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BIOLOGICAL CONTROL IN GREENHOUSE SYSTEMS

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■ **Abstract** The controlled environment of greenhouses, the high value of the crops, and the limited number of registered fungicides offer a unique niche for the biological control of plant diseases. During the past ten years, over 80 biocontrol products have been marketed worldwide. A large percentage of these have been developed for greenhouse crops. Products to control soilborne pathogens such as *Sclerotinia*, *Pythium*, *Rhizoctonia* and *Fusarium* include *Coniothyrium minitans*, species of *Gliocladium*, *Trichoderma*, *Streptomyces*, and *Bacillus*, and nonpathogenic *Fusarium*. Products containing *Trichoderma*, *Ampelomyces quisqualis*, *Bacillus*, and *Ulocladium* are being developed to control the primary foliar diseases, *Botrytis* and powdery mildew. The development of *Pseudomonas* for the control of *Pythium* diseases in hydroponics and *Pseudozyma flocculosa* for the control of powdery mildew by two Canadian research programs is presented. In the future, biological control of diseases in greenhouses could predominate over chemical pesticides, in the same way that biological control of greenhouse insects predominates in the United Kingdom. The limitations in formulation, registration, and commercialization are discussed, along with suggested future research priorities.

CONTENTS

INTRODUCTION

Biological control of plant pathogens is now an established sub-discipline in the science of plant pathology. Although its beginnings can be traced back over 70 years, it was not until the early 1960s that theory and practice came together in the proceedings of one of the first biocontrol meetings (11). Over the past 20 years, the amount of research in this area has increased dramatically. Within the past 10 years, over 40 biocontrol products have appeared on the commercial market, but these are still a small fraction of the total number and sales of chemical fungicides in field, row, and tree crops. In a 1993 report, sales of biofungicides represented less than $1 million, whereas total fungicides sales were then in excess of $5.5 billion. Optimistic estimates projected that sales of biocontrol products could reach $15 million before year 2000 (136). However, agriculture in greenhouses and protected structures offers a unique niche for the development and use of biological control agents. Of the 33 commercial biocontrol products listed by Fravel et al (61), over half have applications in nurseries or greenhouses; and many were specifically developed against the soilborne pathogens *Pythium* and *Rhizoctonia*, which are major greenhouse pathogens. The world's total greenhouse area is 307,000 ha, including both plastic and glass (72), whereas the total land in outside cultivation in 1998 was 1.51 billion ha (58). The use of biocontrol is more prevalent in greenhouse and protected structures than in field crops, even though greenhouses account for only 0.02% of the area used in agriculture. Why has biological control become more integrated into management strategies in protected structures? This chapter addresses this question. We describe some of the disease pressures unique to greenhouses and discuss why biocontrol in greenhouses may have a greater potential use than in field crops. We review some of the products that are commercially available in different parts

of the world and discuss specific case studies of biocontrol of foliar and soilborne pathogens. We draw lessons from these studies for use in the future and speculate on the direction of biocontrol in the greenhouse. What barriers and impediments are faced in the implementation and commercialization of biocontrol in the future?

This chapter focuses only on biological control of fungal diseases, the primary pathogens in most greenhouses. We accept the definition of biological control as defined by Cook & Baker (39) and chose not to cover natural products and the use of non-fungicide products such as silica, sodium bicarbonate, and plant defense inducers.

Unique Disease Problems in Greenhouses

The greenhouse environment presents a unique situation that may make conditions more favorable for diseases [for earlier reviews on diseases of greenhouse crops, see (86, 111, 125)]. First, most pathogens cannot be excluded from the greenhouse environment: Airborne spores enter through doors and screens; soilborne pathogens enter through dust or contaminated soil on shoes, tools, or equipment: and many pathogens are introduced on seeds or contaminated propagating material. Zoosporic pathogens enter through irrigation water, and insects carry fungal inoculum (88) or transmit viruses. The temperature, light, and fertilizer regimes are optimized for maximal plant growth, but these conditions may also be favorable for pathogens. Moreover, warmth and humidity, due to the water vapor transpired by the plants and the lack of air exchange with the outside, provide ideal conditions for foliar pathogens such as *Botrytis* and powdery mildews. Because of high energy costs, ventilation is often reduced to prevent loss of heat. Disinfested soil or soilless substrates such as peat or rockwool lack the microbial diversity and biological buffering present in a natural soil. In this biological vacuum, soilborne pathogens such as *Pythium* and *Rhizoctonia* can quickly grow and spread. In addition, the life stages of plants most commonly found in greenhouse nurseries are seeds, seedlings, and young transplants, all especially susceptible to many pathogens that attack juvenile tissue. High-density planting of greenhouse crops increases the relative humidity and the chances of disease spread, and management practices, including pruning and harvesting, increase the spread and infection through wounds. Hydroponic systems, such as rockwool, nutrient film, or ebb and flow, present another set of disease problems (125). In closed recirculating systems, zoosporic pathogens can easily spread in the water system.

Suitability of Greenhouses for Biological Control

Some of the very conditions that favor disease also favor the management of diseases with biological control agents. Environmental conditions such as temperature and relative humidity can be tightly controlled. Like the pathogen, biocontrol

agents are also sensitive to environmental conditions, and an unfavorable environment in the field has been cited as a reason for failure or inconsistent performance. Conditions in the greenhouse can be optimized for the biocontrol agent. For instance, biocontrol agents of powdery mildews are much more efficient when relative humidity can be maintained above 80%, a condition that is easily monitored under glasshouse conditions (15, 89). The biological vacuum in soil substrates can also favor the establishment of biocontrol agents, provided they are applied before pathogen introduction.

The logistics and economics of applying biocontrol agents in the greenhouse are more favorable than for many field applications. Greenhouse crops have a high economic value, and therefore can absorb higher cost inputs to control disease. The biocontrol agents can be directly applied to the growing mix, in the fertigation system, sprayed on the plants, or applied to high value hybrid seed. They can be applied multiply, which would be uneconomic in most lower value field crops. Because of the reduced area and high density of planting, less inoculum is needed than in treating a field.

Another reason why biocontrol has found a niche in the greenhouse market is because of the absence of registered fungicides. For example, until 1999, there were no fungicides registered in Canada for the control of *Pythium* in greenhouse vegetable crops. High registration and development costs and the lack of return on investment act as deterrents to chemical companies in registering products for the relatively small greenhouse market. Workers are at greater risk of fungicide exposure in the greenhouse because of the intensive nature of crop management. Most fungicides require a re-entry period before the workers can return to a treated crop and there is a harvest interval, a period of time between the last application and harvest. However, many greenhouse crops are continuously harvested and therefore cannot use most fungicides. Breakdown, weathering, and wash-off of chemicals on the leaves or in substrates are all lower in greenhouses than in the field, so fungicides may have a longer residual activity. Finally, the development of fungicide resistance in the pathogen may be exacerbated by the intensive use and limited choice of fungicides in the greenhouse.

There is increasing societal concerns about the environmental and health effects of fungicides. A pesticide-free vegetable or floral product may give greenhouse growers a market advantage, especially during the summer when they are competing with lower-cost field-grown produce. Technology-based management is more prevalent in greenhouse crops, and growers may be more likely to adopt biological control than growers in less managed field crops.

However, greenhouse systems have some constraints that may limit the use of biocontrol. Because of the high value of the crop and emphasis on quality in floriculture, vegetable crops, and ornamentals, there is less acceptance of damage and thresholds for disease are very low. If biocontrol agents cannot perform with the consistency and efficacy of fungicides in these crops, they may not be adopted.

PRODUCTS FOR BIOLOGICAL CONTROL OF SOILBORNE PATHOGENS

Presently, there are over 80 products for biocontrol of pathogens worldwide (159), a significant improvement over the past ten years. Most of these products are formulations either of the fungi *Gliocladium-Trichoderma* or the bacteria *Pseudomonas* and *Bacillus*. However, not all of these products are registered as biocontrol agents, but are marketed as plant growth promoters, plant strengtheners, or soil conditioners. These designations have enabled the products to get to the marketplace with less stringent toxicology or efficacy testing than would be required for plant protectants. However, some countries still have a regulatory framework in place for "plant strengthener." For example, in Germany in 1998, 208 plant strengtheners (Pflanzenstärkungsmittel) were registered with der Biologischen Bundesanstalt, up from 25 in 1991. These products include inorganic compounds such as SiO_2, $NaHCO_3$, organic constituents such as compost, homeopathic compounds, and some containing microorganisms such as *Trichoderma harzianum*, *Bacillus subtilis*, *Pseudomonas*, and *Pythium oligandrum*.

For many of these products, there is a dearth of published scientific data. In the following section, we highlight some of the most important products in use in greenhouses, ones with years of scientific testing and data. Much of this information was derived from a questionnaire and personal communication sent to greenhouse disease pathologists in July 2000. Information was also obtained from the USDA-ARS website http://www.barc.usda.gov/psi/bpdl/bpdlprod/bioprod.html, collated by D. Fravel and Whipps & Davies (159).

Coniothyrium minitans

This mycoparasite destroys sclerotia of *Sclerotinia sclerotiorum* and *S. minor*. The only biological control agent (BCA) registered as a biopesticide in Germany, *Coniothyrium minitans* is used for the control of Sclerotinia wilt of lettuce in greenhouse and rape in the field. It is marketed as a wettable granule, called Contans® WG, by Prophyta Biologischer Pflanzenschutz GmbH, Malchow, Germany. This fungus has been extensively tested on glasshouse lettuce in England. The fungus was produced on a number of solid substrates such as barley, bran-vermiculite, millet, oats, peat-bran, and wheat and tested in a sequence of glasshouse lettuce crops. *C. minitans* reduced the sclerotial populations at the soil surface, survived at least 39 weeks at a density of 10^4–10^5 CFU/g, and spread to infect sclerotia in control plots (116). In another series of glasshouse trials on lettuce, *C. minitans* outperformed *Gliocladium virens*, infecting over 80% of the sclerotia (29). *C. minitans* is also registered in Switzerland and marketed in Hungary under the name of Koni®. Sales reach 30 metric tons per year (P. Leuth, Prophyta, personal communication), but registration is still pending in the United States and most European countries.

Gliocladium virens (=*Trichoderma virens*)

This product was developed by the Biocontrol of Plant Disease Laboratory of the USDA-ARS in Beltsville, MD. Isolated in the late 1980s from a soil in Maryland, the fungus is widely distributed in soil worldwide. It was developed for control of *Pythium ultimum* and *Rhizoctonia solani* in soilless mixes (107). *G. virens* isolate GL-21 was first formulated as an alginate prill (GlioGard®) by W. R. Grace Co. A granular fluid (SoilGard®) was later developed for greenhouse applications, and is presently marketed by Thermo Triology Corp., Columbia, MD. The fungus produces two fungitoxic compounds, glioviren and gliotoxin compounds (85, 108). The lessons learned about commercialization and regulation of this product have been recounted in numerous articles (109, 118, 160).

Trichoderma harzianum Strain T-22

This strain was produced in the late 1980s by protoplast fusion between T-95, a rhizosphere-competent strain of *T. harzianum* originally isolated from a Columbian soil (2), and *T. harzianum* T-12 from New York soil (75, 146). This strain has been extensively tested in greenhouse and field trials (80). This rhizosphere-competent strain can colonize all parts of the root system and persists for a long period when applied as a seed treatment, greenhouse soil drench or granules, or in-furrow drench or granules. T-22 is marketed by Bioworks, Geneva, NY, as a granular formulation (RootShield®) or a water-suspendable drench containing conidia (PlantShield®). The product has been shown to reduce Fusarium crown and root rot of tomatoes grown in potting mix containing T-22 and transplanted into the field (42, 120). In greenhouse trials, T-22 controlled *R. solani* in poinsettia, geraniums, and *Catharanthus*, and *Pythium* on geraniums, impatiens, and petunias. The control it provides is equal to that by fungicides (80), with which it is mostly compatible, but it must be applied as a preventative before disease occurs. *T. harzianum* has multiple mechanisms of action, including mycoparasitism via production of chitinases, ß 1-3 glucanases and ß 1-4 glucanases (106), antibiotics (142), competition (50), solubilization of inorganic plant nutrients (5), induced resistance (10), and inactivation of the pathogen's enzymes involved in the infection process (50, 52). Retail sales in 1999 exceeded $3 million, making it one of the first biocontrol agents in North America to achieve this level of success. Other strains of *T. harzianum* are also marketed in a number of products, including T-35 or Trichodex® from Israel (see section on foliar diseases), Binab T® from Sweden, and Supresivit® from the Czech Republic.

Streptomyces griseoviridis Strain K61

This product is marketed under the name Mycostop® by Kemira Agro Oy, Helsinki, Finland in both Europe and the United States, where it received EPA registration in 1994. It was originally isolated from sphagnum peat and was tested as a biocontrol agent against Fusarium wilt of carnations in commercial greenhouses, where

it reduced the spread of disease and increased yield (102). However, in other published tests, it was not effective in controlling Fusarium wilt of basil (119), Fusarium wilt of carnations (138) or Fusarium root rot of Douglas-fir seedlings (47). Mycostop, with a number of other products, was tested for control of *P. aphanidermatum* in a series of replicated trials in rockwool at the nursery and production stages at the Research Station for Floriculture and Glasshouse Vegetables in Naaldwijk, The Netherlands. Mycostop significantly reduced *P. aphanidermatum* in two of four nursery trials and in one of three production trials (134).

Gliocladium catenulatum Strain J1446

Originally isolated from a Finnish field soil, this isolate is the active ingredient in Primastop®, a product marketed by Kemira Agro Oy, Finland. Primastop® received EPA registration in July 1998 for 55 different crops, but only for greenhouse and indoor use. Target pests include damping-off, seed rot, root rot, and wilt pathogens. It is sold as a wettable powder that can be applied to the soil, roots, or foliage. In glasshouse trials with ornamental bedding plants, application by incorporation into the growing mix or drench reduced damping-off caused by *Pythium* and *Rhizoctonia*. In some cases, *G. catenulatum* was as effective as the fungicides propamocarb or tolclofos (115).

Nonpathogenic *Fusarium oxysporum* Strain Fo47

Originally isolated from a Fusarium suppressive soil in France, this isolate has been investigated for over 25 years by the laboratory of Claude Alabouvette, INRA, Dijon (see 3 for review). It is effective against Fusarium wilt diseases on carnation (133), tomato (64), cyclamen (C. Alabouvette, personal communication), and Fusarium crown and root rot on tomato (103). Mechanisms of action include competition for carbon (104), direct competition with pathogenic strains (56), and induction of host defenses (46, 63). It is marketed by Natural Plant Products, Nogueres, France, as a liquid formulation for soilless culture such as tomato in rockwool, and as a clay formulation for mixing in cyclamen potting mixes. It is also marketed in Germany for use on cyclamens by Klassman-Deilmann GmbH, Westerholfsfelde. Registration by the European Union is currently pending.

Bacillus subtilis var. *amyloliquefaciens* FZB 24

Next to *Pseudomonas*, probably the most widely researched and commercialized biocontrol bacteria have been species in the endospore-forming genus *Bacillus*. For example, *Bacillus subtilis* has been marketed, under the label Kodiak®, for seed and furrow applications on cotton and peanuts by Gustafson, Inc. Over 2 million ha were inoculated in 1994 (9). In 2001 Gustafson will also be releasing a mixture of *Bacillus amyloliquefaciens* and *Bacillus subtilis*, BioYield®, for the greenhouse market (J. Kloepper, personal communication). *Bacillus* spp. have been extensively applied in China as part of a complex so-called "yield increasing

bacteria" (152). In Germany, research from the laboratory of H. Bochow at Humboldt University, Berlin, has focused on the development of *Bacillus* for greenhouse use. One strain, FZB 24, has been developed by FZB Biotechnik GmbH and is marketed in Germany by Bayer as a plant-strengthening agent. It received an EPA registration in January 2000 to control various fungal diseases in non-food crops, but only in greenhouses and for indoor plants. It is marketed in the United States by Taensa Inc., of Fairfield, CT. Of the several strains of *B. subtilis* tested on cucumber and tomato against *P. aphanidermatum* and *Phytophthora nicotianae* in a series of greenhouse trials (71), two strains, FZB 13 and 44, partially compensated for damage caused by these pathogens. FZB 24, the commercialized strain, was not the best in these experiments, but this strain did not show any antifungal activity in vitro against *P. aphanidermatum* (100). However, it did have a growth promotion effect on corn and radish. Another strain, FZB-G, also promoted growth on tomatoes (74). Other strains of *B. subtilis* in this group (FZB C and G) produced peptide antibiotics active against *F. oxysporum* f. sp. *radicis-lycopersici* (45).

PRODUCTS FOR BIOLOGICAL CONTROL OF FOLIAR PATHOGENS

Ampelomyces quisqualis

The fungus *Ampelomyces quisqualis* is the first organism reported to be a hyperparasite of powdery mildews (161) and antagonizes species in the orders Erysiphales, Mucorales, and Perisporiales (57, 91, 148, 150).

In studies of its mode of action, *A. quisqualis* was shown to colonize hyphae and conidiophores of several species of powdery mildew fungi and formed pycnidia within the conidiophores of its hosts (81). Falk et al (57) and Kiss (91) further showed that *A. quisqualis* parasitized cleistothecia of *U. necator* and *B. graminis*, respectively, albeit at low rate of colonization.

Attempts have been made to exploit *A. quisqualis* as a biocontrol agent, because it can be easily found associated with powdery mildew colonies. Under greenhouse or field conditions, this antagonist was reported to be effective only under very high humidity. Jarvis & Slingby (89) therefore proposed the use of water sprays in combination with *A. quisqualis* to alleviate its need for high humidity. However, water sprays alone reduced the severity of *S. fuliginea* under greenhouse conditions, providing moderately good control. In the early 1980s, *A. quisqualis* was shown to be tolerant to some fungicides and could therefore be used in an integrated approach against *S. fuliginea* on greenhouse cucumber, when relative humidity remained high (149). A mixture of *A. quisqualis* with 2% paraffin oil was proposed to control cucumber powdery mildew in the field (130).

The use of *A. quisqualis* to control of powdery mildew has been studied (131, 151). The spores of the fungus were shown to germinate into the hyphae of powdery mildew leading to the collapse of the pathogen (131, 151). A formulated product was registered in the late 1980s in Australia, but the high humidity requirements

of *A. quisqualis* have hampered its efficacy. Recently, Ecogen, Inc. developed a formulation (AQ-10®), sold as water-dispersible granules, based on a new strain that reportedly tolerates lower humidities. Good control of powdery mildew of cucumber with AQ-10® was claimed when disease pressure was moderate (60). Early attempts to introduce AQ-10® into the grape industry failed because of poor formulation and efficacy (83), but now its use is recommended as part of an integrated program and as a preventative measure only. AQ-10® is registered for a number of crops including grapes, vegetables, and other fruits, recommended in conjunction with a wetting/dispersing agent, AddQ, to overcome humidity requirements of the fungus.

Trichoderma harzianum Strain T-39

Trichoderma harzianum strain T-39, developed at the Volcani Center in Israel and marketed as TRICHODEX, 20P by Makhteshim Ltd. (Be'er Sheva, Israel), is targeted at *Botrytis cinerea*. The reported mode of action of *T. harzianum* T39 is competition for nutrients and interference with the production of lytic enzymes by the pathogen (52); thus in addition to slowing the germination of the pathogen's conidia, T39 also prevents the penetration of the host tissue and the maceration process (164).

Although Trichodex was developed primarily for the grape market (48), its efficacy as a biocontrol agent on greenhouse crops has been tested intensively under commercial conditions (53, 54, 73, 112). Trichodex has effectively controlled *Botrytis* diseases in greenhouse crops in Israel (55) and other countries (51) where it is registered for agricultural use. It is the first such product to be introduced commercially to greenhouses.

As with most biocontrol agents, the efficacy of *T. harzianum* T-39 can be influenced by ambient environmental conditions. For this reason, the use of Trichodex in combination or alternating with chemicals has been investigated (50). Also examined was the integration of biological and chemical controls aided by the use of a forecaster to predict *Botrytis* outbreaks. This integrated system for disease management saved 60% of the chemical sprays, which were replaced by Trichodex.

Bacillus subtilis Strain QST713

Serenade®, produced by AgraQuest Inc. (Davis, CA), is the latest product based on strain QST713 of *B. subtilis*. It is currently available as a wettable powder, with a registration for an aqueous suspension formulation pending (Rhapsody®).

The product is advertised to have a spectrum of activity including over 40 plant diseases including common greenhouse diseases such as gray mold (*B. cinerea*), damping-off (*P. ultimum* and *R. solani*), and powdery mildews. The bacterium is presumed to work through a number of modes of action such as competition, parasitism, antibiosis, and induction of systemic acquired resistance (SAR).

Our attempts to obtain scientific data or papers supporting the properties and the efficacy of *B. subtilis* strain QST713 from the company were unsuccessful.

The product is marketed primarily as a tool to prevent resistance to chemicals and as an alternative to chemicals in areas where fungicide-resistant pathogens have developed.

Ulocladium atrum

No biological product based on this organism is currently available, but some European companies have expressed interest in light of promising results of biocontrol of necrotrophs obtained in different laboratories. For instance, applications of spore suspensions of *U. atrum* on greenhouse tomatoes consistently reduced infections by *B. cinerea* (62; D. Yoalem, personal communication). Similar results have been obtained on other greenhouse crops such as cyclamen, geranium, and roses (69, 96). The antagonism of *Ulocladium atrum* against *Botrytis* spp. appears to involve competition in necrotic tissues since no toxins or cell wall degrading enzymes were found (94). Interestingly, among the four antagonists tested, *U. atrum* colonized dead onion leaves the best and also had the strongest antagonism on leaves exposed to field conditions (98). This antagonist also showed a high ecological competence for the habitat of above-ground necrotic plant tissues: It germinated and colonized the substrate under various environmental conditions in the presence of *Botrytis* spp. and other naturally occurring saprophytic fungi (97). Microbial suppression of sporulation on necrotic tissues lower spore load in the crop, which will, in turn, slow progression of disease epidemics, as shown for *Botrytis* spp. in field grown onions (95).

The preceding example is very valuable in demonstrating how ecological processes regulate fungal populations. As suggested previously (24, 99), nutrient competition can be an excellent strategy for microbial suppression of unspecialized necrotrophs such as *Botrytis* spp. On the other hand, the research has somewhat undermined the value of antibiosis and overshadowed the importance of parasitism interfering with the saprophytic phase of a fungus. More recently, Kessel (90) used *U. atrum* against *B. cinerea* in cyclamen and found that the antagonist was as effective as commercial fungicides in suppressing the disease. This success was attributed to the ability of *U. atrum* to exclude *B. cinerea* from necrotic tissue that was a mutual substrate.

BIOLOGICAL CONTROL OF GREENHOUSE DISEASES IN CANADA: A DECADE OF RESEARCH

Biological Control of *Pythium* in Hydroponic Systems

Canada has a sizeable greenhouse industry compared to its relatively small population of 30 million. In 1998, there were 4100 greenhouses in Canada, with a total area of 2583 ha under glass or plastic (145). Greenhouse vegetables were valued at $285 million CN, with tomato the most important ($164 million), followed by cucumbers ($64 million), peppers ($34 million), and lettuce ($13 million). The value

of the greenhouse tomato and cucumber crop has increased by 310% and 116%, respectively, since 1990. Ontario and British Columbia are the largest producers of greenhouse vegetables.

In Canadian greenhouses, *Pythium* is one of the most important root and seedling pathogens, on both vegetables and horticultural crops. In British Columbia, *Pythium aphanidermatum* (Edson) Fitzpatrick, *P. irregulare*, and *Pythium* sp. group G were responsible for root disease and crown rot of greenhouse cucumbers (59). In Quebec, *P. aphanidermatum* and *P. ultimum* were the species most commonly isolated from greenhouse cucumbers (129). Most of the production is on soilless media or in hydroponic systems, such as rockwool, peatbags, sawdust, or nutrient film. Although these media are usually pathogen free, *Pythium* can be reintroduced from contaminated plants, soil on worker's shoes, fungus gnats, or irrigation water. Most *Pythium* spp. produce zoospores that are well adapted for rapid dispersal under aquatic conditions. Environmental restrictions on pollution of groundwater with nitrates have promoted wider use of closed recirculating systems, further exacerbating the problem of rapid dispersal. Although soilless media initially do not contain pathogens, the lack of competing microorganisms in the media make conditions more favorable for *Pythium* spp., which are pioneer colonizers and do not compete well saprophytically. Finally, the host plants are very susceptible, especially at the juvenile stages, and there is no resistance in vegetable cultivars.

How is *Pythium* controlled in the greenhouse? For ornamental plants the systemic fungicide metalaxyl is used as a soil drench, but for greenhouse vegetables, no fungicides were registered for the control of *Pythium* throughout most of the 1990s (126). Small market size and poor economic return for a fungicide manufacturer who went through the registration process were likely responsible. Within the past few years, however, propamocarb hydrochloride (Previcur N) received a minor use registration in Canada (PCP#26288). Cultural control methods based on sterilizing or disinfesting the recirculating hydroponic solution to prevent spread of pathogens were also introduced (117, 125). UV radiation (144), filtration (70), ozonation, and the use of surfactants (143) are among the method tested, although not widely used in the industry. Cultural techniques, such as sanitation, starting with disease-free transplants, and disinfestations of tools and hydroponic systems between crops can also be effective (86).

The lack of adequate control methods for *Pythium* in greenhouse vegetables impelled T. C. Paulitz and coworkers (McGill University, Québec) to initiate a program in 1990 to develop bacterial biocontrol agents against *Pythium*. Biocontrol agents could also be useful in greenhouse floriculture and horticultural crops, which constitute most of the $1.19 billion CN sales in 1998.

Six hundred and four isolates of bacteria were isolated from the rhizospheres of cucumber (*Cucumis sativus* cv. Corona) grown in 34 agriculture and forest soils collected in Quebec. The target fungus was *Pythium aphanidermatum*, the predominant species in Quebec greenhouses and causal agent of root rot and crown rot. Mature, infected plants stressed by fruit production often collapse rapidly.

Various screening techniques were used, but inhibition of zoospore germination and motility was emphasized rather than inhibition of mycelia (129). Strains selected from these inhibition assays were identified and tested on cucumber seedlings growing in nutrient solution in test tubes and mason jars.

Only 15% of the strains inhibited mycelial growth and only 12% inhibited zoospore germination. Of the 35 best strains selected from these screens, all reduced zoospore motility. Most of these strains were *P. fluorescens* subgroups C and E, *P. corrugata* or *Pseudomonas* spp. The 5 best strains in the *in planta* bioassay were also ranked among the top 5 of the total 604 in terms of reducing zoospore germination and motility, indicating the usefulness of these indicators. Three of the five strains also increased plant root growth in the absence of the pathogen, indicating plant growth-promotion (PGPR) activity. These five strains were further tested under simulated commercial conditions in a rockwool hydroponic system, with plants grown through the entire cropping cycle (137). In a spring crop, *P. corrugata* strain 13 and *P. fluorescens* strain 15 produced 88% more marketable fruit than the inoculated control ($0.10 > P > 0.05$). In a fall crop with severe disease pressure due to higher slab temperatures, marketable fruit production was significantly increased (by 600%) with these two strains. Strain 15 also increased fruit production in treatments not inoculated with the pathogen.

Strains 13 and 15 do not produce antifungal compounds detectable by dual culture petri plate techniques or inhibit mycelial growth, but they reduced zoospore germination and chemotaxis. Could they interfere with infection by competing for root exudates on the root surface, thus interfering with the attraction, encystment, and germination of zoospores on the root surface? To test this hypothesis, root exudates were collected from cucumber roots grown in the presence or absence of the five *Pseudomonas* strains. Exudates were filter-sterilized and tested in capillary tubes for chemotaxis. Four of the five strains significantly reduced the number of zoospores that encysted in the capillaries, and all reduced the distance that the zoospores swam in the tubes. The bacteria significantly affected the distribution of zoospores on the roots of cucumber, as viewed by epifluorescence and video microscopy (162). The distribution of zoospores on the root was highly aggregated on nonbacterial roots, but when roots were treated with bacteria, there were fewer of these favorable infection sites with a high density of encysted zoospores. This suggested that colonization of these sites with bacteria made them less attractive to swimming zoospores.

Another possibility for the reduction in disease without apparent antibiotic production was induced resistance, which is "the process of active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents (inducing agents)" (93). The role of induced resistance in biological control of greenhouse diseases has been reviewed (127). The first reports that plant growth-promoting rhizobacteria (PGPR) induced resistance appeared in 1991. Wei et al (158) demonstrated that when cucumber seeds were treated with certain PGPR strains, subsequent inoculation of leaves with *Colletotrichum orbiculare* resulted in less disease. Van Peer et al (157) induced resistance to Fusarium wilt of carnation

by treating roots with *Pseudomonas fluorescens* strain WCS417r. To demonstrate systemic induced resistance against root pathogens such as *Pythium aphanidermatum*, the pathogen and inducing agent must be spatially separated. This can be accomplished by splitting the roots into two separate pots and inoculating one side with the pathogen and the other side with the inducing agent. In split root experiments with strain 13 and 15, most of the bacterial treatments reduced disease severity and incidence, even though the bacteria did not come in contact with the pathogen (163). This was the first demonstration of induced resistance in the root system against a *Pythium* sp.

In 1994, a collaborative research project, SYNERGIE, was initiated to develop biocontrol agents for Canadian greenhouses, in collaboration with industry and university researchers. Paulitz's laboratory focused on bacterial biocontrol products active against root diseases that could be formulated into peat-based growing mixes. This research focused one of the top performing strains, *Pseudomonas fluorescens* 63-28, from a collection of 4000 strains isolated from all over Canada and tested for growth promotion on wheat and canola (92). Strain 63-28 was extensively tested in the greenhouse for control of root diseases (66, 82, 141). This strain has recently been sold to EcoSoil Inc., and an EPA registration was submitted in November 1998 for a product called AtEze, based on strain 63-28, that would be used as a soil drench for greenhouse vegetable crops to control *Pythium* and *Rhizoctonia*.

Since *Pseudomonas* and other gram-negative bacteria are sensitive to drying, a need existed for developing a formulation that would survive in peat for 6 months to 1 year. Strain 63-28 survived best in peat at 100–150% moisture (v/v), but poorly at 45% (the moisture at which peat is shipped) and 25% moisture. Even at the optimum moisture levels, populations declined below the critical threshold of 10^6/g after 1–2 months.

Among the strategies tested to enhance the survival of the bacteria was the addition of carbon sources to the peat at the time of planting that would be selectively utilized by the biocontrol agent, but not the pathogen. Even if the populations of bacteria declined below an active threshold, populations could recover by selective use of the carbon source. Various exotic carbon sources were tested, including nonionic surfactants, but strain 63-28 could not utilize them. Strain 63-28 could utilize mannitol, sorbitol, and trehalose that *Pythium* could not utilize. However, these carbon sources did not enhance biocontrol in pot experiments. Adjuvants were also added to the culture media or peat to enhance survival, including glycerol, paraffin oil, starch, and proline, but none enhanced the survival. Bacteria were adsorbed onto different substrates that were added to peat, including vermiculite, kaolin, talc, and xantham gum. Strain 63-28 survived best when added to vermiculite that was then air dried, but when bacteria-treated vermiculite was added back to peat, survival was not enhanced compared to peat alone. Osmotically stressing or conditioning the bacteria during culturing with polyethylene glycol did not enhance survival either. Freeze drying the bacteria in trehalose or sucrose resulted in the best survival, with high bacterial densities maintained for 6 months to 1 year in dry

peat at room temperature. However, if the freeze-dried bacteria were added to peat at 45% moisture, survival would decline over a 3-month period. Forty-five percent moisture is enough to increase the metabolic activity of the bacteria, leading to decline of populations.

Research in this project also focused on the mechanisms of action of strain 63-28, which produced an antifungal compound on agar culture that inhibited *Pythium ultimum*, *Rhizoctonia solani*, and *Phytophthora cryptogea*. Using thin-layer chromatography and *Cladosporium herbarum* as an indicator (67), three active fractions were identified in chloroform extracts of culture filtrates. These fractions were further separated by high performance liquid chromatograph (HPLC) and compared to standards of known *Pseudomonas* antibiotics such as 2,4 diacetylphloroglucinol, pyrrolnitrin, and pyoluteorin, but they did not match. Two of the compounds were purified and identified with NMR and mass spectroscopy as novel butyrolactone or furanone compounds, never reported in the chemical literature. These compounds were named (Z)-4-hydroxy-4-methyl-2-(1-hexenyl)-2 butenolide and (Z)-4-hydroxymethyl-2-(1-hexenyl)-2 butenolide. Modern furanone nomenclature names them as 3-(1-hexenyl)-5-hydroxy-5-methyl-2,5(H) furanone and 3-(1-hexenyl)-5-hydroxymethyl-2,5(H) furanone. They resemble homoserine lactones used by gram-negative bacteria as quorum sensors (132). They also closely resemble autoinducers produced by *Streptomyces* spp. (84). Another more reduced form of the compound, 3-(1-hexenyl)-5-methyl-2,5(H) furanone, has been identified (128). Antimicrobial 2(5H) furanone compounds are also produced by *Trichoderma* spp. (123), actinomycetes (28), and higher plants (105, 113).

Does strain 63-28, like strain 13, induce resistance in cucumber roots? When one side of a split root system was treated with strain 13 or 63-28, the progress of *P. aphanidermatum* up the opposite side was reduced, so that arrival to the crown was delayed by 3 to 6 days (32). This corroborates the results of Zhou & Paulitz (163), except that the effects were shown to be systemic in the root system. The induction of defense enzymes in the root system of cucumber was also examined. Both 63-28 and strain 13 stimulated higher levels of peroxidase, polyphenoloxidase (PPO) and phenylalanine ammonia lyase (PAL) in cucumber roots without pathogen challenge (34). Roots subsequently challenged with *P. aphanidermatum* seven days after bacterization had even higher levels, and pre-bacterization had no significant influence on the pathogen-induced peak of enzyme production. This same increase of enzyme levels was seen on both sides of a split root, showing that the stimulation of enzyme activity was systemic. Recently, Ongena et al (121, 122) showed that two strains of fluorescent *Pseudomonas* induced the formation of glycosilated conjugated phenolics in cucumber roots and leaves. These aglycones become fungitoxic when acid hydrolyzed. The compounds in the root were different than the recently identified cucumber phytoalexin, p-coumaric methyl ester (41).

What is the role of salicylic acid (SA) in induced systemic resistance (ISR) triggered by PGPR? Its importance in SAR has been demonstrated (147). Both

strains 13 and 63-28 produce SA in culture and higher levels of endogenous SA are induced in cucumber roots 1 to 2 days after treatment with the bacteria (33). This effect is also systemic, based on experiments with a split root system. However, unlike in other systems, exogenous application of SA to the root or injections into the stem did not induce resistance.

What effect does 63-28 have on the ultrastructure of the host and pathogen in planta on the roots? Benhamou et al (20) treated pea roots in culture with 63-28 and *Pythium ultimum*. The bacterium colonized the root surface, epidermis, and intercellular spaces of the cortex. On the surface of bacterized roots, *P. ultimum* hyphae were damaged and collapsed, as viewed by scanning electron microscopy. Hyphae within the cortex showed cytoplasmic disorganization, plasmalemma retraction, organelle damage, and even loss of cytoplasm. However, the cell walls of *P. ultimum* were intact and the cellulose in the wall was not degraded, as shown by labeling with gold-complexed ß 1-4 glucanase. These results were suggestive of an antimicrobial compound produced by the bacterium. In another study with *Fusarium oxysporum* f. sp. *pisi* on peas, hyphae of the pathogen on the surface of bacterized roots were not damaged (19), but were restricted to the root epidermis and outer root cortex. Callose-rich wall appositions and papillae were formed on the inner cell walls of the host in bacterized roots at the site of fungal contact. Aggregated deposits were formed around hyphae in the intercellular spaces, and phenolic compounds occurred in the deposits, based on labeling with gold-complexed laccase. These host responses were not seen in treatments with bacteria only, but the bacteria appeared to induce defenses in the presence of the *Fusarium*. Host cell wall damage in advance of invading hyphae was seen in non-bacterized plants, but not bacterized plants. These results suggest that antibiosis may predominate in *P. ultimum-Pseudomonas aureofaciens* 63-28 interaction on pea roots, but induced host resistance in bacterized roots was responsible for restricting *F. oxysporum* f. sp. *pisi*. A similar induced host resistance was seen in tomato roots treated with 63-28 and *F. oxysporum* f. sp. *radicis-lycopersici* (110).

Biological Control of Powdery Mildews

Powdery mildew fungi are ubiquitous phyllosphere pathogens of numerous field and greenhouse crops. Despite extensive study of their epidemiology and pathogenesis, the diseases they cause remain among the most important problems facing plant pathology worldwide. In greenhouses, powdery mildew diseases are particularly aggressive because constant favorable environmental conditions accelerate their development (13). They attack most plant species and are prominent on the three most important greenhouse crops in Canada: roses, cucumber, and tomato (16, 17). Taken together, these three crops account for more than 50% of the total value of greenhouse sales (145). Although it is difficult to estimate the exact value of losses attributed to powdery mildews, the cost for their control can reach \$6,000 CN ha^{-1} per year. *Pseudozyma flocculosa* (Traquair, L. A. Shaw & Jarvis) Boekhout & Traquairis is the most recently identified and probably the most

efficient natural antagonist of powdery mildew investigated to date. It was discovered along with another closely related species *P. rugulosa* (Traquair, L. A. Shaw & Jarvis) Boekhout & Traquair (154). Traquair et al (154) described both species as yeast-like fungi in the Endomycetaceae, *Stephanoascus flocculosus* Traquair, Shaw & Jarvis (anamorph: *Sporothrix flocculosa* Traquair, Shaw & Jarvis) and *S. rugulosus* Traquair, Shaw & Jarvis (anamorph: *S. rugulosa* Traquair, Shaw & Jarvis). However, they were later redefined as basidiomycetous yeasts related to anamorphs of Ustilaginales belonging to the genus *Pseudozyma* Bandoni emend. Boekhout (25).

Jarvis et al (87) were the first to report that both fungi were powerful antagonists of cucumber powdery mildew, *S. fuliginea. P. flocculosa* was more active than *P. rugulosa* under different environmental conditions. Subsequently, the same two antagonists were shown to be equally effective against *S. pannosa* var. *rosae* and *Erysiphe graminis* f.sp. *tritici*, responsible for rose and wheat powdery mildew, respectively (76, 77). P. *rugulosa* and *T. washingtonensis* have narrower temperature and humidity requirements than *P. flocculosa*.

These results have prompted commercial interest in the development of *P. flocculosa* as a biofungicide. Plant Products Co. Ltd. (Brampton, Canada) has acquired exclusive rights and has underwritten research toward commercialization of a product, Sporodex® (based on conidia of *P. flocculosa*). All corporate investments have been matched by both the provincial (Synergie Program) and the federal (NSERC) governments. Over the past few years, *P. flocculosa* has completed the scientific, legal, and administrative steps necessary for registration of a microorganism. Our laboratory has thereby acquired firsthand experience with the challenges inherent in this process.

How does *P. flocculosa* work? A critical step in biological control studies is the understanding of the properties of the antagonistic agent. Cytological and microscopic studies indicated that *P. flocculosa* does not penetrate its host but rather induces a rapid plasmolysis of powdery mildew cells (78). These results suggested that the antagonist acts by antibiosis rather than by parasitism. Furthermore, extracted culture filtrates of the fungus reproduced the same cell reactions on powdery mildew fungi as *P. flocculosa* (79).

Culture filtrates contained at least four compounds with antifungal activity, three of them being closely related fatty acids (21, 37). Avis et al (8) synthesized two of those acids and demonstrated they duplicated the antagonistic activity of *P. flocculosa*. These compounds act by interfering with membrane fluidity and, as a result, membrane composition should determine the level of specificity. Interestingly, phyllosphere fungi, which are likely to come into contact with *S. flocculosa*, had greater susceptibility to the antagonist than rhizosphere fungi (14). This specificity appears to be linked to the sterol composition in fungal membranes (22), which would explain why *P. flocculosa*, having a high membrane sterol content, is rather insensitive to its own antibiotics.

Can powdery mildew fungi develop resistance to the antibiotics produced by *P. flocculosa*? This question is invariably raised in regard to antibiosis in

biocontrol. A model of activity of the antibiotics in the membranes has recently been proposed (7), based on molecular analyses of membranes from resistant and susceptible fungi. According to this model, a sensitive fungus would have to undergo major structural changes in its membranes to develop resistance, which would, in turn, adversely affect its fitness to compete with its natural community. Despite repeated exposures to the synthesized antibiotics, it has not been possible to date to obtain a resistant strain of *S. fuliginea*. Development of resistance in the field is therefore unlikely because the antibiotics degrade very rapidly in nature.

On the other hand, mutants of *P. flocculosa* deficient in antibiotic production were obtained through plasmid insertion into protoplasts (35, 36). Bioassays have confirmed that these mutants lost both the ability to produce the antibiotics and antagonize powdery mildew fungi.

Can *P. flocculosa* control powdery mildew in the greenhouse? When tested under commercial conditions under a restrictive research permit, fresh spore preparations of *P. flocculosa* controlled rose powdery mildew equal to the commonly used fungicides dodemorph-acetate (Meltatox®) and microfine sulfur (18). In addition, for some cultivars, the biological treatment improved flower quality by eliminating the stress (phytotoxicity) caused by fungicides.

Subsequently, *P. flocculosa* conidia were formulated as a wettable powder (Sporodex®) for use against powdery mildew on greenhouse crops. In two large-scale independent trials, Sporodex® achieved the best level of powdery mildew control on long English cucumber when compared to AQ-10® and fresh preparations of *V. lecanii* (44). However, the formulation left residues on leaves, was hard to dissolve, and was of inconsistent quality.

An improved formulation leaving no residues and dissolving readily in water was developed and tested under commercial conditions in the Netherlands, Canada, and Colombia. In the Netherlands, treatment of a semi-tolerant, long English cucumber cultivar with Sporodex® allowed the crop to be grown pesticide-free for a complete season (16 weeks). In Canada, Sporodex® was compared to myclobutanil in a commercial greenhouse (Agriculture and Agrifoods Canada, Harrow, Ontario, R. Cerkauskas, personal communication). Although absolute control of powdery mildew with Sporodex® was not as good as with the fungicide, it improved cucumber yield by up to 15%. The efficacy of Sporodex® was evaluated against rose powdery mildew in standard commercial greenhouses in Colombia (30). In two separate trials, the product was as effective as fungicide treatments and improved flower quality. Finally, the efficacy of Sporodex® was evaluated in commercial greenhouses in the Netherlands in the spring of 2000 (R. R. Bélanger, unpublished results). Rose growers were satisfied with the trials where Sporodex® controlled powdery mildew as well as chemicals and induced yield of better quality flowers.

Will Sporodex® become available on the commercial market? In the spring 2000, Plant Products Co. Ltd., filed a submission for registration of Sporodex® to be reviewed jointly by the Environmental Protection Agency (EPA) in the United

States and the Pest Management Regulatory Agency (PMRA) in Canada. If successful, registration would be granted simultaneously in both countries. This new initiative by the EPA and PMRA should facilitate and accelerate registration of biofungicides for the North American market.

FUTURE PERSPECTIVES

What is the future for the use of biological agents against diseases in greenhouses? Trends associated with the biological control of insects in greenhouses may be relevant. The use of both conventional pesticides and biological control agents in Great Britain has been assessed since 1968 by the Ministry of Agriculture, Fisheries and Food (68). The use of biological agents on all crops in the United Kingdom increased from 17 ha treated in 1968 to 3,813 in 1981 to 30,889 in 1995—a tenfold increase in 14 years, primarily in insect biocontrol agents because no disease biocontrol agents are registered in the United Kingdom at present. However, most of this increase has been in protected greenhouse crops: 13,960 ha in edible and 7074 ha in ornamental greenhouse crops in 1995. Thus although protected crops represent a small fraction of the total area, they account for two thirds of all biologicals. At the same time, the use of insecticides in greenhouses has declined from 4866 treated ha in 1981 to 2292 ha in 1995. For tomatoes, the most important edible greenhouse crop in the UK, the area treated with insecticides declined from 2497 ha in 1976 to 324 ha in 1995, whereas the area treated with biologicals increased from 406 ha in 1976 to 10,350 ha in 1995, a 23-fold increase. Some of this increase may reflect multiple applications of biologicals, but the actual tomato area treated with biologicals has also increased from 20% to 77% in 1995. At the same time, the total production area of greenhouse tomatoes has decreased by 83% from 1986 to 1995.

What lessons can be learned? A combination of economic, political, and environmental factors has probably contributed to the transition to biologicals: loss of insecticide registrations, insect resistance, and concern for worker safety. Competition from field-grown produce from other countries has diminished the tomato greenhouse industry, but growers now use more intensive and integrated crop management systems than their predecessors did to remain competitive. The exponential increase in the use of biological control of insects is part of this crop management system, but the number of products available to the growers has also increased. In 1985, only four products were available—one bacterium, one fungus, and two predators, whereas ten years later, there were 16 biocontrol species. Greater availability may in turn have fostered the increase in biocontrol use: Did more companies in the marketplace create more demand for biologicals, or did the demand create more companies? Does this demand exist for biocontrol agents for greenhouse diseases only, and why are there not more biological disease control products on the market? We need to look at the present registration system and the greenhouse markets, and pose some basic ecological questions.

Registration, Commercialization, and the Greenhouse Market

Although biocontrol agents are arguably more effective in protected crops, their commercialization can be jeopardized if the market seems too small to justify the expenses inherent in their registration (61). This paradox is largely responsible for the limited number of biofungicides currently available. Meanwhile, there is a flourishing industry of insect biological control agents that dominates the greenhouse market in Europe. Why are microbial agents for disease control lagging behind? Undoubtly, the registration process is one of the main reasons. Registration of insect predators is not subject to the long and costly steps of human and environmental toxicology, formulations, quality assurance of the formulated product, genetic stability, etc. required for microbial pest control agents. The initial investment required to bring beneficial insects to the market is more moderate and therefore not so discouraging to small, specialized companies. There are currently 26 natural enemy producers in Europe, with approximately 65 worldwide (155), and the number of insect biological control agents has grown from 2 in 1970 to almost 100 today (156). These figures attest to the viability of the greenhouse market with regard to biofungicides. Although multinational agrochemical companies have shied away from both insect and microbial pest control agents, smaller companies that have already profited from establishing their niche in the greenhouse market can recognize the potential of the largely unexploited field of disease biocontrol. In fact, promotion of disease biocontrol in the greenhouse environment is attractive from several standpoints. First, as discussed earlier and in several reviews (15, 49, 160), is increased and reproducible efficacy of bicontrol agents under controlled environments. The success enjoyed by insect biocontrol in greenhouses demonstrates convincingly that biological control of pests is easier to achieve when growers can manipulate environmental conditions to best promote the development of the biocontrol agents. Therefore, since the biofungicides industry is still in its infancy, it is important to disseminate positive information to gain credibility among growers.

The second advantage of biologicals over chemicals lies in the registration process. Registration of a biofungicide is notoriously frustrating and legally cumbersome as attested by numerous recent reviews (40, 80, 83). However, regulators in the United States, Canada, and Europe tend to be favorably disposed toward biological pesticides and incentives to encourage companies to register such products. For example, the cost of registration of biofungicides is lower than for chemical pesticides, or can be completely waived. Biological products automatically enter a fast-track review process that speeds up registration. Canada and the United States have recently implemented a joint review process of biological products whereby a registration dossier receives speedier analysis, and approval, when granted, allows commercialization in both countries simultaneously. Details of this joint review process can be found at http://www.hc-sc.gc.ca/pmra-arla/english/ContentPages/InternAcct/Twg_NC.htm and http://www.epa.gov/pesticides/biopesticides/nafta/nafta_joint_review.htm. This initiative will be of particular benefit to North American growers in affording access to a wider

variety of biological products. Furthermore, potential registrants will enjoy a simplified and unified process. A similar protocol to harmonize the registration process of biological products across Europe is currently under consideration.

Microbial pest control agents specifically for the greenhouse market also face lower requirements for environmental toxicology in registration. Indeed, the application of microbial pest control agents within the confined greenhouse environment minimizes exposure to non-target organisms. More operationally advanced commercial greenhouses contain and recycle irrigation waters and reduce environmental release of treated waste materials. Accordingly, since adverse risk to birds, fish, and arthropods is attenuated in these advanced greenhouses, waivers or partial waivers for environmental toxicology testing can be justified, in turn, significantly reducing the registration costs. Under this premise, a company can envision a stepwise strategy whereby a microbial pest control agent is first registered exclusively for greenhouse crops. This approach would be financially expedient because it would minimize start-up costs and expedite the registration process; speedier entry into the marketplace would enhance the product's chance of success; and as the conditions that optimize its efficacy are better understood, the product can be adjusted. Subsequently, the manufacturer can extend the application of the product to field crops by meeting the appropriate environmental standards.

Biocontrol Agents and Ecology

The use of living organisms to combat other living organisms presupposes a thorough knowledge of their ecology. Accordingly, except in the case of induced resistance, a biocontrol agent must occupy an ecological niche similar to that of the plant pathogen and its mode of action (competition, parasitism, antibiosis, induction of SAR) must interfere both spatially and temporally with precise steps in the development of the pathogen. Several excellent reviews have been published on the ecological principles inherent in the success of biological control (12, 23, 24, 65). Nevertheless, the literature is filled with reports of potential antagonists against plant diseases based on in vitro studies, but very few of the hundreds of reported biocontrol agents have lived up to their promises beyond the laboratory. Although ecological factors alone are not responsible, it may be instructive to draw a parallel with insect biocontrol where most commercial successes involve a predator specifically adapted to parasitize a specific insect.

In view of the high costs and the protracted nature of biopesticide registration, and in order to ensure a speedy return on investment, manufacturers will try to (*a*) develop or claim products with a large spectrum of activity and (*b*) market their product for diseases of field crops, which represent a more lucrative market than that for greenhouse crops. Harman (80), for example, suggested recently that only widely adapted and broadly active biocontrol agents can be economically feasible. On the basis of ecological considerations, we depart somewhat from such a strategy. Some biocontrol agents have indeed displayed antagonistic effects against a large number of plant pathogens in *in vitro* or small-scale experiments (1), but evidence that these agents can consistently achieve an acceptable level of wide-spectrum

disease control under commercial situations is not compelling. Biological control can be somewhat successful, in the view of some, when the biocontrol agent possess, in parallel with their antagonistic properties, other attributes that make them ecologically fit to reduce a specific pathogen population (38). Several reviews have described the ecological attributes a biocontrol agent should have in relation to the intrinsic properties of a plant pathogen (6, 23). No single agents can be simultaneously and equally rhizosphere and phyllosphere competent and adapted to control necrotrophs as well as biotrophs. Thus, would growers be better served with a number of products that are each specifically adapted to offer maximum control of a targeted disease, rather than a single (or limited number of) product(s) that offers marginal or inconsistent control of many diseases? This dilemma will eventually be resolved by market forces, but given the limited record of disease control with natural enemies, the latter approach may undermine the credibility of biological control if too many unfulfilled claims arise.

Since biocontrol agents develop optimally within a defined spectrum of environmental conditions, field applications of these agents can give disappointing results if temperature and relative humidity conditions fall outside this spectrum. For instance, high relative humidity requirements of known biocontrol agents of powdery mildews are not as reproducible in the field as in the greenhouse (12), thus reinforcing the concept that biological control in greenhouse systems is more effective than in the field. The insect biocontrol industry is a valid testimony to this reality. However, ecological considerations may be incompatible with market considerations. In our opinion, the best marketing strategy is to promote a product against the disease(s) it best controls consistently for use under the conditions that will guarantee its efficacy. For this reason, the greenhouse market provides the best niche for disease biocontrol. Greenhouse growers have been using insect biocontrol with success for many years and they are extremely receptive to pesticide-free approaches. They understand and can control the conditions that favor biocontrol products. Moreover, the higher costs of biological products are not a deterrent because pest protection represents only about 1% of the overall cost of production of their high value crops (124).

Future Research Priorities

Given the guarded but hopeful prognosis for the future of biological control in the greenhouse, how can the technology be moved from the laboratory to the commercial grower? More scientific efficacy trials with proper replication and statistical analysis are needed under commercial or near-commercial conditions. Ready access to reliable data for growers and extension personnel will be more persuasive than reliance on company advertisements. These data should be accessible in the public domain. Why the dearth of accessible scientific data? Many of the data are probably proprietary, found only in company reports or in registration submissions. Public institutions such as universities and government research labs have moved away from routine testing of products. Results are published in research station bulletins that are not accessible through citation services. Many trials may show

lack of efficacy and are not published. Efficacy trials are generally not reported in the scientific literature because of the perceived lack of innovation. Greenhouse trials should include integration of biological agents for insect and disease control, given the predominance of insect biocontrol. Biocontrol registrations now request data on the interaction of pesticides with beneficial insects. Growers need to know whether new products are compatible with their current pest management strategies.

More studies are needed on the epidemiology and ecology of pathogens in the greenhouse, which may be different from the field (43). This information is especially lacking for soilborne pathogens such as *Pythium* in hydroponic systems. How is the pathogen introduced and how does it spread? What is the relationship between population density and damage? How can the environment be manipulated to favor biological control (one of the main advantages of growing in covered structures)? Low-cost methods are needed for rapid detection of pathogens in the greenhouse, while still below the damage threshold to allow a better chance of control with slower-acting biocontrol agents.

Finally, the challenge of production and formulation of biocontrol agents remains (26, 61, 139), with each organism bringing its own set of problems. Effective production and formulation protocols are usually proprietary, involving substantial investment to develop economic production and a formulation with adequate shelf life, stability, and titer. Even when all these conditions are met, the formulated product may be incompatible with the growers' practice. The mycoherbicide 'Collego' produced by Ecogen, Inc. in the mid-1980s, for example, was sold as a dry powder formulation that was stable for two years. However, actual application of the product was so laborious for the grower (135) that sales plummeted and Ecogen, Inc. eventually stopped manufacture (40). Formulation difficulties may also explain the lack of *Pseudomonas* products, either for soilborne pathogens or foliar pathogens, despite extensive basic research on this group. Based on our experience, we estimate that production and formulation represent at least 50% of the costs of research and commercialization of a biofungicide. Plant pathologists are generally ill-prepared to shoulder these business arrangements. Before any formulated product is marketed, it must first be thoroughly tested by growers, whose comments, critiques, and suggestions for improvement, however drastic, will be crucial in avoiding unsuspected problems and preventing failures.

Adjuvants, either added to the diluted product or incorporated into the formulation, are used as spreaders or anti-dessicants to improve the efficacy of the biofungicides. However, many of these adjuvants have fungicidal and/or insecticidal properties that in themselves will often account for most of the reported activity of the mixed product (15). This trend toward activity from unknown adjuvants could lead to a general depreciation of biological products if growers cannot be convinced of the added benefits of the biocontrol agent. This reiterates the need to conduct rigorous efficacy trials with sound controls that will highlight the properties of the active ingredients.

CONCLUSIONS

Although small by field standards, the greenhouse market still represents over 300,000 ha worldwide, with 50,000 in highly sophisticated production systems. Output can exceed field crops by nearly 40 times for a similar production area (155). Greenhouses offer a privileged environment for disease biocontrol, but implementation is still very limited. However, if we have anything to learn from our entomologist colleagues, it is that this will change. Indeed, from a modest and uneven start in the early 1970s, insect biocontrol has grown to a standardized approach throughout the greenhouse market. Plant pathologists and companies investing in biocontrol products should likewise view the future of biological control of plant diseases in greenhouse systems with optimism. A few products have already been registered and several more should be commercialized within the next few years. Success stories against a number of diseases will be important both to validate biocontrol of plant diseases and, most important, to gain acceptance by growers. Positive reports will, in turn, stimulate research and investments in yet more biological products so that the reduction of chemical fungicides can become a quantifiable reality rather than a pipedream. Greenhouse systems offer the best opportunity of success for disease biocontrol, and the lessons learned from these systems will help in the transition to agronomic and horticulture crops.

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