Fungi on stored Douglas-fir cones-a problem?

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In as little as 1 week fungi grew on Douglasfir cones being air dried before processing. Recommendation: do not store Douglas-fir cones longer than you have to.

Nurserymen often wonder whether the visible fungus growth which frequently appears on cones awaiting processing reduces quality and viability of seed. In an attempt to answer these questions and evaluate cone storage practices at the Wind River Nursery, Carson, Washington, we set up a cone storage-fungicide test.

Many factors affect viability of processed seed, but fungi have long been considered to be very important. Recent research by Gordon (4). Bloomberg (3), Timonin (9), and Jong and Chen (6) suggests the existence of distinct external and internal microfloras of coniferous seed. The origin and significance of the microfloras are still uncertain.

Rediske and Shea (81 reported significant reduction in Douglas-fir seed viability when freshly picked cones of high moisture content (60 percent) were sacked or stored in outside bins in the fall. Immediate, gentle drying or refrigeration maintained viability. They isolated approximately 35 different fungi from Douglas-fir seeds and felt that the particular fungus species present was more important in influencing deterioration than the relative abundance of organisms.

Lavender (7) found no loss in the germinative capacity of Willamette Valley Douglas-fir seed stored in the cones up to 4 months at normal fall temperatures in an unheated warehouse.

Barton (2) found that full viability of extracted Douglas-fir seed was retained for 3 Nears when stored in sealed cans at WF: however, when stored in canvas bags, germination was somewhat reduced within 6 months and seriously reduced after 12 months of storage.

In current cone collecting practice in the Douglas-fir region of the Pacific Northwest, harvested cones arc placed in burlap sacks which are piled on the ground or in a shed while awaiting shipment to the seed extractory. At the extractory, the sacks of cones are usually placed on racks in open sheds to air-dry. This preprocessing air drying may last 3 to 6 months (September to March) before the cones are kiln-dried and the seed extracted and cleaned. During this period, the cones are subject to intervals of high humidity which often lead to molding that may reduce the number of viable seed.

The objectives of our study were:

- To determine if there is a significant fungus-caused loss in the germinative capacity of Douglas-fir seed air-dried in the cones at normal out side fall temperatures for 1, 2, 4, 8, and 16 weeks.
- To determine whether sodium hypochlorite 1 household bleach 1,1) diluted 1:3 (1.3percent solution) or phenacridane chloride' (1) at 100 and 200 p/m would effectively reduce the "fungus load" of stored cones and the subsequent1v extracted seed.
- To determine whether the "fungus load" of cones or seed is correlated with germinative capacity.

Materials and Methods

Five bushels of Douglas-fir cones were picked in mid-September of 1'168 from five areas of different elevations on the Wind River District, Gifford Pinchot National Forest, Washin2ton. Each bushel of cones was from one tree only. As soon as possible after picking (no more than 21 hours), each bushel was divided into 21 equal portions for the 120 treatment-storage combinations of the experiment. The fungicidal treatment soaks were begun at once.

These were:

- One hour soak in 1.3-percent sodium hypochlorite.
- One hour soak in phenacridane chloride-100 p / m.
- One hour soak in phenacridane chloride-200 p/m.
- Controls-no treatment.

Following the 1 hour soak and a draining period of 15 minutes, each cone lot was placed in a small cotton sack. The sacks were then sorted by soak treatment and air-drying periods so that only cones with the same soak treatment and drying period would be stored together. Except for cone lots to be processed immediately, the grouped cotton sacks were then put in large, clean burlap sacks, placed

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1 **Furnished as "Acrizane" by Abbott Laboratories, Scientific Divisions, North Chicago, Illinois 60064.**

ona rack in a covered cone-drying_ shed. and not disturbed until removal at the end of 1. 2. -I. 8. and 16 weeks. respectively. Drying began oil September 18. 1968, fall drying periods) and concluded on January T. 1969 116-week drying period). Immediately after draining, cones of the control treatment (no air-drying) were dried in the Rind River Nursery cone kiln under operational conditions (automatic controls set for 99°F and 17-percent relative humidity) . When the cones were dry and open, the seed was extracted, dewinged, cleaned, and placed in paper sacks for storage at W'F for 26 weeks to simulate nursery practice. The moisture content of seed going into storage was about 5 to 6 percent. These same kiln drying, cleaning. and seed storage procedures were followed at the conclusion of each outside air-drying period .[2] At the end of this storage period (26 weeks, 0°F), seeds were moist-stratified for -l weeks at 34°F and then germinated on moist perlite in a germinator in 16 hours of darkness at 68°F, alternated with 8 hours of light at 86°F. Germinants were counted weekly for 1 weeks.

The "fungus load" of randomly preselected cone and seed replicate lots was assayed as the cones came from air-drying and before the cleaned seed leas placed in cold storage. All treatments and drying periods were represented. but not all replicates. The "fungus load" was estimated by standard dilution plates described by Johnson et al. Surface washings of the cones (diluted 1:10.000) and completely macerated seed (diluted 1:100.000) were plated oil malt-salt agar (3-percent malt: 7.5-percent salt) : colonies were counted after incubation for 1 beck at 68°F.

Results and Conclusions

As the cones were removed front shed storage. fungus growth was visible oil the surface of almost all cones regardless of the length of the drying period or soak treatment. In fact. during preparation of the treatment replicates, we noted that fungi grew on some of the cone lots overnight.

Based on mean percent germination at the end of 4 weeks, no germination benefit resulted from the fungicidal soak treatments (table 11. The generally poor germination performance of the control group suggests that the cones were picked too early and that the seed was immature.

Table 1 shows that regardless of treatment. most of the seed lots showed increases in germination through l weeks of storage in the cones. a slight decrease after -1 weeks. and a sharp decrease between 8- and 16-week storage. It is possible that one or more of three factors was responsible: (1) an afterripening process may have been taking place through the fourth week of cone storage: (2) after the fourth week, fungus degradation may have exceeded any afterripening gains, resulting in reduced germination:(3) the extra 12 weeks of 0°F storage from the 16-week treatment in vapor-pemeable packaging may have also contributed to reduced germination, as suggested by Barton (2).

"The "fungus load" of cones and seed sampled by the cone surface washings and front the extracted. macerated seed varied so erratically that the differences were statistically meaningless (table 2) and were not correlated with germination (table 1) . We can offer no reason for this variation other than sampling error or the possibility that the small inner sacks created differing microenvironments favorable to heavily sporulating genera such as *Penicillium.*

Fungal genera most frequently cultured from cone dilution plates were *Penicillium, Cladosporium, Aspergil*

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2 Seed from 16-week air-drying period stored for an additional 12 weeks (38 weeks total) before stratification due to lack of germinator space.

3 Ten ml sterile water per gram cone or seed; ovendry basis.

Table 1.—Mean germination percent of Douglas-fir seed from cones treated with fungicides and stored for varying periods under outdoor conditions

Cone storage period	Untreated checks	Chlorine bleach 1.3 percent	Phenacridane chloride 100 p/m	Phenacridane chloride 200 p/m	Storage period means
Weeks			*Percent*		
None	55.7	53.3	56.1	49.4	53.6
1	60.5	60.5	67.2	62.6	62.7
2	67.1	60.5	64.9	60.4	63.2
4	68.5	66.9	68.5	70.4	68.6
8	65.4	64.6	63.6	62.2	63.9
16	53.3	38.4	43.2	44.1	44.7
Treatment means	61.8	57.4	60.6	58.2	—

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lus, and *Trichoderma.* Fewer genera were cultured from seed dilution plates; *Penicillium* and yeasts were the most frequently encountered.

Under the conditions of our test, we did not determine whether fungi on stored Douglas-fir cones are a problem. It is clear, however, that seed remaining in cones for *up* to *16* weeks results in decreased germination and fungi are probably responsible for this loss. We recommend that extended storage of Douglas-fir cones be avoided.

The cooperation and interest o f the staff of the Wind River Nursery greatly facilitated this study. Particular thanks are due Frank E. Morby, then Nursery Operation Assistant, now Nurseryman, Lucky Peak Nursery, Boise, Idaho, for his interest and attention to detail far beyond his normal duties.

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PESTICIDE PRECAUTIONARY STATEMENT

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State or Federal agencies before they can be recommended.

Caution: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife-if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

Table 2.—Number of fungal colonies from surface washings of Douglas-fir cones and extracted, macerated seed. Colony count per gram of cone or seed, ovendry basis

Cone storage period	Untreated checks	Chlorine bleach 1.3 percent	Phenacridane chloride 100 p/m	Phenacridane chloride 200 p/m	Storage period means
Weeks			*Number*		
			CONES		
None	25	34	10	5	18
1	223	400	2	502	282
2	289	7	79	154	132
4	619	6	614	29	317
8	1,187	29	281	1,093	648
16	1,737	152	342	573	701
Treatment means	680	105	221	393	—
			SEED		
None	1,460	30	90	60	410
1	70	40	30	50	48
2	40	90	100	1,580	452
4	220	80	30	290	155
8	300	50	140	120	152
16	270	160	210	140	195
Treatment means	393	75	100	373	—