Changing Models for Commercialization and Implementation of Biocontrol in the Developing and the Developed World

Microbially based biocontrol of plant diseases differs fundamentally from chemical control, but pesticide regulations for microbial and chemical pesticides remain similar. In the United States, the Food, Insecticide, Fungicide and Rodenticide Act (FIFRA) (19), like similar laws in other countries, requires any material making a pesticidal claim to be registered. The Act was in response to widespread concern regarding the safety and environmental effects of pesticides. Registration requires significant effort, time, and expense to conduct toxicological, environmental, and in some cases, efficacy testing. However, the nature and advantages of chemicals and microbials are quite different, especially between chemical fungicides and endophytic symbiotic microbes that act through changes in plant gene expression. Further, some organisms with pest control activities were never covered by FIFRA, including obligate symbionts such as rhizobia and mycorrhizal fungi and larger organisms such as nematodes and predatory insects. Furthermore, FIFRA and similar regulations are designed primarily for specific, individual agents, and not for complex mixtures such as composts, manures, and teas made from these largely unregulated sources, even though they have activity against plant pests and their active ingredients may be similar to those in registered pesticides. In addition, pesticide regulations cover only materials that make specific pesticidal claims, i.e., they do not cover agents that make claims for general improvement of plant health and plant quality. Finally, there is great potential for use of microbial agents in the developing world, although full-scale production, marketing, and registration are simply too expensive for these societies. To overcome this economic difficulty, some countries have substituted locally produced products for imported ones (2,55).

There are numerous microbial products listed for the control of various pathogens, insects, or weeds. Copping (14) lists 17 species or strains of fungi or bacteria that have been commercialized for insect control, 37 for control of pathogens or nematodes, and 14 mycoherbicides. This must be an underestimate; Faria and Wraight (20) list 171 commercial mycoinsecticides and mycoacaricides over 12 species or subspecies of fungi. Even this is an underestimate because these authors did not include many nonregistered products or those produced by governmental agencies. In fact, many products or preparations with activity against plant pathogens or other pests are not registered pesticides. Instead, they may be marketed as plant inoculants or plant strengthening agents, which gives these products a marketplace advantage because the time and money necessary for registration are avoided. Moreover, because many microbial agents can be propagated easily, local distribution systems have been developed. As a consequence, biocontrol production, distribution, and methods of use have evolved in diverse ways, each of which has advantages and disadvantages.

There are strong incentives to develop biological products that fall outside the registered pesticide model, and it is likely that more products with pesticidal activity will be sold outside this framework. Much of this paper will deal with biocontrol based on *Trichoderma* strains, but other systems will be discussed as well. Finally, there are safety implications for the different systems that have evolved, but modern taxonomic studies provide insight into this aspect and also will be discussed.

It is important to consider the perceived benefits that microbial agents have in different parts of the world. In the United States and other developed countries, the agents are considered to be useful but not essential. However, in the developing world, as perceived by the Association of Asian Pacific Agricultural Research Institutions (AAPARI), biopesticides and biofertilizers are considered extremely important. Applicators in the developing world may not wear protective clothing (communication in AAPARI meeting cited in Acknowledgments), so there may be serious health hazards to the use of toxic chemical agents. In addition, fertilizers are rapidly becoming too expensive for use by smallholders in many parts of the world, and biofertilizers in their various forms are considered to be essential components of food security in many countries.

Comparison of Chemical Control, Contact Biological, and Endophytic Control Systems

Chemical control. Synthetic chemical disease control products, with a few exceptions, are based on toxicity of the chemicals to target organisms. As chemical methods and products have evolved, they have become much more specific in their modes of action. This specificity has created an increased probability that target microbes will develop resistance to them. Their ability to directly inhibit a high proportion of target organisms means that they can be highly effective; however, their period of efficacy is limited because the amount of toxic substance applied is finite and effects rarely last more than a few weeks. Furthermore, the active chemical control ingredient must be applied to site of infection, either through topical application or via systemic activity of the pesticide.

Contact biological control agents. Some biocontrol agents also must be applied to the site or court of infection or infestation; for example, entomopathogenic fungi must be delivered to a site where they will directly infect the insect pest, since they must infect and sicken the insect to be effective. The same is true for mycoherbicides and their weed hosts. Products based on *Bacillus thuringiensis* also must be delivered to a site where insects will come into contact with the endotoxin(s) in order to be effective.

Some biocontrol agents for plant disease must also be applied directly to the infection court. Examples are products based on *Bacillus subtilis* such as CEASE or Serenade, which deliver both the living organism and antibiotic substances and directly inhibit or

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kill the pathogen. Similarly, some products based on the same microbes described in the next section also must be delivered to the infection court. For example, *Trichoderma* strains designed for control of foliar pathogens may require direct application to the leaf or other tissues where the pathogen will be attacked (18,31,46).

Endophytic symbiotic microbes. A recent advance in our understanding of biocontrol agents is that some clearly are endophytic plant symbionts. Among the more effective agents in this regard are plant growth promoting rhizobacteria (PGPR), mycorrhizal fungi, *Piriformaspora indica,* and free-living rhizosphere competent fungi such as some strains of *Trichoderma* spp. Nonpathogenic Fusaria and binucleate *Rhizoctonia* spp. (*Ceratorhiza* spp.) probably also belong on this list. All of these organisms directly colonize plants internally (11,24,29,39,41,58,63,69). These agents may have much longer periods of efficacy than nonendophytic organisms since many have the ability to grow with plants and in the environment and provide benefits to plants for at least the life of an annual crop (27,29). Other fungi, such as fungal strains endophytic in tropical trees, may colonize shoots, roots, or stems (26,68). Both bacteria and fungi have greater effects directly upon plants (as opposed to direct effects on pathogens or other pests) than do most chemical pesticides. They may increase growth rates, and benefits to plants include: (i) increased growth and yield (12,27,41,45,64–66,72); (ii) increased resistance to water stress (Fig. 1), salt, and/or temperature tolerance (22,27,64); (iii) induced systemic resistance to disease (23,30,64,69–71); (iv) increased nutrient uptake and fertilizer efficiency utilization (27,28,59,72); and (v) increased percentages and rates of seed germination (8,12). *Trichoderma harzianum* strain T22 enhances expression of proteins involved in photosynthesis and starch accumulation, which supports the idea that its effects are due to increased photosynthetic rates in infected plants (27,32,60). Similarly, *P. indica,* PGPR, and mycorrhizal fungi have been shown to increase photosynthetic efficiency (22,25,52). *Trichoderma* strains can alleviate not only extrinsic stresses, but intrinsic stresses as well. Seeds lose vigor as they age, but seed treatments with *Trichoderma* spp. can restore vigor and improve germination, even in the absence of pathogenic organisms (7,61). Mechanisms and effects of the various microbes on plants under stress have been reviewed recently (25,61). Clearly, biocontrol and plant performance-enhancing activities overlap.

Recently, concepts and knowledge of the existence and use of these capabilities have increased markedly. For example, the *Trichoderma* research community originally thought that antibiotic activities and mycoparasitism were the primary modes of action of these fungi (13). However, these sets of mechanisms are clearly not the whole story, although these direct effects on target fungi are real and may be important in some systems (30). Two studies illustrate this point. One of the organisms thought to be most likely to control pathogens via antibiosis was *T. virens*, which produces gliotoxin, an antibiotic with strong activity on a wide range of target fungi (34,48,49). However, Howell and his colleagues (35,36) made a number of mutants that were deficient in their abilities to be mycoparasitic and/or to produce gliotoxin and found no relationship between the abilities of strains to produce the antibiotic or to parasitize target fungi, but observed an extremely high correlation between the abilities of different strains to induce terpenoid phytoalexins in cotton seedlings and the ability to protect the seedlings against *Rhizoctonia solani* (30,35,36). Similarly, *T. harzianum* strain T22 has been shown to be effective for control of seed and seedling diseases caused by *Pythium ultimum*, and we suggested that this biocontrol was due to mycoparasitism because mycoparasitic events could be observed on *Pythium*-like hyphae on seed surfaces (37). *P. ultimum* results in diseases of many plants including *Arabidopsis*, and T22 is effective in controlling this disease; however, if any of five mutants of *Arabidopsis* with disruptions in *NPR1* were tested, biocontrol was lost. Because *NPR1* is essential to induced resistance, and this deletion would have no effect on mycoparasitic events, biocontrol must be due to induced resistance rather than to direct effects on the pathogen by T22 (61). Thus, in two cases considered to be examples of biocontrol via antibiosis or mycoparasitism, respectively, the mechanisms proposed for decades have proven to be incorrect. In both cases, biocontrol is via changes in plant gene expression, which results in induced resistance rather than any direct effect on the pathogen.

Symbiotic microorganisms have effects that extend beyond biocontrol, and detailed studies have been conducted on all of these diverse organisms, which have similar capabilities and mechanisms even though they are of greatly different genetic backgrounds. These capabilities may be a good example of convergent evolution in which various microbes have evolved mechanisms to enhance plant growth and development, which is to their advantage since they live symbiotically in the plants (61).

In summary, many endophytic and symbiotic microorganisms have direct effects on plant physiology. Such abilities may be widespread among plant- and root-colonizing fungi and bacteria, but detailed studies have been conducted on only a few plant–microbe combinations. For this group of organisms, control of biotic stresses, such as plant diseases, is only a subset of their activities and benefits to plants. Control of abiotic stresses—increasing the abilities of plants to use nitrogen, improving photosynthetic efficiency, and control of intrinsic plant physiological stresses—are all possible effects of these organisms. Mechanisms of action involve reprogramming of plant gene expression, and at least in the case of *P. indica* (42,64) and probably *Trichoderma* strains (61), modulation of damaging levels of reactive oxygen species produced in response to stress. Thus, the need for and suitability of FIFRA pesticide regulations is questionable for these organisms even though they are likely to control plant diseases, primarily by inducing systemic plant resistance.

Differing Models for Use of Microbial Agents

There are a number of different economic models for use of biocontrol agents, which we have grouped into four general systems. First, however, effective strains must be selected.

The necessity for strain selection. Microbial strains no doubt differ markedly in their abilities to control diseases, abiotic stresses, and to enhance plant performance and yield. Many of the genera of microbes just discussed as beneficial microorganisms are very common in soil and other plant-related environments. For example, nearly all soils in the world contain 10 to 1,000 CFU of *Trichoderma* spp. per cubic centimeter (40), but application of even very small amounts of selected strains, for example as seed treatments, can provide large benefits (Fig. 2). This is very likely because most strains are poorly able to colonize roots and establish endophytic relationships. Other attributes may be critically important to some uses, such as the ability to grow at high or low temperatures (Fig. 3), or to produce metabolites that are essential to

Fig. 1. Endophytic plant symbiotic fungi can provide benefits to plants that are greater than those provided by disease control alone. Tomato seeds were treated with a newly developed strain of *Trichoderma* (plants on left) or not treated, and planted in a greenhouse potting mix (Cornell mix, which is a mixture of peat, vermiculite, and nutrients). Plants were grown for about 8 weeks and then water was withheld for 2 weeks. Plants grown from treated seeds were more able to resist water deficit stress, an effect no doubt resulting from alterations in plant gene expression. Other strains also have this ability (4). This figure illustrates the advantage of endophytic strains and the requirement of advanced highly selected strains. (Photo courtesy of F. Mastouri and G. E. Harman)

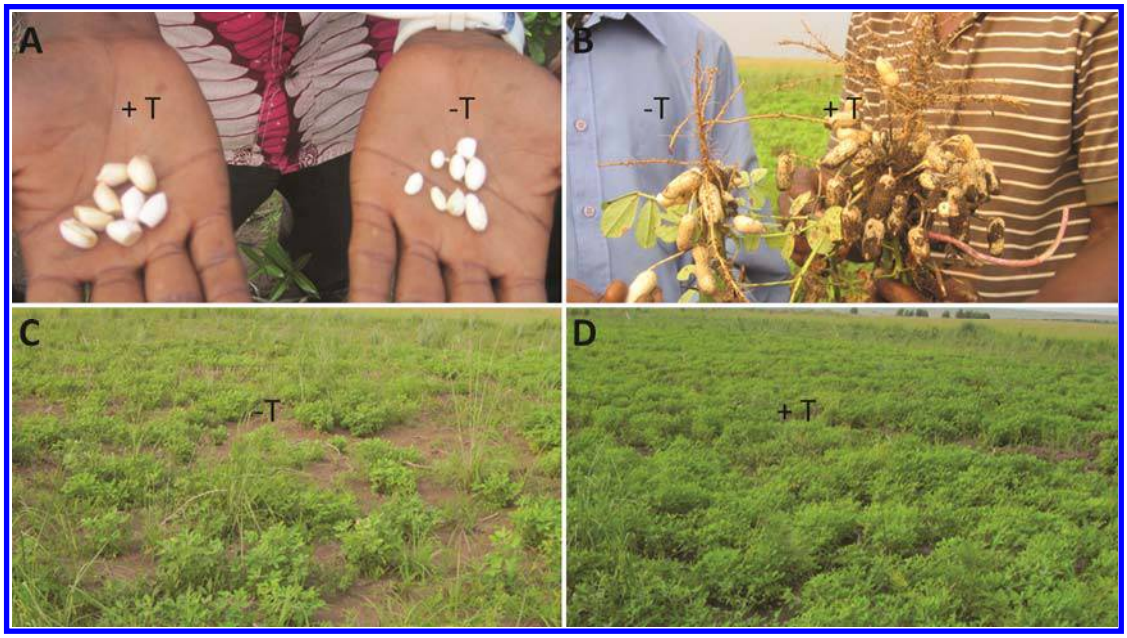

Fig. 2. Advanced strains can provide advantages to growers in developing countries. Shown are results of seed treatments of peanuts in Africa with a mixture of advanced strains (two strains of *Trichoderma harzianum* and one of *T. atroviride*) developed by the first author in cooperation with Advanced Biological Marketing. The seed treatment provided season-long advantages that included **A,** larger peanuts, **B,** more pods, and **C and D,** better strands and greener plants. Economic models that permit such strains to be used in countries and by growers with little available funds need to be developed. Associations of producing companies, governments, and nonprofit organizations may provide such models. Such strains would be particularly useful for small holders, particularly if they could be produced using model 3, local production. (Photos courtesy of Advanced Biological Marketing, Van Wert, OH)

Fig. 3. Local production of biopesticides and biofertilizers based on selected strains of *Trichoderma* developed by the fourth author and that follows model 2 (inoculants, biofertilizers, and plant strengthening agents). On the left is shown basic fermentation facilities in Libya that are operating on relatively large farms. Batteries of fermenters containing different beneficial microbes are installed and connected directly to the irrigation system, thus delivering a liquid mixture of living microbes and metabolites that are freshly made and highly effective. On the right is shown production based on rice, as used in many countries; it is also arranged directly on the farm for internal use or sold locally. The colonized product is either ground and applied as granules, or spores are extracted and used for powder formulation. The fourth author and his colleagues use strains that were selected locally (In Libya, ability to grow at elevated temperatures is important.) or imported and that provide multiple beneficial effects (i.e., stress protection and growth promotion).

success. These strains may be isolated locally, but there appear to be strains that are adapted to a variety of crops and conditions that may be very widely used.

It is important when comparing studies with different strains of even the same species to understand that there are tremendous differences and capabilities in strains and that many or even most studies are conducted with strains that have been selected in rigorous screening over a period of years. In our case, T22 resulted from a screening program of more than 1,000 strains, and the ones that provided the effects shown in Figure 2 were selected because they provided benefits beyond T22. Thus, most strains will not provide the good results shown in this and other papers. Further, the newer selection tools take advantage of the –omics revolution and are based upon extensive data of effects on plants not possible even a few years ago (47).

Model 1. Full registration and marketing—the microbial pesticide model. In the United States, FIFRA requires that any product for which pesticidal claims are made must be registered with the U.S. Environmental Protection Agency. Similar regulations exist in other countries. The word "pesticidal" does not necessarily refer to actual killing or inhibition of a target pest, just that the pest is controlled. Thus, a chemical lure or trap, or a plant induced to be resistant to a pest by a biological or chemical agent (other than conventional plant breeding), may need to be registered, although the path to registration of a transgenic plant differs from that of biological or chemical pesticides. Biological agents that are not themselves toxic or parasitic on a target pathogen, but that induce the plant to become resistant to the pathogen, are, by FIFRA definition, pesticides. The requirements for registration of a biological agent may be substantially less than those for a synthetic chemical, but they will always be difficult. An important point is that the microbial pesticide regulations in FIFRA and similar regulatory frameworks are primarily designed for specific, defined active ingredients. Thus, complex microbial systems such as microbial mixtures, composts, compost teas, and other complex mixtures are poorly suited to these regulations.

The senior author has first-hand knowledge and experience with the process, time, and costs required for EPA registration. In the 1980s, he and his colleagues produced *T. harzianum* strain T22 (62), which was licensed from Cornell University by the Eastman Kodak Company, which developed the toxicity package and environmental studies that resulted in the EPA registration of this organism. At the same time, another strain, *Gliocladium* (now *Trichoderma*) *virens* (Gl21), was identified at the USDA Soilborne Diseases Lab in Beltsville, and it also received EPA registration. In about 1990, Kodak decided to abandon the agricultural pesticide market and gifted the registration of T22 and other data they had generated to the Cornell Research Foundation. Harman and colleagues founded a company, now BioWorks Inc., to develop and market the organism and products (such as RootShield and T22 Planter Box) based on it. The *T. virens* strain was marketed as SoilGard and has had various companies involved with it; currently it is marketed by Certis LLC.

At BioWorks, T22 first had to be produced in large amounts (millions of kilograms of products have been sold) and formulated.

A national, and later international, marketing organization had to be put into place, with special consideration for a product with limited shelf life. Although sales began in 1993, the products based on T22 never gained a significant foothold in agriculture until about 1998, and since then sales have increased about 20% per year. This is a success story shared by products such as those based on *Bacillus* (such as Serenade, Ballad, Sonata, and Quest), and these products have helped make biocontrol an accepted part of commercial agriculture.

Among the impediments to successful products was the registration process. For T22, between Kodak initially and later BioWorks, approximately $12 million was required for product registration and development of a production facility, adequate formulations, and an effective marketing system, before sales of products based upon this organism really began to grow. In addition to the cost, the process is time-consuming; years rather than months are required. For a product that is based on pesticidal claims and especially one started by a new company, such time and expenses are prohibitive. The only reason BioWorks could be successful was because of the unique circumstance of the early Kodak involvement that secured the registration.

The requirements for successful identification, production, registration, and marketing are identified, along with their costs, in Table 1. A likely minimum cost for these steps is $8 million, and three to six years are required, if everything goes well, before even a highly effective product is established in the marketplace using the microbial pesticide model. Given this long time frame, the costs involved, and the market sizes of current products, it will be difficult at best for new products to come through this route.

The situation for registration of biopesticides in European Union countries and Canada is substantially more difficult than in the United States. The regulatory framework there requires not only toxicological and environmental testing, but also efficacy evaluations, which are not required by the EPA when the data package is submitted or for approval, although they do require efficacy data over time. In the EU and Canada, efficacy tests are required for almost every crop–pathogen combination, and these tests limit or even preclude registration of an organism with broad capabilities on many crops or pathogens. Costs are simply too great, especially for entry-level products. There are registered biopesticides in Europe; Contans, based on *Coniothyrium minitans,* is one, and products based on entomopathogenic fungi are others. *C. minitans* differs fundamentally from *Trichoderma* in that it has a limited target range. It is a pathogen of sclerotia of plant-pathogenic fungi and so is registered only for control of *Sclerotinia* spp. *Trichoderma* strains, on the other hand, have very wide capabilities, and the list of crops and pathogens on which it can be used is almost limitless. This makes EU and Canadian registrations nearly impossible from a financial point of view.

Finally, some beneficial microorganisms, including endo- and exo-mycorrhizal fungi, and bacteria such as rhizobia, are not subject to regulatory approval for use in the United States, although efficacy testing may be required by some countries, such as Canada, and in the EU. These organisms are endophytic symbionts, and as described in the first section, mycorrhizae and *Trichoderma* strains may have similar effects on plants.

Model 2. Inoculants, plant strengthening agents, and biofertilizers. In different countries and regions of the world, the descriptions "inoculants," "plant strengthening agents," and "biofertilizers" apply to organisms sold to improve plant performance. These different terms are important because, in some countries, they are written into legal frameworks and definitions.

The fundamentally different nature of "biocontrol" agents allows a different and largely unregulated avenue for use of beneficial microbes. As described in the introduction, organisms such as PGPR, mycorrhizal fungi, and *Trichoderma* spp. have numerous beneficial effects. These organisms, along with others such as rhizobia, can be produced and sold in various agricultural systems without specific pesticide registration if pesticidal claims are not made. These organisms, or mixtures of organisms, may control disease, and the disease-control benefits are well known, but they are not subject to FIFRA unless a claim to control specific diseases is made on the label. Sales of biocontrol agents through this route may be larger than sales of formally registered organisms, although the authors are unaware of any comparative information in this regard.

One of the first widely sold PGPR was a strain of *Bacillus subtilis* marketed by Gustafson, Inc., as Kodiak. This material was applied primarily to seeds; it probably had reasonably good biocontrol abilities, but it was sold as an inoculant to increase yields of field crops. Similarly, T22, although sold as registered pesticide products in the United States, is sold as Trianum in Europe. Trianum is legally a plant strengthening agent, and while it is becoming widely used and its pesticidal activities are known, it has not been sold as a registered pesticide in countries where efficacy testing is required. After many years, the product is just now being registered in Europe. The reason for the delay is simple economics—the return on investment for full European registration is unlikely to occur. Even in the United States, nonregistered products based on T22 have been legally sold. In China, emphasis has been placed on developing entomophagous fungi. For example, more than 70 tons of crude powder of *B. bassiana* were produced and

Table 1. Production and delivery of biocontrol systems in commercial agriculture

System	Steps required	Approximate costs/step	Time to significant market penetration
Full-scale registration and production—the chemical pesticide model	1. Identification of good agent 2. Development of production and formulation system 3. Patenting of strain and/or process 4. Toxicology and other testing 5. Registration 6. Building large-scale production system 7. Nationwide or international marketing	1,2. $100,000 3. Up to $200,000 for international coverage, at least $30,000 for one country 4. At least $500,000 5. $100,000 6. Up to $3-4 million 7. $2-3 million Total: up to $8 million	3 to 6 years
Biofertilizer, inoculant, or plant strengthening agent	1. Discovery of a good agent 2. Development of production and formulation system 3. Patenting of strain and/or process 4. Building large-scale production system 5. Nationwide or international marketing	1,2. $100,000 3. Up to $200,000 for international coverage, at least $30,000 for one country 4. Up to $1 million 5. $0.5 million Total: $1.8 million	1 to 2 years
Local production	Discovery of a good strain	$100,000 Total: $100,000 or less	Less than 1 year
Government sponsored or produced agents	Depends upon governmental direction and philosophy	Unknown	Unknown

sprayed against pine caterpillars on forests as a nonregistered pesticidal product (21). These examples show that niches in disease management that can be filled by endophytic symbiotic microbes are very different than those for which synthetic chemical pesticides are most useful.

The examples given in this section thus far are for seed or soil/planting mix application; however, the concepts are not limited to these uses. For instance, in Italy and other countries, *Trichoderma* strains are being developed as "biofertilizers" (this is the legal defining word), and new formulations for foliar applications have been introduced in the market based on efforts by the fourth author. The need to overcome the constraints of the registration process has stimulated the use and commercialization of biocontrol fungal and bacterial strains based on the multiple, pathogen-independent beneficial effects that they provide (Fig. 3). The system that has been developed and is being sold has the following distinct differences between it and most other products based on biocontrol agents:

- The biofertilizer products are liquid whole culture products prepared in liquid fermenters. Most other products based on biocontrol fungi are dry preparations as either granular or powdered products (Fig. 3).
- The liquid formulation contains the living fungus plus all of the metabolites produced during fermentation, including inducers of plant resistance, enzymes, and antibiotics.
- The liquid preparation can be applied as a foliar spray, in drip irrigation, or in other liquid applications.
- Shelf life of the whole culture products is about six months with refrigeration, which is appropriate for the markets in Europe where it is being sold.
- Use rates for the products sold as foliar sprays are 5 to 10 liters per hectare, and costs are less than those of some other available biological products (Fig. 3).

The cost advantages of this process are several. First, the company manufacturing the product does not have to invest in production systems since there are numerous for-fee fermentation contractors who can efficiently produce the product, or alternatively, the company may acquire used fermentation equipment inexpensively (Fig. 3). The company selling the product also does not have to invest in down-stream equipment and processing (e.g., drying, formulation, and sizing operations). The resulting products can be mixtures of strains selected not only for direct effects on pathogens but also on the ability to promote plant growth and root development, induce systemic resistance (ISR), and other possible benefits. In Europe, in order to meet the registration requirements, these liquid products are mixed with other beneficials, such as *Trichoderma*-compatible strains of *B. subtilis* and mycorrhizal fungi, which permits sales as a biofertilizer under European guidelines. The advantages of using mixtures of organisms are likely to be more than simply a way through the regulatory maze, because combinations of organisms may provide benefits that single organisms do not. This system has found widespread application in several other countries. In these countries, the process includes discovery of local, adapted strains and identification of inexpensive local substrates and fermentation equipment in such a way as to ensure that good growth of the organism(s) occurs, contamination is avoided, and appropriate microbial metabolites are produced. Products based on this system are in operation in Libya, Egypt, Honduras, Chile, Argentina, Brazil, Peru, Columbia, Cuba, Venezuela, and China. These products are used to treat about 150,000 ha; the use of chemical pesticides is almost totally eliminated, crops are well protected, and yields are increased with less cost to growers. In addition to *Trichoderma* strains, entomopathogenic fungi in the genera *Beauveria, Metarhizium*, and *Paecilomyces*, as well as *Lecanicillium lecanii* are being produced. In many cases, materials directly from liquid fermenter vessels are mixed and applied directly in the field.

This model for development of useful products is much quicker and less expensive than the full pesticide registration marketing process described earlier. Assuming that production and formulation systems are known and available, the primary requirement is a good screening system in both the greenhouse and the field. A time frame, once a promising organism is identified, is 1 to 2 years at a cost of not more than $100,000 exclusive of marketing costs; however, production, processing, and formulation may add another $1 to 2 million if these processes are not available in-house or from contractors (Table 1). Thus, this approach is several-fold less expensive than full registration and reduces the time to market by 2 to 3 years. Registrations, for example by EPA, are designed to go more rapidly for biologicals than for synthetic chemical pesticides, but they still typically take several years, and in the United States, each state may require additional registrations that can take years as well.

Products and strains in this category can be patented or not, although patenting is usually preferred. Patent protection may be delayed quite a long time because the response time of the U.S. Patent Office is currently 3 to 5 years.

Model 3. Local production model. The model just described may not be the cheapest or simplest. In many parts of the world, a local production model has developed. In some cases, local production is small and could be considered a cottage industry. The distinctions between the preceding model and this one are not exact. For example, one system in Honduras for liquid fermentation with application through the irrigation system is an example of the local model, although it is fairly large.

There are several general components to this model, and it can be quite inexpensive (Table 1). First, the genera comprising many biocontrol agents occur naturally and are easily cultured. An advantage to this system is that the strains are isolated from an area where they will be used, so they are likely to be well adapted. Second, methods for semi-solid cultivation of these organisms are well known, and usually consist of culture on rice, cassava, wheat bran, or a similar substrate. Liquid fermentation can also be used. Third, they usually are grown just in time for use by local growers. This eliminates the need for extensive processing and formulation. In the United States and elsewhere, sales of composts, which may be disease suppressive, may fall into this category of locally produced products of varying composition that are in actuality entire complex ecosystems.

An example of a successful cottage industry occurs in Costa Rica, and was largely set up and run by the second author (Fig. 4). In this country, local strains were selected and used on different crops (50). In one use, a local strain of *T. atroviride* was selected based on efficacy testing. It is grown on autoclaved rice in a small room for direct use by growers by Asesoramiento Fitosanitario Laboratorio Doctor Obregón (Fig. 4A and B). In addition, *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium lecanii, Streptomyces griseoviridis, Clonostachys rosea, Paecilomyces fumosoroseus, Paecilomyces lilacinus, Pochonia chlamydosporia,* and *Azotobacter* also are produced. Thus, a grower contacts the producers, who grow the required amount to be ready on a specific date. This is produced for use without further processing or drying of the fresh material.

An example of the use of the *Trichoderma* product is on strawberries in Costa Rica. Colonized rice grains (3 g/plant) are placed directly into holes in the plastic-covered raised beds into which plants are placed (Fig. 4C). The plants are grown, and colonized rice grains are spread periodically over the rows (Fig. 1D). Plants grown in the nursery are also grown in the presence of the *Trichoderma*-colonized rice (Fig. 1E). The result has been a total absence of disease with no application of chemical pesticides. Plant roots are healthy and white, and even foliar diseases such as gray mold do not occur, even though environmental conditions are highly conducive to disease development. Costs of the *Trichoderma* product are $7/kg to the grower, and they are applied at a rate of about 12 kg/ha. Growers report that this is about one-sixth the cost of a synthetic chemical–based program (*personal communication*).

Another example is from India. The S. M. Swaminathan Research Foundation is promoting a low-external-input agriculture. Production systems are organized around "biovillages" that create

on-farm and nonfarm employment opportunities. In many cases, biological production was carried out by landless women who were trained by interactive and learning-by-doing techniques. Biological agents that can be produced include *T. viride* and *P. fluorescens*, *Azospirillum*, *Phosphobacter*, and mycorrhizal fungi. Units have been organized around production of specific organisms and have a production capacity of about 1,000 kg/month (57).

Results that can be attained by these local production systems, especially production of active but inexpensive biocontrol products, may not be attainable with more sophisticated production, delivery, and other techniques that are employed in more developed countries.

Small, cottage-scale production is not the only avenue; local production systems can also be used for larger operations. In one case, a grower was producing low-quality biocontrol agents on his own farm in Honduras. He contacted the fourth author, and a liquid fermentation system as described in the preceding section was installed with the financial support of UNIDO (United Nations Industrial Development Organization). The amount of material used was reduced from about 40 kg of semi-solid colonized medium to 3 to 4 liters/ha of liquid fermentation preparations, and is applied directly through the irrigation system. This has dramatically reduced costs and is used on about 5,000 ha of melons.

Brazil, which has been a focus of local production (6), now is requiring registration of biocontrol products. *T. stromaticum* was produced in large quantities for the control of witches'-broom caused by *Moniliophthora perniciosa*. This species of *Trichoderma* is at least very unusual, if not unique, in being host specific, always associated with cacao and the cacao witches'-broom pathogen. Sometimes *T. stromaticum* is found as an endophyte of cacao trunks. The development and testing of the system occurred on a small scale and was highly successful in the hands of growers; however, the Brazilian authorities have stopped the local production of this organism and are requiring that it be registered, so there is no longer any commercial production of this useful organism. The registration process is underway, but cacao is produced mostly by small growers for whom any control is too costly unless the crop price is high, so whether this single-crop use can be economically viable after registration (following the microbial pesticide model) is questionable.

Model 4. Governmental monopolies or state-supported production. Rossert (55) described a circumstance in which an entire nation had to rapidly shift from a conventional agricultural system to semi-organic farming on a large scale. Following the collapse in 1989–90 of trading relationships with the socialist block, Cuba faced a decrease of fertilizer and pesticide imports of more than 80% and a reduction in petroleum availability of about 50%. At the same time, food imports, which accounted for 57% of the caloric intake of the population, fell by more than half. The nation mobilized its scientific infrastructure to produce biopesticides and biofertilizers on a large scale. This, together with integrated pest management, biological pest control, vermiculture, cover cropping, and related ecologically based practices averted a catastrophic shortfall of food availability.

In Cuba, biological control production was of two types. The first was Centers for the Production of Entomophages and Entomopathogens (CREEs) (presumably there also were similar units for control of plant diseases) where "artisanal" production of biocontrol agents takes place. These centers were put into place through funding from a central bank to equip and construct each CREE: a medium-sized house with autoclaves and sterile rooms. They provided products free of charge to state-run coops and sold them to neighboring farms. Salaries and other costs were recovered by these sales. Generally, these are products with high quality standards for high-end markets—former state farms and large coops that produce for export and a lower priced product for local use by campesino level farms. The second type of production was yeast factories that were converted part-time to production of microbial products. Interestingly, the crop yields of small-scale campesino-level farms increased under this biological paradigm, but the larger farms could not adapt to more intensive hand labor practices and fared relatively poorly (55).

More recently, a similar model has developed in Venezuela. Venezuela is directly supporting an "alternative" agriculture system that includes the large-scale implementation of biological disease control methods. A complete biopesticide/biofertilizer develop ment program has been funded and run through the Instituto Nacional de Investigaciones Agricola, which includes selection, formulation, and distribution of strains of *Trichoderma* spp., *Metarhizium* spp., *Bacillus* spp., *Rhizobium* spp., and others. These state-made products (Fig. 5) are distributed widely and freely to farmers, to whom are also offered courses and learning materials focusing on the application concepts. As a result, last year in the southern area of the country alone, the biocontrol products were applied on about 80,000 ha (38). Similar systems were present in the former USSR.

Need for additional economic models. Even though a variety of models are described above, there are still issues not easily addressed by them. Most notably, as described earlier, it is necessary that effective screening and selection systems be in place to pro-

Fig. 4. Elements of the Costa Rican cottage industry. **A,** *Trichoderma* or other biocontrol agents are grown in small rooms on rice and in autoclavable bags. **B,** Upon completion of growth, the rice is thoroughly colonized and is ready for use. **C,** One site of application is strawberry fields on raised beds on plastic. **D,** At the time of planting, a high level (15 g) of colonized rice grains is placed in each planting hole in the plastic, and throughout the season, rice grains colonized by *Trichoderma* are spread by hand over the fields. **E,** Colonized grains also are applied in the nursery beds where roots are very healthy. In this system, this single biocontrol agent used in this way completely controls foliar, fruit, and root diseases in spite of the fact that this is a high rainfall area. Photograph is of fields treated using the system developed by the second author.

vide highly effective strains; without this step none of the models are likely to be effective. This requires time and money. The fourth author has in many cases provided strains, or selected local ones, that are effective for local or regional use (Figs. 3 and 4). In other cases, strains such as T22 and newer strains developed by the first author (Figs. 1 and 2) may be widely adapted and useful in a wide variety of locations and environmental conditions. This probably is a consequence in part of their ability to colonize plants, and thus they are subjected to diverse soil climates and conditions for only a short period of time. However, such strains follow an extended period of selection and development, and are usually patented and sold. Thus, to recoup investment, companies will need to sell the organisms at a profit. In poor countries, such products may be difficult to afford, especially for small holders who have need for the strains.

In many countries, it is considered essential to develop reliable biopesticides and biofertilizers. For this reason, AAPARI advocates public–private consortia to help with the costs of development of

Fig. 5. Result of a government-run program to produce and use *Trichoderma* spp. and other biocontrol agents in agriculture in Venezuela. Products made by public, state-run research and production Institutions are made available free to farmers, along with information and learning material. In the picture are products distributed by the Instituto Nacional de Investigaciones Agricola (INIA) in Venezuela.

useful microbial products, and if required, to develop pesticide registrations. AAPARI also proposes providing government-backed insurance or direct subsidies to further the development of biopesticides and biofertilizers. Companies associated with the first author are seeking to work with nongovernmental organizations to assist in making effective biocontrol systems available. Such systems are unlikely to occur in the United States and in other developed countries. This further exemplifies the disparity in approaches and in views of the importance of microbial agents around the world.

Summary of biocontrol production and marketing systems. The preceding sections have examined four very different models for commercial delivery of biocontrol systems to users, and have indicated the need for additional systems. The four current models are:

- Model 1: Full registration and marketing (the microbial pesticide model);
- Model 2: Inoculants, plant strengthening agents, and biofertilizers;
- Model 3: Local production systems;
- Model 4: Governmental monopolies or state-supported production.

The costs and time required for marketing for the first three are summarized in Table 1; costs for the fourth model are unavailable. A conservative value for the entire development of a registered biopesticide (first model), starting with discovery through significant market penetration, is at least $8 million, and it takes three to six years. Cost and time commitment largely explain why so few biocontrol agents following this model are available.

The biofertilizer/plant strengthening agent model is much less expensive and takes less time to reach the marketplace than the full registration model. Costs exclusive of a dedicated production facility are likely to be around $100,000, which is orders of magnitude less than for a fully registered product in model 1 (full registration and marketing). If a dedicated production facility is required, then costs are greater but still less than for model 1. Model 2 (inoculants, plant strengthening agents, and biofertilizers) requires about 25% as much time as model 1. Model 3 (local production or cottage industries) can be implemented for a low cost but cannot have a national or multinational scope. This model is sufficiently flexible that groups of growers or a single large grower can successfully implement this type of operation. It is very useful and rapid for developing countries, and it is being employed in many places.

Model 4, government monopolies or state-supported production systems, may be similar in size and scope to either model 2 or 3, but growers are provided the biological materials for their operations at low or no cost to them. This is strongly supported in Cuba and Venezuela.

Safety of Different Systems

Reasons for regulation are to ensure product safety to producers and suppliers, safety of the foods and other plant materials produced in the presence of disease control or other agents, and to protect the environment. So, the question becomes: do any of the four general economic models noted above pose more danger than the others? There are no hard data to answer this question, but we can propose some basic considerations. Of course, the microbial pesticides model requires toxicity testing, and so it would be expected to minimize safety considerations.

Generally, microorganisms described in this paper are nontoxic to mammals and nontarget organisms. Toxicity information is available for nine different *Trichoderma*-based products that cover five species, six *Metarhizium*-based products, two *Beauveria* products, *Pseudomonas* spp., and nine *Bacillus* products either for insect or disease control (14). Many have been tested in toxicological assays against various organisms and for nontarget effects, and none are toxic or harmful. For example, in cases where full toxicological data are available for these genera, acute oral toxicity is reported as being >500 to >2,000 mg/kg (meaning that at the highest level tested, no effects were seen) (14). In addition, for registered microbes and products based on them, a U.S. requirement is that any adverse reactions of any sort must be reported. There have been no reportable reactions to any of the cases noted above over many years of extensive use (14). Therefore, there is a significant body of data suggesting that harm is unlikely to arise at least from these organisms.

To take this further, where are risks likely to occur? Primary sources of risk are the organisms involved and the metabolites they produce, and there may be an equal level of risk from contaminating organisms or chemicals that are introduced in the production or formulation processes. For registered products, the actual products being sold must be tested, and so this would be expected to minimize toxic or other deleterious contaminants to the final products. However, for products produced with any of the four models, the testing and processes are only as good as the stringency of the production and quality control processes used by the manufacturer or producer. Products must be free of contaminants, and especially for materials with beneficial metabolites, undergo testing that ensures uniform and consistent products. With any of the four models, contaminating microorganisms can easily be introduced. These pose unknown and significant potential risks. Contamination may occur in a registered or unregistered product, and primarily depends upon the diligence of the producing company.

Some years ago, there was a potential human pathogen concern regarding *Burkholderia cepacia,* which was being considered as a registered biocontrol agent in various countries. However, researchers at the Centers for Disease Control stated, "In the past 2 decades, *Burkholderia cepacia* has emerged as a human pathogen causing numerous outbreaks, particularly among cystic fibrosis (CF) patients. One highly transmissible strain has spread across North America and Britain, and another between hospitalized CF and non-CF patients. Meanwhile, the organism has been developed as a biopesticide for protecting crops against fungal diseases and has potential as a bioremediation agent for breaking down recalcitrant herbicides and pesticides. However, *B. cepacia* is inherently resistant to multiple antibiotics; selection of strains 'safe' for environmental application is not at present possible phenotypically or genotypically; molecular epidemiology and phylogenetic studies demonstrate that highly transmissible strains emerge randomly; and the organism has a capacity for rapid mutation and adaptation (facilitated by numerous insertion sequences), and a large, complex genome divided into separate chromosomes. Therefore, the widespread agricultural use of *B. cepacia* should be approached with caution" (33). This information largely prevented use of this organism over most of the world. Another species of *Burkholderia* (*B. vietnamiensis*) is being marketed successfully for biocontrol of soil fungal pathogens, plant-pathogenic bacteria, and nematodes in Colombia, with the trade name Botrycid, by the company Agrobiologios (1) under model 2 (inoculants, plant strengthening agents, and biofertilizers). This company is well aware of the hazards of *B. cepacia*, and the marketed strain is different from it. The potential for doing harm is of concern to those in biocontrol production companies or organizations, who are unlikely to release or sell organisms that have known health or serious environmental issues. The CDC warning appears to have been sufficient to halt development of *B. cepacia* without any governmental regulatory actions.

There are resources that categorize risk due to specific microorganisms. For example, the American Biological Safety Association (ABSA) is devoted to developing and maintaining professional standards for the field of biological safety and advancing biological safety as a scientific discipline through education and research (3). The ABSA website (3) includes a searchable database that is useful to persons considering development of a plant microbial agent. They categorize risk on a 1 to 4 scale, with 1 being low risk and 4 being very high. In all probability, any organism with a rating of 3 should not be considered as a biological agent for plants, and ones with a rating of 2 are doubtful. ABSA ratings integrate safety ratings from various governmental agencies, such as the U.S. National Institutes of Health. For example, *Burkholderia* spp. are considered to be in group 2 or 3, so those in group 3 should never be used, and those in 2 only with great caution.

This example demonstrates that if potential hazards are not considered, it would be possible to introduce a problematic organism or product. Model 1 (full registration and marketing [the microbial pesticide model]) limits this risk because the registration processes should detect public health issues and prevent wide distribution. However, because many or most strains probably will be marketed under the other models, the potential for harm is present and cannot be avoided completely. Products containing a wide variety of organisms, such as manures or manure teas, may pose the greatest risk because the microbial composition is unlikely to be known and probably will vary from batch to batch.

Among biocontrol organisms, the authors know the most about *Trichoderma*, which can serve as a model of the advantages of modern taxonomic treatments for beneficial microorganisms. If we can identify specific species or groups of strains that may be problematic, then it is easier to avoid these organisms for biocontrol purposes. In the development of a modern taxonomy for *Trichoderma*, and the development of a broader species concept, strains with specific attributes were located in specific species. Until recently, this genus was divided into nine species groups based on growth, colony, and spore characteristics (54). These species groupings had little meaning in terms of ecological or physiological properties since there was tremendous diversity within each species. Over the past 15 years, the taxonomy of *Trichoderma* has been revised and is now based on genetic sequence data. As a consequence, there now are more than 100 species and the taxa are beginning to make more sense in terms of their capabilities and characteristics. A very convenient system of classification is now available. Standard primer sequences have been provided, and once amplification of a DNA segment has been made, the sequence is entered into a *Trichoderma* bar code database, and the presumed species identification returns almost immediately (38). In addition, GenBank contains recent data on gene sequences that has not yet made its way into the bar code database. These efforts have resulted in species definitions that are distinct and unambiguous. Based on modern taxonomy, the potential "bad actors" in the genus belong to species that are genetically different from biocontrol species. Information on the current taxa, with emphasis on the problematic ones, follows. It may be that other genera of biocontrol organisms can be similarly defined, and potentially dangerous species identified.

Mushroom pathogens. Some strains initially identified as *T. harzianum* were able to cause serious diseases of mushrooms, and in the Rifai classification (54) biocontrol strains of *T. harzianum* and mushroom pathogens were all part of *T. harzianum*. Three species, now defined as *T. aggressivum*, *T. pleurotum,* and *T. pleuroticola,* are mushroom pathogens and have now been shown to be genetically distinct from the biocontrol strains of *T. harzianum* (43,56). Thus, the mushroom pathogens can be readily identified and their use avoided in biocontrol and plant growth promotion products. Because of this development, the U.S. Animal and Plant Health Inspection Service (APHIS) has indicated to the senior author that this agency does not wish oversight of strains in his possession if, based on this new molecular criteria, the organism can be proven to be other than *T. aggressivum*. This does not apply to any strains produced transgenically or imported into the United States, which still require standard APHIS import approval. This is a reasonable approach and one that speeds research progress. It should be noted that this lack of oversight was provided to the senior author and others would have to apply separately for such approval.

Trichothecene antibiotics. The most commonly reported species of *Trichoderma*, *T. viride* and *T. harzianum,* are reported to produce the trichothecene-type toxins trichodermin or harzianum A. However, Degenkolb et al. (15) found that strains reported to produce these toxins had been misidentified. They described a completely new lineage, the *T. brevicompactum* complex, where all of the four species produce trichodermin or harzianum A, depending on the species. These authors (15) considered reports of trichothecene production by other species, and they concluded that they were most likely incorrect because of inappropriate methods of biochemical analysis or misidentification of the fungi. Thus, trichothecene antibiotics are produced by *Trichoderma,* but, as defined by modern taxonomic approaches, this production is restricted to a group of closely related species that are not related to any that are used in biological control applications.

Other antibiotics. Other antibiotics produced by *Trichoderma* species, especially *T. virens*, include the epipolythiodioxopiperazine metabolite gliotoxin. Gliotoxin, as noted earlier, has been considered important in the ability of *T. virens* to control soilborne pathogens such as *Fusarium, Sclerotinia, Phytophthora, Pythium,* and *Rhizoctonia.* It is produced by various fungi, including *Aspergillus fumigatus,* but in *Trichoderma* only a subgroup of *T. virens* strains, the "Q" strains (34), produce gliotoxin. Gliotoxin is an immunosuppressive mycotoxin that, when produced by the unrelated mold *Aspergillus fumigatus* in maize silage, can pose a hazard to animal and human health (53). We are not aware of any cases of mycotoxicosis attributed to *T. virens*, although there is a role for gliotoxin in aspergillosis caused by *A. fumigatus* (35). Its production is restricted to *T. virens*.

Other antibiotic compounds, such as the volatile pyrones, have been described for *Trichoderma* (34). The lactone 6-pentyl-alpha pyrone inhibits spore germination in various pathogens including *Botrytis* and *Phytophthora*; thus, strains that produce it could have potential for biological control. This compound has a distinctive and pleasant coconut odor, and the only species in *Trichoderma* that have this odor are clustered in one clade, the *T. viride*/*H. rufa* clade. Because this lactone is present in fruits such as peach, it is used as a flavoring agent despite its antifungal properties (9). Thus, it is not a significant safety hazard.

One group of antibiotics of significant interest is the peptaibols (16,17,51). These compounds are small peptides that form voltage-gated ion channels in lipid membranes. The peptides contain a high level of the rare amino acid α-aminoisobutyric acid, and are not synthesized via ribosomes but instead by a large synthase. A gene encoding an 18 aa peptaibol was 68 kb in size and produced a mixture of similar peptides (67). Some of these compounds can lyse red blood cells. Most strains of *Trichoderma* spp. probably have the ability to produce one or more of these antibiotics, but there is a great deal of diversity. The patterns of peptaibiotics production are specific to species (16). Moreover, in many cases, they are not constitutively expressed, but are only produced when induced by fungal cell walls or other elicitors.

Growth at 37°C. A few *Trichoderma* species are able to grow at temperatures of 37°C or greater, human body temperature. Growth at this temperature is a characteristic of *T. longibrachiatum* and its relatives, and *T. longibrachiatum* is often cited as an opportunistic human pathogen, especially in immunocompromised individuals (44). These species are not commonly cited in the biological control literature, but because of the high incidence of HIV-positive individuals in some developing countries, these species should not be used in biological control schemes. A few individual species of other strains also can grow at 37°C, and screening should be conducted for this property in potential biocontrol strains. *T. reesei*, which is an industrial standard for cellulase production, is a close relative of *T. longibrachiatum*; although it is capable of growing at 37°C, and despite its widespread use in industrial applications, it has never been associated with any human or animal pathogenicity. However, with increasing interest in ethanol production from biomass via cellulase production by *T. reesei* and other molds, care must be taken in handling them because of their potential for infecting immunocompromised individuals.

Hypersensitive reactions. Fungal enzymes can induce hypersensitivity among workers in the animal feeds industry and in indoor environments (5,10). Enzymes from *Trichoderma* are strong sensitizers (10) and must be handled with care. This is particularly significant when inoculum is being prepared for biological control application. It would be best not to dry inoculum in an exposed environment and especially not in an area that circulates air to other parts of the building. People who handle the inoculum should

take special care because of the possibility that sensitivity to *Trichoderma* can develop over time, although, again so far as we are aware, this has not occurred even in facilities where hundreds of thousands of kilograms of *Trichoderma* products are prepared.

To summarize: modern taxonomy can be of great importance in defining species that may be of concern because of the toxins or other metabolites they might produce or because of their biological propensities. In large part, the potentially harmful strains of *Trichoderma* have now been localized in a few well-defined species. If the use of these is avoided in biocontrol products, the potential for harm can be minimized.

Genome sequencing of representative species, together with annotation of genes encoding proteins giving rise to important metabolites of concern for health or that are required for biocontrol, would be of great assistance in determining the genetic potential of species to produce toxins of concern. Three strains of *Trichoderma*, representing *T. virens*, *T. atroviride*, and the cellulase-producing *T. reesei*, have been sequenced and annotation is at different stages for these. However, it is apparent that there is a good deal of diversity in both the genes represented and their arrangement within chromosomes. Given the diversity and the importance of these organisms, and their likelihood for large-scale introduction into the environment by regulated or unregulated mechanisms, much more detailed information must be obtained from sequences of additional strains.

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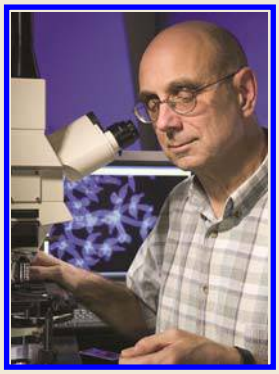
Gary E. Harman

Miguel A. Obregón



Gary J. Samuels

Matteo Lorito

Dr. Harman received his B.S. from Colorado State University in 1966 and his Ph.D. from Oregon State University in 1970. He was a postdoctoral associate at North Carolina State University in 1969 and 1970, and then joined the faculty of Cornell University's New York State Agricultural Experiment Station in Geneva in 1970, as an assistant professor. He became a full professor in 1984 and has held several administrative positions at Cornell. He is a strong proponent of commercialization of biocontrol systems and, in 1993, co-founded TGT Incorporated, which later became BioWorks Inc. He served in various capacities in this company, including CEO for a year, after which he returned to Cornell full time. The company sells several products, many based on *Trichoderma harzianum* T22, which was produced in his lab in 1983. He also is involved with development of improved strains in conjunction with Advanced Biological Marketing of Van Wert, OH. He cofounded another company, Terrenew, LLC, which manufactures a variety of products based on agricultural wastes for use in environmental remediation and horticulture including greenhouses. He has received the Award of Merit in Plant Pathology from the NE Division of APS and is a Fellow of APS.

Dr. Obregón is presently the founder and CEO of a private company that investigates, produces, and commercializes biopesticides and biofertilizers based on beneficial fungi and bacteria, and provides a phytopathological service to farmers in Central and South America. He also serves as a professor of plant pathology (Ph.D. program) at the Instituto Tecnológico de Costa Rica (Technologic Institute of Costa Rica) and formerly was a professor of plant pathology at the Institute of Agriculture in Plovdiv (Bulgaria). He received his Ph.D. in plant pathology in Bulgaria in 1992, and has been a member of the Costa Rica Association of Plant Pathologists and of the APS division Caribe, the representative for Costa Rica at the International Organization of Biological Control (IOBC), the president of the Costa Rica Association for the Development of Biological Alternatives for Agriculture, and research scientist on biological control at the Laboratory of Plant Protection of the National Center on Organic Agriculture (INA) in Costa Rica. He has developed several commercial products based on *Trichoderma* spp., *Metarhizium* spp., *Lecanicillium lecanii, Beauveria* spp., *Pseudomonas fluorescens, Azotobacter,* and *Rhizobium.*

Dr. Samuels received a B.S. with major in botany from Pennsylvania State University in 1966, and an M.A. (1968) and Ph.D. (1971) in biology from Columbia University with emphasis on botany while a graduate fellow at the New York Botanical Garden under the direction of Clark T. Rogerson. He joined the staff of the New Zealand Department of Scientific and Industrial Research, Plant Diseases Division in Auckland in 1973, where he worked until 1985 as a fungal taxonomist. Between 1986 and 1989, he was a research associate at the New Botanical Garden undertaking exploration in tropical America. Since 1989, Dr. Samuels has been on the staff of the U.S. Department of Agriculture, Agricultural Research Service, Systematic Mycology and Microbiology laboratory in Beltsville as a fungal taxonomist emphasizing *Trichoderma*. He has published about 250 peer-reviewed articles on fungal taxonomy, of which approximately 70 concern taxonomy of *Trichoderma* and other fungi used in biological control, and has developed an online interactive key to *Trichoderma* species: http://nt.ars-grin.gov/taxadescriptions/keys/TrichodermaIndex.cfm. He is a Fellow of the Mycological Society of America, which in 2010 honored him as Distinguished Mycologist.

Dr. Lorito is a full professor of plant pathology and of biotechnology applied to plant pathology in the Faculty of Agriculture of the University of Naples Federico II (UNINA) in Italy. He is director of the graduate school in Agro-biology and Agro-chemistry and president of the undergraduate courses in Agricultural Sciences of UNINA. His major interest is in various aspects of biological control, in particular on the fungal agent Trichoderma, of which he has become an internationally recognized authority. His team has discovered molecular factors that regulate plant-pathogen-biocontrol agent interactions and antimicrobial genes useful for increasing plant disease resistance. His research has contributed to the development of new biopesticide and biofertilizer products. He is an APS Fellow and has been given awards by the Organization for Economic Co-operation and Development and the Fulbright Research Program. He is a senior editor of Molecular Plant-Microbe Interactions and an elected member (and treasurer) of the International Society on Molecular Plant-Microbe Interactions board of directors.

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