Biological Control of Root Pathogens in Soilless and Hydroponic Systems

T.C. Paulitz

Department of Plant Science, Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec H9X 3V9, Canada

Hydroponic and soilless systems of plant production are used worldwide to grow flower, foliage, bedding, and vegetable crops. Plants are grown using nutrient solutions with or without solid substrates for root growth. Hydroponic systems without substrates include the nutrient film technique (NFT), the deep flow technique, trough culture, and ebb and flow (Douglas, 1985). Plants can also be cultured on sand, rockwool, or in bags containing peat or sawdust. The nutrient solution can either be recirculated (closed system) or drained after one use (open system). These systems have become popular over the last 20 years in Northern Europe, the United States, and Canada, for the growth of high-value crops in glasshouses. The proportion of glasshouse vegetables produced in hydroponic or soilless systems has been increasing in Europe and Canada, in particular for tomatoes (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), pepper (*Capsicum annum* L.), and spinach (*Spinacia oleracea* L.). For example, seedless cucumbers are the second most important greenhouse vegetable in Canada, with revenues of \$43 million in 1992 (Statistics Canada, 1992). Although initial capital investment is high, these systems permit nutrient regimes and watering to be tailored to fit the physiological age of the crop and environmental conditions. Plant nutrition and the physical environment can be tightly controlled by the grower, resulting in higher yields, better quality, and control of crop scheduling (Resh, 1987). All the elements in the nutrient solution are readily available to the plant, so competition for nutrients can be reduced, and greater plant densities can be used. Another advantage of these growing systems is the avoidance of root diseases. Soilborne pathogens, especially fungi, produce inoculum in soil that can initiate root diseases in greenhouse culture. To avoid this contamination, most greenhouse growers who use soil adopted sterilization and pasteurization procedures to eradicate the inoculum in soil before use. These included the use of steam, aerated steam, soil fumigants, and fungicides (Baker, 1957). Although soil was commonly used 30 to 40 years ago as a growing medium, peat-based potting mixes and other soilless substrates are now more popular because of the elimination of soilborne pathogens.

However, soilless systems are not always free of disease problems and certain types of diseases have become more prominent and devastating. Several characteristics of the soilless system can in fact exacerbate the disease situation. First, in soilless greenhouse systems, the plants are genetically identical, and can be uniformly susceptible. In addition, the dense planting can favor the movement of pathogens from infected to healthy plants. Second, the physical environment, especially temperature and moisture, can be favorable for the pathogen. Third, in closed systems with recirculating water, pathogens can be easily spread from one plant to the other, especially zoosporic fungi that produce swimming spores. A small amount of contamination can lead to substantial infection and disease loss. For example, as few as 20 zoospores of *Pythium aphanidermatum* (Edson) Fitzp. introduced into 100 L of nutrient solution in an NFT system caused significant yield losses in cucumber production (Menzies et al., 1996). Closed systems are becoming more widespread because of ground-water pollution problems associated with open systems. Finally, soilless

substrates lack the microbial diversity and biological "buffering" found in natural soils. In natural soils, many soilborne pathogens are limited by antagonism from other microorganisms, and are subject to nutrient competition and fungistasis (Cook and Baker, 1983). Thus, if a pathogen is introduced into a natural soil, its spread is much slower than if introduced into a substrate that is essentially sterile. Without competition from other microbes, the pathogen may quickly become established in the substrate and cause severe disease.

INTRODUCTION OF PATHOGENS INTO SOILLESS SYSTEMS

Even though soilless systems may start out pathogen-free, severe diseases can occur. How is this inoculum introduced? Jarvis (1992) discusses many of the possible modes of inoculum spread, and how to limit them. Inoculum may come from outside the greenhouse on soil brought in on worker's shoes, tools, equipment, or through dust. Washed river sand or gravel used to cover pathways may also contain pathogens. Some soilborne pathogens, such as *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-lycopersici* W. R. Jarvis & Shoemaker, may produce airborne spores, called conidia, that can be moved into the greenhouse. Inoculum may be introduced on infected seed or propagation material. Peat has been shown to contain pathogens (Favrin et al., 1988). Water, especially reservoir or surface water, may contain zoosporic pathogens such as *Pythium* (Pickett-Popoff and Panter, 1994). Even insects such as fungus gnats (*Bradysia* spp.) and shore flies (*Scatella stagnalis* Fallen.) can vector pathogens in a greenhouse (Gillespie and Menzies, 1993; Goldberg and Stanghellini, 1990; Jarvis et al., 1993). The insects can acquire the pathogen by external contamination, or ingest fungal propagules, such as oospores, and excrete viable spores.

DISEASES IN SOILLESS SYSTEMS

What types of diseases are prevalent on roots in soilless systems? Despite the widespread occurrence of soilborne diseases in agricultural soils, relatively few have been reported in soilless systems. Many of the diseases have been reviewed by Stanghellini and Rasmussen (1994) and Zinnen (1988). The most important fungal pathogens are zoosporic, which produce a flagellated asexual swimming spore that can readily move in water and infect the roots. These zoospores are formed and released from a structure called a sporangium on the surface of the root. Released zoospores swim in the hydroponic solution or in water films surrounding solid substrates and demonstrate chemotaxis, or directed movement toward root exudates. Once they encounter a root, they attach, lose their flagella, encyst by forming a cell wall, and penetrate the root with a germ tube. The most common such species are in the genera *Phytophthora*, *Pythium*, and *Olpidium*. *Olpidium* can also act as a vector of viruses such as lettuce big vein. Recently, a species of *Plasmopara*, a genus of downy mildew normally associated with foliar disease, was shown to infect lettuce roots (Stanghellini et al., 1990a). Zoosporic pathogens can cause root rots, seedling rots, stunting, yield loss, and collapse of the plant if the pathogen moves into the crown region.

The most important nonzoosporic fungi act as wilt pathogens, including forma speciales of *Fusarium oxysporum* Schlechtend.:Fr. and *Verticillium dahliae* Kleb. *Fusarium oxysporum* f. sp. *radicis-lycopersici*, first reported from Canada in 1975 (Jarvis and Shoemaker, 1978), causes a crown and root rot. *Thielaviopsis basicola* (Berk. & Broome) Ferraris (Stanghellini et al., 1990b) and *Colletotrichum coccodes* (Wallr.) S.J. Hughes (Jenkins and Avere,

Received for publication 28 Mar. 1996. Accepted for publication 24 May 1996. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

1983) have also been reported in hydroponic systems. These pathogens form airborne spores called conidia and it appears that the airborne movement rather than movement by infested nutrient solution may be important.

At least three bacteria have been reported in soilless systems. Two are soilborne bacteria, *Clavibacter michiganense* subsp. *michiganensis* (Smith) Davis *et al.* (Staunton and Cormican, 1980) and *Pseudomonas solanacearum* (Smith) Smith (Jenkins and Averre, 1983), causing bacterial canker and wilt of tomato. Jenkins and Averre (1983) also isolated *Erwinia* spp. from diseased tomato plants in commercial hydroponic greenhouses.

DISEASE CONTROL IN SOILLESS SYSTEMS

The use of chemical pesticides, such as fungicides, are a main component of any disease control program in agriculture and horticulture. However, these are mostly used as protectants against foliar diseases. Pathogens in soil are very difficult to control with fungicides, because of the problem of getting the fungicide through the soil to contact the inoculum, and because of losses from the soil by leaching and microbial breakdown. Some soil fungicides are systemic and can be redistributed to the root. Over the past 20 years, systemic fungicides such as benomyl (Benlate, Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate; E.I. duPont de Nemours Co., Wilmington, Del.) and metalaxyl (Ridomil, Subdue, *N*-(2,6-Dimethylphenyl)-*N*-(methoxyacetyl)-alanine methyl ester; Ciba-Geigy, Greensboro, N.C.) have been widely used as soil drenches in the nursery and horticulture industry. However, no fungicides are currently registered for use against root diseases in hydroponic systems in the United States or Canada, for food or horticultural crops. One reason is the possibility of phytotoxicity of fungicides when applied to hydroponic systems. Soil provides a buffer, which may bind fungicides and reduce their uptake by the plant. Microbial breakdown of fungicides also occurs in soil but may be reduced in hydroponic systems. Therefore, there may be a residue problem if the fungicide is taken up by the plant. In addition, greenhouse vegetables such as cucumbers are harvested daily, so the normal preharvest fungicide-free period cannot be observed. Development of resistance to fungicides by pathogens is also a potential problem. For example, resistance to metalaxyl has been reported in *Phytophthora*, downy mildews, and *Pythium* (Staub, 1991). But the biggest reason for the lack of fungicides may be economic. Soilless culture is a small-niche market that is not large enough to justify the expense of developing, testing, and registering new or existing fungicides.

Another means of disease control is to disinfest the nutrient solution by the use of UV radiation (Runia, 1994a; Stanghellini *et al.*, 1984) or filtration (Goldberg *et al.*, 1992). Ozonation is currently used in the Netherlands as a method of disinfecting recirculation water in closed systems (Runia, 1994b). However, there are still questions about the effectiveness of these methods against resistant fungal propagules, problems with interference of organic matter in the water, and problems with adapting these technologies to handle the large amounts of water in many closed systems. However, these methods may become more viable as legislation in Europe prohibits the release of nutrient solutions from greenhouses and more growers convert to closed systems.

Cultural techniques, such as sanitation, can be effective against disease. These include measures to prevent the introduction of soil into the system, disinfestation of tools and worker shoes, screening to prevent insect introduction, and covering reservoir tanks (Jarvis, 1992). Disinfestation of systems with biocides such as sodium hypochlorite between crops is also widely practiced. Starting with disease-free seeds or propagation material is another way of assuring that pathogens are not introduced into the system. The greenhouse environment can also be modified to make conditions less favorable for the pathogen. For example, *Pythium aphanidermatum* is more devastating at temperatures above 25 °C (Gold and Stanghellini, 1985), but in Quebec greenhouses, *Pythium ultimum* Trow is more of a problem at 15 to 25 °C. Surfactants may be a promising method of control. Since the zoospores lack a cell wall and are surrounded only by a membrane, they are sensitive to surfactants. Although surfactants

often may be phytotoxic, recent reports by Rasmussen *et al.* (1995) have demonstrated the effectiveness of nonionic surfactants in controlling the spread of *P. aphanidermatum* in a recirculating system. The use of silicates has also been recently shown to have disease control effects (Bélanger *et al.*, 1995). The mechanisms are unclear, but silicates may induce a defense reaction in the plant against attack by *Pythium* (Chérif *et al.*, 1994). Unfortunately, there are still no resistant cultivars to most of the general root-rotting pathogens, such as *Pythium*.

BIOLOGICAL CONTROL IN SOILLESS SYSTEMS

What is the role of biological control in soilless systems? Biological control would seem to be ideally suited for soilless systems in closed structures. If biocontrol is to be successful anywhere, it will be in closed structures with soilless systems. One of the reasons for many of the failures of biocontrol in field agriculture has been the lack of consistency, often caused by unfavorable environmental conditions. Biocontrol agents are living organisms and are sensitive to temperature, moisture, pH, etc., unlike fungicides. However, the environmental conditions in greenhouses are more uniform and can be adjusted to make conditions favorable for the growth of the biocontrol agent and unfavorable for the pathogen. Another reason for inconsistent biocontrol activity in the field is the failure of the biocontrol agents to establish in a soil that already contains other competitive microbes. However, most soilless systems are essentially sterile at the beginning, and it is easy to establish high populations of biocontrol agents before an increase in the populations of competitors. Biocontrol agents easily can be added to the nutrient solution of a hydroponic system and dispersed. Most hydroponic crops have a high enough economic value to justify the large capital expense of the system and, therefore, would justify the higher costs that may be involved with biocontrol. In addition, the use of biocontrol could give a marketing advantage because consumers may favor crops grown without pesticides.

Despite the advantages of biocontrol in hydroponic systems, a review of the literature from 1985 to present revealed relatively few papers on this method of controlling disease in soilless systems, especially compared to the explosion of disease biocontrol papers in other agricultural systems. Most of these studies involved the use of rhizobacteria such as species of *Pseudomonas* and *Bacillus*. For example, fluorescent pseudomonads have been used against Fusarium wilt of carnation (*Dianthus caryophyllus* L.) in rockwool, possibly due to the effects of siderophores (Van Peer *et al.*, 1990). *Pseudomonas* spp. have been recently shown to also induce a systemic resistance in plants to many pathogens, including Fusarium wilts (Duijff *et al.*, 1993) and foliar pathogens (Wei *et al.*, 1991). *Pseudomonas* sp. reduced cucumber root colonization by *Pythium aphanidermatum* (Moulin *et al.*, 1995) and Pythium disease on cucumbers in closed rockwool systems (Postma *et al.*, 1995). *Bacillus subtilis* (Ehrenberg) Cohn. partially controlled *F. oxysporum* f. sp. *radicis-lycopersici* and Phytophthora diseases on tomatoes in hydroponic culture (Bochow, 1992). Actinomycetes have also been tested, such as a strain of *Streptomyces* sp. currently marketed as a product called Mycostop (Kemira Agro Oy, Vass, Finland). This product has activity against *Fusarium oxysporum* on gerbera (*Gerbera jamesonii* H. Bolus ex Adlam) and Pythium diseases of cucumber in rockwool (Lahdenpera, 1995). *Trichoderma* spp. have been the most widely tested fungal biocontrol, and have activity against *F. oxysporum* f. sp. *radicis-lycopersici* on tomatoes (Hartman and Fletcher, 1991) and *Pythium ultimum* on cucumbers (Wolffhechel and Funck Jensen, 1991). *Gliocladium virens* Miller, Giddens and Foster has been commercialized in the United States as a product called Soilgard (Grace Biopesticides, Columbia, Md.), and controlled Pythium and Rhizoctonia damping-off in peat growing mixes (Lumsden and Locke, 1989). Nonpathogenic forma specialis of *Fusarium oxysporum* have been used to protect plants against wilt-causing pathogenic forma specialis on tomatoes and carnation, and the crown and root rot pathogen of tomatoes (Alabouvette *et al.*, 1993). Mycoparasitic species of *Pythium*, such as *Pythium periplocum* Drechs. have been used against pathogenic species of *Pythium* in cucumber production (Hockenull *et al.*, 1992).

BIOLOGICAL CONTROL OF PYTHIUM DISEASES IN ROCKWOOL-GROWN CUCUMBERS IN QUEBEC

For many of the reasons cited above, we initiated a program to develop biocontrol agents for *Pythium* diseases of cucumbers in rockwool production in Quebec. This project focused on finding naturally occurring rhizobacteria, such as *Pseudomonas* spp., that could be adapted to the rhizosphere of cucumber. We hypothesized that bacteria would be better adapted to the wet conditions of hydroponic systems than fungi. The use of indigenous bacteria would also make the registration process easier. More than 600 bacterial strains were isolated from the rhizosphere of cucumber plants grown in 34 distinct soils collected in Quebec. A screening program was developed to find the most effective biocontrol agents against the target pathogen, *Pythium aphanidermatum*.

The protocol that was developed was a compromise between using *in vitro* and *in planta* techniques. *In vitro* techniques, such as petri plate assays, are easy to perform and can be used to screen many isolates. However, they usually favor antibiotic producers and organisms that may not perform well on the plant. Although *in planta* techniques are more realistic, they require more time and effort. In our screening program, the isolates were tested for their ability to inhibit the mycelial growth of the pathogen on agar and their ability to inhibit the germination and encystment of the zoospores. Zoospores were targeted, since these are the infective units of this pathogen. We also tested the ability of the bacteria to interfere with the chemotaxis of the zoospores, i.e., their ability to sense and swim toward the root.

From this screening of 600 isolates, 35 isolates significantly inhibited zoospore germination and chemotaxis, and were selected for an assay on cucumber seedlings growing in nutrient solution in test tubes (Paulitz et al., 1992). These strains were also tested in a larger experiment with 3-week-old plants in 1-L mason jars aerated with aquarium pumps. Five isolates significantly reduced root rot of seedlings. These five strains of *Pseudomonas* spp., including *Pseudomonas fluorescens* Migula and *P. corrugata* Roberts and Scarlett, were tested in three replicated experiments in rockwool under near-commercial conditions (Rankin and Paulitz, 1994). Cucumber plants were treated with bacteria prior to the cubes being placed on rockwool slabs in the greenhouse. Half of the slabs were inoculated with 100,000 zoospores of *Pythium aphanidermatum*, and half were left noninoculated. Two of the strains significantly increased fruit production in both inoculated and noninoculated treatments. Further testing of these and other strains in greenhouses in Quebec and British Columbia have confirmed these results. Even in the absence of disease pressure, one strain was able to increase yields by 12% to 18%, an increase of six fruit and 3 kg of fruit weight per plant per season (Paulitz, unpublished). Some strains appear to cause a growth response independent of pathogen pressure, and could be classified as plant-growth-promoting rhizobacteria (PGPR; Kloepper et al., 1991).

The mechanisms of action of these bacteria were also investigated. Besides reducing the chemotactic attraction of the root exudates, treatment of roots with bacteria changed the pattern of distribution of encysted zoospores on the root surface (Zhou and Paulitz, 1993). This feature suggests a mechanism of competition, where bacteria occupy microsites on the root, metabolize attractive exudates or preempt potential infection sites for the zoospores. Induced resistance was also implicated (Zhou and Paulitz, 1994), based on the ability of two strains of *Pseudomonas* spp. to alter the disease process when they were spatially separated from the pathogen in a split root system.

FUTURE RESEARCH ON BIOLOGICAL CONTROL IN SOILLESS SYSTEMS

The most successful strategy of using biocontrol agents against root pathogens in soilless systems may be as protectants or prophylactic treatments. If the biocontrol agent can be established before pathogen introduction, can it prevent infection or interfere with the production of pathogen inoculum that could spread through the system? These are the questions that should be addressed experimentally. But, so far, one of the drawbacks of inoculation experiments in greenhouses is that they do not reflect exactly what happens in a

commercial greenhouse. Most systems start out clean, but the pathogen is introduced some time later, probably in low population densities, in a random fashion and possibly many times during the cropping cycle. But in most experiments, the biocontrol agents are added to the soilless system at the start, before adding one massive dose of pathogen inoculum. Under these conditions, the biocontrol agents cannot completely control disease, so most biocontrol treatments have disease severities midway between the inoculated nonprotected control, and the noninoculated, healthy control. Growers expect near-complete disease control and may not accept simple disease reduction. Experiments should be performed with many introductions of low population densities of the pathogen. Under these conditions, the biocontrol agent may prevent the explosive buildup in pathogen populations. The biocontrol agents may delay or reduce the establishment of the pathogen, keeping the pathogen population densities below the economic threshold. We are also unclear on how the initial infection is influenced by the stage of crop development. Are early infestations more important than later infestations, in terms of disease? We also know very little about how environmental and plant factors such as temperature, electrical conductivity, transpiration, and fruit set interact with the biocontrol agents and pathogens to influence disease. Finally, more work should be done on the use of booster applications of the biocontrol agents to the system over the whole cropping cycle. This may overcome the natural decline of some biocontrol agents over time, and may maintain a suppressive state in the system. Given the ease of introducing nutrients into a fertigation system, formulations of bacteria may also be developed that could be added to the injector system for rapid dispersal in rockwool slabs.

In conclusion, biological control may offer another tool for disease management in soilless systems. Given the lack of chemical alternatives and the innovative technology of these systems, many of the future applications of biological control will be in these intensively managed greenhouse systems.

Literature Cited

- Alabouvette, C., P. Lemanceau, and C. Steinberg. 1993. Recent advances in the biological control of fusarium wilts. *Pesticide Science* 37:365-373.
- Baker, K.F. 1957. The U.C. system for producing healthy container-grown plants. Univ. of California Ext. Serv., Manual 23.
- Bélanger, R.R., P.A. Bowen, D.L. Ehret, and J.G. Menzies. 1995. Soluble silicon: Its role in crop and disease management of greenhouse crops. *Plant Dis.* 79:329-336.
- Bochow, H. 1992. Phytosanitary effects of *Bacillus subtilis* as biocontrol agent. Medelelingen Faculteit Landbouww. Rijksuiv. Gent. 57:373-379.
- Chérif, M., A. Asselin, and R. Bélanger. 1994. Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology* 84:236-242.
- Cook, R.J. and K.F. Baker. 1983. The nature and practice of biological control of plant pathogens. Amer. Phytopathol. Soc. Press, St. Paul, Minn.
- Douglas, J.S. 1985. Advanced guide to hydroponics: Soilless cultivation. Pelham Books, London.
- Duijff, B.J., J.W. Meijer, P.A.H.M. Bakker, and B. Schippers. 1993. Siderophore-mediated competition for iron and induced resistance in the suppression of fusarium wilt of carnation by fluorescent *Pseudomonas* spp. *Neth. J. Plant Pathol.* 99:277-289.
- Favrin, R.J., J.E. Rahe, and B. Mauza. 1988. *Pythium* spp. associated with crown rot of cucumbers in British Columbia greenhouses. *Plant Dis.* 72:683-687.
- Gillespie, D.R. and J.G. Menzies. 1993. Fungus gnats vector *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Ann. Appl. Biol.* 123:539-544.
- Gold, S.E. and M.E. Stanghellini. 1985. Effects of temperature on *Pythium* root rot of spinach grown under hydroponic conditions. *Phytopathology* 75:333-337.
- Goldberg, N.P. and M.E. Stanghellini. 1990. Ingestion-egestion and aerial transmission of *Pythium aphanidermatum* by shore flies (Ephydriidae: *Scatella stagnalis*). *Phytopathology* 80:1244-1246.
- Goldberg, N.P., M.E. Stanghellini, and S.L. Rasmussen. 1992. Filtration as a method of controlling *Pythium* root rot of hydroponically grown cucumbers. *Plant Dis.* 76:777-779.
- Hartman, J.R. and J.T. Fletcher. 1991. Fusarium crown and root rot of tomatoes in the UK. *Plant Pathol.* 40:85-90.
- Hockenull, J., D. Funck Jensen, and T. Yudiarti. 1992. The use of *Pythium periplocum* to control damping-off of cucumber seedlings caused by *Pythium aphanidermatum*, p. 203-206. In: E.S. Tjamos et al. (eds.). *Biological control of plant diseases*. Plenum Press, New York.

- Jarvis, W.R. 1992. Managing diseases in greenhouse crops. Amer. Phytopathol. Soc. Press, St. Paul, Minn.
- Jarvis, W.R., J.L. Shipp, and R.B. Gardiner. 1993. Transmission of *Pythium aphanidermatum* to greenhouse cucumbers by the fungus gnat *Bradysia impatiens* (Diptera: Sciaridae). Ann. Appl. Biol. 122:23–29.
- Jarvis, W.R. and R.A. Shoemaker. 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68:1679–1680.
- Jenkins, S.F. and C.W. Averre. 1983. Root diseases of vegetables in hydroponic culture systems in North Carolina greenhouses. Plant Dis. 67:968–970.
- Kloepper, J.W., R.M. Zablotowicz, E.M. Tipping, and R. Lifshitz. 1991. Plant growth promotion mediated by bacterial rhizosphere colonizers, p. 315–326. In: D.L. Keister and P.B. Cregan (eds.). The rhizosphere and plant growth. Kluwer Academic Publishers, Dordrecht.
- Lahdenpera, J.L. 1996. Activity of Mycostop biofungicide in rockwool. Proc. of the 4th IOBC/EFPP Workshop on Biological and Integrated Control of Root Diseases in Soilless Cultures, Sept. 1995, Dijon, France. Bul. 19:109–114.
- Lumsden, R.D. and J.C. Locke. 1989. Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless mix. Phytopathology 79:361–366.
- Menzies, J.G., D.L. Ehret, and S. Stan. 1996. Effect of inoculum density of *Pythium aphanidermatum* on the growth and yield of cucumber plants grown in recirculating nutrient film culture. Can. J. Plant Pathol. 18:50–54.
- Moulin, F., P. Lemanceau, and C. Alabouvette. 1996. Effect of a fluorescent pseudomonad on colonization of cucumber roots by *Pythium aphanidermatum*. Proc. of the 4th IOBC/EFPP Workshop on Biological and Integrated Control of Root Diseases in Soilless Cultures, Sept. 1995, Dijon, France. Bul. 19:77–84.
- Paulitz, T.C., T. Zhou, and L. Rankin. 1992. Selection of rhizosphere bacteria for biological control of *Pythium aphanidermatum* on hydroponically grown cucumber. Biol. Control 2:226–237.
- Pickett-Popoff, L. and K.L. Panter. 1994. Survey of *Pythium* and *Phytophthora* spp. in irrigation water used by Colorado commercial greenhouses to determine source of pathogen introduction. Phytopathology 84:1118. (Abstr.)
- Postma, J., M.J. Willemsen-de Klein, and E.E. Hoogland. 1996. Biocontrol of *Pythium aphanidermatum* in closed culture systems. Proc. of the 4th IOBC/EFPP Workshop on Biological and Integrated Control of Root Diseases in Soilless Cultures, Sept. 1995, Dijon, France. Bul. 19:42–46.
- Rankin, L. and T.C. Paulitz. 1994. Evaluation of rhizosphere bacteria for biological control of *Pythium* root rot of greenhouse cucumbers in hydroponic culture. Plant Dis. 78:447–451.
- Rasmussen, S.L., M.E. Stanghellini, D.H. Kim, and P. Rorabaugh. 1995. Efficacy of surfactants in the control of zoospore root-infecting fungi. Phytopathology 85:1167. (Abstr.)
- Resh, H.M. 1987. Hydroponic food production: A definitive guidebook of soilless food growing methods. 3rd ed. Woodbridge Press Publishing Co., Santa Barbara, Calif.
- Runia, W.T. 1994a. Elimination of root-infection pathogens in recirculation water from closed cultivation systems by ultra-violet radiation. Acta Hort. 361:361–371.
- Runia, W.T. 1994b. Disinfection of recirculation water from closed cultivation systems with ozone. Acta Hort. 361:388–396.
- Stanghellini, M.E., J.E. Adaskaveg, and S.L. Rasmussen. 1990a. Pathogenesis of *Plasmopara lactucae-radicis*, a systemic root pathogen of cultivated lettuce. Plant Dis. 74:173–178.
- Stanghellini, M.E. and S.L. Rasmussen. 1994. Hydroponics: A solution for zoospore pathogens. Plant Dis. 78:1129–1138.
- Stanghellini, M.E., S.L. Rasmussen, and D.J. Barta. 1990b. Thielaviopsis root rot of corn-salad. Plant Dis. 74:81.
- Stanghellini, M.E., L.J. Stowell, and M.L. Bates. 1984. Control of root rot of spinach caused by *Pythium aphanidermatum* in a recirculating hydroponic system by ultraviolet irradiation. Plant Dis. 68:1075–1076.
- Statistics Canada. 1992. Seasonal fruit and vegetable production. Statistics Canada Publ. 22-203.
- Staub, T. 1991. Fungicide resistance: Practical experience with antiresistance strategies and the role of integrated use. Annu. Rev. Phytopathol. 29:424–442.
- Staunton, W.P. and T.P. Cormican. 1980. The effects of pathogens and fungicides on tomatoes in a hydroponic system. Acta Hort. 98:293–297.
- Van Peer, R., A.J. Van Kuik, H. Rattink, and B. Schippers. 1990. Control of Fusarium wilt in carnation grown in rockwool by *Pseudomonas* sp. strain WCS417r and by Fe-EDDHA. Neth. J. Plant Pathol. 96:119–132.
- Wei, G., J.W. Kloepper, and S. Tuzun. 1991. Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacterial. Phytopathology 81:1508–1512.
- Wolffhechel, H. and D. Funck Jensen. 1991. Influence of the water potential of peat on the ability of *Trichoderma harzianum* and *Gliocladium virens* to control *Pythium ultimum*, p. 392–397. In: A.B.R. Beemster et al. (eds.). Biotic interactions and soil-borne diseases, Elsevier, Amsterdam.
- Zhou, T. and T.C. Paulitz. 1993. In vitro effects of *Pseudomonas* spp. on *Pythium aphanidermatum*: Zoospore behavior in exudates and on the rhizoplane of bacteria-treated cucumber roots. Phytopathology 83:872–876.
- Zhou, T. and T.C. Paulitz. 1994. Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp. on cucumber. J. Phytopathol. 142:51–63.
- Zinnen, T.M. 1988. Assessment of plant disease in hydroponic culture. Plant Dis. 72:96–99.

Using Entomopathogenic Nematodes to Control Insects During Stand Establishment

W.R. Martin, Jr.

biosys, Inc., 4131 NW 13th Street, Suite 212, Gainesville, FL 32609

I. INTRODUCTION

Using entomopathogenic nematodes as biological control agents is not a new idea. One of the earliest published accounts of a field study using reared nematodes was published in 1935, using *Steinernema glaseri* (Steiner) to control Japanese beetle (*Popillia japonica* Newman) grubs in soil (Glaser and Farrell, 1935). Recent advances in the development of large-scale, in vitro rearing techniques and formulation technology have prompted the development and commercialization of these nematodes as biological pesticides. Indeed, the potential for these nematodes as biological control agents is very promising,

with proven efficacy against a variety of soil-inhabiting insects, including root weevils (several genera) (Jansson et al., 1990; Schroeder, 1987, 1990), white grubs (*Phyllophaga* spp.) (Kard et al., 1988), mole crickets (*Scapteriscus* spp.) (Parkman et al., 1993), fleas (*Ctenocephalides* spp.) (Henderson et al., 1995; Manweiler, 1994), and fungus gnats (*Bradysia* spp.) (Harris et al., 1995). In addition, under certain conditions, efficacy has been demonstrated against some foliage-feeding insects (Begley, 1990).

The technology for production, storage, and formulation of entomopathogenic nematodes has improved dramatically within the past 10 years. Although nematodes are easily produced in vivo, the high costs of in vivo production make it unsuitable for commercial use. A cost-effective in vitro production technique has been developed and successfully adapted to several species of steinernematids (Friedman et al., 1989). Steinernematid nematodes are now produced consistently in 30,000 to 80,000-L liquid fermenters in a proprietary medium

Received for publication 28 Mar. 1996. Accepted for publication 24 May 1996. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.