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Myths and Dogmas of Biocontrol

Changes in Perceptions Derived from Research on *Trichoderma harzianum* T-22

The biological control of plant diseases has long been an area of fruitful study for plant pathologists, and there are thousands of publications on this topic. However, in spite of this extensive research effort on the part of hundreds of research scientists, there are very few uses of biocontrol in commercial agriculture today.

This statement is correct if we consider biological control to be the use of an added microbial agent to an agricultural system to control pests. It should be pointed out, however, that there are many biocontrol systems in use in agriculture that rely on some manipulation of an environment or ecosystem to reduce diseases. For example, diseases of wheat may be ameliorated by monoculture to induce suppressive soils, together with appropriate tillage, proper fertilizer type and placement, and timely application of herbicides (15,68). Crop rotation with specific alternate crops can reduce nematode levels (77), and composts will become suppressive to various plant pathogens due to the development of appropriate microbial communities if the composting process is properly managed (37,38). These and other cultural systems are based on a sound understanding of the microbiological processes involved and can provide highly effective biological control. However, this article will consider introduced microbial agents or, in the parlance of the Environmental Protection Agency (EPA), microbial pesticides.

I decided more than a decade ago that a priority for my research efforts would be to develop biocontrol systems that are used in commercial agriculture. This effort has included not only research but also other critical issues such as patenting, registrations, and commercial production and development. As a component of this effort, two colleagues and I cofounded a company, TGT Inc., now BioWorks, Inc., to translate biocontrol research into biocontrol reality as defined by sales of commercially useful products. This effort has been largely successful, and now products based on a single strain of *Trichoderma harzianum*, strain T-22 (a.k.a. 1295-22, KRL-AG2, or ATCC 20847), are sold in the greenhouse, row crop, and turf industries. In 1999, retail sales of T-22 products totaled around $3 million, and sales are expected to grow substantially over the next several years. This is one of only a very few biological control products for the control of plant diseases to reach even this modest level of commercial sales and acceptance. In the process of this effort, I have gained an appreciation for the place of biocontrol and its biological and commercial potential. However, I will sound some warnings for those who expect that their research results will ever be used in commercial agriculture.

Nearly all of my research and development effort has focused on *Trichoderma* spp., and the lion's share has dealt with strain T-22. T-22 was produced using protoplast fusion in my lab (73) in an effort to obtain highly rhizosphere competent strains that also possessed substantial ability to compete with spermosphere bacteria. The term rhizosphere competence was introduced in the 1980s by the late Tex Baker and his associates and is defined as "the ability of a microorganism to grow and function in the developing rhizosphere" (1). Strains that were fused were T-95 of *T. harzianum*, which was a rhizosphere competent mutant produced from a strain isolated from a *Rhizoctonia*-suppressive Colombian soil (1), and *T. harzianum* strain T-12, which was more capable of competing with spermosphere bacteria than T-95 under iron-limiting conditions (29); both were strong biocontrol agents. We observed great diversity in progeny strains (73). Strains were selected that were more strongly rhizosphere competent than either parental strain (34,69), that were strongly competitive in the spermosphere environment, and that were broadly effective biocontrol agents (34). Strain T-22 was the most effective of the strains produced (34,69), and research since then has been directed primarily to development, production, and use of this single strain. Over the past few years, this quest has been aided immeasurably by access to data from hundreds of field trials done by commercial and academic cooperators with BioWorks. None of the figures or the table

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Throughout this effort, I have gained some insights that differ from those of many of my colleagues in biocontrol R&D. This article is written to provide the perspective of biocontrol that has developed from immersion in both academic and commercial aspects of biocontrol. It will be colored and exemplified primarily by experiences with *Trichoderma*, but I hope that its underlying message will be useful to those working with other microbes. The paper will deal not only with scientific topics but also with the critical world of commercialization. If biocontrol is ever to live up to expectations, real-world success is essential. But it is by no means guaranteed.

Dogmas and Myths

As I attend meetings, read papers, and review proposals, I am struck frequently by dogmas that have been gaining acceptance within the biocontrol community. Many of these dogmas are not universally applicable:

- Biological control agents (BCAs) are necessarily less effective and reliable than chemical pesticides.
- Single BCAs added to roots or soil cannot affect microbial communities or control root pathogens for long periods of time. As a corollary, biological control is likely to be effective for seed and seedling diseases but not against diseases of the mature crop.
- Single BCAs cannot be effective in diverse environments, on different crops, or against a wide range of plant pathogens. As a corollary, mixtures of BCAs will be required for successful long-term control, since individual components colonize different crops, are adapted to different environments, or have different functions.
- BCAs have simple mechanisms of action that are controlled by one or only a few genes and gene products.
- Registration of BCAs with the U.S. EPA is relatively fast, inexpensive, and simple.

None of these statements are true for the systems and BCAs with which I work. However, if biocontrol researchers accept these concepts as largely or wholly true, these assumptions and concepts can impede biocontrol progress. In this article, I will present evidence that, in the case of the biocontrol systems and agents with which I work, these dogmas are in fact false.

I quote certain papers and articles as evidence that these particular beliefs are widely held. These quotes should not be considered as confrontational to the authors. I quote them because they all have made excellent contributions to biocontrol research and because they are highly respected by others in the field, including myself. In particular, I quote from the recent Plant Disease feature article by Mathre et al. (60), since they clearly have a very different perspective on many aspects of biocontrol than I, and they eloquently state their position.

Dogma 1. Biological control agents are necessarily less effective and reliable than chemical pesticides.

We frequently hear that biologicals have difficulty in acceptance because there is a paradigm of chemical control in the agricultural community. For biologicals, we need to introduce new concepts and uses, i.e., to substitute a biological paradigm for the existing chemical one. However, it frequently is unclear what this means in actual practice.

In my own research, I initially sought to develop biological seed treatments *for seed protection*. These efforts were largely successful as academic projects. We developed strains of biocontrol agents, devised methods and principles, and demonstrated that the biological agents could provide a high level of seed protection, especially when combined with solid matrix priming (also known as biopriming [60]) (32,33, 63,74,75).

To my initial surprise, there was very little interest from companies in commercializing this technology. In hindsight, the reasons are very clear and include the following: (i) there are many highly effective chemical pesticides available for seed protection; (ii) these chemicals frequently are less expensive than biologicals, especially if the BCA requires solid matrix or biopriming; (iii) the shelf life of the chemicals on seeds is superior to biologicals; and (iv) the chemicals protect seeds under a wider range of temperatures and other environmental conditions than biologicals.

In short, we were trying to use our biological agents in a system where chemical fungicides provide a better and more economical fit, i.e., we were trying to fit our BCAs into a chemical paradigm.

However, there are instances where biologicals may be highly attractive to commercial agriculture. Some niches where biocontrol may fit well are given below.

- Replacement of chemical pesticides lost to regulatory action or pest resistance and for which there are no adequate chemical replacements.
- Replacement, or reduction of use, of chemical pesticides in sensitive environments.
- Applications where biologicals accomplish tasks not possible for chemical pesticides.
- Organic applications.

The first niche is perhaps the most obvious and the one most frequently cited as a good reason for use of biologicals and one where there is a shift from "hard" chemicals to much softer alternatives. As an example, control of *Botrytis cinerea* and powdery mildews has become more difficult as fewer chemicals are available and

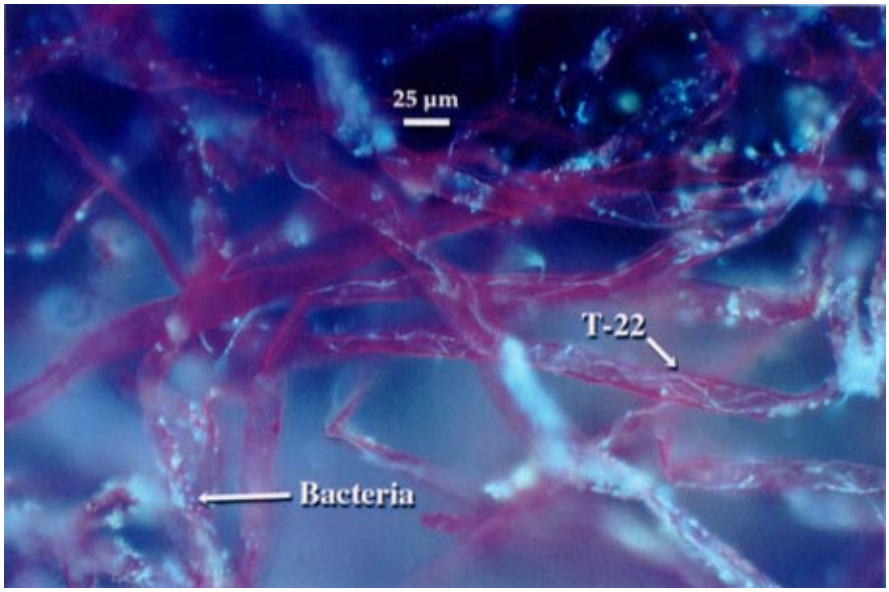

Fig. 1. Colonization of sweet corn root hairs following seed treatment with *Trichoderma harzianum* strain T-22. Root samples from plants grown in greenhouse potting soil were removed and washed. The root surfaces were then oxidized in 1% periodic acid, rinsed, and counterstained for 20 s in Schiff's Reagent. They were then rinsed and mounted in 0.1 µg/µl 4′,6-diamidino-2-phenylindole (DAPI) to stain DNA of inhabiting microbes and observed using epifluorescent microscopy. Fibrillar network of T-22 and bacterial colonies are indicated on photograph. Roots of similar plants grown in absence of T-22 lacked this hyphal network. Photograph courtesy of Thomas Björkman, Cornell University, Geneva, New York. Similar photographs (30) and similar results on creeping bentgrass roots with transgenic mutants of T-22 expressing β-glucuronidase have been published (55).

as pest resistance has increased. A range of biological alternatives is now or is expected to be in the marketplace shortly, including TopShield (strain T-22 of *T. harzianum*, BioWorks, Geneva, NY), AQ10 (*Ampelomyces quisqualis*, Ecogen, Langhorne, PA), and Trichodex (strain T39 of *T. harzianum*, Makhteshim-Agan Chemical Co., Israel). In addition, natural chemicals such as components of cinnamon oil (Mycotech, Butte, MT) and baking soda derivatives (H and J Agritech, Ithaca, NY, and TOAGOSEI Co., Tokyo, Japan) are registered for this use and are just beginning to be offered for sale. This is a niche where biologicals and natural chemicals can establish a presence and provide an alternative to other pest control strategies.

Different agricultural markets differ in the sensitivities of growers or other purchasers of products to the perceived and actual risks of chemical pesticides. Most row crop farmers, unless they cater to the organic trade, use those legal chemical pesticides that provide the most economic control of pests. On the other hand, greenhouse operators are quite sensitive to chemical use issues. EPA-mandated reentry times cause disruptions in their schedules, and they are concerned about worker safety issues. A biological with 0-h reentry and no known health risk to humans, as indicated by an exemption from residue tolerances on foods, is a big advantage to these growers. Consequently, biologicals may have an advantage over chemicals for the same purpose *if they are similarly priced and possess similar efficacy* in enclosed environments. Other examples where sensitivities of end-users to chemicals provide an advantage to biologicals include high public use and urban areas such as golf courses, homeowner properties, sports fields, and parks.

The third niche for biologicals is perhaps the most solid. Biologicals *can* provide advantages over chemical pesticides. Perhaps the best examples of this are strains that are rhizosphere competent and can provide advantages to the subterranean portions of the plant throughout the life of at least annual crops. Commercial biological control agents with this ability are available and will be discussed in the next section. Further, biologicals can be produced on-site, for example using the Bio-Ject systems manufactured by EcoSoil-Systems, and this may confer a perceived advantage over chemicals that must be purchased from a vendor and then applied.

The last niche, use of biologicals by organic farmers and homeowners, has long been a prime target of biocontrol developers. Biologicals may be acceptable to organic certification agencies (T-22 is listed by the Organic Materials Review Institute) and so provide pest protection within an organic framework. It should be noted that microbial pesticides do not automatically qualify for organic agriculture; approval by a certification agency likely will be required for each new agent/product.

Thus, it is important to choose an appropriate system for development of biologicals if commercialization is a goal of the research. Direct competition in a niche where chemicals are readily available and relatively inexpensive, and where there is no perceived premium for use of biologicals in the mind of the user is likely to be unsuccessful.

Dogma 2. Single BCAs added to roots or soil cannot affect microbial communities or control root pathogens for long periods of time. As a corollary, biological control is likely to be effective for seed and seedling diseases but not against diseases of the mature crop.

This perception is widely published; for example, Deacon (20) stated that biological control agents "achieve only transitory localised dominance of the rhizosphere, and in only some soils and seasons…" Similarly, Mathre et al. (60) indicate that nearly all commercialized microorganisms rely upon application of the antagonist "directly and precisely to the infection court when and where needed." Seed protection, therefore, is a logical target, and as a corollary, the most that should be expected from a seed treatment is seed protection and perhaps increased vigor from planted seeds. However, this places the biological squarely into the chemical paradigm where commercial success is difficult to achieve.

This need not be the case. At least two mechanisms, rhizosphere competence and induced systemic acquired resistance (SAR), give long-term protection at a substantial distance from the infection court.

Either fungal or bacterial agents may be rhizosphere competent; for example, *Pseudomonas* species control root diseases of wheat after colonizing roots (60), and *Bacillus subtilis* is sold as Kodiak (Gustafson, Inc., Dallas, TX) for the purpose of increasing plant yields through root colonization.

SAR from bacterial colonization has been well described, for example, by Kloepper and his associates (47). Fungi also can induce SAR, and this will be described in the discussion of Dogma 4. When SAR functions, application of a biocontrol agent to one portion of the plant provides protection to a wide range of pathogens on another part of the plant (51,52); for example, an organism providing SAR applied to roots may cause the plant to be resistant to pathogens on its leaves.

An important point regarding any study of rhizosphere competence is the physiological state of the colonizing microbe. At least in young seedlings, root hairs of corn are colonized by hyphae of T-22 and not by spores (Fig. 1). This is a critical distinction, since spores are quiescent and inactive in biocontrol (49). Unlike many other *Trichoderma* strains (49), T-22 can be added as spores to the soil volume, for example, as in-furrow or greenhouse soil drenches. The strain will then colonize roots, which indicates that the spores germinate and grow in contact with the roots (Fig. 1).

The concept of rhizosphere competence by strain T-22 has been extensively tested on a variety of crops. We have done a substantial amount of root plating experiments and usually find that the entire root is colonized (30,53). BioWorks also conducted root assays; if customers wished to know if T-22 "worked," i.e., colonized roots, they could send samples to the com-

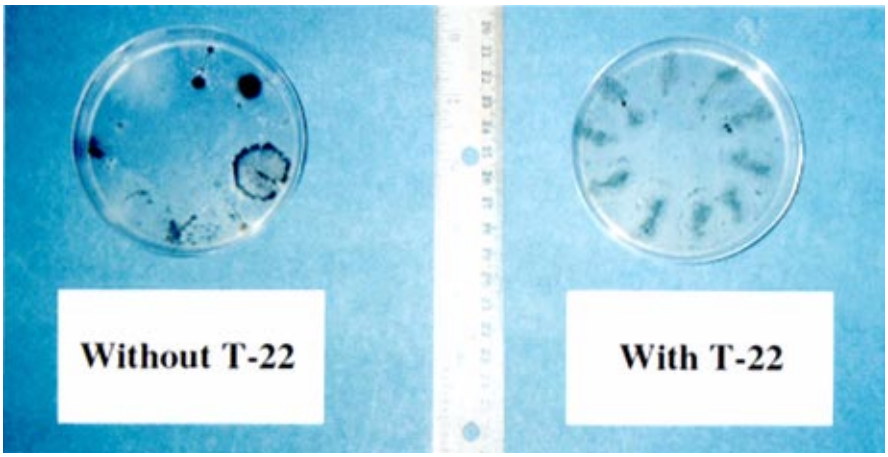

Fig. 2. Standard assay performed by BioWorks in which samples were sectioned into 2.2-cm segments from the upper, central, distal, and lateral portions of entire root system. Root segments were plated onto potato dextrose agar amended with 0.1% Igepal Co-630 as a colony restrictor (71) and chlorotetracycline (50 mg/liter). Numerous fungal types and species grow on this medium, and *Trichoderma* spp. can readily be detected. Typically, 80 to 100% of root segments were colonized by *Trichoderma* spp. over the entire 2.2 cm root length. In some cases, as in this example with poinsettia, T-22 was the primary fungus colonizing roots, while in its absence numerous other fungi were dominant.

pany lab with or without coded controls. Thousands of assays were done on crops ranging from ferns to beans to corn to ornamental flowering plants, and almost without exception, T-22 was found to colonize all parts of root systems. The basic assay technique is shown in Figure 2. These results frequently demonstrated not only that T-22 colonized roots but also that other microflora were displaced, thereby changing the root microfloral composition. Moreover, there was almost no effect of soil type or geographical location on this ability. Probably the only exception to this generalization occurred on cotton roots at the height of the summer from fields outside Phoenix, Arizona. T-22 was present only sporadically on roots obtained from these hot, dry soils. In other studies, T-22 has provided advantages to cotton. Importantly, T-22 has equal ability to colonize roots in both alkaline and acidic soils (53) and across soil types ranging from sandy to heavy and with a wide variation in organic content (30). Further, the method of application was not important. Colonization was obtained over the entire root surface when T-22 was added as a seed treatment, as broadcast granules on the surface of planted soil, as an in-furrow granule or drench, as incorporated granules in greenhouse planting mixes, or as a conidial suspension in greenhouse potting mixes. T-22 grew onto all newly formed root surfaces.

T-22 is not unique in this capability. We obtained strain 41 of *T. virens* from roots of pea plants in an *Aphanomyces*-suppressive soil and discovered it was capable of controlling *Phytophthora* spp. and other pathogenic fungi (71). We conducted a 3-year field study on the control of *Phytophthora* on raspberry. We evaluated raised beds, metalaxyl treatments, metalaxyl + *T. virens*, and *T. virens* added twice a year or once a month during the growing season. We assayed the root and soil fungal populations at monthly intervals over the course of the season. In the absence of application of strain 41, *T. virens* made up only a minor part of the total fungal population. However, when the biocontrol agent was added in any of the regimes noted above (*Trichoderma* spp. generally are resistant to metalaxyl), the *T. virens* populations became established and persisted as the dominant fungus colonizing roots and soil over the entire three growing seasons of the experiment (Fig. 3). The fungus was present in similar numbers over the entire growing season. Clearly, it became the dominant culturable fungus in the soil and persisted extremely well. The behavior of strain 41 differs from that of T-22 in one very important respect: T-22 does not become dominant in the soil but only on the roots. Conversely, strain 41 establishes dominance in both habitats.

In the case of the raspberry study noted above, one of the organisms that *T. virens* was displacing was *T. harzianum*. On roots of turfgrass, however, *T. virens* is the most commonly isolated native microfloral component and makes up the majority of the isolates obtained. If, however, T-22 granules were applied either at high rates or repeatedly, T-22 displaced *T. virens* and other fungi and became the most numerous organism, frequently making up more than 50% of the total fungi isolated from the root zone (K. Ondik and G. E. Harman, *unpublished*).

The consequences of root colonization by *Trichoderma* spp. can be profound in

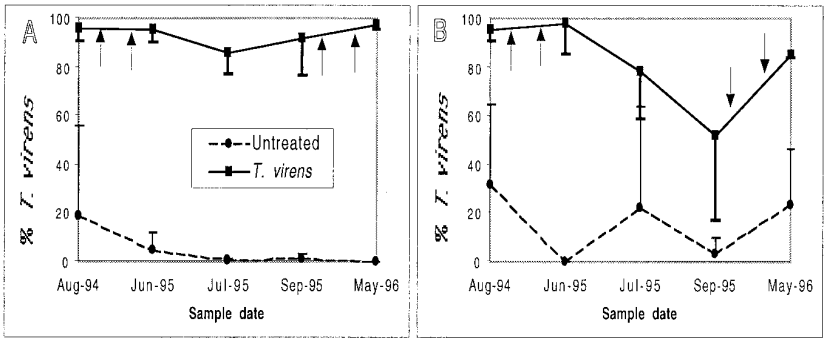

Fig. 3. Replacement of endogenous fungi by application of *Trichoderma virens* strain 41; percentage of total culturable fungi obtained from (A) roots and (B) soil. Strain 41 was applied as a granular application (62 kg/ha) in a strip 1 m wide on rows of raspberries once each in spring and fall. Initial application was at planting, and thereafter granules were banded on soil surface. Two soil cores were taken from each plot and dilution plated onto acidified potato dextrose agar (PDA) and amended with Igepal Co-630 as a colony restrictor (71). Fungi other than *Trichoderma* were easily distinguished on basis of morphology, and *T. virens* could easily be separated from other *Trichoderma* strains on the acid PDA medium. *T. virens* has a pure white coloration on its lower surface, while native *Trichoderma* spp. were tan to brown. Data shown were from plots of cv. Newburgh raspberries on raised beds in a field soil heavily infested with *Phytophthora fragariae* var. *rubri*. Arrows indicate application times. Each treatment was replicated four times, and error bars represent standard deviations. Data are from a larger trial conducted by W. Wilcox, G. Harman, P. Nielsen, and K. Ondik near Geneva, New York.

Fig. 4. Enhanced root development in field crops induced by *Trichoderma harzianum* strain T-22. (A) Roots of sweet corn grown from seeds either treated with T-22 Planter Box or not treated. Although roots colonized by T-22 were more abundant, in this particular trial yields were similar regardless of treatment; conditions of growth were good, and no yield advantage was provided by the improved root system. (B) Soybean plants with roots grown from seeds either treated with T-22 or not. A 123% increase in yield was obtained in this trial as a consequence of treatment with T-22.

terms of plant disease control and plant growth and productivity (6,13). Not only can colonized roots be protected against disease by T-22, but they frequently are larger and more robust (Fig. 4). This increased root development may occur as a consequence of control of clinical or subclinical pathogens, as suggested several years ago for *Pseudomonas* spp. (10). However, T-22 probably also has direct effects upon plant metabolism. In limited studies, we observed that T-22 was as effective as a commercial rooting hormone in inducing rooting of tomato cuttings, although callus tissue was not formed on the base of the cuttings as occurs with commercial hormone preparations (Table 1). Further, T95, which was one of the parental strains used in the fusion that gave rise to T-22, increased plant growth even under axenic conditions (79). Also, cucumber plants grown in axenic hydroponic conditions were larger in the presence of *T. harzianum* strain T-203 than in its absence (81). Therefore, T-22, as well as other similar *Trichoderma* strains, probably enhances root growth and plant development both by displacement and control of deleterious root microflora and by direct effects on plants by as yet unidentified biochemicals.

The length of time that *T. harzianum* strain T-22 can persist and proliferate on roots is rather remarkable, as was already demonstrated with strain 41 of *T. virens*. In an early field trial, we applied broadcast granules of T-22 in October 1993 to a field that had just been seeded to a rye grain cover crop; control plots were seeded to the rye cover crop without T-22. Early next spring, we sampled the roots and found a high population of T-22 (around 10^5 CFU/g dry weight of roots) on the roots where T-22 was broadcast but not on the control areas. We then killed the small rye grain plants with glyphosate and planted sweet corn into the untilled plots. Differences in growth of the corn between the treated and nontreated plots were evident over the entire season and were different at the end of the season (Fig. 5). As would be expected from the size of the plants, the yield of corn was also different, with 1.7 times higher weight of ears being harvested from the T-22–treated plots than from those originally planted to the rye cover crops without T-22.

Cover cropping also was investigated with a quite different system. In the fall of 1997, plots were planted to T-22 treated or nontreated seeds of a Sudan-sorghum (Sudex) hybrid on a highly organic (muck) soil near Oswego, New York. The roots of the Sudex were sampled after the crop became established, and there was a one order of magnitude higher population of *Trichoderma* spp. on Sudex grown from treated seeds than from nontreated seeds. The next spring, onions were planted on the Sudex + T-22 and the Sudex without T-22 plots. Yields were greater in the presence of T-22 than in its absence. The best yields occurred in rows that also received a maneb + metalaxyl (Ridomil) drench to control early-season diseases. With the integrated T-22 + fungicide program, yields were increased 10% relative to the same program without T-22, which was highly significant both economically and statistically.

All of these examples demonstrate that, with T-22 and *T. virens* strain 41 at least, it is possible to achieve much more than "transitory localised dominance of the rhizosphere, and in only some soils and seasons" (20). They indicate that rhizosphere competence is real and can result in long-term root colonization. If so, it should provide quantifiable improvements in plant performance. The following section indicates that substantial and unexpected advantages to plant growth and productivity can be conferred by strongly rhizosphere competent BCAs.

An important question regards the depth and degree of root enhancement with T-22. In 1999, we conducted trials with field corn in a sandy loam grower field. The field was planted to corn either treated or not treated with the commercial formulation of T-22 in alternating bands six rows wide throughout the field. At about the time of tasseling (about 60 days after planting), we dug trenches about 3.2 m deep with a back hoe about 15 cm in front of rows of corn about 2 m tall. The exposed soil profile was washed with a power washer to reveal the roots. We then established 25 × 25 cm grids across and down the soil profile. Each root intercept was marked with a map pin with a differently colored head (different colors for each adjacent grid), the grids were individually photographed, and the pins in each grid were removed, placed in separate boxes, and counted. Numbers of root intercepts were similar in corn with and without T-22 in the upper 25 cm of soil. However, roughly twice as many intercepts were found in the second and third grids when T-22 was present than when it was absent (Fig. 6A and B). This greater deep root density may confer substantial benefits to corn and other crops, especially in dry

Table 1. Enhancement of rooting and root elongation of cuttings of a *Solanum* × *Lycopersicon* hybrid as a consequence of dipping of stem cuttings in Rootone powder (1% indole butryic acid) or in T-22 in the form of RootShield drench

Treatment	Numbers of roots[a]	Length of roots (mm)
None	1.2 ± 0.8	1.8 ± 0.6
Rootone	1.1 ± 0.3	1.6 ± 0.6
T-22	2.5 ± 1.0	2.4 ± 0.6

[a] Thirteen days after treatment just as roots were being initiated.

Fig. 5. Growth of sweet corn without *Trichoderma harzianum* strain T-22 (left) and corn with roots colonized by T-22 (right). A granular formulation of T-22 was applied by broadcast (about 10 kg/ha) at the same time (October 1993) that a rye grain cover crop was seeded. In spring 1994, roots of rye were found to be colonized by T-22, and the rye was killed by spraying with glyphosate. Sweet corn (cv. Jubilee Supersweet with standard fungicide seed treatments) was planted into the field without tillage. Data are from G. Harman, H. Price, and P. Nielsen (pictured), Cornell University.

growing seasons when root colonization by T-22 can reduce sensitivity of crops to drought stress (Fig. 6C).

Further, we sometimes noted that corn plants that grew from seeds treated with T-22 were greener than plants without T-22. Increased greenness in corn frequently is associated with higher levels of nitrogen uptake (66). We began to consider the possibility that one of the effects of T-22 on colonized roots was increased efficacy of use of applied fertilizers, especially nitrogen. In 1998, we established a trial with field corn in a commercial grower's field on a sandy loam. The entire field was planted in bands six rows wide with seed treated with T-22 Planter Box alternating with six rows without the biocontrol agent. We expected that the initial nitrate level would be low, since the field had not received recent manure applications and because corn had been grown the previous season. Pre-sidedress nitrate soil tests (PSNT) verified that the endogenous nitrogen was indeed low, about 20 kg/ha. Nitrogen, in the form of ammonium nitrate, was banded and incorporated beside rows of corn at about the four-leaf stage to give totals, including the 20 kg/ha of residual nitrogen, of 20, 40, 80, 160, and 240 kg of total nitrogen per ha.

Differences were observed almost immediately. Heights of corn were measured 2 weeks after side-dressing, and those with T-22 responded more rapidly and remained larger for most of the growing season than control plots (Fig. 7B and C). At 2 and 4 weeks after nitrogen application, there was no difference in corn greenness as determined by readings with a SPAD meter (66). However, later, at the time of tasseling, the T-22–treated plants were greener in a nitrogen dose–dependent manner (Fig. 7D). At maturity, there was a difference in stem diameter and grain and silage yields (Fig. 7E to G).

Perhaps the most important yield comparisons were the points at which nitrogen no longer gave a yield increase, presumably because the plants had as much nitrogen as they could utilize. The unused nitrogen would not be expected to provide any yield benefit and probably would be volatilized or contaminate groundwater supplies (25). No increase in yield of either silage or grain occurred above 150 kg of N per ha in the presence of T-22. In the absence of T-22, however, the full rate of 240 kg was required for maximum yields (Fig. 7F and G), i.e., maximum yields were obtained with 38% less nitrogen in the presence than in the absence of T-22. The EPA is requiring a plan to reduce hypoxia in the Gulf of Mexico and may mandate a reduction in nitrogen fertilizer use in the Mississippi River Basin (see EPA web site: http://www.epa.gov/msbasin/legis98.html and American Farm Bureau Federation web site, news from 2000 annual meeting: http://www.fb.com/2000annual/amnews/hypoxia.html [Note: web addresses often change]). A microbial agent that increases nitrogen use efficiency by crop plants would appear useful in this regard.

A similar trial was established in the spring of 1999. Soil types and planting conditions were quite similar, but there was a high level of residual nitrogen in the field. PSNT values ranged from 40 to 90 ppm; corn usually does not respond to nitrogen fertilizer if values are above 20 to 30 ppm (35). Under these conditions, there was *no measurable response* of the corn to application of T-22. However, roots were colonized by the fungus, and deep root growth was enhanced; the data in Figure 6 are from this plot. Even in the presence of optimal or supraoptimal levels of N, T-22 apparently enhances corn root development, but unless N is limiting during some phase of the growth cycle of the corn, this enhanced root development does not affect plant growth or yield in the absence of other stress factors such as drought.

More than 70 separate T-22 trials have been done on field corn alone, more than 30 on soybeans, and numerous trials have been done on crops such as wheat, peas, sugar beets, and potatoes. Specific advantages and uses of T-22 on these crops are numerous and diverse. When T-22 is applied as a seed treatment on potatoes, both size (increased grade) and yields frequently are increased. When T-22 or *T. virens* strain 41 was applied in Brazil to wheat

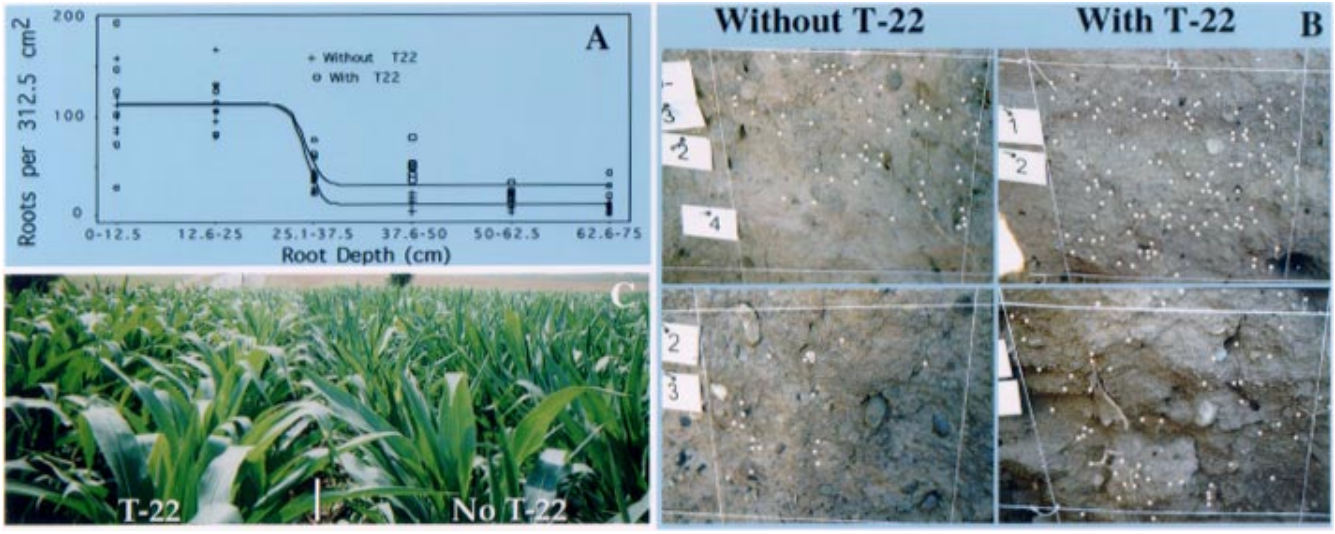

Fig. 6. Enhanced deep rooting in field corn induced by *Trichoderma harzianum* strain T-22. (A) Numbers of roots at different depths of mature (2 m tall) field corn (E238) in field trials near Geneva, New York, in 1999 with and without root colonization by T-22. Separate trenches (about 3.2 m deep × 5 m long) were dug with a backhoe about 15 cm in front of rows of corn. The resulting soil faces were washed with a power washer to expose corn roots, and grids of string were placed over the soil profile. Root intercepts in each square were marked with map pins, each square was photographed, and the number of pins in each square was counted. Data were modeled by logistic curves (72) of the form: Numbers of roots = $a + c/1 - b(\text{depth} - m)$, where a = lower asymptote, $a + c$ = upper asymptote, m = point of inflection and b = slope parameter. Lines are significantly different at $P = 0.006$. Data for the lower four regions of soil were fitted to linear regressions; there was no significant difference in slopes, but elevations were different at $P = 0.001$ (graph not shown). (B) Appearance of root intercepts in the same trial as (A) marked by map pins in single 25 × 25 cm squares 25 to 50 cm (top) or 50 to 75 cm (bottom) below the ground level. Squares shown were chosen because they approximate the average values for each treatment. (C) Drought tolerance in corn induced by T-22 from trials in 1999 in Ohio which probably was a consequence of enhanced deep rooting. Corn without T-22 (right) exhibited the typical leaf curl to reduce transpiration symptomatic of drought stress, while leaves of T-22–treated corn (left) did not. In both New York and Ohio, T-22 was applied as a dust to the seed according to labeled directions for T-22 Planter Box. Data in (A) and (B) are from field trials conducted by R. Petzoldt and G. E. Harman, Cornell University, while (C) is a photo from Ed Winckle, Hymark Consulting, Blanchester, Ohio.

seeds infected with *Pyrenophora tritici-repentis* (17), both stands and yields were increased significantly and substantially (yields increased from 1,666 to 2,166 kg/ha for strain T-22 and to 1,953 kg/ha for strain 41). The same authors obtained similar results over 2 years with corn seeds infected primarily with *Fusarium graminearum* and *F. moniliforme* (W. C. da Luz and G. C. Bergstrom, *unpublished*). Additionally, in 1999, trials were conducted on control of take-all in wheat. Seed treatment with T-22 enhanced spring green-up and significantly reduced white heads caused by *Gaeumannomyces graminis* var. *tritici*

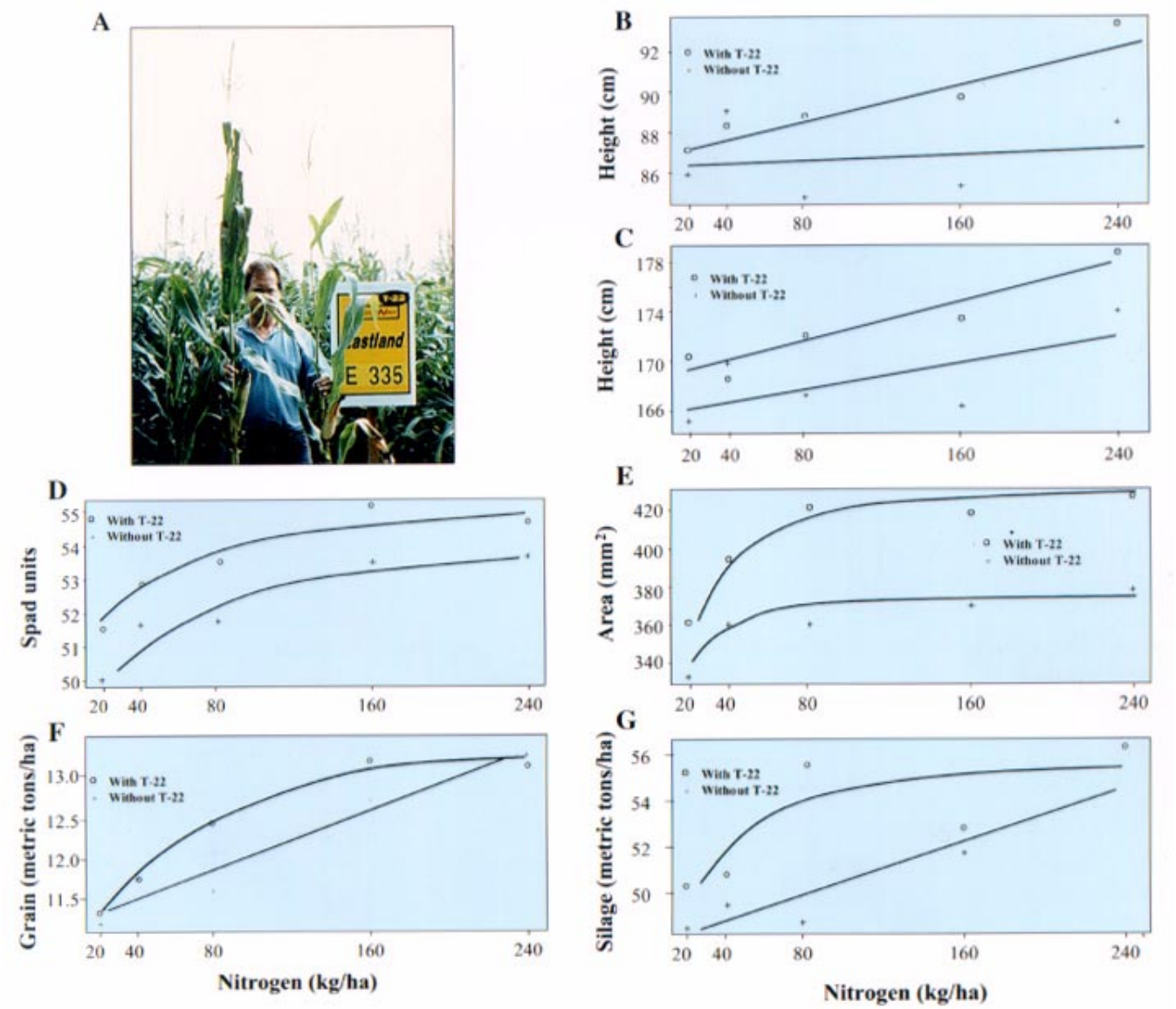

Fig. 7. Interaction of *Trichoderma harzianum* strain T-22 and nitrogen fertilizer on corn growth and yield. (A) Appearance of corn from a grower trial in 1996 produced from T-22–treated plants and from nontreated plants. (B-G) Results from trials in a grower field on deep sandy loam soil at various levels of N fertility. Such treatments have been used for other studies in order to determine nitrogen use efficiency as imposed by various treatments using negative exponential models (46). (B) Corn growth response to different levels of N from T-22–treated and nontreated plots 2 weeks after side-dressing. Plants whose roots were colonized with T-22 responded to N, while those without T-22 did not. Both position and slope were significantly different based on regression analysis (*P* = 0.001 and 0.04, respectively). (C) Corn growth response to N 4 weeks after application of various levels of N. Slopes were similar, but intercept of curve was significantly different (*P* = 0.001). (D) Leaf greenness of corn at tasseling as a function of treatment with T-22 and nitrogen application level as measured with a Minolta SPAD 502 meter. All plants were of similar height, but leaf greenness was significantly different (slope parameters were not significantly different but position was significantly different based on regression analyses at *P* = 0.002). (E) Stalk diameter of corn at harvest as influenced by treatment with T-22 and different levels of N. Slope parameters were similar, but positions of lines were significantly different at *P* < 0.001. (F) Yield of grain as influenced by treatment with T-22 and different levels of N. Analysis of shape of curves gave no evidence for any other than a linear relationship between N level and yield in the absence of T-22, but in its presence a curvilinear relationship was a better fit. Regression analysis indicated no significant difference in shapes or positions of lines generated for T-22 and nontreated corn, but analyses of means using a pooled standard error and a Bonferroni-adjusted 5% least significant difference (62) indicated that there is a significant difference in yields of corn grain between T-22 and nontreated corn at 80 and 160 kg/ha of N. (G) Yield of silage as influenced by presence or absence of T-22 and level of nitrogen fertilizer. Analysis of shape of curves gave no evidence for any other than a linear relationship between N level and yield in the absence of T-22, but in its presence a curvilinear relationship was a better fit. Comparison of shape and position of curves indicated that position of curve generated by T-22–treated corn was significantly higher than that from untreated corn (*P* = 0.055). Photograph in (A) is from BioWorks and shows Christopher Hayes. All other data are from G. E. Harman and R. Petzoldt, Cornell University.

from 32 to 21% (D. Huber, Purdue University, *unpublished*). However, in spite of the results just cited, in other trials no yield increases have been noted. I will provide a rationale for this difference and a description of limitations of T-22 at the end of the discussion of Dogma 3.

In nearly all cases, the best results in direct-seeded field crops are obtained with an integration of chemical seed treatments and T-22. Extensive testing has revealed almost no chemical seed treatments that prevent subsequent root colonization by T-22 (full rates of thiram and tebuconazole are exceptions). This resistance permits the use of both the biological root colonizing agent and chemical seed protectants.

Granules of T-22 frequently are incorporated into potting mixes in greenhouse crops for production of vegetable and flower transplants and also for pot crops such as chrysanthemums and poinsettias.

For example, tomatoes were grown in a potting mix containing the granular formulation of T-22, which permitted roots to become colonized, then transplanted to the field. Fusarium crown and root rot at harvest of mature fruit was reduced (18,64). T-22 was not the only effective organism—the rhizosphere competent *Bacillus subtilis* (the active ingredient in Kodiak) from Gustafson was also effective. The combination of T-22 and the mycorrhizal fungus *Glomus intraradices* was more effective than either organism alone (18). Thus, application of a very small amount of any of several biocontrol organisms at the time of seeding of transplants provided a season-long benefit to tomato health and yield. Again, this is hardly a localized or transitory effect or one confined to seeds, seedlings, or the infection court to which the biocontrol agent was originally applied.

There are similar studies on other transplant vegetables. Pepper seedlings were produced in the greenhouse with or without the addition of RootShield, the greenhouse product containing T-22. The peppers were transplanted into the field under less than ideal conditions. When their roots lacked T-22, fewer pepper plants survived transplanting and early yields were lower (Fig. 8).

A number of trials have focused on ornamentals; generally, T-22 either applied as a drench or incorporated as granules gives adequate disease control over an extended period of time (several months). Further, root development frequently is better in plants where disease is controlled by T-22 rather than by fungicides; many fungicides inhibit root growth to some extent, whereas T-22 enhances root development, as shown for poinsettias (Fig. 9). There have been numerous other demonstrations of similar effects of T-22 on greenhouse ornamentals.

These data clearly demonstrate that T-22, as well as other rhizosphere competent biocontrol agents, can provide long-term protection or other advantages to plants from a single application at the beginning of the season. These biocontrol agents can establish themselves on roots, grow with the developing root system, and remain functional for at least the life of an annual crop.

Thus, biologicals can be *more* effective than chemical pesticides for root protection and plant growth enhancement. However, the assessment of efficacy is dependent upon the particular parameter being measured.

Dogma 3. Single BCAs cannot be effective in diverse environments, on different crops, or against a wide range of plant pathogens. As a corollary, mixtures of BCAs will be required for successful long-term control since individual components colonize different crops, are adapted to different environments, or have different functions.

The dogma that single biocontrol agents cannot be widely effective was very well stated in the recent article by Mathre et al. (60), "One rather daunting principle that applies across all biological methods for disease and pest control with introduced agents…is that, almost invariably, a different agent…is needed for each disease or pest." Similarly, Cook (16) stated that "biological control is widely recognized both scientifically and based on empirical experience as highly pest- or disease-specific." Further, he advocates an approach to biological control that uses mixtures of numerous agents for each pest or disease. This view is inconsistent with our experiences and others, at least with *Trichoderma* spp. A large number of papers indicate that single strains of *Trichoderma* spp. are capable of controlling diverse pathogens, and Chet (14) has summarized some of these.

This is particularly important in light of economic realities. In my view, as I will discuss in the last section of this paper, it probably will be economically impossible to commercialize mixtures of strains, at least until there are significant economic successes in biocontrol. In other words, not only is one strain all that you may need, but perhaps more importantly, one strain is very likely all that you can *afford*! If this view is even remotely true, then it is important to determine what can be accomplished with a single strain, in this case T-22.

Fig. 8. Appearance of pepper plants in a field in New York a few weeks after transplanting from plugs grown in absence (A) and presence (B) of *Trichoderma harzianum* strain T-22 in the form of the commercial product RootShield. (C) Yield data were taken on three cultivars for first and third pickings. Yields for first picking for transplants produced in absence of RootShield were 5.9, 3.6, and 5.4 kg per plot for Merlin Belle, Vanguard Belle, and Bonanza peppers, respectively, while for plants grown in the presence of T-22, yields were 10.2, 8.6, and 9.6 kg per plot. In third picking, yields were similar: in the absence of RootShield, 21.4, 11.7, and 18.1 kg per plot were harvested from the same three cultivars, while in presence of RootShield yields were 24.1, 11.4, and 23.6 kg. Photos and data are from Russell Wallace, BioWorks.

In our first article on T-22 (34), we reported that it controlled *F. graminearum*, *R. solani*, *Pythium ultimum*, and *Sclerotium rolfsii* in laboratory tests. This list has been expanded to include other pathogens of both above- and belowground plant parts in real-world research evaluations.

Control of *R. solani* has been well documented in the greenhouse on a number of different crops (Fig. 10A to C). In many cases, T-22, as its commercial product RootShield, substantially enhances plant quality relative to fungicides. Similar results have been obtained for control of *Pythium* (Figs. 11A to C). In many cases for both pathogens, RootShield is as effective as the competitive chemical fungicides, even when the biocontrol product is applied infrequently and the chemicals are applied more often, as per their label recommendations.

However, other trials have indicated that products based on T-22 are less effective in controlling *Pythium* and *R. solani* than are chemical fungicides. This apparent paradox will be addressed in a subsequent section, but at least two important factors should be mentioned here. Like other biocontrol agents, T-22 can be overwhelmed by heavy disease pressure. Therefore, T-22 must be used strictly as a preventative measure; it cannot cure existing infections. The biocontrol agent must be used within its limits and as part of a total management strategy.

In general, we have not focused on the ability of T-22 to control these pathogens in the field except on golf course turfgrass (53–55), where T-22 reduces disease levels. *Pythium* and *R. solani* on field crops cause seed or seedling disease, and we recommend that these diseases be controlled by appropriate chemical fungicides, for the reasons noted earlier.

Diseases caused by *Fusarium* spp., especially root and crown rot, also are controlled. Earlier in this paper I summarized research (18,64) that demonstrated that a single application of T-22 as RootShield granules in the greenhouse provided protection of the tomato crop against Fusarium crown and root rot of the mature crop. Similarly, a single application of T-22 to red onions at the time of planting resulted in significantly reduced Fusarium basal rot at harvest. An in-furrow drench was more effective in both root colonization and disease control than a seed treatment (Fig. 12A and B).

In the greenhouse, other pathogens such as *Cylindrocladium* and *Myrothecium*, both on *Spathiphyllum*, also were effectively controlled (Fig. 13A and B). However, weekly applications of RootShield were required, as opposed to less frequent applications.

A great need in agriculture, particularly for crops such as vegetables and strawberries in California and Florida, is an effective replacement for methyl bromide. Conceptually, a broadly effective and rhizosphere competent biocontrol agent should be useful. However, there are such diverse and numerous pathogens that a stand-alone biocontrol agent probably cannot be totally effective. Recently, a combination of ozone fumigation followed by T-22 has shown promise on strawberries in California. T-22 alone, ozone alone, and ozone fumigation followed by T-22 were compared in one trial; in another part of the same field, the standard methyl bromide fumigation and no treatment were compared. Yields with

Fig. 9. Appearance of poinsettias grown in a commercial greenhouse in a trial established by Mark Arena, Clemson University, in cooperation with BioWorks. First plant (far left) received no soil fungicide treatment, second received a single treatment of Ban-rot (etridiazole and thiophanate methyl), third plant received a single treatment of Ban-rot plus monthly applications of Subdue (metalaxyl) and Cleary's 3336 (thiophanate methyl), and plant on right received a single early application of RootShield, all according to label directions. This photograph is the property of BioWorks and has been published in GM Pro magazine.

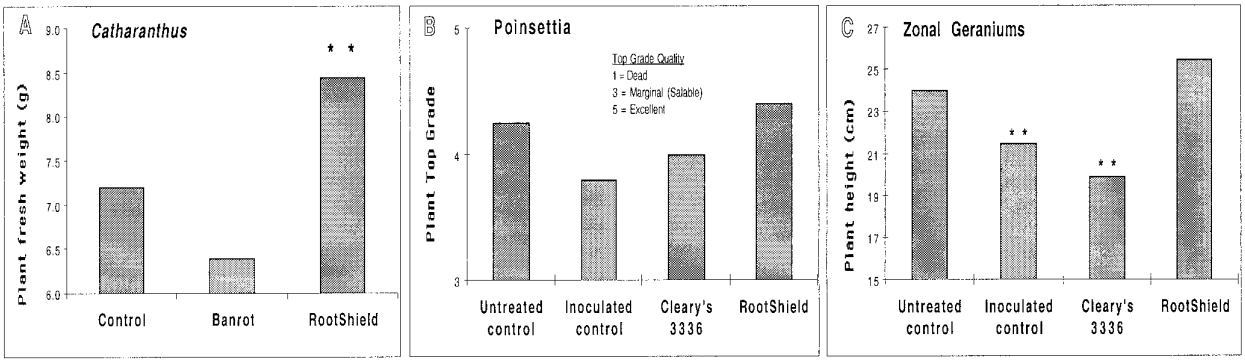

Fig. 10. Control of *Rhizoctonia solani* by *Trichoderma harzianum* strain T-22 added as commercial product RootShield. (A) Fresh weight of *Catharanthus* cv. Peppermint Cooler in the greenhouse in the presence of the pathogen (AG-4) as affected by treatment with Ban-rot (etridiazole and thiophanate methyl) or RootShield. Plants were grown in Earthgro composted soil (George Elliot, University of Connecticut, Storrs, CT, and Wade Elmer, Connecticut Agricultural Experiment Station, New Haven, CT, *unpublished data*). (B) Comparison of effects of a single application of RootShield drench and Cleary's 3336 (thiophanate methyl) on top grade of poinsettias. Pathogen was added to pots with all treatments except untreated control (BioWorks data from A. R. Chase, Chase Research Gardens, Mt. Aukum, CA). (C) Comparison of effects on plant height of zonal geraniums of a single addition of RootShield granules 7 days before inoculation with the pathogen and 10 days before transplanting with Cleary's 3336, applied according to label directions. Data generated by Jean Williams-Woodward, Department of Plant Pathology, University of Georgia, in cooperation with BioWorks. In all cases, asterisks above bars indicate significant differences ($P = 0.05$).

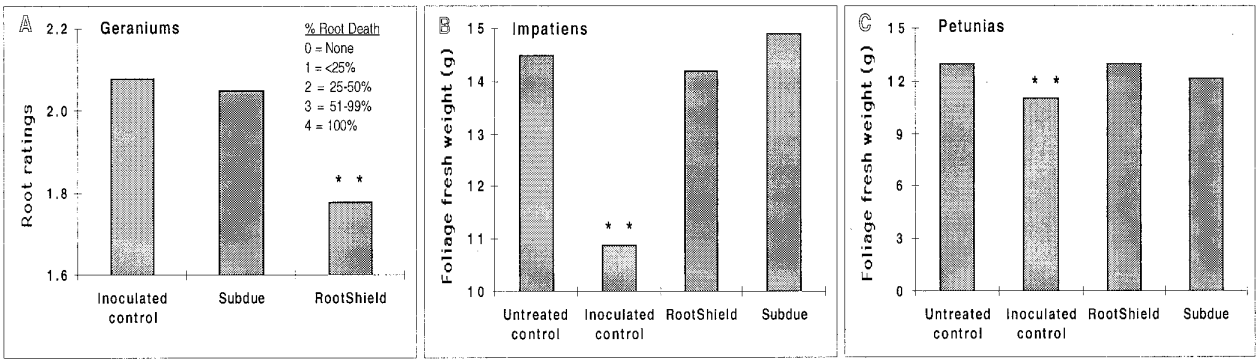

Fig. 11. Control of *Pythium* spp. by *Trichoderma harzianum* strain T-22 added in the form of commercial product RootShield. (A) Comparison of Subdue (metalaxyl) and RootShield drench in control of root rot of geraniums caused by *P. ultimum*. Data generated by Gary Moorman of Pennsylvania State University in cooperation with BioWorks. (B) Comparison of Subdue and RootShield granules in control of Pythium root rot as measured by foliage dry weight in greenhouse impatiens and (C) in petunias. Data generated by Jean Williams-Woodward, University of Georgia, in cooperation with BioWorks. Asterisks placed above bars indicate significant differences ($P \geq 0.05$).

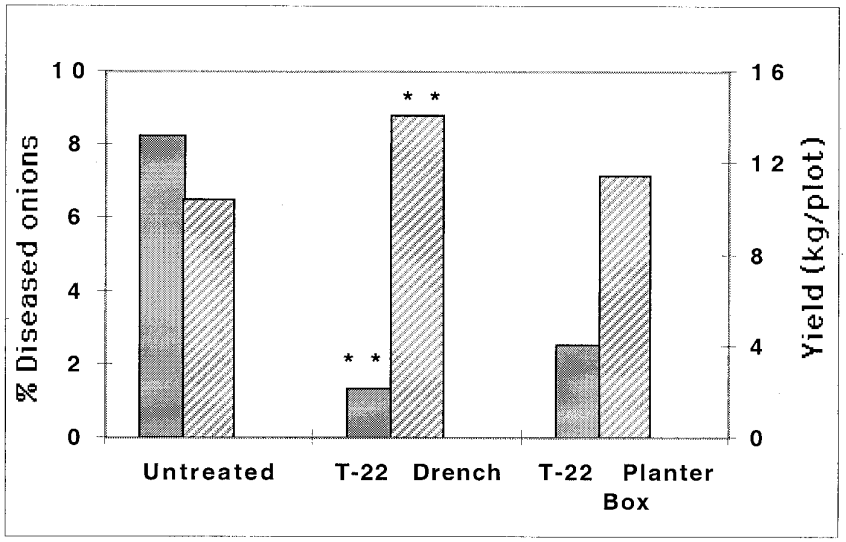

Fig. 12. Incidence of Fusarium basal rot (solid bars) of red onions at end of season and yield (hatched bars) as affected by T-22 PlanterBox seed treatment or an in-furrow drench (1 kg/ha as RootShield drench) of T-22. Onion plants were direct seeded in a muck soil near Oswego, New York, in a randomized complete block design with four replications per treatment. Data generated by J. van der Heide, R. Petzoldt, and G. Harman, Cornell University.

the ozone + T-22 (RootShield granule) treatment or the standard methyl bromide treatment were similar, and the combination was significantly better than either T-22 alone or ozone alone (Fig. 14).

All results discussed above have been directed toward control of root and soil pathogens. However, T-22 also is effective in the control of fruit and foliar diseases when applied as a spray to these plant parts. Diseases controlled include powdery mildews on *Catharanthus* and pumpkins (Fig. 15A and B). Similar formulations are active against *B. cinerea* on greenhouse crops and strawberry (Fig. 16C and D) and against downy mildews (Fig. 16E). T-22 has also demonstrated activity against *B. cinerea* on grape, although results were not always consistent (31), and against turfgrass pathogens such as *R. solani* (brown patch), *Pythium* spp., and *S. homoeocarpa* (dollar spot) (54,55). For these applications, T-22 must be applied frequently, at least once every 10 days when disease pressure is high, since it cannot extensively grow on and colonize newly formed leaf tissues. However, on flowers or fruits, it is highly persistent; an application to either grape or strawberry flowers results in a high percentage of colonization of the immature fruits (*unpublished*). Spores of T-22 germinate on strawberry flowers (G. E. Harman and R. Petzoldt, *unpublished*) and turf leaves (55). However, they may not germinate well on strawberry leaves. The method and adjuvants required for effective control are very important, but this topic is beyond the scope of this article. T-22 is not unique in its ability to control powdery mildews and *B. cinerea*. *T. harzianum* strain T-39, the active ingredient of Trichodex, also controls these pathogens (24).

Further, we have obtained encouraging results with bee delivery of T-22, a concept pioneered by John Sutton and his colleagues at Guelph (65). Conidia of *T. harzianum* are smaller than pollen grains and can adhere to the body of a bee in a similar manner. Bees exiting the hive pass through a device that requires them to come into contact with products containing these spores. They subsequently deliver substantial amounts of T-22 or similar fungi to the strawberry or other flowers. In our hands, this method of delivery is more effective than spray applications for control of *B. cinerea* and has proven as effective, over several years and trials, as standard chemical applications (Fig. 16E) (48).

The last two sections have provided numerous examples of the uses of a single biocontrol agent, *T. harzianum* strain T-22. However, T-22 and the products based upon it are management tools for growers. It is not magical nor is it a "silver bullet" that solves all problems. A summary of limitations follows:

- T-22 is strictly preventative. It cannot control existing diseases, and so a good systemic fungicide must be used if diseases already exist.
- It is less effective against systemic diseases than against more superficial ones. Therefore, it is more effective, for example, against Fusarium crown and root rot than against Fusarium wilts (18,36).
- In conditions of high or very high disease pressure, T-22 should be used as part of an integrated chemical–biological system. For example, for control of *B. cinerea* on strawberries in Florida, its best use is probably as a tank mix or as an alternating spray to reduce, but not eliminate, the chemical fungicide application.
- In other cases, maximum benefit to the crop requires use of both biological and

chemical agents. For example, the combination of chemical seed treatment for maximum seed and seedling protection together with the long-term root protection and enhancement by T-22 is highly effective.

- While T-22 is extremely persistent on root surfaces, it does not persist at biologically significant levels in the absence of roots. Perhaps the only exception to this generalization is in highly organic soils such as those in which onions are grown in upstate New York (see discussion regarding onions and a Sudex cover crop).
- In spite of the pictures presented in the figures, T-22 does not always, or perhaps not even in the majority of situations, give obvious visual enhancement of plant growth or yield. T-22 provides tolerance to a variety of biological and edaphic stresses. If no stresses occur and plants are always growing at near-optimal conditions, T-22 can provide little visual or yield improvement.

Thus, T-22 is a highly versatile biocontrol and plant growth–enhancing agent. It has been the subject of intense public and private sector development for about 15 years. A critical consideration in all of these studies is the mechanisms whereby T-22 and other BCAs exert their beneficial effects.

Dogma 4. Biocontrol agents have simple mechanisms of action that are controlled by one or only a few genes and gene products.

Our expectations of the abilities of biocontrol agents are partially conditioned by our expectations of their mechanisms of action. If we assume that a specific agent (or group of agents such as *Trichoderma* spp.) has a single or a very limited number of mechanisms of action, we could conclude that its activity might be specific to particular crops or pathogens. However, if we determine that there are many different biochemicals, genes, and even general modes of action for a specific BCA, then it is much more reasonable that the particular organism might have manifold and diverse advantages.

Further, the general conceptual framework in which we view the particular organism also affects our thinking about its potential uses. In my view, *Trichoderma* spp. frequently are very numerous and even dominant in agricultural soils because they persist and multiply in the presence of healthy plant roots. In the absence of healthy roots, their numbers are likely to decline. Certainly this is true of T-22. If it is generally true of *T. harzianum*, we need to consider these fungi as opportunistic plant root colonists or even symbionts. If so, then it is reasonable to assume that they will have developed numerous mechanisms to promote their ecological niche, i.e., abundant and healthy plant roots.

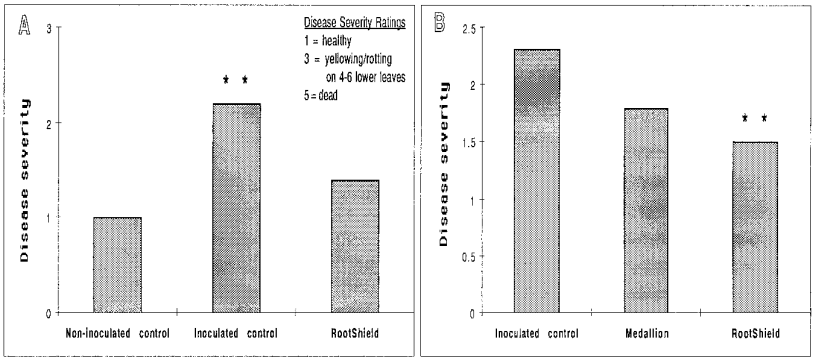

Fig. 13. Comparison of weekly doses of RootShield and the fungicide Medallion on control of (A) Cylindrocladium and (B) Myrothecium petiole rots on *Spathiphyllum*. All experiments were done in randomized complete block designs, and asterisks indicate significant differences (*P* = 0.05). Data from A. R. Chase, Chase Research Gardens, Inc., Mt. Aukum, CA, in cooperation with BioWorks.

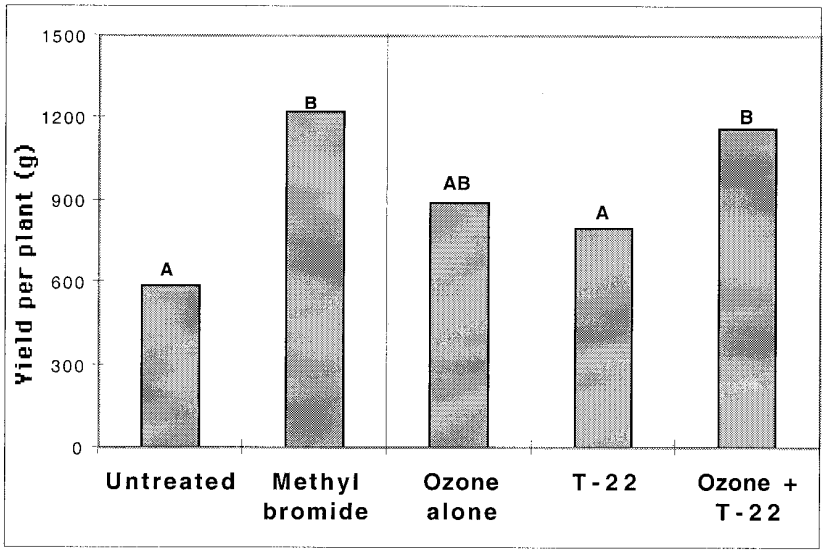

Fig. 14. Cumulative yield of strawberries from a trial in 1997-98 at Monterey Bay Academy, Watsonville, CA, following application of standard rates of methyl bromide: chloropicrin (67:33 at 325 kg/ha), *Trichoderma harzianum* strain T-22 applied as RootShield granules incorporated into the soil at the rate of 135 kg/ha prior to planting, ozone applied at the rate of about 400 kg/ha and combination of preplant application of ozone followed by T-22. Bars with dissimilar letters are significantly different (*P* = 0.05). Methyl bromide + chloropicrin and untreated control data were from other parts of the same field but were not part of the replicated trial that included T-22, ozone, and the combination. Pathogens present in soil included *Verticillium* and *Phytophthora* spp. Data courtesy of John Duniway, University of California, Davis. Other data by J. Duniway indicate significant responses to combinations of T-22 and ozone in other soils and experiments.

The following are the mechanisms by which strains of *T. harzianum* function. The first three are the ones by which these fungi have always been considered to function; those added in the last 1 to 3 years are in italics. In addition, it is extremely likely that other mechanisms exist but have not yet been discovered.

- Mycoparasitism
- Antibiosis
- Competition for nutrients or space
- *Tolerance to stress through enhanced root and plant development*
- *Induced resistance*
- *Solubilization and sequestration of inorganic nutrients*
- *Inactivation of the pathogen's enzymes*

Mycoparasitism. Mycoparasitism has long been considered an important mechanism of action of biocontrol by *Trichoderma* spp. This is a complex process that involves tropic growth of the biocontrol agent toward target fungi, lectin-mediated coiling of attachment of *Trichoderma* hyphae to the pathogen, and finally attack and dissolution of the target fungus' cell wall by the activity of enzymes, which may be associated with physical penetration of the cell wall (14). Probably the killing of target hyphae a short distance away from *Trichoderma* hyphae (50,55) can be considered similar to mycoparasitism. Numerous separate genes and gene products have been proposed to be involved in mycoparasitic

interactions. As recently as 1990, we naively expected to test the involvement of "*the Trichoderma* chitinase and β-1,3-glucanase" in biocontrol by inactivating the genes encoding these two enzymes. We now know that for each of these two functional groups, there are multiple classes of enzymes, and within each class, there are distinctly different enzymes. In 1998, Lorito listed 10 separate chitinolytic enzymes alone (57). Similar levels of diversity exist with β-1,3-glucanases (8). In addition, β-1,6-glucanases (56) and proteases (26) likely also are involved. For mycoparasitism of Pythiaceous fungi, β-1,4-glucanases may also be important (76). To add even more complexity, peptaibol antibiotics are specifically produced by *T. harzianum* in the presence of fungal cell walls, so they probably also should be considered as part of the mycoparasitic complex (67). Adding still further to the complexity is the fact that the regulation of individual genes presumably involved in mycoparasitism differs; for example, the 42-kDa endochitinase is induced before *T. harzianum* comes into contact with *B. cinerea*, while the 72-kDa *N*-acetylhexosaminidase is induced only after the two fungi are in direct contact (82). Therefore, a single step in the mycoparasitic process of *T. harzianum* may involve more than 20 separate genes and gene products under complex regulatory control. Further, most of these gene products are synergistic with one another (see 57 for a summary). Given this entire arsenal of synergistic gene products that are *part of only one mechanism* by which *Trichoderma* species attack and gain nutrition from other fungi, it is not surprising that this genus has been reported to be pathogenic against, and provide control of, very diverse groups of fungi (14).

It should also not be surprising, in view of this complexity, that studies with this organism involving knock-out or overproducing mutants for single genes provide contradictory results. In one recent study, a strain of *T. harzianum* deficient in the ability to produce endochitinase had reduced ability to control *B. cinerea* but increased ability to control *R. solani* (80). In another study, activity of the same enzyme either was deleted or increased in *T. harzianum*, but these changes had no effect on biocontrol ability against *R. solani* or *S. rolfsii* (12). In a third study (4), this same enzyme was disrupted or overproduced in *T. virens*, and the resulting strains were found to have decreased or increased biocontrol ability, respectively, toward *R. solani* relative to the wild strain. Thus, in three studies, almost every conceivable result (ranging from decrease to no effect to increase of biocontrol ability) was obtained by deletion of a presumed biocontrol gene. Finally, transformed plants expressing this same endochitinase have much greater resistance to several plant pathogens than do nonexpressing plants (9,59). This, together with the strong antifungal activity of this enzyme against a variety of plant-pathogenic fungi (57), suggests that this enzyme probably is involved in biocontrol. However, since there are so many different gene products that may also be involved, nonexpression or overexpression of any one product may provide ambiguous results.

Antibiotics. Antibiotics have long been suggested to be involved in biocontrol by *Trichoderma* (78). Sivasithamparam and Ghisalberti (70) list 43 substances produced by *Trichoderma* spp. that have antibiotic activity (enzymes are not included). Of these, alkyl pyrones, isonitriles, polyketides, peptaibols, diketopiperazines, sesquiterpenes, and steroids have frequently been associated with biocontrol activity of some species and strains of *Trichoderma* (40). A substantial number of reports show that mutation to eliminate production of specific antibiotics is associated, in some strains, with a loss of activity against particular pathogens (40). However, particular antibiotics that may be important in activity of one strain may not even be produced by another highly effective biocontrol strain of *Trichoderma*. For example, gliotoxin appears to be necessary for activity of the *T. virens* strain that is the active ingredient of SoilGard, but it is not produced by T-22. Again, to add to the complexity, many of these antibiotics are synergistic with cell wall degrading enzymes (21,58).

Further, as noted regarding the study by Woo et al. (80), some mutants prepared without activity of a specific component expected to be involved in biocontrol may actually be more active than the parental strain. For example, Graeme-Cook and Faull (27) prepared UV mutants of a strain of *T. harzianum* that produced the antibiotic 6-*n*-pentyl pyrone but lacked ability to produce isonitrile antibiotics. Mutants were obtained that possessed unexpected properties, including a newfound ability to produce isonitrile antibiotics. These two classes of antibiotics are different chemically and probably arise from distinctly different pathways (70), so it is unlikely that the UV mutation simply gave rise to a branch in a biosynthetic pathway to produce an alternative product. Instead, as Graeme-Cook and Faull (27) stated, "There are several silent pathways for antibiotic production whose expression has been lost." By this reasoning, the mutation would have induced changes in regulatory controls that activated the "lost" pathway. These results strongly suggest that the parental strain possessed cryptic genes that were not expressed until their activities were in some way released through the mutation process. This alteration of expression indicates that biocontrol strains of *Trichoderma* (and no doubt other microbes) have genes that may only be expressed if some change in regulatory processes occurs. Therefore, studies with knock-out mutants, no matter how carefully the study is done to avoid changes to other than the gene of interest, may identify phenomena that act, by analogy, like "snake's teeth." If a snake's fangs are lost or removed, others take their place. If activity of one gene is deleted and a mechanism of biocontrol is lost, another may take its place.

Competition. Competition for space or nutrients has long been considered one of the "classical" mechanisms of biocontrol by *Trichoderma* spp. (14). However, in many cases, this mechanism was surmised to occur because no evidence for mycoparasitism or antibiosis could be discovered in a particular interaction. Competition very likely is an important mechanism of biocontrol, but it is extremely difficult to prove. Is the displacement of other organisms by either *T. virens* or T-22 a consequence of competition or some other mechanism? Elad et al. (22) presented information regarding biocontrol of *B. cinerea* by *T. harzianum* strain T39. *B.*

Fig. 15. Appearance of *Catharanthus* cv. Parasol plants grown in planting mix at about pH 7 in the absence and presence of *Trichoderma harzianum* strain T-22 applied to the soil mix as RootShield. Experiment conducted by George Elliot, Department of Plant Science, University of Connecticut, Storrs. Photograph by Russell Wallace, BioWorks.

cinerea conidia require external nutrients for germination and infection. When conidia of T-39 were applied to leaves, germination of conidia of the pathogen was slowed, an effect attributed in part to competition (22).

Tolerance to biotic and abiotic stresses through enhanced root development. Another possible mechanism recently gaining credence is tolerance to stress through enhanced root and plant development. The drought tolerance and enhanced nitrogen utilization indicated in Figures 6 and 7 are examples of this mechanism. The enhanced rooting by T-22 probably also induces tolerance to pests that it does not directly control. For example, we do not believe that T-22 has the ability to control *Phytophthora* spp. because it has no mechanism to intercept or attack zoospores. Indeed, in several studies, it was found to have no effect on this pathogen (e.g., 71). However, growers have indicated that *Phytophthora*-attacked plants were larger in the presence of T-22 than in its absence. One possible explanation for this result is that the larger root systems of plants colonized by T-22 were better able to withstand the damaging effects of the pathogen.

Solubilization and sequestration of inorganic plant nutrients. In soil, various plant nutrients undergo complex transitions from soluble to insoluble forms that strongly influence their accessibility and absorption by roots. Microorganisms may strongly influence these transitions (2). Iron and manganese in particular have been investigated with regard to both microbial solubilization of oxidized forms of these elements and their influence on plant disease (28). *Pseudomonas* spp. produce compounds (siderophores) with very high affinities for iron. Iron chelated with these siderophores is unavailable to plant pathogens, so their activity is thereby reduced, but plant roots can take up iron in this form either directly or after reduction of Fe^{3+} to Fe^{2+} (7). Manganese is a microelement essential for diverse physiological functions in plants, including both plant growth and disease resistance (28). Only the Mn^{2+} form of this element is soluble; the more highly oxidized forms are insoluble. Several pathogens, including *Streptomyces scabies* and *G. graminis* var. *tritici*, can oxidize manganese and thereby inactivate a major part of the plant defense mechanisms (43,44).

Earlier, we documented the abilities of strain T-22 to enhance nitrogen use efficiency in corn. However, this strain can also solubilize a number of poorly soluble nutrients. In in vitro studies, T-22 has been shown to solubilize rock phosphate, Zn metal, Mn^{4+}, Fe^{3+}, and Cu^{2+}; these activities are not due to medium acidification or production of organic acids (2). There is no common biochemical mechanism for these abilities. Strain T-22 produces separate substances that reduce and chelate Fe^{3+}, while for Mn^{4+} only reductive materials are produced. The substances that reduce Cu^{2+} or Fe^{3+} are separate biochemical entities (2). Therefore, this strain of *T. harzianum* produces a large number of chemicals involved in nutrient solubilization.

A direct role for the nutrient solubilization and chelating abilities of *Trichoderma* metabolites has not yet been demonstrated, but circumstantial evidence of its ability to solubilize iron and make it usable to plants is available. *Catharanthus* is intolerant of alkaline potting media because of suscepti-

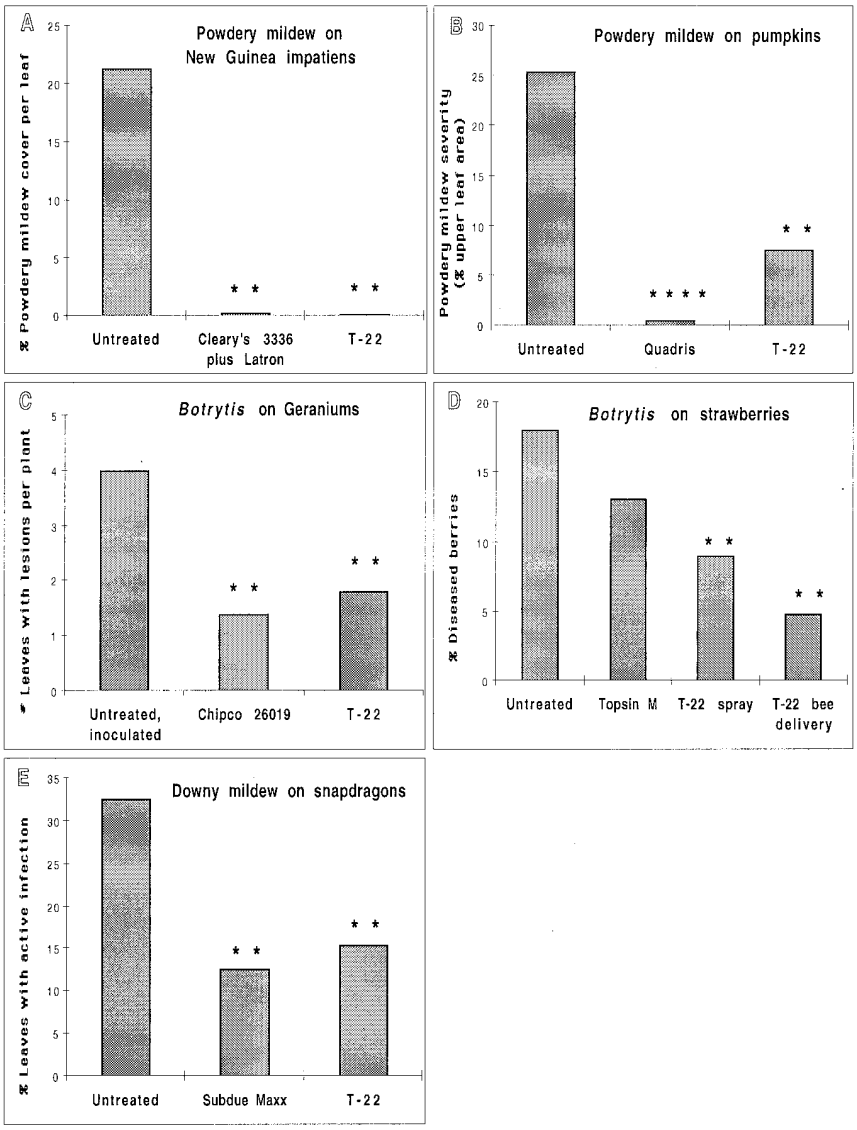

Fig. 16. Control of various foliar and fruit diseases by spray applications of *Trichoderma harzianum* strain T-22 as the prototype commercial formulation TopShield. (A) Comparison of Cleary's 3336 (thiophanate methyl) and T-22, both applied with Latron B-1956 added as an adjuvant in control of powdery mildew on New Guinea impatiens in the greenhouse. Data from M. Daughtrey, Long Island Horticultural Research Laboratory, Cornell University, in cooperation with BioWorks. (B) Control of powdery mildew (*Sphaerotheca fuliginea*) on upper surfaces of field-grown pumpkins with strobilurin fungicide Quadris applied on a 14-day schedule and T-22 applied on a 7-day schedule. This information is part of a larger study (61). (C) Comparison of Chipco 26019 (2.4 lb/liter) and T-22 (7 g/liter) for control of *Botrytis cinerea* on geraniums. Data from M. Daughtrey in cooperation with BioWorks. (D) Comparison of field applications of the chemical fungicide Topsin M with T-22 either as a spray (TopShield) or by bee delivery. For spray applications, T-22 was applied at the rate of 3.2 kg/ha, while for bee delivery, a dry, powdered formulation of T-22 containing about 4×10^8 CFU/g was placed at exit of hive so that bees had to pass through it as they exited (48). (E) A comparison of Subdue Maxx (mefenoxam, 37 mg/liter) and TopShield (3 g/liter) applied to the point of drip on a 7-day schedule for control of downy mildew (*Peronospora antirrhini*) on snapdragon. Infected plants were intermingled with healthy ones, and spores were distributed using a fan throughout the trial. Data are from A. R. Chase, Chase Research Gardens, Inc., Mt. Aukum, CA). In all cases, asterisks indicate that data are significantly different, with four asterisks different from two ($P = 0.05$).

bility to iron deficiency at pH values above about 6.5. *Catharanthus* cv. Parasol plants grown in a soilless potting mix with a pH of about 7 were highly chlorotic in the absence of T-22 but less chlorotic when grown in a medium containing RootShield (Fig. 15). This difference probably is a consequence of enhanced iron uptake, suggesting that T-22 can increase iron availability. Plant tissue culture analyses are underway to test the hypothesis of enhanced iron nutrition (G. Elliot, *personal communication*).

Induced resistance. Elicitation of resistance in plants by *Trichoderma* spp. has been a subject of several papers and is becoming a more researched topic. Studies through 1997 have been reviewed by Bailey and Lumsden (5). Of particular note is the ability of a xylanase or other elicitors from *Trichoderma* spp. to induce resistance (3,5,11).

Some *Trichoderma* strains clearly are potent inducers of SAR-like responses. Strain T39 of *T. harzianum*, which is the active ingredient in Trichodex, can be inoculated onto roots or onto leaves and provide control of disease caused by *B. cinerea* on leaves spatially separated from the site of application of the biocontrol agent (19). Analyses of protected leaves to which T39 was not applied demonstrated that the biocontrol agent was not present. The level of protection afforded by T39 applied to roots was similar to the known bacterial SAR-inducing organism *Pseudomonas aeruginosa* KMPCH (19). Elad and his colleagues (22) consider induced resistance to be the primary method whereby T39 controls powdery mildews. By extension, we would presume that T-22 has a similar mode of action against this pathogen.

In another recent study (81), cucumbers were grown in the presence or absence of strain T-203 in an axenic hydroponic system. Plants were always larger in the presence of T-203 than in its absence and, unlike T-22, this strain penetrated root cortical tissue. Cell walls of the roots were strengthened in the area of penetration. Both chitinase and peroxidase activities were increased in both root and leaf tissues of treated seedlings, which is an indication of SAR (81).

Finally, Howell and his colleagues (41) also have demonstrated the presence of SAR induced by *T. virens*. Effective native strains of *T. virens* usually produce gliotoxin or gliovirin. Mutants were prepared that lacked both mycoparasitic ability and the capacity to produce these or other detectable antibiotics. Surprisingly, many of the mutants were as effective or more effective than the parental strains in biocontrol of *R. solani* (39,42). This high level of protection was associated with significantly enhanced levels of the various terpenoid phytoalexins known to be involved in disease resistance in cotton (41). Levels of phytoalexin production induced by the mutants were significantly greater than the parental antibiotic-producing strains. Further, as a seed protectant, it is superior to our strain T-22, which has no known ability to enhance phytoalexin production in cotton. Thus, at least a portion of the mechanism of action of the mutant strains of *T. virens* is probably SAR (41).

Inactivation of the pathogen's enzymes. Yet another mechanism of biocontrol by *Trichoderma* spp. has been at least tentatively identified. *B. cinerea* depends upon production of pectolytic, cutinolytic, and cellulolytic enzymes to infect living plants (45). However, conidia of two strains of *T. harzianum* (T39 and NCIM1185), when applied to the leaves, produce a serine protease that is capable of degrading the pathogen's plant cell wall degrading enzymes and thereby reducing the ability of the pathogen to infect the plant (23). The biocontrol activity of T39 could be enhanced by adding additional quantities of its protease; further, several protease inhibitors reduced the biocontrol activity of T39. Interestingly, while the proteases of T39 could be detected on leaf surfaces, no activity of chitinolytic or β-1,3-glucanolytic enzymes, which are presumed to be involved in mycoparasitism, could be detected. Thus, there is a solid case for the role of proteases from *T. harzianum* in inactivation of the pathogen's primary modes of ingress into plants. The authors suggest that the proteases may be directly toxic to germination of the pathogen and also may inactivate its enzymes (23).

In conclusion, there is an almost bewildering array of mechanisms by which *Trichoderma* spp. exert biocontrol. Thus, it is not surprising that different strains have very different biocontrol capabilities; even mutants of the same original strain may exhibit very different biocontrol mechanisms. Further, it is not surprising that, in the case of *Trichoderma*, both dogmas one and two are false. With this array of biological weapons, these fungi should be highly versatile and have very little specificity as to the pathogens they control. Definitive studies on single mechanisms of biocontrol are rare because there are so many genes and gene products involved in biocontrol and because these strains can adapt to the loss of one mechanism by turning on another.

Dogma 5. Registration of BCAs with regulatory agencies is relatively fast, inexpensive, and simple. This dogma is a subset of the concept that biocontrol agents of plant pathogens can become both profitable and useful.

Commercialization of biocontrol agents requires several steps, beginning with initial discovery and then proceeding through testing of efficacy, prototype and then commercial production, extensive large-scale field testing, toxicology and environmental tests, registration, and marketing. Nonprofit research institutions are well suited only to the first two steps, and commercial companies are required for the last several steps. For agents used in control of plant disease, these steps have been taken by small to medium-sized companies where the required levels of innovation and technology development have been very substantial. As a consequence, the processes, procedures, and equipment required for economical production of biocontrol agents are jealously guarded and highly proprietary. Together with patents and registrations, this knowledge and capability are among the most valuable components of most of the companies that currently produce biocontrol agents. As a result, these processes are unavailable to academic researchers except in collaboration with the biocontrol companies.

Costs just to reach and validate commercialization steps are expensive (several million dollars) and require a substantial amount of time (two or more years) to accomplish. Funding for commercial biocontrol projects usually comes from investors who are looking for a relatively high payoff of their investment in a relatively short period of time. Unfortunately, increase in sales of most biocontrol products does not ramp up quickly regardless of the product. Success in selling biocontrol products requires that potential users and distributors be educated and convinced about the value of a biological product that is probably more conceptually difficult to use than standard pesticides. Further, one of their chief advantages, that they may have numerous uses in addition to killing pests, adds to the complexity of the biological paradigm. Therefore, adoption of biocontrol technologies by growers proceeds more slowly than if these technologies had simpler bases.

Given the above, it is relatively easy to see why biologicals have never gained a substantial market share. Costs are relatively high, time of development is relatively long, and gaining market share is a slow process. BioWorks and a few other companies are working to change this, but it is extremely difficult.

Time and expenses of complying with EPA, state, or international regulations are significant factors in the development and successful commercialization of biocontrol agents.

I frequently hear statements in meetings, or read in grant proposals and other documents, that EPA registrations are relatively easy and take less than a year to obtain. This is incorrect—in BioWorks' experience (and that of other companies as well), *EPA registrations for microbials, especially for broad-scale uses on food crops, commonly take 3 years or more to obtain* even if there are no negative environmental or health issues of the microbial in question. There are exceptions, but generally this time requirement is correct. The time required

for registration of microbial pesticides has increased significantly since the passage of the Food Quality Protection Act. In many other countries, time to registration is at least as long, or even longer.

This slow pace causes problems for companies that attempt to comply with EPA regulations. Business plans may assume that a registration may be obtained in 1 year or less since this concept has been widely publicized and stated. However, when timelines stretch out, sales do not occur, and companies face bankruptcy. This is a major factor in the lack of biological alternatives to synthetic pesticides in the marketplace.

Companies therefore have a choice. They can cease to exist or they can sell products in violation of EPA regulations, which state that any product that makes pesticidal claims is a pesticide and must be registered before sales can occur. Some companies faced with this dilemma choose to sell their product. Frequently, products are labeled as plant growth promoters, and then the product literature gives pages of data on the biocontrol activity of the active ingredient. I am aware of another approach that states that the product is "EPA registration pending." Either of these cases is in blatant violation of EPA regulations, the latter even stating that it is selling a pesticide without registration! To date, *EPA and state regulators have chosen NOT to enforce EPA and state regulations*, so the number of unregistered products, especially disease-controlling biocontrol products, is becoming very large; perhaps only about 5% of such products offered for sale are EPA registered. This is a disturbing situation for the biocontrol industry. First, companies that comply with regulations are severely penalized by being unable to sell in markets where unregistered microbial pesticides already are gaining a foothold and by being encumbered by the high costs of obtaining appropriate state and federal registrations. More importantly, as the number of unregulated products proliferates, sooner or later it is likely that there will be placed for sale a microbial with health or environmental hazards. The ensuing furor is likely to be extremely damaging to the entire biocontrol industry. This is, in my view, a serious matter that the biocontrol community should consider carefully.

In summary, it will require several years and several to many millions of dollars to bring a single biocontrol agent to market and to become profitable. I frequently read proposals and other documents that decry the "silver bullet" (single biocontrol agent) approach. Therefore, the use of and discovery of multiple agents and mixtures is fashionable. In my view, it will be economically impossible to bring such complex mixtures to market. For EPA registration alone, the time and funds required can be multiplied by the number of agents in the proposed product (unless, of course, EPA continues to avoid enforcement of its own laws).

Therefore, I view the development of widely adapted and broadly active agents as the only economically feasible approach to the development of commercially useful biocontrol. Contrary to frequently stated dogmas of biocontrol, such agents do exist, and they can provide broad-spectrum activity with unique advantages to growers and other users.

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Literature Cited

1. Ahmad, J. S., and Baker, R. 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77:182-189.
2. Altomare, C., Norvell, W. A., Björkman, T., and Harman, G. E. 1999. Solubilization of phosphates and micronutrients by the plant-growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol. 65:2926-2933.
3. Anderson, R. D., Bailey, B. A., Taylor, R., Sharon, A., Avni, A., Mattoo, A. K., and Fuchs, Y. 1993. Fungal xylanase elicits ethylene biosynthesis and other defense responses in tobacco. Pages 197-204 in: Cellular and Molecular Aspects of the Plant Hormone Ethylene. J. C. Pech, A. Latche', and C. Balague, eds. Kluwer Academic Publishing, Dordrecht, Netherlands.
4. Baek, J.-M., Howell, C. R., and Kenerley, C. M. 1999. The role of an extracellular chitinase from *Trichoderma virens* Gv29-8 in the biocontrol of *Rhizoctonia solani*. Curr. Genet. 35:41-50.
5. Bailey, B. A., and Lumsden, R. D. 1998. Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens. Pages 185-204 in: *Trichoderma* and *Gliocladium*, Vol. 2. G. E. Harman and C. P. Kubicek, eds. Taylor and Francis, London.
6. Baker, R., Elad, Y., and Chet, I. 1984. The controlled experiment in the scientific method with special emphasis on biological control. Phytopathology 74:1019-1021.
7. Baker, R., Elad, Y., and Sneh, B. 1986. Physical, biological and host factors in iron competition in soils. Pages 77-84 in: Iron Siderophores and Plant Diseases. T. R. Swinburne, ed. Plenum Press, New York.
8. Benítez, T., Limón, J., Delgado-Jarana, J., and Rey, M. 1998. Glucanolytic and other enzymes and their control. Pages 101-127 in: *Trichoderma* and *Gliocladium*, Vol. 2. G. E. Harman and C. P. Kubicek, eds. Taylor and Francis, London.
9. Bolar, J. P., Norelli, J. L., Wong, K.-W., Hayes, C. K., Harman, G. E., and Aldwinckle, H. S. 2000. Expression of endochitinase from *Trichoderma harzianum* in transgenic apple increases resistance to apple scab and reduces vigor. Phytopathology 90:72-77.
10. Burr, T. J., Schroth, M. N., and Suslow, T. 1978. Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* and *P. putida*. Phytopathology 68:1377-1383.
11. Calderon, A. A., Zapata, J. M., Munoz, R., Pedreno, M. A., and Barcelo, A. R. 1993. Resveratrol production as a part of the hypersensitive-like response of grapevine cells to an elicitor from *Trichoderma viride*. New Phytol. 124:455-463.
12. Carsolio, C., Benhamou, N., Haran, S., Cortes, C., Gutierrez, A., Chet, I., and Herrera-Estrella, A. 1999. Role of the *Trichoderma harzianum* endochitinase gene, *ech42*, in mycoparasitism. Appl. Environ. Microbiol. 65:929-935.
13. Chang, Y.-C., Chang, Y.-C., Baker, R., Kleifeld, O., and Chet, I. 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Dis. 70:145-148.
14. Chet, I. 1987. *Trichoderma*–Application, mode of action, and potential as a biocontrol agent of soilborne plant pathogenic fungi. Pages 137-160 in: Innovative Approaches to Plant Disease Control. I. Chet, ed. John Wiley & Sons, New York.
15. Cook, R. J. 1992. A customized approach to biological control of wheat root diseases. Pages 211-230 in: Biological Control of Plant Diseases: Progress and Challenges for the Future. E. C. Tjamos, G. C. Papavizas, and R. J. Cook, eds. Plenum Press, New York.
16. Cook, R. J. 1993. The role of biological control in the 21st century. Pages 10-20 in: Pest Management: Biologically Based Technologies. R. D. Lumsden and J. L. Vaughn, eds. American Chemical Society, Washington, DC.
17. da Luz, W. C., Bergstrom, G. C., and Stockwell, C. A. 1998. Seed-applied bioprotectants for control of seedborne *Pyrenophora tritici-repentis* and agronomic enhancement of wheat. Can. J. Plant Pathol. 19:384-386.
18. Datnoff, L. E., Nemec, S., and Pernezny, K. 1995. Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biol. Control 5:427-431.
19. De Meyer, G., Bigirimana, J., Elad, Y., and Höfte, M. 1998. Induced systemic resistance in *Trichoderma harzianum* biocontrol of *Botrytis cinerea*. Eur. J. Plant Pathol. 104:279-286.
20. Deacon, J. W. 1994. Rhizosphere constraints affecting biocontrol organisms applied to seeds. Pages 315-326 in: Seed Treatment: Progress and Prospects. T. Martin, ed. British Crop Protection Council, Farnham, UK.
21. Di Pietro, A., Lorito, M., Hayes, C. K., Broadway, R. M., and Harman, G. E. 1993. Endochitinase from *Gliocladium virens*: Isolation, characterization, and synergistic antifungal activity in combination with gliotoxin. Phytopathology 83:308-313.
22. Elad, Y., David, D. R., Levi, T., Kapat, A., Kirshner, B., Guvrin, E., and Levine, A. 1999.

Trichoderma harzianum T39-mechanisms of biocontrol of foliar pathogens. Pages 459-467 in: Modern Fungicides and Antifungal Compounds II. H. Lyr, ed. Intercept Ltd., Andover, Hampshire, UK.
23. Elad, Y., and Kapat, A. 1999. The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea*. Eur. J. Plant Pathol. 105:177-189.
24. Elad, Y., Kirshner, B., Yehuda, N., and Sztejnberg, A. 1998. Management of powdery mildew and gray mold of cucumber by *Trichoderma harzianum* T39 and *Ampelomyces quisqualis* AQ10. BioControl 43:241-251.
25. Environmental Protection Agency. 1990. National Pesticide Survey: Project Summary. U.S.-Environmental Protection Agency, Washington, DC.
26. Geremia, R. A., Goldman, G. H., Jacobs, D., Ardiles, W., Vila, S. B., Van Montagu, M., and Herrera-Estrella, A. 1993. Molecular characterization of the proteinase encoding gene, *prb1*, related to mycoparasitism by *Trichoderma harzianum*. Mol. Microbiol. 8:603-613.
27. Graeme-Cook, K. A., and Faull, J. L. 1991. Effect of ultraviolet-induced mutants of *Trichoderma harzianum* with altered antibiotic production on selected pathogens in vitro. Can. J. Microbiol. 37:659-664.
28. Graham, R. D., and Webb, M. J. 1991. Micronutrients and disease resistance and tolerance in plants. Pages 329-370 in: Micronutrients in Agriculture. R. M. Welch, ed. Soil Science Society of America, Madison, WI.
29. Hadar, Y., Harman, G. E., and Taylor, A. G. 1984. Evaluation of *Trichoderma koningii* and *T. harzianum* from New York soils for biological control of seed rot caused by *Pythium* spp. Phytopathology 74:106-110.
30. Harman, G. E., and Björkman, T. 1998. Potential and existing uses of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement. Pages 229-265 in: *Trichoderma* and *Gliocladium*, Vol. 2. G. E. Harman and C. P. Kubicek, eds. Taylor and Francis, London.
31. Harman, G. E., Latorre, B., Agosin, A., San Martin, R., Riegel, D. G., Nielsen, P. A., Tronsmo, A., and Pearson, R. C. 1996. Biological and integrated control of Botrytis bunch rot of grape using *Trichoderma* spp. Biol. Control 7:259-266.
32. Harman, G. E., and Taylor, A. G. 1988. Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. Phytopathology 78:520-525.
33. Harman, G. E., and Taylor, A. G. 1990. Development of an effective biological seed treatment system. Pages 415-426 in: Biological Control of Soil-borne Plant Pathogens. D. Hornby, ed. CAB International, Oxon, UK.
34. Harman, G. E., Taylor, A. G., and Stasz, T. E. 1989. Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Dis. 73:631-637.
35. Heckman, J. R., Govindasamy, R., Prostak, D. J., Chamberlain, E. A., and Hlubik, W. T. 1996. Corn response to sidedress nitrogen in relation to soil nitrate analysis. Commun. Soil Sci. Plant Anal. 27:575-583.
36. Hervas, A., Landa, B., Datnoff, L. E., and Jimenez-Diaz, R. M. 1998. Effects of commercial and indigenous microorganisms on Fusarium wilt development in chickpea. Biol. Control 13:166-176.
37. Hoitink, H. A. J., Inbar, Y., and Boehm, M. J. 1991. Status of compost-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops. Plant Dis. 75:869-873.
38. Hoitink, H. A. J., Stone, A. G., and Han, D. Y. 1997. Suppression of plant diseases by composts. HortScience 32:184-187.
39. Howell, C. R. 1987. The role of mycoparasitism in the biological control of *Rhizoctonia solani* by *Gliocladium virens*. Phytopathology 77:992-994.
40. Howell, C. R. 1998. The role of antibiosis in biocontrol. Pages 173-184 in: *Trichoderma* and *Gliocladium*, Vol. 2. G. E. Harman and C. P. Kubicek, eds. Taylor and Francis, London.
41. Howell, C. R., Hanson, L. E., Stipanovic, R. D., and Puckhaber, L. S. 1999. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens*. Phytopathology 90:248-252.
42. Howell, C. R., and Stipanovic, R. D. 1995. Mechanisms in the biocontrol of *Rhizoctonia solani*-induced cotton seedling disease by *Gliocladium virens*: Antibiosis. Phytopathology 85:469-472.
43. Huber, D. M., and McCay-Buis, T. S. 1993. A multiple component analysis of the take-all disease of cereals. Plant Dis. 77:437-447.
44. Huber, D. M., and Wilhelm, N. S. 1988. The role of manganese in resistance to plant diseases. Pages 155-173 in: Manganese in Soil and Plants. R. D. Graham, R. D. Hannam, and N. C. Uren, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.
45. Kapat, A., Zimand, G., and Elad, Y. 1998. Effect of two isolates of *Trichoderma harzianum* on the activity of hydrolytic enzymes produced by *Botrytis cinerea*. Physiol. Mol. Plant Pathol. 52:127-137.
46. Klausner, S. D., Kanneganti, V. R., and Bouldin, D. R. 1994. An approach for estimating a decay series for organic nitrogen in animal manure. Agron. J. 86:897-903.
47. Kloepper, J. W., Tuzun, S., Liu, L., and Wei, G. 1993. Plant growth-promoting rhizobacteria as inducers of systemic disease resistance. Pages 156-165 in: Pest Management: Biologically Based Technologies. R. D. Lumsden and J. L. Vaughn, eds. American Chemical Society, Washington, DC.
48. Kovach, J., Petzoldt, R., and Harman, G. E. Using honey bees and bumble bees to disseminate *Trichoderma harzianum* 1295-22 to strawberries for *Botrytis* control. Biol. Control. In press.
49. Lewis, J. A., and Papavizas, G. C. 1984. A new approach to stimulate population proliferation of *Trichoderma* species and other potential biocontrol fungi introduced into natural soils. Phytopathology 74:1240-1244.
50. Lifshitz, R., Windham, M. T., and Baker, R. 1986. Mechanism of biological control of preemergence damping-off of pea by seed treatment with *Trichoderma* spp. Phytopathology 76:720-725.
51. Liu, L., Kloepper, J. W., and Tuzun, S. 1995. Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria. Phytopathology 85:843-847.
52. Liu, L., Kloepper, J. W., and Tuzun, S. 1995. Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology 85:695-698.
53. Lo, C.-T., Nelson, E. B., and Harman, G. E. 1996. Biological control of turfgrass diseases with a rhizosphere competent strain of *Trichoderma harzianum*. Plant Dis. 80:736-741.
54. Lo, C.-T., Nelson, E. B., and Harman, G. E. 1997. Improved biocontrol efficacy of *Trichoderma harzianum* 1295-22 for foliar phases of turf diseases by use of spray applications. Plant Dis. 81:1132-1138.
55. Lo, C.-T., Nelson, E. B., Hayes, C. K., and Harman, G. E. 1998. Ecological studies of transformed *Trichoderma harzianum* strain 1295-22 in the rhizosphere and on the phylloplane of creeping bentgrass. Phytopathology 88:129-136.
56. Lora, J. M., De la Cruz, J., Benitez, T., Llobell, A., and Pintor-Toro, J. A. 1995. Molecular characterization and heterologous expression of an endo-ß-1, 6 glucanase gene from the mycoparasitic fungus *Trichoderma harzianum*. Mol. Gen. Genet. 247:639-645.
57. Lorito, M. 1998. Chitinolytic enzymes and their genes. Pages 73-99 in: *Trichoderma* and *Gliocladium*, Vol. 2. G. E. Harman, and C. P. Kubicek, eds. Taylor and Francis, London.
58. Lorito, M., Woo, S. L., D'Ambrosio, M., Harman, G. E., Hayes, C. K., Kubicek, C. P., and Scala, F. 1996. Synergistic interaction between cell wall degrading enzymes and membrane affecting compounds. Mol. Plant-Microbe Interact. 9:206-213.
59. Lorito, M., Woo, S. L., Garcia Fernandez, I., Colucci, G., Harman, G. E., Pintor-Toro, J. A., Filippone, E., Mucciflora, S., Lawrence, C. B., Zoina, A., Tuzun, S., and Scala, F. 1998. Genes from mycoparasitic fungi as a source for improving plant resistance to fungal pathogens. Proc. Natl. Acad. Sci. USA 95:7860-7865.
60. Mathre, D. E., Cook, R. J., and Callan, N. W. 1999. From discovery to use: Traversing the world of commercializing biocontrol agents for plant disease control. Plant Dis. 83:972-983.
61. McGrath, M. T., Shishkoff, N., and Sieczka, J. B. 1998. Management of powdery mildew and Phytophthora fruit rot, two important cucurbit diseases. 1997 N.Y. State Veg. Proj. Rep. Relating to IPM 123:18-27.
62. Miller, R. G. J. 1991. Simultaneous Statistical Inference. Springer-Verlag, New York.
63. Nelson, E. B. 1988. Biological control of Pythium seed rot and preemergence damping-off of cotton with *Enterobacter cloacae* and *Erwinia herbicola* applied as seed treatments. Plant Dis. 72:140-142.
64. Nemec, S., Datnoff, L. E., and Strandberg, J. 1996. Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetables and citrus. Crop Prot. 15:735-742.
65. Peng, G., Sutton, J. C., and Kevan, P. G. 1992. Effectiveness of honey bees for applying the biocontrol agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea*. Can. J. Plant Pathol. 14:117-129.
66. Piekielek, W. P., and Fox, R. H. 1992. Use of a chlorophyll meter to predict sidedress nitrogen requirements for maize. Agron. J. 84:59-65.
67. Schirmböck, M., Lorito, M., Wang, Y.-L., Hayes, C. K., Artisan-Atac, I., Scala, F., Harman, G. E., and Kubicek, C. P. 1994. Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. Appl. Environ. Microbiol. 60:4364-4370.
68. Simon, A., and Sivasithamparam, K. 1990. Effect of crop rotation, nitrogenous fertilizer and lime on biological suppression of the take-all fungus. Pages 215-226 in: Biological Control of Soil-borne Plant Pathogens. D. Hornby, ed. CAB International, Oxon, UK.
69. Sivan, A., and Harman, G. E. 1991. Improved rhizosphere competence in a protoplast fusion progeny of *Trichoderma harzianum*. J. Gen. Microbiol. 137:23-29.
70. Sivasithamparam, K., and Ghisalberti, E. L. 1998. Secondary metabolism in *Trichoderma* and *Gliocladium*. Pages 139-191 in: *Trichoderma* and *Gliocladium*, Vol. 1. C. P. Kubicek

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and G. E. Harman, eds. Taylor and Francis, London.

71. Smith, V. L., Wilcox, W. F., and Harman, G. E. 1990. Potential for biological control of Phytophthora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. Phytopathology 80:880-885.
72. Snedecor, G. W., and Cochran, W. G. 1986. Statistical Methods. Iowa State University, Ames.
73. Stasz, T. E., Harman, G. E., and Weeden, N. F. 1988. Protoplast preparation and fusion in two biocontrol strains of *Trichoderma harzianum*. Mycologia 80:141-150.
74. Taylor, A. G., and Harman, G. E. 1990. Concepts and technologies of selected seed treatments. Annu. Rev. Phytopathol. 28:321-339.
75. Taylor, A. G., Min, T.-G., Harman, G. E., and Jin, X. 1991. Liquid coating formulation for the application of biological seed treatments of *Trichoderma harzianum*. Biol. Control 1:16-22.
76. Thrane, C., Tronsmo, A., and Jensen, D. F. 1997. Endo-ß-1, 3 glucanase and cellulase from *Trichoderma harzianum*: Biological activity against plant pathogenic *Pythium* spp. Eur. J. Plant Pathol. 103:331-344.
77. Weaver, D. B., Rodriguez-Kabana, R., and Carden, E.-L. 1998. Velvetbean and bahiagrass as rotation crops for management of *Meloidogyne* spp. and *Heterodera glycines* in soybean. J. Nematol. 30:563-568.
78. Weindling, R. 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopathology 24:1153-1179.
79. Windham, M. T., Elad, Y., and Baker, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76:518-521.
80. Woo, S. L., Donzelli, B., Scala, F., Mach, R., Harman, G. E., Kubicek, C. P., Del Sorbo, G., and Lorito, M. 1999. Disruption of the *ech42* (endochitinase-encoding) gene affects biocontrol activity in *Trichoderma harzianum* P1. Mol. Plant-Microbe Interact. 12:419-429.
81. Yedidia, I., Benhamou, N., and Chet, I. 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol. 65:1061-1070.
82. Zeilinger, S., Galhaup, C., Payer, K. L., Woo, S., Mach, R., Fekete-Csaba, L., Lorito, M., and Kubicek, C. P. 1999. Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host. Fungal Genet. Biol. 26:131-140.