Antagonistic Fungi for the Biological Control of Phytophthora Root Rots of Woody Ornamentals

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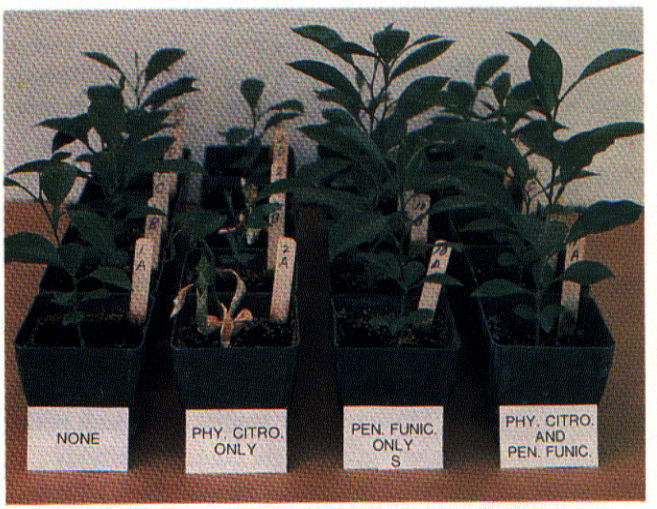
Phytophthora root rot and crown rot of ornamental or landscape shrubs, fruit and forest trees, and other woody perennials are destructive diseases widespread in both nursery and field plantings. The disease often originates on seeds, seedlings, rooted cuttings or other forms of juvenile plants in the nursery and later becomes more serious in the landscape, orchard or forest.

During the past 10 to 15 years, Phytophthora root and crown rots of many ornamental plants have been "successfully managed" in commercial nurseries by the use of chemical fungicides, such as metalaxyl and fosetyl-Al, which are highly specific against *Phytophthora* and related fungi. However, due to the largely fungistatic (instead of fungicidal) nature of these chemicals at the concentrations used, low populations of *Phytophthora* remain in the infested planting mixes or in the infected plant tissues even though the treated plants show little or no detectable above-ground symptoms. In order to keep the pathogens in check and maintain a salable product,

Severity of azalea root rot caused by *Phytophthora parasitica* was reduced by the incorporation of the biocontrol agent, *Penicillium funiculosum*, in the planting mix. Note also the growth stimulation in the treatment receiving *Penicillium funiculosum* only. The photo was taken at 6 weeks.



Severity of citrus root rot caused by *Phytophthora citrophthora* was reduced by the incorporation of the biocontrol agent, *Penicillium funiculosum*, in the planting mix. Note also the growth stimulation in the treatment receiving *Penicillium funiculosum* only. The photo was taken at 6 weeks; two plants were dead in the treatment receiving *Phytophthora citrophthora* only.

Comparison of the biocontrol agent, *Penicillium funiculosum*, with the chemical fungicide metalaxyl at 50 milligrams per liter in the control of azalea root rot caused by *Phytophthora parasitica*.

these fungicides are being applied in many commercial nurseries at sometimes unnecessarily high frequencies or dosages until the container-grown plants have left the nursery and are transplanted in home gardens, orchards, parks, forests, roadsides or other landscape sites.

Since those infected plants cease to receive fungicide applications after transplanting, they soon develop severe below-ground diseases. Root rot severity increases, the plant soon loses most or nearly all feeder roots, and death often occurs if the weakened shrub or tree has suffered from environmental stresses, especially those due to sudden drought, excessive watering or prolonged flooding.

Biological control of root disease fungi, although still in the experimental stages, has been shown to be successful in recent years. However, few studies have dealt with *Phytophthora* fungi or with root rot control in woody perennials. A successful biocontrol program has advantages over chemical soil treatments which are often ineffective, usually expensive and potentially environmentally hazardous. Effective biocontrol agents against Phytophthora root rots of woody perennials will reduce excessive fungicide use and are deemed a useful component in an integrated disease management program in the nursery.

Potentials of Antagonistic Fungi as Biocontrol Agents in Planting Mixes

A large number of beneficial fungi found in natural soils are known to be antagonistic to various *Phytophthora* species, and the use of these antagonistic fungi as potential biocontrol agents of Phytophthora root rots in soilless planting mixes has been under investigation in our laboratory since 1985. Azalea and citrus were used as experimental test plants. Many species of *Aspergillus*, *Penicillium* and *Trichoderma* exhibited various types of antagonism in laboratory tests, and from a large number of soil fungi tested in the greenhouse against Phytophthora root rot of azalea, the following showed promise as potential biocontrol agents: *Aspergillus flavipes*, *A. flavus* and *A. ochraceus*; *Penicillium decumbens*, *P. funiculosum*, *P. janthinellum* and *P. ochrochloron*; and *Trichoderma harzianum* and an unidentified *Trichoderma* species.

Incorporation of these antagonistic fungi into one of several planting mixes, in which azalea rooted cuttings were transplanted, reduced greatly or substantially the severity of azalea root rot caused by either *Phytophthora cinnamomi* or *Ph. parasitica*. Some of the above antagonistic fungi also effectively suppressed citrus root rot caused by *Phytophthora citrophthora* or *Ph. parasitica*. The roots of sweet orange seedlings grown in antagonist-treated peat/perlite planting mix were protected from *Phytophthora* infections, and these treated plants usually grew much better than the untreated control plants. *Pythium nunn*, another antagonistic fungus which had been shown to be an effective biocontrol agent against certain root rots of herbaceous plants, also suppressed citrus root rot caused by *Phytophthora parasitica* in some of our greenhouse tests.

Not all of the above antagonists, however, provided satisfactory root rot control in all of the tests conducted.

In general, root rot suppression was greater when lower levels of *Phytophthora* inoculum were used, planting mix was watered less frequently, and root infection on control plants was less severe. In other words, biocontrol of Phytophthora root rot, as with other forms of control including chemical control, is less effective if conditions are more conducive for Phytophthora infection or the root rot to be controlled is extremely severe.

Penicillium funiculosum as a Biocontrol Agent

Among the above-mentioned potential biocontrol agents, *Penicillium funiculosum* has shown the greatest promise. This antagonistic fungus has performed well in numerous experiments in which the antagonist was first grown in a one-to-one wheat bran/peat moss medium which was then mixed into a three-to-one peat/perlite planting mix at a concentration of 0.35 or 0.7 percent (weight/volume). Rooted azalea cuttings or sweet orange seedlings were then transplanted into the planting mix which we inoculated with a *Phytophthora* species five to seven days later. In most of the tests, *Penicillium funiculosum* effectively suppressed azalea root rot caused by *Ph. parasitica* and sweet orange root rot caused by *Ph. citrophthora*. It also suppressed, to some degree, root rot caused by *Ph. cinnamomi* on azalea and by *Ph. parasitica* on sweet orange. Three subisolates of *Penicillium funiculosum* (T327S, T327H, and T327L) exhibited different degrees of root rot suppression, with T327S consistently providing the best disease control.

Mixing the *Penicillium* bran/peat inoculum into the planting mix was a more effective method for delivering the biocontrol agent than dipping the plant root systems into a *Penicillium* spore suspension before transplanting. *Phytophthora* suppression was also achieved in some tests when the *Penicillium funiculosum* inoculum was reduced to 0.07 percent. We tested the antagonist in a number of different planting mixes and found it to be effective in peat/perlite, peat/vermiculite, or peat/sand, but not in a pinebark/sand mix.

Compatibility with Fungicide Use

The effectiveness of *Penicillium funiculosum* as a biocontrol agent was compared to that of metalaxyl, one of the best fungicides available for the control of *Phytophthora* diseases. Azalea root rot suppression by *Penicillium funiculosum* at a concentration of 0.35 or 0.7 percent was equal to or greater than that provided by the fungicide used at 12.5, 25 or 50 milligrams per liter. Results were similar when the biological agent and chemical agent were compared for citrus root rot control.

Penicillium funiculosum, which belongs to a fungus group in Hyphomycetes, is not sensitive to the chemical metalaxyl, a highly specific fungicide which is inhibitory only against the fungus group in Peronosporales to which *Phytophthora* belongs. Therefore, this biocontrol agent can be combined with this specific fungicide in attempts at integrated control of Phytophthora root rots. Results of our greenhouse experiments showed that the combined treatment of *Penicillium funiculosum* and metalaxyl produced a slightly greater disease control than either agent alone. The combined effect was additive but not synergistic, and has been shown in greenhouse experiments involving the control of Phytophthora root rots of both azalea and sweet orange.

Increased Growth Response in Other Plants

In most of our greenhouse pot experiments, the rooted cuttings of azalea which received the *Penicillium funiculosum*-alone treatment (that is, without *Phytophthora*) grew faster and better (often twice as large), produced greener foliage, and had healthier root systems than the control plants—not only the *Phytophthora*-alone control but also the no-*Phytophthora*/no-*Penicillium* control. This increased growth response was equally as evident on sweet orange seedlings as on azalea, and has also been observed in some experiments involving other antagonistic fungi.

Such a stimulation of plant growth occurred even in the absence of *Phytophthora*, and, therefore, was not simply due to *Phytophthora* suppression. The increased growth response, in the absence of *Phytophthora*, has also been observed on other test plants, such as carnation, stone pine, deodar cedar, holly, myrtle, juniper, and periwinkle in addition to azalea and citrus, both in greenhouse tests and in commercial nursery trials, and in four different planting mixes tested.

Mechanisms of Phytophthora Suppression and Increased Growth Response

The mechanisms of suppression of Phytophthora root rots by *Penicillium funiculosum* were examined in a number of laboratory experiments. Mycoparasitism, antibiosis and lysis were found to be the probable mechanisms. *Penicillium funiculosum* had the ability to destroy *Phytophthora* vegetative and reproductive structures by parasitizing them, to inhibit *Phytophthora* growth by the produc-

tion of potent antibiotic substances, and to dissolve *Phytophthora* cell walls by the production of enzymes.

In addition to exhibiting strong antagonism against *Phytophthora* species, *Penicillium funiculosum* also possesses a broad inhibitory spectrum against many other soil-borne, root-infecting fungi. The extracts from the medium in which *Penicillium funiculosum* had been growing contained potent antibiotic(s) which inhibited the growth of a number of common root-pathogenic fungi, including species of *Cylindrocladium*, *Fusarium*, *Pythium*, *Rhizoctonia*, *Thielaviopsis* and *Verticillium*. It is believed that many other root rot fungi, as yet not tested, may also be sensitive to the antagonistic action of *Penicillium funiculosum*. Many of these root pathogens and other "root-nibblers" are present in the nonsterile planting mixes used in our studies as well as in commercial nurseries. The pronounced increased growth response induced in many test plants by *Penicillium funiculosum* is most likely related to the prevention or reduction in the deleterious effects of these root pathogens. Therefore, it seems that, in a program of root disease management in the nursery, *Penicillium funiculosum* as a biocontrol agent may possess other attributes beyond a specific suppression of Phytophthora root rots of various woody perennials.

Conclusions

Penicillium funiculosum and some other soil fungi are strongly antagonistic to *Phytophthora* and other root pathogens, can effectively suppress Phytophthora root rots in various planting mixes, and induce growth stimulation in many woody perennials. They are promising candidates as potential biocontrol agents against the omnipresent and destructive Phytophthora root and crown rots of woody ornamentals in the nursery. *Penicillium funiculosum* has additional advantages for success in *Phytophthora* control of container-grown nursery plants because a) it can proliferate and is active in an acidic organic substrate or environment (such as in most of the soilless planting mixes containing peat or tree bark) which is known to be unfavorable to *Phytophthora*, and b) it is insensitive to, but compatible with, the Peronosporales-specific fungicides now widely used in commercial nurseries for the control of Phytophthora diseases.

A successful biocontrol program, nonetheless, is not expected to eradicate a root pathogen, to eliminate root infection, or to replace the current effective fungicides. A good biocontrol agent may not work perfectly each time, but can be operative when combined with a tolerant cultivar or rootstock, the judicious and minimal use of an effective chemical fungicide, and an intrinsically suppressive soilless planting mix which enhances maximal feeder root regeneration. It should also be integrated with other time-honored, established cultural methods of sanitation, pathogen exclusion, and proper practices of fertilization and watering which discourage or reduce pathogen proliferation. Our promising results from this project have provided optimism for the prospects of effective biological control of Phytophthora root and crown rots of many woody perennials in an integrated and multidisciplinary management program in the nursery and possibly in the field. Additional studies and a more complete understanding of the behavior and ecology of *Penicillium funiculosum* and other biocontrol agents will make commercial use of biological control of Phytophthora root and crown rots an attainable reality.

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