Microbial Optimisation in Soilless Cultivation, a Replacement for Methyl Bromide

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Abstract

In Europe the soil fumigant methyl bromide is still in use to control soil-borne diseases in greenhouses and open air vegetables. Methyl bromide is extremely toxic and environmental harmful. Countries under the Montreal protocol are demanded to reduce the use of methyl bromide with respect to the average use between 1991 and 1994 resulting in a total phase out from 2005 onwards for all applications except critical uses. For greenhouses replacement of soil grown crops by closed soilless growing systems has significant advantages: conservation of scarce water resources, no leaching of nutrients and pesticides and improved quality of products. A disadvantage of the closed system is the rapid dispersal of soil-borne pathogens by the recirculating nutrient solution. Disinfection of the nutrient solution either by active (sterilisation) or passive (part of the resident microflora survives the treatment) methods may eliminate harmful pathogens, but the hypothesis is that with passive methods a suppressive microflora can be built up, preventing (severe) outbreaks of certain pathogens. A 4-year EU-funded project had the aims to characterise the (suppressive) microflora and metabolites in the nutrient solution, to detect its dynamic behaviour during the cultivation of three crops (tomato, cucumber, gerbera) in a two year period and to demonstrate results to commercial users. Part of the crop was inoculated with *Pythium aphanidermatum* or *Phytophthora cryptogea*, while the nutrient solution was disinfected either with Ultra Violet radiation (active method) or slow sand filtration (passive method) or not at all (control). This paper emphasise on practical aspects of disinfection of the nutrient solution in relation to the presence and behaviour of the microflora. Results indicate that disinfection of the nutrient solution is needed to achieve proper yields. It was not proven that a suppressive microflora could be built up by a passive disinfection method, compared with active disinfection. However, a shift in the composition of the microflora could be detected, but the microflora in the stone wool growing system is mainly plant-driven, realising a microbial balanced system. Application of certain antagonists did also shift the total microflora during cropping, but did not suppress the mentioned pathogens with the exception of a *Trichoderma*-strain.

INTRODUCTION

In Europe the soil fumigant methyl bromide is still in use to control soil-borne diseases in greenhouse cultivation and open air vegetables. Methyl bromide is extremely toxic and environmental harmful (Braun & Supkoff, 1994). In the Industrialised Countries, under the Montreal Protocol, Methyl Bromide will be phased out by 2005 (total ban), because it is depleting the ozone layer. Only critical uses will be accepted. Before that year a reduced use with respect to the average use between 1991 and 1994 is

demanded. Per country legislation is set up to phase-out the use of methyl bromide by 2005. In the Netherlands a step-wise phase out started already in the early eighties after founding remnants of the fumigant in tap water. In some other countries all soil treatments have been phased-out now. In Italy and Spain, but especially the United States methyl bromide is still very much in use, alternatives are being investigated. One of the alternatives for the use of methyl bromide in greenhouse cultivation is the use of hydroponic growing methods.

Most of the present hydroponic systems are open or run-to-waste systems. For environmental reasons these open systems should change into closed soilless systems (Van Os, 1999). The main advantages of closed systems are the savings of scarce water and expensive fertilisers, less leaching of nutrients and pesticides to the environment and better quality of products. Closed soilless systems potentially have a few significant dis-advantages. Apart from the demand for a high quality supply water, there is a risk of rapid dispersal of soil-borne pathogens by the recirculating nutrient solution and accumulation of potential phytotoxic metabolites and organic substances in the recirculating nutrient solution. Where such systems have been adapted commercially, growers attempt to overcome the problems of pathogen dissemination by disinfecting the water by treating it with heat treatment (pasteurisation; Runia et al., 1988), ozone or UV-radiation (active disinfection). Such treatments need a high financial investment. Using these active disinfection methods the natural microflora in the nutrient solution is either suppressed or destroyed. It was demonstrated that the recolonisation of certain pathogens is much faster in sterile growing systems as compared to non-sterilised systems (Postma, 1996; Postma et al., 1996), but recolonisation might be unbalanced. Other research has shown that there is a certain natural suppressiveness by the resident microflora against colonisation by pathogens (McPherson et al., 1995; Postma, 1996; Postma et al., 2000). Several studies indicated that slow (sand) filtration could eliminate pathogens (Wohanka, 1995; Van Os et al., 1997), but, on the other hand, does not destroy the natural microflora. It is a non-sterilising or passive disinfection technique.

Under the acronym MIOPRODIS an EU-funded four years project started in 1999. The objective was to develop a sustainable system for the prevention of root diseases in closed soilless growing systems by optimising microbial suppression in the root environment. The close relationship with presence and behaviour of metabolites will be presented in other venues. The system should be robust, low-tech and inexpensive, so that it can replace the soil grown system in which the soil fumigant methyl bromide is used in Southern Europe and the run-to-waste soilless system in Northern Europe.

In this paper an overview will be given of some of the obtained results with emphasise on practical aspects of disinfection of the nutrient solution in relation to the presence and behaviour of the microflora.

MATERIAL AND METHODS

Treatments

At three experimental sites (Wageningen, Efford (Fig. 1), Geisenheim (Fig. 2)) three crops (respectively cucumber, 'Sudica' and 'Kjell'; tomato, 'Espero'; gerbera, 'Shimony' and 'Kaliki') were grown in a soilless growing system (respectively stonewool slabs, stonewool grow cubes) during two years in two or three cultivation cycles. There were, respectively, 12, 36 and 24 independent hydroponic systems to allow, respectively, 3, 9 and 6 treatments in 4 replications. The main treatment was disinfection of the drainwater either by UV (active method; doses 250 $mJ.cm^{-2}$), slow sand filtration (passive method; grain size 0.1 - 2.0 mm; flow rate 300 $L.m^{-2}.h^{-1}$) or no disinfection (control). Two root pathogens *Phytophthora cryptogea* (tomato, gerbera) and *Pythium aphanidermatum* (cucumber) were introduced in part of the treatments to investigate the effect of disinfection on the development and dispersal of the pathogens and the presence and behaviour of micro-organisms. Inoculation of the pathogen took place either by placing infector plants at the lowest end of the trough (gerbera; 3 plants per system) or by applying inoculum directly to the plants at the stonewool blocks below a dripper

(cucumber, tomato) at the lowest end of an independent system (6 slabs out of 12 were inoculated, cucumber, approx. $2x10^4$ cfu/plant; 2 slabs out of 3 were inoculated, tomato, $4x10^5$ cfu/plant). Additional other micro-organisms were introduced coming from old but healthy solutions of the same crop or as an antagonist [actinomycetes suspension, (6 *Streptomyces*, 1 *Micromonospora*, 1 *Cellulomonas* isolates; *Lysobacter enzymogenes* isolate); *Trichoderma harzianum* T-50 Vitalin; *Pseudomonas* isolate 3992A; *Bacillus subtilis* strain MBI 600]. In table 1 an overview is given of all treatments, introduced pathogens and the analysing methods of the micro-organisms.

The chosen treatments were a result of trials in the first year of the project. Here, the detection methods for micro-organisms and pathogens were optimised (Postma et al., 2000; Van Os & Postma, 2000) and the slow filtration disinfection method was improved and tested for several other filter media (Van Os et al., 2001). The trials in the second and third year, as mentioned in table 1, were meant to enhance microbial suppression of the root pathogens *Phytophthora cryptogea* and *Pythium aphanidermatum* by stimulation and management of the natural microflora and/or introduction of known antagonists.

Measurements

During the trials a number of measurements were done to investigate the influences of the different disinfection methods and other treatments by characterising developments in pathogen dispersal and microflora. Yield and quality of the produce was measured too.

1. Yield. Number, weight and quality of fruits (cucumber, tomato) and number and quality of flowers (gerbera) was registered.

2. Disease Detection

- Number of zoospores: one week before inoculation and thereafter 5 times per cultivation cycle samples were collected of the nutrient solution in the “drain”, directly after flowing out of the trough, and in the container collecting the treated nutrient solution.
- Colony forming units of *Pythium aphanidermatum* and *Phytophthora cryptogea* were enumerated on selective mediums.
- Stem rot and wilting symptoms: number of diseased plants (i.e. brown stem base, wilted or dead plants) was counted separately in all the treatments. In general counting took place once a week.

3. Root Development. At the end of each trial root development and root discoloration was examined around the stonewool slabs (cucumber, tomato) and around the stonewool growing cubes.

4. Microflora. Samples of the nutrient solution were characterised by the following methods:

- Plate counts using semi-selective media: total aerobic bacteria (R2A), fluorescent pseudomonads (KB), filamentous actinomycetes (COA with filter), *Bacillus* (TSA diluted), *Trichoderma* (TSM) and total fungi (1/4 PDA).
- BIOLOG. The ability of all samples to use the 95 substrates in GN plates has been measured after 48 h incubation time. The data were summarised by calculating the average well colony development (AWCD), the area under curve (AUC), by counting the number of substrates that showed a reaction (i.e. optical density larger than in the control, “positive wells”), the diversity in the use of substrates (H’) and by stepwise discriminant analysis (DA).
- DGGE. All samples were concentrated on filters with a pore diameter of 0.2 µm and stored at –20 °C. PCR-DGGE gels of the bacterial population were prepared following the method described in Postma et al. (2000).

RESULTS

All mentioned trials delivered an enormous amount of data. Most of the data are processed now. Below, results will be presented in a summarised way, which means that some general results will be described, illustrated by results from one or two trials.

Yield

In general it appeared that yield was not such a good instrument to answer the questions asked (presence and behaviour of micro-organisms and dispersal of the inoculated pathogen). For cucumber it appeared that in the spring trials it was much easier to get differences in yield between treatments than in autumn, but cropping period should not be too short (trial 2001). There is a higher yield (Table 2) if the recirculating nutrient solution is disinfected (either by UV or slow sand filtration) compared to the control (no disinfection). For tomato, there are no significant differences between treatments in the second crop. In the first crop there were significant differences between open and closed systems in favour of the open systems. However, inoculation took place here in a different way. All systems were inoculated and recirculating in the same way to achieve even pathogen dissemination throughout all the inoculated systems and, after that, individual plots were switched to their individual treatment regime (open systems). For gerbera, there was no difference in yield between treatments in the first crop. However, in the second crop the inoculated control had a lower yield compared to the disinfection treatments. Assessment of leaf area (cucumber and tomato) and leaf length (tomato) show a similar picture as the yield data.

Disease Detection

The number of zoospores in the drainwater varied between plots (Fig. 3). In cucumber about 200 $cfu.l^{-1}$ of *P. aphanidermatum* was obtained within 2-4 weeks after inoculation in the spring trial. In the autumn trial only 100 $cfu.l^{-1}$ could be measured. In tomato levels of 200-300 $cfu.l^{-1}$ of *P. cryptogea* were obtained 12-16 weeks after inoculation. In gerbera similar levels were obtained 3-6 weeks after inoculation (Fig.3), but three inoculations were needed. In all three crops the number of diseased plants was dependent on the disinfection treatment. There was no significant difference between slow sand filtration and UV radiation (Fig. 4). In cucumber, on average, 70% and 40% of the inoculated plants in, respectively spring and autumn, were diseased, while of the disinfected treatments there was hardly any diseased plant. In the first tomato trial there was always about 30% diseased; in the second, disinfection was adequate to avoid diseased plants (difference probably caused by the inoculation method). In gerbera up to 45% was diseased when there was no disinfection. The treatment, at which *Trichoderma* was introduced, showed a remarkable phenomenon. Here, only 10% of the plants were diseased, while there was no disinfection. In all other treatments in all crops there was no significant difference between treatments at which other micro-organisms were introduced (priming with a healthy "old" solution, actinomycetes and *Lysobacter* strain in cucumber; *Pseudomonas* and *Bacillus* strains in tomato; "old" solution in gerbera; see Table 1).

Root Development

At the end of the cultivation cycle plants were examined on root development and discoloration. In general root development was best at the non-inoculated treatments and at the treatments with disinfection (Fig. 5). Oppositely, there was the discoloration or browning of the roots, it was worst at the treatments without disinfection.

Microflora

1. Plate Counts. In all crops numbers of total aerobic bacteria were counted. For cucumber 10^6- 10^7 $cfu.ml^{-1}$ were present in all trials. For tomato and gerbera levels were around 10^5- 10^6 $cfu.ml^{-1}$. In tomato levels were in all treatments rather constant during the crop cycle, while in cucumber and gerbera levels were mostly variable (Fig 6). Between trials and within trials fluorescent pseudomonads showed many fluctuations; decreasing, increasing and constant levels during the crop cycle appear (Fig. 7). Absolute level varies between 10^2 – 10^4 $cfu.ml^{-1}$ for cucumber, between 10^1- 10^4 $cfu.ml^{-1}$ for tomato and between 10^2- 10^5 $cfu.ml^{-1}$ for gerbera. As far as measured (cucumber, tomato) the

filamentous actinomycetes appear in levels between 10^0- 10^1 cfu.ml^{-1}. When they are introduced (cucumber, autumn 2000) levels are 100 – 1000 times higher during the entire trial. Numbers of total fungi mostly increase during the crop cycle. Average levels are between 10^0- 10^2 cfu.ml^{-1} for cucumber, between 10^0- 10^4 cfu.ml^{-1} for tomato and between 10^1- 10^3 cfu.ml^{-1} for gerbera. *Bacillus* spores vary between 10^3 - 10^4 cfu.ml^{-1} for tomato, but are constant during the crop cycle, while for gerbera levels vary between 10^1- 10^3 cfu.ml^{-1}, but they are variable during cropping (in cucumber not measured). Numbers of *Trichoderma* are measured in tomato and gerbera (Fig. 8), levels vary between 10^0- 10^3 cfu.ml^{-1}, while mostly an increasing number can be noticed during the crop cycle. Differences between influent and effluent of the disinfection treatments could be observed in all crops. In cucumber the stonewool slabs were sampled too, which have mostly other levels for total aerobic bacteria, fluorescent pseudomonads, total fungi and filamentous actinomycetes than before (influent) and after (effluent) the disinfection treatment (Fig. 9).

2. BIOLOG. BIOLOG is a method to investigate the potential utilisation of sole carbon sources by the microflora. The method is used for nutrient solutions for the first time and the adaptation and optimisation of the method was part of the project. It appeared in the first year of the project that reading the plates after 48 h is most efficient and saves work compared to reading after 16, 24, 40, 48, 72 or 96 hours. *AUC* and *AWCD* showed similar results, *"Positive Wells"* (wells with absorbency values > 0.5), stepwise discriminant analysis (DA) and *Substrate Diversity* (H') appeared to give the most informative results. For DA different patterns could be obtained between disinfection treatments in cucumber and between different weeks in the cultivation cycle in gerbera.

3. PCR-DGGE. PCR-DGGE is a molecular profiling technique for the microbial population. It gave differences in population where plate counts gave similar results. The method is optimised for taking samples from nutrient solutions. The bacterial profile showed increasing bands (more diversity) during the cucumber crop cycle (Fig. 10). Sometimes UV had less bands than in Control and slow filtration. The actinomycetes profile did not show many changes. Bands in the profile can be compared with known organisms, up to now there was no time to make this shift in population visible.

DISCUSSION

In the first year of the MIOPRODIS project there was a special emphasise on the adaptation and optimising of the methodology to characterise the microflora in a nutrient solution. Consequently, it was possible in the following two years to collect many data, as described partly in this paper, and to analyse those data for crops (cucumber, tomato, gerbera) and treatments (disinfection, antagonist application, open/closed system). The methods to characterise the microflora, are valuable to get information about the population (plate counts) or within the population (BIOLOG, PCR-DGGE). Total aerobic bacteria show stable levels and appeared to be of importance to check the experimental conditions. Fluorescent pseudomonads showed more fluctuations, but because of its rapid growth it is a good indicator of variations in the system. Numbers of total fungi increase in time during the crop cycle and can also be used as an indicator of the situation in the system. Numbers of *Bacillus* or actinomycetes were of less importance to characterise the situation. Additional, other micro-organisms should be counted in specific cases, i.e. as they are introduced as antagonist. BIOLOG and PCR-DGGE may give more specific information about differences in the population whereas plate counts show similar results. However, both methods are labour intensive and, therefore, should only be used as additional information is demanded.

One of the starting hypotheses of MIOPRODIS was that active disinfection (UV radiation, heat treatment, ozone treatment) influenced the present microflora in a different way than passive disinfection (slow sand filtration). With passive disinfection a more balanced microbial population was expected at which suppressiveness of root-borne diseases would become possible. Measurements directly after the disinfection treatment did indeed show the differences between active and passive disinfection. However, the

differences of the microflora in the stonewool slabs and the drainwater, freshly coming out of the slabs, were rather small. Plate counts did not give many differences, but with BIOLOG and PCR-DGGE some differences in the population were found, but small compared to the influence of the sampling time (crop development). The influence of sampling time could be seen in all trials in all crops. As crop development influences the microbial population much more than disinfection treatments the conclusion can be made that the microflora is plant-driven. Between the crops the rather stable level of micro-organisms vary somewhat, cucumber had 10^6- 10^7 $cfu.ml^{-1}$, tomato and gerbera 10^5- 10^6 $cfu.ml^{-1}$. In cucumber the higher level of micro-organisms might be caused by higher levels of exudates and other plant root derived materials. The stable level also indicates that the stonewool system is more balanced than expected. The suppressiveness towards *Pythium* and *Phytophthora* found in the different trials is not enough for growers to avoid problems. The introduced antagonists, such as actinomycetes, priming the solution, *Trichoderma* and *Bacillus*, did have only minor effects. Most clearly was the effect of the *Trichoderma harzianum* (T-50 Vitalin) in gerbera where less disease symptoms could be seen. Therefore, well-adapted organisms should be introduced already at the start of the growth of the plant to get better effects.

Yield measurements appeared to be of minor importance to obtain information about developments in disease and presence and behaviour in microflora. From the growers' point of view yield reduction as a result of a pathogen attack was rather small. Consequently, the risks for the grower are small. However, in cucumber there was a certain dying of plants because of *Pythium* after some time. Here it was concluded that an additional stress factor is needed (i.e. hot weather, skill of the grower) to get an outbreak, while spores of the pathogen were already present for a long time. This may also explain the phenomenon that one grower has always *Pythium* problems, while another one has never problems.

Another aspect of the project was the development of a sustainable closed system, which is robust, low-tech and inexpensive and which can replace the open soilless system and the soil system with the use of methyl bromide. In the trials it appeared that the microflora in the stonewool is plant-driven and more stable than expected. Disinfection methods does not influence the microflora in the slab, besides there is no difference between active and passive methods. It can be concluded that the stonewool closed growing system is a sustainable system but that disinfection of the nutrient solution is needed. Slow sand filtration can be seen as a low-tech method, which is also robust and inexpensive. For Southern European countries this may be a good solution the avoid the use of methyl bromide in greenhouses. A more expensive closed soilless growing system may use UV radiation as disinfection method, this will not influence the microflora in the slab. In the fourth year of the project the closed stonewool system is demonstrated at a commercial nursery for tomato in Spain and at an experimental station for gerbera in Italy. In the latter situation a start has been made with a further screening of antagonistic strains against *P. cryptogea* in gerbera. Results are promising. Besides, a relation will be made between the results described here and the appearance of metabolites.

CONCLUSIONS

The MIOPRODIS project delivered an enormous amount of data after similar trials at three sites with three crops (cucumber, tomato, gerbera). The microflora of a nutrient solution can be characterised by doing plate counts of total aerobic bacteria, fluorescent pseudomonads and total fungi and following the crop in time. BIOLOG and PCR-DGGE should only be used if additional information about specific organisms is demanded. There appeared no difference between active (UV radiation) and passive (slow sand filtration) disinfection methods. Microbial suppression of the pathogens *Pythium aphanidermatum* (cucumber) and *Phytophthora cryptogea* (tomato, gerbera) occurred in a certain extent, but it is too low for commercial application now. Promising results (a Trichoderma strain in gerbera) will have to be developed further. The closed stonewool growing system appeared to be a balanced system at which the microflora is plant-driven,

i.e. the crop creates its own microflora. For greenhouses, a stonewool closed growing system is a sustainable replacement for the soil grown method at which methyl bromide is used as soil fumigant.

ACKNOWLEDGEMENTS

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Tables

Table 1. Overview of treatments in the different crops and the microbial measurements.

	Standard Code [1)]	Disinfection method	Nutrient solution [2)] or antagonist [3)]	Pathogen	Analysing methods
Cucumber-spring 2000	ICO	control	Fresh	*Pythium*	plate BIOLOG DGGE
	ICO+	control	Old	*Pythium*	
	IUV	UV	fresh	*Pythium*	
	IUV+	UV	old	*Pythium*	
	ISF	sand filter	fresh	*Pythium*	
	ISF+	sand filter	old	*Pythium*	
Cucumber-autumn 2000	ICO	control	fresh	*Pythium*	plate DGGE
	ICO+	control	fresh +actino	*Pythium*	
	IUV	UV	fresh	*Pythium*	
	IUV+	UV	fresh +actino	*Pythium*	
	ISF	sand filter	fresh	*Pythium*	
	ISF+	sand filter	fresh +actino	*Pythium*	
Cucumber-spring 2001	ICO	control	fresh	*Pythium*	plate BIOLOG DGGE
	ICO+	control	fresh +T8	*Pythium*	
	IUV	UV	fresh	*Pythium*	
	IUV+	UV	fresh +T8	*Pythium*	
	ISF	sand filter	fresh	*Pythium*	
	ISF+	sand filter	fresh +T8	*Pythium*	
Gerbera-2000	UCO	control	fresh	uninoculated	plate BIOLOG
	UCO+	control	old	uninoculated	
	ICO	control	fresh	*Phytophthora*	
	ICO+	control	old	*Phytophthora*	
	IUV	UV	fresh	*Phytophthora*	
	ISF	sand filter	fresh	*Phytophthora*	
Gerbera-2001	UCO	control	fresh	uninoculated	plate BIOLOG
	ICO	control	fresh	*Phytophthora*	
	UCO+	control	fresh + Trich	uninoculated	
	ICO+	control	fresh + Trich	*Phytophthora*	
	IUV	UV	fresh + Trich	*Phytophthora*	
	ISF	sand filter	fresh + Trich	*Phytophthora*	
Tomato-2000	UCO-o	Control, open	fresh	uninoculated	plate
	UCO	Control	fresh	uninoculated	
	ICO-o	Control, open	fresh	*Phytophthora*	
	ICO	Control	fresh	*Phytophthora*	
	IUV	UV	fresh	*Phytophthora*	
	ISF	sand filter	fresh	*Phytophthora*	
	UCO+	control	fresh + Pseud	uninoculated	
	ICO+	control	fresh + Pseud	*Phytophthora*	
	ICO++	control	fresh + Pseud	*Phytophthora*	
Tomato-2001	UCO-o	control	fresh	uninoculated	plate BIOLOG (treatm. 2,4,5,6)
	UCO	control	fresh	uninoculated	
	ICO-o	control	fresh	*Phytophthora*	
	ICO	control	fresh	*Phytophthora*	
	IUV	UV	fresh	*Phytophthora*	
	ISF	sand filter	fresh	*Phytophthora*	
	UCO+	control	fresh + Bac	uninoculated	
	ICO+	control	fresh + Bac	*Phytophthora*	
	ICO++	control	fresh + Bac	*Phytophthora*	

The code is build up by the following abbreviations:

I = inoculated with pathogen   CO = control, no disinfection   SF = disinfection by slow sand filtration
U = uninoculated, no pathogen   UV = disinfection by UV radiation
-- o = open system, run to waste (all other systems are closed)
+ = nutrient solution primed with an old nutrient solution or with antagonistic isolates
Nutrient solution: fresh = newly prepared nutrient solution; old = old nutrient solution taken from a healthy crop
Added antagonists were:

actino = mix of 8 different actinomycetes isolates (6 *Streptomyces*, 1 *Micromonospora*, 1 *Cellulomonas* isolates)
T8 = *Lysobacter enzymogenes* isolate T8   Trich = *Trichoderma harzianum* T-50 Vitalin
Pseud = *Pseudomonas* isolate 3992A isolated   Bac = *Bacillus subtilis* strain MBI 600

Table 2. Yield of cucumber fruits per m^2 in not inoculated core (disinfection) and additional ("old", addition of actinomycetes) treatments in spring and autumn trial.

		Spring[1]		Autumn[1]	
	Disinfection	"fresh"	"old"	"fresh"	+ actinomycetes
Number	UV	17.0^{c}	16.3^{bc}	21.0^{ab}	22.6^{b}
	Filtration	14.9^{b}	16.8^{c}	22.6^{b}	23.7^{b}
	Control	12.8^{a}	14.8^{b}	22.2^{b}	20.2^{a}
Weight	UV	10.01^{d}	9.27^{cd}	7.72^{a}	8.89^{b}
	Filtration	8.52^{bc}	9.40^{cd}	9.06^{bc}	9.78^{c}
	Control	6.88^{a}	7.83^{ab}	8.95^{b}	7.77^{a}

[1] Means with the same letter within each trial did not differ significantly ($p<0.05$).

Figures

Fig. 1. Experimental site for tomato in Efford (UK).

Fig. 2. Experimental site for gerbera in Geisenheim (D).

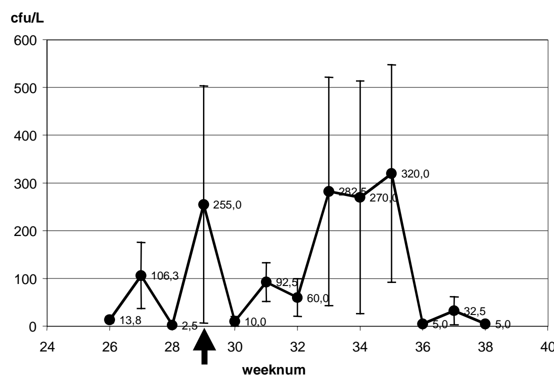

Fig. 3. Concentration of *Phytophthora cryptogea* propagules in the drain of the "inoculated control"-treatment (ICO); means with standard error of 4 repl.; arrow indicates replacement of the first "infector plants", i.e. second inoculation.

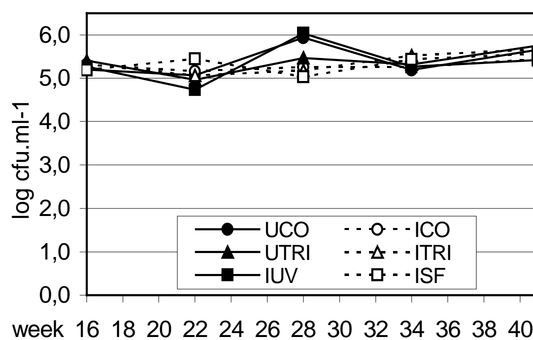

Fig. 6. Development of total bacteria in the drainwater in gerbera, 2001.

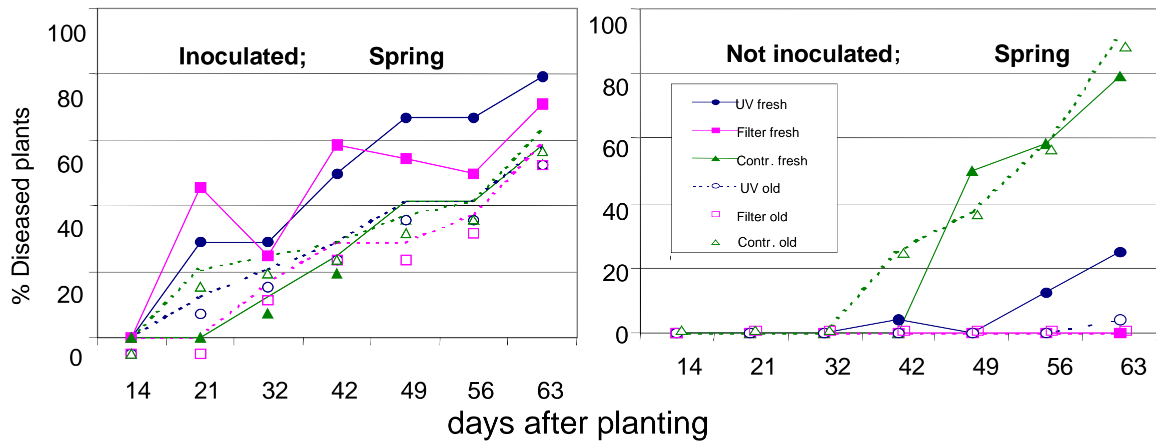

Fig. 4. Number of diseased plants in the inoculated and non-inoculated part per treatment in cucumber spring trial 2000.

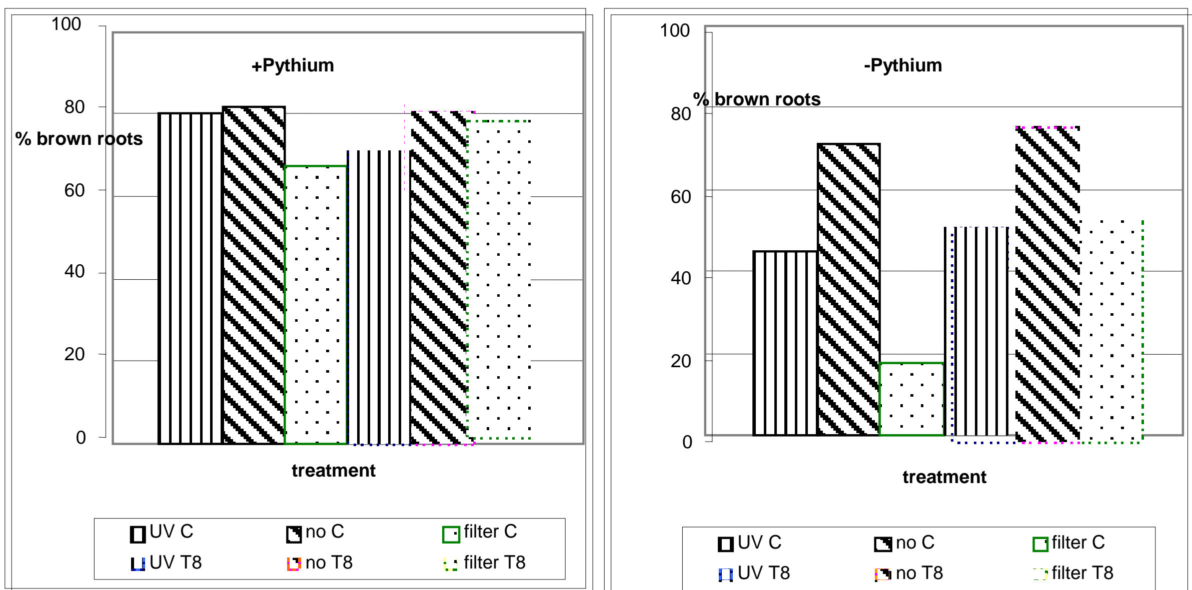

Fig. 5. Percentage of brown roots in treatments with (+ Pythium) or without (- Pythium) inoculation of *P. aphanidermatum*. LSD,0.05 is 19.7 *(spring 2001*).

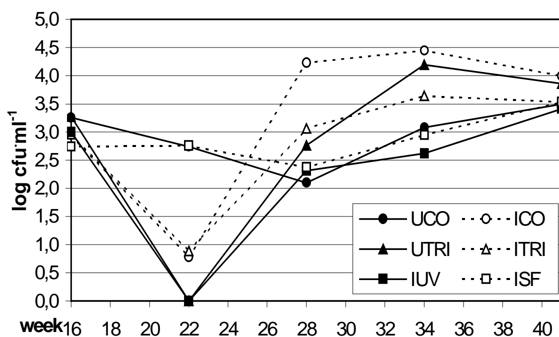

Fig. 7. Development of fluorescent pseudomonads in the drainwater in gerbera, 2001.

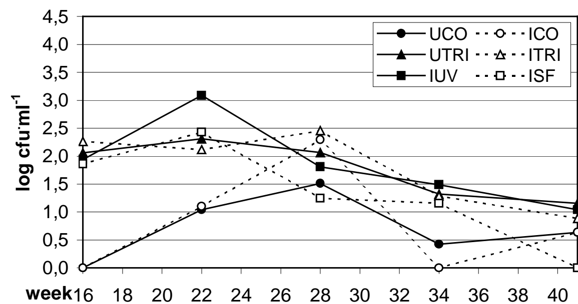

Fig. 8. Development of *Trichoderma* spp. in the drainwater of gerbera, 2001.

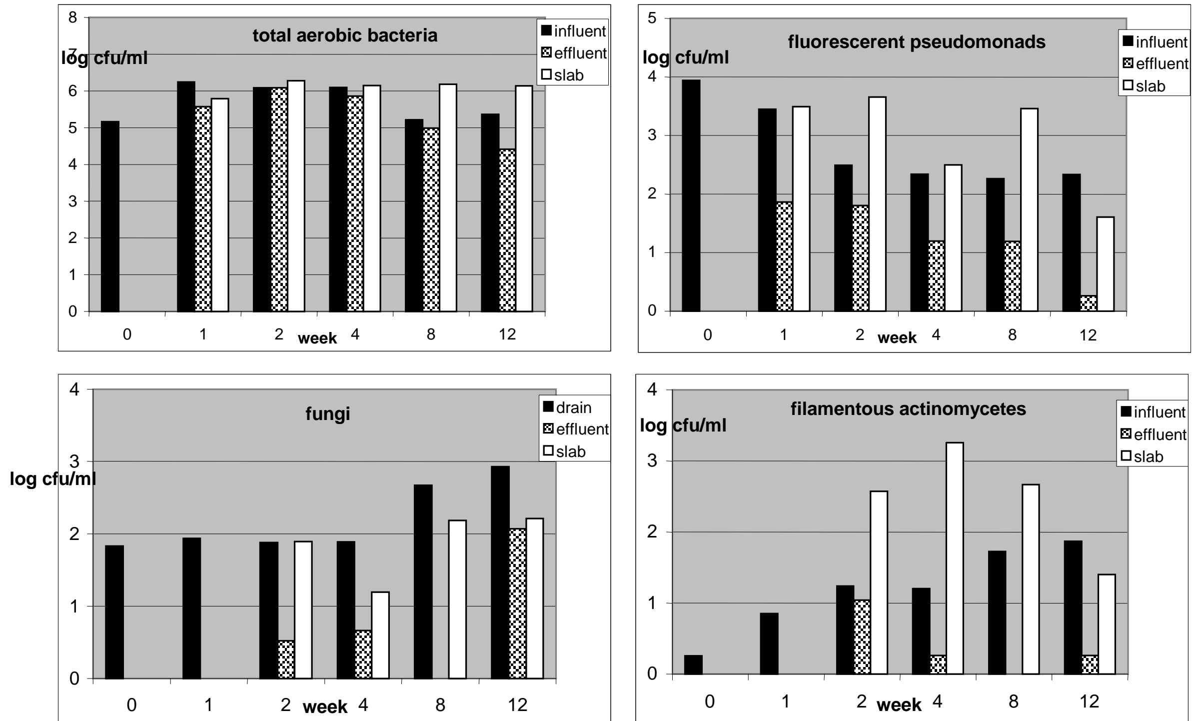

Fig. 9. Plate counts of different microbial groups from rockwool slabs, the influent and the effluent of sand filters in cucumber. $LSD_{0.05}$ values are: 0.46, 1.10, 0.78 and 0.75 for total aerobic bacteria, fluorescent pseudomonads, fungi, and filamentous actinomycetes, respectively.

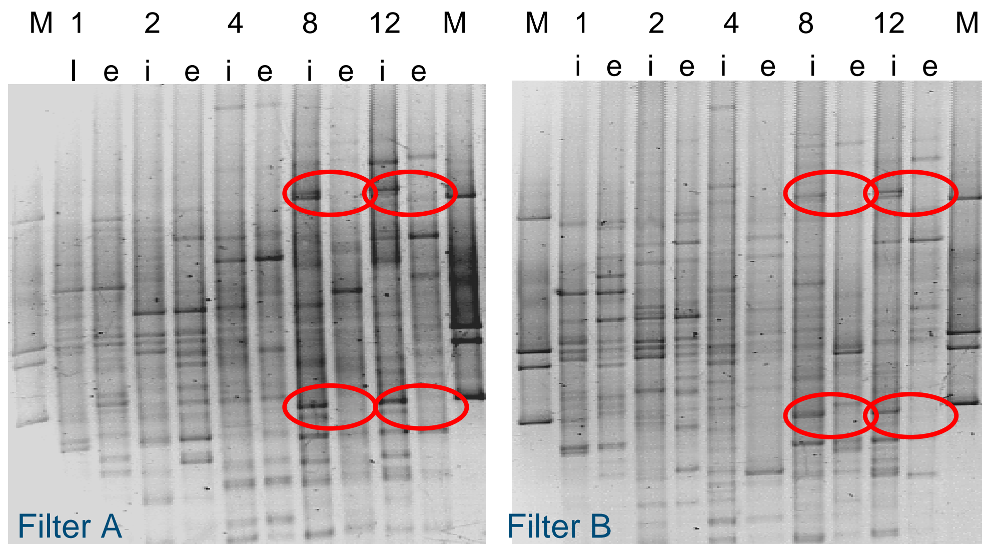

Fig. 10. PCR-DGGE patterns of bacterial populations of the influent (i) and effluent (e) of two slow sand filter systems 1, 2, 4, 8 and 12 weeks after planting in cucumber, spring 2000. M is a mix of four bacteria used as a marker. Red circles indicate differences between influent and effluent.