# Douglas-fir Seed Treatments: Effects on Seed Germination and Seedborne Organisms[1,2]

R. Kasten Dumroese, Robert L. James,
David L. Wenny, and Carma J. Gilligan[3]

Abstract.--Treating Douglas-fir seed prior to stratification with bleach, after stratification with hydrogen peroxide or ethanol, or soaking seed after stratification in 55.5° C water significantly reduced seedborne Fusarium levels while maintaining high cumulative germination.

## INTRODUCTION

Fusarium root disease is an important problem in container nurseries of the Intermountain West. It is especially widespread and damaging to Douglas-fir (Pseudotsuga menziesii var. glauca (Beissn.) Franco). The primary source of inoculum is thought to be infested seed (James 1985a, 1986), although recent observations indicate inoculum is also carried from one year to the next in both styroblocks (James and others 1988a) and Ray Leach® pine cells (James and Gilligan 1988), even those vigorously cleaned.

Many attempts have been made to eliminate or reduce pathogenic organisms on conifer seedcoats. Many of these treatments rely on chemical sterilants, including sodium hypochlorite (Wenny and Dumroese 1987, James and Genz 1981), and hydrogen peroxide (Barnett 1976, Trappe 1961).

Recent work by Sauer and Burroughs (1986) showed corn and wheat seeds treated with 100% ethanol or sodium hypochlorite with lowered pH had decreased levels of Fusarium. Dodds and Roberts (1985) discuss a combination treatment for sterilizing seed for micropropagation. This treatment begins with a 1-3 minute soak in a 70% (v/v) ethanol solution followed by a soak in sodium hypochlorite.

One other approach to reducing seedborne pathogens is hot water treatments (Baker 1962). Hot water treatments have effectively been used on agricultural crops to reduce or eliminate seedborne pathogens while maintaining high germinative capacity without phytotoxic reactions (Neergaard 1977, Walker 1969). A recent innovative approach to hot water treatments is the use of microwaves to heat water to the desired temperature (Lozano and others 1986).

Because of the importance of seedborne inoculum in Fusarium root disease on Douglas-fir, we compared the relative efficacy of control of Fusarium and resultant post-treatment seed germination after use of common chemical sterilants for conifer seed and chemicals now used to sterilize seed in agricultural and micropropagation work. We also evaluated the efficacy of microwave treatments to reduce or eliminate Fusarium from seed coats.

## MATERIALS AND METHODS

For both the chemical and microwave treatments, a northern Idaho source of Douglas-fir seed with known levels of seedborne Fusarium (James and others 1987) was used.

The chemical treatments consisted of six cleaning techniques (table 1), including the control (treatment 6). The control consisted of rinsing the seeds 48 hours in running tap water. Half of the seed was treated prior to cold stratification and the remainder treated after stratification. After half of the seed was treated,

---

[1]Paper presented at the combined meeting of the Western Forest Nursery Council, Intermountain Nursery Association and Forest Nursery Association of British Columbia. [Vernon, British Columbia, Aug. 8-11, 1988].

[2]Idaho Forest Wildlife and Range Experiment Station Contribution No. 405.

[3]R. Kasten Dumroese is a Research Associate and Ph.D. candidate at the University of Idaho, Forest Research Nursery, Moscow, Idaho, 83843.
Robert L. James is Plant Pathologist with the USDA Forest Service, Timber, Cooperative Forestry and Pest Management, Coeur d'Alene, Idaho.
David L. Wenny is Associate Professor of Silviculture and Manager of the University of Idaho Forest Research Nursery, Moscow, Idaho.
Carma J. Gilligan is a Biological Technician with the USDA Forest Service, Timber, Cooperative Forestry and Pest Management, Missoula, Montana.

all seed was rinsed for 48 hours in a running tap water wash to ensure complete imbibition before stratification. For each treatment, seeds were placed into mesh bags to facilitate handling. These were suspended and sealed inside large plastic bags. The plastic bags were then hung inside a cooler and maintained at 2° C for 21 days. After stratification, the remaining seed was treated and all seed was rinsed 24 hours in a running tap water wash.

Table 1. Descriptions of chemical treatments used on Douglas-fir seeds.

| Treatment | Description[1] |
|---|---|
| 1 | Soaked seeds in 95% ethanol for 10 seconds followed by a running tap water rinse. |
| 2 | Soaked seeds 10 minutes in a 2.1% sodium hypochlorite solution (2 parts commercial bleach (5.25% sodium hypochlorite) in 3 parts tap water) followed by a running tap water rinse. |
| 3 | Soaked seeds in a 3% hydrogen peroxide solution for 5 hours followed by a running tap water rinse. |
| 4 | Soaked seeds 10 minutes in a 2.1% sodium hypochlorite solution acidified with HCl to pH 6, followed by a running tap water rinse. |
| 5 | Soaked seeds in 75% (v/v) ethanol for 3 minutes, rinsed three times in tap water, and then soaked seeds in a 2.1% sodium hypochlorite solution for 10 minutes followed by a running tap water rinse. |
| 6 | Seeds rinsed for 48 hours in running tap water (control). |

[1] All seed was rinsed 48 hours in running tap water prior to stratification and 24 hours after stratification.

For the microwave treatments, seeds were rinsed with running tap water for 48 hours prior to 22 days cold stratification at 3° C. Following stratification, seeds were rinsed in running tap water for 24 hours. Seeds were then placed in 300 ml distilled water within a glass beaker. The water-seed mixture was heated to varying temperatures by exposure to microwaves at the full power setting for different time periods. The microwave oven used was a Kenmore model 99701 with 1,400 watts heating power (2,450 MHz). Water temperatures were recorded before and after microwave treatments. Controls consisted of seeds placed in unheated (20° C) water. Following microwave treatments, the water was decanted and the seeds allowed to cool to room temperature before blotted dry on sterile filter paper.

Colonization of seed coats by two groups of fungi (Fusarium, and Trichoderma) was determined by aseptically placing seed on a medium selective for Fusarium (Komada 1975) following treatments. Nineteen replicates of 25 seeds (475 total) were plated on the selective medium for the chemical tests and 9 replicates of 25 seeds (225 total) for the microwave treatments. Plates were incubated at about 22° C under cool fluorescent light for 7 days after which organisms within the two groups, emerging from the seed, were tallied. Percentages of seed colonized with the two groups of organisms were calculated. Several isolates of Fusarium were grown on potato dextrose agar and carnation leaf agar for identification using the taxonomic scheme of Nelson and others (1983).

Four replicates of 100 seeds from each chemical treatment and treatment time were placed into germination trays on moistened absorbent cotton pads. Ten replicates of 15 seeds for each microwave exposure time were also placed on moistened absorbent cotton pads in petri dishes. Trays and petri dishes were incubated under 12 hours of photoperiod at 22-24° C. The containers were examined every seven days for 28 days to determine germination capacity. Seed was considered to be germinated when the radicle was as long as the seed coat.

Treatment effects on germination and the occurrence of seedcoat organisms were evaluated using a one-way analysis of variance. Significant differences among chemical treatment means were located with Duncan's new multiple range test. Tukey's multiple-range comparison test was used for analyzing the microwave treatments. All data underwent arc-sin transformation prior to analysis.

## RESULTS AND DISCUSSION

The chemical treatments significantly affected the cumulative germination of the Douglas-fir seed (table 2). Germination percentages for seed treated prior to stratification (all treatments combined) were significantly lower than germination percentages for seed treated after stratification. Pre-stratification use of treatments 1 and 5 gave large fluctuations in cumulative germination percentage, often 25 to 40 percent differences between replications. Both of these treatments involved ethanol. Perhaps, for seed treated prior to stratification, and especially those treatments with ethanol, seed moisture content may have an influence. Because the seed have very low moisture contents prior to stratification, they may readily imbibe the solution carrying the chemical, resulting in tissue damage and subsequently lower germination. Conversely, the seed treated after

stratification are completely imbibed and cannot readily absorb the chemical solution. Soaking the seed prior to the pre-stratification treatment may remedy this effect.

Table 2. Chemical treatment effects on the cumulative germination of Douglas-fir seed.

| Treatment[1] | Cumulative Germination at 28 Days | |
|---|---|---|
| | Pre-stratification (%) | Post-stratification (%) |
| 1 | 40 d[2] | 88 ab |
| 2 | 88 a | 87 ab |
| 3 | 51 c | 92 a |
| 4 | 74 b | 85 b |
| 5 | 54 c | 84 b |
| 6 | 90 a | 90 ab |
| All treatments | 66 [3] | 88 |

1 See table 1 for descriptions of treatments.

2 Within each column, means followed by the same letter are not significantly different (P = 0.05) using Duncan's new multiple range test.

3 Between pre- and post-stratification means for all treatments, the difference is significant (P = 0.01) using Duncan's new multiple range test.

In all but treatment 1, the chemical sterilants reduced seedborne Fusarium levels (table 3) when compared to the control (treatment 6). Hydrogen peroxide was the most effective chemical in reducing the levels of Fusarium on the seedcoat, supporting work by James and Genz (1981) with ponderosa pine. The combined ethanol-bleach treatment also consistently reduced Fusarium, agreeing with the work of Sauer and Burroughs (1986) on corn.

We devised a ranking procedure to determine which chemical treatment best reduced Fusarium levels, maintained high Trichoderma levels and yielded high germination percentages. In table 4, each treatment, including pre- and post-stratification applications, was given a rank according to germination percentage, Fusarium levels and Trichoderma levels. We multiplied the ranks together to obtain a score. The lowest scores received the highest ranking.

Treating the seed after stratification with hydrogen peroxide was the overall best treatment. Interestingly, our control ranked second because of its high germination capacity and the highest levels of Trichoderma. Treating seed prior to

Table 3. Chemical treatment effects on the occurrence of Fusarium and Trichoderma on seedcoats of Douglas-fir.

| Treatment[1] | Percentage Seedcoat Colonization | | | |
|---|---|---|---|---|
| | Fusarium | | Trichoderma | |
| | Pre[2] | Post[3] | Pre | Post |
| 1 | 6.6 f[4] | 2.1 d | 82 b | 30 c |
| 2 | 1.6 c | 4.2 e | 14 d | 21 d |
| 3 | 0.0 a | 0.2 a | 28 c | 52 b |
| 4 | 2.8 d | 0.9 c | 3 f | 5 f |
| 5 | 0.5 b | 0.9 b | 5 e | 10 e |
| 6 | 4.5 e | 5.2 f | 90 a | 80 a |

1 See table 1 for descriptions of treatments.

2 Pre = treatments performed prior to seed stratification.

3 Post = treatments performed after seed stratification.

4 Within each column, means followed by the same letter are not significantly different (P = 0.05) using Duncan's new multiple range test.

stratification with hydrogen peroxide ranked third because of its complete eradication of Fusarium, but its negative impact on germination reduces its appeal as a treatment. Treating seed prior to stratification with bleach ranked fourth, reducing Fusarium levels by 67%. Post-stratification treatment of seed with 95% ethanol for 10 seconds reduced Fusarium levels by 57% but retained nearly twice the amount of Trichoderma as the bleach treatment. In viewing the data, hydrogen peroxide, applied after stratification, bleach, applied before stratification, and the ethanol quick dip were the three treatments that significantly reduced Fusarium while maintaining the highest germination percentages.

All chemical treatments, and the microwave treatment, were also effective in significantly reducing the levels of Trichoderma on seedcoats (tables 3 and 5). Since Trichoderma are common antagonists against Fusarium spp. (Papavizas 1985), reducing their occurrence on seed may not be desirable. This is especially true if Fusarium inoculum is introduced into containerized seedlings from sources other than seed, such as containers (James 1987, James and others 1988a) or soil mixes (James 1985b).

Effects of microwave hot water treatments on seed germination and Fusarium and Trichoderma levels on Douglas-fir seedcoats are summarized in table 5. No seed germinated after 120 seconds of exposure (66.5°+ C), although germination was not significantly reduced by exposures of 90 seconds

Table 4. Rank of treatment efficacy based on cumulative germination and Fusarium and Trichoderma levels.

| Treatment[1] | Cumulative germination | | Fusarium levels | | Trichoderma levels | | Overall ranking computation | |
|---|---|---|---|---|---|---|---|---|
| | percent[2] | rank | percent | rank | percent | rank | formula | rank |
| 1 pre | 40 f | 11 | 6.6 i | 11 | 82 b | 2 | (11*11*2) = 242 | 8 |
| 1 post | 88 abc | 4 | 2.1 e | 7 | 30 d | 5 | (4*7*5) = 140 | 5 |
| 2 pre | 88 abc | 3 | 1.6 e | 6 | 14 g | 7 | (3*6*7) = 126 | 4 |
| 2 post | 87 bc | 5 | 4.2 g | 9 | 21 f | 6 | (5*9*6) = 270 | 9 |
| 3 pre | 51 e | 10 | 0.0 a | 1 | 28 e | 4 | (10*1*4) = 40 | 3 |
| 3 post | 92 a | 1 | 0.2 b | 2 | 52 c | 3 | (1*2*3) = 6 | 1 |
| 4 pre | 74 d | 8 | 2.8 f | 8 | 3 j | 11 | (8*8*11) = 704 | 11 |
| 4 post | 85 bc | 6 | 0.9 d | 4 | 5 i | 9 | (6*4*9) = 216 | 6 |
| 5 pre | 54 e | 9 | 0.5 c | 3 | 5 i | 10 | (9*3*10) = 270 | 9 |
| 5 post | 84 c | 7 | 0.9 d | 4 | 10 h | 8 | (7*4*8) = 224 | 7 |
| 6 | 90 ab | 2 | 4.8 h | 10 | 85 a | 1 | (2*10*1) = 20 | 2 |

1 See table 1 for descriptions of treatments. Pre = treatments performed prior to seed stratification. Post = treatments performed after seed stratification.

2 Within the percent columns, means followed by the same letter are not significantly different (P = 0.05) using Duncan's Multiple Range Comparison Test.

Table 5. Effects of microwave hot water treatments on occurrence of Fusarium and Trichoderma on Douglas-fir seed, and cumulative germination percentage of Douglas-fir seed[1].

| Exposure time (sec.) | Max. water temperature (degrees C) | Seeds with Fusarium (%) | Seeds with Trichoderma (%) | 28-day cumulative germination (%) |
|---|---|---|---|---|
| 0 | 20.0 | 3.1 a[2] | 98.2 a | 90 a |
| 60 | 43.0 | 1.8 ab | 96.9 a | 87 a |
| 90 | 55.5 | 0.4 b | 46.7 b | 86 a |
| 120 | 66.5 | 0.0 b | 0.4 c | 0 b |
| 150 | 77.0 | 0.0 b | 0.4 c | 0 b |
| 180 | 88.5 | 0.0 b | 0.0 c | 0 b |

1 See James and others (1988b).

2 Within each column, means followed by the same letter are not significantly different (P = 0.05) using Tukey's multiple-range comparison test.

(55.5° C) or less. Unfortunately, the exposure time needed to eliminate all Fusarium from seed also eliminated seed viability. However, the 90 second treatment reduced Fusarium levels to almost negligible amounts (0.4 percent) and did not significantly reduce seed germination. Treatments somewhere between 60 and 90 seconds (43° and 55.5° C) may be best for practical applications. Additional tests are necessary to locate this thermal "window" more precisely.

Treatments of agricultural seeds using vegetable oils, such as sunflower, soybean and maize oils as the medium for heat treatment instead of water, have been effective (Ryndji and others 1987, Zinnen and Sinclair 1982). The major advantage of vegetable oils over water is reduced seed imbibition of the heated medium and resulting toxicity to the embryo. There is currently no information available as to the responses of conifer seeds to such treatments, but evaluations may be beneficial because of the toxicity of hot water to Douglas-fir seed.

It is probable that other conifer species, and also other Douglas-fir seedlots, will respond differently to the chemical and hot water treatments. Larger quantities of seed treated at one time may also react differently. Additional tests are required to establish safe guidelines.

## CONCLUSIONS

Sterilizing Douglas-fir seed before stratification, except with bleach, had a negative impact on germination. Conversely, seed sterilized after stratification maintained high germination percentages. Treating seed prior to stratification with bleach, after stratification with hydrogen peroxide or ethanol, or after stratification in hot water (55.5° C) significantly reduced seedborne Fusarium and Trichoderma levels while maintaining high cumulative germination.

## MANAGEMENT IMPLICATIONS

We need to develop a method for rapid identification of pathogenic Fusarium. Knowing whether the seedborne inoculum is pathogenic or not would help the nursery manager decide if a seed coat sterilization treatment is necessary.

Because of this uncertainty, problems with seed from unknown collection sources, and favorable operational results, the University of Idaho Forest Research Nursery uses the bleach treatment before stratification to reduce seedborne Fusarium levels on pine and Douglas-fir seeds (Wenny and Dumroese 1987). However, for research where nearly complete eradication of Fusarium is essential (i.e. pathogenicity tests), the after stratification hydrogen peroxide treatment appears best. Growers with the benefit of sowing vigorously germinating seedlots with very low levels of seedborne inoculum are probably better off not reducing their seedborne levels of antaganistic Trichoderma with a seed sterilization treatment.

## LITERATURE CITED


Baker, K.F. 1962. Thermotherapy of planting material. Phytopathology 52:1244-1255.

Barnett, J.P. 1976. Sterilizing southern pine seeds with hydrogen peroxide. Tree Planters' Notes 27(3):17-19.

Dodds, J.H. and L.W. Roberts. 1985. Experiments in plant tissue culture. Second edition. 232 p. Cambridge University Press, New York.

James, R.L. 1985a. Pathogenic Fusarium on spruce seed from the Towner Nursery, North Dakota. USDA Forest Service, Northern Region Report 85-23, 9 p. Missoula, MT.

James, R.L. 1985b. Diseases associated with containerized seedling soil mixes. Tree Planters' Notes 36(2):3-5.

James, R.L. 1986. Diseases of conifer seedlings caused by seedborne Fusarium species. pp. 267-271. In: Shearer, R.C. (compiler). Proceedings: Conifer Tree Seed in the Inland Mountain West Symposium. USDA Forest Service General Technical Report INT-203. Ogden, UT.

James, R.L. 1987. Occurrence of Fusarium within styroblock containers - Plum Creek Nursery, Pablo, Montana (Preliminary Report). USDA Forest Service, Northern Region. 2 p. Missoula, MT.

James, R.L. and D. Genz. 1981. Ponderosa pine seed treatments: effects on seed germination and disease incidence. USDA Forest Service, Northern Region Report 81-16, 13 p. Missoula, MT.

James, R.L. and C.J. Gilligan. 1988. Occurrence of Fusarium on Leach pine cells from the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region Report 88-8, 10 p. Missoula, MT.

James, R.L., R.K. Dumroese, D.L. Wenny, J.F. Myers and C.J. Gilligan. 1987. Epidemiology of Fusarium on containerized Douglas-fir seedlings. (1) Seed and seedling infection, symptom production, and disease progression. USDA Forest Service, Northern Region Report 87-13, 22 p. Missoula, MT.

James, R.L., C.J. Gilligan and V. Reedy. 1988a. Evaluation of root diseases of containerized conifer seedlings at the Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region Report 88-11, 21 p. Missoula, MT.

James, R.L., C.J. Gilligan, R.K. Dumroese and D.L. Wenny. 1988b. Microwave treatments to eradicate seedborne fungi on Douglas-fir seed. USDA Forest Service, Northern Region Report 88-7, 8 p. Missoula, MT.

Komada, H. 1975. Development of a selective medium for quantitative isolation of Fusarium oxysporum from natural soil. Review Plant Protection Research (Japan) 8:114-125.

Lozano, J.C., R. Laberry and A. Bermudez. 1986. Microwave treatment to eradicate seedborne pathogens in cassava tree seed. Journal of Phytopathology 117:1-8.

Neergaard, P. 1977. Seed pathology. 1187 p. John Wiley & Sons, New York.

Nelson, P.E., T.A. Toussouw and W.F.O. Marasas. 1983. Fusarium species: An illustrated manual for identification. The Pennsylvania State Univ. Press, University Park. 193 p.

Papavizas, G.C. 1985. Trichoderma and Gliocladium: Biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23:23-54.

Ryndji, M.M., J.B. Sinclair and T. Singh. 1987. Soybean seed thermotherapy with heated vegetable oils. Plant Disease 71:213-216.

Sauer, D.B. and R. Burroughs. 1986. Disinfection of seed surfaces with sodium hypochlorite. Phytopathology 76:745-749.

Trappe, J.M. 1961. Strong hydrogen peroxide for sterilizing coats of tree seed and stimulating germination. Journal of Forestry 59:828-829.

Walker, J.C. 1969. Plant pathology. Third edition. 819 p. McGraw-Hill Book Company, New York.

Wenny, D.L. and R.K. Dumroese. 1987. Germination of conifer seeds surfaced sterilized with bleach. Tree Planters' Notes 38(3):18-21.

Zinnen, T.M. and J.B. Sinclair. 1982. Thermotherapy of soybean seeds to control seedborne fungi. Phytopathology 72:831-834.