Control of *Pythium* spp. Root Colonization in Tomato Soilless Culture Through Chlorination of Water Storage Tank

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Abstract

Fungal and Oomycete plant diseases cause economically important losses in soilless cultures by affecting both yield and quality of productions. In these systems, the irrigation water is the main source of introduction of pathogenic microorganisms whose spreading is enhanced by the recycling of the nutrient solution, increasing thus the risk of root disease. To control the disease development, a chemical treatment through chlorination of water supplies was used in this study. The present experiment was conducted over a period of three years during which water and root samples were collected yearly in 8 to 13 tomato soilless greenhouses (8 greenhouses in year 1 and 13 in years 2 and 3). In control storage tanks, the water added to the nutrient solutions was frequently contaminated by *Pythium* spp. Contamination rates ranged from 5 to 30 *Pythium* cfu/liter but were reduced to 0 to 3 cfu/liter after the chlorination treatment of the water. However, these contamination levels were lower than those detected in the flowing nutrient solution samples: around 50 to 150 *Pythium* cfu/liter. During winter, roots of tomato plants in control conditions were weakly colonized by *Pythium* spp. (below 50 cfu/g of roots). These values dramatically increased during spring and summer; around 200 and 600 to 800 cfu were respectively detected per gramm of roots. No root colonization by *Pythium* spp. was detected in winter after the chlorination treatment in the solutions; the colonization level reached around 50 cfu/g of roots in spring and 150 to 200 cfu/g of roots in summer. In conclusion, the chlorination treatment experienced here has been shown to be effective for the disinfection in the solutions and to have a key impact in reducing and delaying the root colonization by *Pythium* spp. Nevertheless, this treatment has the disadvantage of eliminating not only harmful but also beneficial indigenous microorganisms.

INTRODUCTION

Previous reports have clearly demonstrated that the introduction of pathogens inside hydroponic cultivation systems largely depends on the implemented irrigation system (Ehret et al., 2001; Stanghellini and Rasmussen 1994). In the last decade,

growers have been encouraged to adopt « closed » systems to minimize pollution by re-using the run-off solution. However, such systems increase the risks of pathogen spread in the recycled nutrient solution. For instance, it has been demonstrated that oomycetes of the genus *Pythium*, *Phytophthora*, are particularly well adapted to hydroponics. Zoospores can swim in water supplies and the flowing of nutrient solutions facilitates the root infections (Favrin et al., 1988; McPherson et al., 1995). Minor pathogens, i.e. *Pythium dissotocum* (or *Pythium* group F), and highly pathogenic *Pythium* species, i.e. *Pythium ultimum*, *P. irregulare* and *P. aphanidermatum*, induced higher yield losses compared to infections in field (Rafin and Tirilly, 1995; Rey et al., 1998; Stanghellini and Rasmussen, 1994). As a consequence, preventing pathogenic infections by appropriately disinfecting the nutrient solutions used in soilless cultures has become a major challenge. Several approaches have been developed for disinfecting nutrient solutions, i.e. heat treatment, ozonisation, ultra-violet (UV) radiation (Ehret et al., 2001; Rey et al., 2001). However, although effective, most of these strategies require high investment and operating costs.

In the present paper, experiments have been carried out to assess the potential use of a chemical treatment, i.e. chlorination, to control *Pythium* spp. colonization in several commercial tomato soilless greenhouses. Our aim was to assess the effectiveness of chlorine disinfection to eliminate *Pythium* spp. from the solutions and subsequently to reduce and/or to delay the contamination of tomato roots.

MATERIALS AND METHODS

Experimental design

The experiment was conducted over a 3-year period in 8 to 13 commercial hydroponic greenhouses in Brittany (France), i.e., year n°1: 8 greenhouses, years n°2 and n°3: 13 greenhouses (table 1). Except in control greenhouses (year n°1: 5 greenhouses, years n°2 and n°3: 9 greenhouses) where water supplies were not disinfected, chlorine treatment was used in each of the other greenhouses to disinfect solutions in the storage tanks and in the irrigation systems (year n°1: 3 greenhouses, years n°2 and n°3: 4 greenhouses). The injection of hypochlorous acid (HClO) was proportional to the water flow-rate in order to obtain a concentration of 2 mg per liter of active chlorine.

Root sampling in different commercial soilless greenhouses

Root samples from naturally infected tomato plants grown hydroponically were collected during three consecutive cultural seasons. Depending on the greenhouses investigated, two different kinds of substrates were employed: organic peat or inorganic rockwool. Root samples were taken from greenhouses equipped with either an open or a closed system for nutrient supply.

Root colonization by *Pythium* spp. was determined from samples collected at three different periods, i.e. the first in winter, the second in spring (April or May) and the third in summer (June or July). Each sample consisted of roots taken from 3 randomly chosen peat or rockwool slabs.

Water sampling in different commercial soilless greenhouses

Similarly to root samplings, solution colonization by *Pythium* spp. was determined from samplings carried out at three different periods, i.e. in winter, in

spring (April or May) and in summer (June or July). To assess the effectiveness of the chlorination treatment to destroy *Pythium* spp., solutions were sampled from the irrigation systems and from the storage tanks.

Assessement of tomato roots and water colonization by *Pythium* spp.

Roots were prepared as follows: 3 grams per sample were mixed with 100 ml sterile water in a Stomacher (AES laboratories, France). Subsequently, 200 μl of this solution were plated on a selective *Pythium* isolation medium coded CMA-PARP. This experiment was conducted in duplicate. After 48-hour incubation in the dark at 25°C, *Pythium* thalli were counted. The results were expressed as colony forming unit (cfu) per gram of root.

For solution samplings, 150 ml of irrigation solution flowed through a 0.45μm filter. This was made in duplicate. The filters were then deposited on the selective medium, CMA-PARP. After 48-hour incubation in the dark at 25°C, *Pythium* thalli were counted. The results were expressed as colony forming unit (cfu) per liter of solution.

Statistical analysis of the results

Experimental data were statistically analyzed with the Least Significant Difference's multiple range test (LSD) at P = 0.05 level of confidence by StatGraphics, release 4.0 statistical package (Manugistic Inc., Rockville, USA).

RESULTS

***Pythium* spp. contamination of irrigation solutions and storage tanks**

Solutions taken from control storage tanks were regularly contaminated by *Pythium* spp., whatever the month of sampling. During the cultural season, with the exception of one sample, i.e. print-sample from the year n°1, the oomycete concentrations were stable and ranged from around 10 to 20 cfu/l (Fig. 1). The data in table 2 show the very high efficacy and stability over time of the chlorination treatment at eliminating *Pythium* spp. In the winter and spring times, *Pythium* spp.hyphae were frequently not detected in the water samplings. The highest values were obtained in the summer period with a detection of 2 to 3 cfu/l.

Pythium spp. were regularly detected in the flowing irrigation solutions from control greenhouses. Indeed, the 3-year experimental data indicate that the level of colonization in winter and summer was relatively similar, it ranged from around 10 to 20 cfu/l (Fig. 2). However, the values differ markedly in spring from one year to another.

After chlorination of the irrigation solutions, for the 3 years of the experiment and the 3 periods of sampling, a significant decrease in *Pythium* populations was observed. In year n°1, values were always lower than 3 cfu/ml in the solutions; in year n°2, a few *Pythium* spp. hyphae were detected only in the summer, i.e. around 3 cfu/ml, whereas they were not detected at all in year n°3.

***Pythium* spp. root colonization**

In control greenhouses, the irrigation solutions were not treated with chlorine. From winter to summer, a regular increase in root colonization by *Pythium* spp. was measured. The same trend in *Pythium* spp. root colonization was noticed after treatment of the irrigation solutions with chlorine. However, in the latter case, a marked reduction in root colonization was pointed out (Fig. 3).

In the winter, a few hyphae (around 8 cfu/g) colonized the roots in the control greenhouses in year n°2 and n°3. But, whatever the year of the experiment, hyphae were not detected in the greenhouses with chlorine-treated solutions. In the spring, *Pythium* spp. root colonization ranged from around 100 to 300 cfu/g in control greenhouses and to around 10 to 50 cfu/g in the greenhouses using chlorine-treated solutions. In the summer, around 400 to 1000 cfu/g of roots were measured in the control soilless cultures and around 40 to 110 cfu/g in the greenhouses using chlorine to disinfect the flowing solutions.

DISCUSSION

The experiments reported in this paper evidenced that, from the winter-start to the summer-mid cultural season, solutions from the irrigation systems and the storage tanks of several commercial greenhouses were contaminated by *Pythium* spp. In the storage tanks, contamination rates ranged from 5 to 30 *Pythium* cfu/liter but were reduced to 0 to 3 cfu/liter after the chlorination treatment of the water. However, these contamination levels were lower than those detected in the flowing nutrient solution samples: around 50 to 150 *Pythium* cfu/liter. Again, chlorination treatment induced a dramatic decrease in *Pythium* populations colonizing these solutions. Indeed, *Pythium* counts varied from 0 to 3 cfu/liter.

In a previous experiment, Rey et al. (2001) evidenced two steps in the development of *Pythium* spp. on roots in soilless cultures in Brittany (France). The first one generally took place from winter-crop start to end of spring. *Pythium* spp. were frequently detected, though at a low level. *Pythium* spp. populations dramatically increased in the summer; this increase was sometimes associated with root necrosis and root rot but generally infections were limited to root necrosis and were even symptomless. Our data are in line with the ones obtained by Rey et al. (2001) since we pointed out that during winter, roots of tomato plants in control conditions were weakly colonized by *Pythium* spp. (below 50 cfu/g of roots), then these values regularly increased during spring and summer; i.e. around 200 and 600 to 800 cfu/g of roots, respectively. In our opinion, in the present experiment, the main finding is that chlorination treatment of the solutions has a key impact in reducing and delaying the root colonization by *Pythium* spp. Indeed, no root colonization by *Pythium* spp. was detected in the winter and the colonization level reached around 50 cfu/g of roots in the spring and 150 to 200 cfu/g of roots in the summer.

In the literature, similar results have been reported after using the so-called "active" methods to disinfect the nutrient solutions (Ehret et al., 2001; Runia, 1995). For instance, UV-radiation and heat treatment can eliminate up to 99% of the microflora colonizing the flowing solutions. Tirilly et al. (1997) reported that these disinfecting methods can delay *Pythium* root infection in soilless culture; however, in some cases root colonization is similar to that observed in other non disinfected-greenhouses. In fact, re-contamination of disinfected-nutrient solution has been frequently reported, which nullifies the effect of disinfecting methods (Déniel et al., 1999). According to Van Os et al. (2004), after disinfection of nutrient solutions, the microbial differences in the treated solutions often disappeared after their flow in the rockwool slabs containing plant roots. Such a phenomenon was not observed in our 3-year experiment since a delay in root colonization by *Pythium* spp. was observed each year. However, one may assume that this phenomenon may occur after chlorination treatment, since it can also eliminate the beneficial indigenous microorganisms. Further investigations carried out at determining how chlorination

may affect the potential suppressive microflora colonizing the nutrient solutions and roots of plants grown in soilless cultures (Postma et al., 2005) have to be done.

Literature cited

Déniel, F., Rey P. and Tirilly Y. 1999. Cultures hors-sol : désinfection des solutions recyclées. Fruits Leg. 172:73-75.

Erhet, D.L., Alsanius, B., Wohanka, W., Menzies, J.G. and Utkhede R. 2001. Disinfestation of recirculating nutrient solutions in greenhouse horticulture, Agronomy. 21:323-339.

Favrin R.J., Rahe J.E., and Mauza B. 1988. *Pythium* spp. associated with crown rot of cucumbers in British Columbia greenhouses. Plant Dis. 72 :683-687.

McPherson, G.M., Harriman, M.R. and Pattison D. 1995. The potential for spread of root diseases in recirculating hydroponic systems and their control with disinfection, Med. Fac. Landbouww. Univ. Gent. 60/2b:371-379.

Postma, J., Geraats, B.P.J., Pastoor, R. and van Elsas, J.D. 2005. Characterization of the microbial community involved in the suppression of *Pythium aphanidermatum* in cucumber grown on rockwool, Phytopathology. 95:808-818.

Rafin C. and Tirilly, Y. 1995. Characteristics and pathogenicity of *Pythium* spp. associated with root rot of tomatoes in soilless culture in Brittany, France. Plant Pathol. 44:779-785.

Rey, P., Benhamou, N. and Tirilly, Y. 1998. Ultrastructural and cytochemical investigation of asymptomatic infection by *Pythium* sp. Phytopathology. 88:234-244.

Rey, P., Déniel, F., Vasseur, V., Benhamou, N. and Tirilly, Y. 2001. Evolution of *Pythium* sp. populations in soilless cultures and their control by active disinfecting methods. Acta Hort. 554:341-348.

Runia, W.Th. 1995. A review of possibilities for disinfection of recirculation water from soilless cultures. Acta Hort. 382:221-229.

Stanghellini, M.E. and Rasmussen, S.L. 1994. Hydroponics, a solution for zoosporic pathogens. Plant Dis. 78:1129-1138.

Tirilly, Y. and Letard L. 1997. Maîtrise sanitaire des solutions nutritives en cultures hors sans sol. Infos-Ctifl. 132:35-39.

Van Os, E.A., Bruins, M., Postma, J. and Willemsen-de Klein, M.J.E.I.M. 2004. Investigations on crop developments and microbial suppressiveness of *Pythium aphanidermatum* after disinfection treatments of the circulating nutrient solution, Acta Hort. 644:563-570.

Table

Table 1. Number of greenhouses in these experiment

	First year	Second year	Third year
Control greenhouse	5	9	9
Greenhouse with chlorine treatment	3	4	4

Table 2. Contamination of storage tank solutions by *Pythium* spp. after chlorination treatment of irrigation solutions from soilless greenhouses.

Sampling period	Winter (January/February/ March)	Spring (April/May)	Summer (June/July)
First year (3 greenhouses)	0 ± 0	0 ± 0	0 ± 0
Second year (4 greenhouses)	0 ± 0	0 ± 2	3 ± 7
Third year (4 greenhouses)	1 ± 0	1 ± 2	2 ± 3

Pythium spp. thalli were counted on the selective media coded CMA-PARP after a 48-h incubation of the plates at 25°C in the dark. Results are expressed in cfu/l and they represent the mean ± sd (standard deviation).

Figures

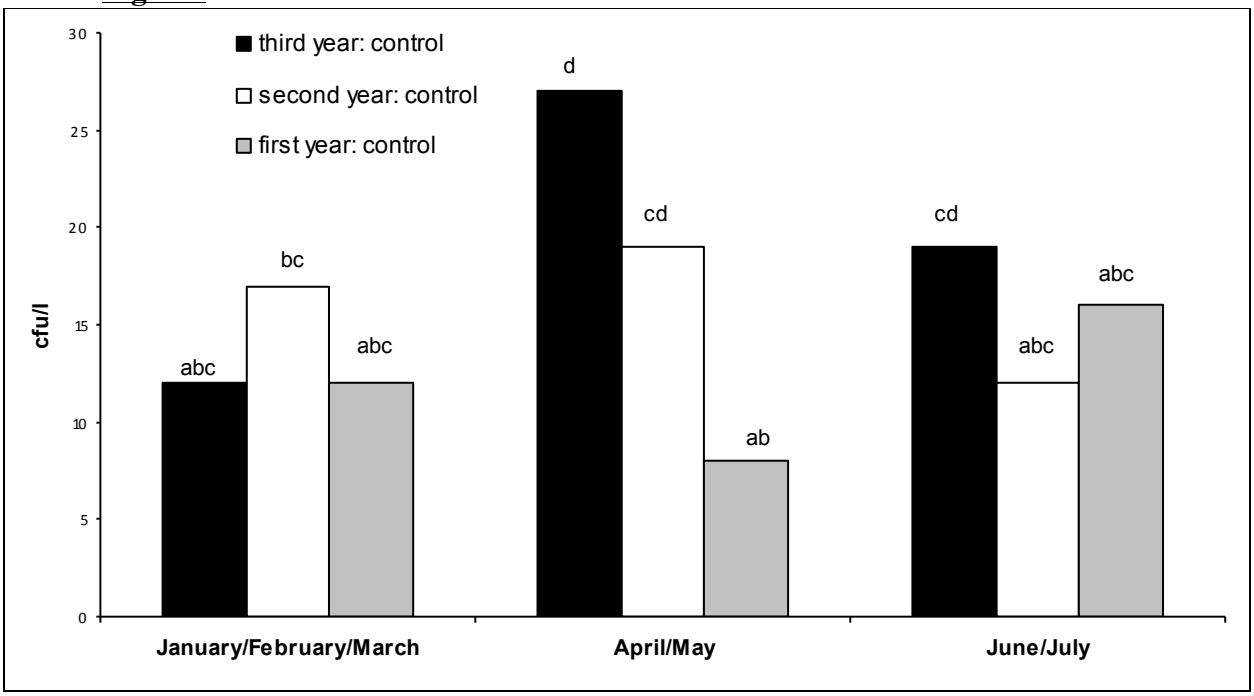

Fig. 1. Contamination of storage tank solutions by *Pythium* spp. in control soilless greenhouses (year n°1: 5 greenhouses, years n°2 and n°3: 9 greenhouses). Results are expressed in cfu/l. Columns with a same letter are not significantly different at p = 0.05% (LSD test).

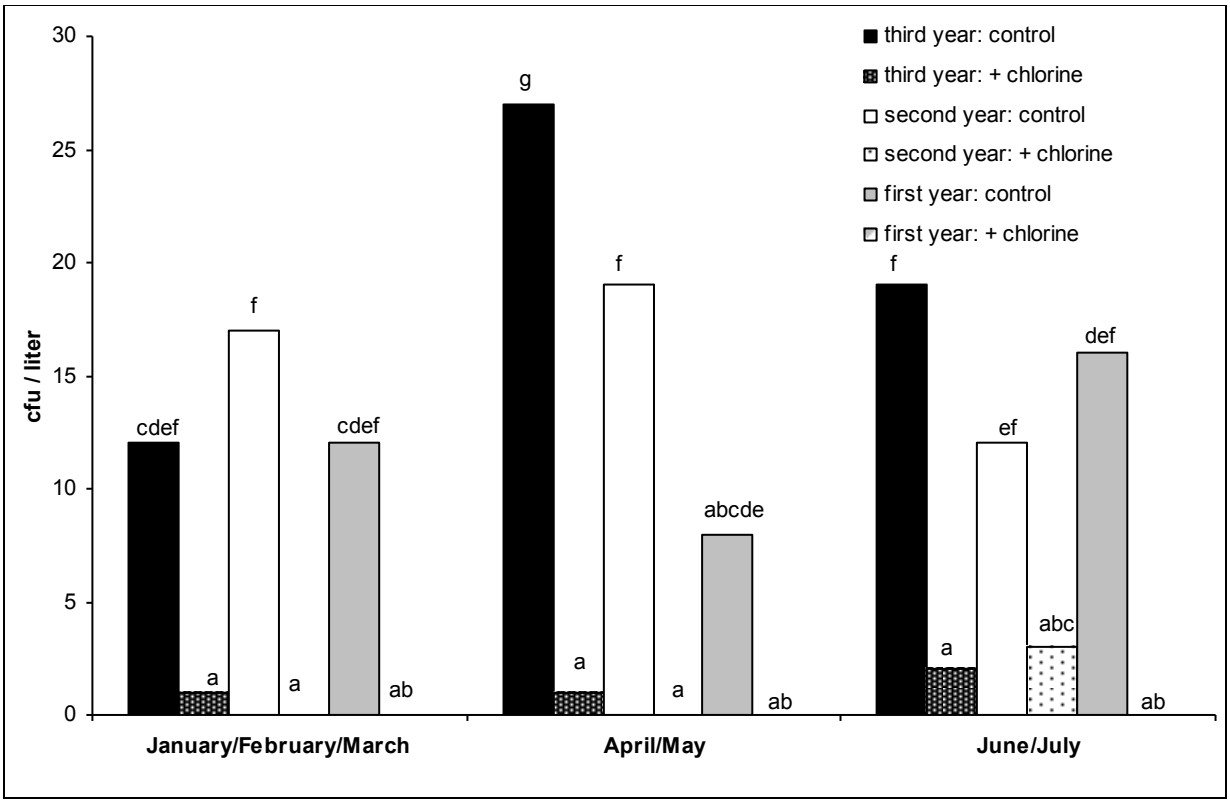

Fig. 2. Contamination by *Pythium* spp. in control- and chlorine irrigation-solutions from soilless greenhouses. Results are expressed in cfu/l. Columns with a same letter are not significantly different at p = 0.05% (LSD test).

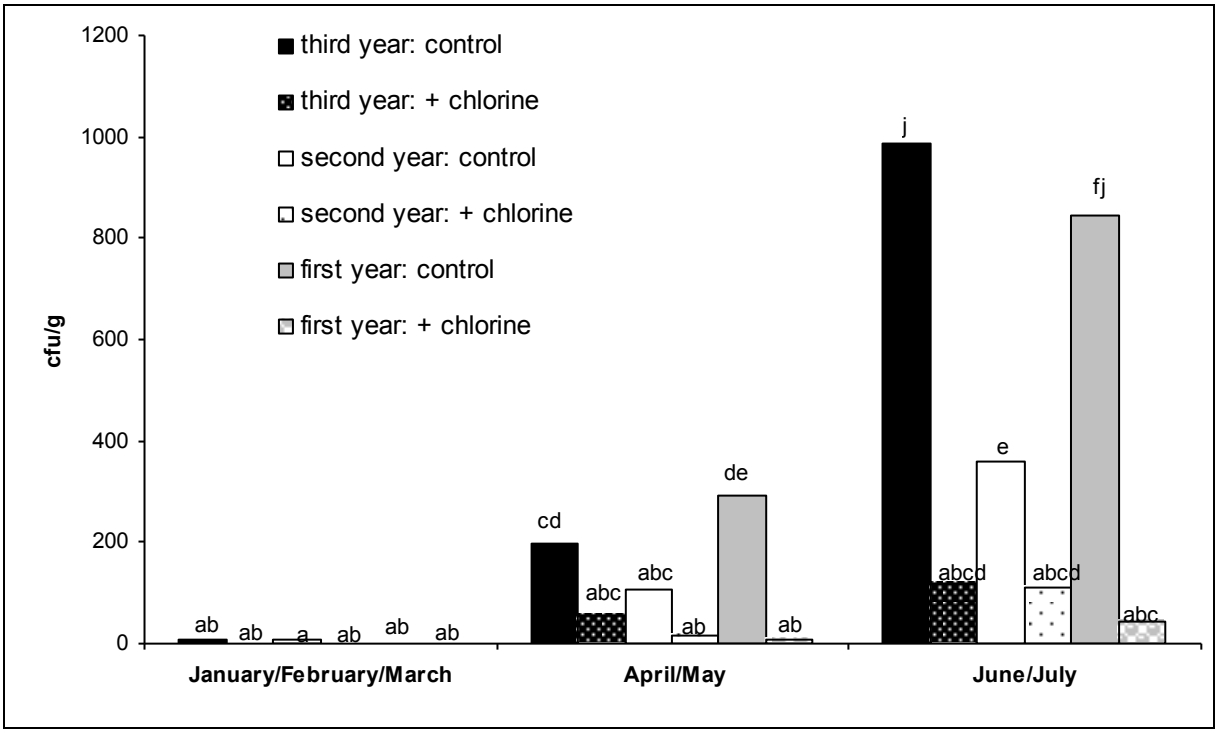

Fig. 3. Root contamination by *Pythium* spp. in soilless greenhouses. The irrigation solutions were either treated with chlorine or not. Results are expressed in cfu/g of roots. Columns with a same letter are not significantly different at p = 0.05% (LSD test).