Principles and Potential for Biocontrol of Diseases in Forest and Conservation Nurseries

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ABSTRACT - Biological control is the reduction of inoculum density or disease-producing activities of plant pathogens by other organisms, accomplished through environmental manipulations or mass introduction of antagonists. Biocontrol agents exert their effects on pathogens by competing for niches or other limited resources, production of antibiotics, exhibiting hyperparasitism, and inducing fungistasis. Developing commercial biocontrol products is a costly, time-consuming process requiring extensive testing at several levels. Very few commercial biocontrol products are currently available to control plant diseases. Although many bacteria and fungi show promise as biocontrol agents, few are available for use or are effective against a wide range of plant pathogens. For biological control to become more widely applicable in forest and conservation nurseries, determined commitments by growers to promote and support greater research and development will be necessary.

INTRODUCTION

Diseases may be important limiting factors in production of high quality seedlings in forest and conservation nurseries. Many pathogens, well adapted to nursery conditions, may quickly cause unacceptable losses. Nursery managers have traditionally relied on chemical pesticides to control diseases. Such reliance has sometimes lead to repeated, widespread applications of several toxic chemicals during the seedling production cycle. Chemical pesticides were initially formulated for effectiveness on many different types of pathogens. This broad spectrum efficacy often resulted in destruction of both beneficial and injurious organisms (Baker and Cook 1974). More recent chemicals have been formulated with more restrictive modes of action, being effective on relatively few targeted organisms (Thomson 1990). However, resistance to these chemicals by pathogens can develop rapidly once introduced into a cropping system (Staub 1991).

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Reliance on chemical pesticides has resulted in greatly expanded production of quality stock when compared to periods before general use. However, recent problems with pest resistance, toxicity to non-target organisms, environmental contamination, and other unforeseen effects have greatly reduced the desirability of chemical pesticides (Campbell 1989). Governmental agencies are increasingly instituting restrictions on registration and use of many chemical pesticides widely used in the past (Harman 1991). Recently, these restrictions resulted in the loss of several important fungicides used in nurseries. Examples include recent withdrawal Of benomyl registration by the producer, restricted use of chlorothalonil because of groundwater contamination, non-registration of captan, and probable future loss of methyl bromide because of its perceived damage to stratospheric ozone. Coupled with increasing governmental regulation is a general public dissatisfaction with chemical use for production of food and fiber crops. Public perceptions may or may not be based on reliable information, but their impact is often sufficient to adversely affect chemical use by growers (Harman 1991). Recent public involvement and disdain for chemicals in agriculture will undoubtedly increase, making use of chemical pesticides difficult at best.

Because of these trends in chemical pesticide use, managers need to look elsewhere for means to control important pests in their nurseries. One of the most acceptable approaches to disease control is use of naturally occurring organisms to reduce or suppress activity of pathogens (Lawson and Dienelt 1989). Biological control exists in most "natural" plant ecosystems and keeps introduced pathogens within check (Baker 1987). However, in our artificial systems of agricultural fields or seedling nurseries, biological control usually fails to function at high levels because of cropping practices and monocultural systems. Therefore, to enhance niccontrol in nurseries, specific steps must be taken to promote a biological balance of organisms so disease will be kept within acceptable limits.

Biological control is defined as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists (Baker 1987; Campbell 1989). Biological control usually has three objectives: 1) reduction of pathogen inoculum through decreased survival between crops, decreased production or release of viable propagules, or decreased syread by mycelial growth; 2) reduction of host infection by the pathogen; and 3) reduction of disease severity (Axelrood 1991).

To more fully understand how biological control can be implemented in nurseries, a few basic concepts should be addressed. Most of .these consider biological balances and how such balances are disrupted in our agroecosystems and nurseries. In general, the greater the complexity of a biological community, the greater its stability (Baker 1987). In natural plant systems, often a myriad of microorganisms reside in the soil or on plant surfaces which tend to "buffer" plants from attack by pathogens. This buffering effect may not always be successful, but the complexity of the system is such that when a pathogen is introduced, particularly to the soil, it has to compete with a great many other organisms and may have difficulty in becoming established. Conversely, in a simplified agricultural community, such as forest and conservation nurseries, an introduced pathogen may successfully establish because of generally reduced competition levels.

The biological world is a vast, interacting network of living populations in a state of dynamic equilibrium, reflecting changes in their physical environment and their relations to each other (Campbell 1989). There is an equilibrium in which an individual organism follows its normal cyclic changes without significantly affecting the whole network, because of compensating changes in other components that maintain the balance. Normally, an organism will increase until the limitations imposed by the biotic and abiotic environment counterbalances the rate of increase (Baker and Cook 1974). With such "checks and balances" most natural ecosystems keep individual species within limits. Another important concept is that plants in their wild state are often adjusted to their pathogens (Campbell 1989). Pressure from pathogens which evolved in conjunction with their hosts has tended to select genotypes that have, through mutations and resultant variability, some measure of resistance.

However, use of chemical fertilizers and pesticides, tillage and seeding practices, and specialized crop varieties, although greatly increasing food and fiber production, has greatly disrupted biological balances (Baker 1987). Complex communities have usually been replaced with simple ones, sometimes resulting in disease problems absent in more balanced ecosystems.

The reminder of this paper will describe mechanisms of biological control, outline procedures for developing biocontrol agents, and give some examples of potential uses of biocontrol agents in forest and, conservation nurseries.

MECHANISMS OF BIOLOGICAL CONTROL

Pathogenic and non-pathogenic microorganisms may occupy the same environmental niches (Axelrood 1991). Whichever becomes established first usually is able to resist colonization by other organisms. For example, if a biological control agent is first to colonize the rhizosphere, pathogens may be excluded if all colonization niches are occupied. Therefore, an important factor in effective biological control is to favor colonization or occupation by non-pathogens by controlling the time of introduction and inoculum potential of biocontrol agents (Campbell 1989). Inoculum potential is defined as the sum of all factors that contribute to the energy available for host infection (Agrios 1969). In a nursery situation, if a biocontrol agent is introduced before seedlings are exposed to pathogens or if enough biocontrol inoculum is present, initial colonization by the biocontrol agent may effectively exclude infection and establishment by pathogens.

Most microorganisms compete with each other for nutrients, water, oxygen, light and space. Competition occurs when two or more organisms require the same thing and use by one reduces the amount available to the other (Baker and Cook 1974; Campbell 1989). From the standpoint of biological control, the goal is to manipulate the growing environment so non-pathogens are favored over pathogens in competition for limiting factors (Campbell 1989).

Antibiotics are produced by a wide variety of microorganisms, particularly those in the soil (Alexander 1971; Griffin 1972). Levels of antibiotics are usually greater in soils or growing media high in organic matter with large populations of microorganisms (Baker 1987). Antibiotic production can also be enhanced by increasing soil carbon sources (Griffin 1972). When host plants are stimulated to produce exudates from their roots, antibiotic production is also increased, probably as a result of competition among microorganisms for this food source (Rovira 1970). Antibiotics are very diverse chemically and either may have specific effects against particular target organisms or may affect a wide range of organisms (Alexander 1971; Campbell 1989). In general, antibiotics cause a reduction or cessation of growth or sporulation of pathogens or reduce spore germination (Jackson 1970). Unfortunately, some potential pathogens are less affected by antibiotics than others. For example, Fusarium spp. are little affected by many antibiotics produced in the soil, compared to Pythium spp. which are usually quite sensitive to antibiotics produced by a wide array of fungi and bacteria (Campbell 1989). Many biocontrol agents are specifically selected for their ability to produce antibiotics when introduced into a cropping system (Baker 1991). Their efficacy against certain target pathogens depends on pathogen responses to their antibiotics as well as soil factors that may influence amounts of antibiotics produced. In some cases, introduced antagonists may themselves be antagonized and made ineffective by the production of antibiotics from other microorganisms, including pathogens (Adams 1990; Barnett 1964; Campbell 1989).

Many potential biocontrol agents exhibit parasitism on a target pathogen. If the biocontrol agent is a fungus, it may be a mycoparasite (parasitic on another fungus). In such cases, the pathogen becomes the food source. Mycoparasites usually produce either chitinase or cellulase, degradative enzymes which break down cell wall components of host fungi (Barnett 1964). Probably the best known mycoparasites are in the genus Trichoderma (Papavizas 1985). These fungi either penetrate resting structures such as sclerotia and chlamydospores or parasitze growing hyphae of pathogens (Papavizas 1985). Some fungi, such as Gliocladium virens, are mycoparasites and produce antibiotics effective in restricting pathogen activity (Barnett 1964; Bryan and McGowan 1945). Mycoparasites may also be useful in invading existing pathogen lesions on hosts, not to control the present infection, but to reduce pathogen spore production and therefore limit inoculum for the next infection cycle (Campbell 1989). Other free-living, soilborne fungal parasites include many species of amoebae, minute insects such as Collembola, and nematodes (Boosalis and Mankau 1970; McE.Kevan 1970). The collective effect of these parasites varies from site to site, although their efficacy can be -ihanced by mass introductions as well as manipulation of the soil environment.

Fungistasis is characteristic of many soils. It is the imposition of dormancy on fungal spores due mostly to nutrient limitation (Griffin 1972; Jackson 1970). Most soil-borne plant pathogens produce resting structures of various kinds that remain dormant in the soil until nutrients are available (Alexander 1971; Griffin 1972). Such nutrients can be supplied by addition of organic matter to soil and by exudates produced from roots of potential host plants (Campbell 1989; Rovira 1970). The saprophytic soil microflora in the soil may reduce available carbon levels and impose fungistasis on pathogens, preventing spore germination and subsequent infection (Jackson 1970). The practical use of fungistasis involves manipulation of carbon status to encourage development of a large component of saprophytes and to limit buildup of pathogens (Campbell 1989).

DEVELOPING BIOLOGICAL CONTROL AGENTS

The first step is isolating potential biocontrol agents. This process usually encompasses screening susceptible host plants without disease even though they are susceptible and the pathogen is present in sufficient numbers to induce disease (Axelrood 1991). Although many potential organisms can be isolated from the rhizosphere of such plants, very few pass the scrutiny of tests required for being considered a viable biocontrol agent (Campbell 1989). Much recent effort has been concentrated on developing biocontrols for root diseases because such diseases are difficult to detect, assay and treat chemically, and have few existing, effective control measures (Baker 1987). Past experience indicates that certain genera of microorganisms have greater potential for being effective biocontrol agents, e.g. the bacterial genera Pseudomonas, Bacillus, and Enterobacter (Hoitink and others 1991; Schroth and Hancock 1982), and the fungal genera Trichoderma, Gliocladium, Penicillium, Chaetomium, and Pvthium (Adams 1990; DiPietro and others 1992; McLaren and others 1989; Papavizas 1985). Isolates selected for further testing should not be adapted to the high nutrient conditions of normal laboratory culture if they are expected to eventually survive and grow in natural environments. Fast growing organisms are usually preferred as well as those

that grow well on relatively cheap media so that at later stages in development there will be no problem finding economic fermentation systems. Most selected isolates are spore forming so the inoculum may have relatively long shelf life (Campbell 1989).

After isolation, selected organisms are screened for their potential as biocontrol agents against selected pathogens. Two types of tests are usually instituted at this stage: in <u>vitro</u> tests which evaluate inhibition or lysis of pathogens in culture plates or. on glass slides, and in <u>vivo</u> tests in which a host plant is introduced into the system (Campbell 1989). In' <u>vitro</u> tests are quick and relatively easy to perform. These tests are suitable for selecting organisms with a particular mode of action. However, they are often poor predictors of the activity of organisms in natural environments. The most widely used test is to identify antibiotic producers by inoculating the pathogen onto an agar plate with the potential antagonist inoculated nearby (Axelrood 1991; Campbell 1989). The degree of inhibition of pathogen growth, in relation to growth in the absence of potential biocontrol agents, is used as a measure of effectiveness. In <u>vivo</u> tests approach a more natural situation. In these tests, a host plant is infected with the pathogen to be controlled and the potential biocontrol agent is applied to the plant (or adjacent environment) after an appropriate incubation. The amount of disease is compared with an unprotected control or with a healthy plant. Organisms that pass both these types of tests are ready for further evaluations.

It is important to find out how, when, where and under what conditions selected biocontrol organisms work (Baker and Cook 1974; Boland 1990; Campbell 1989). To do this, detailed investigations are required. The first step is to properly identify test organisms. This may be relatively easy for most fungi, but often very difficult for bacteria and Actinomycetes because so few of these organisms have been adequately characterized from natural environments (Schroth and Hancock 1982). Once identified, it is important to eliminate any organisms pathogenic on humans, animals or other plants because such organisms could not be used in any practical program of biological control. Selected biocontrol agents have to pass the same environmental tests which chemical pesticides undergo (Campbell 1989). If selected agents have been genetically engineered, there are strict controls mandated for testing and release (Harman 1991). Most genetically engineered organisms for cn ological control would likely have had several changes made; they would be unlikely to occur naturally and their behavior in natural environments unpredictable. Delivery systems also have to be developed for selected biocontrol agents. Several approaches including encapsulation of inoculum in gels, providing nutrients (such as bran), and developing extended viability of material are important (Knudsen and Bin 1990; Lawson and Dienelt 1989). The aim is to preserve microorganism viability while allowing convenient handling and distribution to the correct place. Tests must also be made to quantify inoculum density of the agent for effective biocontrol (Dimond and Horsfall 1970). In addition, fermenter studies are needed to discover the best way to mass produce the organism while maintaining its effectiveness (Campbell 1989). Selected organisms should be genetically stable so that they maintain the desired properties through all testing and production phases (Harman 1991).

After the above tests have been made, the few remaining potential biocontrol agents should be tested in the field under natural conditions to determine their efficacy (Campbell 1989). Such tests are very important to verify that organisms selected work well outside the

laboratory or greenhouse. Field tests are usually conducted on experimental plots established under normal crop growing conditions and often require large numbers of replicates of each treatment because of the great variability encountered under natural conditions. Specialized tests to evaluate effects of environmental factors, inoculum concentration, mixed inocula, cropping systems or other factors are also usually installed in the field. One of the main problems with field tests is knowing if the test organism is still part of the experiment, i.e., that it survived, grew, and colonized host tissues. Precise detection techniques that are designed for a particular organism must be developed (Boland 1990). Although field trials are time consuming, expensive, and often tedious, they are an integral part of the development of biocontrol agents. As might be expected, the number of potential biocontrol agents that are considered excellent in laboratory tests is very large, but the number that are useful in commercial operations is very small.

The final steps in development of biocontrol agents involve patenting, production, and commercial distribution (Campbell 1989). Patenting is important because biological products must be protected from other possible producers so that the research and development money spent can be recovered from sales. Most patenting laws require that the product has novelty and that it has not been previously used, talked about or published in any way which would allow anyone to gain a knowledge of it. Patenting usually allows a monopoly for 20 years. In practice, usually the production process, cultivation techniques for the microbe, and the particular product formulation are patented; the organism itself is often difficult to patent (Campbell 1989).

Only five commercially prepared biological control formulations are currently, or are being registered for use in the United State (table 1). Two of these (GL-21^R and Mycostop^R) have potential for control of root diseases in seedling nurseries.

BIOCONTROL OF DISEASES OF SEEDS AND SEEDLINGS

In this section, we will discuss some actual and potential uses of biological control agents to control diseases of seed and seedlings in agricultural systems. Possible applications to forest and conservation nurseries will be included.

In order to control root diseases in nurseries without use of general biocide fumigants, growers need to promote the formation of pathogen-suppressive soils. These are defined as soils (or growing media) which are inhospitable to some plant pathogens so that

Trade Name	Organism(s)	Diseases and Pathogens Controlled
Galltrol-A	<u>Aqrobacterium</u> <u>radiobacter</u> (Strain 84)	Crown gall caused by <u>Agrobacterium</u> <u>tumefaciens</u> (developed for orna- mentals).
GL-21 Microbial Fungicide	<u>Gliocladium virens</u> (Isolate GL-21)	Root diseases caused by <u>Pythium</u> and <u>Rhizoctonia</u> (developed for bedding plants, ornamentals and food crops).
Binab-T	<u>Trichoderma</u> <u>harzianum</u> <u>Trichoderma</u> <u>polysporum</u>	Decay caused by basidiomycetes on trees (developed as a dry wet- table formulation for applica- tion to tree wounds).
Mycostop	<u>Streptomyces</u> griseoviridis	Damping-off and root disease caused by <u>Fusarium</u> , <u>Phomopsis</u> , and <u>Alternaria</u> (developed as a seed and soil treatment for ornamentals).
Clandosan	Chitin-based polymer	Nematode diseases (stimulates mycoflora to produce chitinase which destroys nematodes; label for ornamentals, greenhouse crops and forest tree nurseries).

Table 1. Commercial formulations of biological control agents registered for use on crop plants in the United States

¹From Locke 1992.

either the pathogens cannot establish, or if they establish, they fail to produce disease, or they establish and cause disease at first but diminish with continued culture of the crop (Huber and Schneider 1982). Suppressiveness is often an inherent characteristic of some soils which can be transferred to other soils or inactivated by treatment at high temperatures or with pesticides (Baker 1987). Unfortunately, a soil suppressive to one pathogen or even one race of a pathogen may not be equally suppressive to other pathogens. Experience has shown that suppressiveness can often be increased by repeated plantings of the same (susceptible) crop so that the disease is at first worse, then gradually lessens (Baker and Cook 1974). Under such circumstances, suppressiveness increases over time and can remain high indefinitely unless soil is treated with heat or pesticides to remove the suppressive characteristic (Baker 1987; Huber and Schneider 1982). Soil suppressiveness is related to total biomass and microbial activity (Baker 1987). Factors such as food source competition, hyperparasitism, antibiotic production, and niche competition are all involved in disease suppression (Baker 1968; Kuack 1989; Liu and Baker 1980).

Another concept sometimes used in biological control is "cross protection". This practice involves protection of a host from disease by inoculating a strain or isolate closely related to the pathogen, such as an avirulent strain

(Baker 1987; Ogawa and Komada 1984). The mechanisms of action involve competition for infection sites and perhaps "induced resistance." Induced resistance is increased host resistance caused by stimulation of host defense systems after inoculation with non-pathogens (Mandeel and Baker 1991; Van Peer and others 1991). Such resistance often entails stimulation of chemical defense mechanisms naturally inherent in plants. Cross protection has been successfully exploited to control Fusarium oxvsporum-caused wilt in a number of crop plants by inoculating them with non-pathogenic strains of the fungus prior to introduction of pathogenic strains (Mandeel and Baker 1991; Ogawa and Komada 1984). The concept :.a r; also been successfully used in protection against certain viral pathogens by inoculating host plants with non-pathogenic strains (Baker and Cook 1974; Campbell 1989). Commercial use of cross-protection is limited by concern that the antagonist may mutate to a virulent form, to a form pathogenic to another crop, or to a strain ineffective in biological control (Baker 1987).

One of the most promising methods of introducing biocontrol agents into cropping systems is on seed (Harman 1991). Many target pathogens attack seed or young germinating seedlings, so that adding biocontrol agents at this stage may be useful. Much of the current technology for seed treatment with biologicals comes from successes of inoculating legume seed with <u>Rhizobium</u> spp. (Harman 1991; Schroth and Hancock 1982). This "bacterization", amending seed with bacterial formulations, has been successful in several agricultural systems. Examples include <u>Bacillus subtilis</u> and <u>Streptomvces</u> sp. applied to wheat seeds to stimulate seedling growth and reduce effects of <u>Rhizoctonia solani</u> (Price and others 1973), and B. <u>subtilis</u> and the fungus <u>Chaetomium</u> <u>globosum</u> tc control <u>Fusarium</u> in corn (Mein and Kommedahl 1968). There have been no reported successes in treating forest tree seed with biologicals to control damping-off or root diseases.

The genera of fungi most commonly evaluated for potential as biological control organisms are <u>Trichoderma</u> and <u>Gliocladium</u>. Several species of <u>Trichoderma</u>, including T_. <u>hamatum</u>, T. <u>harzianum</u> and, to a lesser extent, T. <u>koningii</u>, T. <u>polysoorum</u> and T. <u>viride</u>, have all been used against damping-off caused by several pathogens in the laboratory, greenhouse, and field (Papavizas 1985).

Gliocladium virens has been produced commercially (table 1) and used for control of several pathogens in different agricultural systems. An alginate prill formulation was recently tested against Fusarium-associated diseases of container-grown Douglas-fir seedlings and shown to be ineffective in reducing seedling infection by fusaria (James and others unpublished). These tests also indicated that ,there may be some phytotoxic effects exhibited by G. virens, at least at the inoculum dosages used. This fungus is known to produce metabolites capable of eliciting toxic responses in host plants (Jones and others 1988; Wright 1951). Gliocladium also produces antibiotics (Howell 1991; Howell and Stipanovic 1984) and is parasitic on many other fungi, including some plant pathogens (Bryan 1944; Papavizas 1985; Roberts and Lumsden 1990). Metabolites toxic to

<u>...3</u>⁴ include gliotoxin and gliovirin. These a: capable of directly killing hyphae and propagules of many different fungi in the soil (Howell and Stipanovic 1983). This fungus also produces enzymes which degrade cell walls of certain fungi (Papavizas 1985).

Other fungal genera commonly tested for use as biological control agents include Penicillium (teleomorph: Talaromvces), Chaetomium, Epicoccum, Sporidesmium, and non-pathogenic species of Pvthium. Talaromyces flavus has potential as a biocontrol agent against a wide range of soilborne plant pathogens (McLaren and others 1989; Spink and Rowe 1989). Chaetomium has been shown to effectively control diseases induced by Pythium (DiPietro and others 1992) and Alternaria sp. (Vannacci and Harman 1987). Epicoccum purpurascens has potential as a biocontrol of Sclerotinia diseases (Zhou and Reeleder 1990). Sporodesmium sclerotiorum has also displayed excellent efficacy in controlling Sclerotinia diseases (Adams 1990). Pythium nunn effectively controls pathogenic species of Pythium on several agricultural crops (Adams 1990; Elad and others

1985). Unfortunately, none of these have been tested for efficacy against diseases in forest and conservation nurseries.

Fungi may be introduced into cropping systems on seed (Harman 1991), as spore suspensions (Papavizas 1985) and as pellets or prill directly onto or incorporated within soil or growing media (Lawson and Dienelt 1989). One major problem, particularly with seed treatments, is the induction of rhizosphere competence by biocontrol agents, defined as the ability of the agent to grow and proliferate in the host plant rhizosphere (Gleason and others 1987). This is the zone where protection against pathogens is critical. Most isolates of introduced biocontrol fungi are not rhizosphere competent (Beagle-Ristaino and Papavizas 1985; Chao and others 1986). However, recent work has shown that benomyl-resistant strains of Trichoderma exhibit the ability to proliferate in the rhizosphere (Ahmad and Baker 1987; Baker 1991). This is particularly true if low dosages of benomyl are introduced which reduces competition from other benomyl-tolerant organisms, allowing benomyl-resistant Trichoderma to develop unrestricted, thus offering greater protection against pathogens.

Streptomvces spp. are filamentous bacteria that readily produce a wide variety of antibiotics effective against many soil microorganisms (Alexander 1971). Some members of this genus cause plant diseases, e.g. potato scab caused by **a**. scabies (Agrios 1969). However, mv-y are soil saprophytes and aggressive competitors with other microorganisms (Alexander 1971; Griffin 1972). An isolate of S. griseoviridis obtained from light-colored Sphaqnum peat in Finland has been shown to be antagonistic toward several plant pathogenic fungi (Tahvonen and Avikainen 1987). A commercial preparation of this bacterium is marketed as Mycostop (table 1). It is a powdery formulation produced by fermentation and freeze-drying. This formulation is applied as a seed dressing and in solution as a soil drench. Tests have shown that it is effective in controlling damping-off caused by Fusarium, blight caused by Alternaria, and powdery mildew on foliage (Tahvonen and Avikainen 1987). The formulation has been shown effective on several greenhouse and field crops such as carnation, cauliflower, sweet pepper, and wheat. However, recent trials on container-grown conifer seedlings indicated that at the levels o pathogen and antagonist tested, Mycostop was ineffective in controlling Fusariumassociated damping-off of Douglas-fir (James and others unpublished). Results indicated a possible slight delay of damping-off in Streptomvces-treated seedlings, but no lasting protection was seen.

Other genera of bacteria which have shown promise as biocontrol agents include <u>Bacillus</u>, <u>Enterobacter</u>, and <u>Pseudomonas</u>. <u>Bacillus subtilis</u> is one of the most promising bacterial inoculants

for control of seedling blight on several different crops (Campbell 1989; Loeffler and others 1986). This bacterium often survives soil pasteurization because of its ability to form resistant spores, and shows greatest efficacy when soil moisture levels are high (Baker and Cook 1974). Enterobacter cloacae is a common inhabitant of seed coats of many plants; it has been shown to effectively control Pvthium damping off when inoculated onto seed (Harman 1991). Several species of Pseudomonas show much promise as biocontrol agents. Especially promising are the fluorescent pseudomonads that not only produce antibiotics (Schroth and Hancock 1982), but also successfully compete for available iron, utilizing the mineral and restricting development of pathogens because of iron-limiting conditions (Loper and Buyer 1991). Although several of these bacteria show promise, none have been developed commercially for Hse as biocontrol agents, other than Mycostop .

CONCLUSIONS

Biological control is probably the normal situation in natural, undisturbed plant ecosystems. In most cases, competition among the myriad of organisms associated with plants limit dominance by a few. However, in agroecosystems, this "biological balance" has often been drastically upset either by growing extensive monocultures or by introducing broad-spectrum pesticides that disrupt microorganism populations. Pathogens often find these disrupted ecosystems conducive for their development. Therefore, the primary goal of biological control should be either to reestablish the balanced microbial community or suppress activity by pathogens by introducing organisms that can effectively limit pathogen activity.

Recent restrictions governing the contamination of agricultural environments by chemical pesticides has placed greater emphasis on finding alternative treatments for disease. Biological control, being more "natural", is a widely-accepted alternative to traditional chemical pesticides. Unfortunately there are some disadvantages of biocontrol that should be realized. Biocontrol methods are often not as effective in completely eliminating disease as chemicals have been (Campbell 1989; Baker and Cook 1974). Growers must realize that disease losses may be greater, sometimes significantly greater, under regimes emphasizing biological control. Stock quality may also suffer under non-pesticide growing regimes. This is especially true for bare-root nurseries where there is less control of environmental variables. However, in greenhouse nurseries, reduced chemical usage may be easier to implement while maintaining stock quantity and quality because of more control of the environment and pathogen introductions (Dumroese and others 1990). The concept of "acceptable losses" will become more important as pest management

approaches an integrated system rather than relying strictly on chemicals.

Another serious problem facing implementation of biological control in forest and conservation nurseries is the lack of available organisms for testing. As seen in table 1, very few commercial preparations are available for growers to use. Also, preliminary tests of some of these (GL-21^R and Mycostop^R) indicate that they may be ineffective against pathogens on tree seedlings (James and others unpublished). The most appropriate alternative is to develop biocontrol systems directly from forest seedling systems so that candidate biocontrol organisms would have a greater chance of working in our nurseries. However, research support for nursery diseases in general and biological control in particular is almost totally lacking. Unless more emphasis and resources are directed toward this needed research, we will have to continue to rely on products developed for other agricultural crops. If these products are not effective in forest and conservation nurseries, we should not be surprised since they were not developed with our crop in mind.

Future efforts in developing biologicals for control of plant diseases will most certainly focus on genetic engineering (Baker 1991). Extensive investigations are underway to understand the molecular basis for disease and antagonism by different organisms. Techniques are available to design microorganisms that produce the metabolites needed to effectively control pathogens. These approaches will certainly produce new organisms that work better in controlling pathogens than the ones currently available. However, there will be some inherent problems associated with using such new organisms, such as effective tracking once released into the environment, and reducing the potential for unforeseen side effects (non-target effects) of released organisms (Campbell 1989). However, if these problems are adequately overcome, the future for manufacturing new biological control organisms should be bright.

With regards to forest and conservation nurseries, the future holds promise for greater emphasis on biological control to limit damage from diseases. Our cropping systems are ideally suited for implementing integrated pest management which should include some level of biological control. However, it will take a determined commitment from growers if successes are to be achieved. The extent of that commitment and support for greater research and development will govern the success of biological control in the future.

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