Effect of Different Soilless Growing Systems on Biological Properties of Growth Media in Strawberry

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Abstract
The search for other substrates in soilless growing systems (SGS) can improve the sustainability of these systems. Using a randomized complete block design with three replicates, the following grewed media were compared in three systems: growth media compared were a composted cork thin waste mixed with rice hulls (2:1 v:v) (CCR) and a peat substrate (P) in an open system (O), closed system (C) and closed with disinfection by slow sand filtration systems (CSSF). The microbial activities were determined by the measurement of the β-glucosidase activity and the measurement of the hydrolysis rate of fluorescein diacetate (FDA). The microbial biomass was determined by acridine orange direct counting (AODC). The microbial community profile was determined by dilution plating on semi-selective media. The values of the activity measured by FDA and microbial biomass were higher in CCR than in peat at the beginning of the trial and at two and a half months after transplantation. At the beginning of the trial the activity measured by β-glucosidase was higher in CCR than in P. With regard to growth media chemical properties, the CCR showed a higher pH and electrical conductivity than P, at the beginning and at two and a half months after transplantation. The CSSF showed a lower activity and lower density of copiotrophic bacteria and fungi than the O. The values of the activity measured by β-glucosidase and microbial biomass were higher in the rhizosphere than in non rhizosphere of strawberry plants.

INTRODUCTION
The compost produced from several different types of agricultural residues can be a suitable material for container media or in field soils. In addition, this compost may potentially alleviate disease problems and provide high quality crops.

The level of decomposition of soil organic matter plays an important role in soilborne disease severity (Cook and Baker, 1983). The environment in which the compost is produced, and conditions during curing and utilization determine the potential for recolonization by these microflora and the induction of disease suppression. Peat on the other hand, is a limited resource that cannot be recycled (Hoitink et al., 1996). During recent years protected horticulture has changed from soil-grown systems to soilless systems. Initially, these were run-to-waste soilless systems. Now, soilless systems have to be closed as much as possible. Growers attempt to overcome the problems of pathogen dissemination by disinfecting the water with expensive equipment such as heat treatment, ozone treatment, and UV-radiation. After the sterilization of the nutrient solution, pathogens and beneficial micro-organisms can recolonize the sterilized nutrient solution. It might be better to look for methods to stimulate microbiologically-balanced growing systems by using more specific disinfection methods, such as slow filtration. This method eliminates pathogens, but does not destroy the entire natural microflora (van Os et al., 1997).

Recirculation of nutrient solution in greenhouse growing systems promotes environmental and economic advantages such as saving water and fertilizers. The disadvantages are the greater risk of the spreading of phytopathogens by the nutrient solution and of the accumulation of organic compounds at phytoxic levels. Organic compounds are said to accumulate in the nutrient solution in closed systems (Jensén and Aoalsteinsson, 1992; Waechter-Kristensen et al., 1997). Phenolic acids, as a group among organic acids, are released by plant roots (McCalla and Haskins, 1964) as well as by microorganisms as metabolites or as products of biotransformation (Linch, 1976) in the rhizosphere and in the...
nutrient solution. Current disinfection techniques are high-tech and expensive. The main advantage of slow sand filtration is the stabilizing microflora that remains in the nutrient solution. In comparison to other methods slow sand filtration is non-chemical, cheap and robust.

Its disadvantages are its large area requirements, the cost of cleaning after clogging and the chances of clogging as a result of the turbidity of the water (Wohanka and Woelk, 1989; Wohanka, 1992; Runia et al., 1996; van Os et al., 1999).

The aim of this research was to study the effect of different soilless growing systems on the biological properties of growth media in strawberry.

MATERIAL AND METHODS

Biological Characteristics of Growth Media

Microbial activity and biomass were measured in order to find out the biological properties of the growth media. Microbial activity was estimated by measuring β-glucosidase activity and by the hydrolysis rate of fluorescein diacetate (FDA). β-glucosidase activity was based on the colorimetric determination of the hydrolyzed p-nitrophenol and was measured according to Bandick and Dick (1999). The FDA was measured according to Chen et al. (1988).

The microbial biomass was estimated by the acridine orange direct counting (AODC) technique. This technique was performed by counting cells from an aqueous extraction with an epifluorescence microscope (Kepner and Pratt, 1994). The aqueous extraction was filtered through 0.2 µm pore-size black polycarbonate membranes (Isopore, Millipore Iberica S.A; Madrid, Spain). Counting was performed at 1.250x with a minimum of 400 cells per filter. Controls were also counted in sterile water.

The density of cultivable groups of bacteria and fungi associated with biocontrol phenomena was determined by dilution plating on semi-selective media according to Tuiter et al. (1998). The microorganism populations determined were: copiotrophic bacteria, Bacillus spp., fluorescent Pseudomonas spp., oligotrophic bacteria, oligotrophic actinomycetes, cellulolytic bacteria, cellulolytic actinomycetes and fungi. One sample was taken from the rhizosphere and non rhizosphere respectively and was analyzed for each growth medium and SGS studied systems with the three replicates. The microbial community of two growth media was determined at the beginning and at two and a half months after transplantation.

The values of the activity measured by FDA and β-glucosidase, microbial biomass and microbial community of the growth media were determined from rhizosphere and non rhizosphere samples and were analyzed for each growth medium at the beginning of the trial and at two and a half months after transplantation, and for systems studied at two and a half months after transplantation. All analyses were performed with three replicates.

Electrical conductivity (EC) and pH of growth media were measured in a water extract (2:1; vol/vol), as described by Bunt (1988) and Gabriëls et al. (1991), respectively. Physical and chemical variables of the growth media were determined at the beginning of the bioassay and at two and a half months after transplantation. Two samples were analyzed for each growth medium.

Experimental Design and Statistical Analysis

The experiment was carried out in a metacrilate tunnel type greenhouse in Huelva, Spain. It had a randomized block design with three replicates. Each replicate consisted of a 6 meter hanging cultivation line with 65 strawberry plants per line cultivated in 60 L of growth media along 6 meters. The plants were maintained on the hanging cultivation line with a drip irrigation system (one water emitter for each plant, flow rate of 2.3 L·h⁻¹ in a soilless culture).

The slow sand filtration system was constructed as described by Wohanka (1995). The drain water was pumped into the sand filter to maintain a water layer of 35 cm. The effluent flow rate from the filter was adjusted by a flow-meter located between the filter and the disinfected water storage tank, obtaining a final flow rate of 0.1 to 0.3 m·h⁻¹. We used 6 treatments (2 growth media x 3 cultivation systems). These were applied in the rhizosphere and non rhizosphere of strawberry plants (Fragaria × ananassa Duch., var. Camarosa). The growth media were a composted cork thin waste mixed with rice hulls (1:1, v:v) ratio (CCR).
and a peat substrate (P); and the soilless growing systems (SGS) were: open system (O), closed system (C) and closed with disinfection by slow sand filtration systems (CSSF).

Three-way analysis of variance was performed with growth media, cultivation systems and block as factors; means were separated with mean separation tests such as Tukey’s. All statistical analyses were done with Statgraphics Plus (version 6; Statistical Graphics Corp., Rockville, MD).

RESULTS AND DISCUSSION

The values of the activity measured by FDA, β-glucosidase and microbial biomass were higher in CCR than in P at the beginning of the trial (Table 1). At two and a half months after transplantation, the FDA and the microbial biomass were higher in CCR than in P (Fig.1). Our results are consistent with those studies where compost was found to be a suppressive plant growth medium for several diseases (Hoitink, 1996).

The CCR showed a higher density of copiotrophic bacteria, Bacillus spp., and oligotrophic actinomycetes than P at the beginning of the trial and at two and a half months after transplantation (Table 2). The P showed a higher density of fungi than CCR at the beginning of the trial and at two and a half months after transplantation (Table 2).

Hoitink et al. 1991. suggested that the mixes between horticultural growth media and compost were suppressive of Pythium damping-off when its colonization was more favourable for bacteria than fungi.

Regarding the physicochemical properties of the growth media, the CCR showed a higher pH and electrical conductivity than P, at the beginning and at two and a half months after transplantation (Table 3). This could be favourable because the association of a higher pH and/or Ca with less fusarium wilt was shown by many other investigators in strawberry plants (Jones et al., 1993).

The SGSF showed a lower activity at two and a half months after transplantation (Fig.2) and no different biomass (Fig. 3) than the O. The similar biomass with the lesser activity, suggests a possible accumulation of organic compounds at toxic levels in the nutrient solution in closed systems (Jensen and Aalstensen, 1992). The filter material did not quantitatively influence the remaining bacteria populations after filtration, but the potential activity of microbial communities was found to be less intensive in the filter effluent nutrient solution than in the influent (Postma et al. 1999).

The C displayed a lower biomass than the CSSF (Fig. 3). At two and a half months after transplantation The CSSF exhibited a lower density of copiotrophic bacteria and fungi than the O (Table 4). Postma et al. 1999 suggested that the number of fungi, which were around the detection limit, were probably decreased by the slow filtration. On the other hand, they found that the numbers of specific groups of bacteria did not change significantly under slow filtration. The bacteria numbers were not influenced due to filtration (Van Os et al., 1997).

The values of the activity measured at two and a half months after transplantation by the β-glucosidase and microbial biomass were higher in the rhizosphere than the non rhizosphere of strawberry plants (Table 5). These results suggested that there are nutritive sources available for microorganism in to rhizosphere. These exudates are use by resident microbial populations in the growth media.

Activity measured by FDA, β-glucosidase and microbial biomass was greater in CCR than in P, and the CSSF showed less activity and no different biomass to the O.

In conclusion, both the growth media and the SGS system have a significant effect on the microbiologic characteristics in the surrounding of the root.

Literature Cited


Tables

Table 1. Microbial activity ($\beta$-glucosidase and FDA) and biomass (AODC) of growth media at the beginning of bioassay.
<table>
<thead>
<tr>
<th>Growth media&lt;sup&gt;a&lt;/sup&gt;</th>
<th>β-glucosidase (µg hydrolyzed p-nitrophenol/ml)</th>
<th>FDA (µg hydrolyzed fluorescein diacetate/ml.min)</th>
<th>AODC (Number of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>143.862 b</td>
<td>1.642 b</td>
<td>1.628·10&lt;sup&gt;6&lt;/sup&gt; b</td>
</tr>
<tr>
<td>CCR</td>
<td>350.325 a</td>
<td>2.324 a</td>
<td>3.625·10&lt;sup&gt;6&lt;/sup&gt; a</td>
</tr>
</tbody>
</table>

<sup>a</sup> P: Peat; CCR: Composted Cork thin waste mixed with Rice hulls. Values in each column followed by different letters are significantly different based on Tukey's test at <i>P</i> < 0.05.

Table 2. Microbiological properties of two growth media at the beginning of the trial and two and a half months after transplantation.

<table>
<thead>
<tr>
<th>growth media&lt;sup&gt;a&lt;/sup&gt;</th>
<th>x10&lt;sup&gt;6&lt;/sup&gt;UFC / ml substrate&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>P</td>
<td>0.027 b</td>
</tr>
<tr>
<td>CCR</td>
<td>0.385 a</td>
</tr>
<tr>
<td>Growth media&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Two and a half months after transplantation</td>
</tr>
<tr>
<td>P</td>
<td>7.079</td>
</tr>
<tr>
<td>CCR</td>
<td>5.067</td>
</tr>
</tbody>
</table>

<sup>a</sup> ca: cellulolytic actinomycetes; b: <i>Bacillus</i> spp.; c: copiotrophic bacteria; fp: fluorescent <i>Pseudomonas</i> spp.; cb: cellulolytic bacteria; oa: oligotrophic actinomycetes; ob: oligotrophic bacteria; f: fungi. <sup>b</sup> P: Peat; CCR: Composted Cork thin waste mixed with Rice hulls.

Within each column, values followed by different letters are significantly different based on Tukey's test at <i>P</i> < 0.05. At the beginning of the trial, analysis of variance was performed with transformed data in: Ln(x) for cellulolytic actinomycetes, <i>Bacillus</i> spp., copiotrophic bacteria, cellulolytic bacteria, oligotrophic. At two month and a half after in: Ln(x) for <i>Bacillus</i> spp., (x)<sup>-0.25</sup> for copiotrophic bacteria, (x)<sup>-0.5</sup> for fungi, (x)<sup>-0.2</sup> for oligotrophic actinomycetes.
Table 3. Physico-chemical properties of two growth media at the beginning of bioassay and at two and a half months after transplantation.

<table>
<thead>
<tr>
<th>Growth media</th>
<th>Electrical Conductivity (mS·cm⁻¹)</th>
<th>pH</th>
<th>Electrical Conductivity (mS·cm⁻¹)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0.32 b</td>
<td>5.835 b</td>
<td>2.23</td>
<td>4.7 b</td>
</tr>
<tr>
<td>CCR</td>
<td>1.35 a</td>
<td>7.445 a</td>
<td>2.88</td>
<td>7.7 a</td>
</tr>
</tbody>
</table>

a: P: Peat; CCR: Composted Cork thin waste mixed with Rice hulls. b: The beginning of bioassay. c: Two and a half months after transplantation. Within each column, values followed by different letters are significantly different based on Tukey's test at \( P < 0.05 \).

Table 4. Microbiological properties of soilless growing systems at two and a half months after transplantation.

<table>
<thead>
<tr>
<th>Growing system</th>
<th>x10⁶UFC / ml substratæ</th>
<th>ca</th>
<th>b</th>
<th>c</th>
<th>fp</th>
<th>cb</th>
<th>oa</th>
<th>ob</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>11.389 2.894</td>
<td>183.353 a</td>
<td>c</td>
<td>fpc</td>
<td>3.771</td>
<td>72.520 a</td>
<td>0.249</td>
<td>26.451</td>
<td>3.771</td>
</tr>
<tr>
<td>C</td>
<td>2.565 3.786</td>
<td>602.769 ab</td>
<td>c</td>
<td>fp</td>
<td>45.396</td>
<td>3.501</td>
<td>85.433 a</td>
<td>0.173 ab</td>
<td></td>
</tr>
<tr>
<td>CSSF</td>
<td>4.266 3.767</td>
<td>76.280 b</td>
<td>c</td>
<td>0.036</td>
<td>3.319</td>
<td>6.067</td>
<td>16.334 a</td>
<td>0.124 b</td>
<td></td>
</tr>
</tbody>
</table>

æ ca: cellulolytic actinomycetes; b: Bacillus spp.; c: copiotrophic bacteria; fp: fluorescent Pseudomonas spp.; cb: cellulolytic bacteria; oa: oligotrophic actinomycetes; ob: oligotrophic bacteria; f: fungi. Ò O: Open, C: closed and CSSF: closed with disinfection by slow sand filtration. ÆWithin each column, values followed by different letters are significantly different based on Tukey’s test at \( P < 0.05 \). At two and a half months after transplantation, analysis of variance was performed with transformed data in: \((x)^{-0.25}\) for copiotrophic bacteria, \((x)^{-0.5}\) for fungi and \((x)^{-0.2}\) for oligotrophic bacteria.

Table 5. Microbial activity and biomass in rhizosphere and non rhizosphere of the plant evaluated at two and a half months after transplantation by three different techniques in soilless growing system. Microbial biomass was measured by (AODC).

<table>
<thead>
<tr>
<th>Strawberry plants</th>
<th>β-glucosidase (µg hydrolyzed ( p )-nitrophenol/ml)</th>
<th>FDA (µg hydrolyzed fluorescein diacetate / ml-min)</th>
<th>AODC (Number of cells )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizosphere</td>
<td>508.698 a</td>
<td>2.570</td>
<td>2.976·10⁶ a</td>
</tr>
<tr>
<td>Non Rhizosphere</td>
<td>319.688 b</td>
<td>2.391</td>
<td>1.572·10⁶ b</td>
</tr>
</tbody>
</table>

Within each column, values followed by different letters are significantly different based on Tukey’s test at \( P < 0.05 \). Analysis of variance was performed with transformed data in: Ln(x) for β-glucosidase and \((x)^{a,b}\) for AODC.
Fig. 1. Microbial activity and biomass of CCR and P estimated by FDA and AODC respectively; at two and a half months after transplantation. Analysis of variance was performed with transformed data: Ln(x) for FDA and (x)^0.8 for AODC. Bars with the same letter are not significantly different according to Tukey’s test at P< 0.05. Standard error of the mean is indicated by vertical line.

Fig. 2. Microbial activity of SGS (O, C, and CSSF) estimated by FDA and β-glucosidase at two and a half months after transplantation. Analysis of variance was performed with transformed data: Ln(x) for β-glucosidase and FDA. Bars with the same letter are not significantly different according to Tukey’s test at P< 0.05. Standard error of the mean is indicated by vertical line.

Fig. 3. Microbial biomass of SGS (O, C, and SGSF) estimated by AODC; at two month and a half after transplantation. Analysis of variance was performed with transformed data: (x)^-0.8. Bars with the same letter are not significantly different according to Tukey’s test at P< 0.05. Standard error of the mean is indicated by vertical line.

Fig. 4. Microbial activity and biomass of rhizosphere and no rhizosphere of strawberry plants estimated by β-glucosidase and AODC respectively; at two month and a half after transplantation. Analysis of variance was performed with transformed data: Ln(x) for β-glucosidase and AODC. Bars with the same letter are not significantly different according to Tukey’s test at P< 0.05. Standard error of the mean is indicated by vertical line.