# POLLINATION, POLLEN TUBE DEVELOPMENT AND ORCHARD NURTRIENT STATUS EFFECTS ON CONELET ABORTION IN OPEN POLLINATED LONGLEAF PINE

Early Y. McCall and Robert C. Kellison1/

Abstract.--Possible causes investigated for the 60-90% abortion rate of longleaf pine conelets at the North Carolina Forest Service longleaf clonal seed orchard at Bladen Lakes State Forest were: (1) the absence of pollen or its failure to germinate and (2) possible nutrient deficiencies or excess.

Ovule sections were examined histologically for the presence of pollen, pollen germination, and overall ovule condition. Of the 284 ovules observed, 72% had pollen present in the micropyle. Pollen germination within the micropyle was not inhibited as 78% of those ovules with pollen present had germinated pollen. Thirteen percent of the ovules with germinated pollen were aborted and the incidences significantly increased over time (.01 level using the log-likelihood ratio test). In every instance where aborted ovules were observed the pollen tube appeared normal. Therefore the abortion of conelets of these ovules was apparently not related to lack of pollen or failure of the pollen to germinate.

The possibility of a nutrient problem was investigated by analyzing Bladen Lakes longleaf orchard soil and tissue samples and comparing to standards and symptom descriptions for longleaf and other pine species. Boron was found at deficient levels at the seed orchard and descriptions of fruit drop caused by B deficiency for other species of pine and for fruit and nut trees closely resemble those found at the longleaf orchard.

Keywords: *Pinus palustris*, conelet drop, ovule, ovule abortion, seed orchard management, mineral nutrition.

From 1971 to 1975 about 80 percent of the potential cone crop from the longleaf pine seed orchard of N. C. Forest Service Bladen Lakes State Forest was lost from "conelet abortion". Conelet abortion results from inherent or environmental disorders present within the conelet, tree, or site on which the tree exists. In longleaf pine aborting conelets may appear normal for several weeks after pollination, but internal necrosis develops at the base of the conelet and spreads through the axis toward the apical end. The conelet eventually dessicates and either drops from the tree or may be retained in place for as long as a year. Conelet abortion usually begins about 6 weeks after pollination and is greatest for a short period thereafter, tapering off as the conelet growing season progresses.

The cause of conelet abortion may encompass several different factors in any one orchard or species. Other attempts to define the causes of high conelet

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1/ The authors are respectively; Tree Improvement Forester, ITT Rayonier Inc., Fernandina Beach, Florida and Director, Hardwood Research Program, School of Forest Resources, N. C. State University, Raleigh, N. C. Manuscript based on research conducted by the senior author in partial fulfillment of the Master of Science degree.

abortion at this orchard include controlled pollination studies (White, 1975), biochemical studies (White, 1975), and fertilization studies (Summerville, et al., 1979). Based on their work with radiata pine (*Pinus radiata*) and the work o Sarvas (1962) with Scotch pine (*P. sylvestris*), Sweet and Bollman (1970) concluded that both pollination levels and competition for nutrients and carbohydrates between the shoot and conelet are important factors causing conelet abortion. In this report, pollination levels and macro and micronutrient levels are investigated as potential causal agents of conelet abortion of longleaf pine (*P. palustris*) at the Bladen Lakes seed orchard.

## METHODS AND MATERIALS

The orchard was grafted in 1965 on trees of an existing longleaf plantation situated on a Lakeland soil. The soil is characterized by deep sands with poor nutrient holding capacity, excessive drainage, and low pH.

In 1973, the orchard was split into two sections to study the effect of varying amounts of fertilizer and irrigation on male and female strobilus initiation and retention (Summerville et al., 1979). In each section, three treatments were administered annually: 1)control--nonfertilized, 2) medium rate--400 lbs./acre of 0-20-20 in November, 150 lbs./acre of $NH_4NO_3$ in February, and 250 lbs./acre of $NH_4NO_3$ in July, and 3) heavy rate--500 lbs./acre of 10-10-10 in November, 300 lbs./acre of $NH_4NO_3$ in February, and 300 lbs./acre of $NH_4NO_3$ in July. Equipment failures prevented application of the irrigation treatments. These fertilizer treatments were used to study the effect of nutrient status of orchard trees on conelet abortion and the effect of fertilization on pollen tube development.

### Pollination Studies

Three ramets from each of four clones were chosen for conelet sampling from each of the three fertilizer treatments. Two apparently healthy conelets were sampled from east and west aspects of each tree at the fifth, seventh, ninth and eleventh weeks after pollination occurred during the second week of March in 1976. Each conelet was sectioned for preparation of slides from which two ovules were microscopically observed for the presence of pollen, pollen tubes and an evaluation of ovule health after pollen tube growth (McCall, 1980). Each ovule was classified as follows:

Pollination Class I - no pollen present in the micropyle
Pollination Class II - pollen present but not germin7'?.d
Pollination Class III- pollen present and germinated but ovule aborting
Pollination Class IV - germinated pollen present and ovule apparently healthy

Shortage of conelets at latter sampling dates permitted only 187 of the originally planned 192 conelets to be sampled for a total of 374 ovules. Data were analyzed using the log-likelihood ratio test (G-statistic) which demonstrated the strength of association between sampling parameters and pollination classes. The advantages of using the log-likelihood test of independence over the conventional chi-squared ($X^2$) test are: (1) the computations are less tedious than for $X^2$, (2) it more accurately follows the $X^2$ distribution than does the $X^2$ statistic, and (3) it allows for testing of more than two rows and columns (Sokal and Rohlf, 1969).

Aborting conelets containing some live ovules were also collected. Serial sections were made of two ovules per conelet and pollination classes were compared with sections from apparently healthy conelets to see if lack of pollen might have caused the conelet's ultimate demise.

### Nutrient Status Studies

Three ramets of six clones in each of the three fertilizer treatments were randomly selected for conelet counts. Counts were done separately in the two sections of the fertilizer study and were treated as replications for a total of 108 trees. These counts helped to identify the impact of fertilizer treatments on conelet abortion. Initial counts of female strobili were made in March, 1977 before conelet abortion started, and retained conelets were tallied in October, 1977.

Foliage, conelet, and soil samples were collected and analyzed for dry weight nutrient concentration by the Agronomic Division of the North Carolina Department of Agriculture to determine the nutrient status of the orchard trees. Three ramets were chosen at random from three clones within each of the three existing fertilizer treatments for a total of nine sample trees. Conelet samples were taken in the sixth week after pollination when signs of conelet abortion were becoming apparent and subsequently in the seventh, eighth and tenth weeks. Foliage samples were collected from these same nine trees only in the tenth week after pollination to avoid competition imbalances that may have occurred from removal of foliage before conelet sampling was completed (Sweet and Bollman, 1970).

## RESULTS

### Pollination Studies

For all parameters, except time, no significant association between each parameter and pollination class could be demonstrated. The number of ovules falling into the various pollination classes changed significantly (.01 level) from the fifth to the eleventh week after pollination (Table 1).

Table 1.--Number of ovules of longleaf pine by pollination class at four sampling dates from Bladen Lakes seed orchard.

| Sample Date | Pollination Class[a] | | | |
|---|---|---|---|---|
| (weeks after pollination) | | | | IV |
| Fifth week | 25 | 23 | 1 | 23 |
| Seventh week | 20 | 13 | 1 | 38 |
| Ninth week | 16 | 4 | 6 | 46 |
| Eleventh week | 20 | 6 | 13 | 31 |

a/ Pollination Class I — no pollen present in the micropyle
Pollination Class II - pollen present but not germinated
Pollination Class III - pollen present and germinated but ovule aborting
Pollination Class IV - germinated pollen present and ovule is apparently healthy

As expected, Class I did not change significantly because the number of ovules containing pollen was fixed at the time of pollination. Of all of the ovules assigned to Class I only 21 percent were aborted. Decrease in Class II shows that the pollen grains were still germinating from the fifth through the eleventh weeks. Partitioning of the pollination classes using the log-likelihood analysis showed that increase in germinated pollen grains over time (Class I and II X Class III and IV dependence) was significant (.01 level). Increase in Class III in the ninth and eleventh weeks demonstrated that even though pollen had germinated, incidence of ovule abortion increased. Class IV remained fairly constant although there was a decrease in the number of healthy ovules containing germinated pollen from the ninth to the eleventh week.

When ovules from 42 aborting conelets were compared to those from 187 apparently healthy conelets, 72 percent of the ovules from nonaborted conelets and 59 percent from aborted conelets had at least one pollen grain (Classes II, III, and IV). The difference between aborting and healthy conelets could have been a reflection of the sample size difference, although 42 aborting conelets should have rendered a fair comparison. Only a few pollinated ovules are needed for normal maturation of reproductive structures in shortleaf pine (Bramlett, 1972) and in slash pine, controlled pollinations may produce apparently normal cones with only a few viable seeds.

There also seemed to be adequate germination of pollen, even in aborting conelets (Class III). It appears, therefore, that something other than failure of the pollen to germinate caused the conelets to abort. It also appears that ovule abortion did not directly affect conelet abortion since there was not a large number of aborted ovules in conelets appearing normal even though conelets were collected during the period of maximum conelet abortion.

Conelet Loss Determination within Fertilizer Treatments

Counts of open pollinated female strobili and subsequent conelet abortion from 1971 to 1975 by the N. C. Forest Service showed abortion rates of 26, 96, 88, 100, and 89 percent, respectively. Conelet losses from counts done for this study in 1977 were 56 percent.

Percent retention of conelets in the two replications of this study were best in the nonfertilized plots, although differences among fertilizer treatments are not significant using a covariance analysis. Investigators thus far have found that increased flowering and greater cone and seed yield are obtained from increased fertilization (Shoulders, 1967; Shoulders, 1968; Sweet and Bollman, 1970; Sarvas, 1962; Barns and Bengtson, 1968). However, these results are for species other than longleaf pine. Heavy applications of fertilizers in this longleaf orchard could be causing an adverse effect on conelet retention (Table 2).

Table 2.--Percent of longleaf pine female "flowers" [a] counted at Bladen Lakes seed orchard in March, 1977, retained as conelets in October, 1977, by fertilizer treatment.

| | Nonfertilized | Normal Fertilization | Heavy Fertilization |
|---|---|---|---|
| | % female flowers retained | | |
| Section I | | | |
| Section II | 37 | 24 | 24 |

y owers judged to healthy were scored.

b/ Each percentage figure represents 18 trees. Flowers and conelets were counted on the southeast quadrant of each tree. Branches counted in March were identified with spray paint to minimize errors in the subsequent October conelet count.

## Nutrient Concentration by Fertilizer Treatments

Although P and K had been added to the normal and heavy rate treatments by way of inorganic fertilizers there was no difference in conelet or needle nutrient concentration between the fertilized plots and the controls (Table 3). At the time of this study, fertilizer had been withheld from the control plots for four years. Soil samples from these plots showed that the amount of elemental P and K in the normally fertilized plots was higher than that in the heavily fertilized plots but values of both exceeded the control. This was expected as more fertilizer P and K was actually added to the normally fertilized plots than to the heavily fertilized plots. There was no difference in conelet or needle P and K among treatment plots despite the fact that these nutrients are considered highly mobile within trees (Greulach, 1973).

Table 3.--Nutrient analysis of collections made at four sampling dates by fertilizer treatment at Bladen Lakes longleaf seed orchard.

| | Mean concentration in conelets by dry weight | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | ppm | | | | |
| Treatment | N | P | K | Ca | Mg | Na | Fe | Mn | Zn | Cu | B |
| Heavy Fertilization | 1.93 | .24 | .80 | .13 | .13 | .04 | 21 | 78 | 61 | 5 | 19 |
| Normal Fertilization | 1.80 | .24 | .85 | .12 | .14 | .02 | 21 | 82 | 56 | 6 | 15 |
| Control (nonfertilized) | 1.57 | .23 | .83 | .17 | .14 | .03 | 18 | 97 | 66 | 6 | 14 |

(Table 3 continued)

| Treatment | Mean concentration in needles by dry weight | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | | | | | | ppm | | | | |
| | N | P | K | Ca | Mg | Na | Fe | Mn | Zn | Cu | B |
| Heavy Fertilization | 1.52 | .09 | .44 | .30 | .10 | .03 | 50 | 79 | 25 | 4 | 10 |
| Normal Fertilization | 1.24 | .08 | .40 | .30 | .11 | .04 | 53 | 56 | 25 | 4 | 7 |
| Control (nonfertilized) | 1.07 | .10 | .40 | .33 | .12 | .03 | 49 | 55 | 37 | 4 | 6 |

| Treatment | Mean nutrient levels in soil samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | lbs./acre[a] | | | | ppm | | |
| | pH | P | K | Ca | Mg | Mn | Zn | Cu |
| Heavy Fertilization | 5.0 | 18 | 36 | 559 | 73 | 1.7 | 1.1 | .07 |
| Normal Fertilization | 5.6 | 34 | 43 | 720 | 162 | 1.9 | 1.2 | .22 |
| Control (nonfertilized) | 5.3 | 13 | 16 | 501 | 121 | 1.7 | 1.5 | .15 |

a/ lbs./ac. values are equivalent to kg./ha.

## Nutrient Sufficiency Levels

Nitrogen levels in conelets and needles were directly related to the amount of N applied. Copper and manganese were found at low levels and boron was found at deficient levels in needles collected from the first flush of the previous year's growth (Table 3). Deficiency symptoms usually occur in *Pinus* spp. when needle levels of Cu and Mn drop below approximately 2 and 40 ppm, respectively, and when B levels drop below 10-15 ppm (Stone, 1967).

Low Mn and Cu are not thought to hinder the retention of reproductive structures (Epstein, 1972; Kozlowski, 1971) however, B deficiency in apples (*Malus* spp.) and walnuts (*Juglans regia*) cause visual symptoms and subsequent fruit abortion quite similiar to that at Bladen Lakes seed orchard. Vegetative growth may also be affected by B deficiency in pines, but usually only after reproductive growth is inhibited or abortion occurs (Sprague, 1964). Levels of B were also deficient (mean of 6 ppm) in needle samples collected from a collaborative study involving 30 trees in the Bladen Lakes seed orchard in February, 1977.

Boron may be held unavailable to plants by high levels of K, N, and especially Ca (Epstein, 1972; Chapman, 1966). Calcium has been applied heavily in the form of lime to the Bladen Lakes Orchard especially in the early years of its establishment. Heavy liming has been found to result in B deficiency (Brady, 1974) on deep acid sands such as those found at this orchard. In naturally acid soils B is available largely in a soluble form ($H_3BO_3$) which may be easily leached. Soils in the area surrounding the Bladen Lakes orchard had an average pH of 3.9, therefore much of the soil's B may have been depleted before orchard establishment in 1965. Additions of Ca in the form of lime would help to complex more B. A study was established in 1978 to determine the effect of B on conelet abortion at the Bladen Lakes orchard, but frost damage and limited flowering prevented its completion.

## CONCLUSIONS

Change in pollination classes over time was caused by two factors: 1) increased occurrence of germinated pollen and 2) increased ovule abortion among ovules with germinated pollen. Even though ovules with nongerminated pollen decreased, germination seemed to have been delayed as pollen was still germinating even in the eleventh week of collection. Possibly some growth substance supplied by germinating pollen is produced too late to prevent ovules from aborting. Some investigators have found growth substances being expelled from germinating pollen which are believed to have a role in ovule development and survival (Sweet, 1973; Sweet and Lewis, 1969; Sweet and Lewis, 1971). Sarvas (1962) found that Scotch pine ovules gradually deteriorate after the third week past strobilus receptivity if they are not pollinated. Pollen associated growth substances should be identified and research done to determine if they are produced in time to prevent conelet abortion. Inhibitors and nutrient deficiencies may also cause the pollen to be late in germinating. Boron deficiency has been found to cause poor pollen germination (Stanley and Linskins, 1974), but further studies should be conducted to determine if the pollination delay is caused by B shortage at Bladen Lakes longleaf orchard.

Increased ovule abortion among ovules with germinated pollen was observed during the sample period. In every instance, even where aborted ovules were observed, the pollen tube appeared normal suggesting the ovules would have aborted regardless of the presence or absence of germinated pollen. When ovules within aborting and nonaborting conelets were compared, very few aborted ovules were found in apparently normal conelets, therefore ovule abortion appears to be secondary to conelet abortion. Since most of the conelets were collected before or during the period of maximum conelet loss, conelets with the potential to abort should have been identified by a large number of aborted ovules if ovule deterioration occurred before conelets aborted.

Lack of pollen or failure of pollen to germinate was ruled out as a direct cause of abortion based on the high occurrence of pollen and germinated pollen within ovules. Upon microscopic examination, no mycelial growth was found inside conelets or ovules, also eliminating fungus contamination as a possible cause of abortion.

High doses of NPK fertilizer may have a damaging effect on cone crops, especially in highly leachable soils. A balanced fertilizer, preferably containing a slow release micronutrient mix, should be applied to these sites and foliage nutrient levels monitored to make sure orchards being supplied with all essential nutrients.

More research is needed on effect of competition for carbohydrates between vegetative and reproductive structures in longleaf pine. Conelet abortion begins and is heaviest for a short period of time after the sixth week past the point of maximum flower receptivity. This period of time coincides with occurrence of the second flush of needle growth which is quite demanding on available carbohydrates. By removing needles which would otherwise compete with conelets for these available carbohydrates, Sweet & Bollman (1970) concluded that conelet abortion was reduced. This same approach could be taken to study competion effects at the Bladen Lakes orchard.

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