Preliminary Evaluation of Fungicides for Control of Damping-Off Disease in Container Grown Red Pine Seedlings[1]

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INTRODUCTION

Historically the fungicide benomyl has been widely used by greenhouse managers to control damping- off diseases in container grown conifer seedlings. In 1991, product labeling for benomyl underwent major changes and all greenhouse and ornamental uses were eliminated. Greenhouse managers, no longer able to use benomyl, were faced with the immediate need to identify effective alternative fungicides. This study was undertaken to investigate the effectiveness of several alternative fungicides in controlling damping-off diseases in red pine seedlings, grown under operational greenhouse conditions and naturally occurring disease pressure. The decision to design this study using operational greenhouse conditions and naturally occurring disease pressure was made in response to the immediate need to identify alternative fungicides and transfer this information quickly to greenhouse managers, and the lack of pathological information relating to disease development. Baseline data regarding the species of pathogenic fungi occurring on red pine, and specific disease inoculum levels that incite disease symptoms are unknown, and the determination of such data would have required many months of laboratory and greenhouse testing. Seedlings were grown using cultural practices consistent with common greenhouse operations including the reuse of surface disinfected styroblocks and the sowing of seed which received no surface washing or fungicide seed treatment. The seedlings were grown under conditions of naturally occurring disease pressure and were not artificially inoculated with pathogenic fungi. It was speculated that disease inoculum would be present in cracks and crevices of reused styroblocks and in/on seeds. Damping-off and root rot disease did develop, and disease pressure was sufficient to cause a significant reduction in seedling quality in the untreated control treatment when compared to the fungicide treatments.

The fungicides tested in this study were selected based on their availability for purchase and their efficacy against fungi that commonly incite damping-off disease in container grown conifer seedlings. For example, Banrot has demonstrated efficacy against damping-off and root and stem rot diseases caused by *Pythium, Phytophthora, Fusarium, Rhizoctonia,* and *Thielaviopsis* in ornamental and nursery crops. Cleary's 3336, containing the active ingredient thiophanate-methyl, breaks down to the same active ingredient as benomyl and has activity against species of *Fusarium* and *Rhizoctonia* spp.

MATERIALS AND METHODS

Red pine seeds (lot# 0118-2), collected from seed collection stands in the Chequamegon

National Forest, were used in this study. Seeds were sown in 240 cell styroblocks, with a soil volume of 2.3 cubic inches per cell. Seedlings were maintained in the greenhouse for 4 months and fertilized twice weekly with Peter's Starter (9-45-15) during the first month after germination and weekly thereafter with Peter's Finisher (4-25-35). Fungicide treatments were applied at sowing and at 1 month intervals for a total of 4 applications per treatment. Treatments included: 1) Banrot (8 oz./100 gal.), 2) Cleary 3336 (1.5 lb./200 gal.) and 3) untreated control. Treatments were arranged in a randomized block design, with 3 replications and 240 seedlings per replication.

Seedlings were examined at 2-week intervals to determine seedling survival counts, and all seedlings exhibiting symptoms of damping-off were collected and cultured to determine the presence of root infecting fungi. Four months after planting, seedling height, caliper, and stem and root dry weights were determined on 20 randomly selected seedlings per replication, for a total of 60 seedlings per treatment. Data were analyzed using the statistical program Statistix. Analyses of variance were performed and treatment comparisons were made using the HSD test ($p = 0.05$).

RESULTS

Although no significant differences were observed in seedling survival between treatments (Table 1), significant differences were observed between treatments for several seedling quality parameters measured including seedling height, caliper, stem dry weights and root dry weights (Table 2).

Table 1. Fungicide effectiveness of Banrot and Cleary's 3336, applied as soil drenches, on mean seedling survival of container grown red pine seedlings.

Treatments	Mean Seedling Survival
Banrot 40 WP	238.0 a[1]
Cleary's 3336 50 WP	238.3 a
Untreated Control	230.3 a

[1]means followed by the same letter do not differ significantly (HSD, p=0.05)

Table 2. Fungicide effectiveness of Banrot and Cleary's 3336, applied as soil drenches, on several seedling quality parameters of container grown red pine seedlings.

Treatments	Mean Seedling Height[1]	Mean Stem Caliper[2]	Mean Root Dry Weights[3]	Mean Stem Dry Weights[3]
Banrot 40 WP	8.06 a[4]	1.00 a	0.37 a	1.40 a
Cleary's 3336 50 WP	7.74 a	1.02 a	0.43 a	1.30 a
Untreated Control	5.88 b	0.81 b	0.27 b	0.53 b

[1]measured in cm, [2]measured in mm, [3]measured in grams, [4] means followed by the same letter do not differ significantly (HSD, p=0.0)

All fungicide treatments resulted in significantly taller seedlings than the untreated control treatment. All fungicide treatments resulted in significantly increased stem caliper measurements than the untreated control treatment. All fungicide treatments resulted in significantly increased stem and root dry weights than the untreated control treatment.

Fungi associated with symptomatic seedlings were recorded (Table 3). A total of 38 seedlings exhibited symptoms of damping-off during the 4 month duration of the study, across all treatments. All symptomatic seedlings were cultured and *Pythium* sp. and *Fusarium* sp. were the most frequently isolated fungi. *Pythium* sp. and *Fusarium* sp. were isolated from 82% and 26% of the total number of symptomatic seedlings cultured, respectively.

Table 3. Number of symptomatic seedlings, by treatment, that yielded Pythium and Fusarium sp. in culture.

Treatment	Pythium sp.	Fusarium sp.	Total Number of Symptomatic Seedlings
Banrot 40 WP	4	0	6
Cleary's 3336 50 WP	3	0	3
Untreated Control	24	10	29
Total # of Seedlings	31	10	38
% of Total Seedlings	82	26	100

DISCUSSION

Banrot 40 WP and Cleary's 3336 50 WP, applied as soil drenches at monthly intervals, were effective in producing significantly higher quality seedlings than the untreated control treatment, when seedlings were grown under operational greenhouse conditions and naturally occurring disease pressure. It should be noted that the fertilization and irrigation schedules implemented in this study were designed to promote seedling health and the avoidance of over or under watering.

The fact that seedlings were not challenged with a specific amount of disease inoculum, and disease inoculum levels could vary between individual styroblocks, prevents us from drawing conclusions regarding fungicide efficacy and specific inoculum densities levels. Further studies should be conducted that better control the variability in disease inoculum levels. However, before this next phase of testing can be accomplished, the pathogenic species of Pythium and Fusarium must be identified, and inoculum densities that incite disease must be determined. Care must be taken to determine inoculum densities that result in disease incidence representative of those encountered under operational greenhouse conditions, but that do not overload the system.

SUMMARY

This study provides information on the fungi which contribute to root disease of red pine seedlings growing under operational greenhouse conditions and preliminary efficacy data for two soil-applied fungicides in controlling damping-off disease. Successful management of damping-off diseases in container grown conifer seedlings requires an integration of control strategies that include proper cultural practices and the use of fungicides. Cultural practices must be implemented that reduce the presence of disease inoculum and promote an environment conducive to seedling health. Following is an example of an integrated approach to the management of damping-off diseases in container grown conifer seedlings:

1. Clean and disinfect styroblocks/containers.

- Rinse containers with a high pressure water wash (100 psi) to physically remove planting media, roots, algae and other debris.
- Disinfect containers using a proven method:
  - bleach — 0.5% solution for 10 seconds, buffered to pH 7.0
  - steam — 95^{o}C for 1 minute
  - hot water — 80 ^{o}C for 10 seconds
  - heated soap — 5% solution at 80^{o}C for 10 seconds
  - hydrogen peroxide — 10% solution for 10 seconds
  - sodium metabisulphite — 55% solution for 10 seconds

2. Cleanse seed.

For all of the following methods, place seed in mesh bags that have twice the volume of the bulk seed to ensure adequate movement and agitation of the seed.

- running water	48 hours, with aeration
- bleach	40% solution for 10 minutes. For use only on thick-coated seeds such as pine and Douglas fir. Do not use on seed of larch, spruce, and true firs.
- hydrogen peroxide	3% solution for 3-5 hours, followed by a 48 hour running water rinse.
- ethanol	Not well tested and may inhibit seed germination. 95% solution for 15 seconds was ok on Douglas fir seeds, however, many other species have not been tested.

3. **Implement a fertilization regime that promotes overall seedling health and desired shoot-to-root ratios.**

4. **Implement an irrigation schedule that avoids extremes in soil moisture levels.**

5. **Apply fungicides at sowing and at one month intervals, as needed.**
   - Sowing: use broad spectrum fungicides that have activity against Pythium, Phytophthora, Fusarium, and Rhizoctonia spp. Examples include Banrot G and Banrot WP.
   - Monthly applications: rotate with fungicides that have activity against Fusarium and Rhizoctonia spp. Examples include fungicides that contain thiophanate-methyl such as Cleary's 3336, Fungo, and Domain.

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