DISEASE SUPPRESSIVE SOILLESS CULTURE SYSTEMS; CHARACTERISATION OF ITS MICROFLORA

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Abstract

The trend in glasshouse horticulture has always been to start culture systems as aseptic as possible. However, several root diseases still cause problems under these conditions. The present paper shows the importance of the microflora to suppress *Pythium aphanidermatum*, a fungal root pathogen which is a serious threat in cucumber. Introduced single antagonists as well as the indigenous microflora suppressed pythium root and crown rot. *Pseudomonas fluorescens*, *Streptomyces griseoviridis*, *Pythium oligandrum*, and 2 isolates of *Trichoderma harzianum* reduced the disease occurrence by 60 % or more in several, but not all, of the experiments. The indigenous microflora showed a very constant disease suppression of 50 to 100 %. This was tested in experiments where *P. aphanidermatum* was added to sterilised and non-sterilised rockwool, and to sterilised rockwool that had been recolonised with the original microflora. Suppressiveness correlated with the number of filamentous actinomycetes present in the nutrient solution in the rockwool slabs. If a beneficial microflora is present in the cropping system, it should not be disturbed or eradicated by treatments such as disinfection of the recirculated nutrient solution. Therefore, the effects of different disinfection procedures on the composition of the microflora were compared. Numbers of filamentous actinomycetes in the nutrient solution in the tank after the disinfection treatment were highest without disinfection, intermediate after slow filtration, and lowest after UV treatment. Numbers of actinomycetes in the slabs, i.e. around the roots, were not distinctly different between the treatments. The implication of potential shifts in the microbial populations due to certain treatments for the disease development is not known. Increased knowledge on the beneficial microflora and the treatments that influence the composition of such a microflora, will stimulate the exploitation of microbially balanced and optimised soilless culture systems.

1. Introduction

In general, the strategy in glasshouse horticulture has been to keep the growing systems as clean as possible. However, certain root diseases still cause serious problems. Oomycetes, such as *Pythium* and *Phytophthora* spp., are generally present and well adapted to systems with high water retention capacity (Stanghellini and Rasmussen, 1994). Most of

the oomycetes are able to produce zoospores, which can actively swim to their hosts. *Pythium aphanidermatum* (Edson) Fitzp. is a serious threat in cucumber (*Cucumis sativus* L.) (Moulin *et al*., 1994a; Paternotte, 1992). Under favourable conditions, it spreads, multiplies and infects the crop extremely fast, resulting in severe root loss and crown rot. *Pythium* spp. are difficult to control. In the short term, there are no prospects for breeding resistant cultivars. Fungicides (i.e., propamocarb and metalaxyl) are only effective if used as preventive applications. And, in some countries, these fungicides are not registered for use on vegetable crops cultivated in hydroponic systems. However, *Pythium* spp. are poor competitors relative to other root-colonising organisms (Hendrix and Campbell, 1973; Rankin and Paulitz, 1994). Therefore, it is expected that *Pythium* spp. can be controlled with introduced antagonists or with micro-organisms that are naturally present.

Micro-organisms, such as *Pseudomonas* spp., *Streptomyces* spp., *Trichoderma* spp., *Bacillus* spp., *Enterobacter cloacae*, and *Pythium oligandrum* have the potential to control *P. aphanidermatum* (Bolton, 1980; McCullagh *et al*., 1996; Moulin *et al*., 1994b; Nelson and Craft, 1992; Paulitz *et al*., 1992; Sivan *et al*., 1984; Wölk, 1990). These antagonistic organisms can be introduced as biocontrol agents. But, often, a lack of survival or activity of the introduced organisms results in disappointing biocontrol effects. To overcome this problem, the indigenous microflora can be exploited. Indigenous micro-organisms have several advantages over introduced organisms; they are adapted to the environment, and they form stable (complex) communities. Moreover, combinations of micro-organisms are probably more effective at controlling pythium than single strains (Paulitz *et al*., 1990; Waechter-Kristensen *et al*., 1994).

In a cucumber crop the recirculated nutrient solution is nearly always disinfected. The effect of such a treatment on the composition of the indigenous microflora and, consequently, on pythium crown and root rot is not known. Slow filtration does not destroy the entire natural microflora and is expected to avoid unbalanced recolonisation, probably stimulating more stable and robust cropping systems as compared to a system in which nearly the entire natural microflora is eradicated.

In the present paper, the potential of several antagonists and of the indigenous microflora to suppress *P. aphanidermatum* in soilless culture systems is described. Also the effect of different disinfection treatments of the nutrient solution on the population dynamics of the microflora was studied.

2. Materials and methods

2.1. Biological control of *P. aphanidermatum* with antagonists

During several years, we tested 17 fungal and bacterial antagonists for their ability to control *Pythium aphanidermatum* (Edson) Fitzp. in cucumber (cv. Tyria) on rockwool during nursery and production stage.

At the Research Station for Floriculture and Glasshouse Vegetables (Naaldwijk, The Netherlands) the products of *Streptomyces griseoviridis* (Mycostop, Kemira Agro Oy, Finland), *Gliocladium catenulatum* (Gliomix, Kemira Agro Oy, Finland), *Gliocladium virens* (SoilGard, Grace, USA), and isolates of *Pythium oligandrum* (Polyversum, E. Duskova, Czech Republic), and *Trichoderma harzianum* (MTR35, Makhteshim Chemical Works Ltd, Israel; Supresivit, E. Duskova, Czech Republic; TRI 002, Plant Support, The Netherlands; and 3 isolates from L.D. Orlikowski, Poland) were tested with young plants in an "ebb-and-flood" system (i.e. prolonged nursery stage). Conditions were similar to horticultural practices. Antagonists were added with the nutrient solution to 1 week old cucumber plants grown in rockwool blocks. Antagonist concentrations were 10^7 cfu *T. harzianum* /ml, 250 cfu *P. oligandrum* /ml, or recommended concentrations for the products. *P. aphanidermatum* grown on potato dextrose agar was added with the nutrient solution 1 week later (approx. 25 cfu/ml). Disease symptoms (i.e. brown stem base, wilted and dead plants) were scored when plants were 6 weeks old. The same antagonists were tested for their biocontrol effect during the production stage. They were added twice with

the nutrient solution, first to 1 week old cucumber plants and secondly after placing the 3-week old plants on rockwool slabs with a drip irrigation system. *P. aphanidermatum* was added to the nutrient solution 1 week later (approx. 25 cfu/ml). Disease symptoms (i.e. brown stem base, wilted, and dead plants) were scored until the crop was 3 to 4 months old. All antagonists showing significant disease reductions were tested in repeated experiments during different seasons. Each treatment consisted of five replicates.

At Plant Research International (Wageningen, The Netherlands) antagonists were tested in 32 independent "ebb-and-flood" units under standardised conditions in a closed greenhouse with heating and cooling. Temperature was 19/25 ±1 °C (night/day) and relative humidity 70 % (beginning of the experiment). Humidity was raised up to 90 % when plants grew larger. Additional light was given if daylight was less than 250 Watt/m^2. Isolates of *Pseudomonas fluorescens* (WCS365, B.J.J. Lugtenberg, The Netherlands; Pf15, T.C. Paulitz, Canada; R2f, J.D. van Elsas, The Netherlands), other *Pseudomonas* spp. (P2, J.D. van Elsas, The Netherlands; CH33, C. Alabouvette, France; Pc35 and Pc13, T.C. Paulitz, Canada), *Pythium oligandrum* (Polyversum, E. Duskova, Czech Republic), and *Trichoderma harzianum* (Supresivit, E. Duskova, Czech Republic), and a product containing *Streptomyces griseoviridis* (Mycostop, Kemira Agro Oy, Finland) were added on to the surface of the rockwool blocks or in the nutrient solution just after germination of the seeds and 1 week later. In some experiments, the antagonists were added weekly up to four times to improve their effect. Antagonist concentrations were equivalent to 3 x 10^5 cfu/ml in the nutrient solution. Only for *P. oligandrum* concentration was 10^3 cfu/ml nutrient solution. *P. aphanidermatum* grown in liquid V8-medium was added to the nutrient solution 1 week after sowing resulting in 200 oospores /ml (Postma *et al.*, 1996). The number of plants with disease symptoms (brown stem base, wilted, and dead plants) was determined up to 6 weeks after sowing. Each treatment consisted of four independent units arranged in a randomised block design.

2.2. Suppressive rockwool

Rockwool slabs (approx. 6 cm high) used for the production of cucumbers were collected from growers which did not have pythium symptoms in their crop. The slabs were cut in 15 x 20 cm pieces after removal of the plastic. Part of the slabs was autoclaved (20 min., 121 °C). Some of the autoclaved blocks were placed in contact with saturated non-autoclaved blocks for 1 or 2 days to allow recolonisation with the indigenous microflora. *P. aphanidermatum* inoculum (equivalent to 133 oospores/ml nutrient solution in the system or 3 x 10^5 oospores/plant) was added on the surface of all used rockwool slabs five days after autoclaving. The 6-day old seedlings in rockwool cubes of 4 x 4 x 4 cm were placed on the rockwool slabs one day after the pathogen was added. Experiments were conducted in the "ebb-and-flood" units at Plant Research International as described above with four 15 x 20 cm pieces of the rockwool slabs, each with three seedlings (Postma *et al.*, 2000).

In several treatments, numbers of culturable aerobic bacteria, fluorescent pseudomonads, filamentous actinomycetes and fungi were determined by plate counting. Appropriate dilutions were plated in duplicate on different media using a spiral plater (WASP, Don Whitley Scientific Limited, UK). Aerobic bacteria were enumerated on one tenth strength tryptic soy agar. Fungi were enumerated on one fourth strength potato dextrose agar with triton X-100 to inhibit mycelial growth. Plates were incubated for 7 days at 20 °C. Fluorescent pseudomonads were enumerated on King's B agar (under UV light) after 2 days incubation at 25 °C. Filamentous actinomycetes were enumerated on chitin-oatmeal agar with a 0.2 µm filter (87 mm diameter) placed for 5 days on the agar. Colonies were counted five days after removal of the filter (incubation at 25 °C) (see Postma *et al.*, 1996, 2000 for more details).

2.3. Effect of disinfection on the microflora

A cucumber crop was grown on rockwool at Institute of Agricultural and Environmental Engineering (Wageningen, The Netherlands) in a greenhouse with 12 independent closed systems. The nutrient solution was recirculated without disinfection, after UV treatment (250 mJ/cm^2) or after slow filtration through fine sand (0.15 – 0.35 mm diameter, D_{10} = 0.23 mm). The flow rate through the sand filter was 0.1 m/h. All treatments were applied in four independent closed systems. One week after planting young cucumber plants, *P. aphanidermatum* grown on potato dextrose agar was added to the rockwool blocks (approx. 10^6 cfu/plant). Population dynamics of the microflora at different locations in the system was assessed by plate counting as described above.

3. Results

3.1. Biological control of *P. aphanidermatum* with antagonists

Five of the 17 antagonists tested reduced the disease occurrence during nursery and/or production stage with at least 60 % (Table 1). However, none of these antagonists gave reproducible results during repeated experiments. Temperature influenced the biocontrol effects at the Research Station for Floriculture and Glasshouse Vegetables where none of the antagonists were effective in summer when the temperature was high (above 25 °C). However, variation in the biocontrol effects in the greenhouse of Plant Research International could not be explained by temperature variations, because temperature was very constant through the seasons (temperature deviant of less than 1 °C).

3.2. Suppressive rockwool

All rockwool slabs collected from growers without pythium symptoms in their previous crop were suppressive (Fig. 1). The results were very reproducible (4 years, 9 different rockwool batches) and significant at the P = 0.05 level. Analyses of variance (ANOVA) were followed by calculation of the least significant difference at a significance level of P = 0.05 and were carried out on the disease percentages or on square-root transformed data to stabilise variance. This disease suppression was proven to be due to the indigenous microflora, since the loss of suppressiveness in sterilised rockwool was (partly) restored be adding the original microflora to the sterilised rockwool. Comparing the microflora in rockwool treatments with different levels of suppressiveness showed the highest correlation between suppression and numbers of filamentous actinomycetes.

3.3. Effect of disinfection on the microflora

Disinfection by the UV treatment killed 99.6 % of the total aerobic bacteria. Diversity of the surviving microflora was probably low, since only few morphologically different bacterial colonies were present. Due to disinfection with slow filtration, numbers of total aerobic bacteria decreased with maximally 90 %, still leaving approx. 10^5 bacteria/ml in the nutrient solution. However, the composition of the microflora had changed due to the slow filtration. Numbers of pseudomonads, actinomycetes and fungi were eradicated to a higher extent than the total aerobic bacteria.

The disinfection treatments influenced the composition of the microflora in the container where the nutrient solution was collected. Numbers of filamentous actinomycetes were highest without disinfection, intermediate after slow filtration, and lowest after UV treatment (Fig. 2). Numbers of actinomycetes in the slabs, i.e. around the roots, were not distinctly different. The effect of the treatments on the development of pythium symptoms was not clear, since the disease incidence was low due to the season (autumn).

4. Discussion

The importance of the microflora in suppressing *Pythium aphanidermatum* in cucumber in a soilless system is clearly shown. Introduced as well as indigenous micro-organisms could suppress pythium symptoms significantly. Two bacterial and three fungal antagonists reduced the disease incidence with 60 % or more. Unfortunately, the results were poorly reproducible, and therefore not yet reliable for use in commercial horticultural practice. Insufficient biocontrol of *P. aphanidermatum* by introduced micro-organisms can be due to a lack of survival, activity, or to insufficient colonisation of the infection sites. Most of the antagonists were originally isolated from soil or other crops and are therefore probably not fully adapted to the present soilless system.

In contrast to the application of single introduced antagonists, the indigenous microflora showed a very reproducible suppression of pythium crown and root rot in cucumber. Disease reductions were 50 to 100 %. Whereas in soil and peat the existence of a suppressive microflora towards root pathogens is generally accepted, suppressiveness in soilless systems has only been demonstrated recently (Postma *et al.*, 2000). Only few other studies indicated the disease suppressive potential of the microflora in soilless systems. Artificial substrate suppressive towards fusarium wilt and crown and root rot could be created by growing consecutive infected crops reusing the nutrient solution or the substrate without disinfection (Rattink and Postma, 1996; Rattink unpublished). Also remarkable were the results in NFT (nutrient film technique) systems with *Phytophthora* and *Pythium* sp. in tomato, where closed systems showed less disease than run-to-waste systems (McPherson, 1998; Tu *et al*., 1999). In these studies the role of the microflora was suggested, but not really proven. In the present experiments, disease suppression was proven to be the result of the microflora, since suppressiveness of sterilised rockwool was recovered after its recolonisation with the original microflora.

With the described research we showed the ability of the microflora to suppress disease in soilless systems. This is a new trend, which is breaking with the "sterility" concept commonly used in soilless systems. Introduction or stimulation of a suppressive microflora in soilless systems, might create more stable systems having less problems with diseases. If beneficial micro-organisms are present in the system, they should not be eradicated by treatments such as disinfection of the recirculated nutrient solution. Therefore, the effect of different types of disinfection on the composition of the microflora were compared. First results of this type of research showed a minor impact of the disinfection treatments on the microflora in the rockwool slabs. However, the numbers of filamentous actinomycetes in the nutrient solution in the container after the disinfection treatment were lowest after UV treatment, intermediate after slow filtration, and highest without disinfection. Actinomycetes are suggested to play a role in disease suppression, since their presence correlated with disease suppressiveness in used rockwool, as well as in soil systems (Workneh and van Bruggen, 1994) and compost (Tuitert *et al.*, 1998; Craft and Nelson, 1996). The ultimate answer, however, is the effect of disinfection treatments on disease development, as a consequence of the changes in the microflora.

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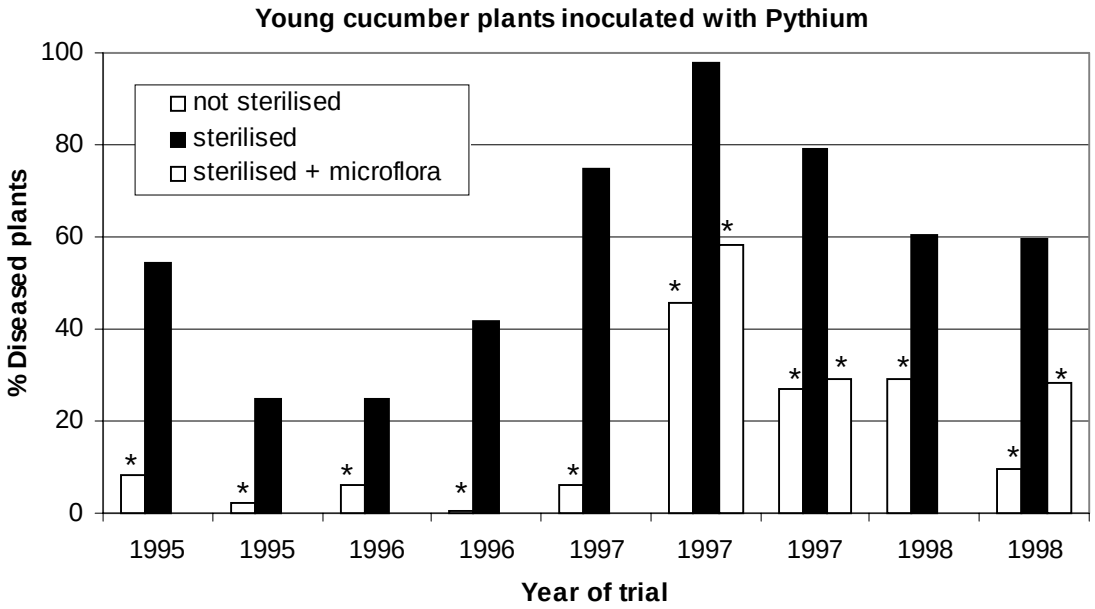
Tables

1. Biocontrol of *Pythium aphanidermatum* in cucumber with five antagonists in repeated experiments during prolonged nursery and/or production stage at Plant Research International (PRI) and/or the Research Station for Floriculture and Glasshouse Vegetables (PBG)

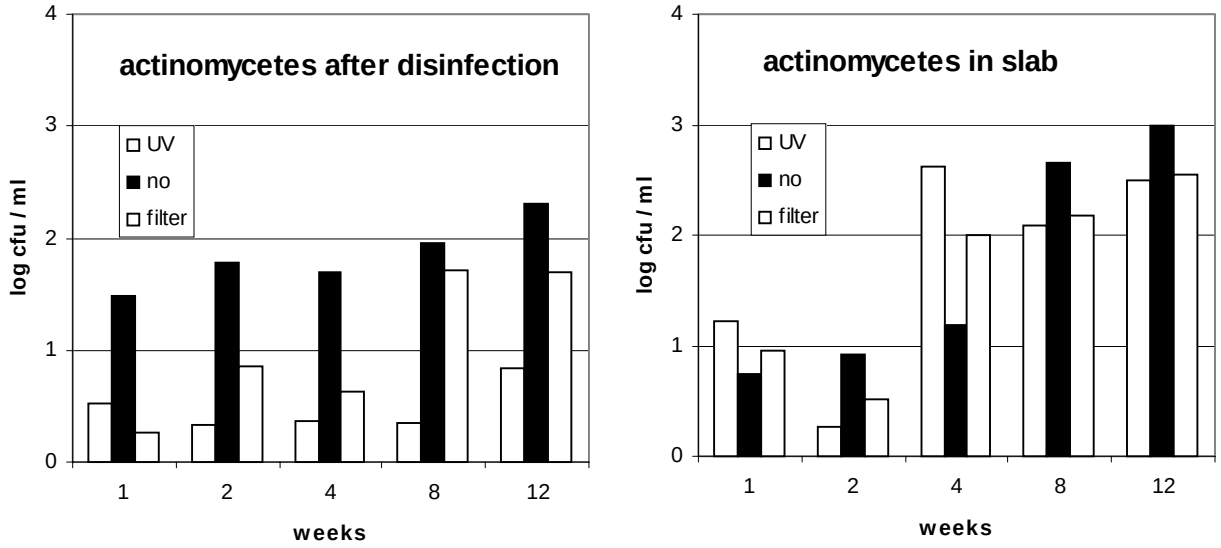
Antagonist	Nursery[PRI]				Nursery[PBG]					Production[PBG]				
Pseudomonas fluorescens WCS365	+	++	±	-										
Pythium oligandrum (Polyversum)	++	-	+		++	-	+	++	+	±	+	++	+	-
Streptomyces griseo-viridis (Mycostop)	+	+	-	±						++	-	±		
Trichoderma harzianum (Poland)					++	-	-	++		+	++	++	-	
Trichoderma harzianum (Supresivit)	+				++	-	-	++	±	±	±	±	++	-

- = no effect, ± = positive trend, + = significant reduction, ++ = > 60% disease reduction

Figures



1. Percentage diseased cucumber plants after adding *Pythium aphanidermatum* to used rockwool which was not sterilised, sterilised, or sterilised followed by recolonisation with the microflora from non-sterilised rockwool. Bars marked with * are significantly different from the highest bar (sterilised used rockwool) according to the least significant difference test (P = 0.05).



2. Numbers of filamentous actinomycetes in the nutrient solution in the container after disinfection and in the rockwool slabs of a cucumber crop grown in a closed system. Treatments were nutrient solution recirculated without disinfection (no), after UV treatment (UV) or after slow filtration (filter). $LSD_{0.05} = 1.04$.