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Efficacy of Chemical and Biological Agents to Suppress *Fusarium* and *Pythium* Damping-Off of Container-Grown Douglas-fir Seedlings

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Abstract

Douglas-fir seedlings are susceptible to *Fusarium* or *Pythium* damping-off that currently is controlled by pre-plant soil fumigation in bareroot nurseries and steam pasteurization or chemical drenches of soilless media in container nurseries. However, because few chemical or biological agents are registered for use on conifer seedlings, we tested several on greenhouse-grown seedlings and found that over-seed applications of Cleary's 3336, Strike, Compass, Compass + Strike, Cygnus, Endura, Medallion, Medallion + Strike, Thiram, and Enzone effectively suppressed pre-emergence damping-off by *Fusarium oxysporum*, but only Cleary's 3336WP and Medallion + Strike also reduced post-emergence damping-off. Compass, Medallion, and Thiram reduced post-emergence damping-off, but not to a statistically significant level. Pre-emergence damping-off by *Pythium irregulare* was reduced only by Ranman, but post-emergence damping-off was reduced by Thiram, Hurricane, Ranman, and Subdue MAXX. Over-seed drenches of biological control agents Companion, Kodiak, Subtilex, Taegro, Primastop, SoilGard, Actinovate, Mycostop, RootShield, and Green-Releaf were ineffective in suppressing either *Fusarium* or *Pythium* diseases, and combining several agents with chemicals did not improve efficacy. These results provide data in support of registration of some of the effective chemicals, but no biological control agents, for the control of conifer seedling damping-off.

Introduction

Seedlings of Douglas-fir [*Pseudotsuga menziesii* (Mirb.)] and other conifer species are susceptible to damping-off and root rot caused by species of *Fusarium* or *Pythium* (2). These diseases can occur on seedlings grown in either containers or in-ground beds of bareroot nurseries, and can be manifested as pre-emergence or post-emergence damping-off, or as stunted seedlings that die due to root rot. Control of these diseases in bareroot nurseries has been primarily by pre-plant fumigation, although *Fusarium* spp. may also be seedborne (3) and thus would be unaffected by soil fumigation. Seed treatments to remove fusaria on seed (1) have been employed by some nurseries using running water or treating seed with chemicals such as sodium hypochlorite (bleach), hydrogen peroxide, or Thiram. Seed treatment with hydrogen peroxide (4) alone effectively removes surface seedborne fusaria, but following that treatment with a bacterial antagonist failed to provide protection against *Fusarium* infection and disease in pathogen-infested medium. However, application of other candidate biocontrol agents could prove this practice useful. Hydrogen peroxide can also cause problems with reduced seed germination and/or phytotoxicity to young germinants.

Recently, some new chemicals and biological control agents (BCAs) have been developed commercially on other crops that could be used to reduce losses from *Fusarium* or *Pythium* infections on conifer seedlings. Several of these agents were tested comparatively in this study as over-seed drench applications.

Preparation of Inoculum and Inoculation Procedure

Fungal inoculum was prepared for all studies using vermiculite infested with *Fusarium oxysporum* Schlect. or *Pythium irregulare* Buisman. Both fungi were shown to be pathogenic on Douglas-fir in preliminary tests. *F. oxysporum* was first grown on acidified potato dextrose agar (25 ml/liter lactic acid), and *P. irregulare* was grown on dilute V8 Juice agar (50 ml/liter clarified V8 juice) in Petri dishes for 14 days at 20°C. Inoculum was prepared by adding clarified V8 broth (7) to dry vermiculite (70% v:v) contained in a 52.5 × 20 × 11.88-cm autoclavable polyethylene bag (12468.75 cm^3), with a contaminant barrier filter patch (Fungi Perfecti, Olympia, WA). Bags were then autoclaved (120°C, 15 psi, 60 min) twice with an overnight cooling period between treatments. Mycelium on agar from two Petri dishes of a desired isolate were cut into approximate 1.5-cm squares and transferred aseptically from 14-day-old culture plates to each autoclaved bag. These were stored in a dark incubator at 20°C for 2 months, with periodic redistribution of contents. Prior to incorporation of vermiculite inoculum into the seedling potting mix, the inoculum was placed in cheesecloth and washed with water to remove excess nutrients and culture metabolites, air dried for 48 h to a moisture level suitable for easy mixing, and tested for viability by plating on selective media.

The Douglas-fir soilless seedling mix was a steam-pasteurized, pathogen-free proprietary blend of peatmoss, perlite, and starter fertilizers, supplied by The Weyerhaeuser Company (Rochester, WA). *Fusarium* and *Pythium* vermiculite inocula were incorporated into the seedling mix at an average rate of 10% by volume. Douglas-fir seedlings were grown in plastic trays designed specifically for seed germination and seedling growth in Weyerhaeuser nurseries. Two days before treatment, a total volume of 2.95 dm^3 of each flat, containing 64 planting cells, was filled with pathogen-infested soilless mix and moistened by misting until gravitational drainage was apparent. One cold-stratified, untreated seed was sown at 0.32-cm depth in each cell. Chemical or biological products were manually applied as drenches just after seeding, following product label or product specialist's recommendations. Specific treatments and replications are designated within each study. All studies were arranged on greenhouse benches in a randomized block design, with *Fusarium* and *Pythium* trays maintained on separate benches to prevent cross-contamination.

Fig. 1. Greenhouse-grown Douglas-fir seedlings in plug trays inoculated (right) or not with *Fusarium oxysporum*, showing pre-emergence damping-off reaction.

Fig. 2. Greenhouse-grown Douglas-fir seedlings, one in center showing post-emergence mortality from infection by *Fusarium oxysporum*.

Greenhouse temperatures were 23/18°C day/night, and after seedlings had germinated lighting was supplemented by high-pressure multi-vapor lamps for 14-h daylengths during November to April. Water was applied as needed, usually thrice-weekly, using a low-pressure sprinkler nozzle. Any control flats without pathogens were removed from the bench set-up and watered apart from the pathogen-infested flats to avoid cross contamination.

Disease Evaluation and Statistical Analysis

Germination was counted three weeks after seeding, and survival was counted weekly for the next six weeks. Then ten seedlings were removed from each treatment replication to assess pathogen recovery from roots. Roots were washed clean of growth medium debris, severed from shoots, and cut into approximate 1-cm segments. Segments were surface-disinfested in 0.03% sodium hypochlorite solution for 3.0 min, followed by immersion in sterile distilled water for 0.5 min. Ten randomly chosen segments were retrieved with forceps, blotted dry on paper towels, and plated on PARP medium (5) for *Pythium* isolation, or Komada's medium (6) for *Fusarium* isolation. Plates were incubated in the dark for 5 to 7 days, after which infected root pieces were counted. The percentage of infected segments was calculated separately for each replicate sample.

At the end of six weeks, ten more seedlings were removed for biomass determination. Seedlings were gently washed free of growth medium debris, roots severed from shoots, and roots and shoots dried at 70°C for 48 h and weighed.

Data were analyzed separately for each experiment. Arcsine-transformed seedling survival data and log-transformed biomass data were analyzed by analysis of variance using Systat 8.0 (SPSS Inc., Evanston, IL). Where appropriate, Fisher's protected least significance test at $P \leq 0.05$ ($FPLSD_{0.05}$) were used to separate treatment means. Untransformed data are presented in all tables.

Chemical and Biological Control Agents, and Method of Application

The chemicals and BCAs we evaluated in 2002-2003, and rates used are shown in Tables 1 and 2, respectively. All were applied as over-seed drenches immediately after sowing seeds in flats, except for Thiram, which was applied as a seed-coat treatment four hours prior to sowing. Reapplication of chemicals followed label or product specialist recommendations, usually at two-week or four-week intervals. All BCAs were reapplied at two-week intervals. Non-chemical control treatments, with and without pathogens, were also included.

Table 1. Chemical products evaluated to determine efficacy in controlling Fusarium or Pythium damping-off of greenhouse-grown Douglas-fir seedlings.[x]

Treatment product	**Product description**	**Rate of product**[y]
3336WP	50% thiophanate-methyl Cleary Chemical Corp, Dayton, NJ	900 µg
Banrot 40%WP	15% etridiozole, 25% thiophanate methyl Scott-Sierra Crop Protection, Marysville, OH	600 µg
BAS 516-04 38WG	Proprietary information BASF Corp., Research Park Triangle, NC	1900 µg
Biophos (Lexx-a-phos)	22.7% di-potassium phosphate, 22.4% di-potassium phosphonate Foliar Nutrients Inc., Cairo, GA	10 µl
Compass	50% trifloxystrobin Bayer Environmental Science, Montvale, NJ	75 µg
Cygnus 50WG	50% kresoxim-methyl BASF Corp., Research Park Triangle, NC	2.4 µl
Endura	Proprietary information BASF Corp., Research Park Triangle, NC	600 µg
Enzone	31.5% sodium tetrathiocarbonate Entek Corp., Elkridge, MD	3.7 µl
Heritage	50% azoxystrobin Syngenta Crop Protection, Greensboro, NC	100 µl
Hurricane	32% fludioxonil, 16% mefenoxam Syngenta Crop Protection, Greensboro, NC	300 µg
Insignia	20% pyraclostrobin BASF Corp., Research Park Triangle, NC	2400 µg
Medallion	50% fludioxonil Syngenta Crop Protection, Greensboro, NC	0.2 µl
Ranman	40% cyazofamid ISK Biosciences, Mentor, OH	0.5 µl
Strike 25WDG	25% triadimefon Olympic Horticultural Products, Mainland, PA	300 µg
Subdue MAXX	22% mefanoxam Syngenta Crop Protection, Greensboro, NC	0.16 µl
Thiram (42-S)	42% tetramethylthiuram disulfide Gustafson LLC, Plano TX	0.024 µg

[x] All products applied as drenches directly after seed-sowing, except Thiram, applied as a seed-coating four hours before sowing. All products applied according to label or product specialist-recommended rates.

[y] Rate of product per ml of solution except for Thiram seed coat (= per gm of seed)

Table 2. Biological products evaluated to determine efficacy in controlling Fusarium or Pythium damping-off of greenhouse-grown Douglas-fir seedlings.[x]

Treatment product	Product description	Rate of product[y]
Actinovate	1% *Streptomyces lydicus* WYEC-108 Natural Industries Inc., Houston, TX	450 µg
Companion	0.03% *Bacillus subtilis* GB-03 Growth Products Ltd., White Plains, NY	1.25 µl
Kodiak	1.37% *Bacillus subtilis* GB-03 Gustafson LLC, Plano TX	1.25 µl
Green-Releaf	0.14% *Bacillis licheniformis* SB-3086 Novozymes Biologicals, Salem, VA	2.8 µl
MycoStop	30% *Streptomyces grisiovirides* K-61 Verdera oy, Fin-02201, Espoo, Finland	300 µg
PrimaStop	37% *Gliocladium catenulatum* J-1446 Verdera oy, Fin-02201, Espoo, Finland	5000 µg
RootShield	*Trichoderma harzianum* T-22 (KRL-AG2) BioWorks Inc., Geneva, NY	375 µg
Soilgard 12G	12% *Gliocladium virens* G-L21 Certis USA LLC, Columbia, MD	2400 µg
Subtilex	2.75% *Bacillus subtilis* MBI-600 Becker Underwood Inc., Ames, IA	600 µg
Taegro	24.5% *Bacillus subtilis* FZB-24 Taensa Inc., Fairfield, CT	200 µg

[x] All products applied as drenches directly after seed-sowing, according to label or product specialist-recommended rates.

[y] Rate of product per ml of solution.

Another experiment in 2004, combining selected chemical products and BCAs, was conducted in late 2004. Three commercial BCAs previously tested — Actinovate, Taegro, and Subtilex — were combined into one treatment (BA1). Chemical products included Endura, BAS 516, Cygnus, Cleary 3336, Heritage (each reapplied every two weeks), Insignia, Hurricane, Ranman (each reapplied every four weeks), and Subdue MAXX (no reapplication). Each of these was tested with and without an application of BA1. The BA1 treatment was prepared by mixing Actinovate, Taegro, and Subtilex into distilled water at label rates, and allowing the mixture to stand 12 h before use.

Since the application of Hurricane had shown some signs of phytotoxicity when both the chemical and pathogen were present, we did another experiment comparing treatments with the chemical alone with the chemical plus *Pythium*. In both cases, Hurricane was applied at 0, 1×, 2×, and 4× the recommended label rate, both over the seed at planting or after 4 weeks of seedling growth.

Efficacy of Chemical and Biological Treatments

The results of a series of experiments using chemical and biological agents to control *Fusarium* or *Pythium* damping-off are shown in Tables 3, 4, and 5. In 2002 (Table 3), both pre- and post-emergent damping-off by *Fusarium* were significantly reduced by chemical applications; seedling total biomass after 10 weeks was not significantly improved. Post-emergence *Pythium* damping-off was effectively controlled by Hurricane, Thiram, Ranman, and Subdue MAXX at 6 weeks. The *Pythium*-inoculated seedlings were not significantly smaller than non-treated, non-inoculated controls, although the pathogen was recoverable. *F. oxysporum* was recovered from 100% of the roots of all inoculated treatments at harvest time, while *P. irregulare* recovery was reduced to near-zero by Hurricane, Ranman, and Subdue MAXX.

Table 3. Effects of chemical application on survival and biomass of Douglas-fir seedlings grown in *Fusarium*- or *Pythium*-infested soilless mix (2002).

Treatment/ product	*Fusarium*			*Pythium*		
	Total dry wt (mg)[x]	**Survival** (%)		**Total dry wt** (mg)	**Survival** (%)	
		3 wks	6 wks		3 wks	6 wks
None minus pathogen	107 a[y]	89 ab	86 a	108 a	93 a	93 a
None plus pathogen	53 b	48 c	18 g	89 ab	88 ab	74 d
3336WP	94 ab	91 ab	86 a	na -	na -	na -
Strike	76 ab	81 b	38 f	na -	na -	na -
Compass	80 ab	89 ab	77 bc	na -	na -	na -
Compass + Strike	82 ab	88 ab	60 d	na -	na -	na -
Cygnus	94 ab	87 ab	56 d	na -	na -	na -
Endura	87 ab	88 ab	56 d	na -	na -	na -
Medallion	90 ab	92 ab	72 c	115 a	79 bc	79 cd
Medallion + Strike	80 ab	95 a	80 ab	na -	na -	na
Thiram	63 ab	82 b	78 b	72 abc	74 c	85 abc
Enzone	59 b	88 ab	46 e	92 ab	84 bc	79 cd
Heritage	na[z] -	na -	na -	31 c	32 d	33 e
Hurricane	na -	na -	na -	60 bc	89 ab	91 ab
Ranman	na -	na -	na -	108 a	93 a	84 bc
Subdue MAXX	na -	na -	na -	94 ab	88 ab	86 abc

[x] Biomass data given as average mg per plant seedling survival as percent.

[y] Mean separation within a column using $FPLSD_{0.05}$. Treatments sharing the same letters are not significantly different. Each value is the average of four replicate blocks with 10 seedlings in each block.

[z] na = product not applied; em-dash indicates inadequate quantity survived for biomass determination no analyses.

Table 4. Effects of biological and chemical agents on survival and biomass of Douglas-fir seedlings grown in *Fusarium*- or *Pythium*-infested soilless mix (2003).

Treatment/ product	*Fusarium*			*Pythium*		
	Total dry wt (mg)[x]	**Survival** (%)		**Total dry wt** (mg)	**Survival** (%)	
		3 wks	6 wks		3 wks	6 wks
None minus pathogen	124 a[y]	89 a	91 a	129 ab	93 a	90 a
None plus pathogen	103 ab	65 b-e	61 cde	105 ab	81 abc	77 ab
Companion	79 a-d	63 b-e	54 def	110 ab	75 bcd	72 bcd
Kodiak	69 bcd	61 b-e	49 ef	119 ab	80 bc	79 ab
Subtilex	86 a-d	59 cde	64 cde	119 ab	78 bc	72 bcd
Taegro	68 bcd	55 de	50 def	110 ab	81 abc	77 ab
Primastop	90 abc	59 cde	57 def	130 a	70 cd	68 cde
SoilGard	84 a-d	73 a-d	67 bc	92a bc	51 e	48 f
Actinovate	65 bcd	50 e	43 f	85 abc	71 cd	63 cde
Mycostop	95 ab	68 b-e	65 bc	104 ab	71 cd	63 cde
RootShield	75 a-d	66 b-e	60 c-f	109 ab	79 cd	74 bc
Green-Releaf	48 cd	21 f	26 g	50 cd	36 f	34 g
3336WP	97 ab	60 ab	78 ab	na -	na -	na -
Insignia	41 d	49 e	47 ef	36 d	36 g	33 g
Endura	122 a	63 b-e	63 cde	na -	na -	na -
Cygnus	85 a-d	75 abc	75 ab	na -	na -	na -
Biophos	94 ab	63 b-e	63 cde	127 ab	85a b	82 ab
Banrot	100 ab	66 b-e	63 cde	104 ab	80 bc	77 ab
Hurricane	na[z]-	na -	na -	84 bc	63 de	59 de
Ranman	na -	na -	na -	106 ab	75 bcd	66 cde
Subdue MAXX	na -	na -	na -	90 abc	63 de	58 ef

[x] Biomass data given as average mg per plant seedling survival as percent.

[y] Mean separation within a column using $FPLSD_{0.05}$. Treatments sharing the same letters are not significantly different. Each value is the average of four replicate blocks with 10 seedlings in each block.

[z] na = product not applied; no analyses.

Table 5. Effects of chemical agents applied alone or in combination with a bacterial antagonist mixture (BA1) on survival and biomass of Douglas-fir seedlings grown in *Fusarium*- or *Pythium*-infested soilless mix (2004).

Treatment / product		*Fusarium*			*Pythium*		
		Total dry wt (mg)[w]	Survival (%)		Total dry wt (mg)	Survival (%)	
			3 wks	6 wks		3 wks	6 wks
Chemical only	None minus pathogen	90 a[x]	88 a	87 a	66 a	86 a	78 a
	None plus pathogen	88 a	72 c	71 c	65 a	54 c	43 c
	3336WP	90 a	83 ab	79 b	na –	–	–
	BAS 516-04F	—[y]	53 d	47 d	na –	–	–
	Endura	82 a	81 b	79 b	na –	–	–
	Insignia	na[z] –	na –	na –	— –	46 d	38 d
	Hurricane	na –	na –	na –	56 a	53 c	60 b
	Ranman	na –	na –	na –	74 a	54 c	60 b
	Subdue MAXX	na –	na –	na –	56 a	60 b	60 b
Chemical plus BA1	None minus pathogen	81 A	92 A	88 A	70 A	78 A	79 A
	None plus pathogen	65 A	73 B	69 C	65 A	49 C	39 C
	3336WP	89 A	83 E	81 B	na –	na –	na –
	BAS 516-04F	— –	48 C	28 D	na –	na –	na –
	Endura	75 A	79 B	75 I	na –	na –	na –
	Insignia	na –	na –	na –	— –	36 C	28 D
	Hurricane	na –	na –	na –	63 A	41 B	50 B
	Ranman	na –	na –	na –	70 A	40 B	52 B
	Subdue MAXX	na –	na –	na –	65 A	54 B	51 B

[w] Biomass given as average mg per plant; seedling survival as percent.

[x] Mean separation within a column using $FPLSD_{0.05}$. Treatments sharing the same letters are not significantly different. Each value is the average of four replicate blocks with 10 seedlings in each block.

[y] "—" indicates too few seedlings survived for biomass determination; no analyses.

[z] na = product not applied; no analyses.

In 2003 (Table 4), *Fusarium* significantly reduced survival of non-treated seedlings, although total biomass was not reduced. Pre-emergent damping off was not effectively controlled by chemical application, but post-emergent attack was reduced by Cleary's 3336WP and Cygnus, while being increased by Actinovate and Green-Releaf. Total seedling biomass was significantly reduced (FPLSD ≤ 0.05) by Green-Releaf and Insignia. Inoculation with *P. irregulare* did not effectively reduce seedling survival, for which we have no explanation, since the pathogen grew out of all inoculum pieces prior to the test. Within this constraint, however, we found a reduction in seedling survival with several chemical treatments. Again, total biomass was significantly reduced by Green-Releaf and Insignia. *F. oxysporum* was recovered from 95 to 100% of the roots of all inoculated treatments at harvest time, and *P. irregulare* was recovered from 13 to 100% of all roots, the lowest being from the Ranman treatment.

Both *Fusarium* and *Pythium* induced significant damping-off in our 2004 study, with or without the presence of BCAs (Table 5). Without BCAs, pre- and post-emergent *Fusarium* damping-off was decreased by Cleary 3336WP, whereas Endura was effective only at pre-emergence. BAS 516 significantly (FPLSD ≤ 0.05) worsened survival, which may have been a phytotoxic effect. *Pythium* damping-off was effectively reduced by Subdue MAXX. Adding BCAs to chemical and non-chemical treatments had no significant effect ($P = 0.05$) on reducing damping-off effects for either pathogen, nor on seedling total biomass.

In the presence of *Fusarium*, Cleary 3336WP again improved seedling survival, while BAS 516 worsened it. In the presence of *Pythium*, Hurricane, Ranman, and Subdue MAXX improved survival. Recovery of the pathogens at harvest was not affected by BCAs, which ranged from 97 to 100% for *F. oxysporum* and 60 to 100% for *P. irregulare*.

Hurricane by itself was not phytotoxic in terms of germination and final stand (Table 6), but there was a significant reduction (FPLSD ≤ 0.05) in total growth at harvest at the 4× rate when the chemical was applied over the seed. No toxicity was detected at any rate when it was applied as a drench over 4-week-old seedlings. Main effects of Hurricane rate, time of application, and *Pythium* inoculation were not significant ($P \leq 0.05$) for germination or survival of seedlings. However, all three factors had very highly signficant effects ($P \leq 0.001$) on growth at harvest, as did their interactions, except for the insignificance ($P \leq 0.05$) of application time × *Pythium* inoculation. In the presence of *Pythium*, however, there was significant (FPLSD ≤ 0.05) growth reduction with Hurricane at 2× and 4× rates with seed application only. These data would suggest that some interaction between Hurricane and *Pythium* occurred, but the nature of that interaction was not explored further.

Table 6. Effects of Hurricane on survival and biomass of Douglas-fir seedlings grown in *Pythium*-infested soilless mix.

Pythium treatment	Hurricane rate	Seed application			Seedling application		
		Total dry wt (mg)[x]	Survival (%)		Total dry wt (mg)	Survival (%)	
			3 wks	6 wks		3 wks	6 wks
None	None	121a[y]	84a	87a	118b	83a	86a
	1x	112a	77a	77a	126ab	86a	86a
	2x	116a	84a	86a	132ab	80a	83a
	4x	88b	87a	84a	129a	86a	86a
Plus	None	105A	87A	87A	107B	88A	90A
	1x	100A	78A	81A	122A	78A	79A
	2x	81B	88A	88A	106B	79A	80A
	4x	87B	82A	84A	104B	81A	81A

[x] Biomass given as average mg per plant; seedling survival as percent.

[y] Mean separation within a column using $FPLSD_{0.05}$. Treatments sharing the same letters are not significantly different. Each value is the average of four replicate blocks with 10 seedlings in each block.

Discussion

A number of chemical agents significantly reduced pre-emergence damping-off (evaluated at 3 weeks after germination) by both pathogens, but were sometimes less effective in reducing post-emergence damping off (evaluated at 6 weeks after germination). The results of over-seed applications of BCAs (Table 4) indicated that none of the agents alone was effective in reducing either *Fusarium* or *Pythium* damping-off. Green-Releaf and Insignia significantly reduced germination and survival as well as biomass of resulting seedlings. Combining chemical treatments with selected BCAs (BA1) did not improve efficacy of the chemical treatments (Table 5). We presume that the lack of efficacy from the BCAs was due to low or slow metabolic activity by the agents in relation to the time and extent of pathogen activity. In results from other unpublished experiments, we attempted to stimulate bacterial growth and activity by adding alfalfa meal extract with the agents, but the additional organic matter enhanced disease by both *F. oxysporum* and *P. irregulare* and not the activity of the BCAs. Perhaps soaking the seed in the BCAs would have improved their efficacy.

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