

# Report 83-6

EVALUATION OF FUNGICIDES TO CONTROL BOTRYTIS BLIGHT IN WESTERN LARCH SEEDBEDS AT THE COEUR D'ALENE NURSERY, IDAHO

by

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## ABSTRACT

Six fungicides were evaluated to control Botrytis blight of western larch in seedbeds at the Coeur d'Alene Nursery. Fungicides were applied at biweekly intervals during the spring of 1982; test seedlings were inoculated with spores of <u>B</u>. <u>cinerea</u> twice during the evaluation period. Seedling mortality and height were recorded in October just prior to lifting. Because of overall low infection levels, no differences in seedling mortality were apparent as a result of fungicide treatment. Low infection was likely due to drier spring weather than when outbreaks of the disease previously occurred. Most fungicides decreased seedling height, although no external indications of phytotoxicity were apparent. Several of the fungicides tested can be considered for use to control Botrytis blight in larch seedbeds.

### INTRODUCTION

Western larch (Larix occidentalis Nutt.) is an important and highly desirable conifer species in the Northern Region. Demand for larch seedlings for outplanting increases each year. Nursery seedling production is often limited by seed availability and other problems such as diseases.

One of the serious diseases of larch in nurseries is blight caused by <u>Botrytis cinerea</u> (Fr.) Pers. This pathogen causes a foliage and twig blight that may result in extensive seedling mortality. Crops at the Coeur d'Alene Nursery in both greenhouses (James et al. 1982) and seedbeds

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(James 1980) are often affected by the disease. Western larch is especially susceptible to Botrytis blight because of the abundance of dead foliage it produces annually. The pathogen readily colonizes this necrotic tissue, and then may spread to adjacent healthy tissues, causing patches of seedling mortality (Jarvis 1980). Damage is often severe under cool, wet conditions, especially when seedling canopies are full and air circulation among plants is reduced (Blakeman 1980; Smith et al. 1973).

Botrytis blight is often controlled in greenhouses by applying fungicides periodically through overhead watering systems (Gillman and James 1980; McCain 1978; McCain and Smith 1978). Several fungicides have commonly been used against this disease. However, the fungus may develop tolerance to some of these chemicals, especially when one particular fungicide is applied repeatedly throughout the growing season (Cooley 1981; Gillman and James 1980; James and Gilligan 1983).

Strategies and schedules for fungicide application to control Botrytis blight in conifer seedbeds have not been developed. Therefore, in this study we evaluated the efficacy of several fungicides commonly used in greenhouses to control the disease in a seedbed environment.

## MATERIALS AND METHODS

Fungicide evaluation tests were conducted on western larch seedlings during their second growing season in seedbeds at the Coeur d'Alene Nursery. Trees of approximate uniform height and density were selected for the evaluation. Six fungicides previously tested in greenhouses (James et al. 1982) were evaluated (table 1). Two formulations of chlorothalonil were tested because both had previously been used operationally at the Nursery and we wanted to find out if there were differences in seedling response to the two formulations. The checks consisted of applying distilled water to test seedlings.

A randomized block design was used for the evaluation. Each treatment block consisted of 0.9 linear meters (3 feet) of seedbed (0.95 m<sup>2</sup>-10.5 ft<sup>2</sup>). A 0.3 m (1 ft.) untreated buffer strip separated each treatment block. Each treatment and the water check were replicated 5 times (totaling 135 blocks). Treatment blocks were delineated on the ground with wooden stakes at each corner and string tied between stakes. The number of test seedlings in each block was recorded at this time. Treatment blocks were clearly marked so that number of test seedlings in each block at the beginning and end of the test could be accurately compared.

Seedlings in each plot were treated with the appropriate fungicide or water six times during the test period at approximately 2-week intervals commencing on March 26, 1982. For each treatment, approximately 9.5 liters (2.5 gal.) of fungicide solution or water were applied to seedlings in each block so that all seedling foliage was thoroughly drenched.

|                |               |   | Application<br>rate per<br>100 gal. |                  |
|----------------|---------------|---|-------------------------------------|------------------|
| Common name    | Trade name    | Chemical name   | water                               | Manufacturer     |
| benomyl        | Tersan 1991®  | Methyl-l-(butylcarbamoyl)-<br>benzimidazole carbarmate                                | 1 1b                                | Dupont           |
| dicloran       | Botran®       | 2,6-Dichloro-4-nitroaniline   | 1-1/3 1b                            | Tuco             |
| chlorothalonil | Bravo 500®    | Tetrachlorosiophthalonitrile  | 2-3/4 pt                            | Diamond-Shamrock |
| chlorothalonil | Daconil 2787® | Tetrachlorosiophthalonitrile  | 1½ 1b                               | Diamond-Shamrock |
| captan         | Captan        | N-(Trichloromethylthio)-4-<br>cyclohexenen-1,2-dicarboximide                          | 2 1b                                | Stauffer         |
| iprodione      | Chipco 26019® | 3-(3,5-dichlorophenyl)-N-<br>(1-methylethyl)-2,4-dioxo-1-<br>imidazolidinecarboximide | 1 1b                                | Rhone-Poulenc    |

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Table 1.--Fungicides tested to control Botrytis blight on seedbed western larch at the Coeur d'Alene Nursery. All test seedlings were inoculated with a spore suspension of <u>Botrytis</u> to provide uniform exposure to the fungus. The <u>B</u>. <u>cinerea</u> isolate used for inoculations (CD-16) was obtained from an infected western larch seedling growing in a greenhouse at the nursery. This isolate had been pathogenic to western larch as indicated in previous greenhouse inoculation-fungicide tests (James et al. 1982). Spore suspensions were prepared by adding 10 ml sterile distilled water to 14-day-old <u>Botrytis</u> cultures growing in petri dishes on potato dextrose agar. Spores were agitated with a sterile camel's hair brush. Spore suspensions were passed through double layers of cheesecloth to remove mycelial fragments. They were then brought to the desired concentration of about 2.8 x 10<sup>6</sup> spores/ml by diluting with sterile distilled water. Test seedlings in each treatment block were sprayed with 20 ml of the spore suspension using a fine mist atomizer.

Seedlings were initially inoculated on April 14-15 after they had been treated with the fungicides twice. Ambient temperature was about  $2^{\circ}C$  and the weather was partly cloudy and windy. A month after this inoculation, there was little indication of infection - this may be due to the dry, windy weather during and shortly after the inoculation period. Therefore, we scheduled a second inoculation to coincide with a period of cool, wet weather. The second inoculation was conducted on May 27 during a light rain with ambient temperatures from 5 to  $10^{\circ}C$ . Light rain continued for 2 days following the second inoculation. The spores used in both inoculations were from the same isolate, and the spore concentrations were also the same.

The evaluation was concluded on October 27-28 just prior to the trees being lifted. The number of live and dead seedlings per plot was tallied. We also measured the height of 100 seedlings in each treatment block. These seedlings were concentrated in the center of each treatment block to minimize possible border effects.

Seedling survival and height data were compared among treatments with an analysis of variance. Significant differences among treatments were located using Duncan's Multiple Range Comparison Test.

#### RESULTS AND DISCUSSION

Our results indicated very high seedling survival in all treatment categories (table 2). Survival was even high (98.6 percent) in the water treatment, indicating very low levels of infection. Seedling survival differences among treatments were not statistically significant (P = 0.05).

| Treatment                      | No. dead<br>seedlings <u>1</u> / | Average<br>percent<br>survival <u>2</u> / |
|--------------------------------|----------------------------------|---|
| water                          | 21                               | 98.6                                      |
| benomyl (Tersan 1991®)         | 30                               | 98.2                                      |
| iprodione (Chipco 26019®)      | 11                               | 99.3                                      |
| chlorothalonil (Daconil 2787®) | 50                               | 97.2                                      |
| captan (Captan)                | 36                               | 97.9                                      |
| dicloran (Botran®)             | 42                               | 96.8                                      |
| chlorothalonil (Bravo 6F®)     | 25                               | 98.3                                      |

# Table 2.--Effects of fungicides on survival of western larch seedlings at the Coeur d'Alene Nursery.

- $\frac{1}{3}$  The number of seedlings that died between the initial count (3/30/82) and the final count (10/27-28/82).
- 2/ Means were not significantly different (P = 0.05) using an analysis of variance. Percentages underwent arc-sin transformations for data analysis.

These low infection levels were disappointing because we had expected significant treatment differences based on previous tests in greenhouses (James et al. 1982). Low infection resulted despite two inoculations of test seedlings. Weather conditions during the spring of 1982 were drier than during the previous 2 years. Since the environment in seedbeds is not as easily controlled as it is in greenhouses, success of our inoculations depended on ambient conditions. The initial inoculation was conducted during a dry, windy period in April. For successful infection, Botrytis spores need to lie in a film of water for at least several hours (Blakeman 1980). Therefore, conditions were not conducive during and shortly after the first inoculation period, resulting in little infection. However, the second inoculation was conducted during a cool, wet period in May and this wet period appeared to have persisted long enough for good infection. Despite these conditions which seemed to be ideal, there was little infection. We know that the spores were viable because they were tested for germinative capacity on laboratory media immediately after inoculation. After the second inoculation, a light rain fell steadily for 2 days, so it is possible that many of the spores were washed from the seedling foliage. Also, the May inoculation was conducted late in the spring. The fungus needs time to become established in infected tissues before onset of drier, summer-like weather. It is possible that the fungus did not have enough time to get established in the host after the May inoculation. In any event, the inoculations resulted in infection much below the desired and expected levels.

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Growth in seedling height during the second growing season was affected by the fungicides (table 3). Using the water check as a guide for normal seedling height, five of the six fungicides tested significantly reduced height growth of western larch. Chlorothalonil (Bravo 6F®) was the only fungicide that stimulated seedling height. However, the height of most of the treated seedlings was well within the standards established by the Nursery. Several of the tested fungicides caused similar effects on seedling height in a previous greenhouse test (James et al. 1982). However, phytotoxicity as evidenced by tissue necrosis or premature needle abscission was not found in either the seedbed or greenhouse tests.

| Treatment                      | Mean height<br>(MM) <u>1</u> / | 95%<br>Confidence interval |  |
|--------------------------------|--------------------------------|----------------------------|--|
| water                          | 351.3 C                        | 343.3 - 359.3              |  |
| benomyl (Tersan 1991®)         | 327.9 B                        | 319.5 - 336.4              |  |
| iprodione (Chipco 26019®)      | 311.3 A                        | 304.4 - 318.2              |  |
| chlorothalonil (Daconil 2787®) | 336.4 B                        | 329.2 - 343.6              |  |
| captan (Captan)                | 328.1 B                        | 320.6 - 335.5              |  |
| dicloran (Botran®)             | 311.6 A                        | 304.4 - 318.9              |  |
| chlorothalonil (Bravo 6F®)     | 370.9 D                        | 362.6 - 379.3              |  |

Table 3.--Effects of selected fungicides on height of western larch seedlings at the Coeur d'Alene Nursery.

1/ Means followed by the same capital letter are not significantly different (P = 0.05) using Duncan's Multiple Range Comparison Test.

Our results indicate that fungicide treatment of seedbeds to control Botrytis blight is necessary only if infection is common and periods of cool, wet weather are expected. We suspect that several of the fungicides tested are effective and could help reduce future losses from <u>Botrytis</u>. The treatment program should include several fungicides used in rotation so that the pathogen will have less opportunity to form tolerant strains (Cooley 1981; Gillman and James 1980).

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