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Strategies for Detecting and Characterizing Systems fo

Interest in biological control of plant pathogens has increased strikingly in the past decade. Wider use of biocontrol, however, has been delayed in part (21) by uncertainty as to how studies can be initiated in this field, particularly methods for finding and testing potential antagonists. By briefly considering how successful biological control systems were discovered and by describing strategies that could be used for other disease situations, we hope the efficiency of finding and exploiting biological control agents will be increased.

Detecting Biocontrol Systems

Although biological control of plant pathogens is common, relatively few examples have been investigated. Every natural soil has potential for some microbiological disease suppression. Failure to recognize instances of disease suppression has precluded their exploitation by plant pathologists.

Biological control can be viewed as a continuum from conspicuous disease suppression through intermediate degrees of suppressiveness/conduciveness (inconspicuous biocontrol) to the extreme of no disease suppression (totally conducive). There are few cases for the latter except under near-sterile conditions, which in itself is strong evidence that biocontrol occurs everywhere.

A prerequisite to detecting biological disease suppression is knowledge of the biology of the pathogen and the disease it causes, its geographic distribution, and the edaphic factors that contribute to disease expression. Growers, farm advisors, disease survey teams, and other field pathologists should be consulted about fields or areas in fields where disease suppression occurs and the conditions under which diseases are suppressed. This preliminary strategy has resulted in the detection and elucidation of most of the following examples.

Fig. 1. Biological suppressiveness of a Queensland avocado grove soil to *Phytophthora cinnamomi* by selective steam treatments. (Left) Untreated soil, (center) soil treated with aerated steam at 60 C for 30 minutes, and (right) soil treated with aerated steam at 100 C for 30 minutes. Each flat was then inoculated with *P. cinnamomi* and seeded to susceptible Jacaranda. Plants are shown after 4 months at 23.5 C. (Courtesy P. Broadbent)

Pathogen introduced but disease absent. *Fusarium*-suppressive soils (Toussoun in 5) have been recognized by scientists and growers for many years by the absence or diminution of disease even though pathogens were introduced to the area or by the absence of pathogens from certain soils (ie, fusaria in forest soils). This knowledge has been significantly exploited by farmers in many areas where the phenomenon occurs. In fact, it is because of the disease suppressiveness that certain crops can even be grown. Thus, vegetables are grown in the Salinas Valley of California, bananas in areas of Central America, beans in areas of Washington, and muskmelons in areas of France without incidence of Fusarium diseases even though the pathogens are present, the crop varieties are susceptible, and the climatic and cultural conditions are favorable for disease development.

Fusarium-suppressive soils have only recently been analyzed to determine why they are suppressive. Sandy soils are known to be generally highly conducive to the vascular wilt diseases caused by formae speciales of *F. oxysporum*, but these diseases rarely occur on clay soils,

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Biological Control of Soilborne Plant Pathogens

especially those rich in montmorillonites. The pathogens generally are present in both suppressive and conducive soils, although in somewhat greater numbers in the latter. Further, the suppressiveness is biological and is largely a potential of the soil before the pathogen is introduced. The clay component apparently has no direct effect on the pathogen but favors the development of an antagonistic microflora that interferes with the activities of the pathogen.

In recent years, the *Fusarium*-suppressive soils of the Châteaurenard region of the Rhône Valley of France have been studied in detail (1). These studies exemplify our strategic approach to understanding and exploiting biocontrol systems. Since ancient times, muskmelons have been grown in that region of France without incidence of Fusarium wilt even though the disease was present a short distance away. Louvet and his colleagues (1) have characterized this natural biocontrol system as highly specific to Fusarium vascular wilts caused by different formae speciales but, interestingly, ineffective against other nonwilt *Fusarium* diseases or diseases caused by other root pathogens. Considerable progress has been made to determine what microorganisms are responsible for the *Fusarium* suppressiveness in these soils. Of the numerous microorganisms tested, many fungi were totally ineffective, but isolates of *F. oxysporum* and *F. solani* (but not *F. roseum*) were highly effective, leading the French investigators to hypothesize that principally fusaria were involved in the suppression phenomenon. They hypothesize that somehow the fusaria responsible for suppression work in concert with associated bacteria to regulate the numbers and activity of the pathogenic *F. oxysporum* population.

Utilization of *Fusarium* suppressiveness is possible without wholly understanding the mechanism, especially if suppressiveness can be transferred to conducive media such as steamed greenhouse potting mixes. The French muskmelon wilt suppressiveness was successfully transferred to such mixes, as was the suppressive factor in Salinas Valley Metz fine sandy loam soils of California. Scher and Baker (20) showed by selective aerated steam treatments that suppressiveness was eliminated in soil steamed at 54 C for 30 minutes and was also very sensitive to pH. Bacteria (especially a *Pseudomonas* sp.) isolated directly from mycelium of the wilt pathogen buried in the suppressive soil induced suppressiveness when added to steamed greenhouse soil.

It has been noted (Toussoun in 5) that *Fusarium* was conspicuously absent from certain soils, even though it had been introduced. For example, nursery-grown seedlings of sugar pine carried a high rhizosphere population of *Fusarium*, but the population dwindled and disappeared a few years after seedlings were outplanted in the forest. Annual plants, which might support *Fusarium* populations away from pine roots, are suppressed by the litter layer in the forest ecosystem. Pine roots become suberized, and specialized rhizosphere microorganisms presumably exclude *Fusarium*, which fails to survive in the absence of plant roots. The microorganisms responsible have been neither identified nor exploited in nursery practice.

Disease absent but pathogen present. *Phytophthora root rot of avocado.* A 1969 request to Australian nurserymen for information concerning situations where disease did not occur even though the environment was very favorable and surrounding plantings sustained severe losses disclosed the Ashburner avocado grove on Tamborine Mountain in Queensland. *Phytophthora cinnamomi* had established in the soil, but root rot of this highly susceptible crop was very rare. The soil was still suppressive after aerated

Fig. 2. Pineapple heart and root rot, caused by *Phytophthora cinnamomi*, controlled (left row) by 1,200 kg/ha of sulfur disked into the infested soil. (Courtesy K. Pegg)

steam treatment at 60 C for 30 minutes, but steaming at 100 C for 30 minutes destroyed suppression (Fig. 1). This showed that the effect appears to be due to spore-forming bacteria and/or actinomycetes. The grove has been maintained for nearly 40 years under conditions of abundant organic matter and calcium, nitrogen in the ammonium form, and a pH near neutrality (2; Broadbent and Baker in 5); this cultural practice has become standard for root rot control in avocado in Australia. Disease control in the Ashburner grove is through diminished zoospore production by the pathogen.

Application of the "Ashburner system" to another farm (Ware) in which dead and dying avocado trees had been pulled before replanting also gave excellent control of root rot. In contrast to the Ashburner grove soil, the population of the pathogen in the Ware farm soil declined rapidly, then stabilized at very low detectable levels. The pathogen produces copious zoospores in this soil, but lytic microorganisms rapidly destroy them (Pegg cited in 2).

Root-knot nematode. Observations in an old peach orchard on Lovell rootstock highly susceptible to root-knot nematode showed the trees were growing well despite the presence of *Meloidogyne* spp. (22). The fungus *Dactylella oviparasitica* was destroying egg masses and providing excellent field control.

Onion white rot. Onion white rot caused by *Sclerotium cepivorum* was first detected in the lower Fraser Valley of British Columbia in 1970. Concern over its spread led to a survey of fields in 1977 to determine where the disease and/or pathogen occurred. The virulent pathogen was found in 10 fields, only one of which produced diseased onions. The absence of white rot in infested fields was not due to unique pH, temperature, or moisture conditions, and it was suggested that levels of fungistasis were higher in the disease-suppressive fields. Splitting sclerotia and plating directly showed that fields without disease had fewer viable sclerotia than fields with disease. Lack of germination was due largely to competing microorganisms, many of which were highly antagonistic to *S. cepivorum*. Several isolates of *Bacillus subtilis* and *Penicillium nigricans* provided levels of control in field experiments comparable to chemical control (23).

Crown gall. Biological control of crown gall is a classic success story with worldwide commercial application. It began when A. Kerr and colleagues noted that the ratio of pathogenic to nonpathogenic *Agrobacterium* in soil was very low around healthy trees and high next to galled trees. By artificially increasing the ratio of nonpathogens to pathogens, they reduced infection on tomato stems. Subsequently, nonpathogenic *A. radiobacter* strain 84, isolated from soil near a galled peach tree, was shown to prevent crown gall when inoculated 1:1 with *A. tumefaciens*. Paradoxically, hundreds of other avirulent strains, whether antibiotic producers or not, failed to provide biocontrol. Kerr stated that, fortunately, K84 was one of the first avirulent strains tested, otherwise biological control might have been overlooked (12,13).

Pathogen decline with monoculture. *Take-all decline.* Take-all disease, caused by *Gaeumannomyces graminis* var. *tritici*, has been steadily disappearing in wheat fields since 1900, and it was suggested that continuous wheat cultivation gradually increased antagonistic soil bacteria that inhibited the fungus (Shipton in 5). Only in the last 15 years, however, has the spontaneous decline of take-all during cereal monoculture been appreciated and investigated. The take-all decline phenomenon has now become a model from which we can extrapolate to many other root-disease situations.

Take-all decline (TAD) has been found in many parts of the world following the peak of disease intensity and has remained at a fairly consistent level as long as monoculture continues. Rotation with certain crops reduces the level of disease suppressiveness. TAD is brought about by a generalized microbiological antagonism between the pathogen and other microorganisms in the soil. This antagonism can be induced only when virulent inoculum of the pathogen infects the host, and not by adding avirulent or killed inoculum. Further, host roots alone will not induce TAD, but they help in some way. Vojinovic (cited by Shipton in 5) found a higher level of suppression in the rhizosphere of diseased plants than in that of healthy plants. In root lesions, where pathogen hyphae and nutrients from leaking host tissues are abundant, the greatest increase occurs in antagonists that specifically inhibit the pathogen. The runner hyphae have reduced branching in the lesion area and there are fewer fine infection hyphae, presumably resulting from exhaustion of nutrients by the abundant microflora or from hyphal lysis. The enhanced antagonism decreases the survival of inoculum. Since organisms involved in TAD are killed by moist heat between 40 and 60 C, the antagonism is due, at least in part, to non-spore-forming bacteria (eg, *Pseudomonas* spp.) or fungi. When small amounts of TAD soil containing these organisms are transferred to conducive soil, TAD will develop under monoculture.

Bean root rot. Burke (6) reported that bean fields in Washington remained free of root rot even though beans had been cultivated there for many years. Tests with these "resistant" soils showed that the resistance was of biological origin, had developed as a result of bean monoculture, but could not be transferred to other soils.

Cyst nematode decline. Populations of the cereal cyst nematode, *Heterodera avenae*, declined to unimportant levels after repeated intensive cropping of cereals in Britain. This decline was caused by the parasitism of females and eggs of *H. avenae* by the fungi *Nematophthora gynophila* and *Verticillium chlamydosporium* (Kerry in 18).

Chestnut blight. Although not a soilborne disease, chestnut blight decline exemplifies an important principle. A. Biraghi's observation for several years of spontaneous healing of cankers on chestnut trees infected with *Endothia parasitica* led to the discovery of hypovirulent strains of the fungus and to application of the strains to biologically control chestnut blight (Grente and Berthelay-Sauret in 14).

Physicochemical treatment affecting antagonist or pathogen growth. *Soil pH change and pineapple root rot.* Because pineapple does not tolerate alkaline soil, the Ashburner system could not be used to control *P. cinnamomi* on that crop. Pegg (cited in 2), however, applied sulfur to Queensland soil and controlled heart and root rot of pineapple (Fig. 2). This treatment lowered soil pH to 3.7, which in turn decreased zoospore formation, perhaps by reducing activity of bacteria that stimulate sporangial formation and by increasing soil ionic concentration. Nitrifying bacteria that convert ammonium to nitrate were inhibited under these conditions, leaving ammonium levels toxic to the pathogen. Furthermore, populations of antagonistic *Trichoderma* spp. were greatly increased.

Crop sequence/fertilizer and bean root rot. In Michigan, the incidence of bean root rot, caused by *F. solani* f. sp. *phaseoli*, decreased after application of nitrate fertilizer or when beans followed corn and increased after application of ammonium fertilizer or when beans followed barley. Disease reduction was correlated with the occurrence of an antagonistic bacterium that caused hyphal lysis of *F. solani* in soil. Bean seedlings aseptically placed in a petri dish with *F. solani* became infected, but not when the antagonistic bacterium was associated with the hyphae (11).

Soil fumigation and Armillaria root rot. Biological control of *Armillaria mellea* by *Trichoderma* spp. was detected when soils were fumigated with sublethal doses of carbon disulfide or methyl bromide. The fumigants apparently prevent the formation of a protective antibiotic produced by *Armillaria*, which then is parasitized by *Trichoderma* (15).

Soil steaming and damping-off. It is generally known that most natural, untreated soils contain antagonistic microorganisms that inhibit some pathogens. These organisms, however, may become apparent only when competing organisms are reduced or removed, as by steaming. Olsen and

Baker (17) showed that antagonists of *Rhizoctonia solani* in natural soil were eliminated by steam at 100 C but not by aerated steam at 60 C (Figs. 3 and 4). More recently, Chet and Baker (7) showed that *R. solani* caused disease on carnation in Colombia in steamed soil but not in nonsteamed soil. Antagonistic microorganisms, especially *Trichoderma hamatum*, had been eliminated by steaming; addition of *T. hamatum* to soil induced suppressiveness.

Baiting for mycoparasites. There are many reports of detecting antagonists by their inhibition of pathogens on soil dilution or isolation plates. Such antagonists are frequently tested in a natural system for their capacity to suppress disease, but the number of successes is very low. In contrast, some of the best antagonists giving biological control have been obtained by various "baiting" methods or by simply isolating them from parasitized propagules recovered from soil.

Mycoparasites of *Sclerotinia sclerotiorum* were detected by adding sclerotia as bait to soil, retrieving them, and isolating potential mycoparasites from their surfaces and interiors (Ayers and Adams in 18). Potential mycoparasites were tested by adding them to soil amended with sclerotia as substrate, and then sieving the sclerotia from the soil and assessing the percentage that were parasitized. Ayers and Adams found *Sporidesmium sclerotivorum* on sclerotia and showed that it parasitized *S. sclerotiorum* and *S. minor* sclerotia. Further, application of 100 and 1,000 *S. sclerotivorum* conidia per gram of soil provided disease control of 40 and 83%, respectively, in four successive crops over 2 years. Disease control was correlated with reduction in *S. minor* inoculum density (Fig. 5).

Characterizing Biocontrol Systems

Where do we look for biocontrol? What are the signs? Procedures that allow disease reduction to be quantified in a comparative way must be developed to answer these key questions about the biocontrol system. The inoculation method must take inoculum density into account. Too little or too much inoculum may mask the effects of suppressive organisms and make it difficult to assess whether the suppressive factor is present and functioning. Environmental conditions must be such that plants are not predisposed by stressful extremes that may mask the suppressiveness of the soil. For example, flooding the soil will temporarily nullify the Ashburner suppressiveness to *Phytophthora*. The form and amount of inoculum and the amount of food base used to grow the inoculum must be considered carefully, especially if the food base serves as the inoculum carrier. The inoculum should be in the most natural form and quantity possible and without excessive nutrients.

Conspicuous signs of biocontrol. Baker and Cook (3) provided the basic principle guiding the search for biocontrol: Look where the disease does not occur but should be expected because the disease occurs elsewhere in the area, all environmental conditions are favorable, and the pathogen has been introduced. Do you or others know of a field or portions of a field where disease does not occur, or where the disease did occur but has declined through the years? Disease suppression may be related to some inoculum. If less disease occurs in the candidate soil, then some suppressive factor is present. If autoclaving or otherwise sterilizing the soil removes this suppressiveness, then the factor is thought to be of microbial origin.

When the answers to the above questions have all suggested a biological suppressiveness, several key experiments can be conducted to identify the suppressive factor(s). These experiments again depend on a critical and quantitative assay to measure disease reduction due to suppressive factors.

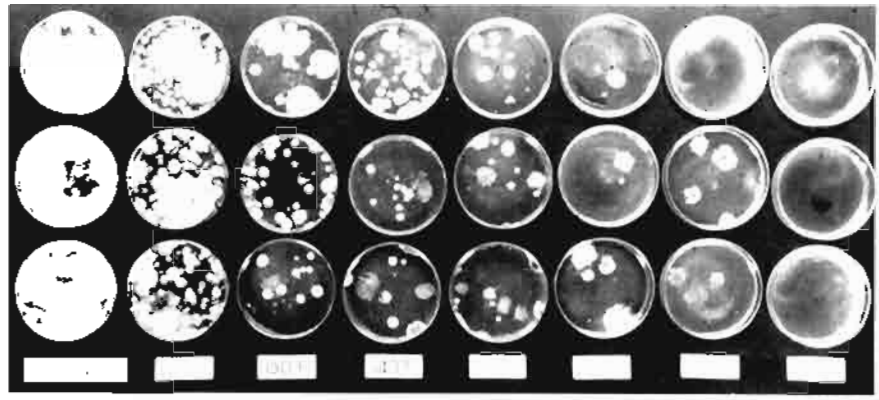

Fig. 3. Effects of selective heat treatment of natural soil on microbial populations. Soils were treated with aerated steam for 30 minutes at indicated temperatures. (Courtesy K. F. Baker)

significant change in cultural or management practice. The Ashburner system of developing *Phytophthora* suppressiveness became known by asking nurserymen if they knew of situations where disease did not occur.

When a potential biocontrol situation is identified, another set of key questions must be answered:

1. Are the plants in the suspected area infected even though the incidence or severity appears less than in other areas? Plants may be less severely infected, or infection may have begun but is developing slowly or is not continuing to develop as in adjacent "conducive" areas.

2. Is the pathogen present in the suspect soil? If so, what is its distribution and population level? The pathogen may be unevenly distributed in the soil or at a lower level than in conducive soils. That may be a significant clue that some biological factor has inhibited pathogen development. The pathogen may be difficult to isolate from suppressive soils or infected plants because of the presence of antagonists; antibiotics may be useful to inhibit the antagonists.

3. Can the pathogen be established in the candidate soil as readily as in conducive soil, and will the added amount of inoculum induce disease comparable to that in the conducive soil? An approach to these questions is to determine disease incidence and severity resulting from autoclaved vs. untreated conducive and candidate suppressive soils amended with the same levels of

1. Is the suppressive factor transferable? In published examples, from 1 to 25% of suppressive soil has been added to live, steamed, or fumigated soil, which is then periodically assayed for induced suppressiveness. Not all suppressive soils have been shown to be transferable to a conducive medium, however. Development of suppression depends on the relative receptiveness of the conducive soil to the biological entities.

2. Can the suppressive factor be isolated from specific fractions of the soil selectively treated thermally (heat or cold) or by desiccation, radiation, or chemicals that selectively kill some segments of the total population? For example, the organisms responsible for take-all decline are killed between 40 and 60 C, indicating their relative temperature sensitivity. The Ashburner *Phytophthora*-suppressive organisms, on the other hand, are not killed at 60 C, indicating they are heat-tolerant organisms. The disease reduction assay is used to indicate what treatments have or have not killed the suppressive organisms (Fig. 1).

3. Can the active organisms be selectively favored by enrichment or otherwise accentuated? At this stage, the biocontrol agent may be isolated, since selective treatments can be used to narrow the range of organisms involved. Selective enrichment tests can be conducted to encourage specific antagonistic organisms to increase faster than others. Onion white rot in which sclerotia of the pathogen served as bait or

substrate to recover antagonists (23) is an example of this strategy. Similarly, Vojinovic (cited by Shipton in 5) exploited take-all lesions as selective enrichment for TAD antagonists, both the pathogen and host lesions acting as bait. Henis et al (10) also concluded that both pathogen and lesions were essential to develop biocontrol of Rhizoctonia root rot of radish.

Organisms from areas that are very likely to contain active antagonists can be isolated by direct plating of plant tissues (lesions) or pathogen propagules or by washing organisms off those tissues or propagules into sterile water, then following standard dilution-plate methods. A general isolation medium may be used if the antagonist group is not known; if the group is known, a selective medium can be used.

Sometimes, hyphae or other propagules of the pathogen can be observed and removed from their natural habitat (11; Ayers and Adams in 18). Occasionally, such propagules are being parasitized and destroyed (lysed) by other microorganisms. As mentioned earlier, effective biocontrol agents have been isolated using this approach (Ayers and Adams in 18; 23).

Once specific organisms are isolated and grown in culture, the temptation is great to test their efficacy against the pathogen in vitro. We urge that bioassays be continued on plants as the primary indicator, with in vitro assays conducted secondarily to gain evidence in support of the plant tests. Furthermore, the bioassay should be conducted in the soil where the organism(s) ultimately will function. Another important consideration is to place the test organism(s) in the potential infection court before the roots are occupied by other competing organisms.

Inconspicuous signs of biocontrol. Biocontrol occurring without conspicuous signs may be missed because we have not looked in the right places or have not recognized the signs for what they really are. Biocontrol is going on in all natural soils in varying degrees. Some soils have a greater potential for disease suppression than others, and only in some does the level become obvious. The less obvious sites of disease suppression may still be sources of biocontrol agents, however. One needs first to recognize them as potential sites. For example, are individual or groups of healthy plants in a field of diseased plants protected because of biocontrol organisms in or on their roots? Admittedly, there are other likely explanations, but biocontrol may be one possibility. Soil from around individual healthy plants should be examined for suppressiveness or isolations should be made directly from roots, lesions, or pathogen propagules on those plants.

A subtle sign is disease spreading slower than expected or spreading rapidly and severely at the beginning of an experiment, then slowing down and eventually stopping. The surviving plants may be infected and viable inoculum is in the soil. Why didn't all the plants die? Did the antagonists equilibrate over time with the pathogen and stop its progress?

In the common observation that some cultural treatments result in less disease, the question infrequently asked is whether the treatment has triggered an increase in the specific antagonists that suppress the disease. One example is addition of sulfur to soil to control pineapple heart and root rot (Pegg cited in 2). Another is the banding application of ammonium and chloride fertilizer to suppress take-all of wheat (R. L. Powelson and T. L. Jackson, *personal communication*).

No signs of biocontrol. Biocontrol agents are in most soils and probably in water and air, usually without producing

Fig. 4. Effects of different aerated steam heat treatments on natural soil inoculated with *Rhizoctonia solani* and planted to peppers. Inoculum was placed in lower left corner of each flat at the time of seeding. (Lower left) Not steamed, (upper left) steamed at 60 C for 30 minutes, (lower right) steamed at 71 C for 30 minutes, and (upper right) steamed at 100 C for 30 minutes. Disease severity increased progressively as more antagonists were killed by higher temperatures. The reduced stand in the untreated flat (lower left) resulted from damping-off by a resident *Pythium* sp. A flat treated at 100 C for 30 minutes but not inoculated with *R. solani* was similar to the flat steamed at 60 C for 30 minutes (upper left). (Courtesy C. M. Olsen)

Fig. 5. Biocontrol of lettuce drop, caused by *Sclerotinia minor*, with sclerotial parasitism by the mycoparasite *Sporidesmium sclerotivorum*. (A) Sporulation of *S. sclerotivorum* on a sclerotium of *S. minor*. (B) Lettuce drop at first lettuce crop harvest 1 year after applying 1,000 *S. sclerotivorum* spores per gram of soil to *S. minor*-infested plot. (C) Control plot without *S. sclerotivorum*. (Courtesy P. B. Adams)

signs that they are or could be involved in biocontrol. When increased by selective enrichment or by selective removal of competing organisms, however, such agents become evident. For example, antagonists that reduced damping-off caused by *Rhizoctonia solani* were shown to be in soil by Olsen and Baker (17) by means of selective aerated-steam treatments. Those antagonists were isolated and tested for biocontrol potential by infesting a flat of steamed (100 C for 30 minutes) soil with the test bacterium and inoculating one corner of the flat with the pathogen at time of seeding with highly susceptible bell pepper (Fig. 4) (4; Ferguson cited in 17). The radial spread of infection, measured by seedling damping-off, indicated the level of suppressiveness of the antagonists. The fact that the pathogen sometimes spread well ahead of the margin of damping-off suggested that the antagonist had affected the hyaline infection cushions and penetration pegs rather than the resistant pigmented runner hyphae.

That antagonists may be airborne was demonstrated by A. G. Watson (cited in 3) with chrysanthemum cuttings rooted in a steamed propagative medium inoculated with a near-sterile organic medium that had been deliberately exposed to airborne contaminants. Some contaminants proved to be antagonists that provided good protection against *Pythium* when cuttings were transplanted to infested soil.

In biocontrol of brown blotch of mushrooms, several hundred bacteria were isolated from distilled water dilutions of mushroom casing peat and soil (16). Each isolate was tested for ability to cause blotch on 1-cm^2 blocks of aseptic mushroom tissue. Isolates that did not cause blotching were combined individually with *Pseudomonas tolaasii*, and serial dilutions of this mixture were inoculated on mushroom blocks. Three isolates that provided the best protection against blotching were tested further. They neither inhibited growth nor caused direct lysis of *P. tolaasii* in culture. When the antagonists and pathogen were mixed at a ratio of 80:1 and added to unsterilized casing peat, however, blotching in commercial mushroom beds was only 7.6–11.1%, compared with 100% blotching in the absence of antagonists. Competition for nutrients is suspected as the mode of action (16).

Management of microorganisms in biocontrol systems. The strategies employed up to the time of isolating potential antagonists will not identify microorganisms with disease control potential without additional knowledge of the factors favoring potentially antagonistic organisms. The "best" organisms selected from in vitro tests often fail miserably in greenhouse or field trials. Why? It may mean we have chosen the wrong organisms or have not provided the conditions necessary for them to function. "I tried them but they didn't work" is often heard. Unfortunately, few investigators stay with a study long enough to find out why they failed.

Several types of critical information are useful in the final testing stages of a potential biocontrol agent, including the type, organic matter content, pH, nutrient level, and moisture level of the soil from which the potential biocontrol agent came. Since the organism was working in that soil, it may function only in tests that nearly match those conditions. The easiest way is to use the same soil for the tests, but conducting other experiments with the soil variables to see how they affect the performance of the biocontrol system is also useful. Certain variables are known to nullify biocontrol, and success in our tests could be precluded by ignoring some of these critical factors.

Failure to demonstrate a positive response with tested biocontrol agents may result from application of the organism(s) to the wrong place or at the wrong time. Biocontrol agents are living entities with certain environmental requirements. Biocontrol of a pathogen is the result of normal metabolism by the antagonist and thus is slower acting and usually has less potential than chemicals to eradicate the pathogen. The antagonist's greatest biocontrol potential is as a preventive, and thus it should be applied before the pathogen is introduced. For example, the K84 control agent is most effective when established at the wound infection site before the crown gall pathogen. Application of K84 as a soil drench after transplanting nursery stock into the field does not give biocontrol. Should a potential antagonist recovered from an acidic forest soil and tested in an alkaline soil low in organic matter be expected to work? Altering soil pH by one unit can drastically affect the efficacy of a test organism (20). We need to "manage" biocontrol systems if we expect them to maintain effectiveness. The biocontrol organisms must be applied and remain viable, possibly under special conditions, to be effective.

Improvement of biocontrol agents by genetic manipulation. Genetic techniques now available may prove very useful in developing new microbial antagonists. Existing desirable traits may be enhanced by simple selection for spontaneous or drug-induced mutations. For example, a mutant of *A. radiobacter* K84 that produced greater amounts of agrocin 84 was developed with the use of a chemical mutagen (8). Antibiotic-resistant or fungicide-resistant mutants of biological control agents may be selected and used in an integrated program with chemical controls (19). Techniques are also available to move genes from one organism to another, permitting combination of desirable traits from different organisms into one. Transfer of the bacteriocinogenic plasmid from *A. radiobacter* K84 into different background strains, however, resulted in an effective biological control agent only when the background strain was able to grow vigorously at the wound site (9).

Genes for antibiotic production or other mechanisms of biological control may be introduced into organisms that already grow and colonize the site of infection. For example, one could select an organism that normally lives in the xylem of a plant species and then introduce genes for antibiotic production to control a vascular wilt pathogen. Likewise, an inhabitant from the rhizosphere of the plant on which you wish to control a root pathogen may be selected and then engineered to produce antibiotics.

The Challenge of Biocontrol

The potential for biological control of plant pathogens is great but has yet to be widely exploited. As pointed out by Baker and Cook (3), "Biological control is not inherently spectacular, and its successes tend to be overlooked or attributed to other factors."

Motivation to seek useful biocontrol systems comes in part from consideration by pathologists and growers of some advantages over other control measures. While biocontrol systems do not provide the rapid, effective control of some chemical toxicants, they have a longer residual activity. They are part of an environment that is more stable than those unbalanced systems resulting from "overkill" treatments. Biocontrol systems have a high degree of pathogen specificity for which there appears to be little resistance. The results from a stable biocontrol system often are more economical in the long run than those from short-term traditional controls.

Application and management of biocontrol systems will have to be developed on a case-by-case basis that takes into account the crops and cultural systems. It may be difficult or not cost-effective to establish and maintain a biocontrol system under certain conditions where environmental control is not possible, but in some stages in the production scheme, environmental controls are less significant. The introduction of soil/rhizosphere organisms as antagonists is difficult if the soil is already biologically buffered or the rhizosphere is already fully occupied. Thus, introducing the antagonist into the soil immediately after fumigation or steaming or establishing antagonists in the rhizosphere/rhizoplane at the time of rooting of cuttings, germination of seeds, or transplanting of seedlings may be useful strategies to preempt the potential infection site.

It is important to realize that the utility of a biocontrol system does not

necessarily depend on identifying specific organisms or on having a commercial product of single or combined organisms. A natural mixture that is transferable can be used. The inclination for many investigators is first to identify the antagonistic agent(s) or organism(s), but field testing by the scientist or commercial exploitation of the system should not await identification. In many cases, biocontrol will be achieved through cultural management of resident microorganisms.

It is also important to recognize and appreciate the specificity of many, if not most, biocontrol systems. Many aspects of this specificity are little known or understood. This is why we advocate the use of conditions similar to those where the system is working. This approach reduces the effects of those specificities as variables. To duplicate the natural system may be difficult in commercial production, but it has been accomplished in several situations.

Investigators of biological control of plant pathogens need to develop tenacity and to avoid becoming discouraged and frustrated. Perhaps the courage to persist may come from the knowledge that somewhere there is a working biocontrol system for nearly all plant diseases. The challenge is to find it, make it work, and then understand it.

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