COMMERCIALIZATION AND IMPLEMENTATION OF BIOCONTROL

D.R. Fravel
Vegetable Laboratory, USDA-ARS, BARC-West, Beltsville, Maryland 20705;
email: fraveld@ba.ars.usda.gov

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Abstract  Although the number of biocontrol products is increasing, these products still represent only about 1% of agricultural chemical sales. Yet these are important contributions because biocontrol agents offer disease management alternatives with different mechanisms of action than chemical pesticides. Trends in research include the increased use of biorational screening processes to identify microorganisms with potential for biocontrol, increased testing under semicommercial and commercial production conditions, increased emphasis on combining biocontrol strains with each other and with other control methods, integrating biocontrol into an overall system.

INTRODUCTION

The goal of biocontrol research is to provide additional tools for disease management. To place these tools in the growers' hands, products must be commercialized. This article discusses the process of commercialization and commercial products currently available in the United States. Also discussed is implementation of biocontrol, particularly use and testing of commercial products and testing new biocontrol agents under commercial or semicommercial conditions.

There is no single, accepted definition of biocontrol. This article discusses primarily the use of living microorganisms (including viruses) for the amelioration of plant diseases with an emphasis on recent literature on commercialization, commercial products, and commercial use. Although some examples of mycoherbicides are included, the article does not review literature on mycoherbicides. Even though the long-term beneficial effects of many cultural practices, including crop rotation and green manures, as well as other practices such as solarization and biofumigation, are due to biological control, these are not covered here (17, 34, 53, 74).

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With such a limited definition of biocontrol, the overall contribution of biocontrol of plant diseases to plant health management is currently relatively small (16), representing about 1% of agricultural chemical sales (67). For comparison, fungicides represent approximately 15% of pesticide sales (http://www.epa.gov). Nevertheless, the contributions of biopesticides are important because they offer different modes of action from chemical pesticides and can, therefore, be applied in rotation with pesticides to reduce the possible development of pathogen resistance. Pathogen resistance to fungicides has promoted interest in development of biocontrol agents (111). Biocontrol can also be used in situations where currently no control is available, where conventional pesticides cannot be used due to reentry or residue concerns, or where the product must be certified organic. Biocontrol can also be used in combination with reduced rates of pesticides. We do not know what challenges may face us in the future, and it seems prudent to develop a variety of options for disease control.

DEVELOPMENT AND COMMERCIALIZATION OF BIOCONTROL AGENTS

Unlike biocontrol of insects, biocontrol of plant diseases is relatively new. The first bacterium, *Agrobacterium radiobacter* strain K84, was registered with the United States Environmental Protection Agency (EPA) for control of crown gall in 1979 (Table 1). Ten years later the first fungus, *Trichoderma harzianum ATCC 20476*, was registered with the EPA for control of a plant disease. A total of 14 bacteria and 12 fungi have been registered with EPA for control of plant diseases, and one bacterium (88) has been removed from the EPA list. Most of these are currently sold commercially as one or more products. This technology is still emerging. Sixty-five percent of the EPA-registered organisms have been registered within the past 10 years, with 36% registered over the past 5 years. Many technological problems were overcome and shifts in thinking occurred for these products to reach the shelves.

Choice of the Problem

From a scientific viewpoint, biocontrol is more likely to be successful in some pathosystems than others. Systems (or particular times in the cropping cycle) with low biological diversity and at least some degree of environmental control are logical targets (20, 47, 89). Diseases with a limited window of opportunity for infection, as well as those with monocyclic disease cycles or slow rates of disease progress, also have been targeted. Classical concepts in control should also be remembered, such as determining when and where the pathogen is most vulnerable.

Lidert has pointed out that some reasons why biocontrol agents fail to become products are that scientists outside industry often (a) overestimate the power of
### Table 1

Microorganisms registered with the U.S. Environmental Protection Agency as biopesticides

<table>
<thead>
<tr>
<th>Biocontrol agent</th>
<th>EPA registration number</th>
<th>Year registered</th>
<th>Target organism or disease</th>
<th>Crops or use</th>
<th>Product</th>
<th>Manufacturer or distributor</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agrobacterium radiobacter</em> strain K84</td>
<td>11,4201</td>
<td>1979</td>
<td><em>Agrobacterium tumefaciens</em></td>
<td>Ornamentals, fruits, nuts</td>
<td>Galltrol</td>
<td>AgBioChem, USA</td>
</tr>
<tr>
<td><em>Agrobacterium radiobacter</em> strain K1026</td>
<td>00,6474</td>
<td>1999</td>
<td><em>Agrobacterium tumefaciens</em> and <em>A. rhizogenes</em></td>
<td>Ornamentals, fruits, nuts</td>
<td>Nogall</td>
<td>Bio-Care Technology, Australia</td>
</tr>
<tr>
<td><em>Anapelmocyes quisqualis</em> isolate M-10</td>
<td>02,1007</td>
<td>1994</td>
<td>Powdery mildew</td>
<td>Fruit, vegetable, and ornamental crops</td>
<td>AQ10 BioFungicide</td>
<td>Ecogen, USA</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> strain AF36</td>
<td>00,6456</td>
<td>2003</td>
<td><em>Aspergillus flavus</em></td>
<td>Cotton</td>
<td>Aspergillus flavus AF36</td>
<td>Arizona Cotton Research and Protection Council, USA</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> NRRL 21,882</td>
<td>00,6500</td>
<td>2004</td>
<td><em>Aspergillus flavus</em></td>
<td>Peanut</td>
<td>Afla-guard</td>
<td>Circle One Global, USA</td>
</tr>
<tr>
<td><em>Bacillus licheniformis</em> strain SB3086</td>
<td>00,6492</td>
<td>2003</td>
<td>Foliar pathogens and blights</td>
<td>Ornamental plants and ornamental turf</td>
<td>EcoGuard; Novozymes Biofungicide Green Relief</td>
<td>Novozymes Biologicals, USA</td>
</tr>
</tbody>
</table>

(Continued)
TABLE 1  (Continued)

<table>
<thead>
<tr>
<th>Biocontrol agent</th>
<th>EPA registration number</th>
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<th>Product</th>
<th>Manufacturer or distributor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus pumilus strain GB 34</td>
<td>00,6493</td>
<td>2002</td>
<td>Rhizoctonia, Fusarium</td>
<td>Soybean</td>
<td>GB34 Concentrate Biological Fungicide</td>
<td>Gustafson, USA</td>
</tr>
<tr>
<td>Bacillus subtilis GBO3</td>
<td>12,9068</td>
<td>1992</td>
<td>Rhizoctonia, Fusarium, Aspergillus, and others</td>
<td>Crop seeds, including seeds for cotton, peanuts, soybeans, wheat, barley, peas, and beans</td>
<td>Kodiak; Companion</td>
<td>Gustafson, USA; Growth Products, USA</td>
</tr>
<tr>
<td>Bacillus subtilis MBI 600</td>
<td>12,9082</td>
<td>1994</td>
<td>Fusarium, Rhizoctonia, Alternaria, and Aspergillus</td>
<td>Cotton, beans, barley, wheat, com, peas, peanuts and soybeans</td>
<td>Subtilex; Histick N/T</td>
<td>Becker Underwood; Premier Horticulture, USA</td>
</tr>
<tr>
<td>Bacillus subtilis strain QST 713</td>
<td>00,6479</td>
<td>2000</td>
<td>Foliar pathogens, rots, and blights</td>
<td>Cherries, cucurbits, grapes, leafy vegetables, peppers, potatoes, tomatoes, and walnuts</td>
<td>Serenade; Rhapsody</td>
<td>AgraQuest, USA</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td><strong>Strain</strong></td>
<td><strong>Year</strong></td>
<td><strong>Diseases</strong></td>
<td><strong>Applications</strong></td>
<td><strong>Company</strong></td>
<td><strong>Country</strong></td>
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<tr>
<td><strong>Bacillus subtilis</strong> var. amyloliquefaciens strain FZB24</td>
<td>00,6480</td>
<td>2000</td>
<td><em>Rhizoctonia</em> and <em>Fusarium</em></td>
<td>Shade and forest tree seedlings, ornamentals, and shrubs</td>
<td>Taego</td>
<td>USA</td>
</tr>
<tr>
<td><strong>Candida oleophila</strong> isolate I-182</td>
<td>02,1008</td>
<td></td>
<td>Postharvest diseases</td>
<td>Various fruits, vegetables, flowers, ornamentals, other plants</td>
<td>Aspire</td>
<td>USA</td>
</tr>
<tr>
<td><strong>Coniothyrium minitans</strong> CON/M/91-08</td>
<td>02,8836</td>
<td>2001</td>
<td><em>Sclerotinia sclerotiorum</em> and <em>Sclerotinia minor</em></td>
<td>Agricultural soil</td>
<td>Contans WG; Intercept</td>
<td>USA</td>
</tr>
<tr>
<td><strong>Gliocladium catenulatum</strong> strain J1446</td>
<td>02,1009</td>
<td>1998</td>
<td>Soilborne pathogens</td>
<td>Vegetables, herbs and spices, turf, ornamentals, tree, and shrub seedlings</td>
<td>Primastop</td>
<td>USA</td>
</tr>
<tr>
<td><strong>Gliocladium virens GL-21</strong></td>
<td>12,9000</td>
<td>1990</td>
<td>Soilborne pathogens</td>
<td>Ornamentals, vegetables, cotton</td>
<td>Soilgard</td>
<td>USA</td>
</tr>
<tr>
<td>Killed <em>Myrothecium verrucaria</em>: fermentation solids and solubles</td>
<td>11,9204</td>
<td>1996</td>
<td>Plant parasitic nematodes</td>
<td>All food, fiber, and ornamental crops</td>
<td>DiTera</td>
<td>USA</td>
</tr>
</tbody>
</table>

*Continued*
### TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>Biocontrol agent</th>
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<th>Target organism or disease</th>
<th>Crops or use</th>
<th>Product</th>
<th>Manufacturer or distributor</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas chlororaphis</em> strain 63-28</td>
<td>00,6478</td>
<td>2001</td>
<td><em>Pythium, Rhizoctonia solani, Fusarium oxysporum</em></td>
<td>Vegetables and ornamentals in greenhouses</td>
<td>AtEze</td>
<td>EcoSoil Systems, USA</td>
</tr>
<tr>
<td><em>Pseudomonas aureofaciens</em> strain Tx-1</td>
<td>00,6473</td>
<td>1999</td>
<td><em>Sclerotinia homeocarpa, Colletotrichum graminicola, Pythium aphanidermatum, Microdochium nivale</em></td>
<td>Golf course turf</td>
<td>Bio-Ject Spot-Less</td>
<td>EcoSoil Systems, USA</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> A506</td>
<td>00,6438</td>
<td>1992</td>
<td>Frost damage, fire blight, bunch rot</td>
<td>Fruit crops, as well as almond, potato, and tomato crops</td>
<td>BlighBan A506; Frostban</td>
<td>Frost Technology Corporation, USA; Plant Health Technologies, USA</td>
</tr>
<tr>
<td><em>Pseudomonas syringae</em> strain ESC-10</td>
<td>00,6441</td>
<td>1995</td>
<td>Postharvest diseases</td>
<td>Apples, pears, lemons, oranges, or grapefruit after the fruit is harvested</td>
<td>Bio-Save 10LP</td>
<td>EcoScience Produce Systems Division, USA</td>
</tr>
<tr>
<td><strong>Pseudozyma flocculosa strain PF-A22 UL</strong></td>
<td>11,9196</td>
<td>2002</td>
<td>Powdery mildew</td>
<td>Roses and cucumbers in greenhouses</td>
<td>Sporodex L Plant Products Co., Canada; Technology Sciences Group, USA</td>
<td></td>
</tr>
<tr>
<td><strong>Streptomyces griseoviridis strain K61</strong></td>
<td>12,9069</td>
<td>1993</td>
<td>Soilborne pathogens</td>
<td>Food crops, ornamentals, and tree seedlings</td>
<td>Mycostop Kemira Oy, Finland</td>
<td></td>
</tr>
<tr>
<td><strong>Trichoderma harzianum ATCC 20,476</strong></td>
<td>12,8903</td>
<td>1989</td>
<td>Tree wound pathogens</td>
<td>Wounds in ornamental, shade, and forest trees</td>
<td>Binab T BINAB Bio-Innovation AB, Sweden</td>
<td></td>
</tr>
<tr>
<td><strong>Trichoderma harzianum strain T-22</strong></td>
<td>11,9202</td>
<td>1990</td>
<td>Soilborne pathogens</td>
<td>Greenhouses, nurseries, turf, home gardens, planting boxes, and outdoor soil</td>
<td>Root Shield; Plant Shield Bioworks, USA</td>
<td></td>
</tr>
<tr>
<td><strong>Trichoderma harzianum strain T-39</strong></td>
<td>11,9200</td>
<td>1996</td>
<td>Botrytis cinerea</td>
<td>Most food crops</td>
<td>Trichodex Makhteshim Agan of North America, USA</td>
<td></td>
</tr>
</tbody>
</table>
environmental concerns as economic drivers; (b) lack sufficient knowledge of grower needs, registration strategy and competitive forces; (c) have naïve ideas about positioning and market strategy; and (d) underestimate registration costs and difficulties (67). He adds that insufficient cost-performance and poor shelf-life are other reasons why promising microorganisms do not become products. Others representatives from industry have made similar remarks, indicating that researchers choose problems for markets that are financially too small to justify development costs or problems that are “so expansive that it is impossible to clearly define what the product must do to be commercially acceptable” (80). A “product concept” should be developed early in the overall development process (40, 80). Industry must also be able to protect its investment through patents or other forms of protection (40).

Screening

The ultimate success of biocontrol depends on how well the searching and screening process is done. There is no single, correct way to search or screen. Both depend on the target pathogen, the crop, and the cropping system. Cook & Baker (17) and Schisler & Slininger (99), as well as others, provide some insight as to where to look for antagonists. For example, biocontrol agents have been isolated from suppressive soils (64). Where you look and how you screen depends on what the most logical strategy appears to be. For example, finding microorganisms to protect postharvest fruit would likely require screening for microorganisms that colonize the surface of the fruit quickly and thoroughly to exclude pathogens or those that compete well for nutrients against pathogens requiring nutrients for germination (47). Similarly, in some cases it may advantageous to colonize the rhizosphere (e.g., 2). Destruction of overwintering inoculum may be appropriate for pathogens with monocyclic disease cycles. In this case, appropriate screening may consist of burying sclerotia and retrieving them to identify mycoparasites. Someone working with steamed, solarized, or fumigated soil may look for an excluder that colonizes soil quickly or, even better, one that colonizes soil quickly and can be put in place before the heat treatment or sublethal fumigation. Berg et al. screened bacteria isolated from the rhizospheres using three different screening methods (3). They analyzed in vitro antagonism toward *Verticillium dahliae* and other plant-pathogenic fungi, production of fungal cell wall–degrading enzymes, and plant growth–promoting effects on strawberry seedlings. Strains selected by this triple screening method performed better in greenhouse tests than commercial biocontrol products.

Although many useful biocontrol agents were first identified through in vitro inhibition tests (i.e., evaluating inhibition of a target pathogen on an agar medium), several researchers have reported no correlation between in vitro inhibition tests and field performance of biocontrol agents. For example, Burr et al. found no correlation between the ability of bacteria and yeasts to inhibit *Venturia inequalis* in vitro and the ability to control apple scab (7). Similarly, Milus & Rothrock reported that bacteria showing the highest levels of inhibition in vitro were not effective in the field in controlling Pythium root rot of wheat (79).
In the past several years, there has been an increase in the use of screening procedures that mimic conditions under which the agent will be used. Wilson et al. screened isolates of *Pseudomonas syringae* and *P. fluorescens* for control of bacterial speck of tomato in the greenhouse (112). Strains providing the best control in the greenhouse were then evaluated in ten field tests in the United States and Canada.

Baiting techniques are often useful in identifying mycoparasites. Krauss & Soberanis used basiodiocarps of *Crinipellis pernicosa*, causal agent of witches’ broom of cacao, to bait for mycoparasites (59). They identified mycoparasites effective against *C. pernicosa* as well as against two other cacao pathogens. Baiting was also used to identify mycoparasites for use against Rosellinia root rot of cacao (35).

**Effects of Environmental Parameters**

A frequent criticism of biocontrol is that the extent of control may differ with varying environmental parameters. For example, Huang et al. attributed inconsistent performance of biocontrol agents against white mold of bean between years of field testing to environmental differences (45). Unless the control is being developed for a system with a relatively constant environment, it is important to determine to what extent temperature, moisture, soil type, host cultivar, and other factors affect biocontrol performance. For example, both temperature and light significantly affected biocontrol efficiency by nonpathogenic *Fusarium oxysporum* (63).

In an evaluation of mycoparasites for use against Rosellinia, Mendoza Garcia et al. found that high soil organic matter favored that pathogen more than the biocontrol agents and that biocontrol was most efficient at higher pH values (77).

**Mechanisms and Ecology**

Understanding how the biocontrol agent works can facilitate optimization of control, as well as help to screen for more efficient strains of the agent. Mechanisms of some biocontrol agents are now understood in detail (e.g., 30, 108, 114, 115). Our understanding of ecology and microecology has increased also (e.g., 84). A more thorough understanding of ecology can help us figure out which problems to work on, how to approach them, when and where to apply the biocontrol agent, and predict situations in which control would not be expected to work. For example, the use of biosensors can provide information about the nutritional status of biocontrol agents on plant surfaces that can be used to enhance biological control. *Pseudomonas fluorescens* strain A506, which needs iron to form an antibiotic toxic to the fireblight pathogen, was transformed to act as a biosensor for iron (106, 110). The transformed bacterium colonized apple and pear flowers well, but flowers were an iron-limited environment unless treated with iron in the form of FeEDDHA. FeEDDHA is one of several iron compounds that can be applied to avert iron chlorosis. The microecology of fruit has also been studied. Castoria et al. determined that reactive oxygen species generated by wounding apples were
damaging to some biocontrol yeasts and that resistance to oxidative stress enhanced colonization ability of the yeast (9). Behavior of *Bacillus subtilis* was modeled on leaves with and without 1% β-glucan (12). Higher populations of vegetative cells were more likely to be present after 14 days in the presence of 1% β-glucan, and populations were more aggregated without β-glucan. Thus, the distribution of the biocontrol agent on the leaf can be manipulated.

**Field Testing**

The bottom line for biocontrol is whether it works under production conditions. Volume 1 of *Biological and Cultural Tests* (1986) contained 69 reports, 11 of which (16%) evaluated biocontrol agents. All of the agents evaluated were experimental microorganisms, some of which later became commercial products. Nearly 20 years later in 2004, *Biological and Cultural Tests* contained 100 reports, 16 of which (still 16%) reported evaluation of microorganisms for control of plant diseases. Most of these were commercial products being tested for new uses, but reports also included experimental microorganisms as well as tests for disease control using products sold commercially as microbial inoculants or growth promoters (and thus not registered as biopesticides). A greater diversity of biocontrol agents was being tested in 2004 than in 1986. Biocontrol agents are also being tested in combination with one another. For example, mixtures of plant growth-promoting rhizobacteria usually provided better control of disease than single strains in several pathosystems (49).

Biocontrol agents are being tested more often in the production system for which they are intended, rather than relying solely on experiments done in vitro, on detached leaves, on plantlets, or in the greenhouse on non-greenhouse crops. They are often being tested in multiple locations and seasons and tested in locations naturally infested with pathogens. In a field naturally infested with *Verticillium dahliae* and *Phytophthora cactorum*, the chitinolytic bacterium *Serratia plymuthica* suppressed disease caused by both pathogens and increased strawberry yield by 60% compared with the nontreated control (61). *T. harzianum* and the mycorrhizal fungus *Glomus intraradices* were tested in a field naturally infested with the tomato root and crown rot pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (19). Although no differences in yield were noted, both *T. harzianum* and *G. intraradices*, as well as the combination of the two, significantly reduced disease severity. In a five-year test rotating four crops, the mycoparasite *Coniothyrium minitans* was evaluated in a field naturally infested with *Sclerotinia sclerotiorum* (36). At the end of seven years, the number of sclerotia in plots treated with *C. minitans* was lower than at the beginning of the trial even where two highly susceptible bean crops had been planted. *Clonostachys rosea* applied to the upper parts of alfalfa plants significantly reduced pod and seed rot caused by *Botrytis cinerea* in each of three years of a field test (66). Antagonistic bacteria and a fungus applied to apples in the field reduced postharvest diseases as well as a chemical fungicide (65). A benign viral satellite RNA was used to modulate a mild strain of *Cucumber mosaic virus* (CMV) (82). In field tests, this combination was used to preinoculate...
pepper and melon against two severe strains of CMV. Preinoculated plants were nearly completely protected from challenge inoculations with the severe strains three weeks later.

**Production of Biocontrol Agents**

One factor limiting commercial interest in biocontrol is the high cost of production for most biocontrol agents (32). This may be due to high cost of substrate, low biomass productivity, or limited economies of scale.

The purpose of production is to produce the greatest quantity of efficacious propagules in the shortest period of time. Processes that produce the most propagules are not always those that produce the best type of propagule for formulation or the most efficacious propagules (5, 46). For some biocontrol agents, we know a great deal about how to manipulate the production medium to induce production of the desired propagules. Factors that are often important include carbon source, osmotic potential, temperature, and pH. For example, conidiation in Trichoderma can be induced through carbon manipulation (1). A C:N ratio of 14:1 consistently produced the conidia with the longest shelf life (28).

Large-scale production of *Gliocladium virens* involves starting the fungus in a commercial medium in shake flasks, then transferring to a proprietary medium in a seed fermentor, before transferring to fermentors up to 4000 L (29). Droby et al. used fermentation with a cheap industrial waste material to scale-up production of *Pichia guilliermondii* for biocontrol of postharvest decay of citrus (25). They went on to conduct tests in packinghouses where *P. guilliermondii* plus one tenth the normal rate of fungicide in commonly used waxes was equivalent to the full rate of the fungicide in controlling disease.

**Formulation**

Formulation can affect many aspects of biocontrol performance, shelf life, and safety. Formulation of biocontrol agents has been reviewed recently (6, 31, 109). A recent review that includes biocontrol of postharvest disease of pear stresses the importance of evaluating biocontrol microorganisms in the formulation, since the formulation can enhance or diminish control (71). A reasonable amount of literature on formulation notwithstanding (41), many believe that most of the knowledge in this area is proprietary and thus not generally accessible.

As with any biological system, three parameters that greatly affect success are water, food, and environment. Water activity can profoundly affect survival of biocontrol agents in formulations (13). A dry product is less weight to ship and at lower risk of possible contamination. Some biocontrol agents form life stages that are relatively simple to formulate, such as bacterial endospores, yeasts, and the resting-spore stages of many fungi. In these cases, revival of the spores must be considered. Some organisms, such as yeasts, dry well.

Hjeljord et al. demonstrated that conidia of *Trichoderma* spp. formulated in commercial products were significantly slower to germinate and colonize...
senescent strawberry leaves than fresh conidia, even though there was no difference in germination on laboratory media (43). Rehydration of microorganisms may require some care. Collins et al. developed a method for germinating endospores of Bacillus subtilis prior to spraying (11). Connick et al. developed an improved invert emulsion with high water retention (14).

Survival of Arthrobotrys dactyloides was poor in a kaolin, vermiculite, and gum arabic granule made from liquid biomass, but was greatly improved by inclusion of a solid-phase incubation for 3 days at 25°C under sterile conditions (104). Sabaratnam & Traquair found that populations of Streptomyces sp. were stable in talcum powder and starch granules over the 10–14-week test period and that they were more stable at 4°C than at 24°C (97). Minuto et al. found that antagonistic strains of Fusarium spp. effectively reduced Fusarium wilt of cyclamen when applied as chlamydomspores dispersed in t alc or as a conidial suspension, but not when applied in an alginate-kaolin formulation (81).

McGuire & Hagenmaier developed a shellac latex formulation and a dissolved shellac/shellac ester formulation that significantly improved survival of Candida oleophila for protection of postharvest protection of grapefruit from Penicillium digitatum (76). Although the constituents of many commercial coatings used post harvest on grapefruit were fungicidal to yeasts, three were identified that were satisfactory carriers for yeast used as biocontrol agents (75).

Delivery

Delivery systems that are well thought-out as to time and place can greatly reduce the amount of biocontrol agent needed. Where and when to deliver the biocontrol agent depends on the biocontrol agent, the pathosystem, and the cropping system. Researchers are now looking beyond the basics of whether to apply an agent as seed coat or spray, and have begun to fine-tune delivery in some systems. Freeman et al. evaluated 2-, 7-, and 10-day spray schedules for single and multiple isolates of Trichoderma strains for control of strawberry anthracnose and gray mold (33). Steddom & Menge determined that ten repetitive applications of Pseudomonas putida at low concentrations through irrigation water resulted in soil populations similar to those from a single application at a tenfold greater concentration (102). Microsphaeropsis ochracea was evaluated in the field for control of apple scab (8). Naturally infected, scabbed leaves were sprayed with M. ochracea at seven times from August to November and then overwintered in the field. Spraying the biocontrol agent in August or September reduced ascospore production by 61% to 96%.

Under low disease pressure, Ulocladium atrum reduced gray mold on strawberry at harvest in four of seven experiments (4). Sprays starting at transplant provided better control than sprays initiated at flowering in only one of five experiments, and thus flowering is considered to be the best time to start. Bacillus subtilis applied 1–5 days before infection by Cercospora increased control (11).

Unlike chemical pesticides, biocontrol agents may lend themselves to delivery by insects. Bees have been used to disseminate bacterial and fungal biocontrol
agents to specific sites such as flowers (50, 70, 90, 107, 113). Populations of T. harzianum on strawberry flowers were half as large when delivered by bumblebees or honeybees than by spray applications, but the bee-delivered inoculum provided better control in a 4-year field study (58). Further, the number of seeds per berry and berry weight were significantly greater in bee-delivered treatments. These were significantly reduced in treatments that were both bee-visited and fungicide-treated at bloom, possibly indicating the impact of fungicides at bloom on pollination and yield. Honeybees vector the plant pathogen Monilinia vaccinii-corymbosi, causal agent of mummy berry disease on blueberry. In a 3-year field study, beehives were equipped with dispensers containing the commercial product Serenade (Bacillus subtilis) (22). This treatment significantly reduced disease levels. Pusey examined dispersal of P. agglomerans, used to control fire blight, under conditions that limited or permitted natural dispersal of the biocontrol agent by honeybee activity (91). He concluded that natural dispersal of biocontrol agents may obscure differences in efficacy among treatments and that treatments in orchards should be widely separated in large-scale trials.

Ants have been used to deliver a prospective mycoherbicide to the root zone of plants (38). Ants had a preference for formulations containing oils, perhaps because they perceived these granules as seeds.

Registration

In the United States, three pieces of legislation have a great effect on how quickly biocontrol can get to the market and whether it stays there: The Federal Food, Drug, and Cosmetic Act (FFDCA), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), and the Food Quality Protection Act FQPA. Reregistration under FIFRA and the loss of crops from the labels of some pesticides under FQPA have reduced management options in many cases (94). Microbial products that are sold for control of plant diseases must be registered as biopesticides with the EPA. The EPA website contains information on how to register a biopesticide, actions affecting registration, and toxicological and other data from products that have already been registered (http://www.epa.gov).

Two important factors in registration are toxicity and environmental fate. While most toxicity data are not developed by plant pathologists, they do contribute to the risk assessment process (18, 68, 95). Cook et al. provided a framework for evaluation of safety of biocontrol microorganisms (18). Some of the factors to be addressed in assessment of safety of biocontrol agents to humans include pathogenicity, toxicity, and allergenicity of the fungus and its products to humans (85). Care should be taken in manufacturing and application of Trichoderma to avoid aerial contamination by conidia (85).

More studies are also needed to determine the environmental fate of biocontrol agents. De Leij et al. found that field release of wild-type and genetically modified P. fluorescens resulted in transient perturbations of some of the culturable microflora (21). Défago et al. found that heavy rainfall could distribute P. fluorescens
CHA0 into deeper soil layers (23). Harris & Adkins found that two biocontrol isolates of binucleate Rhizoctonia isolates survived saprophytically for at least 13 months in pasteurized potting medium (41). Recently developed genetic tools should facilitate accurate tracking of biocontrol agents.

TECHNOLOGY TRANSFER TO GROWERS

Industry will conduct marketing assessment and test marketing (69), but public-sector plant pathologists also have roles in transferring technology to growers, including additional field testing both alone and in combination with other agents or pesticides, integrating biocontrol into the production system, and in including proven biocontrol methods in extension materials. Even after the product is on the shelf, the role of the plant pathologist does not end.

My perusal of state extension web sites found that most states that list recommendations for disease problems include biocontrol options when they are available. Some also include educational material about the nature of biocontrol. The Organic Materials Review Institute (OMRI) provides independent review of materials that can be applied in organic production systems. Their website lists these products, including biocontrol products for plant diseases and the suppliers of these products (http://www.omri.org). The website maintained by B. McSpadden-Gardener lists biocontrol products sold worldwide (http://www.oardc.ohio-state.edu/apsbcc/productlist2003USA.htm).

Testing Commercial Agents

Serenade was identified as having potential for control of blueberry mummy disease (98). Commercial preparations of Kodiak HB (Bacillus subtilis) and T-22G (Trichoderma harzianum) were tested alone and in combination with a nonpathogenic Fusarium oxysporum for control of fusarium wilt of chickpea (42). The combination of the two commercial agents was as effective as either alone, whereas the combination of the nonpathogenic Fusarium with B. subtilis was ineffective even though each antagonist alone was effective in reducing wilt. Proyeast (Metschnikowia fructicola) applied to table grapes 24 h before harvest significantly reduced the number of decayed fruit (52).

Combined with Pesticides and Other Chemicals

Trichoderma virens was combined with metalaxyl for field control of cotton seedling diseases (44). The most consistent control of avocado black spot was obtained by integrating a chemical fungicide with B. subtilis sprays (57). The biofungicide AQ10 (Ampelomyces quisqualis) alone did not reduce the size of colonies of powdery mildew colonies on detached leaves but did reduce the amount of inoculum produced by the pathogen (100). AQ10 was compatible with the chemical pesticide triadimefon and the adjuvant AddQ. A synergistic effect in the
control of *Rhizoctonia solani* on rosemary by combining *Laetisaria arvalis* and a foliar spray of half the rate of an experimental fungicide (15). In this same system, synergism was not observed for *T. harzianum* plus iprodione at the label rate. Increasingly, biocontrol agents are being applied with reduced rates of chemical fungicides or bactericides for control of disease in the field. For example, Mew et al. used seed bacterization with biocontrol agents in combination with reduced rates of fungicides (78). Quin & Tian combined the antagonistic yeast *Cryptococcus laurentii* with reduced rates of fungicides for control of postharvest diseases of jujube fruit (93).

Improved control of Rhizopus rot on nectarine was obtained by combining the yeast *Pichia membranefaciens* with either iprodione or CaCl (92). Similarly, the ability of *Candida sake* to reduce postharvest blue mold on pears was enhanced by NH₄-Mo (86).

Biocontrol agents have also been combined with SAR inducers. In three field tests on tomato, a mixture of six bacterophages was applied alone or in combination with harpin protein, acibenzolar-S-methyl, or a standard bactericide treatment of copper hydroxide plus mancozeb (87). While not increasing yield, treatments with bacteriophage-treated plots showed significantly better disease control and total yield of marketable fruit.

**Combined with Cultural and Physical Practices**

In a three-year field microplot study of an integrated control for Fusarium wilt of chickpea, the effect of the biocontrol agents on disease was less in January than in February or March sowings demonstrating the inconsistency due to environmental effects that can been seen in biocontrol (62). A combination of four biocontrol fungi plus fertilization was more effective and more economical for control of three cacao diseases than chemical control (60). The combination of the yeast *Debaryomyces hansenii* with ultraviolet light-C was as effective as chemical pesticides in reducing storage rots of fruits and vegetables (103). Heat-treating potting medium enhanced control of Rhizoctonia and weakened control of Pythium by commercial biocontrol agents (54).

**Integrated Systems**

An integrated strategy for the control of *Botrytis cinerea* in greenhouse vegetables was developed using a weather-based disease forecasting system and sprays of *T. harzianum* T39 (Trichodex) or a chemical fungicide (101). The chemical fungicide was used under expected high disease pressure, no spray when slow or no disease progress was expected, and application of biocontrol in all cases. Timing of sprays of *P. fluorescens* (Blight Ban A506) and *P. agglomerans* to control fire blight on pear and apple blossoms was optimized by adapting fire blight forecasting concepts (51). Martin & Bull concluded that while single microbial inoculants may provide some control for specific diseases of strawberry, they cannot provide the broad-spectrum control needed to replace methyl bromide (73). They further
indicate that cost effective biocontrol strategies will likely require development of an integrated systems approach. Martin points out that longer-term goals for methyl bromide replacement have focused on more sustainable systems that incorporate cultural practices, host resistance, and biological approaches (72).

Commercial and Semicommercial Testing and Use

In a field experiments with potted plants and in a poplar plantation, Mycostop (Streptomyces sp.) and other strains of Streptomyces spp. significantly reduced Septoria leaf spot (39). Tests under commercial greenhouse conditions demonstrated that U. atrum and Gliocladium roseum were as effective as the standard chemical fungicide program in preventing Botrytis leaf rot on cyclamen (55). In five of six commercial greenhouses using different production systems, U. atrum controlled Botrytis on cyclamen as well as did fungicides (56). In one greenhouse, disease pressure was very high and neither fungicides nor U. atrum controlled the disease. In tests in a commercial greenhouse, Clonostachys rosea reduced sporulation of B. cinerea, but did not consistently reduce disease incidence, possibly due to heavy disease pressure and no sanitation in nontreated plots (83).

Prestop (Gliocladium catenulatum), RootShield (T. harzianum), Mycostop (Streptomyces griseoviridis), and composted dairy solids reduced the severity of Fusarium root and stem rot of cucumber under semicommercial greenhouse conditions (96).

Biocontrol of postharvest diseases has been successful under commercial conditions, particularly using yeasts as biocontrol agents (26). In a test in a commercial packinghouse, a combination of the yeasts Cryptococcus laurentii and C. infirmominutus controlled blue mold of pear as well as a high label rate of thiabendazole (10). This combination was also effective in controlling blue mold of apple, as was the thiabendazole. Combining C. laurentii with half the label rate of thiabendazole was significantly more effective than the label rate of the fungicide whenever thiabendazole-resistant spores of the pathogen were present. In another packinghouse trial, Aspire (Candida oleophila) plus 100 ppm thiabendazole controlled decay of pear as well as 569 ppm of thiabendazole, the maximum label rate (105).

The combination of Candida saitoana plus 0.2% glycolchitosan was comparable or superior to thiabendazole in reducing postharvest decay of several cultivars of apples under semicommercial conditions (27). The combination also controlled decay of oranges and one lemon cultivar as well as imazalil. A combination of Aspire (C. oleophila) and thiabendazole was evaluated in commercial packinghouse tests (24). This combination controlled green and blue molds as well as a conventional fungicide treatment. In addition, it reduced sour rot, a disease not controlled by fungicides. However, incidence of decay in Aspire-treated fruit was 1% greater in shipped fruit compared with the fungicide treatment. Apple fruit with stem pulls resulting from a mechanical harvester is more susceptible to blue mold decay than apples without stem pulls (48). BioSave (P. syringae) significantly reduced the incidence of blue mold in apples with stem pulls.
FUTURE RESEARCH DIRECTIONS

Entire books have been written on biocontrol of crops ranging from rice, wheat, cotton, tobacco, peanut, sugarcane, potato, soybean, tomato, apple, turfgrass, and postharvest citrus (37). Although biocontrol still represents a very small portion of disease management, some of these uses, such as Agrobacterium for management of crown gall, are significant. If the goal of biocontrol research is to place biocontrol products in the growers’ hands, then perhaps there needs to be more communication between public researchers and industry in the early stages of development. Over the past 25 year, the approach to biocontrol research has evolved toward being more ecologically holistic and more oriented toward both production systems and industry’s concerns. This evolution is likely to continue. Because the science is still young, research is needed in many areas. In particular, research in production, formulation, and delivery could greatly assist in commercialization of biocontrol agents. More research is needed in integrating biocontrol agents into production systems, such as in rotating biocontrol with chemical pesticides and in calculating these into forecast models to choose whether to apply a chemical pesticide or biocontrol. Continued research in biocontrol is needed to contribute to the movement toward sustainable agriculture and simply to ensure that alternatives are available if other management tools fail or are lost.

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LITERATURE CITED


63. Larkin RP, Fravel DR. 2002. Effects of varying environmental conditions on
biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. *Phytopathology* 92:1160–66
76. McGuire RG, Hagemmaier RD. 1996. Shellac coatings for grapefruits that favor biological control of *Penicillium digitatum* by *Candida oleophila*. *Biol. Control* 7:100–6


100. Shishkoff N, McGrath MT. 2002. AQ10 biofungicide combined with chemical fungicides or AddQ spray adjuvant for control of cucurbit powdery mildew in detached leaf culture. *Plant Dis.* 86:915–18


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