SWEETGUM POLLEN TESTING

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Abstract. --Freshly extracted sweetgum pollen germinated within 4 hours on hanging drops of Nygaard's medium. Ninety to 100 percent of fresh pollen germinated, but after forced-air extraction and refrigerated storage, germination varied from 4 to 71 percent. With the procedure described, pollen can be rapidly and conveniently tested for viability prior to controlled pollinations.

Additional keywords: Germination medium, pollen application, pollen extraction, pollen germination, Liquidambar styraciflua.

Fast, simple, and accurate techniques for testing pollen germination are needed if viability is to be checked before making controlled pollinations. With the technique described, freshly collected sweetgum (Liquidambar styraciflua L.) pollen germinated in 4 hours on a medium that is simple to prepare and needs no autoclaving.

METHODS

Catkin-bearing branches were collected from three trees on the Harrison Experimental Forest near Gulfport, Mississippi, during the last week in March 1970. Anthers were mature at the time of collection. Pollen was collected in three ways: (1) branches inserted into water-filled bottles in the laboratory and pollen collected as anthers dehisced; (2) mature catkins (one layer thick) dried in kraft bags and pollen extracted by shaking and screening through voile cloth; and (3) the forced-air techniques described by Snyder (1961) for pine pollen. Extracted pollen (2 and 3 above) was stored at 4° C in cotton-stoppered vials at 50 percent relative humidity.

Germination was tested in the laboratory at 22° to 24° C using Nygaard's (1969) medium, designated BKPS, This medium was prepared by dissolving 100 mg H_3BO_3, 300 mg $Ca(NO_3)_2 \cdot 4H_2O$, 200 mg $MgSO_4 \cdot 7H_2O$, 300 mg KH_2PO_4, and 100 g sucrose in 1 liter of distilled water, and adjusting to pH 5.2 with 0.01 N NaOH, Culture chambers similar to those described by Fechner (1958) were prepared by pouring paraffin to a depth of 5 mm in 60 x 20 mm disposable petri dishes. Twelve holes were cut with a No. 3 cork borer as the paraffin solidified, Two drops of medium filled each hole sufficiently to allow the drop to hang with minimum convexity when inverted. When applying pollen the dish cover was removed and a mylar tube, 48 x 200 mm long, inserted to

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enclose the holes. A dehiscing catkin or a camel hair brush filled with pollen was inserted at the top of the tube. A gentle tap sent pollen falling onto the medium in a well-distributed pattern. There were no convection disturbances. The dish was in verted immediately after replacing the cover. Viability was estimated after 4, 18, and 21 hours by counting the number of pollen grains out of 100 with intact pollen tubes whose length was twice the diameter of the grain.

RESULTS AND DISCUSSION

Fresh pollen.--All pollen grains from two of the trees germinated. Germination from the third tree averaged 98 percent and ranged from 90 to 100 percent. Germination was complete in 4 hours, In one test pollen from tree No, 1 was mixed into the medium rather than dusted on the surface. Mixing depressed germination percent (mean 55 percent, range 14 to 100 percent) and increased germination time to 21 hours.

Stored pollen.--Drying pollen in kraft bags in the laboratory depressed germination slightly. After 40 hours of drying and 30 hours of refrigerated storage, germination ranged from 80 to 97 percent. Drying for 64 hours and storage for 6 hours resulted in slightly better germination--89 to 98 percent. Time required for complete germination of stored pollen increased to 18 hours.

Forced-air drying for 96 hours followed by refrigerated storage for 7 hours was devastating. Germination ranged from 4 to 71 percent and time for complete germination increased to 21 hours.

Pollen should be dusted onto the surface rather than mixed into solution with the medium. Surface germination simulates natural conditions under which pollen grains adhere to stigmatic surfaces and receive nutrients, moisture, and aeration. Clumping of grains toward the center is also reduced when pollen is on the surface.

The treatment described here--surface application of pollen onto a nearly complete nutrient medium in a specialized hanging-drop chamber--has not been reported previously. Duffield (1954) and Worsley (1959) tested conifer pollens in hanging drops of distilled water. Nygaard (1969) used BKPS, but not in a hanging drop, to study germination in _Pinus mugo_, Fechner (1958), testing four Rocky Mountain conifers, mixed pollen in 2 percent sucrose solution in a hanging-drop chamber similar to the one reported in this paper. Tucovic (1972) [2/] germinated Quercus robur pollen in hanging drops of 15 percent sucrose solution.

[2/] Tucovic, Aleksandar. Develop breeding techniques for oaks. In PL 480 Final Technical Report (Project E 30-FS-6) (Unpublished), p. 10-18. Institute for Forestry and Wood Industry, Kneza Viseslava 3, Belgrade, Yugoslavia. 1972.

Germination of fresh sweetgum pollen from dehiscing anthers can be quickly, easily, and accurately tested in modified hanging drops of BKPS culture medium. No autoclaving is necessary because the test is completed within 4 hours (before fungi develop). The medium can be stored in a refrigerator at 4^{0} C for 1 week and drops placed into pre-prepared culture dishes as needed. Clumping of grains is minimized because of the short culture time and low convexity of the hanging drop surface. Grains are observed directly through the petri dish at 40X magnification without inverting or uncovering.

The successful use of hanging drop germination techniques and nutrient culture media used by others suggests that the techniques and medium reported in this paper may be effective for testing pollen from other species of hardwoods and conifers.

Extracted and stored pollen has lower germination and requires more time on the medium. Forced-air extraction as accomplished in this study destroys much of the viability.

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