.01 b	12.33	12.33	12.62 a	0.8167
AW	8.59	9.18	2173	149.34	263.06	5.11 a	10.55	1.21 b	6.47 ab	14.91	14.91	11.22 a	0.8139
AWS	6.55	9.08	1760	128.41	223.92	4.20 ab	9.33	1.22 b	6.96 ab	11.97	11.97	10.82 a	0.8066
AWC	7.97	9.85	1817	143.40	228.86	4.84 ab	9.50	1.21 b	7.49 ab	14.02	14.02	9.73 a	0.7977
Probability level													
Irrigation treatment	NS ^x	NS	0.0006	NS	NS	0.04	NS	0.02	0.02	0.01	0.01	0.05	0.019
Inoculation treatment	NS	NS	NS	NS	NS	NS	NS	0.03	NS	NS	NS	NS	0.012
Interaction	NS	NS	NS	NS	NS	0.04	NS	0.001	0.003	NS	NS	0.003	NS

^z1 g = 0.0353 oz, 1 cm² = 0.1550 in².

^yAny two means within a column not followed by the same letter are significantly different at $P \leq 0.05$ according to Tukey's multiple range test.

^xNonsignificant at $\alpha = 0.05$.

inoculation with *P. ultimum* (15.15 to 18.74 µg fluorescein per gram of soil per hour), irrigation treatments with treated water (18.37 to 16.37 µg fluorescein per gram of soil per hour), or both had no significant effect on the total microbial activity in the tomato rhizosphere.

Discussion

In areas where water resources are scarce, recycled and treated wastewater is being used increasingly for crop irrigation (Angelakis et al., 1999). The accumulation of organic compounds or the microbial population in the recycled nutrient solution treated by passive biological systems such as slow filter or AWs may play an important role for root disease suppression, and at the same time, may have harmful effects on plant growth and development. For greenhouse AW-treated waters, as minerals such as SO_4^{2-} and Na^+ may reach detrimental concentrations for plant growth when the AW design is not optimized for these specific high ion concentrations (N. Gruyer, unpublished data), treated waters have to be blended with freshwater to obtain recommended well-balanced nutrient solution as done in our study. This dilution, however, may reduce the disease suppression potential of the treated water.

In our in vitro assays, we observed that the disease suppressive potential of treated water is the most expressed when the AWs are supplemented with sugar. This suppressive agent is water soluble as it is present in the membrane filtered solution, but is strengthened in the presence of the indigenous microflora (crude-treated water). The biological properties of the treated irrigation water played a major role in the *Pythium* sp. suppression compared with the significantly, but less important role played by their chemical and physical properties. This is shown by the lower inhibition potential of sterilized (4.07% to 7.56%) and membrane filtered (1.80% to 10.13%) treated waters compared with crude treated water (17.99% to 90.25%). The microflora's potential for suppression of root diseases may result from antagonist microorganisms and their compounds produced (Berger et al., 1996; Chen et al., 1998; McPherson et al., 1995; Postma et al., 2000, 2005; Stewart-Wide, 2011). Effluents from slow filters and

artificial wetlands are generally rich in *Bacillus* and *Pseudomonas* sp. (Dénél et al., 2004; Gruyer et al., 2011), which may have an antagonist effect against *Pythium* sp. (Dénél et al., 2004). The main difference between the control nutrient solution (tap water) and treated water nutrient solutions (AW, AWS, and AWC) was the level of total organic carbon (TOC) and DOC. The organic compounds originated from common cattail root exudates, wetland filter media, and the wetland microbial activity. In our study, the plant density was relatively high (nine plants per unit area of 0.08 m²) resulting in a build-up of root exudates in treated water. Furthermore, in contrast to our previous study (Lévesque et al., 2011), the wastewater was treated by only one wetland unit, which limits the wetland efficiency in terms of ions (e.g., SO_4^{2-}) and organic compound removal compared with three units placed in series. To pinpoint the causal agents, chemical characterization of the organic fraction as well as the microbial community composition is needed. In the present study, no qualitative analysis on organic compounds was made. The organic compounds excreted by the plant or by microorganisms could act either antagonistic, neutral, or phytotoxic, depending on concentration and composition. For example, 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid, and pyoluteorin are all antibiotics produced by microorganisms with antagonistic potential, which were found in the nutrient solution of closed tomato systems (Jung et al., 2002). Closed systems equipped with slow filters showed higher amounts of benzoic acid, p-hydroxybenzoic acid, and 2,4-diacetylphloroglucinol. Secondary metabolites such as hydrolytic exoenzymes are also often correlated with the biocontrol of plant pathogen (Brand and Alsanius, 2004; Whipps 2001; Woo et al., 1999).

Our in vivo assay showed for the first time that the use of AW-treated water to irrigate tomato plants significantly reduced the root *Pythium* population (by up to 100% for AWC-treated water) compared with inoculated control plants. Similar results have been reported for closed systems using a slow filter, with the total microflora having a suppressive effect on plant pathogens (Postma et al.,

1999). However, with respect to root colonization of *P. ultimum*, these results do not support the finding of our in vitro assay. When the in vivo and in vitro experiments were compared, no relationship was observed between root colonization by *P. ultimum* and its plate inhibition. This might partly be attributable to the chosen microbiological medium (PDA) in the agar test, which provides a favorable environment for the plant pathogen. However, for inhibition to occur, the causal agent and the pathogen have to be settled in the same site (Whipps 2001). Furthermore, the site of colonization—the rhizosphere—is a dynamic site with respect to carbon economics resulting in multilateral interactions (Lynch and Whipps, 1990). However, when we compared the treated water from two added carbon sources, we observed that a simple C source (sucrose) inhibited *P. ultimum* 90% compared with a complex C source (25%).

These results suggest that treating water through wetlands provided to the wastewater some chemical and biological properties, constituting a preventive means toward *P. ultimum* infection.

In our study, leaf and stem dry weight, as well as LAR, were unaffected by the *P. ultimum* inoculation. This result showed that the microflora inhabiting treated water associated with *P. ultimum* had no significant negative effect on canopy growth. Similar results were obtained in study with tomato plants when both *P. ultimum* and a strain of *Pseudomonas fluorescens* with reinforced capacity for 2,4-diacetylphloroglucinol formation were present (Khalil, 2001). However, plant growth of tomato was affected by the use of AW-treated water. Regardless of the *P. ultimum* inoculation treatments, all plants irrigated with treated water had a higher shoot:root ratio than control plants because of smaller roots. Control plants had a similar shoot:root ratio to those reported in the literature (Heuvelink and Dorais, 2005). Although typical ranges of *Chl a* fluorescence were measured (Bolhar-Nordenkamp and Öquist, 1993), we observed a negative effect of the inoculated and AWC-treated water treatments on the Fv:Fm ratio of dark-adapted leaves, which represents the maximum quantum efficiency of photosystem II. From these results,

we can conclude that *P. ultimum* inoculation, as well as plant irrigation with AW-treated water negatively affected root growth and development of young tomato plants compared with uninoculated control. It is well known that root development is essential for optimal production since a poor root system limits water and nutrient uptake over time. Jung et al. (2004), Waechter-Kristensen et al. (1999), and Yu and Matsui (1994) showed that the presence of organic compounds, such as high concentrations of various phenolic acids, affected root development and growth of tomato. The qualitative and quantitative composition of the organic fraction in the added effluents as well as microbial community composition needs to be characterized to explain the observed root growth limitation in depth.

On the other hand, reducing the organic compounds in treated water that are released from root exudates through the use of several wetland units may decrease the organic effluent (e.g., phenolic compounds) load and consequently the negative impact of treated water on plant growth. However, the reduction in the organic compounds released from the crop and wetland effluents could reduce the disease suppression potential of treated water by up to 10% as shown by a significant effect of sterilized and membrane filtered water on *P. ultimum* (Table 2). In addition, organic compounds from root exudates, along with the C sources added to the wetlands, could also affect the microbial communities and their activities. It is necessary to have a specific knowledge of the compounds that are present and prevailing the environmental conditions (Alsanius et al., 2011). Further studies should be undertaken to establish the metabolic profile of these biological systems and to identify and monitor the microbial community changes from the viewpoint of composition and functionality, so as to obtain scientific knowledge needed for a closed biological and sustainable production system.

Conclusion

This study clearly showed the potential that wetland-treated waters have for the suppression of *P. ultimum*. However, treated water had a negative effect on growth and development of young tomato plants, as evidenced

mainly expressed by root dry weight and consequently by an unbalanced shoot:root ratio.

From our results, we can conclude that AWs-treated greenhouse effluent blended with freshwater and appropriate nutrients can be a sustainable way to manage greenhouse crop irrigation. The potential and the benefits of using AWs as a low-cost and efficient alternative approach for removing nutrient pollutants and pathogens (*Pythium* sp. and *Fusarium* sp.) from greenhouse effluents have been clearly demonstrated (Gruyer et al., 2011; Lévesque et al., 2011). Commercial validations test using AWs-treated water need to be conducted to confirm that there will be no negative effects on long-term crop growth and productivity. Adapted wetland designs in which several units are placed in series and the plant populations are optimized have shown promising results (Lévesque et al., 2011) indicating that this sustainable technology can be applied to the greenhouse industry and horticultural crops.

Literature cited

Adam, G. and H. Duncan. 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.* 33:943–951.

Alsanius, B.W., V. Jung, M. Hultberg, S. Khalil, A.K. Gustafsson, and S. Burleigh. 2011. Sustainable greenhouse systems – The potential of microorganisms. *Acta Hort.* 893:155–167.

Alsanius, B.W., L. Nilsson, P. Jensen, and W. Wohanka. 2001. Microbial communities in slow filters. *Acta Hort.* 548:591–601.

Angelakis, A.N., M.H.F. Marecos do Monte, L. Bontoux, and T. Asano. 1999. The status of wastewater reuse practice in the Mediterranean basin: Need for guidelines. *Water Res.* 10:2201–2217.

Berger, F., H. Li, D. White, R. Frazer, and C. Leifert. 1996. Effect of pathogen inoculum, antagonist density, and plant species on biological control of *Phytophthora* and *Pythium* damping-off by *Bacillus subtilis* Cot1 in high-humidity fogging glass-houses. *Phytopathology* 86:428–433.

Bolhar-Nordenkamp, H.R. and G. Öquist. 1993. Chlorophyll fluorescence as a tool in photosynthesis research, p. 193–206. In: D.O. Hall, J.M.O. Scurlock, H.R. Bolhar-Nordenkamp, R.C. Leegood and S.P. Long (eds.). *Photosynthesis and production in a changing environment:*

A field and laboratory manual. 1st ed. Chapman & Hall, New York.

Brand, T. and B. Alsanius. 2004. Cell wall degrading enzymes in slow filters of closed hydroponic systems. *J. Hort. Sci. Biotechnol.* 79:228–233.

Chen, C., R.R. Bélanger, N. Benhamou, and T.C. Paulitz. 1998. Induced systemic resistance (ISR) by *Pseudomonas* spp. impairs pre- and post-infection development of *Pythium aphanidermatum* on cucumber roots. *Eur. J. Plant Pathol.* 104:877–886.

Chérif, M., Y. Tirilly, and R.R. Bélanger. 1997. Effect of oxygen concentration on plant growth, lipid peroxidation, and receptivity of tomato roots to *Pythium* F under hydroponic conditions. *Eur. J. Plant Pathol.* 103:255–264.

Cocos, I.A., G.J. Zagury, B. Clément, and R. Samson. 2002. Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment. *Water Res.* 36:167–177.

Déniel, F., P. Rey, M. Chérif, A. Guillou, and Y. Tirilly. 2004. Indigenous bacteria with antagonistic and plant-growth-promoting activities improve slow-filtration efficiency in soilless cultivation. *Can. J. Microbiol.* 50:499–508.

Dorais, M. and Y. Dubé. 2011. Closed greenhouse systems: Multiphase recycling of solid and liquid organic waste. *Chron. Hort.* 51:12–19.

Dorais, M., A.P. Papadopoulos, and A. Gosselin. 2001. Influence of electrical conductivity management on greenhouse tomato yield and fruit quality. *Agronomie* 21:367–383.

Ehret, D.L., B.W. Alsanius, W. Wohanka, J.G. Menzies, and R. Utkhede. 2001. Disinfestation of recirculating nutrient solutions in greenhouse horticulture. *Agronomie* 21:323–339.

Gravel, V., C. Ménard, and M. Dorais. 2009. *Pythium* root rot and growth responses of organically grown geranium plants to beneficial microorganisms. *HortScience* 44:1622–1627.

Gruyer, N., M. Dorais, V. Gravel, and G.J. Zagury. 2011. Removal of *Pythium ultimum* propagules in recycled greenhouse nutrient solution using artificial wetlands. *Acta Hort.* 893:1179–1183.

Heuvelink, E. and M. Dorais. 2005. Crop growth and yield, p. 85–144. In E. Heuvelink (ed.). *Tomatoes* (Crop production science in horticulture). CABI Publishing, Cambridge, UK.

Jeffers, S.N. and S.B. Martin. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Dis.* 70:1038–1043.

- Jung, V., L. Chimuka, J.-A. Jonson, N. Niedack, P. Bowens, and B. Alsanius. 2002. Supported liquid membrane extraction for identification of phenolic compounds in the nutrient solution of closed hydroponic growing system for tomato. *Anal. Chim. Acta* 474:49–57.
- Jung, V., E. Olsson, S. Caspersen, H. Asp, P. Jensen, and B.W. Alsanius. 2004. Response of young hydroponically grown tomato plants to phenolic acids. *Sci. Hort.* 100:23–37.
- Kadlec, R.H. and S.C. Wallace. 2009. *Treatment wetlands*. 2nd ed. CRC Press, Boca Raton, FL.
- Khalil, S. 2001. Microflora in the root environment of hydroponically grown tomato: Methods for assessment and effects of introduced bacteria and *Pythium ultimum*. Swedish University of Agricultural Sciences, Uppsala, Sweden, PhD Thesis, Agraria 263.
- Lequillec, S. 2002. La gestion des effluents des cultures légumières sur substrat. Centre technique interprofessionnel des fruits et légumes (CTIFL), Paris.
- Lévesque, V., M. Dorais, V. Gravel, C. Ménard, H. Antoun, P. Rochette, and S. Roy. 2011. The use of artificial wetlands to treat greenhouse effluents. *Acta Hort.* 893:1185–1194.
- Lin, Y.F., S.R. Jing, T.W. Wang, and D.Y. Lee. 2002. Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. *Environ. Pollut.* 119:413–420.
- Lynch, J.M. and J.M. Whipps. 1990. Substrate flow in the rhizosphere. *Plant Soil* 129:1–10.
- McPherson, G., M.R. Harriman, and D. Pattison. 1995. The potential for spread of root diseases in recirculating hydroponic systems and their control with disinfection. *Medicine Faculty Landbouww Universiteit Gent* 60(2b): 371–379.
- Neculita, C.M. and G.J. Zagury. 2008. Biological treatment of highly contaminated acid mine drainage in batch reactors: Long-term treatment and reactive mixture characterization. *J. Hazard. Mater.* 157:358–366.
- Park, J.B.K., R.J. Craggs, and J.P.S. Sukias. 2008. Treatment of hydroponic wastewater by denitrification filters using plant prunings as the organic carbon source. *Bioresour. Technol.* 99:2711–2716.
- Postma, J., B.P.J. Geraats, R. Pastoor, and J.D. van Elsas. 2005. Characterization of the microbial community involved in the suppression of *Pythium aphanidermatum* in cucumber grown on rockwool. *Phytopathology* 95:808–818.
- Postma, J., E.A. van Os, and G. Kritzman. 1999. Prevention of root diseases in closed soilless growing systems by microbial optimization. *Med. Fac. Landbouww. Univ. Gent* 64:431–440.
- Postma, J., M.J. Willemsen-de Kleinand, and J.D. van Elsas. 2000. Effect of the indigenous microflora on the development of root and crown rot caused by *Pythium aphanidermatum* in cucumber grown in rockwool. *Phytopathology* 90: 125–133.
- Prystay, W. and K.V. Lo. 2001. Treatment of greenhouse wastewater using constructed wetlands. *J. Environ. Sci. Health* 36:341–353.
- Raviv, M. 2011. Suppressing soil-borne diseases of container-grown plants using compost. *Acta Hort.* 893:169–182.
- Stanghellini, M.E. and S.L. Rasmussen. 1994. Hydroponics: A solution for zoospore pathogens. *Plant Dis.* 78:1129–1138.
- Stewart-Wide, S.M. 2011. Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: Their detection and management. *Irr. Sci.* 29:267–297.
- Stottmeister, U., A. Wiessner, P. Kusch, U. Kappelmeyer, M. Kästner, O. Bederski, R.A. Müller, and H. Moormann. 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. *Biotechnol. Adv.* 22:93–117.
- van Kooten, O. and J.F.H. Snel. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosyn. Res.* 25:147–150.
- Voogt, W., W.J.M. Cuijpers, P.H.E. de Visser, G.J.H.M. van de Burgt, and A. van Winkel. 2011. Nutrient management in organic greenhouse production: Navigation between constraints. *Acta Hort.* (in press).
- Wächter-Kristensen, B., S. Caspersen, S. Adalsteinsson, P. Sundin, and P. Jensen. 1999. Organic compounds and microorganisms in closed hydroponic culture: Occurrence and effects on plant growth and mineral nutrition. *Acta Hort.* 481: 197–204.
- Whipps, J.M. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Expt. Bot.* 52:487–511.
- Whitmire, S.L. and S.K. Hamilton. 2005. Rapid removal of nitrate and sulfate in freshwater wetland sediments. *J. Environ. Qual.* 34:2062–2071.
- Wohanka, W. 1995. Disinfection of recirculating nutrient solution by slow sand filtration. *Acta Hort.* 382:246–251.
- Woo, S.L., D. Donzelli, F. Scala, R. Mach, G.E. Harman, C.P. Kubicek, G. Del Sorbo, and M. Lorito. 1999. Disruption of the ech42 (endochitinase-encoding) gene affects biocontrol activity in *Trichoderma harzianum* P1. *Mol. Plant Microbe Interact.* 12:419–429.
- Xue, Y., D.A. Kovacic, M.B. David, L.E. Gentry, R.L. Mulvaney, and C.W. Lindau. 1999. In situ measurements of denitrification in constructed wetlands. *J. Environ. Qual.* 28:263–269.
- Yu, J.Q. and Y. Matsui. 1994. Phytotoxic substances in root exudates of cucumber (*Cucumis sativus* L.). *J. Chem. Ecol.* 20: 21–31.
- Zagury, G.J., V.I. Kulnieks, and C.M. Neculita. 2006. Characterization and reactivity assessment of organic substrates for sulphate-reducing bacteria in acid mine drainage treatment. *Chemosphere* 64:944–954.