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COMMENTARY

Safety of Microorganisms Intended for Pest and Plant Disease Control: A Framework for Scientific Evaluation

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Microorganisms are enormous but largely untapped natural resources for biological control of pests and diseases. There are two primary reasons for their underployment for pest or disease control: (1) the technical difficulties of using microorganisms for biological control, owing to a lack of fundamental information on them and their ecology, and (2) the costs of product development and regulatory approvals required for each strain, formulation, and use. Agriculture and forestry benefit greatly from the resident communities of microorganisms responsible for naturally occurring biological control of pest species, but additional benefits are achieved by introducing/applying them when or where needed. This can be done as (1) an inoculative release, (2) an augmentative application, or (3) an inundative application. Because of their specificity, different microbial biocontrol agents typically are needed to control different pests or the same pest in different environments. Four potential adverse effects are identified as safety issues (hazards) associated with the use of microorganisms for the biological control of plant pests and diseases. These are: (1) displacement of nontarget microorganisms, (2) allergenicity to humans and other animals, (3) toxigenicity to nontarget organisms, and (4) pathogenicity to nontarget organisms. Except for allergenicity, these are the same attributes that contribute to the efficacy of microbial biocontrol agents toward the target pest species. The probability of occurrence of a particular adverse nontarget effect of a microbial biocontrol agent may be a function of geographic origin or a specific trait genetically added or modified, but the safety issues are the still the same, including whether the microorganism intended for pest or disease control is indigenous, nonindigenous (imported and released), or genetically modified by traditional or recombinant DNA (rDNA) technology. Likewise, the probability of occurrence of a particular adverse nontarget effect may vary with method of application, e.g., whether as an aerosol, soil treatment, baits, or seed treatment, and may increase with increased scale of use, but the safety issues are still the same, including whether the microorganism is used for an inoculative release or augmentative or inundative application. Existing practices for managing microorganisms in the environment (e.g., plant pathogens, *Rhizobium*, plant inoculants) provide experience and options for managing the risks of microorganisms applied for pest and disease control. Moreover, experience to date indicates that any adverse nontarget effects, should they occur, are likely to be short-term or transitory effects that can, if significant, be eliminated by terminating use of the microbial biocontrol agent. In contrast, production agriculture as currently practiced, such as the use of tillage and crop rotations, has significant and long-term effects on nontarget organisms, including the intentional and unintentional displacement of microorganisms. Even the decision to leave plant pests and diseases unmanaged could have significant long-term environmental effects on nontarget organisms. Potential safety issues associated with the use of microbial biocontrol must therefore be properly identified and compared with the impact of other options for managing the pest or leaving the pest unmanaged. This paper provides a scientific framework for this process.

KEY WORDS: **biological control; entomopathogens; microorganisms; weeds; arthropod pests; plant-parasitic nematodes; plant pathogens; biosafety; antagonists.**

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INTRODUCTION

Microorganisms[1] are vast but largely untapped natural resources for use in biological control of pests[2] and plant diseases.[3] Microbial biocontrol agents include natural enemies[4] and antagonists[5] of pests. Although more than 850 beneficial arthropods (natural enemies) have been used in agriculture, forestry, or other managed ecosystems in the United States as parasitoids and predators of arthropod pests and herbivores of weeds, only about 30 species of microbial biocontrol agents have been used for pest and disease control in the United States. About one-third of these microorganisms are *Bacillus* species and insect viruses (Table 1).

There are two primary reasons for the underemployment of microbial agents introduced/applied for pest or disease control: (1) the technical difficulties of using microorganisms in this way, owing to a lack of fundamental information on them and their ecology; and (2) the cost to laboratories, agencies, or companies of product development and obtaining regulatory approvals, which commonly cannot be justified because the pest- and/or environment-specific nature of these agents limits their use to niche markets (Cook, 1993a; Schippers *et al.*, 1995).

Microbial biocontrol potentially includes a wide range of approaches. For example, some microbial pathogens of insect pests and weeds have been imported as natural enemies from the native home of the pest and released (introduced) with approvals in the same way that parasitoids and predators of pest species have been imported and released for biological control. The use of microbial pathogens in this manner, where the pathogen is introduced and establishes in the environment in a density-dependent relationship with the targeted pest species, illustrates the concept of classical biological control (DeBach, 1964). Strains of *Bacillus thuringiensis* Berliner (Bt), on the other hand, which produce crystalline proteins lethal to certain insects, are applied as insecticidal sprays using formulations based on these proteins. These two examples represent the extremes of a continuum for use of microorganisms for biological control.

Regardless of the approach, risk is the combination of hazard and exposure. Thus, the risk of using an agent with some known hazard can be reduced by limiting the exposure, and the use of an agent with no known hazards and high exposure presents little or no risk. Unfortunately, the hazards associated with microorganisms often are not properly identified or evaluated and the resulting risk/benefit analysis is therefore inaccurate. In this article, we attempt to identify the potential adverse effects (hazards) associated with microorganisms when used to control pests and plant diseases, the level of risk that might be expected from the use of microorganisms in various control strategies, and some options available to manage any risks that might be identified. Comprehensive reviews on the risks and benefits of biological control have recently been published (Hokkanen and Lynch, 1995; National Research Council, 1995).

This document is restricted to safety considerations of live microorganisms introduced for and claimed to provide biological control. It takes into account indigenous, nonindigenous,[6] and genetically altered microorganisms, including: (1) viruses and viroids that can infect plants (weeds or parasitic plants), plant-pathogenic fungi, plant-pathogenic bacteria, plant-parasitic nematodes, or arthropod pests; (2) bacteria and other prokaryotes that can infect plants (weeds or parasitic plants), plant-parasitic nematodes, or arthropod pests, or compete with or inhibit (antagonize) plant-pathogenic fungi, bacteria, nematodes, or arthropod pests (e.g., bacterial endophytes); (3) fungi that can infect plants (weeds or parasitic plants), arthropod pests, plant-parasitic nematodes, other fungi, or complete with or inhibit plant-pathogenic microorganisms, plant-parasitic nematodes, or arthropod pests (e.g., fungal endophytes); (4) protozoa and other microfauna that infect arthropod pests and plant-parasitic nematodes.

Some of the fundamental premises of classical biological control have recently been questioned (e.g., Howarth, 1991; Lockwood, 1993a, b; Miller and Aplet, 1993). Our report makes no attempt to list the pros and cons of using biological control in agriculture. Rather, we identify and discuss the safety issues identified with the use

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[1] "Microorganism" refers to (i) viruses of plant, fungi, arthropods, bacteria, and nematodes; (ii) bacteria, including mycoplasmas and spiroplasmas; (iii) fungi; and (iv) protozoa.

[2] "Pest" refers to weeds; plant parasitic nematodes; arthropod pests of plants, animals, and people; plant pathogenic viruses; prokaryotes (including bacteria, mycoplasma-like organisms [MLOs], and spiroplasmas); and fungi. Pests are organisms at population densities that cause death or injury, or constitute a nuisance to crops, livestock, pets, people, or the environment.

[3] "Plant disease" refers to infectious diseases caused by plant pathogenic viruses, viroids, bacteria, mycoplasma-like organisms (MLOs), spiroplasma, fungi, and nematodes. Disease is a process that results from a compatible interaction between virulent pathogen and susceptible plant.

[4] "Natural enemy" is used in this report to include the microbial pathogens of weeds, arthropod pests, and plant parasitic nematodes. In the broadest sense, natural enemies also include arthropod parasitoids and predators of these pests.

[5] "Antagonist," a kind of natural enemy, is used to mean a microorganism with potential to interfere in the life processes of another organism. Antagonists of insect pests include the endophytic bacteria and fungi with ability to discourage or disrupt insect feeding on plants through production of toxic substances. Antagonists of plant pathogens include microorganisms with ability to exert their effect through competition, antibiosis, or direct exploitation of pathogenic inoculum.

[6] "Nonindigenous," as used in this document, refers to any isolate of a microorganism from outside of North America.

TABLE 1

Microbial Biocontrol Agents Currently Registered in the United States[a]

		Products registered		
EPA microbial pesticide A.I.	OPP P.C. No.	Year	No.	Pest/disease controlled
Bacteria				
Agrobacterium radiobacter				
● K84	114201	1979	2	*Agrobacterium tumefaciens* (crown gall)
Bacillus popilliae & *B. lentimorbus*	054501	1948	2	Japanese beetle larvae
Bacillus thuringiensis				
subsp. *aizawai*	006403	1992	2	lepidopteran larvae
● GC-91	006426	1992	2	lepidopteran larvae
subsp. *israelensis*	006401	1981	25	dipteran larvae
subsp. *kurstaki*	006402	1961	133	lepidopteran larvae
● EG 2348	006424	1989	4	lepidopteran larvae
● EG2424	006422	1989	1	lepidopteran larvae
● EG2371	006423	1990	3	lepidopteran larvae
● BMP123	006407	1993	5	lepidopteran larvae
● EG7673	006448	1995	2	Colorado potato beetle
subsp. *tenebrionis*[b]	128946	1988	1	coleopteran larvae
subsp. *tenebrionis*	006405	1988	6	coleopteran larvae
Bacillus sphaericus	119801	1991	1	dipteran larvae
Bacillus subtilis				
● GBO3	129068	1992	3	damping-off disease
● MBI 600	129082	1994	1	damping-off disease
Pseudomonas cepacia				
type Wisconsin	006419	1992	2	damping-off disease & nematodes
Pseudomonas fluorescens				
● EG1053	006440	1988	2	damping-off disease
● A506	006438	1992	2	ice-crystallizing *Pseudomonas* species
● 1629RS	006439	1992	2	ice-crystallizing *Pseudomonas* species
● NCIB 12089	006429	1994	1	bacterial blotch of mushrooms
● *Bt* var. *tenebrionis*[b] d-endotoxin in killed *P. fluorescens*	006409	1991	2	lepidopteran larvae
● *Bt* subsp. *kurstaki* d-endotoxin in killed *P. fluorescens*	006410	1991	1	coleopteran larvae
Pseudomonas syringae				
● 742RS	006411	1992	2	ice-crystallizing *Pseudomonas* species
● ESC 10	006441	1995	2	post-harvest decay-causing pathogens of fruit
● ESC 11	006451	1995	2	post-harvest decay-causing pathogens of fruit
Streptomyces griseoviridis				
● K61	129069	1993	5	damping-off disease
Yeasts				
Candida oleophila				
● I-182	021008	1995	2	post-harvest decay-causing pathogens of fruit
Filamentous fungi				
Ampelomyces quisqualis				
● M10	021007	1994	2	powdery mildew
Beauveria bassiana				
● GHA	128924	1995	6	grasshopper, crickets, locusts, whiteflies
Phytophthora palmivora				
● MVW	111301	1981	1	citrus strangler vine
Colletotrichum gloeosporioides f.sp. *aeschynomene*				
● ATCC 20358	226300	1982	1	northern joint vetch
Gliocladium virens				
● GL-21	129000	1990	2	*Pythium, Rhizoctonia*
Lagenidium giganteum	12984	1991	3	mosquito larvae
Metarhizium anisopliae				
● ESF-1	129056	1993	7	cockroaches, flies, termites
Puccinia canaliculata				
● ATCC 400199	129085	1993	1	yellow nutsedge
Trichoderma harzianum				
● ATCC 20476	128903	1989	1	tree wound decay
● RL-AG2	119202	1990	4	damping-off disease
Trichoderma polysporum				
● ATCC 20475	128902	1989	1	wood rot
Protozoa				
Nosema locustae	117001	1980	3	grasshoppers
Viruses				
Autographa californica NPV[c]	128885	1994	1	alfalfa looper larvae
Heliothis NPV	107301	1975	1	cotton bollworm, budworm
Douglas fir tussock moth NPV	107302	1976	1	Douglas fir tussock moth larvae
Gypsy moth NPV	107303	1978	3	gypsy moth larvae
Beet armyworm NPV	129078	1993	1	beet armyworm larvae

TABLE 1

Microbial Biocontrol Agents Currently Registered in the United States[a]

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[a] This listing is current as of May 1995 and indicates all "active ingredients" (A.I.) of microbial biocontrol agents defined and registered by the U.S. Environmental Protection Agency (EPA) as "microbial pesticides." The microorganisms are listed alphabetically by general organismal type, with specific strain registrations prefixed by a bullet (●); the information provided for each registered microbe includes the EPA, Office of Pesticide Programs Pesticide Chemical number (OPP P.D. No.), year of first registration of each active ingredient, number of registered products including this active ingredient, and the target organisms or diseases for which use of the active ingredient is registered.

[b] Registered as subsp. *san diego*, now recognized as subsp. *tenebrionis*.

[c] NPV, nuclear polyhedrosis virus.

of microorganisms in various approaches to biological control, including classical biological control with microorganisms. Our intent is to frame the issues pertinent to the safe use and management of microorganisms introduced or applied for biological control of pests. This report is intended for use/consideration by academia, industry, State and Federal government agencies, and others interested in the safe use of microorganisms for pest control.

We do not address: (1) conservation or enhancement of microbial biocontrol by resident naturally occurring agents, such as achieved by some cultural practices, other than to illustrate concepts; (2) the efficacy of microorganisms introduced/applied for pest control; (3) the inert materials used as carriers or to facilitate introduction, application, or survival of microorganisms for pest control; (4) contaminants that may occur with microbial biocontrol agents; (5) substances extracted from microorganisms, such as antibiotics or other biologically active metabolites or gene products applied directly as natural products (pesticides) for pest control, other than Bt; (6) control of plant virus diseases by cross protection using mild strains (Fulton, 1986), or by coat-protein-mediated resistance or other use of virus genes as transgenes in plants (Beachy *et al.*, 1990); and (7) the use of genes from microorganisms as transgenes expressed in plants for pest control (Vaeck *et al.*, 1987).

SAFETY ISSUES

In this section, we identify four safety issues as the potential unintended adverse effects of microbial biocontrol agents on nontarget organisms. In these examples, humans, domesticated animals, and wildlife are included as organisms. The potential safety issues are:

I. Competitive displacement

II. Allergenicity

III. Toxigenicity of antibiotics and other biologically active metabolites

IV. Pathogenicity

Of these potential unintended adverse effects, competitive displacement, toxigenicity, and pathogenicity are also intended effects to target organisms (see below). These four safety issues represent the unintended adverse effects on nontarget organisms whether the microorganism is indigenous or nonindigenous, naturally occurring or modified by classical genetic or recombinant DNA (rDNA) techniques. Similar safety issues were listed by Burges (1981b).

Gene transfer offers a means to introduce specific traits for new or more precise intended effects or to eliminate traits for potential adverse effects. Traits of microbial biocontrol agents potentially are also transferred naturally to other microorganisms in the environment by gene transfer. Such gene transfer could result in a new genotype of naturally occurring microorganism less able, more able, or of the same ability as the source microorganism to establish and maintain its population in competition with other microorganisms. A safety issue would arise if the gene transfer resulted in a microorganism with potential to have one of the four unintended adverse effects listed above. Any risks would depend on many factors, including the biology of the recipient organism, nature of the trait transferred, and the environment. There is no safety issue inherent in the process of gene transfer in itself, whether it occurs naturally or is done deliberately.

Competitive Displacement—Target Effect

Competitive displacement is used here to mean an array of effects resulting from microbe–microbe interactions, including preemption, exclusion, and other outcomes with potential over time to allow a microorganism introduced or applied for biological control to assume the habitat of a nontarget native organism. For example, application of the saprophytic fungus *Phlebia gigantea* (Fr.:Fr.) Donk as a spore suspension to the freshly cut surface of a pine stump allows this fungus to become established in advance of the arrival of airborne spores of *Heterobasidion annosum* (Fr.:Fr.) Bref., the cause of annosus root rot of pine (Rishbeth, 1975). Without the prior colonization of the stump surface by *P. gigantea, H. annosum* is capable of colonizing the entire stump, and thereafter attacking pine trees with roots naturally grafted to those of the colonized stump. Biological control results from preemption of a foodbase needed by the pathogen to infect pine roots. The spores of *P. gigantea* can be suspended in a bucket of water and brushed on the freshly cut pine stump, or they can be suspended in the oil used to lubricate the chain saw (Artman, 1972).

Some microorganisms, e.g., yeasts and bacteria, have potential to protect wounds and other infection sites on fruit by prior-establishment and competition with pathogens for sites and nutrients. These kinds of microorganisms are currently under study for potential use in biological control of fruit rots during storage (Roberts, 1990; Wilson and Wisniewski, 1989; Wisniewski *et al.*, 1991). *Candida oleophila* Montrocher (Aspire) and two isolates of *Pseudomonas syringae* van Hall with this ability (Bio-Save 10 and Bio-Save 11) were registered with the U.S. Environmental Protection Agency (EPA) in 1995 (Table 1).

As another example of competitive effects, a nonpathogenic strain of *P. syringae* rendered unable to nucleate ice formation (ice-minus) in supercooled water (water cooled to −2 to −9°C but still liquid) can be applied to newly forming leaves of frost-sensitive plants such as tomato and potato to preempt the subsequent natural establishment of ice-nucleation-active (INA) bacteria

(Lindow, 1983). Biological control results from prior establishment of the ice-minus strain, which prevents establishment of the INA strain through competition for nutrients. It is worth noting that an INA strain of *P. syringae* has been used commercially in the U.S. as Sno-Max, applied as an aerosol of bacterial cells to produce snow in ski areas, with no evidence of risk to people or the environment having thus far been identified.

Nonpathogenic bacteria that produce siderophores (natural iron-chelating compounds), if established in adequate populations in the rhizosphere, provide biological control of certain root pathogens by robbing them of needed iron (Kloepper *et al.,* 1980; Schippers *et al.*, 1987). Biological control can also result from rhizosphere-inhabiting nonpathogens out-competing the pathogen for carbon and energy or nitrogenous compounds (Baker, 1968).

Biological control through competitive displacement using strains closely related and ecologically similar to pathogens has great potential for plant disease management. This includes either naturally occurring nonpathogenic relatives of the pathogen (Ogawa and Komada, 1984) or pathogens rendered nonpathogenic by deletion or modification of critical genes (Freeman and Rodriguez, 1993).

Competitive Displacement—Nontarget Effect

Microorganisms introduced for biological control purposes potentially could preempt or displace nontarget microorganisms as one of many microbe–microbe interactions mediated through competition for sites or nutrients. For example, the early and deliberate establishment of the saprophytic *P. gigantea* on the freshly cut stumps of pine to preempt establishment of the annosus root rot fungus, theoretically, could also preempt the establishment of some other wood-colonizing saprophyte. This kind of effect is no different from the effects of many other kinds of temporal and spatial displacements of nontarget microorganisms in the rhizosphere, within crop residue, on plants, or elsewhere in the environment associated with many common agricultural practices. Moreover, if the preempted/displaced nontarget saprophyte is widespread in nature, with ability to colonize other substrates, its unintentional preemption along with a target pathogen as a colonist of stumps would seem inconsequential to the ecology of the nontarget microorganism. Unfortunately, there is no reliable way to monitor and document the effects of competitive displacement on the ecology of nontarget microorganisms. It might be instructive to determine the extent to which preemption of *H. annosum* as a colonist of freshly cut stumps has impacted the ecology of this fungus in forest ecosystems.

Few if any examples of competitive displacement of insects as a consequence of the use of microbes are recorded. In the UK, an induced viral infection eliminated a rabbit population and consequently changed the local vegetation patterns to cause extinction of a local population of the large blue butterfly, *Maculina arion* L. (Moore, 1989). Howarth (1991) and Lockwood (1993a,b) discuss hypothetically how the ecosystem-level nontarget effects might eventually lead to extinctions of populations of nontarget organisms.

Allergenicity—Target Effect

There are no intended target effects for allergenicity as a mechanism of microbial biocontrol.

Allergenicity—Nontarget Effect

Certain kinds of pollen and airborne fungal spores are inevitably present in the air we breath and cause allergies in sensitive people, domestic animals, and wildlife. Only a very small proportion of fungal species produce spores that cause allergies or allergic reactions (Latge and Paris, 1991). Potentially, a biocontrol microorganism released into the air could cause allergies or elicit allergic reactions in humans. Workers in production facilities exposed repeatedly to high concentrations of spores of fungi such as *Beauveria* or *Metarhizium* spp. may develop hypersensitive reactions (York, 1958), although such reactions are not known for people living in application areas. Allergenicity is therefore a potential safety concern as a result of direct exposure by workers at the production center or the application site but is not likely to be a public health issue. Exposure to allergenic particles of all types is common in agricultural settings, and allergies will not be a new problem because of the use of microbial biocontrol agents, but should be addressed as a safety issue during development and application.

Toxigenicity—Target Effect

Antibiosis is the inhibition or disruption of the behavior of one organism by the metabolite of another organism (Cook and Baker, 1983). Microorganisms produce many kinds of compounds inhibitory to the growth, development, or behavior of other organisms.

Some microorganisms, known as endophytes, live within leaves or other plant parts where they derive benefit from their host while also producing chemicals disruptive to feeding by insects. For example, species of *Acremonium* in the leaves of ryegrass and fescues produce alkaloids toxic to aphids and other insect herbivores (Siegel *et al.*, 1987). If the plant produced these alkaloids, this would be an example of host-plant resistance to insects by antibiosis. Instead, the fungus is the source of the antibiosis. Seed companies in the southern states where this endophyte is especially common certify the level of natural endophyte infection of grass seed rather than inoculate seeds with the endophyte.

Agrobacterium radiobacter (Beijerinck and van Delden) Conn strain K84, a nonpathogenic bacterium closely related to the crown-gall *Agrobacterium tumefaciens* (Smith and Townsend) Conn, inhibits *A. tumefaciens* by production of an antibiotic. Simply dipping the bare roots of transplant trees or shrubs into a bucket of strain K84 cells suspended in water is sufficient to protect the roots against infection by the soil-inhabiting *A. tumefaciens* when the transplants are planted into the natural soil (Kerr, 1980). A single dip of the young tree or shrub before transplanting protects the plant for life. This strain is now in use for biological control of crown gall worldwide (Moore, 1979).

A derivative of K84 that has been genetically altered by rDNA techniques to sustain the effectiveness of this biocontrol agent is used in Australia (Ryder and Jones, 1990). Strain K84 has one genetic mechanism for production of the antibiotic and another closely linked genetic mechanism for insensitivity to its own antibiotic. Through natural matings with the pathogen in soil or on roots, it is possible that strain K84 could transfer the genetic mechanism for insensitivity to the antibiotic to the pathogen, whereupon the pathogen would acquire resistance to the biological control agent. Scientists in Australia developed the derivative of K84, strain K1026, which cannot transfer to the pathogen the trait for insensitivity to the antibiotic. Deletion of the gene(s) for ability to transfer the trait for insensitivity to the antibiotic does not affect biocontrol activity (Ryder and Jones, 1990).

The antibiotic, gliotoxin, has been implicated in the biological control of pythium and rhizoctonia damping-off diseases by the soil-inhabiting fungus *Gliocladium virens* Miller, Giddens & Foster. A product based on this fungus (Gliogard) has been registered for use against pythium and rhizoctonia damping off (Table 1). Gliotoxin was one of the first antibiotics associated with a soil fungus and implicated in plant disease control in the 1930s (Lumsden *et al.*, 1992). Use of root-associated microorganisms that protect roots by their production of antibiotics represents major opportunities for greater use of microbial biocontrol (Weller and Thomashow, 1993). Typical of antibiotic-producing microorganisms more generally, *G. virens* produces its antibiotic only after inoculum has been introduced into soil and in the inoculum (product) handled by workers.

The bacterium, *B. thuringiensis*, produces a number of toxins that kill many species of Lepidoptera, Coleoptera, and Diptera (Faust and Bulla, 1982). These spore/toxin mixtures are the active ingredient in a number of registered products that are used worldwide in pest management (Beegle and Yamamoto, 1992) (Table 1). One U.S. company has registered a product consisting of the bacterium *Pseudomonas fluorescens* with the Bt gene for production of the protein endotoxin introduced by DNA technology. The genetically modified *P. fluorescens* is killed before marketing, but the activity of the endotoxin remains unaffected.

Toxigenicity—Nontarget Effect

Substances such as alkaloids produced by endophytes in leaves of ryegrass and fescue that provide protection from insect pests of these grasses also cause ryegrass staggers and fescue toxicosis in livestock allowed to graze on these infected plants (Siegel *et al.*, 1987). Presumably deer and other wild-life that feed on grasses could also be affected by endophytes established in grasses used for gold courses, lawns, and landscapes.

The various toxins of Bt affect a number of arthropod species that are not pests, but are considered harmless for other animals. The safety of these toxins to vertebrates is established by a combination of the knowledge about the mode of action of the toxin and data on safety accumulated through years of testing (Rogoff, 1982; Laird *et al.*, 1990). In what may be the most comprehensive risk assessment ever made for microbial biocontrol, the Environmental Impact Statement for gypsy moth management in the United States concludes that (a) Bt subsp. *kurstaki* de Barjac & Lemille may be associated with irritant effects for some people “under extreme conditions,” that (b) no adverse effects are expected for humans because of the use of Gypchek, the NPV for gypsy moth, and (c) the only agent likely to cause adverse health effects “under routine conditions of exposures” is the gypsy moth itself (USDA, 1995).

The antibiotics produced by microorganisms introduced into soil or other habitats or with the planting material for biological control potentially could be toxic to nontarget microorganisms naturally present in these habitats. While the potential exists, there are no known or documented examples of such nontarget effects, possibly because of the minute quantities of these compounds required for biocontrol activity and/or because of the small-scale use of such biocontrol. *Pseudomonas fluorescens* strain 2–79 is a bacterium with biocontrol activity against wheat take-all [*Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *tritici* Walker] as the result of its ability to inhibit the pathogen through production of phenazine-1-carboxylate. Thomashow *et al.* (1990) reported that this strain produced phenazine-1-carboxylate at less than 100 mg/ha in the field on wheat at the three-leaf stage. Paulitz and Linderman (1989) showed that this phenazine produced in the rhizosphere had no effect on establishment of mycorrhizal fungi.

Antibiosis is a universal phenomenon in habitats occupied by microorganisms. Furthermore, certain antibiotic-producing traits are highly conserved in bacteria (Cook *et al.*, 1995). As an example, ability to produce the antibiotic 2,4-diacetylphloroglucinol is a trait of bacteria associated with the natural protection of roots of wheat against take-all in Washington (Vincent *et al.*, 1991), sugar beets against *Pythium* infections in Ireland (Shanahan *et al.*, 1992), and tobacco against black root rot in Switzerland (Défago *et al.*, 1991). Apparently certain antibiotic-producing traits are conserved in bacteria (Cook *et al.*, 1995). Mazzola *et al.* (1992) showed that antibiotic-producing ability is a natural survival mechanism of bacteria in the rhizosphere.

Some plant-associated microorganisms applied to seeds can cause injury to the germinating seed, expressed as stand failure or stunted plants (D. M. Weller and R. J. Cook, unpublished). These injuries are caused by the toxic effects of metabolites produced by the microorganism during germination of the seed. Such nontarget effects would typically be identified early in the research and development phase and would usually result in attempts to eliminate or manage the effect or terminate further effort to develop the microbe for biocontrol. There are no known examples of documented effects to animals or people that might eat microbially treated plant parts.

Pathogenicity—Target Effect

Some of the first and greatest uses of microorganisms introduced/applied for biological control have been as pathogens of targeted pest species. The classic example is the bacterium *Bacillus popilliae* Dutky, a pathogen of the Japanese beetle, first registered in the U.S. in 1948 for use in biological control and in continuous commercial use since. *Bacillus sphaericus* Neide, a mosquito pathogen, was registered in 1991 for use in the U.S. but is not yet available commercially.

Four fungal pathogens of insects have been registered by the EPA for commercial use in the United States. One, *Hirsutella thompsonii* Fisher, a pathogen of the citrus rust mite (McCoy, 1981), is no longer available in the United States for economic reasons. Another, *Lagenidium giganteum* Couch, was registered for control of mosquito larvae but is not yet available commercially (Kerwin, 1992). A third fungus, *Metarhizium anisopliae* (Metsch.) Sorok, was registered in 1993 as BioPath for biological control of cockroaches and in 1995 as BioBlast for biological control of termites (Table 1). A fourth fungus, *Beauveria bassiana* (Bals.) Vuill. (Mycotrol), was registered in 1995 for control of grasshopper, locusts, Mormon crickets, whiteflies, aphids, thrips, psyllids, mealybugs, and plant hoppers (Lord, 1995). *Paecilomyces fumosoroseus* (Wize) Brown & Smith, selected for control of white flies and thrips, is expected to be registered (Vandenberg, 1993). In other countries, *M. anisopliae* is used throughout Brazil against spittlebugs on alfalfa and sugarcane (Alves, 1986) and *Beauveria brongniartii* (Sacc.) Petch is used in Switzerland and adjacent areas of central Europe against scarabaeid beetles (Keller, 1992).

The nuclear polyhedrosis virus (NPV) from *Anticarsia gemmatalis* Hübner has been used very successfully to control this insect on soybeans in Brazil. Beginning in 1982, when 2000 ha were treated with virus produced by farm cooperatives, its use has expanded to 500,000 ha by 1988. In 1989 the production and formulation technologies were transferred to six private companies in Brazil (Moscardi, 1993). In the People's Republic of China, a semiautomated production facility was built by a farm cooperative that could produce enough *Heliothis* NPV to treat 75 ha per day. The virus was used on the crops of the producing cooperative and also sold to neighboring cooperatives (Ignoffo, 1989). Other NPVs are commercially available in the UK, France, and Finland (Payne, 1989). These viruses each infect only a very limited number of insect species.

The U.S. Forest Service has registered the NPV of *Lymantria dispar* L., *Orgyia pseudotsugata* McDunnough, and *Neodiprion sertifer* Geoffroy with the EPA. However, the registration of the *N. sertifer* NPV has been allowed to lapse because of the small market and high cost for *in vivo* production. Currently, some 7–10,000 ha of forest are treated annually with NPV (Gypchek) by the U.S. Forest Service and the USDA Animal Plant Health and Inspection Service (APHIS). The *Helicoverpa zea* Boddie NPV, registered in 1975, was marketed for the control of *H. zea* on cotton. In 1993 the *Spodoptera exigua* Hübner NPV and in 1994 the *Autographa californica* Speyer NPV were registered with the EPA by a company intending to market them in the United States and other countries for use on a variety of food crops. The *A. californica* NPV is the first of the "broad" host range viruses to be registered in the United States (Table 1). NPVs typically infect one or only a few closely related insect species (Payne, 1988).

The microsporidium, *Nosema locustae* Canning, a pathogen of grasshoppers, is commercially used on rangeland in the United States. Like *M. anisopliae* (BioPath) for biological control of cockroaches, *N. locustae* is used together with grasshopper bait (Henry and Onsager, 1982). Other examples of insect control with insect pathogens are given in Roberts *et al.* (1991), Tanada and Kaya (1993), Ferron (1985), Lacey and Undeen (1986), and Lüthy (1986).

Three fungal pathogens of weeds are registered in the United States (Charudattan, 1991) (Table 1). These are *Puccina canaliculata* (Schwein.) Lagerh., a rust fungus, registered as Dr. Biosedge for biological control of yellow nutsedge (*Cyperus esculentus* L.); *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. f. sp. *aeschynomene* as Collego, for biological control of northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.]; and *Phytophthora palmivora* (E. J. Butler) E. J. Butler as Devine, for biological control of strangler vine [*Morrenia odorata* (H. & A.) Lindl.] in citrus. *Phythothora palmivora* is applied in an inundative fashion but apparently can establish in soil after a single application. In Canada, the pathogen *C. gloeosporoides* f.sp. *malvae* has been registered by Agriculture and Agri-Food Canada as BioMal for control of round-leaf mallow (*Malva pusilla* Sm.). Two other fungi, mutants of *Sclerotinia sclerotiom* (Lib.) de Bary and special formulations of *Myrothecium veurucaria* (Albertini & Schwein.) Ditmar:Fr., have been studied for use as broad-spectrum "bioherbicides" in selected environments (Sands *et al.*, 1990; Yang and Jong, 1995).

Several nonindigenous pathogens have been used for biological weed control initiated as inoculative releases (see next section: Use of Microbial Biocontrol: Strategies with Examples). This is a relatively new field, but there has been success and a growing interest in this strategy (Watson, 1991). Each of these pathogens has been studied extensively for host specificity, either in containment or at overseas locations, before release. These fungi were selected for evaluation because, as obligate parasites, there is a high probability for host specificity and they are wind-disseminated and therefore they are likely to spread rapidly. These applications fit with the concept of classical biological control.

Arthrobotrys robusta Duddington, a nematode-trapping fungus, has been sold in Europe for control of nematodes on mushrooms, and another *Arthrobotrys* species is used there to manage nematodes on tomato (Cook and Baker, 1983). The fungus, *Paecilomyces lilacinus* (Thom) Samson, has been used in the Philippines as a parasite of nematode eggs (Davide, 1991). The bacterial pathogen of nematodes, *Pasteuria penetrans* (Thorne) Sayer & Starr, would probably be used worldwide today for biological control of nematodes if an economical method to mass produce this agent were available (Stirling, 1991). Each of these agents is, or would be, applied according to an augmentative or inundative strategy (Stirling, 1991).

Pathogenicity—Nontarget Effect

Microbial biocontrol agents used as pathogens or potential pathogens could also be pathogenic to nontarget organisms. An unwanted pathogenic effect would include: (1) a microorganism applied as a pathogen for biological control of an arthropod, nematode, or weed that also affected beneficial arthropods, beneficial nematodes, vertebrates, crop plants or native plants; and (2) a microorganism applied to plants to induce disease resistance, if this also caused disease in other plants either alone or through synergy with another pathogen in the inoculated plants.

The ability of a microbial biocontrol agent to infect hosts other than the targeted pest is undoubtedly the most important and potentially controversial issue relative to the use of microbial pathogens as biological control agents. Most microbial biocontrol agents used as pathogens (viruses, bacteria, protozoa, and fungi) are relatively host specific. Because the host range may be more narrow in the field than indicated by studies in the laboratory or greenhouse, scientists are faced with the dilemma of estimating the ecological host range from the results of host-range experiments carried out in the laboratory or greenhouse. Whether the pathogen is indigenous or nonindigenous can also influence how host specificity data are used in predicting a nontarget effect.

Some species of microorganisms with potential for use as biological control agents can also be opportunistic pathogens of humans. These species, such as *Aspergillus ochraceus* Wilhelm (Sinski, 1975), are generally well known and are therefore usually not considered for use as biological control agents. Such microorganisms would need special attention if they were to be considered as biological control agents.

Although Bt has been shown to depress populations of nontarget lepidopterans (Miller, 1990), the *Bacillus* spp. used worldwide for insect control appear to have little potential for pathogenicity to vertebrates. Registered strains have been tested extensively for possible effects on nontarget organisms, including mammals, as part of the registration requirements, with no harmful effects reported (Rogoff, 1982; Laird *et al.*, 1990).

The likelihood that arthropod viruses used in pest management may be or become pathogenic to vertebrates is remote. The viruses most commonly considered for pest control are the baculoviruses specific to arthropods (Payne, 1988). These viruses are enclosed in a protein that is solubilized in the insect gut to release the infectious virions. In the mammalian stomach, the freed virions are destroyed by the acid condition, providing one of several physiological barriers to the infection of mammals. In addition, significant and repetitive data have been accumulated from past safety tests required for each registration. For a review of this work, readers are referred to Gröner (1986).

Fungal biocontrol agents of arthropod pests could potentially affect predators or parasitoids of those same pests indirectly, either by depleting the host population or competing with them (in the case of parasitoids) in the host tissue. Several studies have demonstrated the compatibility of fungi with parasitoids or predators in integrated pest management programs (Goettel *et al.*, 1990). In South Africa, an epizootic in *Plutella maculipennis* Curtis by the fungus *Zoophthora radicans* (Brefeld) Batko reportedly depressed the populations of other natural enemies of *P. maculipennis* sufficiently to allow this pest to become more damaging the next season (Ullyett and Schonken, 1940). In many cases, good synchronization actually will result in joint action against the host and prevent antagonism between these two types of biological control agents.

Although *Beauveria bassiana* has been recorded as infecting over 700 species of arthropods (Li, 1988), laboratory studies have shown that different isolates of this species are generally most virulent to the host from which they were first isolated (Goettel *et al.*, 1990; Feng *et al.*, 1994). Some arthropods can be infected readily in the laboratory by fungi not known to attack them naturally, and some strains of insect pathogens can exhibit high pathogenicity to previously unencountered hosts (Prior, 1990; Feng *et al.*, 1994).

Individual isolates of fungal pathogens of arthropods are frequently more host-specific than may be indicated by the aggregate host range based on all published reports of that pathogen (Goettel *et al.*, 1990). Similarly, isolate specificity in the field is typically narrower than that demonstrable in laboratory bioassay studies (Goettel *et al.*, 1990). There are several reports of fungi attacking only one host even though closely related susceptible species are present. No candidate fungal biocontrol agent has been reported to cause an epizootic among honey bees, even though these same (or similar) fungi may show some pathogenicity to honey bees in laboratory studies.

Similar observations have been made for plant pathogens of weeds; greenhouse evaluations indicate that under optimal conditions, species not normally susceptible in nature can develop symptoms of infection. Rust fungi tested to date rarely can be maintained on nontarget species, and then only under the optimal test conditions (Bruckart and Shishkoff, 1993). The issues of nontarget effects and physiological (versus ecological) host range have been discussed elsewhere for plant pathogens of weeds (Watson, 1986).

Host range of the fungal pathogen, *C. gloeosporioides* f. sp. *aeschynomene,* registered as Collego for control of northern jointvetch in rice, includes certain other, but not all, leguminous plants (TeBeest, 1982). Those mildly or moderately susceptible to this pathogen include perennial sweet pea (*Lathyrus latifolius* L.) and certain other *Lathyrus* spp., white lupine (*Lupinus densiflorus* Benth.) and Texas bluebonnet (*Lupinus subcarnosus* Hook.), broad bean (*Vicia faba* L.), and 23 of 26 cultivars of pea (*Pisum sativum* L.). Disease was not evident in 25 other genera of legumes nor in any of the nonlegumes representing nine families tested. Even among the susceptible legumes, the northern jointvetch targeted for control by this fungal pathogen was the most susceptible.

Knowledge from a well-studied group of grass pathogens has helped to clarify the potential for change within the rust fungi intended for use in biological control of weeds. Attempts to cross strains (biotypes) of *Puccinia graminis* that are specific to different grass hosts generally were unsuccessful; many of the hybrids were infertile, and those few that were virulent caused very weak infections, although they had acquired the ability to infect both parent plant species (Johnson, 1949). Anikster (1985) indicated that crosses (somatic or sexual) are rare among rust fungi in nature and the progeny do not persist.

USE OF MICROBIAL BIOCONTROL: STRATEGIES WITH EXAMPLES

Virtually all pest species and plant diseases are subject to some level of natural biological control imposed by the pathogenic and other antagonistic effects of microorganisms already present in the environment and interactive with pest agents. Crop rotations and organic amendments are examples of farming practices designed to take advantage of or enhance the activities of resident populations of microbial biocontrol agents without having to introduce them. “Suppressive soils” (Baker and Cook, 1974) are so-named because the microorganisms naturally present in these soils provide an unusually high level of biological control of a broad variety of pests, including root-infecting fungi (Schneider, 1982), plant parasitic nametodes (Stirling, 1991), and soil-inhabiting insect pests (Ko *et al.*, 1982). Other soils become microbiologically suppressive to pests in response to the cropping practice (Cook and Baker, 1983). Agriculture and forestry benefit greatly from the resident communities of microorganisms pathogenic or inhibitory to pest species. However, this background level of biological control is seldom adequate, by itself, but can be greatly enhanced by introducing/applying additional microorganisms when and where needed.

Strategies

There are three strategies for use of microorganisms introduced/applied for biological control. These are inoculative release, augmentative application, and inundative application. While the intent in choosing a strategy could be to reduce cost, limit exposure of nontarget organisms, or optimize efficiency, the strategy is usually dictated by the biology of the microbial biocontrol agent, the target pest, or both.

Inoculative releases seek to introduce the agent once or only occasionally into the environment, with the intent that it will establish as a sustained population and impose some level of biological control. This strategy, followed for biological control of an established nonindigenous pest species with a nonindigenous natural enemy (pathogen) of that pest species, is defined as “classical biological control” (DeBach, 1964).

Augmentative applications seek to supplement the resident population of a microbial biocontrol agent by applying a microorganism already present, either naturally or because of a previous introduction/application.

Biological control results from the subsequent increase of the microbial population to an effective density prior to economic damage caused by the target pest.

Inundative applications seek to elevate the population of a microbial biocontrol agent to an instantly very high and timely population density to insure maximum and rapid suppression or kill of the target pest species.

There is nothing inherent in the strategy itself (inoculative, augmentative, or inundative) that raises a safety issue. We are still only concerned with the four basic safety issues discussed above: competitive displacement, allergenicity, toxigenicity, and pathogenicity. If there is no concern with one or more of these safety issues, the strategy for use is not a concern.

Consideration should be given to how the microbial biocontrol agent is applied. As an example, an agent with potential to cause an allergy would more likely raise a question of risk if applied aerially (e.g., as an aerosol or dusting) than if applied directly to soil, seeds, or water. An agent with known or suspected toxigenic properties would more likely raise a question of risk if used to treat plant parts consumed by people, livestock, or wildlife than if introduced into soil or applied as a seed treatment or root-dip for transplants. Management to reduce any risks associated with the use of microorganisms for biological control is discussed near the end of this report.

Examples

Examples of successful inoculative releases of fungal pathogens into susceptible target insect populations include *Zoophthora radicans* from Israel against spotted alfalfa aphid [*Therioaphis trifolii* (Monell) var. *maculata* Buckton] in Australia (Milner *et al.*, 1982), and *Entomophaga maimaiga* Humber, Shimazu & Soper from Japan against gypsy moth in North America (Hajek *et al.*, 1990; Elkinton *et al.*, 1991; Smitley *et al.*, 1995). These are examples of nonindigenous pathogens used to control nonindigenous pests. More recently, a nonindigenous *Entomophaga* species from Australia has been introduced against indigenous North American grasshoppers (Carruthers and Onsager, 1993) although not without controversy (Bidochka *et al.*, 1996; Lockwood, 1993a,b).

The European spruce sawfly (*Gilpinia hercyniae* Hartig) was accidentally introduced into Canada without any of the natural enemies that controlled it in Europe and, in 1938, caused extensive tree damage. About this time, an NPV of uncertain origin developed in the spruce sawfly population in Canada, and, by 1940, the insect population had declined to the point where no significant damage was caused. Viral epizootics followed the spread of the insect into the northeastern United States where the virus continued to suppress the population without further human intervention (Steinhaus, 1949).

Examples of successful biological control of weeds using inoculative releases include rush skeletonweed (*Chondrilla juncea* Hartig) in California (Supkoff *et al.*, 1988) and in Australia (Cullen, 1986) by the Eurasian rust fungus, *Puccinia chondrillina* Bubák & Syd.; hamakua pamakani (*Ageratina riparia* K. & R.) by the Caribbean smut fungus, *Entyloma compositarum* Farlow (=*Cercosporella* sp.), in Hawaii (Trujillo, 1986); and European blackberry (*Rubus fruticosus* L. aggregate) in Australia by a North American strain of the rust fungus, *Phragmidium violaceum* (Schaltz) Winter (Bruzzese and Hasan, 1986).

Mycoparasites, i.e., parasites of fungi, also have potential through inoculative releases to establish and provide biological control of target fungal populations (Cook, 1993b). *Sporidesmium sclerotivorum* Uecker, Ayers & Adams may be such a mycoparasite (Adams and Fravel, 1990). This fungus behaves as an obligate parasite of sclerotia of *Sclerotinia sclerotiorum* and *S. minor* Jagger, two closely related pathogens of a wide array of dicotyledonous crops. When introduced into soil, the population density of this mycoparasite increases or decreases in response to the numbers of sclerotia of these plant pathogens.

Evidence also has been presented that the fungal pathogen of nematodes, *Hirsutella rhossiliensis* Minter & Brady, is maintained in a density-dependent relationship on target plant parasitic nematodes following an inoculative release into soil (Jaffee, 1992; Jaffee *et al.*, 1992).

Microorganisms introduced with planting material such as seeds, and expected to then spread downward on roots or upward in stems or onto leaves with plant development, are another kind of "inoculative release." Rather than spreading over a landscape, these microbial biocontrol agents spread over and within the plant. An example is the endophytic bacterium *Clavibacter xyli* Davis, Gillaspie, Vidaver & Harris, modified by rDNA technology to produce the Bt toxin, and that spreads in the xylem of the developing corn plant following inoculation of the seed (Turner *et al.*, 1991).

Examples of augmentative or inundative applications of pathogens against target insect populations include those of the fungi *Verticillium lecanii* (A. Zimmerm.) Viégas against a broad range of aphids, whiteflies, and thrips in greenhouses (Hall, 1981); *Metarhizium flavoviride* Gams & Rozsypal against locusts (Prior and Greathead, 1989); *M. anisopliae* in Tasmania against pasture scarabs (Rath, 1992); and *B. bassiana* against numerous insect pests (Hajek *et al.*, 1987; Feng *et al.*, 1994). A growing population of gypsy moths contains a low level of NPV that will eventually cause the population to collapse after it reaches a high and ecologically damaging level; control of this insect pest can be achieved by timely application of additional virus, which causes the population to collapse before it reaches a damaging size (Podgwaite, 1981). In the case of weeds, augmentative applications of the rust fungus *Puccinia canaliculata* (Schwein.) Lagerh. are used to control yellow nutsedge (*Cyperus esculentus* L.) in the U.S. (Bruckart *et al.*, 1988; Phatak *et al.*, 1983), and an inundative application of the fungal pathogen *C. gloeosporioides* f.sp. *aeschynomene*, as the "mycoherbicide" Collego, is used to control northern jointvetch in rice in Arkansas (Charudattan, 1991). Inundative applications also may be used for control of plant diseases; spores of a saprophytic or weakly parasitic fungus "brushed" as a water suspension onto pruning wounds of trees can be used to preempt infection by wound pathogens of that tree (Cook and Baker, 1983; Kommedahl and Windels, 1981).

The inoculative, augmentative, and inundative strategies each can be implemented in many different ways. As one approach (tactic), the pest species may be lured or baited by pheromones or food sources to come into contact with the microbial biocontrol agent, such as the BioPath method to lure cockroaches into contact with spores of *M. anisopliae* (Vandenberg, 1993). As another approach, the microbial biocontrol agent may be delivered as infectious units (spores, cells, virus particles) to make contact with the pest agent. As still another approach, the microbial biocontrol agents may be released or applied as point-sources of inoculum to spread naturally or be carried by nematodes, bees, advancing root tips, or other means to the sites where needed (Sutton and Peng, 1993).

Induced resistance is a phenomenon whereby a microorganism applied to seeds, leaves, or roots of plants elicits a response in those plants that results in a disease-resistant phenotype. Induced resistance has been reported for both insect pests (Karban *et al.*, 1987) and plant diseases (Kuć, 1987). Microbially induced resistance in plants to pests and diseases (Kuć, 1987) will most likely involve an inundative application of an inducing agent. It may also involve an augmentative application in the form of a "booster" to the original inundative application. The inducing agent may be applied to seed prior to planting, in-furrow or as a band during planting, or to the foliage of seedlings or maturing plants. One example is the use of select strains of plant growth-promoting rhizosphere microorganisms applied to seed. These microorganisms induce systemic resistance in the entire plant to subsequent attack by certain foliar pathogens (Wei *et al.*, 1991).

POTENTIAL DURATION OF NONTARGET EFFECTS

Microorganisms released into the environment have raised special concern because of their potential to establish and spread. In actual practice, it has proved very difficult to maintain the population of an introduced microorganism at a density greater than occurs naturally, or to successfully establish a microorganism where it does not already occur (Garrett, 1965; Baker and Cook, 1974). Populations of microorganisms applied to the environment commonly decline to a density naturally sustainable within that environment, often to undetectable levels (Podgwaite, 1981; TeBeest, 1982). For plant-associated microorganisms introduced as biocontrol agents into the rhizosphere or phyllosphere, the population of the microbial biocontrol agent declines to background levels when the supporting plant dies, and it must be applied again with the next planting of that crop (Weller, 1984; Cook *et al.*, 1991).

Regardless of the potential duration (permanence) of a nontarget effect, the safety issues are the same, namely, competitive displacement of nontarget microorganisms, allergenicity, toxigenicity, or pathogenicity. Of these unintended effects, pathogenicity is, potentially, the most likely to cause a significant long-term nontarget effect. Such a long-term effect would be limited to plants and to nontarget invertebrates and would not directly affect humans or other vertebrates.

A pathogen applied for biocontrol of a weed potentially could harm a susceptible crop plant related to that weed and grown within the range of dissemination of that pathogen. An endophyte introduced into a new variety of forage grass could induce toxicoses to cattle fed on that grass. A dry-spore fungal preparation applied as a seed treatment could cause allergies in workers handling the treated seed. A microorganism introduced into the rhizosphere for biological control of a root disease could preempt or displace naturally occurring fungal colonists of the rhizosphere (Kloepper and Schroth, 1981). These are mainly immediate effects that can, if significant, be eliminated upon termination of use of the offensive microbial biocontrol agent.

Long-term effects of microorganisms on ecosystems could result if these microorganisms are (or became) pathogenic to nontarget native plants or arthropods by killing or rendering them less competitive in their native environments. The advisability and long-term environmental effects of this and other introductions of nonindigenous biocontrol agents against indigenous pests has stimulated a philosophical controversy (Howarth, 1991; Lockwood, 1993a,b).

The best-documented cases of long-term ecosystem effects are indirect effects of microbial biocontrol agents on the predators and parasitoids of the target pest through host depletion (Goettel *et al.*, 1990). Some concerns have been expressed over the use of Bt preparations in forests to control gypsy moth, because the insecticidal protein kills other lepidopteran larvae that are food sources for birds (Miller, 1990). However, there is evidence that the effects are short-term and subtle and that nontarget populations stabilize relatively quickly after application is discontinued (Peacock *et al.*, 1993; Wagner *et al.*, 1996).

The probability for occurrence of significant adverse effects on nontarget organisms will be influenced in most cases by the scale of use of the microbial biocontrol agent, i.e., the greater the geographic area treated or number of workers exposed, the more likely the occurrence of an unwanted adverse effect. Some microbial biocontrol agents such as *B. thuringiensis* are applied directly to thousands of hectares worldwide. Many other microbial biocontrol agents will be used on a much smaller scale but in high concentration. For example, *Agrobacterium radiobacter* K84, although used worldwide for biological control of crown gall caused by *A. tumefaciens,* is applied on only a very small area; virtually all inoculum within any one country or region is provided by a single supplier, in some cases by a local agricultural experiment station. In other cases, a small amount of introduced inoculum of an insect or weed pathogen has the potential to spread over and occupy large areas infested by the target pest (host).

On the other hand, no new safety issue is raised because of the amount of inoculum applied or scale of area potentially affected by a microorganism introduced/applied for pest or disease control; the safety issues (competitive displacement, allergenicity, toxigenicity, and pathogenicity) are still the same. A microorganism introduced to inundate a target pest organism potentially could increase the risk of one or more of the four potential adverse effects on other organisms, because of the amount of inoculum added and therefore the extent of exposure.

It would be pure speculation to suggest that competitive, toxigenic, allergenic, or pathogenic microorganisms introduced to control pests or diseases could have long-term adverse effects on the ecosystem, either directly or indirectly, except by imposing a standard on biocontrol that is not imposed on other methods of pest control or other ecosystem management practices (see Appendix 1). In general, it is not possible to reduce a pest population without affecting another component of the ecosystem, regardless of the strategy used.

It must also be kept constantly in mind how very expensive it can be to obtain meaningful safety data and accurate estimates of risks to human health and the environment, let alone provide solid assurances of acceptable risk-management strategies. Common sense must therefore continue to contribute toward answering the question “How safe is safe?” The answer to this question is critical to the full development of microbial biocontrol. This issue is discussed below.

MANAGEMENT OF MICROORGANISMS INTENDED FOR PEST AND DISEASE CONTROL

As indicated at the outset, the primary intent of this report is to frame the issues pertinent to the safe use of microorganisms for biological control of pests and diseases. “Safe use” includes not only assessment but also management of any risks or potential risks that may be identified. Organisms known or suspected to cause unacceptable adverse effects are usually eliminated in the initial stages of research projects, but some may be tested further or used commercially depending on the benefits and whether the organism or its unintended adverse effects can be reasonably managed. There are many steps in the research and development process and subsequent commercial use whereby knowledge of and experience with the microorganism are accumulated that can be used to manage adverse effects. Safety to workers should be assured at all stages of the research and development process by good laboratory practices. The discussion below is intended only to illustrate principles and is not an exhaustive treatment of management practices.

Management Based on Knowledge of the Organism

One of the useful functions of taxonomy is its predictive value. If a microorganism is known to have certain properties, then a taxonomically related organism will frequently have similar properties. While this does not preclude the need to study each organism, it does mean that general predictions can be made about organisms, and studies can be focused to test those predictions. As more information is gained about a genus, each species does not have to be treated as if it was a completely unknown organism, except possibly to gain a better understanding of its real or potential hosts and geographic ranges. Relevant knowledge of the organism may be derived from information provided for purposes of registration of related microbial biocontrol agents.

Management Based on Knowledge of the Environment

The great majority of introductions/applications of microbial biocontrol agents will be made into managed environments. These can include: managed nonagricultural environments, such as urban areas, parks, lakes and waterways, and forests; agricultural environments for perennial and annual crops, including ranges, pastures, orchards, open fields, and woodlands; and contained environments such as commercial greenhouses, households, and processing and storage facilities. Each of these environments, in turn, offers some unique as well as some common options and challenges for management of microbial biocontrol agents.

Management Based on Experience with Other Microorganisms

A great deal of experience exists for management of microorganisms or their adverse effects in the environment. This includes the experience with the management of economically important plant pathogens (Leonard and Fry, 1986, 1989) and beneficial or economically important microorganisms such as *Rhizobium* spp. and mycorrhizal fungi. The same principles and methods for management of these microorganisms would apply to the management of unintended adverse effects of microbial biocontrol agents in the environment.

Management during Basic Research in the Field

It is necessary, during the course of conducting research with microbial biocontrol agents, to carry out experiments in the field. It is assumed that prior studies of the microorganism under controlled (or contained) conditions indicated no unmanageable detrimental characteristics and a realistic potential for use in biocontrol. Some pertinent information about safety as well as performance may require conducting small-scale preliminary field trials. Examples could include experiments to obtain more information on survival/persistence, dispersal/dissemination, and interactions with other organisms. Several studies have been conducted during the past 5 years with microorganisms genetically marked as a means to track them and therefore learn more about their ability to spread and survive in nature (Kleupfel, 1993). Such studies have confirmed that plant-associated microorganisms introduced into soil remain virtually in the row where introduced and decline to undetectable populations soon after and sometimes before the supporting plant completes its life cycle (Cook *et al.*, 1991).

It is likely during research in the field that the main safety issue with microorganisms will be their pathogenicity to nontarget organisms. The potential for such an outcome is remote, since such experiments with nonindigenous microorganisms are carried out only when judgments based on results from studies in the greenhouse/growth chamber, experience in other countries, or reports in the scientific literature indicate with reasonable certainty that the microorganism is safe. There is no known scientific basis to consider nontarget toxigenic or allergenic effects during basic research other than for worker safety, which can be adequately managed as part of good laboratory practices. Microorganisms with known potential to spread and to multiply as pathogens may require special management during the course of basic studies in the field.

There are several approaches to management of microbial biocontrol agents intended for use on plants and for which there is insufficient preliminary information to be assured of safety. For example, the field experiment can be conducted in an isolated area, or the site can be protected with buffer strips of the same or different plants. Microorganisms introduced into soil and for which there are safety concerns can be eliminated at the end of the experiment by soil fumigation. In most cases, plant-associated microorganisms can be effectively managed by no longer growing the supporting plant species, including the use of fallow or crop rotation if necessary.

Management during Production and Formulation

Production and formulation phases of research and development typically are carried out within enclosed facilities. This virtually eliminates the chances for adverse pathogenic effects on nontarget plants and animals but increases the chances for worker exposure to microorganisms with known or suspected toxigenic or allergenic effects. These safety issues can be managed effectively by use of good laboratory practices, including appropriate filters on the equipment and facilities and the use of dust masks and protective clothing by the workers as appropriate. It would also make sense not to use microorganisms such as *Aspergillus fumigatus* Fres. that can be opportunistic human pathogens (Sinski, 1975).

Management during Application or Release

Much or most of the safety of use of microbial biocontrol agents will be addressed during introduction/application of the microorganism. Workers can be protected by wearing appropriate clothing and gloves to prevent exposure to the skin or dust masks if airborne spores are involved. The potential for nontarget effects can be further managed by timing of the applications. Potential problems such as drift and other unwanted dissemination can be managed by site-directed methods of application and timing of application.

Postapplication Management

In the great majority of cases, potential unintended adverse effects of microbial biocontrol agents will have been eliminated or prevented by interventions based on experimental data or scientific literature *before* introduction/application of the microorganism into the environment. However, some risks may exist after the introduction/application is made. Most of the principles of disease and pest management, including integrated pest management, are relevant to management of unwanted or unintended adverse effects of microbial biocontrol agents *after* introduction/application into the field. The practices include the use of crop rotations and tillage, as examples. In rare cases or emergencies, chemical pesticides may be needed.

Management with Public Oversight

It is axiomatic that no responsible scientist involved in the development and implementation of microbial biocontrol would deliberately introduce or apply inoculum of a microorganism with known potential for an unmanageable adverse effect on humans or the environment. Further, professional standards of scientific conduct are established and continually improved through the informal but highly effective procedures of peer review. Most countries also depend on formal oversight by way of statutory requirements for permits and approvals. Unfortunately, requirements for microorganisms intended for pest or disease control have been based on requirements developed for chemical pesticides and have not been particularly applicable or appropriate for microorganisms. Regulation of microbial biocontrol agents in the U.S. is further complicated by a lack of consistently applied clear definitions for the terms "indigenous" and "nonindigenous." In the U.S., efforts have been made in recent years to improve the procedures for safe use of nonindigenous microbial biocontrol agents (Coulson and Soper, 1989; Coulson *et al.*, 1991; Charudattan and Browning, 1992).

In the U.S., microorganisms intended for use in pest control can be approved by the Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) under statutes of the Federal Plant Pest Act for field testing in plots involving a particular microorganism against a particular pest on not more than 10 acres (4 ha) of land or not more than one surface-acre (0.4 ha) of water. However, nonindigenous microorganisms and those modified by recombinant DNA (rDNA) technologies are exceptions to this rule; APHIS must provide a permit to bring the nonindigenous microorganism into the U.S., and the EPA must be notified prior to field testing of these microorganisms. For tests of any microorganism to control pests on plots of more than 10 acres of land or one surface-acre of water, an Experimental Use Permit (EUP) must be obtained from EPA prior to the testing. The U.S. guidelines for the registration of microbials are published in the Pesticide Assessment Guidelines Subdivision M (revised July, 1989). The data requirements are published in 40 CFR Part 158.170.

In certain situations, U.S. investigators also may have to comply with the Lacey Act as amended (Public Law 97-79), which prohibits the importation, possession, or transport of fish, wildlife, or plants in violation of any state, tribal, foreign, or U.S. law. This law will apply, for example, if a scientist discovers a microorganism having potential value in pest management in a foreign country with laws restricting or prohibiting the export of germplasm, including microbial germplasm. The Lacey Act requires that the scientist must have documented permission from the appropriate authority in that country to import the microorganism into the United States. Microorganisms must be imported through a designated port of entry or advance permission to use another port of entry must be obtained from the U.S. Department of Interior, Fish and Wildlife Service.

EPILOG

To our knowledge, this is the first comprehensive report on microbial biocontrol developed jointly by representatives of insect pathology, plant pathology, nematology, and weed science. Our personal research programs include the use of viruses, bacteria, and/or fungi for weed, insect, plant disease, or nematode control in agriculture and forestry. This report is the product of more than 2 years of discussions and analyses and represents our collective experiences with and knowledge of microbial biocontrol. We are concerned that, in spite of extensive basic research over several decades, too little use is made of microbial biocontrol in pest and plant disease management systems. This report is intended to provide a scientific framework for making greater use of microbial biocontrol, illustrated with examples. We have focused on a scientific framework for identification and evaluation of safety issues in hopes that this will lead to more appropriate questions and further discussions on risks and risk management relative to the use of microbial biocontrol. While our focus has been on microorganisms intended for pest and disease control, the principles set forth could apply to any environmental or industrial use of beneficial microorganisms.

APPENDIX 1

Environmental Effects of Common Pest Control Options

General. By its very nature, agriculture affects the "natural" ecological processes and species distribution within the agroecosystem and, in some cases, within natural ecosystems well beyond the agroecosystem where the management is applied. Processes such as land preparation, pesticide application, planting, irrigating, fertilizing, and harvesting all have effects on the ecology of the managed area, including the temporal and spatial dynamics of plant, animal, microbial, and arthropod species. Even a decision to withhold the application of some management tool (whether it be chemical, cultural, or use of host-plant resistance) influences what species will prevail in that environment. The risk to ecosystem stability by altering species composition is theoretically no greater—and may be less—an issue for biological control than for other management practices.

Tillage is possibly the most widely used method for control of weeds as well as some insect pests and soilborne plant pathogens that carryover from crop to crop in infested crop residue. Tillage is also the single greatest reason for loss of soil organic matter and soil structure, loss of soil by erosion from wind and water, and loss of soil microbial biomass and earthworms, as examples. Tillage makes soil vulnerable to movement by wind that gives rise to dust storms or by water that gives rise to nonpoint sources of pollution, sediment, and siltation in lakes, streams, rivers, and other waterways well beyond the agricultural environment. Tillage may well be the single greatest threat to the sustainability of agriculture in the United States. Tillage also increases exposure of humans to microorganisms and particulates as dust.

Crop rotation is an important method for managing soil-inhabiting pests, including plant parasitic nematodes, soil insect pests, and soilborne plant pathogens. Crop rotation allows time for resident or soil-inhabiting biocontrol agents to lower the pest population of one crop while growing a different and taxonomically unrelated crop. Rotating crops also causes shifts in populations of many nontarget soil organisms associated with those crops (Baker and Cook, 1974). The crops grown in rotations can also have effects on wildlife, nontarget arthropods, and populations of airborne fungi, including fungi with potential to cause allergies.

Host-plant resistance is an important method for managing many arthropod pests, pathogens, and plant parasitic nematodes. This approach has been especially effective against the more “specialized” pests, i.e., those highly selective in host preference. Most of the major leaf diseases caused by fungi and several leaf-attacking arthropod pests of the eight to ten most important U.S. crops and many minor crops are now effectively managed or will soon be managed by host plant resistance already available or under development. However, each crop cultivar released with resistance to a disease, nematode, or arthropod pest, regardless of the source of that resistance or method of plant breeding, if grown extensively, tends eventually to select for a population of the pest with ability to attack that cultivar. Thus, breeding for host-plant resistance, like all other approaches to pest management, is a never-ending process required to stay ahead of the ever-changing pest populations.

Compost or other organic matter added directly and in large quantities (several tons per acre) to soil as an amendment has potential for control of many soilborne plant pathogens, including plant parasitic nematodes, by stimulating the activities of resident naturally-occurring microbial biocontrol agents (Baker and Cook, 1974; Papavizas and Lumsden, 1980; Stirling, 1991). Composting is used commercially in the ornamental-plants industry to produce rooting media naturally suppressive to root pathogens of container-grown plants (Hoitink et al., 1991). The first attempts at biological control of soilborne plant pathogens during the early part of this century were based on stimulation of resident antagonists in the soil with organic manural treatments (Baker and Cook, 1974). However, these treatments, e.g., chicken manure added to control take-all of wheat (Fellows and Ficke, 1934), while effective, like crop rotations, predictably cause major shifts in microbial populations of the soil. Such large quantities of organic materials also add large quantities of organic nitrogen, which, following mineralization, can lead to nitrate leaching. There could also be toxigenic or allergenic responses to mold spores produced in compost.

Chemical pesticides can provide highly effective pest control, but like host-plant resistance, chemicals tend to select for resistant or insensitive pest populations. Chemicals can also have nontarget effects on beneficial organisms, such as insecticidal effects on bees and natural enemies of arthropod pests and fungicidal effects on yeasts and filamentous fungi competitive with pathogens on leaves (Fokkema, 1988). Some chemical pesticides, especially some nematicides and soil-applied herbicides, have turned up as contaminants in ground water supplies. Many chemical nematicides (Stirling, 1991), insecticides, and fungicides (Pimentel et al., 1991) can have adverse effects on mammalian and avian health. Some chemical pesticides may have toxigenic and allergenic effects for nontarget organisms, especially those in production/formulation facilities and applicators. Some herbicides residual in soil have been shown to predispose the subsequent crop to greater damage from root pathogens (Rovira and McDonald, 1986). Methyl bromide used as a soil fumigant has been implicated in damage to the ozone layer.

No inputs. Leaving pests and diseases uncontrolled is another option, but this contributes to fertilizers left unused in the soil, irrigation water wasted, stand failures and unthrifty plants that allow greater establishment of weeds and elimination of valuable trees from forests or landscapes. The lack of disease and pest control can also have major economic effects. In the United States alone, approximately seven billion dollars in agricultural losses per year are attributed to the lack of control of nematode pests. The U.S. wheat crop is diminished by an estimated 400 million bushels of wheat annually because of inadequate nationwide control of root diseases (R. J. Cook, unpublished data). The estimated annual monetary cost of weeds with current control strategies in 46 crops is $4.1 billion (Anonymous, 1992). Without the currently available herbicides, this cost in unrealized yield increases to $19.6 billion. The lack of disease and pest control can also require that more land be used to produce the necessary food and other products of agriculture, thereby leading to more destruction of wildlife habitat, more drainage of wetlands critical to certain ecosystem functions, and other destructive effects on the environment and natural ecosystems.

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