Europ.J.Hort.Sci., **68** (2), 67–76, 2003, ISSN 1611–4426. © Verlag Eugen Ulmer GmbH & Co. Stuttgart

Dynamics in Functional Diversity of Heterotrophic Microorganisms in a Closed Hydroponic Commercial Nursery Stock Plant Production System

Beatrix W. Alsanius and Victoria Jung
(Department of Crop Science, Swedish University of Agricultural Sciences, Alnarp, Sweden)

Summary

As an introductory survey for assessment of the risk for dispersal of plant diseases in a closed hydroponic outdoor cultivation system for nursery stock production the dynamics of the microflora inhabiting the roots of selected crops and inhabiting different localities within the irrigation system were studied using sole carbon source utilization. *Chamaecyparis lawsoniana*, *Ribes sanguineum* and *Symphoricarpos* were used as target crops. Analyses of irrigation water focused on three layers in the irrigation pond (surface, epilimnion; mid layer at 40 cm depth, metalimnion; bottom at 200 cm depth, hypolimnion). Root and water samples were collected with five replicates at three cardinal incidents during the vegetation period (autumn; spring; summer). Additional samples were taken from other water sources feeding into the irrigation pond, namely the three wells (spring and summer) and the retained irrigation effluent (summer).

The present study indicated shifts in functional diversity during the vegetation period, regarding both the grown crops and location within the irrigation system. It also showed differences between the chosen habitats. Differences also occurred between the sampling incidents with respect to utilization of *Pythium* specific carbon sources. With respect to carbon sources specific for *Pythium* ssp., utilization was high for the microflora of all three crops and started after a short lag-phase. Further, also utilization of *Pythium* specific carbon sources by the microflora inhabiting the studied water habitats was substantial. Discriminant analysis (DA) of the functional diversity at the chosen sampling sites with respect to sampling incidents was dominated by carbon sources specific for *Pythium*. However, utilization of precursors for phenolic compound metabolism, malonic acid and phenylalanine, was low in most cases. An negative correlation between α-D-glucose, D-trehalose and cis-aconitic acid to phenylalanine was found for the root samples during the second sampling. Some compounds (D-fructose, α-D-lactose, D-raffinose, L-aspartic acid, glycyl-L-aspartic acid, urocanic acid, glycerol, glucose-1-phosphate, glucose-6-phosphate) were found as discriminating between the samplings occurred for several of the studied habitats.

Zusammenfassung

Dynamik der funktionellen Diversität heterotropher Mikroorganismen in einem geschlossenen hydroponischen Baumschulsystem. In einer einführenden Studie zum Verständnis der Risiken der Verbreitung von Krankheitserregern in geschlossenen Kultursystemen im Freiland wurde die Dynamik der Mikroflora an ausgewählten Habitaten innerhalb eines geschlossenen Baumschulsystems anhand von Verwendungsmustern einzelner Kohlenstoffquellen (SCSU; Microlog GN-Panel) untersucht. Die Analyse konzentrierte sich auf die wurzelkolonisierende Mikroflora von drei Kulturen (*Chamaecyparis lawsoniana, Ribes sanguineum, Symphoricarpos*) und auf die Mikroflora, die verschiedene Schichten des Bewässerungsteiches besiedelt (Epi-, Meta- und Hypolimnion in 200 cm Tiefe) mit Probenentnahme zu drei Zeitpunkten während der Vegetationsperiode (Herbst, Frühjahr, Sommer). Ausserdem wurden zusätzliche Wasserquellen (Brunnen 1 + 3, Brunnen 2, rezirkuliertes Bewässerungswasser) innerhalb eines Erwerbsbaumschulsystems an zwei (Frühjahr, Sommer) bzw. einem Zeitpunkt (Sommer) während der Vegetationsperiode untersucht. Proben aus sämtlichen Habitaten wurden mit fünf Wiederholungen gezogen.

Die vorliegende Studie weist auf Veränderungen in der funktionellen Diversität während der Vegetationsperiode zwischen den Kulturen und zwischen den Probenentnahmezeitpunkten hin. Ausserdem wurden Unterschiede zwischen den gewählten Probeentnahmeplätzen im Bewässerungssystem gefunden. Signifikante Unterschiede zwischen den Probenentnahmezeitpunkten traten auch hinsichtlich der Verwendung *Pythium*-spezifischer Kohlenstoffquellen auf. Bezüglich der Verwendung *Pythium*-spezifischer Quellen stieg die Verwendung besonders schnell in den Wurzelproben an. Aber auch in den untersuchten Habitaten innerhalb des Bewässerungssystems kam eine bedeutende Verwendung *Pythium*-spezifischer Quellen vor. Die Analyse der Habitate in Bezug auf Probenentnahmezeitpunkte mit Hilfe der Diskriminantanalyse (DA) wurde von *Pythium*-spezifischen Kohlenstoffquellen dominiert. Die Verwendung von Substanzen, die die Vorstufen für die Produktion von phenolischen Stoffen sind (Malonsäure, Phenylalanin) war in den meisten Fällen ge-

ring und trat erst nach längerer Inkubationszeit auf. Eine negative Korrelation konnte zwischen einigen *Pythium*-spezifischen Substanzen (α-D-Glukose, D-Trehalose and cis-Aconitonsäure) und Pheylalanin bei der zweiten Probenentnahme festgestellt werden. Einige Substanzen (D-Fruktose, α-D-Laktose, D-Raffinose, L-Asparaginsäure, Glycyl-L-Asparaginsäure, Harnsäure, Glyzerol, Glukose-1-Phosphat, Glukose-6-Phosphat) zeichneten in der DA als determinierend für mehrere der untersuchten Habitate aus.

Key words. Biolog GN-panel – *Chamaecyparis lawsoniana* – irrigation system inhabiting microflora – *Ribes sanguineum* – root microflora – sole carbon source utilization (SCSU) – substrate microflora – *Symphoricarpos*

Introduction

Eutrophication of surface and ground water and concomitantly increased vegetation, oxygen depletion and death of heterotrophic organisms in lakes and cost near aquatic habitats has become an increasing problem in many areas (RAVEN et al. 1995). Due to legislative intervention and water conservation programs in different European countries, the release of nutrient enriched effluent from horticultural and agricultural production sites has to be stopped. For greenhouse market gardens, closed systems have increasingly been established, replenishing the recollected effluent with nutrients and water according to the plants' uptake and evaporation. But also intensive nursery stock plant production in containers is a source of nutrient leakage to the ground water. Due to the basic design of outdoor crop production, the production area has to be prepared with efficient drainage systems to secure the recollection of the irrigation effluent. Other differences to indoor crop production are the choice of fertilizers, the technical design of irrigation system, the higher diversity of propagated plant species and the environmental impact throughout propagation period (KRÜSSMANN 1999). Further, water quality is less controllable due to an abundance of sources for pollution (herbicides, pesticides).

Recollection of irrigation effluent may be a feasible solution for the environment surrounding an outdoor production site, but inhabits a couple of biological problems for the growing crop. Hydroponics is an ideal environment for zoosporic fungi (STANGHELLINI and RASMUSSEN 1994), among these are common plant pathogenic species, such as *Pythium* sp., *Phytophthora* spp. and the virus-vector *Olpidium*. But also other plant pathogenic fungi may be spread by the irrigation water. Due to the difference in the technical design in outdoor systems, desinfection technologies as applied indoors (EHRET et al. 2001) are not always considered as appropriate. Further, organic compounds, deleterious to plant growth may accumulate in the recollected water (YU and MATSUI 1993; ASAO et al. 1999). The situation for outdoor nursery systems is in particular problematic as many different species with different nutritional demands are grown within the same site, which may both predispose for plant disease attack and on physiological and qualitative aspects. Nationally and internationally, studies have predominantly emphasized on questions concerning herbicides and eutrophication (see MOLITOR et al. 1994) while microbial and plant pathogenic aspects have been less highlighted.

For evaluation of the potential in a closed growing system to suppress plant disease, understanding of the microbial background within the system is an important precondition. Microbial communities may be described from different perspectives. Molecular and chemical techniques, such as DGGE/TGGE-PCR (denaturing gradient gel electrophoresis/temperature gradient gel electrophoresis coupled polymerase chain reaction bias) or phospholipid fatty acid profiles, give a picture on the structural composition of the studied microflora (PARKES 1987; RATLEDGE and WILKINSON 1988; VESTAL and WHITE 1989; DUINEVELD et al. 1998; GELSOMINO et al. 1999). In contrast, sole carbon source utilization (SCSU) patterns give information on the function of the heterotrophic aerobic microorganisms within the studied microbial community. As the function of the microflora is decisive for assessment of suppressiveness and conducive conditions for plant disease attack, we elected to use SCSU for the present study. This technique has been a successful tool for description of differences between crops, plant disease attack, seed or substrate inoculation with plant growth-promoting bacteria, growing medium as well as dynamics in microbial communities of crops grown in closed hydroponic greenhouse systems (KHALIL et al. 2001; ALSANIUS et al. 2001, 2002a, b; KHALIL and ALSANIUS 2001), but the approach is novel to closed hydroponic outdoor nursery stock production systems.

The objective of the present investigation was to follow the dynamics of microbial communities with respect to biochemical qualities in crucial parts of the production system during the vegetation period with specific emphasis on biochemical compounds characteristic for *Pythium* and precursors for phenolic compound metabolism.

Material and Methods

A commercial nursery site (Splendor Plant, Svanshall, Jonstorp, Sweden) producing shrubs and woody plants for wholesale to minor nurseries, garden centers and communal park services was chosen for the present study. At this production site, irrigation water had been

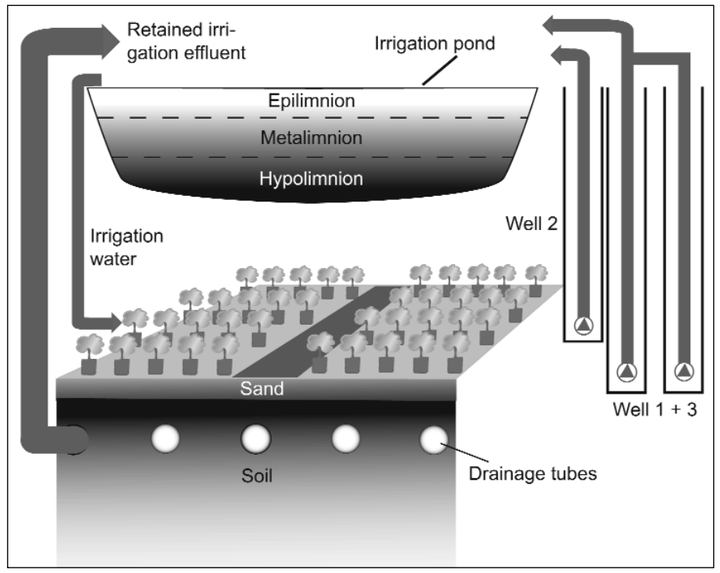


Fig. 1. Schematic view on the production beds (illustration: B. Alsanius).

recollected since 1982. The nursery is situated in a coast near site, in the vicinity of the sea bay, Skälderviken, in the Southwest of Sweden and had a production area of 3.1 ha. The production beds (Figure 1) were built up of a 1–4 cm thick sand layer (inclination: 3 %) covered with Mypex fabric to inhibit growth of weeds between the containers. Under each bed irrigation effluent was retained by drainage tubes in the upper soil layer (humus enriched sand) and resupplied to a collection pond (1500 m^3). In order to secure water supply during the dry part of the summer besides the collected irrigation effluent and precipitation, the pond could be refilled from three wells with a total depth of 118 m (well 1), 171 m (well 2) and 196 m (well 3) and a pump depth of 55 m, 150 m and 160 m respectively.

Plants were propagated in containers using peat as a growing medium amended with slowly released fertilizers Nutricote T70, T 100 and T140. Because of the choice of fertilizers, fertigation was randomly used. The plants were irrigated in some parts of the nursery by drop and in others by sprinkle irrigation from irrigation ramps. Three species were chosen as target species for the present study

(i) *Chamaecyparis lawsoniana* 'Columnaris',
(ii) *Ribes sanguineum* 'Pulborough Scarlet' and
(iii) *Symphoricarpos* 'Arvid'.

The deciduous shrubs had been propagated from cuttings in a greenhouse 0.5 year before the start of the study and were transferred to outdoor conditions eight weeks later. *Chaemaecyparis* had been propagated two years before the start of the study and was kept under greenhouse conditions during the first 6 months before being transferred to outdoor conditions and was stored under controlled cold temperature regime during the winter periods before the start of the study.

To examine the dynamics in functional diversity within the closed nursery system, samples were taken in autumn at mellowing (sampling incident 1), in spring at leaf sprouting (sampling incident 2) and in summer (sampling incident 3). Samplings were concentrated to the following sites:

(i) three layers in the irrigation pond (surface, epilimnion; mid layer at 40 cm of depth, metalimnion; bottom layer at 200 cm of depth, hypolimnion),
(ii) wells (well 1/3; well 2),
(iii) retained irrigation effluent,
(iv) root environment of the three target species (*Chamaecyparis lawsoniana* 'Columnaris', *Ribes sanguineum* 'Pulborough Scarlet', *Symphoricarpos* 'Arvid') and
(v) the growing medium of in the containers from the sampled plants.

Analyses of the growing medium was only conducted in the first sampling incident and were not regarded any further as no differences were found with respect to general parameters. Analysis of the retained water was only conducted during the third sampling incident as the irrigation system was not used during the time of the autumn and spring sampling incident. For analyses of root samples, all substrate fibres were removed from the roots. To collect the microflora from substrate and root samples, 20 g of the sample were shaken in 50 ml of detergent (0.2 % Na-hexametaphosphate, 0.1 % peptone (Oxoid) (w/v) per 1000 ml ultra pure water (Elga Maxima ultra pure water) for 20 min on a rotary shaker (200 rpm). Coarse particles were removed from the suspension by partite centrifugation for 4 min centrifuged at 1250 X*g*, sealing through autoclaved fabric with a pore size of 70 µm and continued centrifugation at 1250 X*g* for 40 min. The supernatant was discarded and the pellet was washed by repeated resuspension in 5 ml of Ringer solution and spinning 1250 X*g*. In the final step, 0.85 NaCl was used for resuspension. The suspension from the substrate and root samples was then adjusted spectrophotometrically to an optical density (590 nm) of 0.100 ± 0.005 whereas samples from differ-

ent habitats within the irrigation system were adjusted to OD_{590} 0.010.

Functional diversities were assessed by measurement of microbial sole carbon source utilization using Microlog GN-panel (Biolog, Hayward, CA., USA). The 95 wells of the microtitre plates contain a low concentrated, buffered nutrient medium, a carbon source and the redox dye tetrazolium. For the substrate in the control well, no carbon source is added. According to GARLAND & MILLS (1991) the carbon compounds in the GN panel may be divided into 11 groups: alcohols, amides, amines, amino acids, aromatic compounds, brominated compounds, carbohydrates, carboxylic acids, esters, phosphorylated compounds and polymers. The redox dye tetrazolium is soluble in water in its oxidized state and appears colorless. By microbial respiration tetrazolium is reduced to the insoluble compound formazan. The purple formazan complex is irreversibly deposited inside the bacterial cell (BOCHNER and SAUVAGEAU 1977).

The suspensions were then inoculated on the microtitre plates. Utilization of the single compounds was measured spectrophotometrically by color change of the redox dye tetrazolium violet on a computer integrated microtitre plate reader (Asys, Linz, Austria; software: Digiwin) at 590 nm. Readings were done directly after inoculation to the microtitre plates and and 4 h, 18 h, 24 h, 30 h, 36 h, 48 h, 60 h, 72 h and 96 h after inoculation.

Statistical analysis

For analysis of the carbon utilization, no adjustment for negative values was done. The measured values from carbon utilization may be separated into three hierarchical levels; the whole plate, groups of compounds and single carbon sources. "Average well colour development" (AWCD), expressing the sum of absorbance units of all of the 96 wells divided by the total number of wells, and "number of positive wells" with an absorbance level >0.5 as well as the increase in AWCD were analyzed for each treatment and reading time by one way analysis of variance (ANOVA, Minitab version 12, 1999), considering the least significant difference (LSD) with $p<0.05$ as significant. The incubation time which best separated the treatments was found by maximizing the ratio "sum of squares for model"/"total sum of squares" ($=R^2$). Single carbon sources characteristic for *Pythium* (α-D-glucose, D-trehalose, cis-aconitic acid, D-gluconic acid, D-saccharic acid, L-asparagine, L-aspartic acid; ALSANIUS et al. 2001) or precursors for formation of phenolics (malonic acid, phenylalanine) were analyzed using ANOVA. The results of SCSU was studied by multivariate statistical methods, using discriminant analysis (DA, SAS version 6.12, 1996) on the basis of canonical function 2 and canonical function 3 (KHALIL et al. 2001).

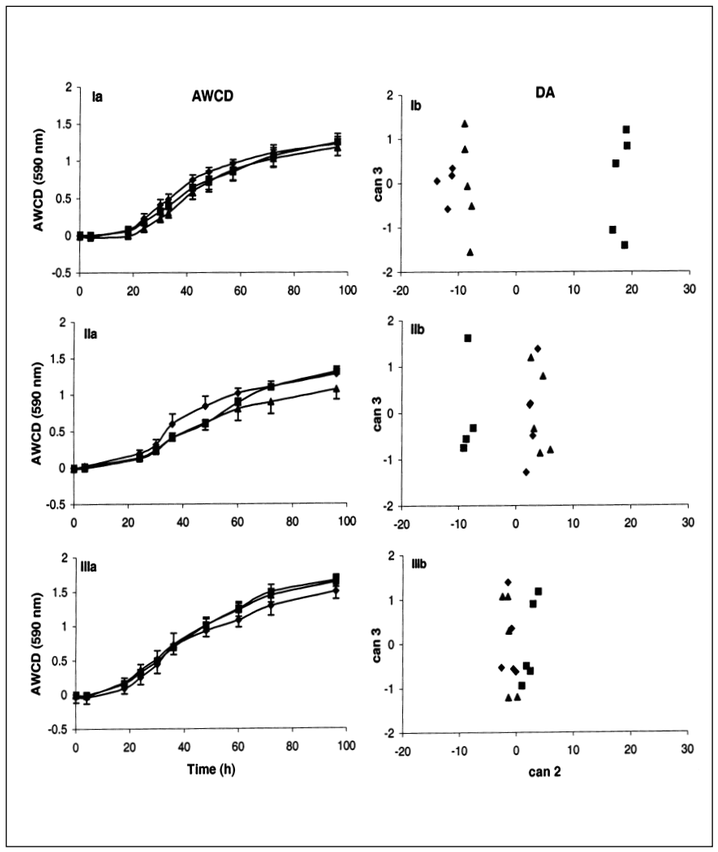


Fig. 2. Sole carbon source utilization by the root microflora inhabiting three crops (◆: *Chamaecyparis lawsoniana* 'Columnaris'; ■: *Ribes sanguineum* 'Pulborough Scarlet'; ▲: *Symphoricarpos* 'Arvid') in a closed hydroponic nursery stock production systems as assessed using Biolog GN-panel (Biolog Hayward CA, USA). The profiles were based on the average well color development (AWCD; a) expressing the ratio of sum of color change at 590 nm to number of wells and and discriminant analysis (DA; b) standardized to ten decisive carbon sources. DA were based on canonical function 2 and canonical function 3. Root samples were taken with five parallels within each systems during fall at mellowing (I^1), during spring (leaf sprouting) (II^2) and during summer (III^3).

[1] Discriminating compounds: glycogen, D-galactose, D-psicose, xylitol, methylpyruvate, D-glucuronic acid, β-hydroxybutyric acid, itaconic acid, D-serine, thymidine;

[2] Discriminating compounds: citric acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, malonic acid, bromo succinic acid, L-alanine, L-proline, D,L-carnitine, 2,3-butnediol,;

[3] Discriminating compounds: tween 80, α-D-glucose, m-inositol, monomethyl succinate, α-keto butyric acid, propionic acid, succinamic acid, D-alanine, L-treonine, γ-amino butyric acid.

Results

The results obtained from the different habitats, root, irrigation pond, wells and retained irrigation effluent, show on the basis of AWCD, number of positive wells and DA that the function of the microflora inhabiting these habitats varies during the vegetation period.

Root samples from all three target crops showed an increasing utilization of carbon sources during the observation period. Utilization of carbon sources was most rapid (positive wells) and highest (AWCD) in the summer sample and significant differences could be obtained between all three incidents (Fig. 2) compared to the two earlier samples. For both *Ribes* and *Symphoricarpos*, differences appeared after 48 h of incubation as opposed to *Chamaecyparis*, where differences were first obtained after 60 h (positive wells) and 72 h (AWCD) of incubation. Using multivariate statistical methods, canonical function 2 discriminated between the three samplings for all three crops. However, quantitative and qualitative differences between the three crops occurred. Quantitative differences between the sampling incidents were most expressed for *Symphoricarpos* when scrutinized after 48 h of incubation, whereas separation for *Chamaecyparis* was most expressed after 72 h of incubation. Qualitative differences were found for the ten carbon compounds discriminating between the sampling incidents (*Chamaecyparis*: α-cyclodextrin, D-raffinose, D-gluconic acid, p-hydroxy phenylacetic acid, formic acid, propionic acid, L-aspartic acid, putrescine, glycerol, D,L-α-glycerol phosphate; *Ribes*: L-arabinose, D-fructose, D-raffinose, D-sorbitol, D-alanine, L-aspartic acid, α-aminobutyric acid, urocanic acid, 2-aminoethanol, 2,3 butanediol; *Symphoricarpos*: glycogen, N-acetylglucoseamine, D-mannitol, D-raffinose, L-rhamnose, β-hydroxybutyric acid, L-phenylalanine, γ-aminobutyric acid, urocanic acid, glucose-1-phosphate). Canonical function 2 discriminated between the sampling incidents in all three crops. Compared to differences between the samplings, differences within the sampling between the crops were lower. No significant differences between the crops within the sampling incident could be found for samples collected during fall and summer. In the spring sample, however, significant differences in utilization pace and selectivity of carbon sources were obtained between the root microflora of *Chamaecyparis* and *Symphoricarpos* for the exponential phase. Subsequently, also differentiation between the three crops using DA was comparably low (Fig. 2). Differences in the carbon source utilization pattern was most expressed for the autumn sample. In all three samplings the utilization patterns of the root microflora from *Chamaecyparis* and *Symphoricarpos* were more similar to each other than the one from *Ribes*. Gradual changes, however, occurred during the vegetation period, resulting in very similar patterns during the summer sample (Fig. 3). The three sets of discriminating carbon sources were dominated by sugars and organic acids (Sampling 1: glycogen, D-

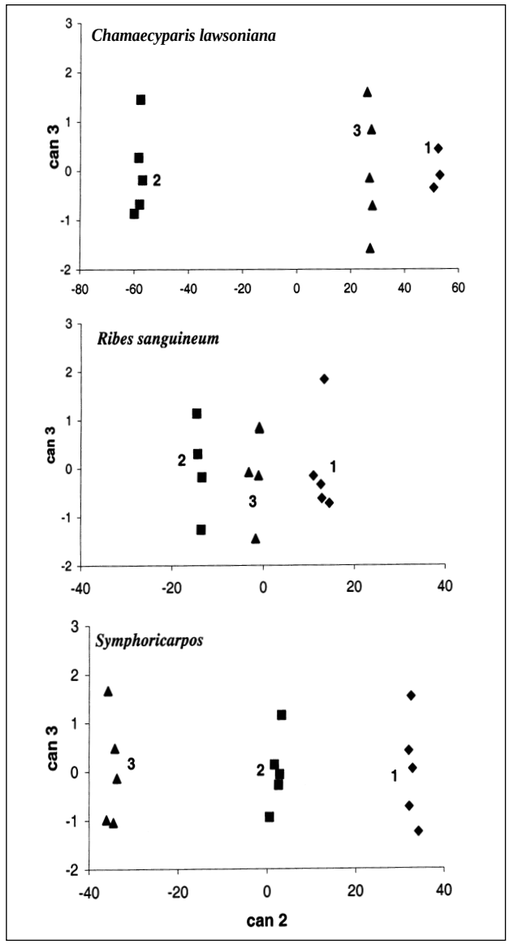


Figure 3. Functional diversity of the root microflora inhabiting three crops (*Chamaecyparis lawsoniana* 'Columnaris'[1]; *Ribes sanguineum* 'Pulborough Scarlet'[2]; *Symphoricarpos* 'Arvid'[3]) in a closed hydroponic nursery stock production systems using discriminant analysis (DA), standardized to ten decisive carbon sources. DA was performed for *Ribes* and *Symphoricarpos* after 48 h and for *Chamaecyparis* after 72 h of incubation on the Biolog GN-panel. Root samples were taken at a density of five parallels during fall, during spring and during summer. Utilization of the carbon sources was assessed indirectly by color change of the redox dye tetrazolium blue using Biolog GN-panel (Biolog Hayward CA, USA). DA were based on canonical function 2 and canonical function 3.

[1] Discriminating compounds: α-cyclodextrin, D-raffinose, D-gluconic acid, p-hydroxy phenylacetic acid, formic acid, propionic acid, L-aspartic acid, putrescine, glycerol, D,L-α-glycerol phosphate;

[2] Discriminating compounds: L-arabinose, D-fructose, D-raffinose, D-sorbitol, D-alanine, L-aspartic acid, γ-aminobutyric acid, urocanic acid, 2-aminoethanol, 2,3 butanediol;

[3] Discriminating compounds: glycogen, N-acetylglucoseamine, D-mannitol, D-raffinose, L-rhamnose, βγ-hydroxybutyric acid, L-phenylalanine, γ-aminobutyric acid, urocanic acid, glucose-1-phosphate.

Table 1. Utilization of *Pythium*-specific compounds by the microflora inhabiting the root of *Chamaecyparis lawsoniana*, *Ribes sanguineum* and *Symphoricarpos* after 48 h of incubation on Biolog GN-panel as assessed by color change of tetrazolium blue at 590 nm. Samples were collected at three incidents (autumn, spring and summer) during the vegetation period.

	Autumn			Spring			Summer		
Compound[1]	*Chamaecyparis lawsonniae*	*Ribes sanguineum*	*Symphoricarpos*	*Chamaecyparis lawsonniae*	*Ribes sanguineum*	*Symphoricarpos*	*Chamaecyparis lawsonniae*	*Ribes sanguineum*	*Symphoricarpos*
C 18	1.178 a[2]	0.927 a	0.927 a	1.714 a	0.946 b	0.786 b	1.405 a	1.236 a	1.290 a
C 32	1.822 a	1.297 a	1.498 a	2.215 a	1.336 b	0.145 c	2.068 a	1.890 a	1.893 a
C 38	1.817 a	1.655 a	1.720 a	1.612 a	1.222 ab	0.802 b	1.522 a	1.725 ab	1.800 b
C 43	1.535 a	1.256 a	1.237 a	1.333 a	1.245 a	1.418 a	1.338 a	1.424 a	1.520 a
C 58	1.303 a	1.264 a	1.313 a	1.304 a	0.975 a	1.077 a	1.050 a	1.464 b	1.350 ab
C 68	1.741 a	1.735 a	1.631 a	1.971 a	1.511 ab	1.062 b	1.804 a	2.027 b	1.973 ab
C 69	1.756 a	1.517 a	1.546 a	1.713 a	1.342 ab	1.043 b	1.793 a	2.047 a	2.022 a

[1] C 18: α-D-glucose, C 32: D-trehalose, C 38: cis-aconitic acid, C 43: D-gluconic acid, C 58: D-saccharic acid, C 68: L-asparagine, C 69: L-aspartic acid.
[2] Values within the same row and crop followed by different letters are significantly different at $p<0.05$ according to Tukey test. N=5.

galactose, D-psicose, xylitol, methylpyruvate, D-glucuronic acid, β-hydroxybutyric acid, itaconic acid, D-serine, thymidine; sampling 2: citric acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, malonic acid, bromo succinic acid, L-alanine, L-proline, D,L-carnitine, 2,3-butnediol; sampling 3: tween 80, α-D-glucose, m-inositol, mono-methyl succinate, α-keto butyric acid, propionic acid, succinamic acid, D-alanine, L-treonine, γ-amino butyric acid).

The plant root-inhabiting microflora generally started to utilize *Pythium*-specific compounds already after a short lag-phase. Differences occurred between the plant species in the first and second sampling where *Chaemacyparis* inhabiting root microflora utilized *Pythium*-specific compounds more rapidly and to a higher extent than the one inhabiting *Symphoricarpos* (Table 1). A gradual shortage of lag-phase could be observed between the samplings and in the summer sampling, no differences between the crops was found. With respect to the two examined precursors for phenolic compound metabolism, no differences between the crops were observed in the first and third sampling (data not shown). However, in the second one, the ability to metabolize malonic acid and phenylalanine was significantly higher in for the root microflora inhabiting *Symphoricarpos* compared to the one of *Chamaecyparis* and a negative correlation could be stated for α-D-glucose, D-trehalose and cis-aconitic acid to phenylalanine.

Also for the water samples, differences in utilization pattern between the incidences were found. In the autumn sample the microflora inhabiting the hypolimnion of the irrigation pond showed the quickest utilization on the basis of AWCD and number of positive wells, whereas it had the slowest utilization during spring and summer. Microbial sole carbon source utilization of the samples from the irrigation pond collected during fall varied substantially; therefore no significant differences between the three assessed layers were found. With respect to AWCD and number of positive wells, the metalimnion of the irrigation pond showed the quickest utilization, whereas the epilimnion took an intermediate position between the mid layer and the bottom layer when sampled during spring and summer. In these two incidents, differences in SCSU by the inhabiting microflora was significant between the mid and bottom layer and, during the exponential phase, also between the epi- and metalimnion. The differences in utilization were most expressed when assessed after 48 h of incubation with the sole carbon sources (Fig 4). The utilization pace increased during the vegetation period and significant differences were found between the one sampled in autumn and the one collected during summer. Regarding multivariate analyses of sole carbon source utilization on the basis of DA, canonical function 2 separated between the sampling incidents of the samples from the irrigation pond (data not shown). Also here, qualitative and quantitative differences were found between the position in the irrigation pond. The biggest differences were found for the samples from the metalimnion. The carbon sources tween 40, gentibiose, α-D-lactose, D-mannose, D-psicose, D-raffinose, glycyl-L-aspartic acid, L-serine, phenylethylamine, glucose-6-phosphate discriminated between the three sampling incidents for the epilimnion, whereas the carbon sources fructose, α-D-lactose, D-melbiose, β-methyl-D-glucoside, turanose, acetic acid, L-alanyl glycine, glycyl-L-aspartic acid, L-histidine and glycerol separated between the samplings of the metalimnion and fructose, D-sorbitol, mono-methylsuccinate, formic acid, α-hydroxy butyric acid, D-saccharic acid, L-leucine, D-L-carnitine, urocanic acid and glucose-6-phosphate between the ones of the hypolimnion, respectively.

Comparison of the three studied layers in the irrigation pond using DA of SCSU resulted in a marked discrimination between the samples from the metalimnion and the upper and lower sampling layer at all three sampling incidents by the second canonical function (Fig. 4). There was a gradual change in SCSU pattern of the hypo- and epilimnion, from high similarity on the basis of the ten chosen carbon sources in the autumn sample (α-cyclodextrin, Tween 80, L-fucose, D-melibiose, cis-aconitic acid, citric acid, γ-hydroxybutyric acid, L-alanyl-glycine, phenylethylamine, glucose-6-phosphate), to close, but distinguishable in the spring sample (dextrin, L-arabinose, D-arabitol, cellobiose, lactulose, D-melibiose, dis-aconitic acid, citric acid, D-glucosaminic acid, glucoronamide, L-alanyl glycine, pehnylethylamine) and finally, to low similarity in the sample collected in summer (α-cyclodextrin, tween 40, xylitol, acetic acid, D-gluconic acid, sebacic acid, L-asparagine, glycyl-aspartic acid, L-threonine).

Regarding the positions in the irrigation pond, differences in utilization of *Pythium*-specific compounds were found between the samples and between the sampling incidents (Table 2). In contrast to utilzation of *Pythium*-specific compounds by the root microflora, the lag-phase was in general more extended for samples from the irrigation pond. However, the lag phase diminished from the first to the third sampling incident. *Pythium*-specific compounds were utilized most efficiently by the microflora inhabiting the metalimnion,

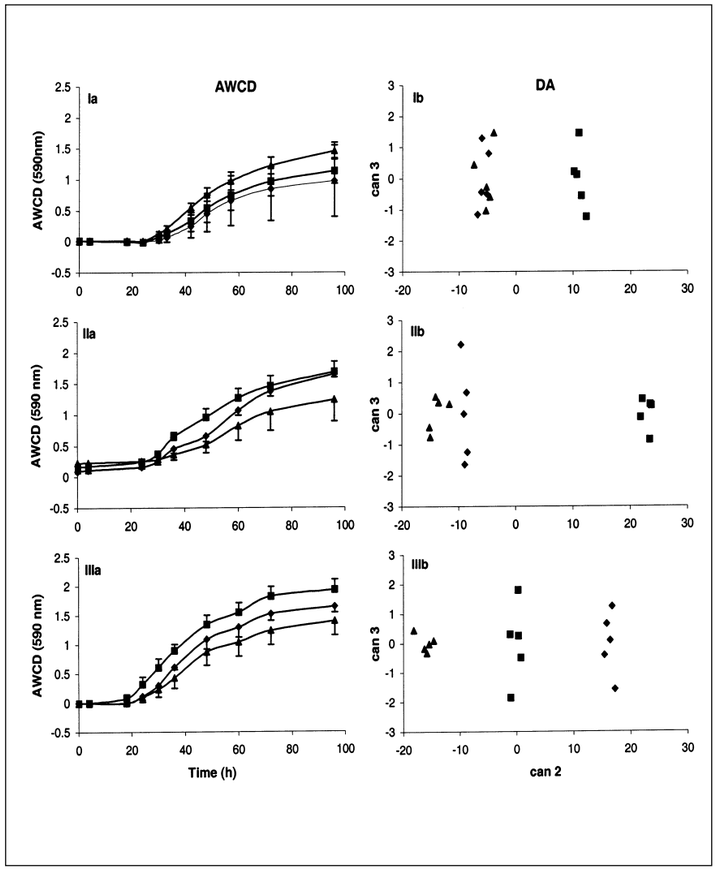


Fig. 4. Functional profiles of the hetertrophic microflora inhabiting the irrigation pond at three depth (◆: epilimnion; ■: metalimnion; ▲: hypolimnion) integrated into a closed hydroponic nursery stock production systems using sole carbon source utilization. Biolog GN-panel (Biolog Hayward CA, USA) was adopted for assessment of sole carbon source utilization. The profiles were based on the average well color development (AWCD; a) expressing the ratio of sum of color change at 590 nm to number of wells and discriminant analysis (DA; b) standardized to ten decisive carbon sources. DA were based on canonical function 2 and canonical function 3. Water samples were taken with five parallels within each systems during fall (I[1]), during spring (II[2]) and during summer (III[3]). (n=5).
[1] Discriminating compounds: α-cyclodextrin, Tween 80, L-fucose, D-melibiose, cis-aconitic acid, citric acid, γ-hydroxybutyric acid, L-alanyl-glycine, phenylethylamine, glucose-6-phosphate;
[2] Discriminating compounds: dextrin, L-arabinose, D-arabitol, cellobiose, lactulose, D-melibiose, dis-aconitic acid, citric acid, D-glucosaminic acid, glucoronamide, L-alanyl glycine, phenylethylamine);
[3] Discriminating compounds: α-cyclodextrin, tween 40, xylitol, acetic acid, D-gluconic acid, sebacic acid, L-asparagine, glycyl-aspartic acid, L-threonine.

Table 2. Utilization of *Pythium*-specific compounds by the microflora inhabiting the epilimnion, metalimnion and hypolimnion of an irrigation pond integrated to a commercial closed hydroponic system for outdoor nursery stock production. Utilization was assessed after 48 h of incubation on Biolog GN-panel by using color change of tetrazolium blue at 590 nm as an indicator of utilization. Samples were collected at three incidents (autumn, spring and summer) during the vegetation period.

	Autumn			Spring			Summer		
Compound[1]	Epi-limnion	Meta-limnion	Hypo-limnion	Epi-limnion	Meta-limnion	Hypo-limnion	Epi-limnion	Metal-limnion	Hypo-limnion
C 18	0.118 a[2]	0.535 b	0.289 ab	0.273 a	0.378 a	0.028 b	0.862 a	1.453 b	0.780 a
C 32	0.044 a	0.259 a	0.273 a	0.231 a	0.358 a	0.064 b	0.604 a	1.531 b	0.565 a
C 38	0.110 a	0.400 a	0.141 a	0.394 a	0.460 a	−0.015 b	1.022 ab	1.376 a	0.369 b
C 43	0.087 a	0.215 a	0.407 a	0.210 a	0.476 b	0.051 c	0.671 a	1.429 b	0.650 a
C 58	0.031 a	0.137 a	0.111 a	0.067 a	0.171 b	−0.007 c	0.727 ab	0.917 a	0.231 b
C 68	0.243 a	0.370 a	0.286 a	0.393 a	0.507 b	0.011 c	1.008 ab	1.427 a	0.423 b
C 69	0.090 a	0.240 a	0.160 a	0.296 a	0.309 a	−0.006 b	1.093 a	1.501 a	0.234 b

[1] C 18: α-D-glucose, C 32: D-trehalose, C 38: cis-aconitic acid, C 43: D-gluconic acid, C 58: D-saccharic acid, C 68: L-asparagine, C 69: L-aspartic acid.
[2] Values within the same row and sampling followed by different letters are significantly different at $p<0.05$ according to Tukey test. N=5.

whereas the lowest utilization was found for the ones inhabiting the hypolimnion. Also with respect to phenolic metabolism precursors, differences were obtained between the sampling incidents and samples. Utilization of malonic acid was low by all three studied positions in the irrigation pond during all three occasions. The microflora inhabiting the surface and the metalimnion showed a substantial capacity for utilization of phenylalanine in the spring sample (data not shown).

No differences were obtained between well 1+3 and well 2 both regarding AWCD and number of positive wells. The same trend was obtained for both samplings (data not shown). However, significant differences occurred between the two sampling incidents due to an increased start emission value. The retained irrigation effluent, which was sampled only once, had a significantly quicker and higher utilization of the offered sole carbon sources than the two wells. Its utilization pattern regarding AWCD and number of positive wells had a certain similarity to the one of the metalimnion samples from the irrigation pond.

Discussion

The present study indicated shifts in functional diversity during the vegetation period, regarding both the grown crops and locality within the irrigation system. It also indicated differences between the chosen habitats. However, the selected microbial habitats, root and water, should not be compared inbetween as the density of the water samples was 10-fold lower than the one of the root samples. Therefore, the longer lag-phase found for the water samples was expected. In the light of this, it is, however, striking that the lag phase of the retained irrigation effluent is much shorter than the one of the other water samples.

Interpretation of SCSU patterns may be performed on different hierarchical levels. Regarding the level of the entire panel, both the level of the utilization rate and the length of lag phase give interesting information on the status of the growing system. The length of lag phase on the level of the entire panel gives information on the activity of the heterotrophic aerobic microflora. The utilization path of single carbon sources is a reflection of their occurrence in the studied habitat. In this context, a short lag-phase and a steep exponential phase indicates that the resident microflora is adapted to utilization of the compound. An extended lag phase, on the contrary, expresses that the ambient heterotrophic aerobic microflora is not adapted to the compound, but has the capacity to utilize it.

In contrast to the compounds discriminating between the crops or layers within the irrigation pond, the compounds discriminating between the sampling incidents of either root or water samples were dominated by *Pythium*-specific compounds (ALSANIUS et al. 2001) or compounds fulfilling nutritional requirements or favoring the development of *Pythium* species (MCQUILKENS et al. 1992; DONALDSON and DEACON 1993; ALSANIUS et al. 1998). This gap may be explained by the fact that these compounds kept comparable levels within the sampling incidents. This also indicates that the predisposition for *Pythium* was comparable for samples within the same habitat within the same sampling incident.

ALSANIUS et al. (2001) demonstrated that the microflora of tomato seedlings infected with *Pythium ultimum* had a substantially shorter lag phase than the uninfected ones regarding both AWCD and number of positive wells. A similar picture could be found for hydroponically grown tomato small plants and older tomato plants in the presence of *P. ultimum*. In the present study a gradual shortage in lag phase regarding both AWCD and number of positive wells was found from the autumn sample to the one collected during summer. Further, a gradually shortened lag phase was also found for *Pythium*-specific compounds from the first to the last sampling incident. This might indicate that the studied microbial communities get more adapted to utilization of *Pythium*-specific compounds. This is also strengthened by the fact that also compounds, earlier characterized as specific for the presence of *Pythium* (ALSANIUS et al. 2001) or as utilized by *Pythium* (ALSANIUS et al. 1998) or as nutritional requirements for encystment or spore germination of *Pythium* species (MCQUILKENS et al. 1992; DONALDSON and DEACON 1993), were decisive for discrimination between the sampling incidents.

Microbial community shifts in the studied layers of the irrigation pond might appear peculiar. However, in fresh water lakes, a distinctly stratified pattern develops during the summer months (JACKSON and JACKSON 1996). The upper layer, epilimnion, contains warmer and less dense water. The lower layer, hypolimnion, contains cooler and denser water. These two are separated by the metalimnion, a zone with rapid temperature changes. The epilimnion is depleted of nutrients whereas the hypolimnion is depleted of oxygen due to microbial break down of organic material. During autumn and spring, the temperature of the epilimnion reaches the same level of the one in the hypolimnion which enables wind-induced verticle mixing of the water layers and oxygenation of the hypolimnion. This might explain the higher microbial activity of the samples collected from the bottom of the irrigation pond during autumn and the similarity of the microbial communities in the epi- and hypolimnion during spring and autumn.

Compounds with a phenolic basic structure are of interest for the interaction between microorganisms. Phenolic acids, such as caffeic acid (KASENBERG and TRAQUEIRA 1988), have shown to have inhibitory effect on some fungal pathogens. Also, antibiotic compounds with a phenolic basic structure, such as phenazine-1-carboxylic acid, 2,4-diacetyl phloroglucinol, pyoluteorin, play a role in biocontrol of fungal pathogens. Precursors for the phenolic acid metabolisms (shikimic acid pathway and malonic acid pathway) are malonic acid and phenylalanine. We assume that phenolic compound precursors would be utilized extensively under conditions of high production of phenolic compounds. Apart from the spring sample from *Symphoricarpos*, microbial utilization of either phenylalanine or malonic acid was low and in most cases, also the capacity to adapt to one of these compounds as a sole carbon source was low. This might indicate, that there also was a low capacity to antagonize fungal pathogens. This assumption is supported by the fact that only few fluorescent pseudomonads, isolated from the selected habitats in the present study, were able to inhibit

growth of either *Phytophthora cinnamomi*, *Pythium irregulare* or *Pythium sylvaticum* (JUNG 1999; ALSANIUS & JUNG 2001). Further, a negative correlation between the utilization of some *Pythium*-specific compounds and phenylalanine was stated. It is too early to draw any conclusions from this finding, but it would be of interest to further look into the interaction between these compounds.

From the present survey we draw the conclusion that the microbial communities in different parts of a closed hydroponic nursery stock growing system varies during the vegetation period and that the predisposition for *Pythium* attack increases. However, the present study was performed by indirect measurement of the conditions for *Pythium*. At condition that this is confirmed by direct analyses of the occurrence of different pythiacious fungi and by assessment of crop losses due to theses diseases, assessment of the functional diversity of the heterotrophic microflora might become a useful tool to recognize predisposition for and to foresee disease attack by pythiacious fungi in closed outdoor systems.

Acknowledgment

The authors would like to thank the owners of the nursery, in particular Mr. Niels Andersén, for opening his nursery and giving us the opportunity to collect samples as well as for fruitful discussion. Further, we would like to thank Dr. Peter Sundin, Department of Environmental Assessment, SLU, Uppsala, and MSc Sofia Windstam, Department of Crop Science, SLU, Alnarp, for critical reading of the manuscript. The present study was funded by the Royal Academy of Forestry and Agricultural Sciences, Stockholm, Sweden, which is gratefully acknowledged.

References

ALSANIUS, B.W., S. KHALIL and M. HULTBERG 1998: Biochemical and chemical chararcterization of *Pythium ultimum*. Med. Fac Landbouww. Univ. Gent **63/3a**, 891–897.

ALSANIUS, B.W. and V. JUNG 2001: Risk för att återanvända bevattningsvattnet på friland? Hortica September 2001, 5–8 (in Swedish).

ALSANIUS, B.W., M. HULTBERG and S. KHALIL 2001: Microorganisms – tools for closed hydroponic greenhouse systems? Acta Horticulturae **548**, 207–214.

ALSANIUS, B.W., S. KHALIL, M. HULTBERG, J-E. ENGLUND and P. SUNDIN 2002a: The influence of introduced *Pseudomonas* isolates on the rhizobacterial community of hydroponically cultivated tomato: a comparison of different methods of assessment. Soil Biol. Biochem. (accepted).

ALSANIUS, B.W., S. KHALIL, M. HULTBERG, L. HAGTORN and T. BRAND 2002b: Comparative study of plant performance and root microbial communities of tomato grown in closed NFT greenhouse systems as affected by seed inoculation with Pseudomonas fluorescens 5.014 and 5-2/4 (Submitted manuscript).

ASAO, T., H.R. PRAMANIK, K. TOMITA, Y. OHBA, K. OHTA, T. HOSOKI and Y. MATSUI 1999: Influences of phenolics isolated from the nutrient solution nourishing growing cucumber (*Cucumis sativus* L.) plants on fruit yield. J. Japan. Hort. Sci. **68**, 847–853.

BOCHNER, B.R. and M.A. SAVAGEAU 1977: Generalized indicator plate for genetic, metabolic and taxanomic studies of microorganisms. Applied and Environmental Microbiology **33**, 434–444.

DONALDSON, S.P. and J.W. DEACON 1993: Effects of amino acids and sugars on zoospore taxis, encystment and cyst germination in *Pythium aphanidermatum* (Edson) Fitzp., *P. catenulatum* Matthews and *P. dissotocum* Drechs. New Phytol. **123**, 289–295.

DUINEVELD, B., A.S. ROSADO, J.D. VAN ELSAS and J.A. VAN VEEN 1998: Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. Applied and Environmental Microbiology **64**, 4950–4957.

EHRET, D.S., B.W. ALSANIUS, W. WOHANKA, J.G. MENZIES and R.S. UTKHEDE 2001: Disease control in closed nutrient systems. Agronomie **21**, 323–339.

GARLAND, J.L. and A.L. MILLS 1991: Classification and characterization of heterotrophic microbial communities in the basis of patterns of community-level sole-carbon-source utilization. Applied and Environmental Microbiology **57**, 2351–2359.

GELSOMINO, A., A.C. KEIJZER-WOLTERS, G. CACCO and J.D. VAN ELSAS 1999: Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. Journal of Microbiological Methods **38**, 1–15.

JACKSON, A.R.W. and J.M. JACKSON 1996: Environmental Science. Longman, Harlow.

JUNG, V. 1999: Recirkulering av vatten vid friland-sodling – risk för sjukdomsspridning? MSc-Thesis, SLU, Alnarp, 1999, 8 (ISSN 1403-0993) (in Swedish).

KASENBERG, T.R. and J.A. TRAQUEIRA 1988: Effects of phenolics on growth of *Fusarium oxysporum* f.sp. *radicis-lycopersici in vitro*. Canadian Journal of Botany **66**, 1174–1177.

KHALIL, S. and B. W. ALSANIUS 2001: Dynamics of the indigenous microflora inhabiting the root zone and the nutrient solution of tomato in a commercial closed greenhouse system. Gartenbauwissenschaften **66**, 188–198.

KHALIL, S., B.W. ALSANIUS, E. BÅÅTH, J-E. ENGLUND, U. E. GERTSSON, P. SUNDIN and P. JENSÉN 2001: A comparison of sole carbon source utilization patterns and phospholipid fatty acid profiles to detect changes in the root microflora of hydroponically grown crops. Canadian Journal of Microbiology **47,** 302–308.

KRÜSSMANN, G. 1997: Die Baumschule. 6th ed., P. Parey, Berlin.

MCQUILKENS, M.P., J.M. WHIPPS and R.C. COOKE 1992: Nutritional and environmental factors affecting biomass and oospore production of the biocontrol agent *Pythium oligandrum*. Enzyme Microb. Technol. **14**, 106–111.

MINITAB 1999: Minitab for Windows, Release 12.23. Minitab Inc.

MOLITOR, H.-D., W. WOHANKA and H. WIENHAUS 1994: Erfahrungen mit geschlossenen Bewässerungssystemen im Zierpflanzenbau. II. Eriken in Fließrinnen. KTBL-Arbeitsblatt Lfd Nr. **0674**.

PARKES, R.J. 1987: Analysis of microbial communities

within sediments using biomarkers. In: M. FLETCHER, T. P. G. GRAY and J. G. JONES (Eds): Ecology of Microbial Communities, Cambridge University Press, London, 147–178.

RATLEDGE, C. and S. G. WILKINSON 1988: Lipids in plants and microbes. Academic Press, London, 3–16; 23–26; 117–119; 299–300; 869–885.

RAVEN, P. H., L. R. BERG and G. B. JOHNSON 1995: Environment. Saunders College Publishing, Fort Worth.

SAS, 1996. The SAS System for Windows, Release 6.12, SAS Institute Inc. Cary, NC, 27513 USA.

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

VESTAL, J. R. and D. C. WHITE 1989: Lipid analysis in microbial ecology. BioScience **39**, 535–540.

YU, J. Q. and Y. MATSUI 1993: Extraction and identification of phytotoxic substances accumulated in nutrient solution for the hydroponic culture of tomato. Soil Sci, Plant Nutr. **39**, 691–700.

Received December 19, 2001 / Accepted February 6, 2003

Addresses of authors: Beatrix W. Alsanius and Victoria Jung, Department of Crop Science, SLU, P.O. Box 44, SE-23053 Alnarp, Sweden, e-mail: beatrix.alsanius@vv.slu.se.