LOBLOLLY PINE POLLEN GRAIN COUNTS BY OVULE DISSECTION

Fred R. Matthews and Tony E. Blalock [1/]

Abstract.--A procedure for counting pollen grains on the nucellar tissue of loblolly pine conelets after ovule dissection is described. The technique involves harvest of conelets 2 weeks after maximum pollen receptivity and the removal of individual ovule-bearing scales. After the integument is excised, the nucellus with adhering pollen grains is placed in a drop of stain on a microscope slide and the pollen grains counted. Conelets may be frozen in water for dissection later. The technique can be used to evaluate efficiency of controlled pollinations or supplemental pollination, and in studies involving flower receptivity and pollen quantity and quality.

Additional keywords: Pinus taeda, pollination, ovulate strobilus

The number of pollen grains that enter individual ovules in conifer conelets is of concern to tree breeders. The success of pollination of an ovulate strobilus depends upon the number of pollen grains entrapped in the pollen chamber of each fertile ovule. Theoretically, the more viable pollen grains within the chamber, the better the chance of subsequent fertilization and development of viable seed. In wind-pollinated conelets, multiple pollen grains per ovule can reduce the impact of self pollination and increase the chances for genetic outbreeding. The absence of pollen in the pine ovule, resulting from inadequate pollen supply or failure of the pollen to reach the nucellus, has been shown to cause first-year ovule abortion which is a major factor in reduction of seed yields (Sarvas 1962, McWilliam 1959).

Pollen grains in conifer ovules traditionally have been counted by standard histological procedures which involve paraffin embedding of the detached ovule, serial sectioning, staining, and microscopic examination (Sarvas 1962, Bramlett and Johnson 1975). This process requires considerable time and equipment, difficulties occur in recognizing individual grains in the serial sections, and grains may be lost during the procedure. A method in which pollen grains within the pollen chambers of fresh ovules could be quickly and accurately counted would overcome the limitations inherent in the histological procedures. Lill (1974) presented data on total pollen counts in and around the micropyles of fresh ovules of Pinus radiata D. Don, and Brown (1971) counted the number of pollen grains within the ovules of P. sylvestris L. However, no details of the procedures used were presented in these reports.

During the development and evaluation of a new pollinator (Matthews and Bramlett 1981) and in studies involving pollen quantities in controlled pollinations, it was apparent that there was a need for a practical method to determine the number of pollen grains in a large number of loblolly pine (P. taeda L.) ovules. This paper reports details on such a method.

[1/] Plant Pathologist and Biological Technician, Southeastern Forest Experiment Station, USDA Forest Service, Athens, Georgia

METHODS

Open and control-pollinated loblolly pine conelets were collected from one ramet of each of three clones located at the Arrowhead Seed Orchard in Pulaski County, Georgia. The conelets were collected 2, 4, and 6 weeks after maximum strobilus receptibity and were stored either in plastic bags at 3C or - 15C, or in water-filled plastic tubes at - 15C until use.

Conelets were dissected on the stage of a dissecting microscope equipped with cool (fiber-optics) illumination. Basal infertile scales were removed with a micro-scalpel until the first course of fertile scales appeared. Fertile scales were recognized by the presence of normal-appearing ovules and remnants of attached micropylar arms. Beginning at a point one-two scales above the first recognizable fertile scale, 10 sample scales were randomly selected to represent the entire fertile portion of the conelet. Each sample scale was removed from the conelet axis by a basal incision with the micro-scalpel. The detached scale was then held by self-closing forceps, and an L - shaped incision was made in the top of the ovule integument with the micro-scalpel. The loose flap of integument was folded back to expose the under-lying nucellus and the pollen chamber. The nucellus and the adhering pollen grains on its tip were excised from the underlying ovular tissue with a hooked micro teasing needle. Both ovules on each sample scale were dissected.

The nucellus was then placed in a drop of 0.5% acid fuchsin in saturated chloral hydrate on a microscope slide. A cover slip was placed over the speci-men and pressed lightly to separate the pollen grains and to enhance micro-scopic examination. Acid fuchsin stained the pollen germ tube red, while the chloral hydrate dissolved the resinous material in which the pollen grains adhere to the nucellar tip. The periphery of the nucellus and the walls of the pollen grain were also stained slightly. Both nucelli from the scale may be placed in the same drop of stain if they are kept separated.

The specimen was then examined at 100 X magnification on a compound micro-scope and the number of grains counted. Approximately 20 ovules per hour can be dissected and the grains counted by an experienced technician.

RESULTS AND DISCUSSION

Pollen grain counts in 100 ovules of each of the three clones of wind-pollinated loblolly pine showed a mean of 4.0 grains per ovule with a range of 0-7. Germination of these grains was 82%. The counts were influenced by the relative positions of the 10 sample scales on the conelet. The two lower-most and two upper-most fertile scales averaged about 30% fewer pollen grains than did scales from the middle of the conelet. Lill (1974) reported that micropylar (pollen chamber) capacity of P. radiata limited the amount of pollen that reached the nucellus, with the capacity being smaller on the basal scales. Although no measurements were made, it appeared that the ovules of loblolly pine were smaller in the apical region than those in the middle and basal regions of the conelet. Lower grain counts in the basal ovules may be caused by a shortened exposure to pollen due to the developmental progression of strobilus receptivity.

Conelet collections made 2 weeks after maximum strobilus receptivity were superior to those made 4 or 6 weeks after receptivity. In collections at 2

weeks, the pollen grains were more easily separated from the resinous deposit on the nucellar tip than in the 4 and 6 week collections in which the grains adhered tightly to the nucellus possibly due to germ tube penetration. It should be noted, however, that macroscopic evidence of the beginning stages of ovule abortion due to lack of pollen was apparent only in conelets collected 6 weeks after maximum receptivity.

Freshly collected conelets may be frozen in water if long-term storage is required. Conelets have been stored in this manner for up to 1 year with no detrimental effect on the dissection and grain counting procedures. Dry freezing or storage at 3-5C in plastic bags will suffice for periods up to 1 week.

This technique has applicability for evaluating: (1) the efficiency of controlled pollinations, (2) the effect of supplemental pollination, (3) flower receptivity, and (4) pollen quality and quantity.

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