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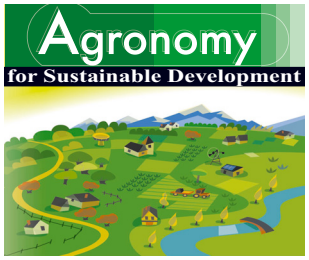

Review article

Pathogenic and beneficial microorganisms in soilless cultures

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Abstract – Soilless cultures were originally developed to control soilborne diseases. Soilless cultures provide several advantages for growers such as greater production of crops, reduced energy consumption, better control of growth and independence of soil quality. However, diseases specific to hydroponics have been reported. For instance, zoospore-producing microorganisms such as *Pythium* and *Phytophthora* spp. are particularly well adapted to aquatic environments. Their growth in soilless substrates is favoured by the recirculation of the nutrient solution. These pathogenic microorganisms are usually controlled by disinfection methods but such methods are only effective as a preventive measure. Contrary to biofiltration, active treatments such as UV, heat and ozonisation have the disadvantage of eliminating not only the harmful microorganisms but also the beneficial indigenous microorganisms. Here, we review microbial populations that colonise ecological niches of hydroponic greenhouse systems. Three topics are discussed: (1) the general microflora; (2) the pathogenic microflora that are typical to hydroponic systems; and (3) the non-pathogenic and possibly beneficial microflora, and their use in the control of plant diseases in soilless greenhouse systems. Technical, economic and environmental concerns are forcing the adoption of new sustainable methods such as the use of microbial antagonists. Thus, increased attention is now focused on the role of natural microflora in suppressing certain diseases. Managing disease suppression in hydroponics represents a promising way of controlling pathogens. Three main strategies can be used: (1) increasing the level of suppressiveness by the addition of antagonistic microorganisms; (2) using a mix of microorganisms with complementary ecological traits and antagonistic abilities, combined with disinfection techniques; and (3) amending substrates to favour the development of a suppressive microflora. Increasing our knowledge on beneficial microflora, their ecology and treatments that influence their composition will help to commercialise new, ready-to-use substrates microbiologically optimised to protect plants in sustainable management systems.

antagonistic agents / biological control / microbial ecology / disinfection methods / hydroponics / recirculating solutions / root rots / suppressive microflora / wilting / zoosporic pathogens

Contents

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1. INTRODUCTION

Soilless cultures are used worldwide. Depending on the country, growers use a variety of complex technologies, all of which offer advantages making them appropriate alternatives to traditional soil culture (Fig. 1A). In cases where the soil is polluted by chemical residues or contaminated by pathogens which colonise and persist in the soil for years or when excessive salinity causes water problems, soilless cultures can be an alternative. The main advantage of soilless cultures is that plants grow in a controlled environment. For instance, nutrient solution supply, electrical conductivity, pH and temperature are monitored and regulated by the grower. It provides an ideal environment for growth and development of plants and a greater yield is frequently obtained than with traditional cultural methods. The majority of greenhouse crops are grown using artificial substrates (Fig. 1B), which improves control of water, aeration, nutrition and root distribution. These systems were originally developed as open systems and excess nutrient solution was disposed of outside the greenhouse. In recent years, closed hydroponic systems have been developed to minimise pollution. In a closed system, the nutrient solution is recovered, replenished with nutrients and water, depending on plant uptake, and the pH adjusted before resupplying to the plants.

Microbial contamination of the root system in these culture systems can arise from many sources: plant material, growth media, and water from lakes, rivers and wells (Stanghellini and Rasmussen, 1994). Root colonisation by fungi and bacteria is favoured by at least three factors: (i) genetically uniform host plants, (ii) environmental conditions, i.e. suitable temperature and moisture regime, and (iii) rapid dispersal of root-colonising agents throughout the cultural system via the recycled nutrient solution.

The activity of microorganisms, however, may be pathogenic or protective, so two scenarios are possible. (1) One of the reasons for developing soilless culture was to prevent root diseases caused by soil-pathogenic microorganisms. Although a decrease in the diversity of root-infecting microorganisms has been reported, root diseases still occur frequently in hydroponics and disease outbreaks are sometimes greater than in soil (Stanghellini and Rasmussen, 1994). Some minor infections have become threats in soilless culture, indicating that unique diseases are observed with this method of plant cultivation. (2) The role of natural microflora in suppressing certain diseases was demonstrated by comparing systems with and without their original microflora (Postma et al., 2000; Minuto et al., 2007). Indeed, it has been shown that the natural microflora can suppress diseases (Berger et al., 1996; Chen et al., 1998) and that a high density of bacteria in the rhizosphere can limit pathogenic attacks on roots (Tu et al., 1999). From these observations ensued the hypothesis that indigenous bacteria were involved in disease biosuppression.

In this study we focused on the microbial communities colonising the root systems of plants growing in soilless cultures and highlighted the specificity of microbes in this type of cultivation system. Three topics were reviewed: (i) the general microflora; (ii) the pathogenic microflora of typical diseases related to hydroponics; and (iii) the non-pathogenic and possibly beneficial microflora and their use in the control of plant diseases in soilless greenhouse systems.

2. ECOLOGY OF THE MICROFLORA IN SOILLESS SYSTEMS

Soon after the start of a soilless culture, a microflora rapidly colonises three ecological niches: the substrate, the nutrient solutions and the rhizosphere of the cultivated plants. The density and diversity of this microflora are affected by the type of substrate (organic or inorganic), the nutrients in the solutions and the age and cultivar of the plant species.

Cultural methods have been used to characterise this microflora, but in recent years other methods based on sole-carbon-source utilisation (Khalil and Alsanius, 2001; Khalil et al., 2001b), phospholipid fatty acid profiling (Waechter-Kristensen, 1996; Khalil and Alsanius, 2001; Khalil et al., 2001a, b) and molecular fingerprinting (Postma et al., 2000; Calvo-Bado et al., 2003, 2006) have provided structural and functional analysis of the soilless microflora. Recent studies on microflora have provided key information on the microbial diversity and dynamics of soilless systems.

2.1. Influence of the kind of substrate on microflora

In soilless cultures a microflora rapidly develops soon after the start of the culture via the plants and the water supply, even though inorganic substrates contain few microbes. Once plants are introduced into greenhouses, extensive colonisation of rockwool substrates by bacteria and fungi rapidly occurs (Price, 1980; Carlile and Wilson, 1991). Inorganic substrates are mainly colonised by bacteria while organic substrates are colonised by fungi (Koohakan et al., 2004). In the case of crops of tomatoes, for instance, bacteria including fluorescent pseudomonads were higher in rockwool than in peat substrates and

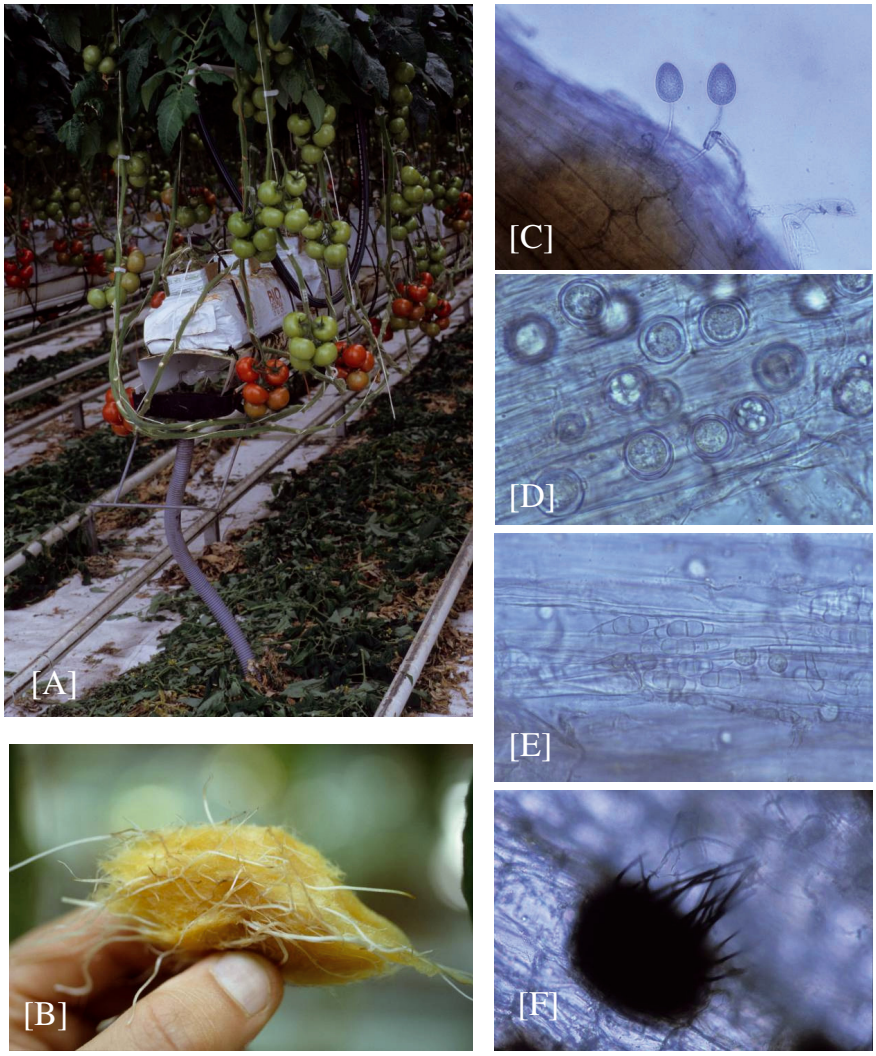

Figure 1. Tomato soilless culture and the main associated fungal pathogens. Suspended substrate in a tomato soilless culture (A), rockwool containing healthy and altered roots (B), *Phytophthora cryptogea* sporangia on the surface of a necrosed root (C), *Pythium aphanidermatum* oospores (round with a thick wall) in the root cortex cells (D), macroconidia of *Fusarium oxysporum* f. sp. *radicis-lycopersici* with chlamydospores in formation (E), *Colletotrichum coccodes* acervulus with black seta (F).

the reverse was observed for fungi, actinomycetes and *Trichoderma* spp. (Khalil and Alsanius, 2001). This might be due to the presence of available organic compounds within the organic substrates which may modify the microbial equilibrium through reduced competition (Koohakan et al., 2004). The level of conduciveness to the diseases caused by a given pathogenic agent might be determined by the nature (structure, composition) of the growth substrate of the crop. For instance, rockwool is more conducive to *Pythium* root rot and crown rot in cucumber culture than coir dust, pumice and perlite (van der Gaag and Wever, 2005). Temperature and oxygen concentration did not explain the differences between the media but the higher incidence of disease on rockwool was associated with a much greater water content than in the three others. When the height of the rockwool slabs was increased, the percentage of diseased plants decreased. These results indicated that water content plays a major role in the development of root and stem rot and that the type and height of substrate are important tools for decreasing yield losses.

2.2. Root system and nutrient solution microflora

Microorganisms multiply rapidly on roots and in nutrient solutions. Large populations of heterotrophic bacteria (10^5–10^6 cfu mL^{-1}) developed in the circulating nutrient solutions 20 h after planting tomatoes (Berkelmann et al., 1994). The number of bacteria on young tomato roots can be as high as 10^{10} cfu g^{-1} of fresh roots (Waechter-Kristensen et al., 1994). However, there are differences between microbial communities colonising roots and nutrient solutions; more fungi and bacteria were detected on roots than in nutrient solutions

(Koohakan et al., 2004). Besides the densities, the structure and the diversity of bacterial communities, as assessed by a molecular fingerprint method (Single-Strand Conformation Polymorphism, SSCP), were also different on roots and nutrient solutions (Renault, 2007).

The cultural systems (inorganic and organic media, deep flow technique and nutrient film technique) favoured in different ways the growth of unique indigenous microorganisms (Koohakan et al., 2004). Fungi and *Fusarium* spp. were found to colonise preferentially roots grown in a coconut-fibre system (organic medium) compared with a rockwool system (inorganic medium). *Pythium* spp. were mainly detected in nutrient solutions and on roots from the nutrient film technique system. Among the non-specific bacterial genera, aerobic bacteria seemed predominant on roots and in nutrient solutions, with only slight differences between the four systems (inorganic and organic media, deep flow technique and nutrient film technique). Whatever the system, fluorescent pseudomonads were frequently detected on roots and in nutrient solutions, which was consistent with previous findings showing that 40% of the cultivable bacteria belonged to the genus *Pseudomonas*, known to contain potentially antagonistic agents toward pathogens (Berkelmann et al., 1994). Similar results were obtained in the recycled nutrient solution during a six-month experiment in a soilless tomato greenhouse (Déniel et al., 2004). These findings might be explained by the fact that the temperature, high nitrogen content and oxygen concentration of the nutrient solutions offer an optimal growth environment for this genus.

2.3. Influence of the rhizosphere on the microbial communities

There is a clear relationship between cultivated plants and the establishment of the rhizosphere microflora. In closed hydroponic systems, it results from the release of organic compounds by the roots (Waechter-Kristensen et al., 1997). Passive or active leakage of carbon sources from plant roots differs in quantity and quality depending on plant species, plant cultivar and environmental factors such as light, climate, nutrient source, pH, humidity, etc. Whatever the hydroponic habitat, the diversity of microorganisms depends on their ability to metabolise the available carbon sources. Although a nutrient film technique system is much simpler than a soil-based culture system, SSCP analyses showed the bacterial diversity of the rhizoplane to be as high as that of the rhizosphere in soil (Chave et al., 2008). However, further studies comparing the microorganisms colonising soil and soilless cultures are needed to draw any conclusion.

2.4. Evolution of microbial communities

As mentioned above, biological processes in the rhizosphere are strongly affected by plant root exudates that attract specific microbial populations and stimulate their growth and evolution. Based on viable counts, aerobic bacteria colonising the rhizosphere of four types of soilless tomato production systems (inorganic substrate: rockwool; organic substrate: coconut-fibre; deep flow technique, nutrient film technique) were found to become stable at 10^{10} cfu g^{-1} (of fresh roots) in all systems investigated, contrary to fungi, that tended to increase throughout the experiment (Koohakan et al., 2004). However, changes in the composition of the microflora have been demonstrated by molecular and biochemical analyses. For instance, Khalil et al. (2001b) highlighted the differences between the microflora of two supposedly identical hydroponic cultivations by comparing sole-carbon-source utilisation (SCSU) patterns and phospholipid fatty acid profiles (PLFA). In tomato soilless cultures, Renault et al. (2008) also observed a temporal shift over a cropping season in the bacterial composition both in the nutrient solution and on the roots. Indeed, community-level physiological profiles (CLPPs) indicated that bacterial metabolism in nutrient solutions progressively shifted from carbohydrates towards the degradation of specific amino acids and carboxylic acids.

There is no consensus about whether shifts in the rhizosphere microflora can result from pathogenic attacks. Indeed, changes in the microbial communities of the rhizosphere could be a consequence of both root damage caused by pathogens such as *P. ultimum* and secondary colonisation due to the resulting nutrient leakage (Naseby et al., 2000; Hagn et al., 2008). On the other hand, it has been reported that the microbial communities established early on the roots of tomatoes grown in soilless systems were robust and resistant to the effect(s) of the introduction of oomycete pathogens or of switching from a recirculating to a run-to-waste nutrient supply (Calvo-Bado et al., 2006). However, this assumption, arising from experiments conducted over only 1.5 months, is contradicted by the observation of changes in the microbial communities of tomato plants grown hydroponically over the 6-month experiments of Vallance et al. (2009). SSCP analyses of three different DNA regions indicated increases in the complexity and size of the fungal microflora as the cropping season progressed. Nevertheless, both studies suggest that there are no substantial changes in the genetic structure of the indigenous rhizospheric fungal community after root inoculation with the non-pathogenic oomycete *P. oligandrum* or the pathogenic oomycetes *Pythium* group F, *P. aphanidermatum* and *P. cryptogea*.

3. UNIQUE DISEASE PROBLEMS IN SOILLESS CULTURES

3.1. Infections by zoosporic oomycetes

Among the pathogenic microorganisms frequently detected in hydroponic cultures, those producing zoospores, i.e. *Pythium* spp. and *Phytophthora* spp., are particularly well adapted to these cultivation systems (Favrin et al., 1988; Rafin and Tirilly, 1995) (Figs. 1C, 1D). As zoospores can swim, recycling facilitates rapid dissemination and subsequent root

infection of the whole culture (McPherson et al., 1995). Disease epidemics can occur, particularly in periods of stress, because of high temperatures and the low concentrations of dissolved oxygen in the nutrient solution (Gold and Stanghellini 1985; Stanghellini and Rasmussen 1994; Chérif et al., 1997). Highly pathogenic *Pythium* species, i.e. *Pythium ultimum*, *P. irregulare* and *P. aphanidermatum* (Blancard et al., 1992; Jenkins and Averre, 1983; Linde et al., 1994), caused root rot and wilting.

In Brittany (France), two stages in root infection by *Pythium* spp. in commercial tomato greenhouses were observed by Rey et al. (2001). The first is generally from the start of the winter crop (February) to June. A small population of *Pythium* spp. is frequently detected. The population then dramatically increases between July-August and the end of the cropping season (October–November); this increase is sometimes associated with root necrosis and root rot, but generally infections are limited to root necroses and are even symptomless. This pattern was particularly observed in greenhouses with organic (peat) and, to a lesser extent, inorganic substrates (rockwool). With a nutrient film technique system, *Pythium* spp. invasion was earlier and more severe than in other cultures, but with no amplification of symptoms.

A DNA macroarray for the detection and identification of more than 100 *Pythium* species was developed to assess the number and diversity of *Pythium* species on a single root sample (Tambong et al., 2006). This technology has the advantage of combining DNA amplification with the screening capability of DNA arrays, resulting in a high degree of sensitivity and multiple species specificity. The results of the DNA array tests confirmed that the substrate was almost free of oomycetes at the start of plant culture. *P. dissotocum* (or *Pythium* group F) was spontaneously detected on roots throughout the growing period but other *Pythium* species (*P. intermedium*, *P. ultimum* and *P. sylvaticum*) were sporadically detected (Le Floch et al., 2007). The relative predominance of *P. dissotocum* (or *Pythium* group F) and the low diversity of *Pythium* species confirm the results of previous studies conducted in soilless cultures (Herrero et al., 2003; Moorman et al., 2002; Moulin et al., 1994; Rafin and Tirilly, 1995; Rey et al., 1997).

3.2. Complex of pathogens on necrotic roots

A variety of fungal complexes and oomycetes are responsible for root necroses. A three-year experiment in tomato soilless cultures in France revealed that the distribution of the fungi and of the oomycetes was region-dependent (Blancard, unpubl. data). In the South-West, between two and five fungi and oomycetes were frequently found on roots, whereas in the five other regions (Brittany, the Eastern Pyrenees, Nantes region, Orleans region, the South-East), up to three different microorganisms were isolated from the samples. Some fungi, including *Fusarium oxysporum* f. sp. *radicis lycopersici*, and oomycetes, such as *Pythium* species, were found in all the greenhouses investigated in the six French regions (Fig. 1E). Other fungi, i.e. *Colletotrichum coccodes, Rhizoctonia solani* and *Thielaviopsis basicola*, or oomycetes such as *Phythophthora* spp. were only found on roots in some of the greenhouses (Fig. 1F).

3.3. Symptomless and minor pathogen infections on roots

Asymptomatic root colonisation in hydroponic cultures can be correlated with yield loss (Rey et al., 1997; Stanghellini and Kronland, 1986). *Pythium dissotocum* caused yield reductions of up to 54% in hydroponically grown lettuce although there was no visible damage (Stanghellini and Kronland, 1986). Such infections might be more common in soilless greenhouse systems than originally thought, because of the lack of root symptoms (Favrin et al., 1988). Immunoenzymatic staining procedures showed that *Pythium* spp. were the most frequent fungal invaders in asymptomatic roots of hydroponically grown tomato plants. *Pythium* spp. represented around 40% of the colonised segments as opposed to 12% for the other fungi. *Pythium* group F accounted for 75 to 90% of all the *Pythium* isolates from the loose or dense mycelia of *Pythium* spp. on the root epidermis (Rafin and Tirilly, 1995; Rey et al., 1997). Certain strains produce large numbers of zoospores (Rafin, 1993), possibly facilitating the spread and the development of *Pythium* group F in soilless cultures. When plants were grown under optimal conditions *Pythium* group F-infected roots were symptomless. However, roots looked generally macroscopically healthy but the oomycete caused limited changes in the root cortex (Rey et al., 1998) and produced metabolites that may facilitate *Pythium* group F infections (Rey et al., 2001). Moreover, due to high *Pythium* group F populations over the cropping season, limited but repeated damage to root cortexes could lead to slight yield reductions (Rey et al., 1997). Severe damage, such as root rot, only occurs when plants are placed under physiological stress conditions, i.e. lack of oxygen in nutrient solutions (Chérif et al., 1997). The nature of *Pythium* group F is still unclear. The taxonomic position of this oomycete has only become clearer in recent years with the increased interest in *Pythium* group F. Van der Plaats-Niterink (1981) used the term group F because oomycetes of this group only produce non-inflated filamentous sporangia on traditional culture media and sexual structures are not observed. However, after molecular characterisation of *Pythium* group F isolates by ribosomal and intermicrosatellite-DNA regions analysis, Vasseur et al. (2005) suggested that *Pythium* group F isolates could be *P. dissotocum*-like isolates unable to form sexual structures on traditional media. Moreover, Lévesque and De Cock (2004) suggested that *Pythium* group F could be related to *P. dissotocum* because of the complete homology of ITS sequences.

3.4. Other potentially pathogenic microorganisms in soilless cultures

The pathogenicity of a few microorganisms (*Humicola* sp., *Olpidium brassicae* and *Plectosporium tabacinum*) (Figs. 2A–2E) needs to be determined because some root

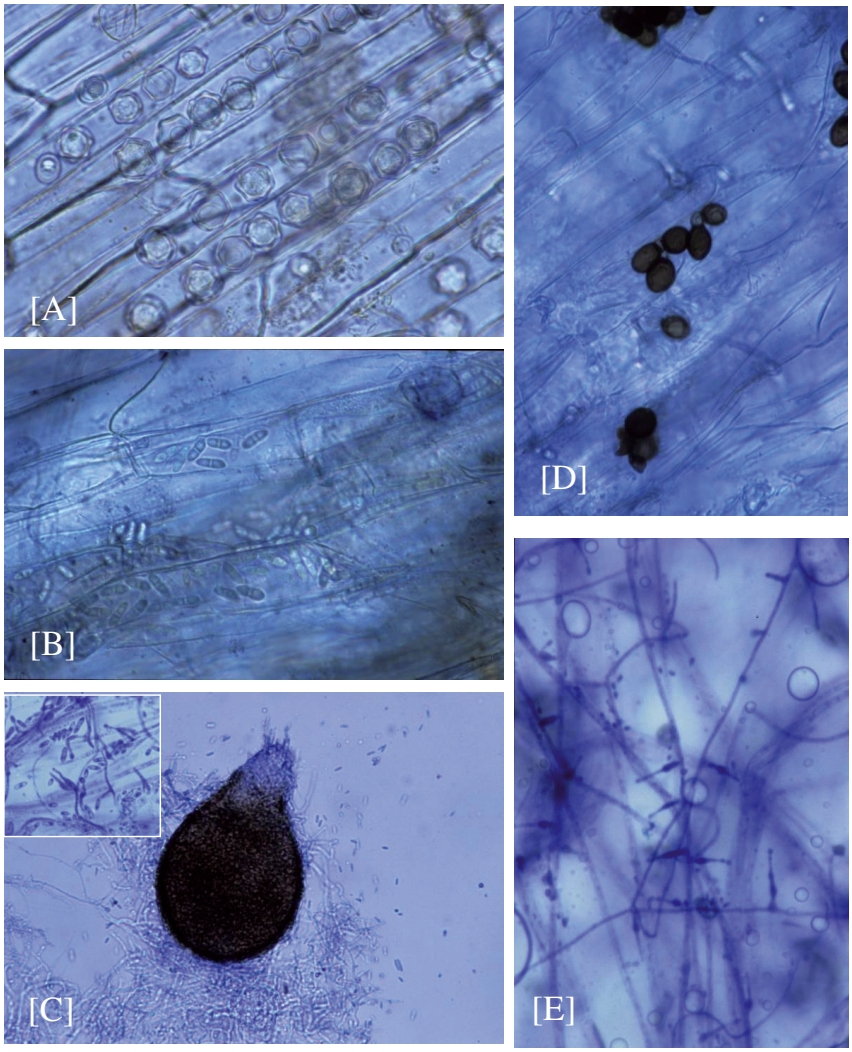

Figure 2. In situ and in vitro appearance of three fungi sometimes associated with root rot in tomato soilless culture but whose aggressiveness has never been proven on this Solanaceae. *Olpidium brassicae* resting spores aligned in several root cells (A), *Plectosporium tabacinum* bicellular conidia within root cortex cells (B), phialides of *Plectosporium tabacinum* (C), aleuriospores (dark brown) of *Humicola* sp. (D), phialides of *Humicola* sp. perpendicular to the mycelium; they form chains of conidia (E).

microorganisms of minor importance in soils have become of major economic importance in hydroponic cultures (Stanghellini & Rasmussen, 1994). Hydroponics, for instance, have favoured the development of *Phytophthora cryptogea* on lettuce, whereas, in the field, no attacks by this fungus have been reported. *Plectosporium tabacinum* (formerly *Fusarium tabacinum*), frequently isolated from soilless tomato cultures in France (Blancard, unpublished data) is a possible pathogen. It is detected on a variety of soil-grown plants, i.e. melon (Soran and Ozel, 1985), sunflower (Mirza et al., 1995) and basil (Minuto et al., 1997). Matta (1978) and Pascoe et al. (1984) reported that it caused necrotic lesions on young leaves in tomato plants and El-Gindy (1991) noticed necrosis and root rot in plantlets. Such symptoms have never been observed on tomato plants grown hydroponically. However, considering the pathogenic potential of *P. tabacinum* and its frequency in greenhouses, its pathogenicity in hydroponics needs to be assessed. Another example is *Humicola fuscoatra*. Gruyter et al. (1992) reported the association of *H. fuscoatra* with corky root symptoms in wilted glasshouse tomatoes. However, Menzies et al. (1998) pointed out that *Humicola fuscoatra* colonised roots, but did not cause necrosis and was, therefore, not pathogenic in tomato plants. These findings highlight the difficulties in distinguishing minor pathogens from other fungi, as both frequently colonise roots in soilless cultures.

4. EFFECT OF DISINFECTION TECHNIQUES ON THE MICROFLORA OF SOILLESS SYSTEMS

Closed hydroponic systems increase the risk of pathogen attack by using water contaminated with pathogenic microorganisms (McPherson et al., 1995; van Os, 1999). Therefore,

prevention of these infections has become a major challenge in the last decade (Runia, 1995; Ehret et al., 2001).

4.1. Active methods

The so-called "active" methods disinfect the nutrient solutions and are very effective (Ehret et al., 2001; Goldberg et al., 1992; Rey et al., 2001; Runia, 1995; Steinberg et al., 1994); for example, UV radiation and heat treatment can eliminate up to 99% of the microflora colonising the flowing solutions. UV irradiation of recirculating solution was effective in controlling *Pythium* spp.-induced root rot in tomato and cucumber plants (Postma et al., 2001; Zhang and Tu, 2000). Tirilly et al. (1997) reported a delay in *Pythium* root infection in soilless culture with this method; however, in several cases there was no difference in root colonisation from non-disinfected greenhouses. Moreover, re-contamination of the disinfected nutrient solution nullified the effect of disinfection (Déniel et al., 1999). Such drastic treatments create a microbiological vacuum in which microbial pioneers spread more easily because of the lack of competition (Paulitz and Bélanger, 2001; Postma, 2004). The microbial differences in solutions treated with UV and slow filtration often disappeared once they flowed through the rockwool slabs containing plant roots (van Os et al., 2004). Chlorination is effective in disinfecting water in storage tanks and reduces and delays root colonisation by *Pythium* spp. (Déniel et al., in press). However, this treatment has the disadvantage of eliminating not only harmful but also beneficial indigenous microorganisms; a weakness of "active" methods of disinfection. Zhang and Tu (2000) imputed the lack of control of *P. aphanidermatum* on tomato roots to the reduction of bacterial communities caused by UV radiation.

4.2. Passive method: slow filtration

The traditional technique of slow filtration, used for more than 100 years for water disinfection (Graham and Collins, 1996; Ellis, 1985), has been adapted for horticulture over the last decade (Ehret et al., 2001). Water flows slowly through a bed of substrate, i.e. sand, rockwool or pozzolana; mechanical and biological factors are thought to be responsible for the efficacy of the system (Ellis, 1985; Weber-Shirk and Dick, 1997). Experiments to improve slow filtration efficacy have focused on the determination of flow rates through the filter unit as well as on the nature and the optimal depth of substrates in filter tubes (Wohanka et al., 1999). Further investigations showed that the formation of bacterial microcolonies or biofilms on substrates enhanced efficiency. Indeed, after sterilising a filtering column, a dramatic loss in *Xanthomonas campestris* pv. *pelargonii* elimination has been reported (Brand and Wohanka, 2001). *Pseudomonas* was the predominant genus (50%) from the cultivable bacteria colonising the filtering media, especially the top layers of sand filters, and 10% of isolates were identified as *Bacillus* (Brand, 2000; Calvo-Bado et al., 2003). The *Bacillus* and *Pseudomonas* genera were recently reported to account for 42 to 86% of the total cultivable bacterial flora in a biocenosis film of pozzolana grains used as filtering medium (Déniel et al., 2004).

Pathogens eliminated efficiently by this technique include zoosporic fungi, i.e. *Phytophthora* spp., bacteria, i.e. *Xanthomonas campestris*, nematodes and even viruses (Ehret et al., 2001; van Os et al., 1999). During a 3-year experiment in a commercial greenhouse, Déniel et al. (2006) reported that a biofilter eliminated more fungi than bacteria under tomato production conditions. The efficiency of elimination of pathogenic fungi was genus-dependent. *Pythium* spp. were more effectively eliminated (99%) than *Fusarium oxysporum* (92.7 to 99.3%). The high percentage of *Pythium* spp. elimination was correlated with low root colonisation by these pathogens. Effluents of filtering columns have been shown to be colonised by a considerable natural bacterial microflora (10^2–10^4 cfu mL^{-1}) (Déniel et al., 2004, 2006; Renault, 2007). Moreover, molecular fingerprinting analyses of the total microflora (denaturing gradient gel electrophoresis, DGGE, and SSCP) pointed out clear changes in bacterial communities after the passage of the nutrient solution through the filter unit (Postma et al., 1999; Renault, 2007). Thus, slow filtration preserved part of the natural microflora, because it is harmless to specific groups of bacteria which are assumed to preserve microbial ecosystems in the plant rhizosphere. Furthermore, resident bacteria of nutrient solutions were shown to reduce *Pythium* root rot in closed soilless systems (Tu et al., 1999). The potential benefit of microflora in soilless cultures thus has to be taken into account.

5. DISEASE SUPPRESSION IN SOILLESS SYSTEMS

Pathogen-suppressive soils have been defined as "soils in which (i) the pathogen does not establish or persist; (ii) establishes but causes little or no damage; or (iii) establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil" (Borneman and Becker, 2007). Soils suppressive to several pathogens have been widely described and investigated (Alabouvette et al., 1979; Jager et al., 1979; Lifshitz et al., 1984; Garibaldi et al., 1989; Whipps and Lumsden, 1991), while the first studies of suppressiveness in soilless systems were by McPherson et al. (1995) and Tu et al. (1999). Both studies demonstrated the potential of the indigenous microflora to inhibit root diseases in hydroponic cultures. In soilless cultures, the term "suppressiveness" referred to the cases where (i) the pathogen does not establish or persist; or (ii) establishes but causes little or no damage. McPherson et al. (1995) described the spread of *Phytophthora cryptogea* in tomato nutrient film technique systems. In closed systems, the pathogen caused less damage than in the parallel run-to-waste ones; they therefore suggested that the potentially beneficial microflora colonising the recycled nutrient solution were responsible for disease suppression. They also suggested that the method of disinfection, i.e. "active" or "passive"

(by total or partial elimination of the microflora) could be important in the maintenance of the disease suppression. Tu et al. (1999) also showed that *Pythium* root rot disease was less severe in closed rockwool systems than in open culture due to the greater numbers of bacteria in closed systems. They found a strong correlation between the resident bacteria and the biosuppression of *Pythium*.

The presence of microflora suppressing *Pythium aphanidermatum* in cucumber rockwool substrate has been reported and some of the microorganisms involved in the suppressiveness identified (Postma et al., 2000, 2004, 2005). *Pythium* damage was lower in non-autoclaved than in autoclaved rockwool; the disease incidence was reduced by 50 to 100%. Suppressiveness could be restored in sterilised rockwool substrates by re-introducing the original microflora through contact with untreated rockwool or through the nutrient solution taken from untreated slabs. These results indicate that disease suppression is of biological origin and is transferable. Experiments on the microbial communities of rockwool showed a positive association between disease suppressiveness and the composition and diversity of bacteria and culturable filamentous actinomycetes. Actinomycetes may prevent the colonisation of dead root fragments by *Pythium* zoospores, whilst bacteria may secrete antibiotics, surfactants, etc. preventing colonisation of fresh root fragments.

Suppression of *Fusarium oxysporum* f. sp. *radicis lycopersici* has also been demonstrated. The incidence of *Fusarium oxysporum* f. sp. *radicis lycopersici* on tomato seedlings was significantly reduced with recycled, non-disinfected rockwool compared with new rockwool (Minuto et al., 2007); and in tomato soilless culture, by the re-use of perlite and perlite-peat substrates (Clematis et al., 2008). The indigenous microorganisms colonising these recycled substrates were considered responsible for the suppressive effects.

How the suppressive microflora becomes established is relatively unknown, but it has been suggested that pathogens themselves might influence suppressiveness. For instance, a study showed that *P. ultimum* induced shifts in cucumber indigenous microflora, favouring groups known to include potential biocontrol agents (Hagn et al., 2008). However, knowledge of structural and functional interactions and synergisms between the microorganisms of the suppressive microflora is limited and the influence of the plant and the pathogens on the whole system needs further investigation (Weller et al., 2002; Burdon et al., 2006).

6. MANAGEMENT OF THE SOILLESS MICROFLORA FOR DISEASE SUPPRESSION

Factors influencing disease suppression such as the activity of the total microflora, the diversity of the microbial communities and the presence of specific antagonists are not fully understood (Postma, 2004). Nevertheless, managing disease suppression in hydroponics represents a promising way of controlling pathogens. Three main strategies can be used: (i) increasing the level of suppressiveness by the addition of antagonistic microorganisms; (ii) using a mixed culture of microorganisms with complementary ecological traits and antagonistic abilities combined with disinfection techniques; and (iii) amending substrates to favour the development of the suppressive microflora.

6.1. Increasing the level of suppressiveness by the addition of antagonistic microorganisms

Environmental conditions in greenhouses are controlled and can be optimised to suit antagonistic agents. The biological vacuum and the limited volume of the matrix of the soilless substrates are thought to facilitate the introduction, establishment and interaction of the biocontrol agent with the root environment (Paulitz and Bélanger, 2001; Postma, 2004). Thus, representatives of a range of bacterial (*Pseudomonas*, *Burkholderia*, *Bacillus*, *Serratia*, Actinomycetes), fungal (*Trichoderma*, *Penicillium*, *Gliocladium*, non-pathogenic *Fusarium*) and oomycete (*Pythium*) groups have been tested as biocontrol agents in soilless cropping systems. The antagonistic activities of these microorganisms can be divided into several categories: competition for nutrients and space, parasitism, antibiosis and systemic induced resistance (Garbeva et al., 2004; Alabouvette et al., 2006; Lemanceau et al., 2006). Nevertheless, biocontrol of root diseases is often inefficient and only a few antagonists are available commercially.

The lack of efficiency is due to unsuitable methods of selection of antagonistic microorganisms. Results from in vitro studies did not always correlate with the antagonistic activity of the biocontrol agent once they were introduced into greenhouses (Fravel, 2005; Alabouvette et al., 2006; Georgakopoulos et al., 2002). These results also demonstrated the importance of the medium used for doing the in vitro tests; it has to be as close as possible to the environment into which the antagonists will be introduced. Even then, the colonisation, survival and antagonistic activity of the biocontrol agent may be insufficient and/or inconsistent at the infection site because the antagonist is not adapted to the soilless environment. The use of microorganisms selected from the indigenous suppressive microflora and not from a suppressive soil or a different crop might solve this problem: the microorganisms would be better adapted to the soilless crop environment and the ecological niche where their interaction with the pathogens will take place.

For example, the pathogenic fungi or oomycetes most frequently involved in root diseases in soilless cultures are those producing zoospores, such as *Pythium* spp. and *Phytophthora* spp., making them particularly well adapted to the aquatic environment of hydroponics. The use of an antagonist belonging to the same taxonomic group (i.e. oomycetes), with the same life cycle and similar properties, is of particular interest. An example of such an antagonist is the oomycete *P. oligandrum* (Rey et al., 2008; Vallance et al., 2009); it has been widely reported as an effective biocontrol agent (Foley et al., 1986; Jones and Deacon, 1995; Benhamou et al., 1997; Rey et al., 1998, 2005; Wulff et al., 1998). The beneficial effects of *P. oligandrum* are due to its potential to colonise roots without

damaging the host plant cells and to survive in the rhizosphere. *P. oligandrum* biocontrol in the rhizosphere is a complex process including direct control of pathogens by mycoparasitism, antibiosis or competition for nutrients and space; and/or indirect control via the plant, i.e. induction of resistance and growth promotion (Le Floch et al., 2005; Rey et al., 2008). Persistent root colonisation by *P. oligandrum* strains may be associated with an increase in tomato yield in soilless cultures (Le Floch et al., 2003), a transient increase (Le Floch et al., 2007) or not (Vallance et al., 2009).

When root colonisation by *P. oligandrum* is assessed, results from molecular (DNA macroarray and real-time PCR) and culture-dependent methods may be contradictory. Indeed, in the experiment of Le Floch et al. (2007), *P. oligandrum* was detected throughout the growing season (6 months) with molecular methods, but only for three months with plate counting on semi-selective media. These findings have important implications for biocontrol strategies aimed at protecting plants. Indeed, two different strategies could be envisaged: (i) based on cultural data, *P. oligandrum* inoculation on roots should be repeated three months after the first application; or (ii) conversely, based on molecular results, reinoculation is unnecessary because *P. oligandrum* is still present. In conclusion, the second strategy probably represents the true pattern of root colonisation by the antagonist, because detection by DNA array and real-time PCR is more accurate. Appropriate methods should therefore be used to detect the antagonistic agent(s) in assessment of biocontrol.

A strategy for increasing suppressiveness and therefore making biocontrol more successful might be to associate several antagonistic agents with complementary and/or synergistic modes of action against one or several pathogens (Spadaro et al., 2005). This is the case in naturally suppressive soils, where suppression is the result of complex interactions between several microorganisms acting together. Known examples are soils suppressive to *Fusarium* wilts where non-pathogenic *Fusarium* and fluorescent *Pseudomonas* were identified as the main antagonists (Alabouvette and Lemanceau, 1999). The non-pathogenic *Fusarium* competes for carbon sources while bacterial antagonists produce siderophores competing for iron. In soilless cropping systems, the association of the non-pathogenic *Fusarium* strain Fo47 and fluorescent *Pseudomonas* strain C7R12 controlled fusarium diseases better than single inoculations of each antagonistic microorganism (Eparvier et al., 1991). Another strategy was to combine inoculation of *Lysobacter enzymogenes* with chitosan. Chitosan enhanced the biocontrol efficacy of *L. enzymogenes* in the control of *P. apahidermatum* in cucumber soilless greenhouse systems. Chitosan either served as a C and N source for the antagonist, induced antagonistic gene expression, or both (Postma et al., 2009).

6.2. Use of a mixed culture of antagonistic microorganisms with disinfection techniques

A more complex strategy consists of combining nutrient solution disinfection methods with biocontrol agents to colonise and protect the roots from pathogenic attack. One of the first experiments of this type combined slow filtration and *P. oligandrum* inoculation on roots in a tomato soilless greenhouse system (Rey et al., 1999). Then, the association of slow sand filtration and antagonistic strains of *Fusarium* spp. and *Trichoderma* spp. isolated from a gerbera rhizosphere was successfully tested (Garibaldi et al., 2003). A similar experiment also reported that slow filtration and antagonistic fungi (*Fusarium* spp. and *Trichoderma* spp.) operated synergistically to significantly reduce the incidence of *P. cryptogea* root rot in gerbera crops (Grasso et al., 2003). Another strategy with slow filtration is to enhance efficiency by biological activation of the filtering columns with bacteria with suppressive traits, i.e. antagonistic activities, or siderophore and auxin production (Déniel et al., 2004). These bacteria, i.e. *Bacillus* and *Pseudomonas* strains, were isolated from a mature tomato hydroponic slow filtration unit and then inoculated into a new filter (Renault et al., 2007). Further investigations showed that the six-month period for the control filter to reach maximum efficiency against *F. oxysporum* was shortened in the bacteria-amended filter; in addition, filtration was highly efficient from the first month. Fast colonisation of pozzolana grains by selected bacteria and their subsequent interaction with *F. oxysporum* is probably responsible for filter efficiency. *Pseudomonas* spp. are supposed to act by competing for nutrients and *Bacillus* spp. by antibiosis and/or direct parasitism (Déniel et al., 2004). However, after nine months of operation, bacteria from the genera *Pseudomonas* and *Bacillus*, used to inoculate the filters, were not recovered in significant numbers from substrates in these filtering columns (Renault, 2007). Therefore, although early bacterial inoculations promote filter efficacy and induce a significant shift in microbial communities, the inoculated bacteria do not colonise the filtering substrates for long periods.

6.3. Nutritional amendments

Although physico-chemical factors influence the prevalence of *Pythium* diseases in certain substrates (van der Gaag and Wever, 2005), the main factor regulating disease suppression in hydroponic cultures is the microflora. The rhizosphere competency of potential biocontrol agents is often limited due to a lack of available organic nutrients in soilless growth media. Indeed, the main source of nutrients for the microflora on inorganic substrates is the plant roots, i.e. exudates, mucigel, sloughed root cells, etc. In conventional agriculture many other sources are available: organic amendments such as compost can be used as fertilisers or to improve the physical structure of the soil. Composted organic amendments are also substrates capable of suppressing plant diseases caused by a wide range of pathogens and pests, including bacteria, fungi and nematode species (Hoitink and Boehm, 1999; Alabouvette et al., 2006; Termorshuizen et al., 2006). Therefore, to maintain a critical threshold population of antagonistic microorganisms in soilless substrates, two approaches (similar to those in conventional cultures) could be considered: (a) the use of a different organic material, i.e. compost, as an alternative substrate

for greenhouse production, and (b) the introduction of a food base for the biocontrol agent to sustain its antagonistic activity without stimulating that of the pathogen.

(a) Composted organic amendments have been tested as alternative substrates to peat in soilless systems to preserve peat bogs. Two different types of citrus compost and their water extracts were investigated as partial peat substitutes for melon seedlings in greenhouse nurseries. Compared with peat, both composts (containing plant nutrients and auxin- and cytokinin-like compounds) enhanced the plant growth; biocontrol of *Fusarium oxysporum* was also achieved due to the biotic component. Water extracts had no effect on plant yield but their biocontrol ability was similar to that of their solid matrices (Bernal-Vicente et al., 2008). Another study showed that the suppressiveness of compost is related to the ability of its microflora to degrade organic compounds. The microbial communities associated with three substrates with varying capacities of *Fusarium* wilt suppression were characterised: peat (conducive to wilt), cork (moderately suppressive) and grape marc (very suppressive). The nature and composition of the plant growth medium determined the microbial communities: in suppressive media, the microflora preferentially metabolised less easily biodegradable compounds such as carboxylic acids, amino acids, amines, phenolic compounds and polymers; while the microflora of peat used mostly sugars (Borrero et al., 2006).

(b) As the availability of nutrients is a limiting factor for the growth of the microbial communities in various plant habitats, the use of nutritional amendments has been studied to selectively increase the communities' size and the biocontrol efficacy of a target biocontrol agent. The feasibility of selective enhancement and maintenance of desired populations of naturally-occurring biocontrol agents such as *Pseudomonas putida* by amending the nutrient solution with a nitrogen stabiliser, N-Serve®, has been demonstrated. Both active and inert ingredients in N-Serve® were involved in the suppression of root disease of pepper and cucumber caused by *Phytophthora capsici* and *P. aphanidermatum*. Xylene and 1,2,4-trimethylbenzene, the constituents of the inert fraction of N-Serve®, served as carbon sources for the selective enhancement of the pseudomonad populations, and nitrapyin, the active ingredient, reduced the vegetative growth of both pathogens (Pagliaccia et al., 2007, 2008).

7. CONCLUSION

The last three decades have convincingly shown that, in soilless culture, the initial goal of growing plants free of soilborne microorganism attacks was not realistic. Diseases specific to this type of cultivation have been frequently reported; indeed, the elimination of the soil did not remove the pathogenic issue but has simply moved it. For instance, in comparison with soil, some diseases are only observed or have taken on a greater importance in soilless cultures. In that context, control methods have to be adapted to soilless greenhouses. One of the main options that has gradually emerged in recent years has been the use of non-pathogenic microflora. This assumption was based on the finding that if hydroponics is a "solution" for the development and spread of pathogenic zoosporic fungi and oomycetes, much evidence indicates that it can also be one for the management of the plant protective microflora. The development of sustainable control methods such as classical biological control but also new kinds of experiments, i.e. the re-use of substrates (with their suppressive microflora) or the use of suppressive ready-to-use substrates, is a must for soilless cultures. As numerous environmental parameters are controlled, managing the microflora is much easier in soilless culture than on soil. It will be a testing ground on which the results could be used for transfer to more complex systems such as soil.

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