United States
Department of
Agriculture

Forest
Service

**Northern
Region**

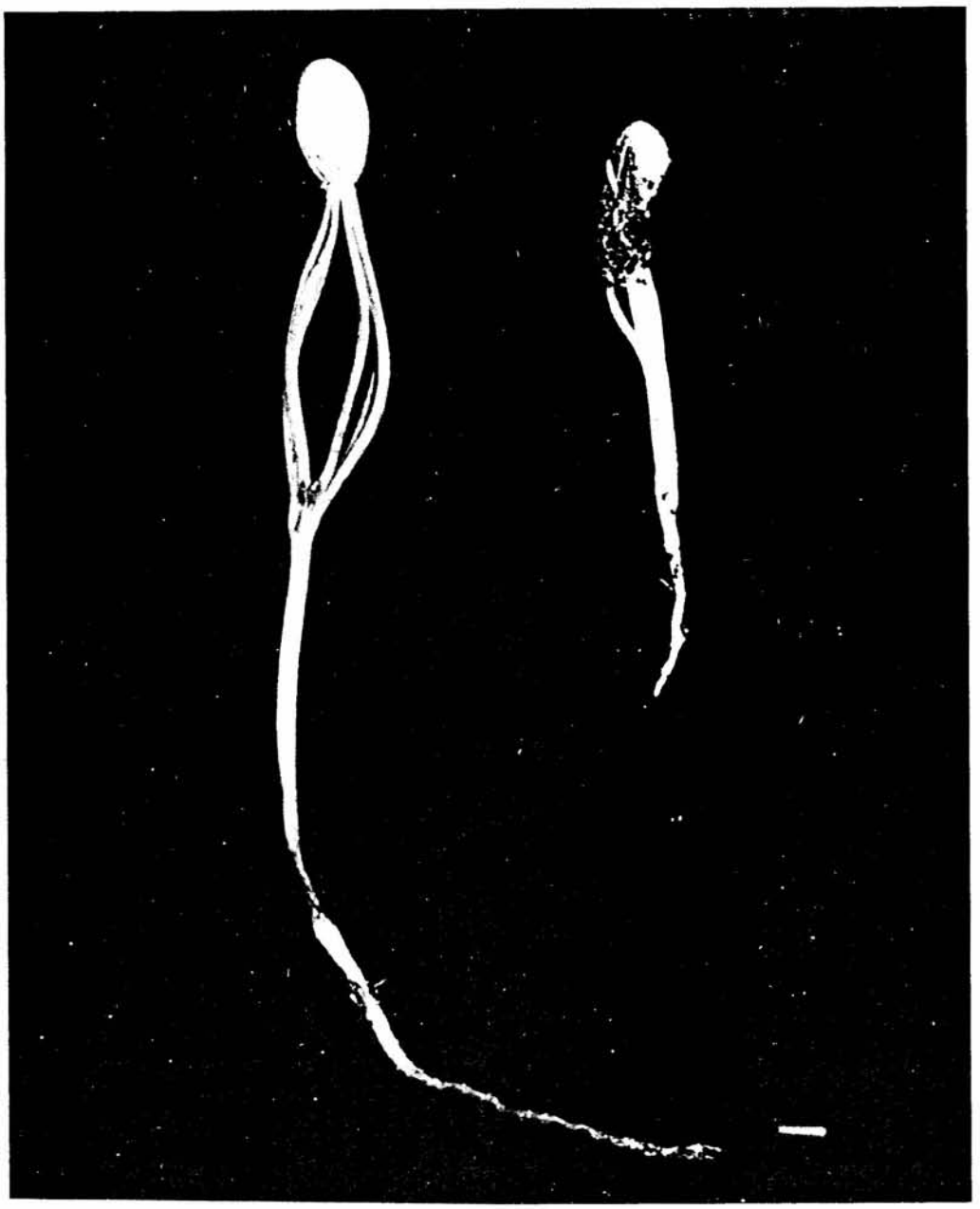
**Forest Pest
Management**

Report No. 81-16

Ponderosa Pine Seed Treatments

Effects on Seed Germination and Disease Incidence

R.L. James & Daryl Genz



EVALUATION OF PONDEROSA PINE SEED TREATMENTS: EFFECTS ON SEED GERMINATION AND DISEASE INCIDENCE

By

R. L. James, Plant Pathologist
Forest Pest Management, Missoula, MT

and

Daryl Genz, Nursery Supervisor,
Champion Timberlands Nursery, Plains, MT

ABSTRACT

Four treatments were tested on two ponderosa pine seedlots to determine effects on seed germination and occurrence of disease on seeds and emerging hypocotyls. Treatment with hydrogen peroxide was most satisfactory in protecting seeds and enhancing germination. Sodium hypochlorite (Clorox ^{R}) was less effective, but generally superior to water treatments. A running water bath resulted in fewer disease problems than a still water soak. Major fungal associates of diseased seed and hypocotyls were Fusarium, Trichothecium, Penicillium, and Aspergillus.

INTRODUCTION

Seed germination tests conducted under standard conditions within incubation chambers are used to evaluate performance of different seedlots. Expected germinative capacity of seed within seedbeds or containers can be ascertained from such tests. Recent experience at the Champion International Nursery (Plains, Montana) indicated relatively low germination of ponderosa pine (Pinus ponderosa Laws.) seed, thought to be the result of extensive fungal contamination. Certain seedlots were especially prone to colonization by molds during germination tests. Fungi commonly colonized seedcoats and attacked emerging radicles.

Previous work (8, 19, 22) indicates that several seed treatments commonly reduce fungal contamination and may improve germination and seedling survival. Therefore, trials were conducted to evaluate four seed treatments with the hope of establishing standard procedures for improving seed germination and reducing disease occurrence at the Plains nursery.

MATERIALS AND METHODS

Two representative ponderosa pine seedlots (designated 3-21-26 and 25-14-17) were tested. Cones for both seedlots were obtained from squirrel caches on Champion International Timberlands in northwestern Montana during the fall of 1980. Cones were dried in burlap bags at about 35° C for 24-36 hours in a dry kiln. Extracted seed was selected randomly for testing from each seedlot.

Seed treatments (table 1) were conducted prior to stratification. Following treatment, seeds were aseptically placed in sterile, square, plastic petri dishes (95 mm diameter) on a wetted sterile cotton matting. Four replicates of 50 seeds each were evaluated for each of the four treatments for each seedlot (800 seeds tested per seedlot). Dishes with seeds were placed in a stratification chamber and incubated for 30 days at about 0-2° C.

Immediately following stratification, seed were analyzed for:

1. Percentage germination (indicated by emergence of a radicle through the seedcoat).

2. Percentage of emergent radicles/hypocotyls that were diseased (characterized by necrotic lesions, water-soaking, and growth of mold and bacteria).

3. Percentage of ungerminated seed with clean seedcoats (without mold and bacterial growth).

4. Percentage of ungerminated seed with mold and bacterial growth on seedcoats.

5. Percentage of ungerminated seed with open seedcoats. These seed apparently began to germinate, but their endosperm was invaded by mold and bacteria. Mold and bacterial growth usually occurred at the point of seedcoat opening.

Table 1. Ponderosa pine seed treatments evaluated for effects on germination and disease occurrence.

Treatment designation	Description
A	Soaked in regular tapwater for 12 hours; no rinse.
B	Soaked in a continuous tapwater bath (running water) for 48 hours.
C	Soaked in 5.25 percent sodium hypochlorite (concentrated Clorox ®) for 5 minutes; rinsed with tapwater three times for 5 minutes each.
D	Soaked in 0.03 percent hydrogen peroxide for 5 hours; rinsed with tapwater three times for 5 minutes each.

All ungerminated seeds were also dissected and examined under the binocular microscope (10-25x) for presence and condition of the endosperm. Endosperms were classified as either healthy or diseased. Diseased endosperms had noticeable deterioration or decay; they were often off-white to yellow and had a milky consistency rather than being firm. Empty seeds without endosperms were also noted.

Associated fungi on seedcoats and radicles were identified by culturing eight diseased seedlings per treatment for each seedlot. Selected seedlings were incubated on 2 percent water agar for 7-14 days at about 24° C. Emerging fungi were aseptically transferred to potato dextrose agar slants.

Colony morphology, growth habits, and microscopic characteristics of sporulation were used to identify fungi according to standard taxonomic guides (2, 4, 12).

Treatment effects and differences between seedlots were tested using one-way analysis of variance. If differences existed, Tukey's test for block comparisons was used to locate significantly different treatments.

RESULTS

Hydrogen peroxide significantly (P=0.05) improved seed germination (table 2); both tested seedlots were similarly affected. No other treatments improved seed germination as well as hydrogen peroxide. Both seedlots had several empty seeds without endosperms (table 3). This contributed to the relatively low levels of resulting germination.

Treatment effects on disease incidence (colonization of the hypocotyl with bacteria or fungi) are summarized in table 4. Results indicated that no treatment was completely effective in removing contaminating organisms. The Clorox® treatment best protected emerging hypocotyls. Other treatments were less satisfactory.

Table 5 summarizes treatment effects on seedcoat colonization by fungi and bacteria. The water soak was least effective in removing seedcoat organisms; hydrogen peroxide was most effective, followed by Clorox® and running water.

Hydrogen peroxide treatment also resulted in significantly fewer (P=0.05) diseased endosperms (see Materials and Methods for description) in ungerminated seeds (table 6). Most seeds with diseased endosperms also had open seedcoats (table 5) through which rotting fungi and bacteria entered. Fungi associated with diseased hypocotyls and contaminated seedcoats are listed in table 7. Species of *Fusarium*, *Trichothecium*, *Penicillium*, and *Aspergillus* were most common.

Table 2. Effects of selected seed treatments on germination of ponderosa pine seeds

Treatment[1/]	Seedlots					
	3-21-26 Percent germination[2/]		25-14-17 Percent germination[2/]		Both Percent germination[2/]	
	1	2	1	2	1[3/]	2
A	22.5	24.2	20.5	21.8	21.5C	23.0
B	18.5	20.0	38.0	43.2	28.2B	31.3
C	25.0	28.0	20.0	27.4	22.5C	28.2
D	34.5	37.5	38.0	42.5	36.3A	39.9
Average	25.1[4/]	27.6	29.1[4/]	39.6	27.1	33.0

1/ See table 1 for treatment descriptions.

2/ Values in column 1 are for all seeds; those in column 2 are for only seeds with endosperm (empty seeds omitted)--see table 3.

3/ Within this column, values followed by the same capital letter are not significantly different (P=0.05) using Tukey's test for multiple comparisons.

4/ Average percent germination of different seed sources tested were not significantly different (P=0.05) using one-way analysis of variance.

Table 3. Percentage of empty seed (without endosperm) in two tested ponderosa pine seedlots

Seedlot	Percent of empty seed
3-21-26	9.0
25-14-17	13.9
Both	11.4

Table 4. Effects of selected seed treatments on disease incidence in hypocotyls of germinated ponderosa pine seeds.

Treatment [1]	Seedlots		
	3-21-26	25-14-17	Both
	Percent germinated seeds with hypocotyls diseased [2]	Percent germinated seeds with hypocotyls diseased [2]	Percent germinated seeds with hypocotyls diseased [2] [3]
A	100.0	90.2	95.3 A
B	86.5	72.4	77.0 A
C	66.0	52.5	60.0 B
D	76.8	76.3	76.6 A
Averages	81.1 [4]	73.4 [4]	77.0

1/ See table 1 for treatment descriptions.
2/ Diseased hypocotyls had characteristic necrotic lesions, watersoaking, mold growth, bacterial slime or other pathological symptoms.
3/ Within this column, values followed by the same capital letter were not significantly different (P=0.05) using Tukey's test for multiple comparisons.
4/ Average hypocotyl disease incidences of different seed sources tested were not significantly different (P=0.05) using one-way analysis of variance.

Table 5. Effects of selected treatments on seedcoat colonization by mold and bacteria on ungerminated ponderosa pine seeds.

Treatments[1]	Seedlots								
	3-21-26			25-14-17			Both		
	% seed w/clean seedcoat	% seed w/mold on seedcoat	% seed w/open seedcoat[2]	% seed w/clean seedcoat	% seed w/mold on seedcoat	% seed w/open seedcoat[2]	% seed w/clean seedcoat[3]	% seed w/mold on seedcoat[3]	% seed w/open seedcoat[2]
A	16.1	31.6	52.3	28.3	13.2	58.5	22.3 A	22.3 A	55.4 A
B	31.3	26.4	42.3	47.6	12.9	39.5	38.3 B	20.6 A	41.1 B
C	54.7	12.7	32.7	53.1	5.0	41.9	53.9 C	8.7 B	37.4 B
D	64.1	16.0	19.8	44.4	7.3	48.4	54.5 C	11.8 B	33.7 C
Averages	40.4[4]	22.0[5]	37.6[4]	43.0[4]	9.5[5]	47.7[4]	41.7	16.0	42.4

1/ See table 1 for treatment descriptions.
2/ Indicate seeds that started to germinate but mold and/or bacteria colonized endosperm. Most of these seeds had diseased endosperms (table 6).
3/ Within each column, values followed by the same capital letter are not significantly different (P=0.05) using Tukey's test for multiple comparisons.
4/ Averages of different seed sources tested are not significantly different (P=0.05) using one-way analysis of variance.
5/ Averages of different seed sources tested are significantly different (P=0.05) using one-way analysis of variance.

Table 6. Effects of selected treatments on endosperm disease incidence within ungerminated ponderosa pine seeds. 1/

Treatments 2/	Seedlots					
	3-21-26		25-14-17		Both	
	% seed w/healthy endosperm	% seed w/diseased endosperm	% seed w/healthy endosperm	% seed w/diseased endosperm	% seed w/healthy endosperm	% seed w/diseased endosperm
A	27.1	63.9	31.4	61.0	29.3 A	62.4 A
B	46.6	44.2	40.3	40.3	43.9 B	42.5 B
C	40.7	41.3	23.8	42.5	31.9 A	41.9 B
D	61.1	26.7	35.5.	47.6	48.6 B	36.9 C
Averages	43.2 4/	44.7 5/	32.1 4/	48.3 5/	37.8	46.5

1/ Diseased endosperms were those with noticeable deterioration or decay, off-white to yellow discoloration, and milky consistency when dissected. Empty seeds (those without endosperms) are excluded--see table 3.
2/ See table 1 for treatment descriptions.
3/ Within each column, values followed by the same capital letter are not significantly different (P=0.05) using Tukey's test for multiple comparisons.
4/ Averages of different seed sources tested are significantly different (P=0.05) using one-way analysis of variance.
5/ Averages of different seed sources tested are not significantly different (P=0.05) using one-way analysis of variance.

Table 7. Fungi isolated from ponderosa pine seedcoats and emerging radicles.

Associated fungi	Comments 1/
Fusarium roseum Link emend. Sny. & Han.	Found in all treatments
Fusarium oxysporum Schlecht. ex Fr.	Found in all treatments
Trichoderma sp.	Most common in treatment A
Verticillium sp.	Most common in treatments A & B
Gliocladium sp.	Most common in treatments A & B
Trichothecium sp.	Found in all treatments
Aspergillus sp.	Most common in treatments A & B
Penicillium sp.	Found in all treatments
Coniothyrium sp.	Most common in treatment A
Mucor sp.	Most common in treatment A
Graphium sp.	Found only in treatment B
Dictyostelium sp. (slime mold)	Found only in treatment A

1/ See table 1 for treatment descriptions.

DISCUSSION

Treatment of conifer seeds to reduce or eliminate contaminating pathogens is a common practice in many forest nurseries (16, 29). Without treatment, seedling losses are often substantial.

Several chemicals have effectively reduced seed transmission of pathogens. However, detrimental effects on seed viability may result following treatment (8, 35). Hydrogen peroxide effectively sterilizes seeds and improves germination (7, 15, 33); germination also occurs more rapidly following treatment with the chemical (9). Our tests showed that treatment with relatively low concentrations of hydrogen peroxide improved seed germination and reduced seedcoat contamination compared to the other treatments. Also, more ungerminated seeds had healthy endosperms when treated with hydrogen peroxide. Sodium hypochlorite (Clorox ®), also reported as an effective seed sterilant (17), was less satisfactory than hydrogen peroxide in our tests.

Tested concentrations of both sodium hypochlorite and hydrogen peroxide were low; exposure times were short. This may account for the poorer protection of seeds from contaminating fungi and bacteria than was expected. Only about half of the seed treated with either chemical were not colonized by fungi. Other studies (3, 15, 17) using higher chemical dosages resulted in better seed protection. However, phytotoxicity often accompanies high chemical concentrations (9, 33, 35).

in reducing seed contamination at the Coeur d'Alene Nursery in Idaho (J. Y. Woo, personal communication). Our results were less satisfactory, although running water was more effective than a stagnant water soak. Unusually high fungal contamination of collected seed may partially account for differences between our results and those reported by others (17, 22).

Several common fungal genera were identified from diseased ponderosa pine hypocotyls and colonized seedcoats. Although many were probably saprophytes, several may be pathogenic to seedlings under certain conditions. Fusarium oxysporum Schlect. ex Fr. is especially notorious as a pathogen causing damping-off (5, 24). Another Fusarium commonly isolated was identified as F. roseum Link emend. Sny. & Han. using the taxonomic scheme of Snyder and Hansen (26). These fungi were common on seed from all treatments and tested seedlots.

Another possible pathogen commonly isolated was Trichothecium sp. This fungus is common on seeds of many different plants (14, 20) and may rot seeds of several forest trees (21, 34). Aspergillus and Penicillium, common colonizers of stored seed (11, 22), were also isolated from several diseased hypocotyls. Aspergillus is most common on stored grain where it may produce powerful toxins (10, 37); Penicillium commonly causes seed rot of several plant species (22, 30).

Other possible pathogenic fungi isolated from diseased hypocotyls include Coniothyrium and Verticillium. Coniothyrium has been isolated from forest tree seed (1, 22). Verticillium is a common soilborne fungus, frequently contaminating seeds (25). Most other fungi obtained from isolations were probably saprophytic. Gliocladium is a common seed inhabitant which may often be antagonistic toward other seedborne fungi (22, 36). Trichoderma is a common mycoparasite and colonizer of organic matter in soil (23); it often parasitizes plant pathogenic fungi (13, 18). Mucor is a common saprophytic soil inhabitant (27), Graphium often colonizes wood (28), and Dictyostelium is an epiphytic cellular slime mold (6).

Actual roles of any of these fungi in causing disease to ponderosa pine seeds and hypocotyls cannot be determined without pathogenicity tests involving inoculation of seeds. Based on previous experience, the most probable pathogens likely are Fusarium, Trichothecium, and possibly Aspergillus and Penicillium. Although the other fungi may be involved in disease initiation, they probably play a secondary role.

Several factors may have contributed to the relatively low levels of seed germination obtained. Our levels were much less than the normal germinative capacity previously reported (31, 32). A large percentage of the ungerminated seed had open seedcoats and probably would have germinated if fungi had not invaded their endosperms. Also, more than 11 percent of the seed evaluated were empty (without endosperms) and never would have germinated. These seeds either did not develop properly or were parasitized by insects or fungi during their development.

If more effective treatments can be developed to reduce contamination by pathogenic fungi, seed germinative capacity and subsequent survival of seedlings should improve. Testing other chemicals or higher doses of hydrogen peroxide and sodium hypochlorite would be beneficial. More careful selection of seed sources may be necessary; fungal contamination is especially severe on seed from squirrel caches (29).

LITERATURE CITED

1. Andersen, H. and P. Neergaard.
   1956. Statens Plantetilsyn vedr. fropatologisk kontrol, 1952-1953. Tidsskr. Plavl. 59:867-876.

2. Barnett, H. L.
   1960. Illustrated genera of imperfect fungi. Burgess Publ. Co. Minneapolis. 225 p.

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

4. Bessey, E. A.
   1965. Morphology and taxonomy of fungi. Hafner Publ. Co., New York. 791 p.

5. Bloomberg, W. J.
   1971. Diseases of Douglas-fir seedlings caused by Fusarium oxysporum. Phytopathology 61:467-470.

6. Bonner, J. T.
   1959. The cellular slime molds. Princeton University Press, Princeton. 150 p.

7. Carter, M. C. and L. Jones.
   1962. The effect of hydrogen peroxide on the germination of loblolly and slash pine seed. USDA-For. Serv., Southeastern For. Expt. Stn., Res. Paper SE-141. 12 p.

8. Cayford, J. H. and R. M. Waldron.
   1967. Effects of captan on the germination of white spruce, jack and red pine seed. For. Chron. 43:381-384.

9. Ching, T. M. and M. C. Parker.
   1958. Hydrogen peroxide for rapid viability tests of some coniferous tree seeds. For. Sci. 4:128-134.

10. Christensen, C. M. and H. H. Kaufmann.
    1965. Deterioration of stored grains by fungi. Ann. Rev. Phytopath. 3:69-84.

11. Christensen, C. M. and F. Lopez.
    1963. Pathology of stored seeds. Proc. Int. Seed Test. Assoc. 28:701-711.

12. Clements, F. E. and C. L. Shear.
    1971. The genera of fungi. Hafner Publ. Co., New York. 496 p.

13. Dennis C., and J. Webster.
1971. Antagonistic properties of species-groups of Trichoderma. II. Production of volatile antibiotics. Trans. Brit. Mycol. Soc. 57:41-48.

14. Doyer, L. C.
1938. Manual for the determination of seed-borne diseases. Int. Seed Testing Assoc., Wageningen. 59 p.

15. Edwards, D. G. W. and J. R. Sutherland.
1979. Hydrogen peroxide treatment of Abies seeds. Can. For. Serv. Bi-monthly Res. Notes 35:3-4.

16. Filer, T. H., Jr. and G. W. Peterson.
1975. Damping-off. In Peterson, G. W. and R. S. Smith, Jr, (Tech. Coord.). Forest Nursery diseases in the United States. USDA-For. Serv. Agr. Handbook 470. pp 6-8.

17. Harvey, G. M. and L. R. Carpenter.
1975. Fungi on stored Douglas-fir cones - a problem? USDA-For. Serv. Tree Planters' Notes 26(4):16-17, 22.

18. Haskins, R. H. and N. R. Gardner.
1978. Effects of Trichoderma on sexual reproduction of some species of Pythium and Phytophthora. Can. J. Bot. 56:1651-1654.

19. Lock, W., J. R. Sutherland, and L. J. Sluggett.
1975. Fungicide treatment of seeds for damping-off control in British Columbia forest nurseries. USDA-For. Serv. Tree Planters' Notes 26(3): 16-18.

20. Malone, J. E. and A. E. Muskett.
1964. Seed-borne fungi. Description of 77 fungus species. Proc. Int. Seed Test. Assoc. 29:179-384.

21. Mason, G. N. and E. P. Van Arsdel.
1978. Fungi associated with Pinus taeda seed development. Plant Reptr. 62:864-867.

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

23. Norton, D. C.
1954. Antagonism in soil between Macrophomina phaseoli and selected soil inhibiting organisms. Phytopathology 44:522-524.

24. Pawuk, W. H.
1978. Damping-off of container-grown longleaf pine seedlings by seed-borne fusaria. Plant Dis. Reptr. 62:82-84.

25. Pegg, G. F.
1974. Verticillium diseases. Rev. Pl. Path. 53:157-182.

26. Snyder, W. C. and T. A. Toussoun.
1965. Current status of taxonomy in Fusarium species and their perfect stages. Phytopathology 55:833-837.

27. Stevens, R. B.
1974. Mycology guidebook. University of Washington Press. Seattle. 703 p.

28. Stewart, E. L., M. E. Palm, J. G. Palmer, and W. E. Eslyn.
1979. Deuteromycetes and selected Ascomycetes that occur on or in wood: an indexed bibliography. USDA-For. Serv. Gen. Tech. Rept. FPL-24. 165 p.

29. Sutherland, J. R. and E. Van Eerden.
1980. Diseases and insect pests in British Columbia forest nurseries. Can. For. Serv. - British Columbia Min. of For. Joint Rept. No. 12. 55 p.

30. Tarr, S. A. J.
1962. Diseases of sorghum suden grass and broom corn. Commonwealth. Mycol. Inst. Ken, Surrey. 380 p.

31. Toomey, J. W. and C. F. Korstian.
1942. Seeding and planting in the practice of forestry. John Wiley & Sons, Inc., New York. 520 p.

32. Toomey, J. W. and C. L. Stevens.
1928. The testing of coniferous tree seeds at the School of Forestry, Yale University, 1906-1928. Yale Univ. School of Forestry Bull. 21. 46 p.

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

34. Urosevic, B.
1961. The influence of saprophytic and semi-parasitic fungi on the germination of Norway spruce and Scots pine seeds. Proc. Int. Seed Test. Assoc. 26:537-556.

35. Vaartaja, O.
1956. Screening fungicides for controlling damping-off of tree seedlings. Phytopathology 46:387-390.

36. Weindling, R. and H. S. Fawcett.
1936. Experiments in the control of Rhizoctonia damping-off of citrus seedlings. Hilgardia 10:1-16.

37. Wogan, G. N.
1966. Chemical nature and biological effects of the aflatoxins. Bact. Review 30:460-470.