Phytopathol. Mediterr. (2003) 42, 55–64

Selected microbial strains suppress *Phytophthora cryptogea* in gerbera crops produced in open and closed soilless systems

Valeria Grasso, Andrea Minuto and Angelo Garibaldi

Centre of Competence for the Innovation in the Agro-environmental Sector, AGRINNOVA, Università, Via Leonardo da Vinci 44, 10095 Grugliasco (Torino), Italy

Summary. Several micro-organisms (*Fusarium* spp., *Trichoderma* spp., Oomycetes and bacteria) were isolated from the rhizosphere of gerbera plants grown in an open soilless system. Their capacity to suppress root rot caused by *Phytophthora cryptogea* was evaluated in 2000–2001 in three independent trials in open soilless systems. Different strains reduced disease incidence and/or increased flower production. The most effective strains (three of *Fusarium* and three of *Trichoderma*) were also tested in a closed soilless system in combination with slow sand filtration, in order to determine whether these two techniques together would be more effective in reducing *P. cryptogea* root rot. The results showed that slow sand filtration with antagonistic micro-organisms operated synergistically to significantly reduce disease incidence.

Key words: root rot, ornamental crop, biological control, nutrient solution, slow sand filtration.

Introduction

An intense use of methyl bromide (MB) has been made in Italy for soil fumigation, especially due to intensive production of vegetables and ornamentals in this country (Garibaldi and Gullino, 1995; Minuto *et al.*, 2002). In recent years, concern about damage to the ozone layer has led to MB being included in the list of ozone-depleting substances controlled by the Montreal Protocol (Bell *et al.*, 1996; Ristaino and Thomas, 1998). The strict rules that now limit its usage, before it is phased-out, have stimulated the search for methods to reduce dosage and consequently emission of MB into the atmosphere (Gamliel *et al.*, 1997; Minuto *et al.*, 1999).

The soilless growing system represents a viable way to reduce MB use (Garibaldi and Gullino, 1995; Van Os, 1999). Since it renders crop management more expensive, soilless cultivation is generally most suitable for high-value crops such as rose, carnation, gerbera, basil, tomato, lettuce, sweet pepper and strawberry (Serra, 1994; Tognoni and Serra, 1994). In Italy soilless cultures are being increasingly adopted for ornamental crops such as rose, gerbera and carnation (Tognoni, 2003).

Soilless systems are often open, without recirculation of the nutrient solution. One of the main constraints of "run-to-waste" systems is their high environmental impact, since large amounts of water and fertilizers are drained into the environment. Closed systems seem to offer a better solu-

Corresponding author: V. Grasso
Fax: +39 011 6708541
E-mail: valeria.grasso@unito.it

tion, but are not realistic without some strategies to prevent dispersal of root-infecting pathogens through the recirculating nutrient solution (Garibaldi *et al.*, 2001).

Zoosporic fungi such as species of *Pythium* and *Phytophthora* cause major crop losses in soilless cultivations, since these systems foster the dissemination and spread of such water-borne micro-organisms (Stanghellini and Rasmussen, 1994). Gerbera is one of the main ornamental crops grown in soilless conditions in Italy and *Phytophthora cryptogea* is responsible for a severe root rot that can cause serious losses in this crop (Garibaldi, 1978; Garibaldi *et al.*, 2000).

In the last few years, researchers have become aware that some micro-organisms present in the nutrient solution may have a role in suppressing diseases (Postma *et al.*, 1999 and 2000; Tu *et al.*, 1999; Van Os and Postma, 2000). If a biological vacuum occurs (for example, after disinfection of the nutrient solution by UV or heating), the nutrient solution may lose this suppressiveness and an explosive outbreak of disease may be a consequence. It is therefore good practice to foster the resident microflora and to utilize disinfection methods that do not harm it (Postma *et al.*, 2001). Moreover, soilless growing systems normally have a low microbial buffering capacity because they are composed of inert materials; they thus favour the establishment of biocontrol agents applied at the start of the cropping season (Paulitz and Bélanger, 2001). Antagonists for use in soilless cultures should be selected from the microflora indigenous to these growing systems, and should be adapted to the conditions used for cultivating the crop. Micro-organisms have particular niches to which they are adapted: if they have to control root-infecting pathogens such as *P. cryptogea*, antagonists should be able to live in the rhizosphere and interact with the pathogens at the infection sites on the root surface: such ability is often called rhizosphere competence (Ahmad and Baker, 1987; Nemec *et al.*, 1996).

The aims of the present study were (1) to select from the rhizosphere of gerbera plants micro-organisms well adapted to soilless conditions, and (2) to evaluate the capacity of these organisms to suppress *P. cryptogea*, responsible for root and crown rot on gerbera grown in open systems. The most effective antagonistic strains were then further tested in a closed system in combination with slow sand filtration, to determine whether this combined disinfestation procedure would be more effective in reducing *P. cryptogea* root rot than the antagonistic approach alone.

Materials and methods

Isolation of micro-organisms from gerbera rhizosphere

Several gerbera plants, 150 days old and grown on a pumice and peat substrate (80:20 v:v of granulated pumice, size 3–7 mm, and Irish peat, size "tout-venant" 0–30 mm) previously steam-disinfected at 80°C for 15 min, were grown in open soilless systems at the Agricultural Experimental Centre of Albenga (northern Italy). From these systems, three types of plants (three plants per type) were collected: healthy plants grown in a soilless system and *P. cryptogea*-free; plants inoculated with *P. cryptogea* but apparently healthy; and inoculated plants showing clear root-rot symptoms.

Micro-organisms were extracted from the rhizosphere and the rhizoplane by shaking 20 g of roots from each of these plants on a rotary shaker for 20 min in a flask containing 200 ml of sterile deionized water to which was added 0.3 ml l^{-1} of Agral, a non-ionic surfactant based on nonilphenol condensed with ethylen oxide (a.i. 12%). After shaking, a 200 ml aliquot of serial diluted suspension, in the range of 10^{-2} to 10^{-5}, was plated onto Petri dishes (9 cm diameter) containing 10 ml of media that were selective for *Fusarium* (Komada, 1975), *Trichoderma* (Elad *et al.*, 1981) or oomycetes (Masago *et al.*, 1977). Moreover, potato dextrose agar (PDA; 39 g PDA in 1 l of deionized water, autoclaved at 120°C for 15 min), amended after autoclaving with 0.5 g of terramycin, was used to isolate the fungi, and nutrient yeast dextrose agar (NYDA, 20 g agar, 8 g nutrient broth, 4 g yeast extract, 1.5 g glucose in 1 l of deionized water, autoclaved at 120°C for 15 min) was used to isolate bacteria. Five replicate plates of each medium were inoculated for each dilution; plates were incubated at 28°C. After 48 hours, different single colonies were selected randomly from these agar plates and stored at 4°C in tubes on an appropriate medium. The isolates were assigned to the genera *Fusarium* or *Trichoderma* or to the class of oomycetes on the basis of their colony morphology and

the selective medium from which they were isolated. For bacterial isolates genera were not determined. All strains were divided in four groups ("F" for *Fusarium*, "T" for *Trichoderma*, "OO" for oomycetes and "B" for bacteria) and, in each group, single isolates were numbered in progressive order: from F1 to F26, from T1 to T15, from OO1 to OO12, from B1 to B17.

Greenhouse trials for evaluation of the antagonistic effect of isolated micro-organisms

Trials in open soilless systems

All isolates were screened individually in three different trials (Table 1) to identify potential antagonists of *P. cryptogea*. In the first trial, 12 *Trichoderma* (T1–T12), 12 *Fusarium* (F1–F12), 4 oomycetes (OO1–OO4) and 3 bacterial (B1–B3) isolates were tested. In the second trial the effectiveness of 3 *Trichoderma* (T13–T15), 14 *Fusarium* (F13–F26), 8 oomycetes (OO5–OO12) and 14 bacteria (B4–B17) was evaluated. The most effective strains found were tested again in the third trial. These trials were carried out *in vivo* on gerbera plants (cv. Alberino for the first trial; cv. Orange Dino for the second trial; cv. Amazzone for the third trial) grown in plastic pots (container type, 19 cm diameter, 3.5 l volume) in an open soilless growing system.

Fusarium and *Trichoderma* strains were propagated in flasks on wheat (300 g of wheat kernels in 320 ml of deionized water, autoclaved at 120°C for 30 min); while oomycetes were propagated on wheat plus hemp (200 g of wheat kernels and 100 g of hemp kernels in 320 ml of deionized water, autoclaved at 120°C for 30 min). All strains were incubated for at least 20 days at 20°C. Bacterial isolates were grown in flasks each containing 250 ml of a liquid medium (10 g bacto-tryptone, 5 g yeast extract and 10 g NaCl, pH 7.5, autoclaved at 120°C for 15 min) and shaken for 48 hours.

To test the antagonistic activity of *Fusarium*, *Trichoderma* and oomycetes isolates, 1 kg of fungal formulation was added to 100 l of substrate, obtained by mixing 80:20 v:v of granulated pumice with peat, previously steam-disinfected at 80°C for 15 min. Gerbera plants, 60–75 days old, grown in peat pots of 4×4×5 cm, were transplanted 3–12 days after the addition of micro-organisms to the growth medium. To test the antagonistic activity of the bacterial strains, gerbera roots were dipped in a bacterial suspension (5×10^8 cfu ml^{-1}) before being transplanted to pots filled with the steam-sterilised substrate mixture. Treatment with each fungal and bacterial strain was randomised within three independent blocks and consisted of 10 plants per replicate. Thirty plants not treated with any micro-organisms were used as control. The plants were grown in an open soilless system on greenhouse benches and irrigated/fertilized by a drip irrigation system (one water emitter per plant; flow rate 6 l h^{-1}). The crop was irrigated two or three times a day and fertilised with 0.5 g l^{-1} of a 1:1:1 ($N:P_2O_5:K_2O$) nutrient solution. All pots were suspended on plastic containers 5 cm high to collect the drain water easily without risk of contact between gerbera pots.

Pathogen inoculation

Phytophthora cryptogea used to inoculate plants was previously isolated on a selective medium for oomycetes (Masago *et al.*, 1977) from a naturally infected gerbera plant. Specific morphological structures were observed under the light microscope. After re-inoculation in healthy plants, this isolate reproduced the typical symptoms of *P. cryptogea* root rot. The pathogen was inoculated once a week starting 4–6 weeks after transplanting, by putting a zoospore suspension ($0.5–1\times10^2$ cfu ml^{-1})

Table 1. Dates of gerbera transplantation, applications of the antagonists and pathogen inoculations in the open and closed soilless system trials (Albenga, 2000–2001).

System	Trial No.	Application of antagonists	Gerbera transplantation	Pathogen inoculation
Open	1	10.03.2000	20.03.2000	02.05.2000
	2	10.05.2000	13.05.2000	15.06.2000
	3	12.04.2001	24.04.2001	25.05.2001
Closed	1	20.01.2001, 30.03.2001	19.12.2000	30.01.2001, 16.03.2001

directly into the drip irrigation system. Each pot, previously irrigated with 100 ml of water, was inoculated with 50 ml of zoospore suspension and then irrigated again with 50 ml of water. This suspension was obtained by incubating at 24°C for 3–4 days mycelium of *P. cryptogea* in Petri plates (20 cm diameter) with a soil broth. To obtain this broth, 250 g of soil was suspended in 1 l of deionised water and shaken for 12 h. The suspension was filtered and centrifuged at 11,000 *g* for 15 min. The supernatant was autoclaved at 120°C for 15 min and the pH was adjusted to 5.0–5.5 with nitric acid.

Assessment of disease severity and flower production

The effect of micro-organisms on disease incidence (percentage of diseased plants), crop yield (flowers per healthy plant) or both (flowers per m^2) was assessed. The antagonistic effect against *P. cryptogea* was evaluated by counting the diseased plants. The flowers produced by each plant were harvested and counted every 10–15 days as well. Crown sections of diseased plants were surface-disinfected and plated on a selective medium for oomycetes (Masago *et al.*, 1977) at 28°C for 48 h to check for *P. cryptogea*.

Trial in closed soilless systems

Gerbera plants cv. Red Dino, 75 days old, grown in peat pots of 4×4×5 cm, were transplanted to plastic pots (19 cm diameter, 3.5 l volume) filled with a substrate consisting of a mixture of 80:20 v:v of granulated pumice and peat, previously steam-disinfected at 80°C for 15 min. The plants were placed on concrete benches with drip irrigation (one water emitter per plant, flow rate 6 l h^{-1}) in a closed system. Details on water, fertilizers and nutrient solution used in the trial are shown in Table 2.

Sand filtration

A slow sand filtration system (Fig. 1) was constructed as described by Wohanka (1995) and was tested for its efficacy in disinfecting the recirculated nutrient solution. The sand was obtained from a mine deposit crushed and sieved and not from a riverbed. The drain water was pumped into the sand filter only while the plants were being irrigated, maintaining a water layer of 35 cm. The effluent flow rate from the filter was adjusted by a flow-meter located between the filter and the disinfected-water storage tank, obtaining a final flow rate of 200 l $(m^2\ h)^{-1}$.

Further trials of most effective antagonistic strains

Three *Fusarium* (F7, F10, F19) and three *Trichoderma* (T5, T6, T13) strains, previously selected in open soilless system trials for their potential antagonistic activity against *P. cryptogea*, were tested in a closed soilless system provided with a sand filter.

The antagonistic strains were incubated on casein liquid medium for 10–15 days. The *Fusarium* isolates were cultured on a rotary shaker and then centrifuged (10,000 *g* for 25 min). Before application, the microbial isolates were mixed together: F7 with F10 and F19, and T5 with T6 and T13. These antagonistic suspensions were applied twice: first in the filtered solution storage tank (200 l of water, Fig. 1) to obtain a final concentration of 1×10^5 cfu ml^{-1}; and then by suspending the antagonists in 50 l of water (final concentration 5×10^6 cfu ml^{-1}) and applying the suspension directly to each cultivation pot (0.5 l per pot). The treatment with each microbial mixture was randomised within three independent cultural rows and consisted of 30 plants per replicate. Details on gerbera transplantation, applications of antagonists and pathogen inoculation are shown in Table 1.

Table 2. Details on water, fertilizers and nutrient solution used in the closed soilless system trial (Albenga, 2000–2001).

Nutrient solution (1 g l^{-1}) [a]		Mineral content in the water[b] and EC[c]
Fertilizer (%)	N total, 23	HCO^{3-}, 4.1 mmol l^{-1}
	NO_3, 11.5	Ca + Mg, 2.6 mmol l^{-1}
	NH_4, 11.5	NO_3-N, 0.3 mmol l^{-1}
	P_2O_5, 6	Na, 0.4 mmol l^{-1}
	K_2O, 10	Ca, 2.0 mmol l^{-1}
	MgO, 2.5	Mg, 0.7 mmol l^{-1}
		Cl, 0.3 mmol l^{-1}
pH	6.75	SO_4, 0.5 mmol l^{-1}
EC[c]	1.15 dS/m	Zn, 1.1 µmol l^{-1}
		Cu, 0.0 µmol l^{-1}
		EC[c], 0.51 dS/m

[a] Scotts Universol, The Scotts Company, Marysville, Ohio, USA.
[b] Analysis provided by Dr Wohanka and Dr Molitor, Geisenheim Research Station, Geisenheim, Germany (24.01.01).
[c] EC, electrical conductivity.

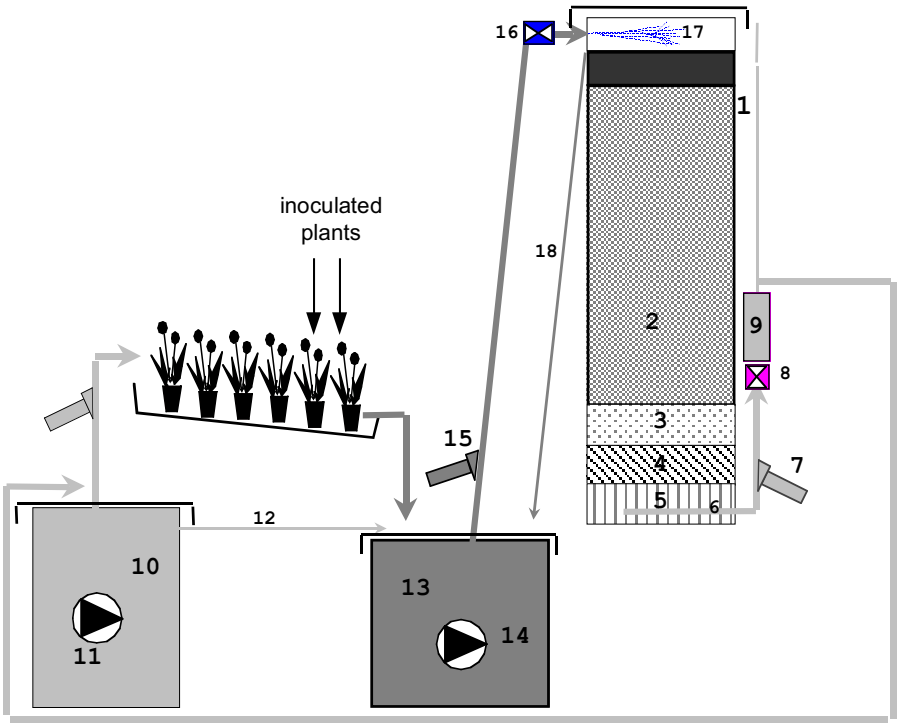

Fig. 1. Technical characteristics of the sand filter.

1. Sand filter container, PVC pipe, 1.5 m high, 0.4 m ø.
2. Filtering layer 80–100 cm, sand size 0.2–2 mm; effective grain size (d10 - sieve opening through which 10% by weight of the grains will pass) 0.08678 mm; uniformity coefficient (UC - d60/d10) 3,0617; density 2.6 g $(cm^3)^{-1}$; $SiO_2 > 96\%$.
3. Drain layer 15–20 cm, sand size 2–3 mm; absolute density 2.6 g $(cm^3)^{-1}$; SiO_2>94%.
4. Drain layer 15–20 cm, sand size 8–12 mm; absolute density 2.6 g $(cm^3)^{-1}$; SiO_2>94%.
5. Drain layer 15–20 cm, sand size 20–40 mm; absolute density 2.6 g $(cm^3)^{-1}$; SiO_2>99%.
6. PVC (ø 3/4") output diffuser (effluent) 35 cm length with 140 holes ø 2 mm.
7. Filter to remove inorganic (quartz) dust from sand filter layers (ø 3/4").
8. Stainless steel adjust (ø 1/2") valve for flow meter.
9. Flow meter min-max flow rate 6.5–65 l h^{-1} (connection with pipe ø 8 mm for filter water level monitoring).
10. Polyethylene storage tank of effluent from sand filter (200 l).
11. Irrigation pump (effluent) connected with pipe PN 4 ø 20 mm with water emitter ø 0.9 mm (50 cm length) (H max 16 m). Valve between pump and water emitter pipe to avoid flow back.
12. Polyethylene (PN 6 ø 16 mm) storage tank of effluent overflow.
13. Polyethylene storage tank of drainwater from the gerbera crop (200 l).
14. Input pump (influent) connected with input pipe PN 6 ø 16 mm (H max 6.5 m).
15. Disk filter to remove organic and inorganic dust from the gerbera crop (ø 3/4", 120 mesh, 130 µm).
16. Polyethylene adjust valve (ø 1/2") for input diffuser (influent).
17. Polyethylene (PN 6 ø 16 mm) input diffuser (influent) 35 cm length with 40 holes ø 2 mm.
18. Polyethylene (PN 6 ø 32 mm) input overflow (influent).

Pathogen inoculation

One month after gerbera plants were transplanted to the closed soilless system, the same isolate of *P. cryptogea* used in open system trials, artificially propagated on wheat and hemp kernels (200 g of wheat kernels and 100 g of hemp kernels in 320 ml of deionized water, autoclaved at 120°C for 30 min), was inoculated into the system. To obtain an efficient natural zoospore spread, 95–100 g of *P. cryptogea*-infected kernels per plant were inoculated near the basal foliar stems. Only two plants in each row were inoculated and placed immediately before the storage tank, so that the pathogen would not spread in the drain water to other pots (arrows in Fig. 1). When these plants became completely collapsed, they were replaced with new inoculated ones.

Assessment of disease severity and flower production

The effect of micro-organisms on disease incidence (percentage of diseased plants), crop yield (flowers per healthy plant) or both (flowers per m^2) was evaluated in the same way as in the open system trials.

Statistical analysis

Analysis of variance was carried out with the statistical programme SPSS. The significance level was evaluated at P=0.05. Mean separation was determined with Duncan's Multiple Range Test.

Results

In the first trial in the open system, the greatest antagonistic activity against *P. cryptogea* was shown by *Fusarium* F10 and F11, *Trichoderma* T5 and bacterium B3 (Table 3). *Trichoderma* T5 had a slight negative effect on flower production, but F10, F11 and B3 did not affect crop yield very much (Table 4 and 5). Strain F7 increased flower production (Table 4 and 5), but did not reduce disease incidence compared to the control (Table 3).

In the second trial three *Fusarium* strains (F14, F15, F19) and three oomycete strains (OO9, OO10, OO11) were most effective in greatly reducing root rot incidence (Table 3). The oomycete OO9 was most strongly suppressive against *P. cryptogea* (13.7% disease incidence, data not shown in tables) compared to the control (37%). These strains (especially F19 and OO9) also produced a small increase in crop yield (Table 4 and 5). Other strains (F18, F26, B8) increased yield but did not reduce disease severity.

In the third trial, carried out to confirm the beneficial effect of the strains found effective in the previous two trials, *Trichoderma* T5 again reduced the percentage of diseased plants (Table 3). Moreover, T5 did not reduce yield in this trial (Table 4 and 5). Also, the oomycetes OO9 and OO10, *Fusarium* F19, bacterium B3 and *Trichoderma* T6 con-

Table 3. Reduction of *P. cryptogea* root rot of gerbera achieved by selected strains of *Fusarium*, *Trichoderma*, oomycetes and bacteria applied by soil mixing (fungi) or root dipping (bacteria) in open soilless systems (first and second trial, Albenga, 2000; third trial, Albenga, 2001).

Disease suppression (%)[a]	Antagonistic effectiveness of strains[b, c]		
	Trial 1	Trial 2	Trial 3
>50		OO9, OO10	
>25	F10, F11, T5, B3	F14, F15, F19, T14, OO5, OO8, OO11, OO12, B10, B13	T5, B8
<25	F3, F8, F9, T1, T2, T7, T9, OO2, OO3	F13, F17, F20, F23, F24, B6, B11	F9, F19, T1, T6, T12, OO4, OO9, OO10, B3
No effect	F1, F2, F4, F5, F6, F7, F12, T3, T4, T6, T8, T10, T11, T12	F16, F18, F21, F22, F25, F26, T13, T15, OO6, OO7, B4, B5, B7, B8, B9, B12, B14, B15, B16, B17	F1, F3, F6, F7, F10, F12, F18, T2, T3, T4, T8, OO1, OO2, OO3, OO7, OO11, B1, B7, B9, B17

[a] Root rot in control plants (%): Trial 1, 56.7; Trial 2, 37; Trial 3, 53.3.
[b] F, *Fusarium*; T, *Trichoderma*; OO, oomycete; B, bacterium.
[c] Days after transplanting: Trial 1, 184; Trial 2, 181; Trial 3, 117.

firmed their antagonistic potential against the pathogen. These strains did not reduce yield (OO10, B3) and sometimes even increased it (F19, T5, T6, OO9).

In the closed system trial, the plots treated with *Fusarium* and *Trichoderma* strains showed a significantly lower disease incidence than did plots in the closed system with a sand filter but without

Table 4. The effect of selected strains of *Fusarium*, *Trichoderma*, oomycetes and bacteria applied by soil mixing (fungi) or root dipping (bacteria), on the number of flowers per healthy gerbera plant grown in open soilless systems (first and second trial, Albenga, 2000; third trial, Albenga, 2001).

Increase in No. of flowers per healthy plant (%)[a]	Effect of strains[b] on production of flowers per healthy plant[c]		
	Trial 1	Trial 2	Trial 3
>50			
>25			OO11
<25	F6, F7, F10, T2, T6, T8, OO1, B1	F13, F15, F17, F18, F19, F22, F24, F26, T13, OO5, OO7, OO8, OO9, OO10, OO11, B5, B6, B7, B8, B9, B12, B14, B16, B17	F1, F6, F7, F9, F12, F19, T3, T4, T5, T6, T8, T12, OO1, OO2, OO3, OO4, OO7, OO9
No effect	F1, F2, F3, F4, F5, F8, F9, F11, F12, T1, T3, T4, T5, T7, T9, T10, T11, T12, OO2, OO3, OO4, B2, B3	F14, F16, F20, F21, F23, F25, T14, T15, OO6, OO12, B4, B10, B11, B13, B15	F3, F10, F18, T1, T2, OO10, B1, B3, B7, B8, B9, B17

[a] No. of flowers per healthy control plant: Trial 1, 9.8; Trial 2, 4.8; Trial 3, 4.1.
[b] See Table 3.
[c] Days after transplanting: Trial 1, 170; Trial 2, 181; Trial 3, 117.

Table 5. The effect of selected strains of *Fusarium*, *Trichoderma*, oomycetes and bacteria applied by soil mixing (fungi) or root dipping (bacteria), on production of flowers per m^2 in gerbera plants grown in open soilless systems (first and second trial, Albenga, 2000; third trial, Albenga, 2001).

Increase in No. of flowers per m^2 (%)[a]	Effect of strains[b] on production of flowers per m^2[c, d]		
	Trial 1	Trial 2	Trial 3
> 50			
> 25		F19, OO9	T6, OO4, OO9
< 25	F7,T2, B3	F13, F14, F15, F17, F18, F24, F26, T13, OO5, OO8, OO10, OO11, OO12, B6, B7, B8, B9, B10, B17	F7, F9, F19, T3, T4, T5, T8, T12, OO1, OO3, OO10, OO11, B8
No effect	F1, F2, F3, F4, F5, F6, F8, F9, F10, F11, F12, T1, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, OO1, OO2, OO3, OO4, B1, B2	F16, F20, F21, F22, F23, F25, T14, T15, OO6, OO7, B4, B5, B11, B12, B13, B14, B15, B16	F1, F3, F6, F10, F12, F18, T1, T2, OO2, OO7, B1, B3, B7, B9, B17

[a] No. of flowers per m^2 in control plants: Trial 1, 101.8; Trial 2, 47; Trial 3, 22.9.
[b] See Table 3.
[c] Days after transplanting: Trial 1, 170; Trial 2, 181; Trial 3, 117.
[d] Crop density: Trial 1 and 2, 12 plants per m^2; Trial 3, 8 plants per m^2.

antagonists (Table 6). The highest percentage of diseased plants was observed in the closed system not provided with any disinfection treatment. There were hardly any significant differences among treatments in the number of flowers per healthy plant or the number of flowers per m^2 (Table 7 and 8). The closed system in which the nutrient solution was not disinfected showed the lowest crop yield.

Table 6. The effect of selected strains of *Fusarium* and *Trichoderma* on *P. cryptogea* root rot of gerbera grown in different closed soilless systems (Albenga, 2001).

Treatment			Diseased plants at different dates (%)			
Soilless system	Slow sand filtration	Addition of antagonists	29.01.01	16.03.01	17.04.01	23.05.01
Closed	Yes	No	0 a[a]	0 a	5.6 ab	11.1 b
Open	No	No	0 a	3.2 ab	5.3 ab	12.6 b
Closed	Yes	*Fusaria*	0 a	0 a	0 a	1.9 a
Closed	No	No	0 a	7.6 b	9.1 b	22.8 c
Closed	Yes	*Trichoderma*	0 a	0 a	0 a	2.1 a

[a] In each column values followed by the same letter do not differ significantly according to Duncan's Multiple Range Test (significance level at P=0.05).

Table 7. The effect of selected strains of *Fusarium* and *Trichoderma* on the production of flowers per healthy gerbera plant grown in different closed soilless systems (Albenga, 2001).

Treatment			Number of flowers per healthy plant at different dates					
Soilless system	Slow sand filtration	Addition of antagonists	23.01.01	05.02.01	01.03.01	30.03.01	23.04.01	10.05.01
Closed	Yes	No	1.5 a[a]	2.5 a	4.2 a	6.9 a	15.9 a	17.8 a
Open	No	No	1.5 a	2.4 a	4.2 a	7.5 a	12.9 a	14.8 a
Closed	Yes	*Fusaria*	1.4 a	2.3 a	4.7 a	7.3 a	10 a	11.8 b
Closed	No	No	1.2 a	2.1 a	3.6 a	5.7 a	8.4 b	9.4 b
Closed	Yes	*Trichoderma*	1.7 a	2.7 a	4.9 a	7.6 a	10.5 a	11.9 b

[a] In each column values followed by the same letter do not differ significantly according to Duncan's Multiple Range Test (significance level at P=0.05).

Table 8. The effect of selected strains of *Fusarium* and *Trichoderma* on the production of flowers per m^2 in gerbera plants grown in different closed soilless systems (Albenga, 2001).

Treatment			Number of flowers per m^2 at different dates					
Soilless system	Slow sand filtration	Addition of antagonists	10.01.01	05.02.01	01.03.01	30.03.01	23.04.01	10.05.01
Closed	Yes	No	3.5 a[a]	12.1 a	19.1 a	27.9 a	35.1 a	40.7 a
Open	No	No	2.7 a	11 a	17.3 a	28.1 a	39.9 a	47.4 a
Closed	Yes	*Fusaria*	4.2 a	10.8 a	20.4 a	30.3 a	40.8 a	47.8 a
Closed	No	No	2.6 a	9 a	12.3 b	16.6 b	21.5 b	24 b
Closed	Yes	*Trichoderma*	3.3 a	12.3 a	19.5 a	28.2 a	37.4 a	42 a

[a] In each column values followed by the same letter do not differ significantly according to Duncan's Multiple Range Test (significance level at P=0.05).

Discussion

The results on gerbera in the three trials on open soilless systems suggest that some micro-organisms may boost flower production and reduce disease incidence in plants that are infected with *P. cryptogea*. Of the *Fusarium* strains, F19, isolated from healthy *P. cryptogea*-free gerberas grown in a soilless system, gave the best results, with a great increase in yield. The most strongly antagonistic *Trichoderma* strains were T2 and T5, both from healthy *P. cryptogea*-free gerbera plants grown in a soilless system, and T6, isolated from plants grown in a soilless system that were apparently healthy but had been artificially infected with *P. cryptogea*. The three oomycetes OO9, OO10 and OO11, isolated from apparently healthy gerberas artificially infected with *P. cryptogea* and grown in a soilless system, also yielded very interesting results: they were highly disease-suppressive and, also, increased flower production. The most effective bacterial strain was B3, isolated from healthy *P. cryptogea*-free gerbera plants grown in a soilless system.

The results over two years suggest that some microbial strains, that are well adapted to soilless conditions, may increase gerbera plants yield and reduce root rot incidence. No correlation was found between strain effect and strain origin. Among the most effective strains, only a few were isolated from diseased gerbera plants grown in soilless system artificially infected by *P. cryptogea*; most came from healthy *P. cryptogea*-free gerbera plants grown in a soilless system or from apparently healthy gerberas artificially inoculated with *P. cryptogea*. This could be due to the occurence of a protective microflora in the rhizosphere of plants that survive in a system where the pathogen is present.

Other researchers have reported that the microflora in soilless systems is important for the suppression of root pathogens (Postma *et al.*, 1999 and 2000). However, more knowledge about the mechanisms of action and the micro-organisms involved in suppression is needed before these micro-organisms can be stimulated or enhanced in practice. There is also a need for better formulation and application procedures and a clearer understanding of the environmental conditions under which these antagonists are most effective.

The trial in the closed soilless system showed that is feasible to combine selected antagonistic micro-organisms in soilless cultivations with the passive disinfection of the recirculating nutrient solution (slow sand filtration). Periodical assessments of disease severity and flower production showed that the incidence of *P. cryptogea* root rot in the cultural rows with the antagonists was significantly less than that in the control using only slow sand filtration without the antagonists.

Slow sand filtration largely eliminates some pathogens, such as *Pythium* and *Phytophthora*, but others, such as viruses and nematodes, are only partly eliminated (Wohanka, 1995; Runia *et al.*, 1997; Van Os *et al.*, 2000). It is less expensive than active disinfestation techniques such as heat or UV treatment and for this reason is suitable in countries where economic constraints are greater. Slow sand filtration does not kill the entire indigenous microflora in the nutrient solution, but allows useful microbial populations to develop on the upper layer of the sand filter (filter skin). This technique can therefore be used in combination with selected antagonistic strains in order to enhance the natural suppressiveness of the filter (Van Os and Postma, 2000).

Future work will focus on how to improve disinfection treatments, and particularly slow sand filtration, which may be able to keep the closed soilless system in microbial balance, and on how to enhance microbial activity by stimulating the indigenous microflora and/or applying particular antagonistic strains to the closed soilless system. Moreover, since the main purpose of this work was to demonstrate the feasibility of selecting suppressive strains from plant rhizosphere, due to the lack of knowledge concerning the identification of the these strains, based until now only on selective media and colony morphology, the next step will be the study of the mechanism of action of the most effective strains, their identification to genus and species level, and the feasibility of developing a semi-commercial formulation well adapted to soilless systems.

Acknowledgements

This work has been carried out with the financial support of the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD programme, CT98-4309, "Microbial Optimisation to Prevent Root Diseases" (MIOPRO-

DIS). The authors thank Ing. E.A. van Os for critically reviewing the manuscript.

Literature cited

Ahmad J.S. and R. Baker, 1987. Rhizosphere competence of *Trichoderma harzianum*. *Phytopathology* 77, 182–189.

Bell C.H., N. Price and B. Chakrabarti (ed.), 1996. *The Methyl Bromide Issue*. Wiley, New York, NY, USA, 400 pp.

Elad Y., I. Chet and Y. Henis, 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. *Phytoparasitica* 9, 59–67.

Gamliel A., A. Grinstein and J. Katan, 1997. Improved technologies to reduce emissions of methyl bromide from fumigated soil. *Phytoparasitica* 25 (Supplement), 21S–30S.

Garibaldi A., 1978. Fungal and bacterial diseases of carnation and gerbera. In: *Proceedings of the Eucarpia Meeting on Carnation and Gerbera*, 1978, Alassio, Italy, 69–88.

Garibaldi A. and M.L. Gullino, 1995. Focus on critical issues in soil and substrates disinfestation towards the year 2000. *Acta Horticulturae* 382, 21–36.

Garibaldi A., M.L. Gullino and V. Lisa, 2000. *Malattie delle Piante Ornamentali: Malattie da Funghi, Batteri, Virus, Viroidi, Fitoplasmi e da Cause Abiotiche*. Calderini Edagricole, Bologna, Italy, 574 pp.

Garibaldi A., A. Minuto and D. Salvi, 2001. Disinfection of nutrient solution in closed soilless systems in Italy. *Acta Horticulturae* (in press).

Komada H., 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review of Plant Protection Research* 8, 114–125.

Masago H., M. Yoshikawa, M. Fuhada and N. Nakanishi, 1977. Selective inhibition of *Pythium* spp. on a medium for direct isolation of *Phytophthora* spp. from soil and plants. *Phytopathology* 67, 425–428.

Minuto A., G. Gilardi, M.L. Gullino and A. Garibaldi, 1999. Reduced dosages of methyl bromide applied under gas-impermeable plastic films for controlling soilborne pathogens of vegetable crops. *Crop Protection* 18, 365–371.

Minuto A., A. Camponogara, A. Garibaldi and M.L. Gullino, 2002. Bromuro di metile: ormai prossimi all'eliminazione, l'uso si fa "critico". *Informatore Agrario* 58(12), 77–85.

Nemec S., L.E. Datnoff and J. Strandberg, 1996. Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetables and citrus. *Crop Protection* 15, 735–742.

Paulitz T.C. and R.R. Bélanger, 2001. Biological control in greenhouse systems. *Annual Review of Phytopathology* 39, 103–133.

Postma J., E.A. van Os and G. Kritzman, 1999. Prevention of root diseases in closed soilless growing systems by microbial optimization. *Mededeling Faculteit Landbouwwetenschappen Universiteit Gent* 64/3b, 431–440.

Postma J., M.J.E.I.M. Willemsen-de Klein 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 on rockwool. *Phytopathology* 90, 125–133.

Postma J., M.J.E.I.M. Willemsen-de Klein, H. Rattink and E.A. van Os, 2001. Disease suppressive soilless culture systems; characterisation of its microflora. *Acta Horticulturae* 554, 323–229.

Ristaino J. and W. Thomas, 1998. Agriculture, methyl bromide, and the ozone hole: Can we fill the gap? *Plant Disease* 81, 964–977.

Runia W.Th., M.G.P.J. Michielsen., A.J. van Kuik and E.A. van Os, 1997. Elimination of root infecting pathogens in recirculation water by slow sand filtration. In: *Proceedings of the 9th International Congress on Soilless Culture*, St. Helier, Jersey, UK. International Society of Soilless Culture, Wageningen, The Netherlands, 395–408.

Serra G., 1994. Innovation in cultivation techniques of greenhouse ornamentals with particular regard to low energy input and pollution reduction. *Acta Horticulturae* 353, 149–163.

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

Tognoni F., 2003. Le colture fuori suolo: situazione in Italia e prospettive per il futuro. *Informatore Fitopatologico*, 53(2), 7–12.

Tognoni F. and G. Serra, 1994. New technologies for protected cultivations to face environmental constraints and to meet consumer's requirements. *Acta Horticulturae* 361, 31–38.

Tu J.C., A.P. Papadopoulos, X. Hao and J. Zheng, 1999. The relationship of *Pythium* root rot and rhizosphere micro-organisms in a closed circulating and an open system in rockwool culture of tomato. *Acta Horticulturae* 481, 577–583.

Van Os E.A., 1999. Closed soilless growing systems: a sustainable solution for Dutch greenhouse horticulture. *Water Science and Technology* 39, 105–112.

Van Os, E.A. and J. Postma, 2000. Prevention of root diseases in closed soilless growing systems by microbial optimisation and slow sand filtration. *Acta Horticulturae* 532, 97–102.

Van Os E.A., W. Wohanka, M.A. Bruins and R. Seidel, 2000. Slow filtration: a technique to minimise the risks of spreading root-infecting pathogens in closed hydroponic systems. *Acta Horticulturae* 559, 495–502.

Wohanka W., 1995. Disinfection of recirculating nutrient solutions by slow sand filtration. *Acta Horticulturae*, 481, 577–583.

Accepted for publication: February 20, 2003