IS BIOLOGICAL CONTROL OF COTTON ROOT ROT POSSIBLE?

Thomas Isakeit
Professor and Extension Plant Pathologist, Texas A&M University, College Station

Root rot, caused by *Phymatotrichopsis omnivora*, a fungus, continues to be a serious problem of cotton in many areas of Texas. After a century of research, there are no effective, economical cultural, chemical, or varietal control measures. What about biological control, using antagonistic microbes added to soil?

Background:

Antagonism between microbes was recognized by numerous microbiologists since the late 19th century. It is very easy to demonstrate when growing cultures on a Petri dish (Fig. 1).

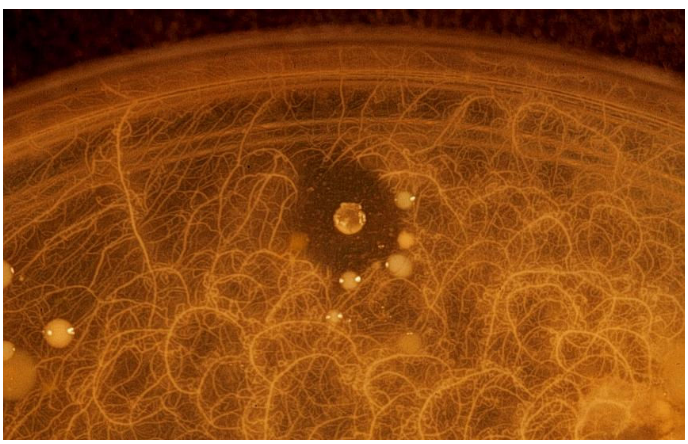

Fig. 1. Microbial antagonism as seen in a Petri dish. The colony is producing a chemical that prevents growth of other microbes, showing a zone of growth inhibition.

A most famous example is Alexander Fleming's discovery of the antibiotic, penicillin, which originated from the observation of growth inhibition of bacteria in a Petri dish by a contaminating fungus.

However, it has not been easy to transfer the growth inhibition as seen on a Petri dish to the use of microbes to control plant diseases, particularly those caused by root-infecting fungi. Studies on the use of microbes to inhibit plant pathogens began in the 1920s and continue to this day. There are now many microbes that have been commercialized for biological control, but none of those can reliably control root-infecting fungi, ***particularly those causing diseases in field soils***.

There are some examples of successful, commercialized biocontrol agents, for example, Galltrol-A, used to control crown gall, and atoxigenic *Aspergillus flavus* strains (AF36 and Aflaguard), used to control aflatoxin in cottonseed and peanut. With successful examples of biological control, the site of action is either not in soil (e.g. the developing seed of a cotton plant, for aflatoxin control), or if a root-infecting fungal pathogen is targeted, the target site involves soilless media (hydroponics, rockwool), potting soils (Fig. 2), or soils that have been previously sterilized.

Fig. 2. An experiment showing biological control of Pythium damping-off in a peat-vermiculite mix. Tomato seed in the left container was treated with an antagonistic bacterium (*Lysobacter enzymogenes*); control on the right.

In contrast, successes using commercial

biocontrol agents introduced into field soils for control of root-infecting fungal pathogens are lacking, although there are at least two dozen commercial products available world-wide that target such pathogens. The reason for this is simple and was pointed out in 1974, in the landmark book of K.F. Baker and R.J. Cook, "Biological Control of Plant Pathogens". That is, the composition of a microbial community of a typical field soil is stable and resists change. The consequence of adding large numbers of microbes to soils is that these microbes either disappear (they starve to death or become the food of other microbes) or they remain, but are not effective at controlling disease (Fig. 3).

Fig. 3. Imprint of pepper roots in a Petri dish, showing extensive colonization by the biocontrol bacterium, *Burkholderia cepacia* (Deny). In a field experiment, I readily isolated this bacterium from cantaloup roots killed by the fungus, *Monosporascus cannonballus*.

In a field soil, an introduced biocontrol agent must not only contend with the fungal pathogen, it must also survive an onslaught of the "restless natives": bacteria, fungi, protozoans, and nematodes. Introduced microbes do not fare well in the environment of a field soil.

***Streptomyces* for biological control:**

Many species of bacteria and fungi have been evaluated for biological control. *Streptomyces* is a genus of spore-forming, filamentous bacteria found in most agricultural soils. A few species are plant pathogens (e.g. potato scab), but many species produce antibiotics or have enzymes that degrade fungal cell walls. To my knowledge, there are two *Streptomyces* species sold as biocontrol treatments: Mycostop (*S. griseoviridis* strain K61) and Actinovate (*S. lydicus* strain WYEC 108). During 1991-1992, I had evaluated Mycostop in a greenhouse experiment to control Fusarium wilt of carnations growing in pots and did not see any control of the disease.

Actinovate:

Streptomyces lydicus WYEC 108 was isolated from flax root soil in England, as part of a larger study that screened potential antagonists against *Pythium ultimum,* using Petri dish testing (Crawford *et al*., **A**pplied and **E**nvironmental **M**icrobiology 58:3899, 1993). Additional studies documented antagonism against *Phymatotrichopsis omnivora* (the cotton root rot fungus) in Petri dish testing, noting that it was not as strongly inhibited by WYEC 108 as other fungi were, and it was not killed by the exposure to WYEC 108 (Yuan and Crawford, **AEM** 61:3119, 1995). The authors also documented a reduction of damping off of pea and cotton, caused by *P. ultimum,* in a growth chamber experiment maintained at 82º F, evaluating both a sterilized and non-sterilized silt loam soil, pH 7.4.

There have been nine reports of experiments with Actinovate published in **B**iological and **C**ultural **T**ests for Control of Plant Diseases (APS Press, St. Paul, MN) from 2001 to 2006. The work was done in Connecticut with greenhouse ornamentals and the diseases were: Fusarium corm rot of gladiolus, Fusarium wilt of cyclamen, Pythium blackleg of geranium, and Rhizoctonia root and crown rot of Vinca. The growing medium for these plants was potting soil. In one experiment with cyclamen, Actinovate reduced Fusarium wilt, as did the other biocontrol agents tested (except Mycostop), and worked just as well as the fungicide treatments (**BCT**17:O07). Actinovate reduced Rhizoctonia root rot of vinca at one site, but not at another site (**BCT**18:O015). The other seven reports showed no reduction in disease with Actinovate treatment, as compared with the control (**BCT** 16:O042; 17:O06; 17:O10; 19:O007; 20:O010; 21:O004; 21:O014).

In an outdoor location in Florida, Actinovate and another biocontrol product, AtEze (*Pseudomonas chlororaphis*), did not control Rhizoctonia root and crown blight of impatiens

(McGovern *et al*., Plant Disease 86:1388, 2002). As the authors explained, "These biocontrols may have failed to persist in the root systems, may have been out-competed by indigenous bacteria, or in the case of nematode suppression, may have been inherently ineffective".

In a joint Florida-Connecticut study of Fusarium wilt of cyclamen, biocontrol products, including Actinovate, were ineffective as a stand-alone protective treatment, but there was a reduction in disease when these materials were used in combination with a fungicide such as fludioxonil (Elmer and McGovern, Crop Protection 23:909, 2004).

In a greenhouse experiment done in Idaho, an Actinovate treatment did not reduce Verticillium wilt of potato, as compared with the control (Entry *et al*., Biocontrol Science and Technology 10:677, 2000). However, the disease was reduced when Actinovate was part of a wood chip-polyacrylamide medium incorporated into soil.

Finally, in reviewing published studies on the effectiveness of Actinovate for control of plant diseases, I will mention my own study, done in collaboration with plant pathologist, Marvin Miller, at the Texas A&M Agricultural Experiment Station in Weslaco (**BCT** 11:108, 1996). This study appears to be the first published, independent evaluation of Actinovate. We evaluated Actinovate as a seed treatment and transplant dip for control of pink root of onions (Fig. 4), in an area of the Station with a history of pink root, using a replicated, randomized experimental design (Fig. 5).

Fig. 4. Symptoms of pink root of onion, caused by the fungus, *Phoma terrestris.*

We did not see any reduction in disease, nor did we see any increase in yield, with the exception of one grade of one out of the four cultivars evaluated.

Fig. 5. Experiment to evaluate Actinovate for control of pink root of onion. Texas A&M, Weslaco. 1994-1995.

Actinovate for Cotton Root Rot?

I have not seen any data indicating that Actinovate can control cotton root rot. By data, I mean measurements of plants in randomized, replicated experiments. Based on the aforementioned reports, it appears that Actinovate may work against some diseases in a greenhouse environment, where the soil has been previously sterilized or amended with some organic nutrient to support growth of the bacterium. By amendment with organic nutrient, I don't mean ounces or pounds per acre, I mean ***tons*** per acre. There is nothing published that suggests that Actinovate could have some activity in non-amended field soils such as those used to grow cotton. Furthermore, our work with pink root of onion suggests that Actinovate will not work as a stand-alone material in a field soil.

Testing Biocontrol Agents:

I am not inclined to devote limited time and resources to testing or doing result demonstrations with biocontrol products, unless there is previous data from replicated experiments done in the field, with some positive results. Chemical companies have R&D departments to do the initial screening of their new fungicides; they do not expect universities to do this screening for them. Moreover, I do not recommend that growers use untested products for managing diseases.

Biocontrol of Cotton Root Rot?

Researchers from the 1930s and 1940s felt that suppression of root rot with manure was caused by antagonistic microbes, but they never analyzed the reason for suppression. There was also research at Texas A&M University during the 1980s specifically directed at finding antagonistic fungi that could kill the survival structures (sclerotia) of the cotton root rot fungus. This work was not further pursued.

I do feel there is potential for biological control to work by managing the antagonistic microbes that are already part of the soil community. Most agricultural soils have some degree of disease suppression imposed by naturally-occurring microbes (Fig. 6). The objective of my future work is to figure out a means to enhance that suppression for control of CRR.

Fig. 6. Experiment showing natural suppression by native soil microbes of damping-off of marigold, caused by the fungus, *Rhizoctonia solani*. The soil on left had been heat-treated, killing most of the native microbes, prior to planting and adding the pathogen. Disease is much greater than in the soil on the right, which had not been treated (control). Re-inoculating heat-treated soils can restore disease suppressiveness.

PHOTO CREDITS:

These are all my photos, based on various research studies that I had done since 1980.

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