ESTIMATING POLLEN CONTAMINATION IN
LOBLOLLY PINE SEED ORCHARDS BY POLLEN TRAPPING

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Abstract.--The construction of an inexpensive, easy-to-build pollen trap is described, which rotates to keep the trap oriented into the wind at all times. These traps have been deployed for several years, both within and surrounding four loblolly pine (*Pinus taeda* L.) seed orchards ranging in age from 7 to 25y. A comparison of pollen caught on both the orchard and background traps permits an estimate of how much pollen the orchard is producing relative to background. The resulting estimates of background contamination are very similar to those obtained with other methods.

Keywords: pollen contamination, pollination, seed orchards, *Pinus taeda*.

Loblolly pine seed orchards of the North Carolina State and Western Gulf Cooperative Tree Improvement Programs produced over 60 tons of improved loblolly pine seed in 1982, enough to grow about 1 billion seedlings (Talbert et al.., 1985; Byram et al., 1985), After genetic roguing of the orchards, the predicted volume gain at rotation from these improved seedlings is about 12%. However, these gains assume that there is no pollen contamination from outside the seed orchards. Squillace and Long (1981), citing several types of estimates of background contamination, suggest that contamination may range from 30 to over 80%, even in mature orchards surrounded by isolation zones. Friedman and Adams (1981), estimate that outside contamination in a 16-year old loblolly pine seed orchard was 28%, based on detection of several allozyme gene markers *via* electrophoresis. Pollen contamination of 30 or 80% will reduce a projected 12% gain to 10.2 or 8.2% respectively. Given the amount of seed now being produced by seed orchards, minimizing the impact of background pollen should be a major concern. Therefore, measuring orchard pollen production and the contribution of background pollen should be a routine part of quality control.

The most accurate way of assessing background contamination is by using genetic markers for such traits as allozymes or monoterpenes. But these methods, although potentially precise, require relatively sophisticated equipment and methods. A simple and possibly quite accurate method of determining background contamination is to compare pollen production within the orchard to background pollen trapped nearby. Koski (1970) used a similar method for estimating background contribution to total pollination in Scotch pine plantations in Finland. Here we describe a simple method of measuring the relative amount of pollen contributed by both the orchard and background.

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Contamination is then estimated as a simple ratio of background production to total orchard production. Estimates of background contamination by pollen trapping are being verified by comparison with estimates made by analysis of allozymes.

METHODS

Pollen flight in four Weyerhaeuser Co. loblolly seed orchards was assessed by means of pollen traps changed at 24-hour intervals throughout the period of pollen flight. The daily peak in pollen flight was detected by changing traps at hourly intervals on 4/11/80 (see Fig. 1). Each trap consists of a glass microscope slide bearing a piece of double coated tape (about 1cm^2), held at a 45° angle by a clothespin mounted on a vane which keeps the trap oriented into the wind at all times (see Fig. 1, inset).

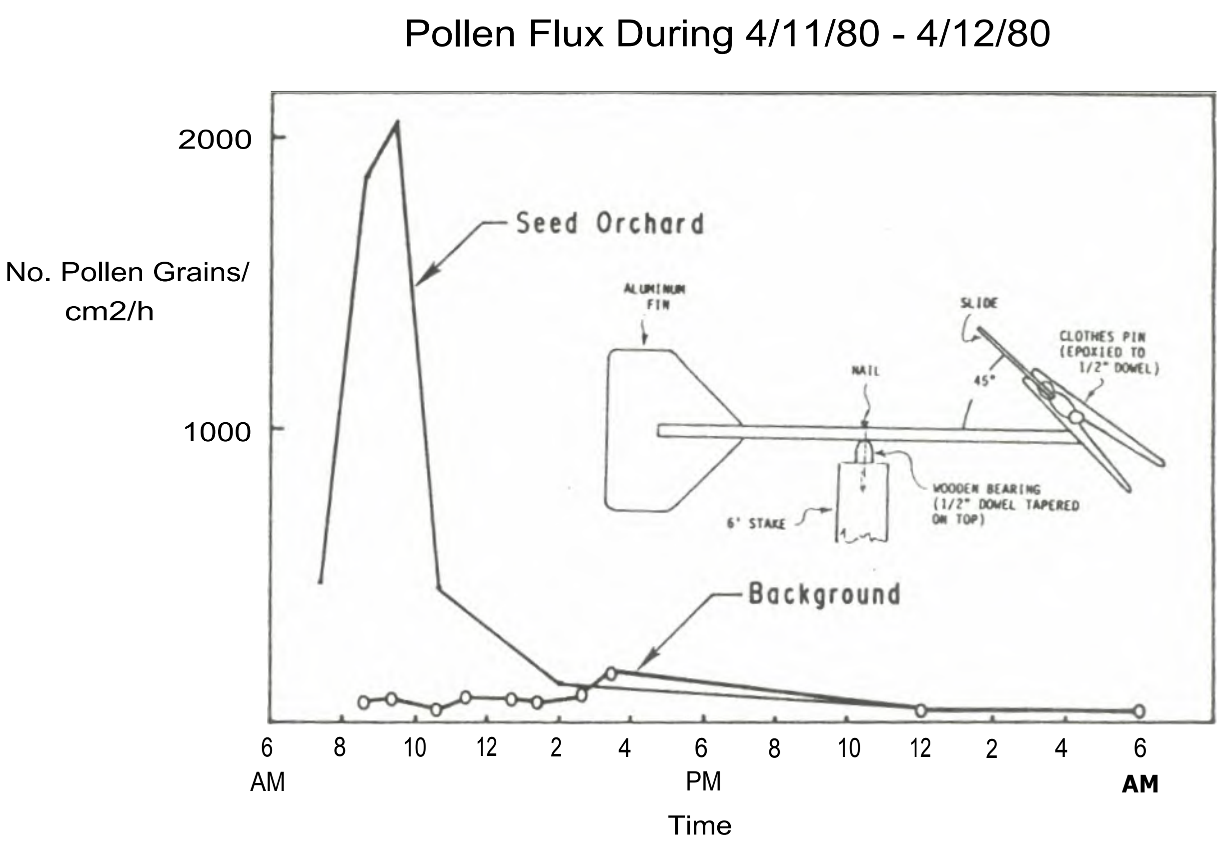

Figure 1.--Diurnal pattern of seed orchard and background pollen flight, diagram of pollen trap.

Materials required to construct the vane-type pollen trap are: 1) 0.5 in. diameter wooden dowels; 2) wooden clothespins; 3) aluminum nails (2.5 in. long x .12 in. dia.); 4) aluminum sheets (very thin aluminum plates used for the printing of newspapers, when cleaned, work very well and are cheap); 5)

2.5x7.6 cm (1x3 in.) glass microscope slides with frosted end; 6) Scotch brand #666 double coated tape with liner (0.5 in. width). The 0.5 in. wooden dowels are cut into 1-ft. lengths. One end of the dowel is then cut at a 45° angle to serve as the attachment point for the wooden clothespin. A thin slit, approximately 2 inches long, is cut in the other end of the dowel for attachment of the aluminum vane. The clothespin is glued to the dowel using contact cement or some other waterproof glue. The clothespin is oriented so that the glass slide, when held by the clothespin, is angled toward the vane end of the dowel. At this point, the dowel with clothespin attached can be painted. The aluminum vane, when cut to a desired size and shape, is inserted into the slit and attached to the dowel with aluminum nails. To avoid splitting the dowels, the heads should be cut from the nails to allow them to be mounted into a drill for insertion. Once the aluminum vane is attached, the excess nail should be cut off and filed flush with the surface of the dowel. To determine where to drill the hole in the dowel for attachment to a wooden stake, a slide is mounted on the clothespin and the balance point for the pollen trap is located. A hole large enough to allow rotation of the trap about the aluminum nail is drilled in the dowel at the balance point. A 1.25-inch segment of dowel with one end rounded serves as a bearing for pollen trap rotation (see Fig. 1).

Keeping the traps oriented into the wind resulted in up to a 7-fold increase in pollen catch over a trap fixed in a SW direction. Whenever possible, the traps were changed before rainfall, since rain will wash off significant quantities of pollen (0.6 cm of rain washed off over 60% of pollen trapped previously). After drying, however, the adhesive properties of the tape were restored.

In order to monitor pollen flight within the seed orchard, the traps were placed in open areas where trees had been removed, to minimize the impact of individual trees. Although Koski (1970) reports maximum pollen catch at crown level in Scotch pine, mounting traps in tree crowns is very inconvenient. During heavy pollen flight in the J. P. Weyerhaeuser seed orchard, we compared pollen catch on traps within the crowns of 5 different trees at a height of about 12M, with a trap located near each tree approximately 1M from the ground. The mean catch over a 24-hour period for the crown traps was about 3100 grains/cm compared with 2,800 for the ground traps. Since the difference was small, we adopted the easier option of deploying traps on 1.8M tall stakes so they could be easily reached.

Background pollen was trapped in open areas at least 300M from the edge of the orchard or any non-orchard sources of loblolly pine pollen. At one seed orchard, wind direction and velocity were recorded with a vane anemometer, while at the other three, the wind direction during orchard pollen flight (which usually occurs in the morning) was noted visually and recorded. At least four background traps were located approximately north, south, east, and west of the orchard. Traps located downwind during heavy production of orchard pollen could then be excluded from background averages. Ten traps were located throughout the seed orchard in open areas where trees had been removed.

Several transects, consisting of 4 traps 30M apart, were also placed downwind from the edge of the orchard to assess how pollen catch changes with distance from the orchard. At Lyons, background traps were also located in the center of a 300-acre field about a mile from the orchard site (the Cato traps - see Fig. 2).

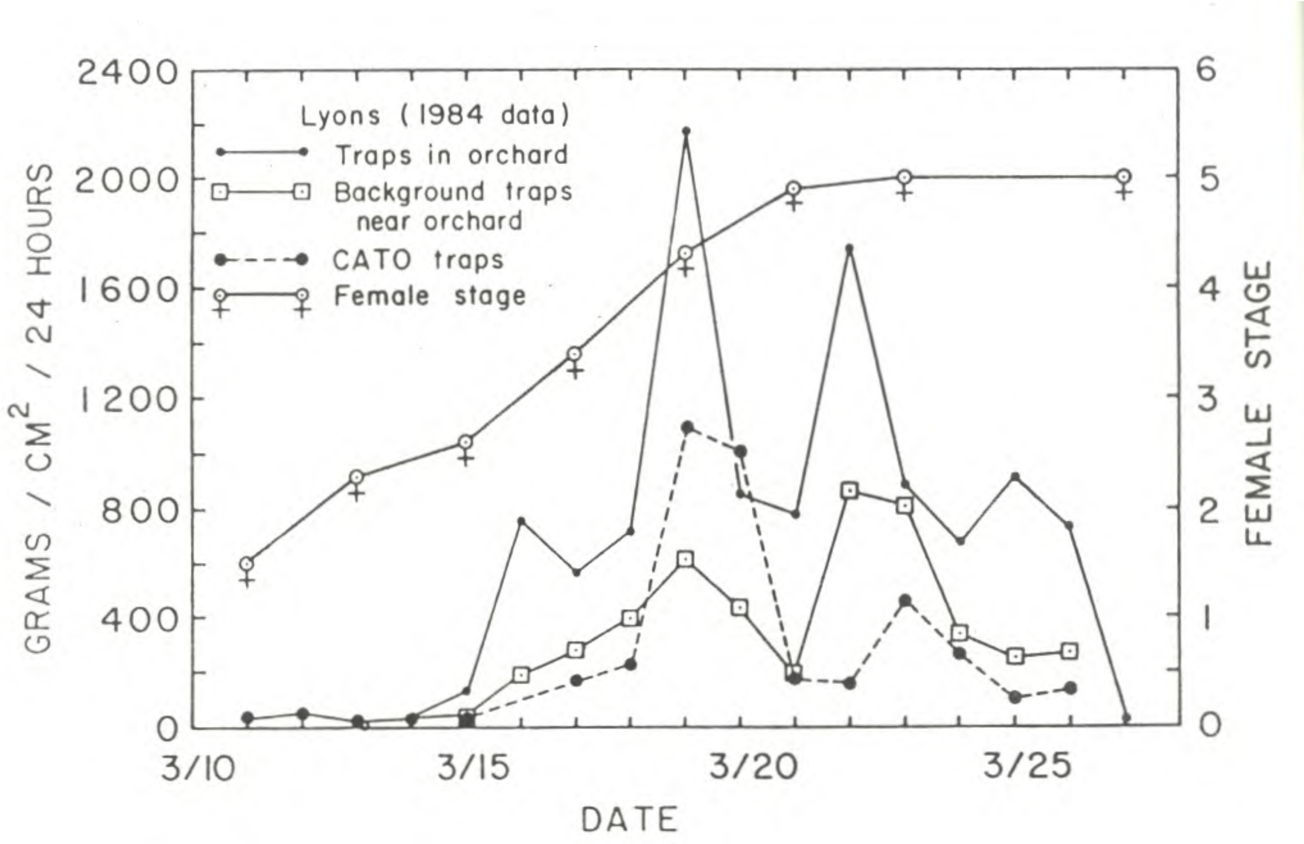

Figure 2.--Seasonal pattern of pollen flight for orchard located in Lyons, GA. Background trapped near the orchard and in a 300 acre field about 1 mile from the orchard (Cato) is also shown.

The traps were collected at the same time every day (either early morning or late afternoon, depending on the location), and the tape was covered with a plastic cover slip to prevent contamination. Number of pollen grains trapped per cm^2 super cestimated by counting 10 separate fields of view at 100x or 430x using a compound microscope. The area of the field of view was calculated after measuring its diameter with a stage micrometer. The results are presented as number of pollen grains/cm /24h.

Stage of female development was estimated for 10 clones, representing early, late, and average occurrence of female receptivity. Fifty or more strobili were observed from a lift truck on each of 3 ramets/clone every one or two days, and stage of development (Bramlett and O'Gwynn, 1980) was recorded for each.

RESULTS and DISCUSSION

After several years of observation, the orchard pollen almost always flies in mid to late morning after the catkins have dried out and when the wind is sufficiently strong to move the branch (see Fig. 1). A similar diurnal pattern is observed with other wind pollinated species (Ogden et al., 1969). Occasional afternoon pollen flights have been seen if morning rain was followed by a period of clearing and drying. Virtually no pollen is shed on rainy days. A comparison of hourly pollen catch for both an orchard and nearby background trap (upwind from the orchard) during heavy orchard pollen flight is shown in Figure 1. Note the great bulk of orchard pollen flew between 9 and 11 a.m., while background catch was fairly uniform, showing a slight peak between 3 and 4 p.m. Little pollen was trapped between 3 p.m. and 12 midnight, or 12 midnight to 6 a.m. the next day.

Since the background catch shown in Fig. 1 did not peak at the same time of day as the orchard, we can be reasonably certain that the background trap, located upwind from the orchard, was not receiving residual pollen from the seed orchard. Nonetheless, on a daily basis, background peaks, whether adjacent to the orchard or a mile away, do occur at roughly the same time as orchard peaks (see Figure 2). This suggests that the background pollen trapped was probably shed the same day as the orchard pollen. If we assume 1) that background pollen was shed at the same time as the orchard pollen; 2) that the wind velocity averaged from 10-15 mph on 04/11/80; and 3) that background catch peaks at 3 p.m. (about 6 hours after peak shed in the orchard see Fig. 1), then some of the pollen trapped late in the day could have traveled 60-90 miles.

To **assess** the possible impact of orchard pollen on background traps located downwind from the orchard, transects were located in open areas normally downwind from the orchards. Table 1 shows the catch averaged over several days versus distance from the edge of the orchard (Table 1).

Table 1.--Impact of orchard pollen on background estimates - pollen catch vs. distance downwind from orchard.

Location	Orchard	30M	60M	90M	120M	Background
J.P. Weyerhaeuser[a/]	4723	3159	2372	1979	2037	1112
Comfort[b/]	2464	2440	1846	1947	1428	909
Lyons[c/]	908	647	593	614	402	351

a/ Results from 2 transects over 8 days.
b/ Results from 2 transects over 4 days.
c/ Results from 4 transects over 12 days.

At 120M downwind from the orchard, the pollen catch diminished considerably from that in the orchard but was still 13 to 45% higher than the mean of background traps not located downwind. Wang et al. (1960) showed that while pollen catch from a single tree declines logarithmically with distance, the decline from a stand is much less steep and appears somewhat linear. Background traps that are downwind from the orchard on a given date should be excluded from background estimates. We recommend that background traps be located at least 300M from the periphery of the orchard or any local source of pollen.

A comparison of pollen trapped throughout the pollination season both inside and outside four seed orchards, over two successive years, is shown in Table 2. Note that the older seed orchards produce the most pollen, but there is considerable variation by year. Also, total background catch is not correlated well with orchard production (total orchard minus background). Variation in orchard production only explains 15% of the variation in background catch across all seed orchards and years (r =0.15), so the magnitude of background catches shown here are not significantly related to seed orchard pollen production.

The patterns of pollen flight and female receptivity shown in Fig. 2 are representative of all orchards studied here. Both background and orchard pollen flies during the receptive period of most female strobili within the orchard.

Pollination in loblolly pine is a two-step process, the first being accumulation of pollen on the micropylar horns, the second being transfer of the grains to the nucellus by rain or the pollen drop (Brown, 1984; Greenwood, 1985). Any pollen grain reaching the micropylar horns, whether it arrives early or late, has an equal chance of reaching the nucellus and presumably germinating (Greenwood, 1985). An estimate of the contribution of background pollen to total pollination (in the orchard) should, therefore, be equal to the ratio of total background to total orchard (the latter includes both background and orchard). Estimates of contamination expressed as % total orchard pollination contributed by background are shown in the last column of Table 2.

Table 2.--Background pollen catch as percent total catch in 4 seed orchards, for two yeas each. Both background and orchard pollen were trapped throughout pollination period.

Orchard	Year	TOTAL TRAPPED Background	TOTAL TRAPPED Total Orchard	Background as % Total Orchard
Washington, NC.	1983	10,868	34,362	32%
Est. 1959	1984	15,935	50,968	31%
Comfort, NC.	1983	16,342	27,086	60%
Est. 1974	1984	7,579	19,299	39%
Lyons, GA.	1983	8,372	11,209	75%
Est. 1978	1984	4,782	11,330	42%
Magnolia, AR.	1982	11,345	12,838	88%
Est. 1972	1983	16,130	23,783	68%

Estimates of contamination range from 31 to 88%, similar to the range presented by Squillace and Long (1981). As expected, the oldest orchard showed the least contamination. However, the Magnolia orchard has sustained very high contamination because background there was very high both years. On the other hand, background was consistently low for both years at Lyons. Clearly, orchard location can significantly affect background load, especially when the orchard is young.

We are currently verifying the estimation of background contamination at the Washington, N.C., seed orchard with allozyme markers, and our first results show close similarity to those presented here (N. C. Wheeler and M. S. Greenwood, unpublished data). As mentioned earlier, other workers, also using biochemical genetic markers, have obtained a comparable range of estimates for southern pine seed orchards.

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