Biological Control of *Pythium* Damping-off and Root Rot of Greenhouse-Grown Geraniums and Poinsettias

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***Pythium ultimum*, which causes root rot and damping-off of many floricultural crops grown in Oklahoma greenhouses, produces oospores for survival and to initiate disease. Strains of *Actinoplanes* spp. that are hyperparasites of oospores were evaluated for their biological control of *Pythium* root rot of plants grown in a greenhouse. In soil-less potting mix infested with 10^3 oospores of *P. ultimum* /g mix, strains W57, W257, or 25844 of *Actinoplanes* spp. on clay granules applied at 5% or 0.5% w/w to the mix 5 d prior to replanting geranium or poinsettia seedlings reduced root rot severity and increased plant stand compared to nontreated plants after 6 wk. Granules of W257 applied at 5% w/w to mix 7 d prior to replanting poinsettia seedlings reduced root rot of the plants after 6 wk, whereas seedling dips in a suspension of macerated mycelium of W257 (10^9 colony-forming units/mL) had no effect. Strain W257 applied as granules (1% w/w) or as a root dip (8×10^8 colony-forming units/mL) 7 d after replanting poinsettia seedlings was as effective as a fungicide (metalaxyl) in reducing root rot after 9 weeks, but no treatment increased plant stand. Colonization of roots by *P. ultimum* was reduced by metalaxyl or W257 compared to roots from nontreated, pathogen-infested mix. *Actinoplanes* spp. show promise in the biological control of *P. ultimum* in greenhouse floricultural crops.**

INTRODUCTION

Production of floriculture crops grown in greenhouses is frequently limited by *Pythium* spp. that cause seed damping-off and root rot (*1-3*). In Oklahoma, *Pythium* spp. attacked 10% of the flower and house plant samples submitted in 1992 to the Oklahoma State University Plant Disease Diagnostic Laboratory (Jacobs, unpublished data), causing an estimated $1 million annual loss to the Oklahoma ornamental and nursery industry. Genetic resistance to *Pythium* diseases is low in several important greenhouse crops, such as geraniums and poinsettias. Presently available fungicides are often costly to use and may become environmentally unacceptable in the future. In addition, fungicides have little effect against oospores, which are the small, hardy spores of *Pythium* spp. that may persist in dry soil or soilless media for years and still cause disease. Current microbial-based biological controls (*1*, *4-6*) are an alternative to fungicides, but none destroys oospores.

Actinoplanes spp. are a novel group of filamentous bacteria that produce minute sporangia (*7*), which when hydrated release motile spores capable of parasitizing *Pythium* spp. or related fungi (*8-10*). A clay pellet formulation that was developed for applying sporangia to soil has been shown to reduce *Pythium* root rot of bush beans and table beets in field microplots (*11*). Little is known, however, about the potential of *Actinoplanes* spp. to control diseases of *Pythium* spp. in floricultural crops in greenhouses. Therefore, we evaluated the effectiveness of *Actinoplanes* spp. to biologically suppress the damping-off and root rot caused by *P. ultimum* Trow, which is a troublesome fungal pathogen in greenhouses, in geraniums (*Pelargonium x hortorum* Bailey) and poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch).

MATERIALS and METHODS

Preparation of *Pythium*-Infested Potting Mix and *Actinoplanes* spp. Application: Oospores of *P. ultimum* were grown in dilute corn meal broth, harvested, (*8*) and mixed into potting mix (peat/ perlite/ vermiculite; v/v/v; limed to pH 6.8) at 10^3 oospores/g of mix. Strains A25844 (*A. brasiliensis*), W57, and W257 of *Actinoplanes* spp. were evaluated for biological control because of their high degree of parasitism of oospores of *Pythium* spp. (*8*). *Actinoplanes* spp. strains were applied as sporangia on clay granules (*11*) or as a root dip in a suspension of comminuted mycelia in 1% carboxymethylcellulose (CMC) as a sticker (*12*). All experiments were conducted in greenhouses under conditions similar to commercial greenhouse culture (*2, 3*). Plants were grown in peat /perlite /vermiculite potting mix, in 10-cm diameter pots, unless otherwise stated.

Effectiveness of *Actinoplanes* spp. Granules: Clay granules with *Actinoplanes* spp. were mixed into moist, *P. ultimum*-infested potting mix at 5% or 0.5% w/w in plastic bags. After 5 d incubation at 24°C to allow parasitism of oospores, the contents of the bags were transferred to pots. Red Orbit geraniums from seed and Guthier V-14 Glory poinsettias from cuttings in Oasis™ (Oasis, Smithers-Oasis, Kent, OH) blocks were replanted into the pots. There were 10 replicates per treatment per pathogen per host. Controls consisted of plants in nontreated, infested potting mix, and in infested mix treated with 5% granules without *Actinoplanes* sp. After 6 wk, plant stands were counted, and the roots harvested and washed in running tap water. Root rot severity in geraniums was rated using an index of 1-6, where 1= healthy root, 2 = 1-25% root rot, 3 = 26-50% rot , 4 = 51-75% rot, 5 = >75% root rot, and 6 = nonrecoverable root. Root rot severity in poinsettias was rated, using a 1-5 index, where 1 = healthy root, 2 = 1-25% root rot, 3 = 26-75% rot, 4 = >75% root rot, and 5 = nonrecoverable root.

Granules of W257 Compared to Root Dipping in a W257 Suspension: Potting mix infested with *P. ultimum* was amended with 5% w/w granules of W257 in plastic bags as described above and incubated for 7 d at 25°C prior to dispensing into pots. Poinsettia seedlings in Oasis blocks were then planted into the pots. Oasis blocks with poinsettia roots also were dipped into a W257 suspension (10^9 colony-forming units/mL) and immediately planted into pots with infested mix. There were 20 replicates per treatment. Controls were poinsettia roots dipped in CMC and poinsettia seedlings in nontreated, infested potting mix. Stand and root rot severity were determined 6 wk later.

Efficacy of W257 Applied as Granules or a Root Dip Compared to a Fungicide: Granules of W257 (1% w/w) and a root dip of W257 (8×10^8 colony-forming units /mL) were compared to a fungicide drench of metalaxyl (150 mL/pot of 0.2 cc Subdue™ /6L; Novartis, Inc., Greensboro, NC) for suppressing damping-off of poinsettia plants. Granules of W257 were added to infested potting mix in 15-cm diameter pots 7 d prior to planting. Root dips were planted immediately after treatment. Controls were 1% w/w granules without *Actinoplanes* sp.; root dips in CMC; and nontreated, pathogen-infested potting mix. There were 15 replicates per treatment. Root rot severity was measured 9 wk later. Pieces of roots from five randomly selected plants were planted on a *Pythium* selective medium (*13*) to assess the effect of treatments on root colonization by *P. ultimum.*

Data Analyses: Percentage plant emergence, root rot severity, and percentage recovery of *P. ultimum* from roots were arcsine transformed prior to ANOVA, and means were compared with the Student Newman Keul's test ($P \leq 0.05$).

RESULTS

Effectiveness of *Actinoplanes* spp. Granules: Granules of strains A25844, W57, and W257 of *Actinoplanes* spp. applied at 5% or 0.5% (w/w) to root medium infested with *P. ultimum* oospores reduced root rot severity of geranium and poinsettia seedlings compared to untreated plants (Table 1). Suppression or root rot was greater with the 5% (w/w) treatment than with the 0.5% treatment. Clay granules without *Actinoplanes* spp. (5% w/w) did not reduce root rot severity, indicating that *Actinoplanes* spp. were the active agents of disease control.

Actinoplanes spp. also significantly increased stand after 6 wk. In control pots only 30-40% of plants survived, whereas 100% of geraniums survived in pots receiving granules with *Actinoplanes* spp. strains (5% w/w) (data not presented). At 0.5% w/w surviving geranium plants in pots treated with A25844, W57 or W257 were 80%, 100%, and 80%, respectively, of initial plantings. There was 100% survival of poinsettia plants in pots treated with either 0.5 or 5.0% (w/w) of *Actinoplanes* spp., but this survival was not significantly different ($P = 0.05$) than the 70% survival in the untreated pots.

Granules of W257 Compared to Root Dipping in a W257 Suspension: Poinsettias in root medium treated with strain W257 applied as 5% granules had reduced severity of root rot (Table 2) compared to the untreated control; however, root dips in a suspension of W257 did not decrease severity. The CMC treatment sticker did not reduce root rot symptoms compared to the untreated control, suggesting that disease suppression was due to *Actinoplanes* spp. No increase (P= 0.05) in poinsettia plant survival was found in *Actinoplanes*-treated pots (75-85%) compared to the controls (50%).

Efficacy of W257 Applied as Granules or a Root Dip Compared to a Fungicide: Poinsettias in pots that were treated with 1% w/w granules of W257, root dipping in a W257 suspension, or metalaxyl had less root rot than the control treatments (Table 3). Root rot control by W257 was

TABLE 1. Severity of root rot geraniums and poinsettias grown in potting mix infested with 10^3 oospores/g of *P. ultimum* and treated or not treated with two levels (w/w) of clay granules bearing sporangia of *Actinoplanes* spp. strains.

Granule treatment	Severity of root rot of host [1]	
	Geranium	Poinsettia
A25844 (0.5% w/w)	3.2 b	2.4 b
W57 (0.5% w/w)	2.7 b	2.3 b
W257 (0.5% w/w)	3.4 b	2.7 b
A25844 (5.0% w/w)	1.6 a	1.8 a
W57 (5.0% w/w)	1.9 a	1.7 a
W257 (5.0% w/w)	2.1 a	1.6 a
No *Actinoplanes* sp. (5.0% w/w) [2]	5.0 c	3.6 c
No clay granules	5.5 c	4.0 c

[1] Severity was assessed at 6 wk after exposure to *P. ultimum*, using rating indices of 1-6 or 1-5 for geranium or poinsettia, respectively, and where 1= a healthy root and 6 or 5 = an nonrecoverable root,respectively. Values were the means of 10 replicates. Means in a column followed by the same letter were not different ($P < 0.05$) using the Student Newman Keul's Test.
[2] Clay granules without *Actinoplanes* sp.

TABLE 2. Severity of root rot of poinsettias grown in potting mix infested with 10^3 oospores/g of *P. ultimum* and treated or not treated with strain W257 applied a granules or as a root dip.

Treatment	Severity of root rot [1]
Granules (5.0 % w/w)	3.1 a
Dip (10^9 cfu/ml CMC) [2]	3.7 ab
CMC only	4.3 b
None	4.5 b

[1] Values were assessed at 6 wk after exposure to *P. ultimum* using a rating index of 1-5, where 1 = a healthy root and 5 = an nonrecoverable root. Values were the means of 20 replicates. Means in a column followed by the same letter were not different ($P < 0.05$), using the Student Newman Keul's Test.
[2] CMC = carboxymethylcellulose (1% w/v in water).

TABLE 3. Severity of root rot of and pathogen recovery from poinsettias grown in potting mix infested with 10^3 oopsores/g of *P. ultimum* and treated with strain W257 as granules or as a dip compared to a metalaxyl drench.

Treatment	Severity of root rot [1]	Recovery from roots (%) [2]
Granules (1.0% w/w)	2.2 a	30 ab
Dip (8 x 10^8 cfu/ ml CMC) [3]	2.1 a	28 ab
Metalaxyl [4]	2.3 a	23 a
Granules only (1.0% w/w)	2.9 b	55 c
CMC only	2.9 b	49 bc
None	3.1 b	59 c

[1] Values were assessed at 9 wk after exposure to *P. ultimum* using a rating index of 1-5, where 1 = a healthy root and 5 = an nonrecoverable root. Values were the means of 15 replicates. Means in a column followed by the same letter were not different ($P < 0.05$), using the Student Newman Keul's Test.
[2] Five pieces of roots were plated on 5 dishes of an agar medium. Number of *P. ultimum* colonies found were expressed as a percentage of pieces plated per treatment. Means followed by the same letter in a column were not different ($P < 0.05$).
[3] CMC = carboxymethylcellulose (1% w/v in water).
[4] Applied as 150 ml/pot of 0.2 cc Subdue™/6L of water.

as effective as metalaxyl. None of the *Actinoplanes* or metalaxyl treatments increased plant survival after 9 wk compared to the control.

Recovery of *P. ultimum* from poinsettia roots in root medium treated with metalaxyl or with strain W257 was less frequent than that from roots in untreated, pathogen-infested medium (Table 3). Recovery from roots in medium treated with granules or CMC was not different from the untreated control. Less *P. ultimum* was recovered from root medium treated with granules of W257 than from root medium treated with unamended granules. Percentage recovery from roots in medium treated with W257 was not different from roots in medium treated with metalaxyl. These results indicate that the colonization of roots by *P. ultimum* was suppressed by strain W257.

DISCUSSION

Results from this study suggest that *Actinoplanes* spp. may have promise for reducing *Pythium* root rot in floricultural plants in the greenhouse. *Actinoplanes* strain W257 applied as granules or as a root dip was as effective as the fungicide metalaxyl in reducing root rot after 9 wk (Table 3). Colonization of poinsettia roots by *P. ultimum* was reduced by either metalaxyl or W257 compared to roots from untreated, pathogen-infested medium.

Realizing the potential of *Actinoplanes* depends on developing a practical application method and inoculation time. Application of *Actinoplanes* spp. on granules to root growing medium is a method compatible with greenhouse operation; however, the lowest granular rate (0.5% w/w) of *Actinoplanes* spp. effective in our study may not be an economical level to apply. A rate of 0.01-0.1% (w/w) would be more practical for growers. A lighter carrier than the currently used clay granules or an increase in sporulation density on the carrier could reduce the granular rate needed. Further study may produce an effective yet economical granular rate. In addition, microbial parasites need time to find their host and infect. In our experiments we allowed 5-7 d of prior incubation of *Pythium*-infested medium with *Actinoplanes* spp. granules. We need to determine the minimum incubation time for control.

Our results with root dips as an application method for *Actinoplanes* were mixed; nevertheless, further study of this method of protecting roots against *Pythium* spp. is warranted. Root dips, especially roots encased in foam blocks which may absorb and retain biocontrol agents, are a method compatible with the greenhouse culture of several floricultural crops. The results with root dips also raises the question of mode of action, because roots are replanted immediately after dipping, and the incubation time needed for parasitism of oospores most likely would be limited. *Actinoplanes* spp. are known (*14*) to produce antibiotics, so antibiosis may play a role. However, little is known about the use of antibiotic-producing strains of *Actinoplanes* spp. in the biocontrol of *Pythium* spp., and further research is also warranted in this regard.

Other biocontrol agents, such as *Trichoderma harzianum (4)* or composted amendments (*1*) inhibit hyphal growth of *P. ultimum* in soil, but they have little effect against oospores of the pathogen. Biocontrol agents that effectively parasitize oospores in planting mix would reduce the survival of *P. ultimum* in soil and greenhouse root medium. Moreover, because the oospore is the sexual stage where genetic recombination occurs, reducing oospore populations may help maintain the efficacy of current anti-Pythium fungicides by reducing the frequency of new fungicide-resistant isolates (*15*). In addition, the combination of *Actinoplanes* spp. which are known parasites of oospores (*8-10*), with other biocontrol agents that inhibit hyphal growth may provide greater control than either agent used separately.

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