BIOLOGICAL CONTROL OF PHTHIACEOUS FUNGI CAUSING DEATH TO LONGLEAF PINE SEEDLINGS IN COLD STORAGE

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In some ways biological control, when defined as the control of disease by the introduction of a controlling organism, is the "Holy Grail" of plant pathology. Like the "Holy Grail" it usually eludes its pursuers. The difficulty is that plant diseases are serious only when environmental conditions heavily favor the pathogen; to effect biological control one must either select a control organisms which is also effective under those conditions, change conditions to favor the biological control organism, or overwhelm the pathogen system with initially high levels of the biological control organism. In other words, proper environmental parameters in combination with an effective organism are necessary for biological control to be effective. Under natural field conditions this combination can be attained only with difficulty and, once attained, difficult to maintain. The system we worked with is essentially a closed, stable ecosystem which is at once artificial and an industry norm.

Longleaf pine seed are planted in the nursery in March at a density of 25 ft^2 of which about 10-15 survive until lifting. They are lifted from the nursery during the following winter, placed in kraft paper bags in bundles of 1000, and stored in a cold room (3°C) until they can be outplanted. The seedlings can be subjected to storage times of up to six weeks prior to planting. This procedure has not been problematic for other southern pine species such as loblolly or slash pine, but longleaf tended not to survive even a few days of storage. Longleaf, while a generally highly desirable species, has not been used extensively in plantation establishment because of its reputation for being difficult to handle but interest in it has been growing during the last few years. We decided to look at the storage problem some years ago and quickly found indications that *Pythium* spp. were the principal cause of seedling mortality during storage. In initial studies investigating the cause of longleaf seedling mortality, *Pythium dimorphum* was found naturally occurring at high levels in seedling root systems (Jones et al. 1992). That was not the case in the work presented here due largely, we believe, to the subsequent use of fungicides in the nursery. It proved to be a fairly straightforward matter to control the problem with fungicides (Table 1). Since this system seemed to present a good opportunity for relatively exact control of seedling environment during storage, we also decided that it would be a good system for investigating biological control.

Trichoderma spp. could be statistically associated with seedling survival, so we decided to concentrate on that genus to find biological candidate organisms. We screened several thousand isolates, selecting the 280 that grew fastest at 10°C on Corn Meal Agar. These were then screened for their ability to kill or inhibit 14 randomly selected *Pythium* isolates. Twenty-one of these isolates killed all 14 *Pythium* and were screened against an additional 105 *Pythium* isolates; one *Gliocladium* virens and nine Trichoderma isolates were able to kill all 119 *Pythium* isolates. Nine *Pythium* isolates selected for their ability to cause lesions on four day old slash pine seedling roots, five randomly selected isolates, and four Trichoderma isolates were used for initial inoculation studies during 1993. The bare root seedlings were dipped in a clay slurry containing 108 mls/1 of wheat bran on which the test fungus had been incubated for 3-5 days and cold stored for 4 weeks. The seedling survival rate was significantly greater than zero for only one *Pythium* isolate, and significantly less

than control for only one *Trichoderma* isolate (Table 2). This confirmed the ability of *Pythium dimorphum* to kill longleaf seedlings under these conditions, but the *Trichoderma* spp. in general had no statistical effect on seedling survival. The results also indicated that the natural levels *of Pythium* present were quite low, so it was decided that coinoculation with P. *dimorphum* would be necessary to provide sufficient disease pressure to test for bio-control efficacy. A titration study was used to determine the level *of Pythium* inoculum necessary to cause approximately 70% mortality among the longleaf seedlings (Table 3), and in the 1994-95 growing season thirteen *Trichoderma* spp. and one *Gliocladium wrens* were used in a coinoculation study with *Pythium dimorphum* (Table 4).

We found that the manner of inoculation of the *Trichoderma/*wheat bran inoculum was critical to achievement of effective biological control. The best procedure was to mix the inoculum into the clay slurry and then dip the seedling roots into the slurry. Sprinkling the inoculum of *Trichoderma* onto roots after they had been dipped into a clay slurry occasionally resulted in slightly higher levels *of Trichoderma* recovery, but substantially higher rates of seedling mortality. In the experiment presented in Table 4, *Trichoderma* inoculum had been applied to the root systems one week prior to inoculation with P. *dimorphum.* We also applied another inoculum load of *Trichoderma* at the time of *Pythium* inoculation. The results clearly indicate that this procedure did result in useful control levels for those seedlings dipped in a clay slurry/inoculum mix.

The ability of *Trichoderma* spp. to effect biological control was not dependent upon species so much as the specific isolate. This is not surprising in that it is possible that morphologically le isolates may indeed be different species (Kuhls *et al.* 1997). In any case, survival rates ranged from 71% to 98% for the different isolates when seedling roots were dipped in the inoculum and from 43% to 88% when they were sprinkled with inoculum. The long term growth, as well as survival, *of* fungicide treated longleaf seedlings was improved significantly (Brissette *et al.* 1996). The long term effects of biological control measures have yet to be determined. Future research using this system should address refining timing and rates of *Trichoderma* inoculation In our work we basically saturated the system with an overload of *Trichoderma* to insure a degree *of* significant control. It might also be possible to inoculate the nursery beds with *Trichoderma* and effect control. It would also be desirable to establish a disease nursery so that natural pathogen levels could build up to test control regimes without necessitating adding of pathogen inoculum It is possible that the reported biological control was possible only because the pathogen was not established as it would be under natural conditions. The research reported here utilized seedling produced in only one nursery; in other nurseries it is possible that other genera or species of pathogen are of primary importance.

Literature Cited

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Table I . Outplanting survival rates of longleaf pine seedlings and isolation rates of *Pythium* and *Trichoderma* spp. after cold storage of up to six weeks[1].

	Survival (%)			Pythium levels[2]			*Trichoderma* levels[2]		
Treatment	0 wk[3]	3 wk	6 wk	0 wk	3 wk	6 wk	0 wk	3 wk	6 wk
Control	96a	72a	l8a	5a	31a	39a	57a	l8a	17a
Benlate	96a	92b	95b	2a	0b	0b	lb	lb	0b
Ridomil	98a	96b	97b	3a	2b	0b	39a	7a	26a

[1]Column values followed by differently letters are significantly different (p=0.05) according to Duncan's Multiple Range Test
[2]Isolation rates (0/9) from 3 replicates of 25 1 cm root segments/replicate
[3]Storage interval, in weeks

Table 2. *Trichoderma* and *Pythium* recovery rates from inoculated longleaf pine roots before and after cold storage and seedling survival after 4 weeks *of* cold storage in the 1993 field trial

	Isolation Rates[a]		Seedling Survival[b]
Isolate[c]	*Trichoderma*	*Pythium*	
P. dimorphum 155	57.8b[d]	68.9b	0.0c
P. dimorphum 150	8.9d	100a	0.0c
P. dimorphum 81	60.0b	95.6a	0.0c
Pythium spp. 115	ll.le	93.3a	0.0c
Pythium spp. 29	86.7a	100a	0.0c
P. dimorphum I 10	8.9d	100a	1.7c
Pythium spp. 146	31.1c	97.8a	0.0c
Pythium dimorphum 76	15.6cd	77.8b	3.4c
P. dimorphum I I 10	22.2cd	75.6b	0.0c
Pythium spp. 1173	55.5b	77.8b	43.4b
P. dimorphum 1122	57.8b	100a	0.0c
Trichoderma hamatum 3	100a	8.9d	80.0a
T. harzianum 2	100a	4.4d	51.7b
T. piluliferum	100a	2.2d	81.7a
T. hamatum I	97.8a	22.2c	85.0a
Control	17.7cd	22.2c	75.0a
Non-stored control			71.5a

[a]Percentage *of* recovery of the isolate from 25 one centimeter root pieces.
[b]Percentage *of* survivng seedlings/20 seedlings planted
[c]Wheat bran inoculum (108 mls/l) *of* each fungal isolate was delivered to roots in clay slurry.
[d]Column means followed by different letters are different at p=0.05 according to Duncan's Multiple Range Test

Table 3. Inoculum levels, recovery rates of Pythium dimorphum and seedling survival rates in 1994.

Inoculum[a]	PRR[b]	SSR[c]
6.8 ml/l Pythium dimorphum	73.3a[d]	28.0a
13.5 ml/l P. dimorphum	75.8a	25.0a
27.0 ml/l P. dimorphum	64.8ab	12.0b
54.0 ml/l P. dimorphum	61.1b	2.0c

[a]As wheat bran added to clay slurry
[b]as percentage recovered from 25 one centimeter root pieces/replicate
[c]Percent seedling survival from 20 seedlings/replicate
[d]Column mews followed by different letters are different at p=0.05 according to Duncan's Multiple Range Test

Table 4. Trichoderma and Pythium recovery and seedling survival of coinoculated seedlings after cold storage

Inoculant	Trichoderma Recovery Rate[a]		Pythium recovery rates[a]		Seedling Survival Rates[a]	
	Dipped[c]	Sprinkled[d]	Dipped	Sprinkled	Dipped	Sprinkled
T. hamatum I	77.8cd[e]	77.8d	0.0e	0.0c	88.5abc	66.5bcdefg
T. hamatum 3	71.1de	15.6f	0.0e	6.7bc	91.5ab	43.3gh
T. hamatum 4	84.4bc	82.2cd	6.7de	0.0c	88.5abc	81.5abc
T. hamatum 7	51.1f	86.7bcd	33.3b	11.1bc	71.5d	45.0fgh
T. viride 5	97.8a,	100.0a	33.3b	2.2c	93.Oab	60.0cdefg
T. koningii 6	77.8cd	57.7e	8.9de	11.1bc	91.5ab	61.5cdefg
T. pseudokoningii	97.8a.	100.0a	8.9de	4.4bc	91.5ab	76.5abcd
G. Wrens 20	93.3ab	97.8ab	15.5cd	0.0c	96.5a.	71.5bcde
T. harzianum 2	97.8a	97.8ab	8.9de	4.4bc	98.6a	51.5efg
T. harzianum 9	100.0a.	100.0a	6.7de	6.7bc	88.5abc	70.0bcde
T. harzianum I I	100.0a	100.0a	6.7de	2.2c	83.5bc	75.0abcde
T. harzianum 12	100.0a	100.0a	11.1cde	6.7bc	91.5ab	88.5ab
T. piluliferum 15	100.0a	100.0a	22.bc	11.1bc	91.5ab	78.0abc
T. piluliferum 19	100.0a	97.8ab	2.2e	8.8bc	76.5cd	88.5ab
P. dimorphum control	6.7g	2.2g	73.3a.	88.9bc	28.0e	28.0h
Sterile Wheat Bran	64.4a	2.2g	15.6cd	8.9bc	98.0a.	96.5a

[a]As percent recovered from 25 one centimeter root pieces/replicate
[b]Percent seedling survival from 20 seedlings/replicate
[c]Seedlings dipped in 108 mls wheat bran-inoculant/l clay slurry, stored one week then dipped in 6.8mI/l Pythium wheat bran inoculant/l clay slurry plus another 108 mls wheat bran-inoculant/l clay slurry
[d]Seedlings dipped in clay slurry, then sprinkled with 400 mls wheat bran-inoculant, stored one week then dipped in 6.8 ml Pythium-wheat bran/l clay slurry and sprinkled again with 400 mls of *wheat bran-inoculant.*
[e]Column means followed by different letters are different at p=0.05 according to Duncan's Multiple Range Test