APPLICATION OF RADIOGRAPHY TO SEED VIABILITY PROBLEMS IN YELLOW-POPLAR SEED ORCHARDS

Russell A. Cox[1/]

Abstract. --Cross-pollinated samaras from 25 clones in a yellow-poplar breeding orchard were subjected to radiograph and germination analysis. A high correlation was found between filled samaras identified by radiographs and samaras that germinated. Using clones as females, significant differences were observed among clones for percent filled samaras; no such differences were present among pollen parents. Viability tended to be higher when clones were cross-pollinated than when the same clones were open-pollinated.

The large number of seed of low viability (normally less than ten percent) produced by yellow-poplar (Liriodendron tulipifera L.) is perhaps the biggest problem face in a seed orchard made up of this species. The main objective of this study was to explore a method of nondestructive viability testing that could result in a reliable estimate of viable samaras for this species.

Pioneer work in the use of radiography in forest tree seed testing began in Sweden in the early 1950's. These researchers studied the usefullness and the effects of x-rays on the seeds of various forest species. Several properties of the seed sample, including percentage of filled seed and percentage of seed lacking embryos, could be diagnosed without destroying the seed sample (Simak and Gustafsson, 1953).

Practical application of techniques and refinement of methods during the 1950's transformed radiography from a laboratory oddity to a useful tool for testing forest tree seed. For instance, Klaehn and Wheeler (1960) reported on the possibility of radiography being of use in determining seed quality and observing embryo development of seed produced by various breeding methods. Taft (1962) used x-ray analysis to study the effect of controlled pollination on yellow-poplar seed quality. Many tree species that were difficult to analyse by other methods were readily adaptable to radiography. Proponents of this method of analysis have claimed that it is superior to other methods of viability testing due to its quickness and nondestructive characteristics.

The primary advantage to using radiography with yellow-poplar samaras is the conservation of the seed sample. This is particularly valuable when the seed subject to sampling are not available in quantity or are the result of carefully performed cross-pollination. X-ray negatives show clearly whether or not there is endosperm present within the samara. It has been established that if endosperm is present an embryo is also present (Wean and Guard, 1940). Therefore, it can be assumed that if endosperm is present, the samara is viable.

[1/] Graduate Research Assistant, Forestry Department, The University of Tennesse Knoxville.

METHODS AND PROCEDURES

Pollination of Flowers and Harvesting of Samaras

The technique used to acquire cross-pollinated samaras for this study was similar to that described by Wilcox and Taft (1969) and consists of emasculating an unopened flower bud and pollinating the exposed gynoecium with pollen from a desired source. Emasculation is carried out by choosing unopened buds, preferably just prior to natural opening, and removing the sheath of petals and stamens while leaving the gynoecium attached to the peduncle. At this stage receptivity of the stigmas is evident -- they appear slightly swollen and erect. Pollen from the desired clone is then brushed onto the exposed stigmas by using a detached and depetaled flower which is actively dehiscing.

Five pollinations were attempted for each cross on the same mother tree. Where five flowers were not available on one tree another ramet of the same clone was used. Crossing was done during the springs of 1970, 1971, and 1972 at the yellow-poplar seed orchard at The University of Tennessee.

Harvesting of samaras from controlled crosses was undertaken during the latter part of September for each of the three seasons encompassed by the study. Each group consisting of five pollinations (one cross) was collected and combined to provide a more reliable sample.

Radiograph Analysis

A sample of samaras was taken from each cross and placed in small cloth bags for stratification. Samples were soaked overnight, allowed to drain, and placed in cold storage at 32°-36°F for approximately 90 days. Following stratification each sample was mounted on an index card to allow for easy observation through the germination procedure. Stratified samaras were radiographed utilizing five by seven inch x-ray film under a General Electric Maxitron 300 x-ray unit. Best results were obtained using Dupont Cronex NDT 45 industrial film and x-ray unit settings of 70 KVp, 5 mA, and a focal distance of 75 cm. An aluminum filter (0.50 mm) was used to intercept hard x-rays that might have damaged the samaras. These specifications allowed for a dosage of 1.9 to 2.0 Roentgens, far below what is considered damaging radiation levels.

Data collection from the three seasons consisted of determining from the radiographs the percentage of filled samaras in each cross-pollinated sample. Each sample was then placed in a mist bench so that a measure of germination percentage could be obtained. At the end of a 50 day period the total number of germinations per cross were tallied and correlated with the number of filled samaras indicated by radiography.

In order to observe the effect of ionizing radiation on germination a study was undertaken which entailed exposing open-pollinated samaras to a wide range of doses of gamma radiation. Gamma radiation was used because of the similarity of its effect to x-radiation (Casarett, 1968) and the controllability of the cobalt 60 unit available. Twenty-four samples consisting of 500 samaras each from two clones were treated with six exposures ranging from 0 to 62500 Roentgens. After irradiation the samples were placed in a mist bench according to a split-plot design and allowed to germinate for 100 days.

RESULTS

Effect of Radiation

As illustrated by Figure 1, there is little difference in germination among the control, the 100R, 500R, and the 2500R treatments. However, somewhere between 2500R and 12500R there is a dosage that results in total death of the embryos. Exposures of this magnitude have little practical significance for radiography work because low doses of radiation are all that is necessary to produce a readable x-ray negative. Indeed, no adverse effect with respect to germination was noted at exposures up to and including 2500R (analysis of variance revealed no significant difference among the first four treatments). In fact, there seemed to be a slight stimulation of germination (not significant) as has been noted by previous authors (Simak and Gustafsson, 1953).

Reliability of Radiography

Approximately 1500 open-pollinated samaras on 15 seed mounts were examined by radiography. Each samara could be located on its seed mount by its position on the corresponding radiograph. All samaras were cut to determine if they were filled with endosperm. The reliability of the x-ray technique was tested by using regression analysis to obtain a sample correlation coefficient (r). For each of the 15 mounts, the number of predicted filled samaras found by x-ray was compared to the number of actually filled samaras found by cutting. The resulting correlation coefficient was 0.99, suggesting that one may consider the detection of filled samaras by radiograph as being very reliable; even those carpels filled with fungal mycelia did not register as full on the x-ray negatives.

Variation within the Gynoecium

Five ripe, but still entire, gynoecia were collected from each of three open-pollinated clones. Samaras from these clones were mounted on index cards in order of their occurrence from the topmost samara to the last basal samara **on** the central spike. Ten zones were established for each seed mount: Zone one consisted of the topmost ten percent of samaras from a gynoecium and likewise down the central spike including the basal samaras. This arrangement placed the great majority of basal samaras in zone ten.

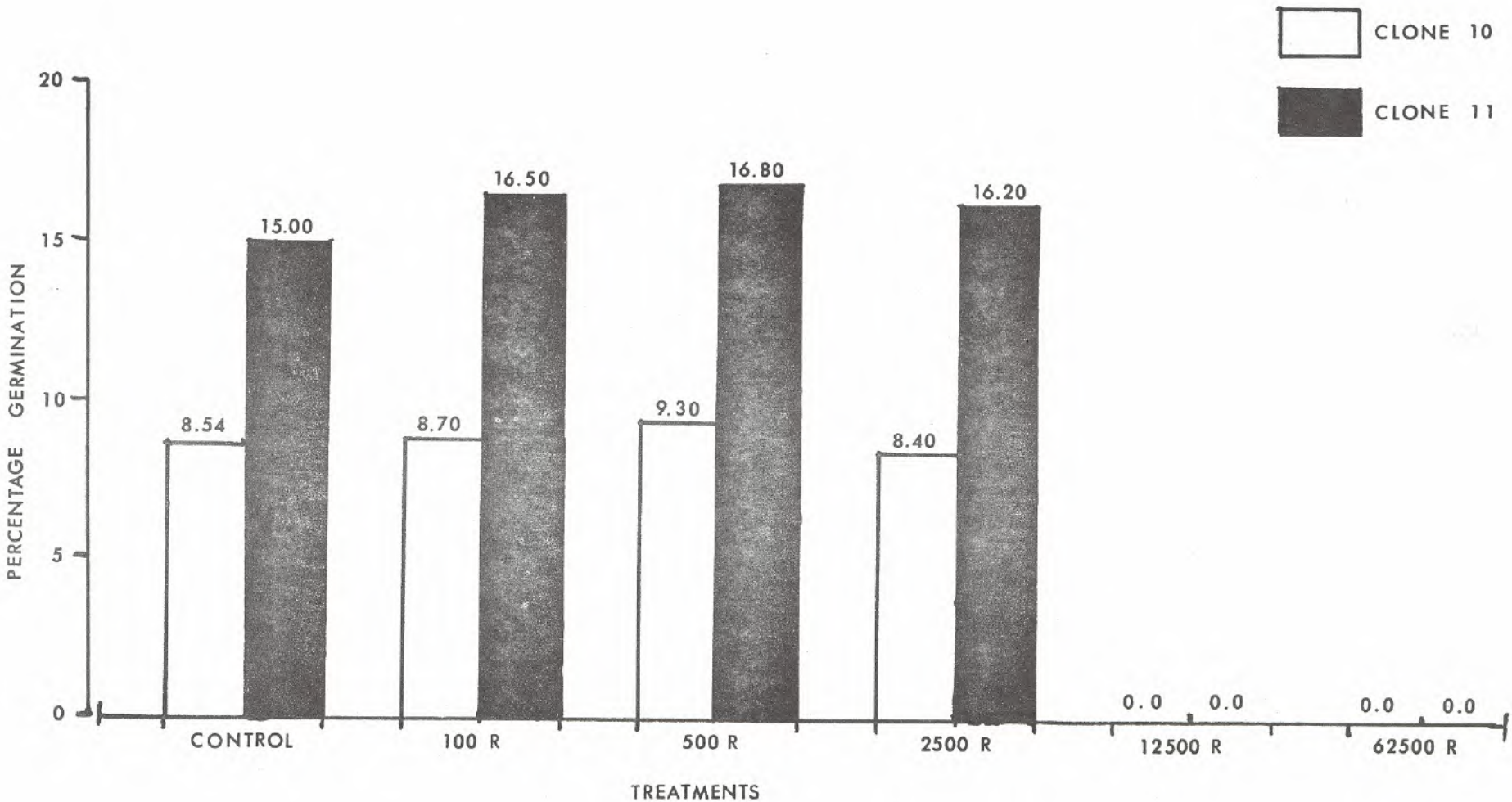

Figure 1.--Mean effect of gamma radiation on the germination of yellow-poplar samaras from two clones.

The number of filled samaras were counted from radiographs and compiled for each zone on all gynoecia. Figure 2 pictures the results of compiling the zonal means for all three clones. Zone one rarely contains viable samaras and zone ten can be considered as consistently sterile in this study. There seemed to be little tendency toward higher or lower viability within the remaining zones.

Radiography of Crosses and Results of Analysis

Data from radiograph analysis and subsequent germination were collected for two seasons. The sample correlation coefficient (r) for the first season was 0.803 (r^2 = 0.644) which indicates that 64.4 percent of the variance in the sample was due to the regression of filled samaras on samaras that germinated (Snedecor and Cochran, 1967). The same analysis for the second season resulted in an "r" value of 0.869 (r^2 = 0.756) indicating that 75.6 percent of the sample variation for that season was explained by the described regression.

One of the more outstanding factors explaining the somewhat low "r" values for these two seasons is the tendency for yellow-poplar samaras to fail to break dormancy after the first cold storage treatment. In fact, under natural conditions samaras have been recorded to have remained viable for four years in forest litter (Clark and Boyce, 1964).

Although the harvest of the third season of the study was not germinated in the mist bench, the various crosses were radiographed following the procedure of the previous year. This provided the study with three years of data with respect to filled samaras. Examination of these data revealed that 15 out of the 25 clones used during the three seasons were crossed with sufficient regularity to be used in an analysis of variance. The mean percentage filled samaras of each mother parent crossed by several distinct pollen parents was computed. These means are given in Table 1.

Using the three years as replications and the different clones used as females as treatments a simple analysis of variance was performed. The results indicated no significant difference among years but a highly significant difference among clones.

An analysis of variance was also performed for those clones used regularly during the three seasons as pollen parents (as it turned out 12 of the 25 clones were used during all seasons). The results of this analysis revealed no significant difference among years or clones. A further difference between the female and the pollen parent clones is the range of viability. Three year means for female clones range from 4.6 percent to 34.5 percent, comparable with results obtained by Thor (1966). Pollen parent clones range from 10.3 percent to 19.3 percent (Table 1). Considering these findings, one is inclined to conclude that the viability level of a given cross is primarily controlled by maternal factors. This is in agreement with the suggestion of other authors that incompatability and subsequent low seed viability is caused by a chemical incompatability between the style of the mother parent and the pollen tube of the pollen parent (Kaeiser and Boyce, 1962).

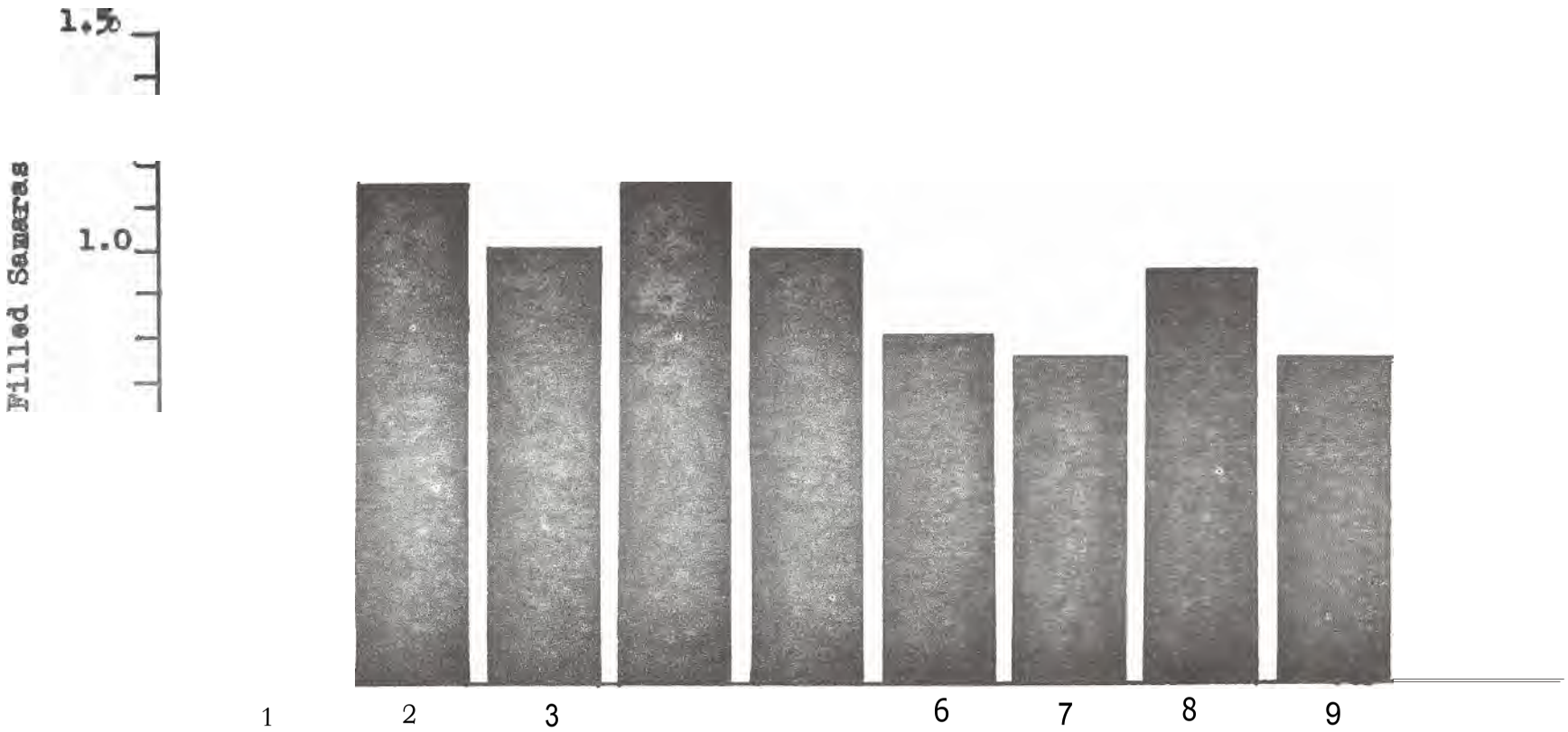

Figure 2.--Zonal means for all gynoecia
from three open-pollinated clones°

Table 1.--Mean percentage filled samaras obtained from controlled crosses in three seasons

Clone	1970		1971		1972		Means for 3 seasons	
	♀	♂	♀	♂	♀	♂	♀	♂
	---------- Percentage ----------							
1	14.0	-	14.1	-	12.3	-	13.5	-
2	8.5	17.0	8.9	6.9	10.5	18.6	9.3	14.1
3	11.5	12.6	1.6	7.5	10.9	23.2	8.0	14.4
4	0.5	13.2	5.1	17.7	10.0	17.4	5.2	16.1
5	24.7	17.8	23.1	8.2	24.6	11.2	24.1	12.4
6	4.0	-	3.4	-	6.5	-	4.6	-
11	34.6	17.2	32.4	22.0	36.4	18.8	34.5	19.3
12	2.0	-	22.4	-	15.6	-	13.3	-
13	27.4	8.1	21.0	8.0	13.4	17.7	20.6	11.3
16	10.8	12.4	11.7	16.4	14.3	27.7	12.2	18.9
22	11.3	17.2	4.2	20.8	5.2	11.2	6.9	16.4
27	9.0	18.4	6.9	19.6	14.1	11.4	10.0	16.5
31	22.8	11.7	9.7	4.8	11.0	17.8	14.5	11.4
35	-	7.0	-	15.4	-	8.7	-	10.4
39	10.0	-	12.1	-	14.4	-	12.2	-
108	14.1	10.0	15.7	9.0	15.1	18.6	14.9	12.5
Means	13.7	13.6	12.9	13.0	14.3	16.8	13.6	14.0

An analysis similar to that done for the means of cross-pollinated clones was performed for eleven open-pollinated clones found in the same orchard. Th results of this analysis indicate no significant difference among years at the ten percent level but significant difference among clones at the same level. Clonal means varied from a low of two percent filled samaras to a high of 18 percent, with a mean of 8.6 percent. This mean is considerably lower than the 13.7 percent obtained for the cross-pollinated samaras. However, the clones producing samaras with high viability when cross-pollinated also produced a high percentage of filled samaras when open-pollinated.

CONCLUSIONS

The findings of this study indicate that radiography can serve as a useful tool to the tree improver. For instance, the analyses of variance indicat that there is enough difference among clones in the orchard to warrent removal of some clones with lower seed viability. Removing the poorest one quarter of the clones from this orchard will increase percentage filled samaras from 8.6 to 10.3; an increase of nearly 20 percent.