Cross Response of Slow Filters to Dual Pathogen Inoculation in Closed Hydroponic Growing Systems

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Abstract: The efficacy of slow filters for horticultural purposes is suggested to be the result of multiple factors. Physical and biological properties of the filter column and the nutrient solution are likely to influence on filter performance. It has previously been shown that enzyme activity on the filter skin is a main parameter which will explain roughly 50% of filter efficacy. Since enzyme activity is enhanced by the addition of fungal cell walls to the filter skin, presence of a fungal root pathogen might affect enzyme activity and filter efficacy. In this study, slow filters integrated into closed NFT systems with a tomato crop were inoculated with mycelia from *Pythium aphanidermatum*. Enzyme activity, microbial colonisation, polysaccharide content and DNA content of the filter skin as well as microbial flora and total organic carbon in the nutrient solution were monitored. Tests of filter efficacy against *Fusarium oxysporum* f.sp. *cyclaminis* showed no significant influence on filter efficacy by the addition of *P. aphanidermatum*. Enzyme activities in the filter skin were not affected by the addition of *P. aphanidermatum.* A negative correlation was found between biofilm polysaccharide content and activity of xylanase activity on the filter skin. Filter efficacy was high (>99.9%) throughout the study. No damage to the crop as effect of pathogenic fungi was seen. We concluded that abundance of an oomycete pathogen in a closed hydroponic system does not affect filter efficacy, and that the filter skin microflora function, but not composition, is affected by this pathogen.

Keywords: *Fusarium oxysporum,* hydroponics, polysaccharide, *Pythium aphanidermatum*, slow filtration, TOC, tomato, viable count.

INTRODUCTION

Statutory requirements to reduce nutrient leakage, and the benefits of reducing water and fertiliser use have encouraged the use of closed hydroponic growing systems in greenhouse horticulture. However, closed systems carry a higher risk of root-borne pathogens spreading in the crop than open systems [1]. To prevent dispersal of diseases with the recirculated nutrient solution, drained nutrient solution is disinfestated before reuse by techniques such as pasteurisation, UV radiation, ozonisation or slow filtration are developed for this purpose [2].

Slow filtration is as a low-cost technique with good efficacy towards significant root pathogens in such as *Phytophthora*, *Pythium* and *Fusarium* in closed systems [3-6] and appears therefore to be a suitable alternative for greenhouse industries with small production areas. The disinfesting ability of slow filtration is based on a combination of physical, chemical and biological factors. The uppermost layer of the filter column, the filter skin (*"Schmutzdecke"*) is a microbially densely colonised layer, that plays a major role in pathogen removal [7]. To improve confidence in slow filtration as a reliable method, growers have been asking for tools for monitoring the efficacy of slow filtration during operation.

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For experimental purposes, the efficacy of disinfection equipment is commonly assessed by artificial massive inoculation with plant pathogens of relevance for horticultural cropping systems. Such pathogens include *Pythium* spp. [8], *Fusarium oxysporum* [9,10], *Xanthomonas campestris* [11] and Tomato Mosaic Virus [12].

However, this method is not applicable in operating commercial systems. Brand & Alsanius [13] showed that enzyme activity explained approximately 50% of filter efficacy. They also showed that enzyme activity on the filter skin is induced by the addition of fungal cell wall preparations (FCWP) from *Fusarium oxysporum* f.sp. *cyclaminis*. A recent study reported that chitinase and cellulase activity of the filter skin is induced by both *Fusarium oxysporum* and *Pythium aphanidermatum* [14]. According to *in vitro*-assays, biofilm polysaccharides (bPs) and total polysaccharides (tPs) might have potential as additional predictors of filter efficacy [15]. However, no full-scale trials have been performed. As root infection by pathogens results in increasing release of organic carbon to the nutrient solution due to degradation of root tissue and increased leakage of root exudates from the wounded roots [16], total organic carbon (TOC) and carbon turnover are also of interest.

These state variables (enzyme activity, bPs, tPs and TOC) are also related to the indigenous microflora in the cropping system. Fluorescent pseudomonads, which are abundant in the filter skin, are efficient utilisers of organic compounds, [7, 17] and producers of antimicrobial metabolites [18]. The possible connection between TOC of the nutrient solution and colonisation of the filter skin by

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fluorescent pseudomonads suggests that TOC is of interest both for indicating infection and for predicting filter efficacy.

Our starting hypotheses were that i) filter efficacy can be predicted using the parameters bPs and tPs, and that suboptimal factors will favour disease development, ii) the presence of *Pythium* on the filter skin will increase cellulase and protease activity, iii) TOC and microflora in the nutrient solution and on the filter skin will increase in the presence of *Pythium*, and iv) the efficacy of the filter will be affected by *Pythium* occurrence.

MATERIALS AND METHODOLOGY

Greenhouse Experiment

Experiments were carried out in a 90 m^2 greenhouse chamber at the SLU research facility, Alnarp, Sweden. Tomato plants (*Lycopersicon esculentum,* cv. Aromata) were grown in six independent systems using Nutrient Film Technique (NFT), each connected to two slow filters. Plastic gutters with a slope of 3% and a length of 6 m were covered inside with plastic film. Greenhouse temperature was set to 22 ºC day/21 ºC night, respectively. Four-week old plantlets (15 plants $gutter^{-1}$) were transferred into the system in mid-February. Artificial lighting was used for the first three months of the experiment (SON-T, 200 W m^{-2}). Nutrient solution was prepared according to Sonneveld and Straver [19] with (per 1000 L of water), KNO_3 810 g, NH_4NO_3 48 g, $MgSO_4$ 225.75 g, KH_2PO_4 205 g, $Ca(NO_3)_2$ 690 g, FeEDTA 6.875 g, $MnSO_4$* 2 H_2O 2.338 g, $ZnSO_4$ * 7 H_2O 1.798 g, H_3BO_3 1.932 g, $CuCl_2$ * 2 H_2O 0.16 g, and Na_2MoO_4 * 2 H_2O 0.151 g). Electric conductivity was set to 2.5 ±0.2 mS cm^{-1} and pH was set to 5.9 ±0.1 by the addition of 2M sulphuric acid or 2M potassium hydroxide and manually adjusted once a day. Tap water was supplied continuously with respect to depletion. Nutrient solution flow rate was set to 96 L h^{-1}. Nutrient solution effluent from the gutters was collected in 100 L plastic collection tanks and pumped from there into the slow filter. The filtered solution was returned to the NFT-gutters after passing a through a flow meter and flow control valve.

Slow Filter

Slow filters (surface area 0.22 m^2, filter bed depth of 0.4 m + 0.1 m drainage layer) were constructed from plastic containers (200 L) filled with rockwool granulate (Grodan® 14556, Roermond, the Netherlands) at a density of 190 kg m^{-3}. The flow rate of the filter was set to 300 L m^{-2} h^{-1}. The microbial population in the slow filters were natural colonization. The filter skin was enriched weekly with fungal cell wall preparations, (FCWP, 10.5 g m^{-2} filter surface area) from *Fusarium oxysporum* f.sp. *cyclaminis* produced according to Sivan and Chet [20]. Lyophilised FCWP pellets were resuspended in water using a high-frequency mixer. The filter was stopped and FCWP-suspension was added and allowed to settle for 20 minutes.

In three out of the six systems in the experiment, living mycelium mats from *Pythium aphanidermatum* were supplied to the filter skin weekly.

Inoculum Preparation

Mycelium mats from *Pythium aphanidermatum* were grown on V8 medium according to Postma *et al.* [21]. Pieces of agar with *Pythium* hyphs where placed in petri dishes with 20 mL of V8 medium. After 5 days of incubation at 20 °C, a mycelium mat was formed. The mats were washed with detergent (0.1% peptone from meat, Fluka 70175, 0.2% sodium hexametaphosphate, Fluka 71600, in aqua dest.) and autoclaved tap water to induce zoospore production [22].

For the efficacy tests, *Fusarium oxysporum* f.sp. *cyclaminis* was grown on PDA agar for two weeks and then 10 mL sterile NaCl-solution (0.85% w/v) were added to petri dishes and mycelium was abrased from the agar, and the resulting conidial suspension was filtered through cheesecloth.

ANALYSES

Efficacy Test of the Slow Filter

The efficacy of the filter towards the pathogen *Fusarium oxysporum* f.sp. *cyclaminis* was tested three times during the experiment. The flow through the filter was stopped and a conidial suspension of *Fusarium oxysporum* f.sp. *cyclaminis* was added to the collection tank at a final concentration of approximately 10^4 spores mL^{-1}.

After thoroughly mixing the conidial suspension with the nutrient solution, the system was restarted and samples from the nutrient solution before (supernatant) and after filtration (effluent) were taken two times per hour for two hours (supernatant) or for five hours (effluent). The content of *Fusarium oxysporum* in the samples was evaluated using semi-selective agar medium Komada [23]. Komada plates were inoculated with 100 µL sample suspension and incubated for five days. Five or three replicate plates were used for the supernatant and effluent, respectively. Colonies were counted on plates containing 30-300 colonies. Efficacy was calculated using the equation

$$\text{Efficacy (\%)} = 1-(cfu_{\text{after filtration}}/cfu_{\text{before filtration}}) \times 100 \quad (1)$$

Sample Collection

Samples of nutrient solution were collected from the filter supernatant and effluent, and filter skin material were collected on four occasions at three-week intervals, starting when the culture was six weeks old. Aliquots of 100 mL nutrient solution were sampled in sterile bottles. Microbial analyses were performed immediately, while samples for TOC analyses were stored at -20°C. For the filter skin, small pieces of filter material were taken aseptically and immediately frozen at -80°C for 30 min before lyophilisation for 48 h and storage at -20°C.

Enzyme Activity

Enzyme activity on the filter skin was analysed with respect to five enzymes (chitinase, protease, β-1,3-glucanase, cellulase and xylanase) using the method described by Brand & Alsanius [24]. In brief, lyophilised samples from the filter skin were incubated with enzyme substrate (Blue substrates, Göttingen, Germany, CM-chitin-RBB, Gelatine-RBB, CM-curdlan-RBB, CM-cellulose-RBB, and CM-xylan-RBB for

chitinase, protease, β-1,3-glucanase, cellulase and xylanase, respectively) at 37 ºC for two hours. Aliquots of 150 µL from the incubated enzyme substrate were transferred to a microtitre plate, where reaction was terminated by the addition of 150 µL 2M HCl. After centrifugation (3250 x g, 15 min), 150 µL of the supernatant solution were transferred to an additional plate. The light extinction of the solution was measured spectrophotometrically at 550 nm (chitinase) or 590 nm (protease, β-1,3-glucanase, cellulase and xylanase) (Asyshitec, Linz, Austria, software Digiwin). Samples prepared as described above but with reaction terminated without incubation were used as controls. Enzyme activity was calculated by the equation

$$\text{Enzyme activity (mU)} = (E_s\text{-}E_c) \times 1000 \times t^{-1} \quad (2)$$

with E_s = extinction of the sample, E_c = extinction of the control and t = incubation time (min).

Polysaccharide Analysis

The content of total exo-polysaccharides (tPs) in the filter skin was analysed using the phenolic method of Dubois *et al.* [25] modified by Furtner *et al.* [15]. Lyophilised filter skin samples (20 mg dry matter) were incubated with 0.8 mL of 5% phenol and 4 mL of H_2SO_4 (95-97%) for 30 minutes at 25ºC. After centrifugation (3000g for 5 minutes, 25ºC), 3 mL of the supernatant solution were transferred to plastic cuvettes and absorbance was measured at a wavelength of 488 nm (Cary 50, Varian Inc. Palo Alto, USA). Content of total exo-polysaccharides was calculated from a calibration curve made by glucose at 10 different concentrations (0.02-0.11 mg glucose) and expressed as mg per g of filter skin.

The filterskin Biofilm polysaccharide (bPs) content was measured as described by Furtner *et al.* [15]. In brief, Biofilm polysaccharides were extracted from the lyophilised filter material using a mixture of 0.23% formaldehyde (v/v) and 0.8 % sodium chloride (w/v) during 1 hour of incubation at 4ºC. After addition of sodium hydroxide solution (1 M) the solution was incubated for a further three hours at 4ºC. The mixture was centrifuged (20,000 x g, 20 min.) and supernatant solutions was filtered through a membrane filter (0.2 µm, Minisart, Sartorius, Germany). This solution was subjected to polysaccharide analysis as described for tPs.

DNA Analysis

The proportion of DNA on the filter skin was determined using the fluorescent dye method, to ensure that cell lysis during polysaccharide extraction was low [26]. Biofilm extraction was performed as described for bPs analysis, but using wet samples (3.4 g). Aliquots of 2 µL of the extracted solution was transferred to a quartz cuvette with 2 mL of TN8-buffer, and measured photometrically (Hoefer DyNA Quant 200, Hoefer Scientific Instruments, San Francisco, USA) with a standard curve from DNA solution (Sigma D-4764, 1 mg mL^{-1}). DNA content was expressed as mg g^{-1}.

Organic Matter

Total organic carbon (TOC) content in the nutrient solution was measured using a method by Hach-Lange GmbH, Germany. Sample suspension was added to a tube with an oxidiser. During a two-hour incubation period at 100 ºC, the organic carbon in the solution was oxidised to carbon dioxide, which was carbon captured in a container with a chemo-chromatic solution. The extinction of the solution was measured using a photometer (Xion 500, Hach-Lange GmbH, Düsseldorf, Germany) and the TOC-content was calculated by the instrument.

Organic matter content of the filter skin was determined at the end of experiment. Pieces of the filter skin (200 cm^2 from each filter) were carefully lifted and transferred to glass petri dishes. Samples were dried for four days at 70°C. Organic carbon content was determined through incineration, with unused mineral rock wool served as the control. Organic matter (OM) content of the filter skin was calculated as

$$\text{OM (\%)} = 1 - \text{Weight sample}_{\text{after incineration}} / \text{Weight sample}_{\text{before incineration}} \times 100 \quad (3)$$

These analyses were performed by LMI AB, Helsingborg, Sweden.

As a supporting variable, dissolved oxygen content in the nutrient solution was measured weekly using an oxygen meter (OxyGuard handy Mk I, OxyGuard a/s, Birkeröd, Denmark, before and after filtration) seven days after addition of FCWP. The temperature of the nutrient solution at the inflow to the filter was recorded every 30 minutes using dataloggers (HOBO H8, sensor TMC6-H, Onset computer corp., Bourne, USA).

Microbial Analysis

The nutrient solution before and after filtration and the filter skin were analysed with respect to microbial factors using a spread-plate technique as earlier described by Khalil [27]. The general bacterial flora was analysed using R2A agar (Difco 218236), incubation at 25°C for 3 days, general fungal flora using diluted malt extract agar (per L. of water; 10 g Malt Extract (Difco 218630), 20 g Bacto agar (Difco 0140-01), incubation at 25°C for 4 days, fluorescent pseudomonads using King's medium B [28], incubation at 25°C for 2 days and *Pythium aphanidermatum* was monitored using PDA (Difco 213400) incubated for 18 h at 35°C. supplemented with rifampicin and kanamycin (Alsanius *et al.*, submitted manuscript). Each sample was analysed on two replicate plates.

Crop Development

Crop development was monitored once a week. Plant length, total leaf length, stem diameter, number of trusses, number of leaves, number of flowers per truss and truss development were recorded. Plant length was recorded through measurements of apical shoot growth. Total leaf length was measured on the first leaf under the flowering truss. The stem diameter was measured at two diametrically opposed positions on the stem, at the point where apex was positioned seven days before. The numbers of fully developed leaves (under the flowering truss) were enumerated. The numbers of flower buds on each truss were counted.

Statistical Analyses

The experiment was carried out with three independent systems with two replicate filters each per treatment. The

results of the viable counts are expressed as mean ± SD after log transformation [29]. The data were analysed with ANOVA, $p<0.05$ considered significant, and step-wise regression, were the level of significance was $\alpha=0.05$. Two-sample T-tests were used to decide on significance for levels of TOC, dissolved oxygen and crop development. Calculations were performed with software Minitab 15 (ver. 1.1.0, Minitab inc., State College, USA).

RESULTS

Biochemical and Chemical Assessment

Chitinase activity in the filter skin was low at the beginning of the experiment, increased for the following three weeks and then declined during the last six weeks of the experiment. This was most apparent for the filters without addition of *Pythium aphanidermatum*. For the cellulase, similar trends were observed. Glucanase activity was significantly higher at the second sampling compared with the first in the systems without *P. aphanidermatum*. For the protease, activity was higher at the first sampling than at subsequent samplings, irrespective of treatment (Fig. **1**).

The tPs-values were around 4 mg g^{-1}, no differences were found between treatments or over time. The bPs content increased on the last sampling occasion (data not shown). This increase in bPs during the end of the experiment was correlated with the decrease in xylanase (R^2 0.938, $p=0.001$, $R^2=0.711$, $p=0.000$ with PA/without PA, respectively).

Filter efficacy against *Fusarium oxysporum* f.sp. *cyclaminis* was high (99.9%) on all test occasions. No differences were found between treatments.

The levels of DNA on the filter skin decreased during the experiment in the treatment with PA, but there were no significant differences between treatments (data not shown). In the supernatant solution from the filters (before filtration), the general bacterial flora (cfu log 3.90±0.76), fungal flora (cfu log 0.75±0.37) and levels of fluorescent pseudomonads (cfu log 0.78±0.55) remained unchanged during the experiment. *Pythium aphanidermatum* was not observed in the supernatant.

In the effluent solution (after filtration) no differences were observed neither for general bacterial flora (cfu log 3.35±0.64), the general fungal flora (cfu log 0.67±0.46) nor fluorescent pseudomonads (cfu log 1.47±0.83). In general, microbial counts appeared to fluctuate more in the effluent. *Pythium aphanidermatum* did not occur in the effluent solution at any sampling.

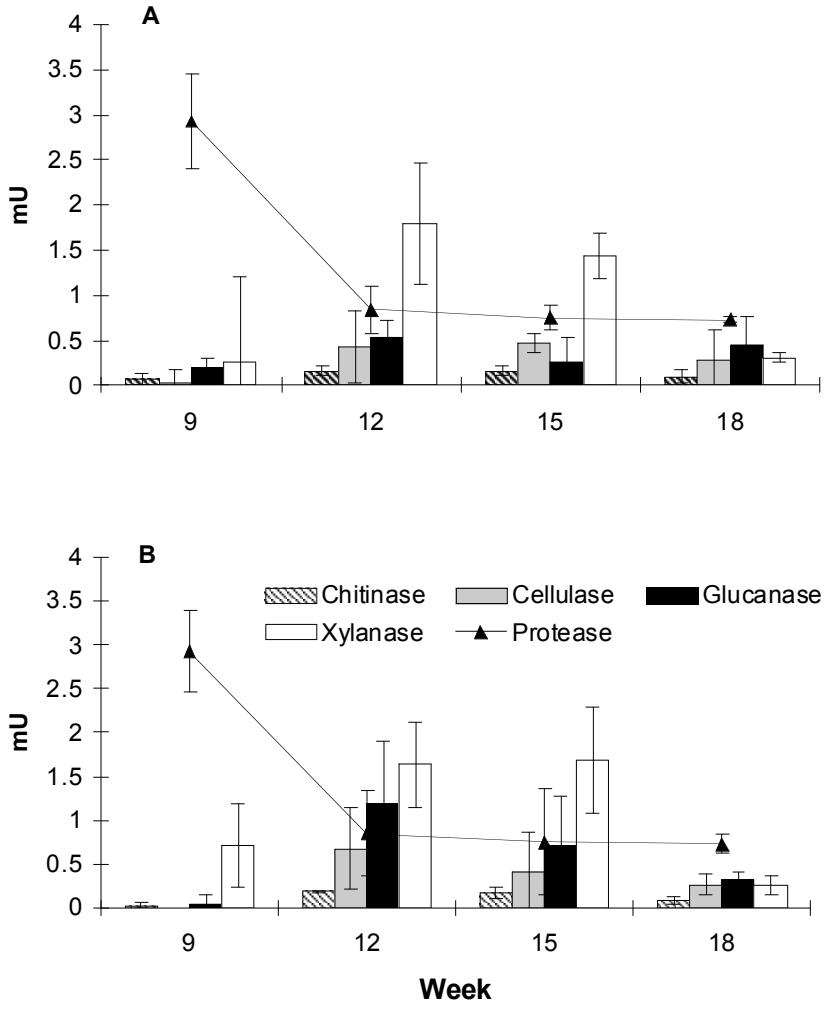

Fig. (1). Activity of five enzymes on the filter skin of slow filters in closed hydroponic systems with (**A**) or without (**B**) addition of *P. aphanidermatum*.

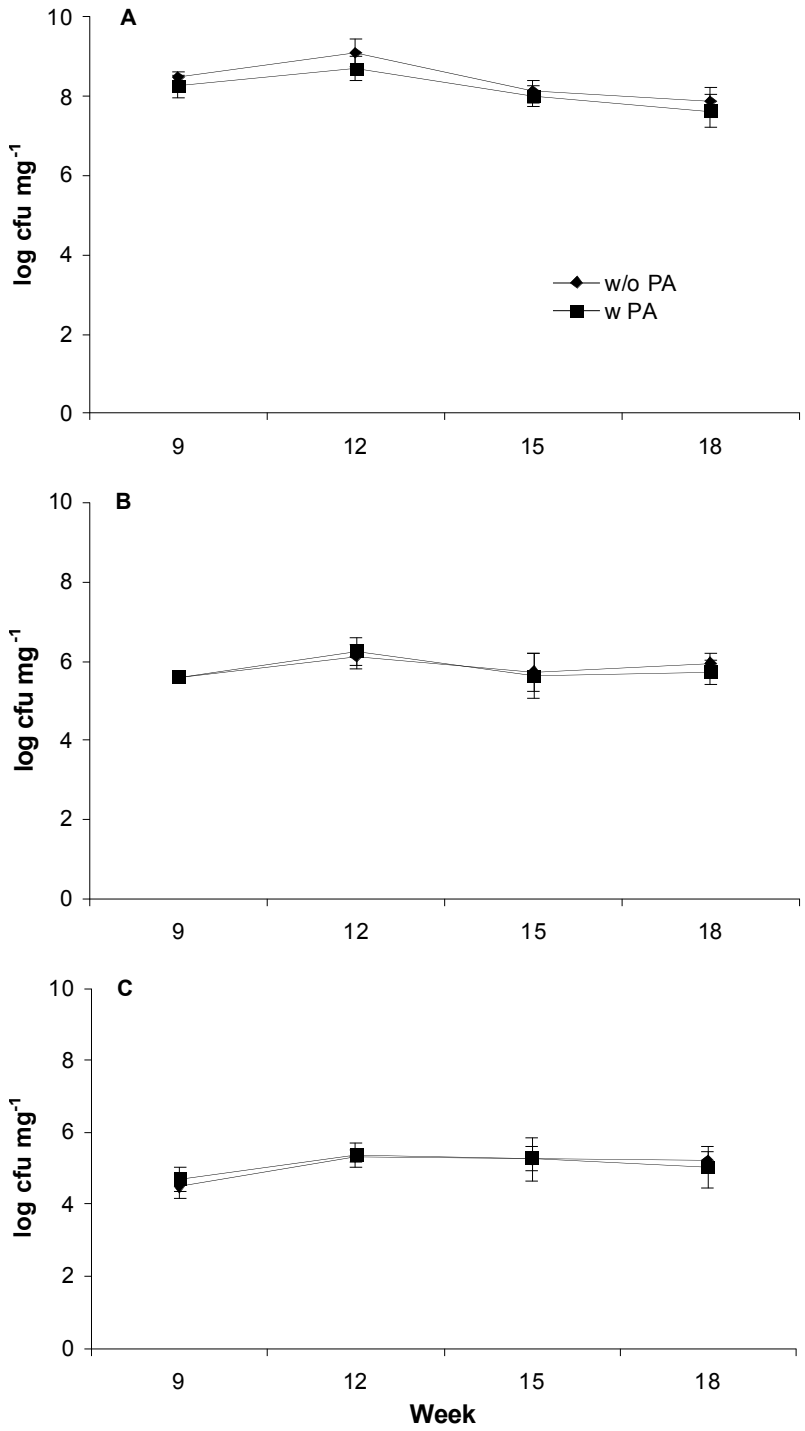

Fig. (2). (**A**): Bacterial flora, (**B**): Fungal flora, (**C**): Fluorescent pseudomonads on the filter skin of slow filters with addition of *P. aphanidermatum* ('w PA') or with no addition ('w/o PA'). Samples were taken from end of February to beginning of May.

For the filter skin, the general bacterial flora (analysed on R2A agar) on the filter skin decreased slightly during the experiment irrespective of treatment. For general fungal flora and fluorescent pseudomonads no differences were found (Fig. **2**). The ratio of fluorescent pseudomonads to general bacterial flora in the non-filtered nutrient solution at the beginning of the experiment was approx. 1:10, with a tendency to increase to 2:10 at the end of the experiment in the *Pythium*-enriched treatment. *Pythium aphanidermatum* was sporadically found on the filter skin.

Interactions between biological/biochemical factors and enzyme activity were found, described by the equations:

$$Chit = - 0.144 + 0.0239\ R2A - 0.0287\ MA + 0.0589\ KB + 0.00561\ tPs - 0.00509\ bPs - 0.128\ DNA \quad (R^2=0.509,\ p=0.037) \quad (4)$$

$$Prot = 2.47 + 0.362\ R2A - 0.237\ MA - 0.670\ KB - 0.078\ tPs + 0.0343\ bPs + 3.34\ DNA \quad (R^2=0.564,\ p=0.016) \quad (5)$$

$$Xyl = 0.68 + 0.025\ R2A - 0.344\ MA + 0.507\ KB + 0.104\ tPs - 0.00509\ bPs - 0.128\ DNA \quad (R^2=0.473,\ p=0.035) \quad (6)$$

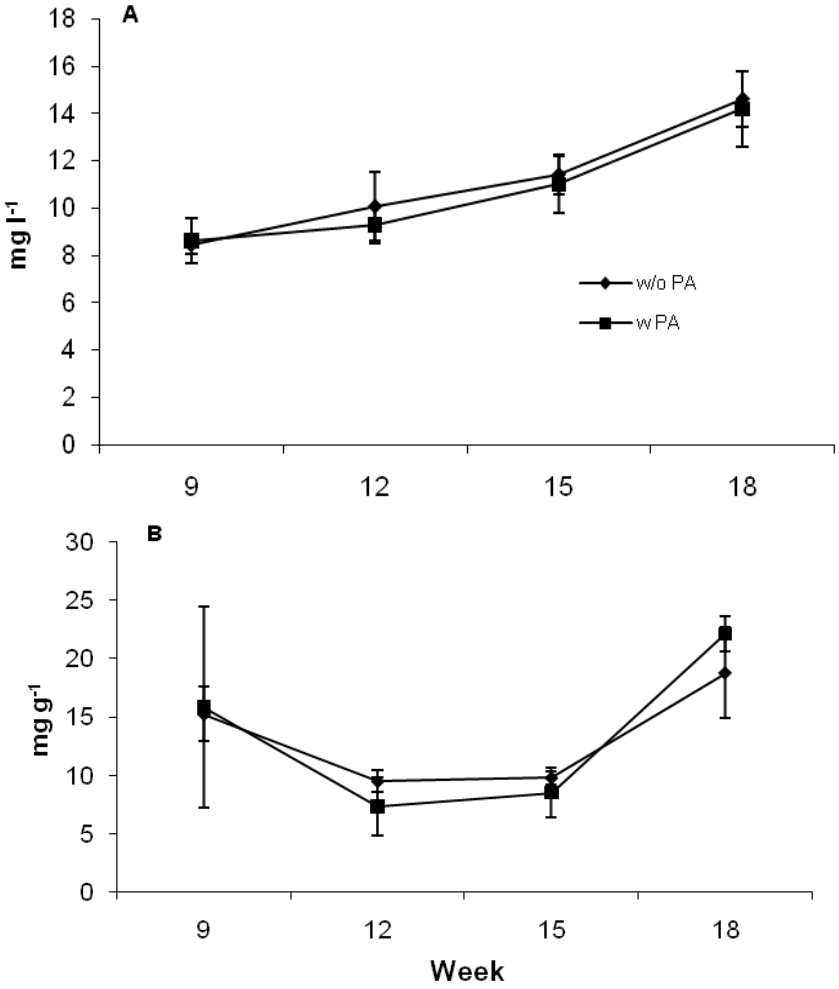

Fig. (3). Concentration of total organic carbon (**A**) in the nutrient solution from closed hydroponic systems with slow filters, and biofilm polysaccharides (**B**) from the filter skin of the slow filters. 'w PA' denotes weekly enrichment of the filter skin with mycelium from *P. aphanidermatum*, 'w/o PA' denotes no addition of mycelium of *P. aphanidermatum*.

bPs = 23.1 – 38.6 Chit – 1.27 Prot + 2.67 Cell + 0.65 Gluc – 4.42 Xyl (R^2=0.514, p=0.016) (**7**)

where Chit, Prot, Cell, Gluc, and Xyl represent chitinase, protease, cellulase, β-1,3-glucanase and xylanase activitiy in the filter skin, respectively. tPs represent total polysaccharides, bPs biofilm polysaccharides and R2A, MA and KB represent plate counts from general bacterial and fungal flora and fluorescent pseudomonads in the filter skin, respectively. DNA denotes DNA amount in the filter skin.

Chemical and Physical Assessment

Total organic carbon accumulated in the nutrient solution during the experiment. This trend accelerated at the end of the experiment with a significant increase in TOC-levels during the last three weeks of the experiments (Fig. **3**). No differences between the treatments could be stated. Organic of the filter skin was on average 1.4% of dry matter at the end of experiment and again, no differences were found between treatments.

Levels of dissolved oxygen were significantly higher in both supernatant and effluent solution in the system without addition of *P. aphanidermatum*, compared with the system with *P. aphanidermatum* (p=0.002, p=0.004, respectively, data not shown). The average temperature of the nutrient solution was 23.4°C.

Crop Development

Plant height described a linear regression at R^2 = 0.99 (Fig. **4**). Stem diameter reached a maximum around four weeks after the start of the experiment and declined slowly for the remaining 12 weeks (Fig. **4**). Leaf length increased during the first five weeks of the experiment and developed irregularly for the remainder of the experiment. The number of leaves increased, for the first four weeks almost linearly, for the following five weeks more irregularly, and then stabilising. Numbers of trusses described a similar pattern, with a regular increase for the first eight weeks after flowering, and then decreasing as harvest began. Step-wise regression revealed crop development to be a predictor of TOC content in the nutrient solution and protease activity of the filter skin, described by the equations (8) and (9):

TOC = 6.66 + 0.38 nt + 0.545 g (R^2= 0.715, p<0.001) (**8**)

Prot= 0.91 – 0.32 nt + 0.13 g (R^2=0.737, p<0.001) (**9**)

with nt=number of trusses and g=growth

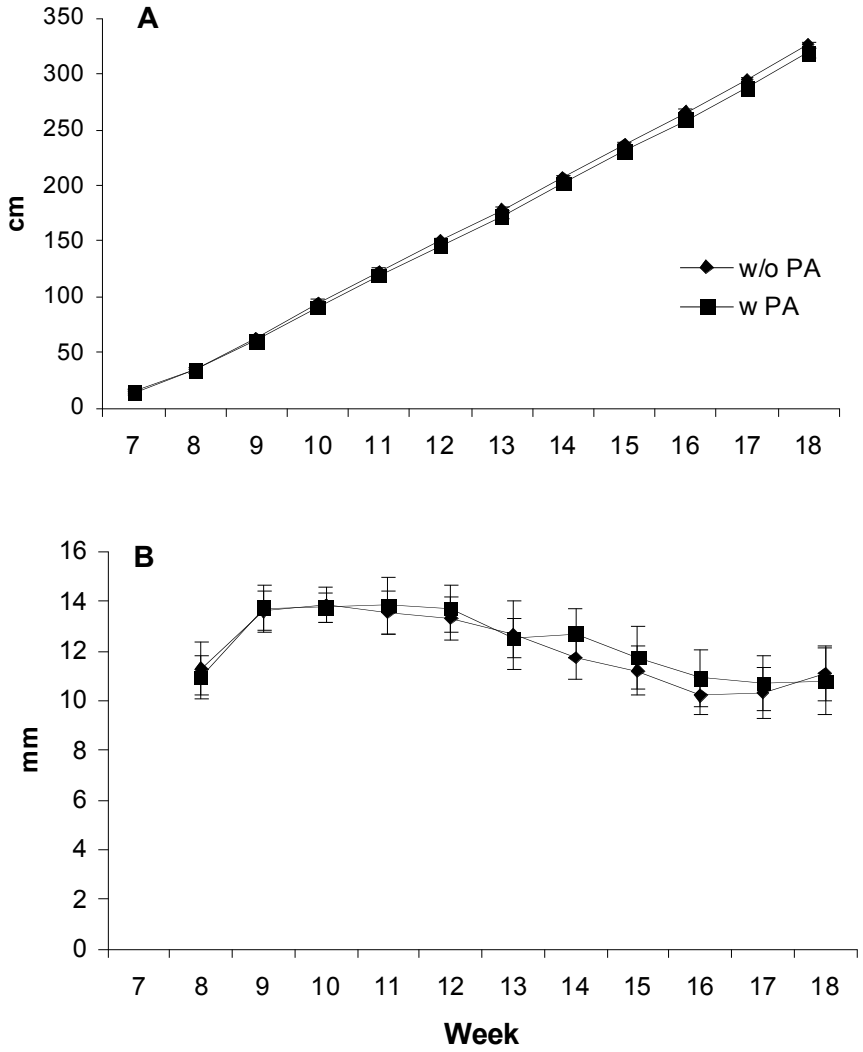

Fig. (4). Crop development of a tomato crop cultivated in closed hydroponic NFT-systems with slow filtration, where '**A**' denotes plant length and '**B**' stem diameter. Slow filters were amended (w PA) or without (w/o PA) *P. aphanidermatum* once a week.

Numbers of flowers per truss was the highest for truss no. 4 and then decreased slowly (data not shown). Addition of *P. aphanidermatum* to the filters did not affect crop development.

DISCUSSION

Enzyme activity in the filter skin of slow filters is enhanced by the addition of dead *F. oxysporum, Pythium ultimum* and constituents of fungal cell walls [14, 24]. In this study, living *P. aphanidermatum* was added to the filter skin but, no significant increase in enzyme activity was occurred. This was in contrast with our hypothesis. This might be explained by the time interval between amendment of *P. aphanidermatum* and sample incident. The samplings for enzyme activity were always performed five days after *P. aphanidermatum* addition. As Alsanius *et al.* [14] reported a peak in enzyme activity 48 to 72 hours after the addition of FCWP of *P. ultimum*, it might be possible that enzyme activity was higher immediately after the addition also in the present study. Furthermore, it is tempting to speculate that the microflora adapts to consecutive additions of the same compound by increased degradation pace as described by Alexander [30].

Total organic carbon content in the nutrient solution was increasing during the experiment. This was expected due to accumulation of dissolved organic compounds released from the roots [31, 32], due to increase of release of such compounds with increasing plant age [33], and a mismatch between factors and mechanisms responsible for degrading organic compounds in the nutrient solution [34]. No differences in the colony forming units of monitored microorganisms were found in the filter skin or nutrient solution irrespective of treatment or sampling occasion. However, the changes in enzyme activities indicated that the function of the microflora was altered, an event which is also supported by decreased dissolved oxygen levels in the nutrient solution when mycelium of *P. aphanidermatum* was added.

No effect was observed on tPs due to addition of living *P. aphanidermatum,* in agreement with Furtner *et al.* [15], who found that addition of FCWP from *F. oxysporum* did not affect tPs build-up on the filter skin during a 6-month operation period. However, Furtner *et al.* [15] found a correlation between bPs and efficacy, a relationship not

found in this study due to the high and consistent filter efficacy.

As filter efficacies in this experiment was high and consistent irrespective of treatment, no correlations between filter efficacy and other factors was obtained. It is possible that sub optimizing filter operation conditions further would reveal differences not obvious during the conditions employed in this experiment.

Furtner *et al.* [15] found xylanase activities ranging from not detectable up to 0.8 mU, whereas substantially higher values were found in this study. Results similar to those found in this study were also found by Alsanius *et al.* [35]. As xylan is a component of plant cells but not fungal cells [36] we speculate that plant cells deriving from the NFT-system was substrate for the microbially produced xylanase activity. Xylan is a component of plant cells [36] but not is as common in fungal cell walls. It is tempting to speculate that xylanase activity is due to plant cells (i.e. root pieces) from the NFT-system settling on the filter skin. The hypothesis that filter efficacy could be predicted using bPs and tPs was not confirmed by the experiment. The negative correlation between xylanase activity and bPs in this study was unexpected. We suggest that the decrease of bPs in connection with high xylanase activities is due to an adaptation of the microflora. This should be confirmed in future experiments.

Enzyme activity was not affected by the addition of *P. aphanidermatum*. Filter efficacy was high and no damage to the crop was evident. This shows that massive inoculation with pathogens does not negatively affect filter efficacy. It is possible that not only enzyme activities, but other microbial metabolites such as biosurfactants and other antimicrobial compounds, are produced by the filter skin microflora and that these compounds contribute to efficacy. For example, Jung [37] found increasing levels of benzoic acid in the nutrient solution from a tomato crop when *P. ultimum* was added. The ability of the filter skin microflora to produce biosurfactants has been demonstrated by Hultberg *et al.* [38, 39]. In future studies it would be of interest to examine the occurrence of these compounds.

CONCLUSION

The present study further supports the claim that slow filtration is a reliable disinfestation technique for hydroponic systems. Correlations were found between enzyme activity and microbial flora, bPs, tPs, and DNA on the filter skin. Also a correlation between TOC in the nutrient solution and plant development was observed. However, the aim of linking filter efficacy to selected parameters as enzyme activity, microbial flora, tPs and bPs was not fulfilled.

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