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An evaluation of yellow-poplar samaras by radiography

Russell Alfred Cox

To the Graduate Council:

I am submitting herewith a thesis written by Russell Alfred Cox entitled "An evaluation of yellow-poplar samaras by radiography." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

Eyvind Thor, Major Professor

We have read this thesis and recommend its acceptance:

Kingsley Taft, Edward Buckner, Vernon Reich

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

October 24, 1973

To the Graduate Council:

I am submitting herewith a thesis written by Russell Alfred Cox entitled "An Evaluation of Yellow-Poplar Samaras by Radiography." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

Eyvind Thor
Major Professor

We have read this thesis and recommend its acceptance:

Edward R. Buckner

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Accepted for the Council:

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AN EVALUATION OF YELLOW-POPLAR SAMARAS BY RADIOGRAPHY

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Russell Alfred Cox

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ABSTRACT

Control-pollinated samaras from 25 clones in a yellow-poplar breeding orchard at the University of Tennessee, Knoxville, were subjected to radiograph and germination analysis. A high correlation was found between filled samaras identified from radiographs and samaras that germinated. Significant differences were observed among clones used as females for percentage 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.

Analysis of 62 crosses and their reciprocals indicated significant difference between the two, thus, precluding a partial diallel analysis for viability and indicating a strong maternal effect.

An irradiation (gamma) study revealed that there is no significant effect with respect to germination and seedling dry weight from exposure to doses of radiation up to and including 2500R. Seedling length showed significant differences among treatments below 2500R with the two half-sib families used in the study reacting somewhat differently to the radiation treatment.

Statistical analysis of ten arbitrarily assigned zones of viability within several ripe gynoecia revealed that the topmost and lowermost (basal) samaras on the yellow-poplar cone are generally nonviable.

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CHAPTER I

INTRODUCTION

Yellow-poplar (Liriodendron tulipifera L.) is considered the most utilized hardwood in the United States for general purposes. This species' straight bole, exurrent branching and the excellent workability of its wood make yellow-poplar prime for lumber and veneer, and the length of its fibers are acceptable for hardwood pulp. In addition, this species is handsome enough in appearance to make an attractive yard tree.

Various authors have reported that many of the traits attributed to yellow-poplar (bole straightness, branch angle, pruning ability, wood specific gravity, etc.) are either genetically transferable or are suspected to be so. It would also seem that most of the desirable traits are of a quantitative nature. Furthermore, it has been found that there is considerable natural variation in yellow-poplar within its natural range (racial) as well as within stands (among families). These factors provide the attributes necessary for a species to benefit from forest genetics work.

However, as is the case with many desirable tree species, yellow-poplar has quite a few inherent problems, particularly with respect to its seed. Although this species produces an abundance of seed most years, the percentage viability is extremely low (usually from two to ten percent). Since

parthenocarpy is the rule rather than the exception with the fruit of this tree, visual inspection is useless in most instances. Furthermore, yellow-poplar samaras defy most standard viability tests due to the diminuative size of its embryo and the difficulty of breaking dormancy with standard stratification techniques. Since this seed viability problem is considered to be one of the greatest drawbacks in yellow-poplar tree improvement, the primary purpose of this study is to explore the use of radiography in viability tests and prediction for yellow-poplar samaras. Additional objectives of this study are to investigate methods for improving the percentage viability of seed lots and examine the effect of hard radiation on seed germinability and early growth characteristics of yellow-poplar seedlings.

CHAPTER II

REVIEW OF LITERATURE

I. SEED GERMINATION AND VIABILITY TESTING IN FOREST TREE SEEDS

Seed viability is defined as the percentage of seed capable of germination when exposed to favorable conditions (Woody-Plant Seed Manual, 1948). Thorbjornsen (1956) expanded on this concept by grouping viability tests into three categories according to length of time and environment involved in the test. These categories are: (1) Germinative Capacity or Final Germination Percentage—the percentage of seed germinating regardless of the time involved; (2) Germination Percentage—the percentage of seed germinating within a given time interval; and (3) Germinative Energy—the germination percentage under optimum conditions.

At the time that the Woody-Plant Seed Manual was published the methods used to determine viability consisted of either direct methods which involved germination tests or indirect methods which involved cutting tests, growth of excised embryos, flotation, size determination, color observation, and certain biochemical methods such as embryo staining and measurement of enzyme activity. However, most of

these methods are destructive and are, therefore, prohibitive if the amount of seed available is small.

For some species (Picea spp. and some members of Pinus) the direct approach for determining viability has proven to be the most reliable method. This method, which involves the actual germination of a sample of seed in a mist bench or Jacobsen apparatus, is ideal for those species that exhibit no seed dormancy. Many species that do require an "afterripening" period are adaptable to this method if the dormancy of their seed can be broken quickly. A proper combination of light, temperature, and moisture (the combination varies widely with species) can considerably shorten equilibration time (Heit, 1961).

However, this method falls far short of reliability when yellow-poplar seed is to be tested. This species is notorious for its slow seed germination. Under natural conditions yellow-poplar seed may fail to germinate but remain viable in forest litter for as long as four years (Clark and Boyce, 1964). Even under greenhouse conditions complete germination of all viable seed is not usually attained during the first growing season (Boyce and Hosner, 1963). Although some researchers have been able to greatly reduce the time necessary to obtain total germination in this species by alternating storage temperatures, the minimum time necessary seems to be about six months. Needless to say, this period is far too long for most purposes.

Another method of viability testing entails the excision of the embryos of a sample of seed and "growing" them in vitro. According to Heit (1959), who was a great proponent of embryo excision, this method was pioneered by Flemion (1948) and involves removing the embryo from the seed after soaking the seed in water for from one to five days. Embryos are then placed into Petri dishes on a moist substratum in the presence of light at favorable temperatures (62-72°F, depending on species). Embryos from viable seed will germinate, show some greening, exhibit spreading of cotyledons, or remain firm and white. Conversely, embryos of nonviable seed, i.e., seed that are weak or dead, will discolor, severely mold, or decay (Heit, 1959). Reliable results can be secured in five to fifteen days utilizing this method on a species which may require one to five months to test via direct germination techniques.

The embryo excision method has been found to be "much safer and more reliable . . . than any type of cutting test. . . . This test was found applicable to any kind of seed from nondormant to hard-coated seed with extremely dormant immature embryos" (Heit, 1955). However, this method is limited by the size of the embryo, i.e., the smaller the embryo the more difficult and unreliable the test. Since yellow-poplar has one of the smallest embryos of the commercially important forest trees, this method must be considered unreliable and overly time consuming for this species.

Two techniques, using different chemicals, will serve to illustrate the method of embryo staining. The first technique involves removing the embryos after the seed have been treated with a sodium selenite solution. In a study using Ponderosa pine seed, Thorbjornsen (1956) found that best results were obtained with a three percent aqueous solution in which the seed were soaked for three days. Formaldehyde (five percent solution) was added as a fixing agent when the staining was complete. After treatment, viable embryos are stained red (the darkness of the stain and the area of the embryo that stains determines the "degree" of viability) and are easily differentiated from nonviable embryos. The second staining technique involves the application of tetrazolium chloride (2,3,5-triphenyltetrazolium-chloride). Grano (1958) described the reaction involved as being based on the action of reducing enzymes (dehydrogenases) which are found only in living tissues. Seed are cut so as to expose the embryo and soaked in a one percent solution of tetrazolium chloride in distilled water for 24 hours in the presence of total darkness. Embryos are then excised and studied under a microscope. Viable embryos tend to be stained a shade of red.

Both of these techniques were and are considered much better than the older conventional methods in that they are quicker, require less space, and account for abnormal germination. However, the disadvantage in limitation of embryo

size remains. Utilizing these techniques with species such as yellow-poplar offers long, tedious work to the researcher because of minute embryos. Furthermore, these and the other techniques so far mentioned are destructive in nature—a distinct disadvantage when testing small lots of valuable seed.

II. USE OF RADIOGRAPHY IN FOREST TREE SEED TESTING

The history of radiography in forest tree seed testing is relatively short. However, the need for a nondestructive method has long been recognized, and in the early 1950's the search began for a suitable x-ray procedure.

Pioneer work in this field began in Sweden when Simak and Gustafsson (1953) studied the usefulness and the effects of x-ray "photography" on the seed of Scots pine (Pinus sylvestris), Norway spruce (Picea abies), western redcedar (Thuja plicata), a species of larch (Larix sibirica), and two species of alder (Alnus glutinosa and A. incana). Through their work several properties of a seed sample, including percentage of filled seed and percentage of seed lacking embryos, could be diagnosed without destroying the seed in the sample.

Additional work was done until procedure and technique were perfected to the point that the seed of some conifers could be placed into various "viability groups" via

radiography. Ehrenberg et al. (1955) found that seeds of pine and spruce could be classified into five embryo types and two endosperm classes. The five embryo types, which also took the presence of endosperm into consideration, ranged from "0" (no embryo, no endosperm) to "IV" (one fully developed embryo and endosperm). The endosperm classes consisted of class "A" and class "B" which denoted the quantity of endosperm present within the seed. This classification was found to be valid as a ranking of quality in subsequent experiments; the "0" type exhibited no germination and the "IV" type 94 percent germination (Ehrenberg et al., 1955).

The first study of this sort in the United States was performed as part of a Master's thesis by Thorbjornsen in 1956. He tested the viability of ponderosa pine (Pinus ponderosa) seed via radiography using a classification scheme similar to the one devised by Ehrenberg et al. (1955) and found a high correlation between x-ray classification and subsequent germination tests.

Additional work done in the 1950's consisted of refining techniques, viz. finding suitable contrast agents to increase clarity of radiographs (Simak, 1957) and x-ray analysis to determine seed quality and predict germination rate (Simak and Gustafsson, 1959).

The 1960's ushered in the practical application of forest tree seed radiography and x-ray negatives became a

tool rather than a laboratory oddity. For instance, Klaehn and Wheeler (1961) reported on the possibility of radiography being of use in determining seed quality and observing embryo and endosperm development of seed produced by various breeding methods in spruce. Taft (1962) used x-ray analysis to study the effect of controlled pollination and honeybees on yellow-poplar seed quality and elaborated on the advantages of radiography over other methods of determining seed viability. Seed damage determination via x-ray also came into being (Kamra, 1963a) in conjunction with the development of better contrast methods (Kamra, 1963b).

Many tree species that could not be analyzed by the "old methods" were readily adaptable to radiography. Both yellow-poplar (Taft, 1962) and redwood (Hanson and Muelder, 1963) are species which defy all or most of the conventional methods of nondestructive viability testing but are well suited to x-ray analysis. Proponents of this method of analysis have stated that it is far superior to all other methods of viability testing of forest tree seed (Simak and Kamra, 1963; Kriebal, 1965; Belcher and Hitt, 1965; Belcher, 1968; Kamra, 1971). The chief advantages mentioned are the nondestructive properties and the quickness of radiography.

III. BIOLOGICAL RESPONSE TO IONIZING RADIATION

It is necessary to consider the absorption of x-radiation in order to understand what damage, if any, will occur to exposed material. If a radiograph of a living specimen is desired one needs to know if the dosage necessary to produce a readable negative is damaging to that specimen and how radiation damages chromosomes and DNA strands.

It has been known for some time that a beam of x-radiation looses energy as it traverses matter. According to Bragg and Bragg (1924) part of this loss is due to the scattering action of electrons and other particles through which the beam passes. Another fraction of the energy is transferred into the energy of x-rays of different wavelengths and of electrons in motion. The entire energy of the original beam may eventually be converted to heat through the action of electrons set into motion by the ionization which such electrons produce. Unless the atomic weight of the specimen is small the scattering effect is negligible in comparison to the transformation effect. In later years these theories have been corroborated; additional research has shown that it is the high speed electrons set into motion by the x-radiation that produce a biological effect (Easley, 1969).

After a beam of ionizing radiation enters an organism the first event is the production of ionization which represents the absorption of a great deal of energy in a short period of time and in a small volume (Evans, 1961). This event is followed by the excitation of atoms, activation of molecules, and the production of active molecular radicals. This step represents the absorption of a smaller amount of energy per unit volume but over a larger volume and over a longer period of time. The formation of these active radicals results in recombination and subsequent production of toxic materials; this change representing the absorption of still less energy per unit volume and lasts for a longer period of time. These latter processes are greatly affected by linear energy transfer, oxygen tension, and many other factors which alter the rate of metabolism in tissues.

Radiation may be applied to a specimen in one of two ways: either by fractionation (alternating periods of exposure and quiescence) or by protraction (continual exposure for a preset time). In general, the variables which have been found to be important in determining biological response to fractionation or protraction are: (1) total dose, (2) time interval(s), (3) total exposure time, (4) dose rate, and (5) energy level (Hekhuis, 1961). Hekhuis (1961) suggests that these variables modify the biological response in different ways, and that one biological response is modified by some of the variables and not by others.

The cell nucleus is the major site of radiation damage leading to cell death, chromosomal aberrations, and mutations. Researchers have calculated that over 1,000,000R* of radiation are necessary to inactivate certain critical enzyme systems in cells; doses of 1000R or more are required to damage membranes, while only chromosomal aberrations and mutations are produced at relatively low exposures (Casarett, 1968). Since only a few hundred roentgens are necessary to produce a high degree of lethality in many organisms, the nuclear changes produced by these doses are probably responsible for cell death and still lower doses are responsible for chromosomal aberrations and mutations.

Radiation Effect on Cell Function and Structure

All molecules can be altered by sufficiently large amounts of ionizing radiation; massive doses can break down and "destroy" components of a cell. Lesser doses can also result in cell death, but by less obvious mechanisms. Radiation may result in such widely varied effects as increased permeability of membranes, gross chromosome structural changes, and subtle chemical changes in the structure of DNA molecules (Casarett, 1968).

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*One R (roentgen) unit is 2×10^9 ionizations per cm^3 of air under standard conditions and is considered a unit of exposure as opposed to the rad (r); a unit of absorption.

Higher plants may show the effect of radiation by stunted growth, inability to set fruit, and similar aberrations. There are good indications that exposure to significant amounts of radiation increases the incidence of somatic mutations (Casarett, 1968). Stairs and Jeffers (1967) studied the effects of gamma-radiation on juvenile and mature cuttings of quaking aspen; they found that the radiation treatments utilized resulted in reduced growth rate and lessened rooting ability. A later study by Rudolph (1971) indicated that physiologically active plants are more radiosensitive than plants of the same species in dormant or lower metabolic states. He found that irradiated seedlings of several gymnosperm species showed a sharp response over a relatively narrow range of exposures. Shoot dry weight at 50 and 130 days, number of leaves at 50 days, and survival at 130 days were all drastically reduced (and remained low) at exposures between 600 and 900R.

Radiation Effect on Pollen and Developing Plant Embryos

When fresh pollen is irradiated massive doses are required to inhibit subsequent germination. There is an apparent correlation between radiosensitivity and pollen size, viz. large grains are generally more sensitive than small grains (Rudolph, 1965). This relationship could be due to the multiplying effect of a greater amount of moisture

surrounding the nuclei of the larger pollen grains (Osborne and Lunden, 1965).

The degree of radiosensitivity of developing plant embryos depends largely on the species, the stage of development of the embryo at the time of irradiation, and the criteria used to measure the effect. Moderate exposures (500-1000 roentgen) of zygotes or early "proembryos" of barley will greatly reduce the number that will develop into mature plants. Many will fail to develop into seed or seed that do form will fail to germinate. Mid- or late-proembryos are somewhat less radiosensitive than the earlier stage and show a different pattern of response. Seed are usually formed but the incidence of nongermination is high and the plants that do form may have specific morphological abnormalities (Casarett, 1968).

Irradiation of differentiating embryo tissue often results in the formation of abnormalities in the developing plant. Embryos in the final stages of maturation are somewhat less sensitive, although exposure during these later stages may result in inhibition of growth in roots and shoots in the developing plant (Casarett, 1968). Embryos seem to be the most sensitive to the effects of ionizing radiation during times of active growth and differentiation. One can easily imagine that this might be due to the presence of actively pairing and duplicating chromosomes during this time (Rudolph, 1971).

IV. EFFECT OF X-RADIATION ON FOREST TREE SEED

Perhaps one of the first authors to study the effect of radioactivity on plant seed was Gager (1907). He stated that:

> Experiments lead to the conclusion that the rays of radium act as a stimulus to the physiological processes of plants, accelerating, retarding, or inhibiting, either germination, growth, respiration, fermentation, cell division, starch formation, (or) sensitiveness to gravity, according to the strength of the radium salt employed, the duration and distance of exposure, the intervention of screens, the nature of the tissue, and, possibly, the species of plant.

Although this statement tends to sound extreme, many of his conclusions were confirmed by later research. As a matter of fact, the harmful physiological effects of moderate and severe doses of radiation have been completly established for many species, but the response to lower doses is still under investigation (Thorbjornsen, 1956).

The initiation of studies concerning the effects of severe doses of ionizing radiation on forest tree seed came during 1953 in the same project that gave birth to forest tree seed radiography (Simak and Gustafsson, 1953). They found that different species exhibited varying degrees of radiosensitivity; the hardwood species tested (Alnus spp.) could withstand more exposure to radiation than the coniferous species used in the study (Pinus spp. and Picea spp.). At doses of 5000R the alder seed showed 70 percent

germination whereas the pine and spruce seed were rendered inviable; lower doses (200R) seemed to have a stimulative effect on alder seed (Simak and Gustafsson, 1953).

Other authors have reported improved germination at low doses for many other species, suggesting that such low dosage treatments might have a slight stimulative effect on seed germination. However, some other investigators believe these conclusions to be the result of insufficient data or misinterpretation (Thorbjornsen, 1956). Stimulative effects were more or less refuted in a later paper by Gustafsson and Simak (1958) on the effects of x- and gamma-radiation on conifer seed. Kaeiser and Boyce (1962b) supported this conclusion in a study involving yellow-poplar radiography; no visual effects on seed germination were observed after low exposures to radiation. Indeed, Gustafsson and Simak (1958) found that x-radiation had a depressing effect with respect to germinability and rate of germination at exposures higher than 150-300R.

Heaslip (1959) irradiated the dormant seed of 18 forest tree species native to the eastern deciduous forest with a cobalt-60 gamma source. He found that this exposure affected germination of seed, seedling growth, and survival of each species with varying degrees of magnitude. Survival of seeds and seedlings from seeds exposed to 10,000R was less than 30 percent for one-half of the species tested in the

laboratory and less than 30 percent for almost two-thirds of the species in field tests. Sweetgum (Liquidambar styraciflua) and white ash (Fraxinus americana) were the only species that exhibited seed germination and had any surviving seedlings following a 100,000R exposure.

Apparently there is great variability in radiosensitivity among species (Simak and Gustafsson, 1953; Heaslip, 1959; Casarett, 1968) and, in some cases, variability in moisture content of seed within the same species. Radiosensitivity may also be genetically controlled (Simak et al., 1961). Comparatively little work has been done concerning the genetic aspect of radiosensitivity, but much has been written on the effect of moisture levels on this phenomenon.

The water content of seed has been shown to markedly influence radiosensitivity. Gustafsson and Simak (1958) noted that the seed of Norway spruce at low moisture content seemed to be more sensitive to x-radiation than seed at higher moisture levels. Conversely, many other authors have found that seed at high moisture levels are more radiosensitive. These disagreements have, no doubt, caused some confusion in past years. However, Casarett (1968) indicates that radiation has a minimum effect on seed and plant functions when the exposed seed are in an air dried condition (10-15 percent moisture content). Above and below this moisture level radiation has much more of an inhibitory effect. This

inhibition applies to germination of seed, survival of seedlings, and seed production of plants originating from irradiated seed.

Micke (1965) explains this variability in radiosensitivity in relation to the number of free molecular radicals present in the seed at time of irradiation. The number of radicals present in this free state is independent of moisture content, but the "survival time" of free radicals is greatly influenced by the degree of hydration of the tissues within the seed. Very dry seed have high radiosensitivity, especially in the presence of quantities of oxygen. It appears that, under these conditions, free radicals are quite stable for several days, i.e., they do not recombine readily to form harmless products of metabolism. If these radicals are exposed to water, however, they recombine in a matter of a few hours. Evidently the exposure of the unattached radicals to ionizing radiation render the seed more radiosensitive by some means. It could very well be that these stray molecular particles have a magnifying effect on the ionization of molecules by providing more "loose" particles and thus adding "fuel to the fire" so to speak. Evans (1961) suggests that these free radicals might recombine with radicals created by ionization to form toxic materials.

At high moisture contents free radicals recombine in a matter of a few seconds. One may wonder then why presoaked seed are more radiosensitive than air dried seed, or why seed that have been presoaked then dried back to air dried moisture levels are equally radiosensitive to those that are irradiated wet. Some authors have inferred that since soaking permits a leaching of materials into the water, a permanent change associated with the loss of leached amino acids, sugars, and organic substances occurs; thus, the seed may become more radiosensitive (Casarett, 1968).

The research of various authors has shown that conifers are generally more radiosensitive than hardwood species. As previously mentioned, Simak and Gustafsson (1953) noted that while doses of 25,000R resulted in complete lack of germination in Scots pine a few of the alder seed in the same study survived. Alder seed exhibited 70 percent germination at 5000R, whereas 1000R reduced germination of Scots pine seed to 30 percent. Gustafsson and Simak (1958) observed a depressing effect on germination at 150-300R with Scots pine, and Snyder et al. (1961) determined that a dose of 2000R was lethal to longleaf (Pinus palustris) and slash pine (P. elliotii) and 4000R was lethal to loblolly (P. taeda) and shortleaf pines (P. echinata); LD-50* for loblolly seed has

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*Lethal dose for 50 percent of the sample population with respect to one year survival.

been set as low as 1000R. On the other hand, sweetgum and white ash seeds have been recorded to have germinated and the subsequent seedlings to have survived after exposure to 100,000R (g) (Heaslip, 1959). Another example is furnished by MacDonald et al. (1962) who noted that doses of 2800R had little effect on the germination of American chestnut (Castana dentata) and that the LD-50 for this species was 5600R.

It is evident then that some forest tree seed seem to be able to tolerate high levels of radiation. For instance, Thorbjorsen (1956) observed little effect on ponderosa pine seed germination with a dose of 780R and Ohba and Simak (1961) recorded that exposures lower than 2400R did not affect the germinability of seed samples of Scots pine. In conclusion, one may assume that the low exposures involved in radiography (usually less than three roentgens) have little or no effect on the germination of forest tree seed.

V. EMBRYOLOGY AND SEED CHARACTERISTICS OF YELLOW-POPLAR

The Flower

The flower of yellow-poplar is solitary, pedunculate, showy, and perfect and occurs at the tips of slender lateral branches. Flower bud scales consist of modified stipules. The three sepals become reflexed as the flower opens, the

campanulate (expanding from a short and rounded base into a spreading border—bellshaped) corolla consists of two whorls of three petals each with conspicuous nectaries on bright orange spots near the base (Chapman, 1860; Kaeiser and Boyce, 1962a; Wilcox and Taft, 1969). Stamens are numerous with linear extrorce anthers (face outward toward the petals). Pistils are flat, narrow and imbricated, and are numerous and free; often totaling more than 100 per infloresence (Chapman, 1860; Kaeiser and Boyce, 1962a). These pistils cohere in an elongated cone which becomes an aggregate of samaras as the fruit ripens.

Under natural conditions flowers may begin to appear when the plant is in its fifteenth year (Thor, 1966). Flowering tends to occur annually from late March to June depending on geographical location and weather conditions (Wilcox and Taft, 1969). There is much variation in time of flowering among trees within a stand and the flowering period for an individual may vary from two to six weeks (Thor, 1966).

The Fruit and Seed

The fruit of the yellow-poplar is an erect, conelike aggregate of samaras which ripens in the early autumn (Thor, 1966). This aggregate is the dehydrated gynoecium of the flower. When ripe the samaras dehise from the receptacle

(the receptacle may persist until the next year) and scatter after the fashion of other "winged" seed.

In yellow-poplar parthenocarpy is the rule rather than the exception, i.e., fullsized cones and samaras develop from unpollinated flowers but the seed are empty (Boyce and Kaeiser, 1961). Although total viability of a crop of seed tends to be low, seed is produced in great abundance so that there is usually ample viable seed to produce a crop of seedlings (Thor, 1966). According to Wean and Guard (1940) each samara may contain two seed, but one seed per samara is the general rule; double-seeded samaras are rare.

At the time the seed are shed the embryo consists of a hypocotyl with two well-developed cotyledons and an undeveloped plumule (Wean and Guard, 1940). There is no development beyond this point until just prior to emergence at which time the cotyledons increase in size and the plumule begins to develop. The embryo is minute, measuring one to one and one-half millimeters long and approximately one-half millimeter wide (Boyce and Kaeiser, 1961).

Pollination

Under natural conditions yellow-poplar must be considered a strictly insect pollinated species. According to Kaeiser and Boyce (1962a) the pollen is covered with a mucilagenous substance; therefore, it is doubtful that any wind

dissemination occurs. This lack of movement of pollen by air currents coupled with low insect populations has been cited as a major cause of the low viability of seed of this species (Kaeiser and Boyce, 1962a; Taft, 1965; Thor, 1966), i.e., cross-pollination via insects appears to be rather inefficient. These authors also suggest that another factor contributing to this inefficiency is the high degree of selfing caused by insects moving from flower to flower on the same tree.

When controlled pollinations of yellow-poplar flowers are performed a great increase in viability usually results. As early as 1950 Carpenter and Guard noted this increase. More recent works by Taft (1965) and Thor (1966) have substantiated this. The reason for this large gain in viability is probably related to the incompatibility of yellow-poplar to selfing or to crossing with closely related genotypes (Carpenter and Guard, 1950; Kaeiser and Boyce, 1962; Taft, 1965).

Viability and Incompatibility

Yellow-poplar produces seed of low viability with respect to total seed production because of inefficient pollination which results in a small number of fertilized egg cells. Pollen and styles of the same tree have been found to be generally incompatible but some cross-

pollinations are no more effective than selfs (Boyce and Kaeiser, 1962a). There appears to be relatively little outcrossing in the wild due to the inefficiency of insect pollination and the failure of compatible trees to grow adjacent to one another (Kaeiser and Boyce, 1962b). Other possible causes for low viability include the short receptivity period of the stigmas (12-24 daylight hours) and the negative effect that inclement weather (rain, etc.) has on pollen availability.*

According to Boyce and Kaeiser (1961) there are no irregularities in the developmental processes of yellow-poplar that could result in nonviability of seed. In their study, they found the development of pollen, ovules, egg sacs, and eggs were "normal and regular"; and that no chromosomas irregularities were present which would interfere with fertilization (of compatible trees) or stop the formation of embryo or endosperm tissues. Furthermore, there was no evidence that large numbers of embryos deteriorated before the seed matured. Therefore, if flowers are pollinated with compatible pollen when stigmas are receptive viable seed will be produced (Boyce and Kaeiser, 1961; Kaeiser and Boyce, 1962a).

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*Personal experience.

VI. RADIOGRAPHY AND YELLOW-POPLAR SEED TESTING

It has long been established that if endosperm is present in the ovules of yellow-poplar samaras that embryos are also present (Wean and Guard, 1940; Foster and Gifford, 1959). Kaeiser and Boyce (1962b) noted this and developed a method for identifying samaras with filled seed. The method consisted of placing 100 to 200 samaras over an x-ray film and exposing the samaras and film to a low dosage of soft x-rays (10 KVp, 5 mA, 24 inches, 10 minutes). Negatives obtained by this method clearly revealed the presence or absence of endosperm within an ovule. No visual effects of the x-radiation on the seed with respect to germination or growth were noted. Seedlings from x-rayed seed did not show aberrent forms and grew as well as those exposed to background radiation only. Further testing of this method over a three year period indicated a close correlation with the percent of seed which the negatives showed to be full and those which were found to be full when they were disected (Kaeiser and Boyce, 1962b).

The primary advantage to the use of radiography is the conservation of the seed sample (Taft, 1962). This form of viability testing allows the seed to remain viable and intact, i.e., radiography is a true form of nondestructive testing. This is particularly valuable when the seed are the result of carefully performed crosses and may not be

available in quantity. In cases such as this, the advantage of this nondestructive test far outweighs the additional cost involved.

VII. HERITABILITY TESTS FOR YELLOW-POPLAR

Relatively little work has been done to obtain heritability estimates for yellow-poplar, but this is understandable in view of the site sensitivity of the species. In order to perform a valid heritability investigation a large, uniform site must be used that will provide for good growth. Such a test involving yellow-poplar entails the use of small plots and many replications.* These factors tend to make heritability testing of yellow-poplar complicated and the yield of reliable information low.

However, both Taft (1965) and Thor (1966) have found considerable variation in certain characteristics of yellow-poplar—enough to make heritability tests warrented as well as desirable. For instance, Thorbjornsen (1961) found large among tree variance for wood specific gravity in Tennessee yellow-poplars. Kellison (1967) found branch angle and leaf, fruit, and seed characteristics to be inherited. In addition, total height after four months has been found to be consistently higher for controlled crosses than for open-pollinated

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*Personal communication with E. Thor.

seedlings, and reciprocal crosses have yielded almost uniform increases in vigor (Wilcox and Taft, 1969). According to Wilcox and Taft (1969) there are excellent opportunities to exploit heterosis for growth in progeny from crosses among parents from different stands.

CHAPTER III

METHODS AND PROCEDURES

I. THE DIALLEL CROSSING SCHEME

The diallel crossing scheme used in this study includes 25 clones representing three broad physiographic regions in the State of Tennessee, viz., East, Cumberland, and West. Clones from the eastern region were taken from nine ortets in three counties in East Tennessee: four from Monroe County, Four from Sevier County, and one from Cocke County. The nine Cumberland ortets are located in Morgan County (Tennessee) and Bell County, Kentucky; eight from the former and one from the latter. Western ortets were found in four counties of West Tennessee: four from Lauderdale County, and one each from Obion, Hardeman, and Lawrence counties. Ramets from these 25 clones, along with several other clones that may be included in future schemes, are located in two orchards: the East Tennessee seed orchard and the Tennessee Breeding Orchard. Both orchards are situated on the University of Tennessee Plant Science Farm adjacent to Fort Loudon Lake.

In setting up a diallel design there are two purposes that one must keep in mind: (1) the use of the crossing scheme as a vehicle in obtaining an estimate of variance,

and (2) the use of the scheme for the basis of a progeny test. However, one of the prime objectives of any yellow-poplar orchard is to increase the viability of the seed produced. In fact, the first selection within these orchards will be based on the seed viability of the clones; i.e., clones with the highest viability will be retained while those exhibiting low viability will be rogued from the orchard.

Since economic considerations are paramount, clones within the orchards are not a random sample of the yellow-poplar population, but are representative of sueprior phenotypes from this population. Thus, heritability estimates, which requires that crosses be made from randomly selected parents, cannot be obtained. However, the progeny test—a method of determining the relative superiority of one genotype over another—is one valid purpose of the orchards used in this study. The diallel scheme is also useful in determining the general and specific combining ability for each clone (general combining ability of a parent refers to the high average performance of progenies as compared to progenies of other parents in the same test; whereas, specific combining ability refers to the product of progeny from a single cross which performs better than expected on the basis of parental averages).

Progress with the diallel used in conjunction with the yellow-poplar orchards at the University of Tennessee is as yet hard to measure. One objective of the crossing scheme was the detection of "interprovenance hybrid-vigor", that is, to determine if there is any advantage to crossing among clones from different regions of the state (viz. eastern, Cumberland, and western regions). Since the orchards are rather young (establishment started in 1964), some clones have not yet started flowering. Crossing has been carried out for four seasons thus far. To start with, all possible crosses were made among flowering trees—excluding selfs. As more trees reached maturity, however, the discovery was soon made that this complete diallel scheme was too ambitious. One shortcoming of the scheme was that the manpower available to do the crossing is far deficient in relation to the relatively short period of time that yellow-poplar flowers are receptive to pollen. To date there are 25 clones which are actively flowering in the orchards (given a good season) and additional clones are added each year.

A complete diallel scheme could easily become prohibitively cumbersome if a large number of clones are included. The number of crosses necessary may be reduced by using male testers. These testers should consist of clones which are flowering abundantly so as to supply enough pollen to facilitate making the necessary crosses. For instance,

given the situation that there are six days available for cross-pollination in which an optimum amount of pollen and receptive gynoecia are available and that there are two men available to do the crossing; this amounts to 12 mandays. If weather permits each man can complete 15 crosses per day, each cross consisting of five pollinations per mother parent, or 180 crosses per season.

Applying these figures to the situation of the orchards concerned in this study, one can best utilize the 180 crosses by selecting two pollen parents (testers) from each broad physiographic region and ten mother clones from each region—30 mother parents in all. By ignoring selfs and reciprocal crosses, a partial diallel can be completed within one season.

However, this scheme cannot be applied to the orchards involved in this study as yet because there are not enough clones flowering with sufficient abundance to facilitate any type of diallel analysis. Furthermore, before attempting a diallel of this type one should be sure that there is no significant reciprocal effect.

II. POLLINATION AND HARVESTING OF SEED

The flowering period for the yellow-poplar varies from two to six weeks depending on the size and age of the trees, but variation in flower development on an individual tree

may be great (Taft, 1962; Thor, 1966). However, according to personal observation, one to three weeks (three weeks being the projected maximum) seems to be more realistic with respect to orchard trees.

Controlled crossing in yellow-poplar orchards is still in the developmental stages as far as technique is concerned; therefore, there is no "standard" procedure in artificial pollination. Basically, the technique used in this study is similar to that described by Taft (1962) and Wilcox and Taft (1969). This technique consists of emasculating an unopened flower bud and pollinating the bare gynoecium with pollen from a desired clone.

Emasculation is carried out by choosing buds at the right stage of development (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 the receptivity of the stigmas is evident; they appear slightly swollen and erect at full receptivity. Pollen from the desired clone is brushed on these exposed stigmas by utilizing a natural "pollen brush" consisting of a detached and depetalled flower from the pollen parent. The ideal time for selecting flowers for the pollen source seems to be a few hours to a day after the flower opens, but this time interval depends upon weather conditions; a hot, dry day will cause anthers to dehydrate and pollen to begin to dehicse within two or three hours after the flower opens.

The procedure used in this study varies from Taft's procedure in the collection of pollen. Taft collected the unopened buds from clones he wished to use as a pollen source and "forced" the buds open in the greenhouse, whereas, for the most part, in this study flowers were taken directly from the orchard tree and pollen immediately transferred to the receptive bud. Both techniques have obvious advantages and disadvantages. Taft's procedure insures a ready source of pollen in spite of weather conditions and is applicable to trees in the wild, but is somewhat time consuming in the orchard. The technique used in this study is quick, easily executed, and readily adaptable for orchard use, but cannot be easily used for trees in the wild and is difficult to use in windy or rainy weather since pollen is blown or washed from the pollen source before the pollinator has an opportunity to use the flowers. Both of these techniques were used during the third season of pollinating (1972) due to a scarcity of pollen that year. No difference between the two techniques with respect to seed viability was observed.

Some modification of the described pollination technique was made during the 1972 season by the introduction of "pollinating" tubes: plastic tubes were placed over the pollinated gynoecia and sealed with cotton and masking tape to prevent entrance of insects carrying undesirable pollen. These tubes were removed after two or three days when

stigmata on the gynoecia had turned brown in color indicating loss of receptivity. No significant difference was found among seasons concerning samara viability so one might conclude that these tubes had no deleterious effect. This technique should be adopted if further investigations reveal that significant stray pollen is effective in fertilization of unprotected gynoecia.

Five pollinations were attempted for each cross on the same mother parent. Where five flowers were not available on the same ramet of the mother parent another ramet of the same clone was used. Of course, more than one cross was made on ramets that were flowering abundantly, i.e., a copiously producing ramet may accommodate five to ten crosses. Each gynoecium was tagged with its respective mother and pollen parent clone numbers as it was pollinated to facilitate record keeping and relocation at harvest time. Pollination was usually carried out during the first part of May (1971: 4 May to 21 May; 1972: 5 May to 18 May).

Harvesting of the controlled crosses was undertaken during the latter part of September. Each group of five "cones" (one cross) was put in a paper bag; the bags were labeled, and closed to guard against mixture with other crosses and taken to the greenhouse where the cones were allowed to dry. After a period of two or three weeks, the cones were broken apart into component samaras and samples

of these samaras taken by randomly selecting approximately 100 samaras per sample.

III. EQUILIBRATION OF SAMARAS

The equilibration procedure used for this study is quite similar to that described by Bonner (1970). Step one entailed placing the individual samples of seed into small cloth bags equipped with drawstrings. These bags were tagged with the respective clone numbers of the cross. Step two consisted of emersing these sample bags in water for approximately 24 hours and allowing them to drain for a few hours to remove excess moisture. Lastly, the samaras were placed in a refrigerator set to allow for a reasonably constant temperature range of 34-36°F for about 90 days. This temperature and time period agree closely with Bonner's "overwintering" suggestions.

IV. RADIOGRAPH ANALYSIS PROCEDURE AND MIST BENCH GERMINATION

First Year

The radiograph analysis for the first year's harvest (samaras collected from the crosses made in the spring of 1970) served for development of procedures to be used for the following two years of the study. Refinement of technique and procurement of more suitable x-ray film enabled

the author to obtain radiographs which were more readable in subsequent seasons; thus, producing more reliable viability estimates. However, x-ray negatives from the 1970 harvest were sharp enough in contrast to make reasonably successful viability predictions.

After the yellow-poplar samaras were equilibrated, samples from each cross were mounted with masking tape on five by seven inch index cards. These materials were chosen because it was reasoned that they would not interfere with germination while still allowing for the observation of the germination of individual samaras.

Radiographs of these mounted seed were produced utilizing a General Electric Maxitron 300 Medical X-ray unit. Film used for the first year's radiographs was a standard type five by seven inch medical x-ray film (Kodak Type R, single coated SR54) which required the use of lead backed film holders. This type of film proved cumbersome as each sheet had to be loaded into the holders in the darkroom before the radiographs could be produced. In order to obtain the correct dosage a "trial and error" process was used which entailed working with the several controllable variables integral to radiograph production: milliamperage (mA), kilovolt peak (KVp), distance (focal length) exposure time, and filter combination. By varying the combination of these factors the settings and the filters which produced

the best clarity on the x-ray negative were obtained for the type of film used. These settings were: 100 KVp, 15 mA, a focal length of 75 cm, a 0.20 mm copper filter, and an exposure time of 7.5 seconds. These specifications resulted in a dose ranging from 2.3 to 2.6R depending on the temperature and barometric pressure of the x-ray laboratory at the time of exposure. Radiographs were developed using standard x-ray developing procedures.

Following radiography the mounted samaras were placed in mist benches and allowed to germinate for 50 days. At the end of this 50 day period the total number of germinations per cross were tallied and correlated with the number of filled samaras indicated by radiography.

Second Year

The procedure used in radiographing the samaras harvested in the fall of 1971 was basically the same as that used during the previous year. Improvements in the technique were based on the introduction of a different type of film: Dupont Cronex NDT 45 Industrial X-ray film in "day pack" containers. This film is individually packaged in light-proof wrapping, eliminating the need for film holders and decreasing the dosage necessary to produce a readable negative. In addition, the industrial film is of a finer grain which allows for better contrast and more clarity. Another reason for the

change in film is that the yellow-poplar samaras are of such low density relative to the film holders used with the medical type film that producing sharp images was next to impossible without reducing filtration on the x-ray unit (this would increase the amount of "hard" x-rays bombarding the seed, thus, increasing the chances of radiation damage). In comparison, the day pack film is wrapped in a relatively low density paper which allows for sharp images with a reduction in total radiation exposure. An additional advantage attained in switching films was the decrease in KVp and mA output of the unit, thus, reducing the amount of hard radiation generated by the x-ray machine. This reduction in KVp and mA settings eliminated the need for the severe filtration necessary the previous season.

The settings used for the second year of radiography were: 70 KVp, 5 mA, 75 cm focal length, 11 to 15 seconds exposure time, and a filter of 0.50 mm aluminum. The exposure time varied according to temperature and barometric pressure within the x-ray laboratory. Attempts were made to hold the dosage to 1.9 to 2.0R because the clearest negatives were produced within this range.

Third Year

Samaras harvested during the fall of 1972 were handled similarly to those of the two previous seasons with two

exceptions. These samaras were not equilibrated before they were radiographed and rather than restricting the sample size to 100 samaras, a random sample of samaras was selected for each cross by merely scattering a handful directly onto the x-ray film package. Care was taken to spread the samaras evenly enough to allow for easy counting.

Setting specifications for the x-ray unit were the same for the third season as during the second season. Some clarity was lost due to the lack of moisture in the samaras relative to those that were equilibrated. However, the negatives were clear enough to determine the number of filled samaras per sample.

Data collection for all three seasons consisted of determining the percentage of samaras with at least one filled seed in each sample. Mist bench germination of the samples was only carried out for the first two seasons.

V. IRRADIATION PROCEDURE

In order to acquire some degree of knowledge concerning the effects of ionizing radiation on the seed of yellow-poplar, a small irradiation experiment was undertaken. Twenty-four 500 samara lots of open-pollinated samaras from two clones in the University of Tennessee East Tennessee Seed Orchard were used in a split plot design including the two families as split-plot treatments. Treatments were

placed randomly within two replications on a mist bench. The open-pollinated samaras were harvested and placed into equilibration during the fall of 1971.

The six main treatments consisted of five levels of radiation and one control. The 24 lots of seed were placed in small polyethylene bags and sealed to prevent excess moisture loss. In April of 1972 these samples were transported to the UT-AEC* facilities in Oak Ridge, Tennessee, where they underwent treatment with a Cobalt-60 gamma source at 318R per minute exposure rate. The treatments and exposure rates were as follows:

Treatment	R Dose	Duration of Exposure
1	100	19 Seconds
2	500	94 Seconds
3	2500	472 Seconds
4	12500	2358 Seconds
5	65200	12300 Seconds
6 (Control)	0	0

The treatments were set up in increasing multiples of five with respect to exposure in order to cover the widest range of exposures in the limited space available for the experiment. Gamma radiation was used because of its similarity to x-radiation in its effects on cellular and subcellular structures and functions (Casarett, 1968) and the

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*University of Tennessee-Atomic Energy Commission. The author appreciates the enthusiastic cooperation of Dr. Milton J. Constantin, Professor of Radiobiology.

controlability of the particular source available through UT-AEC.

After irradiation the samples were placed in a mist bench in flats filled with mason grade sand and approximately one half inch of sterile white sand was used to cover the samaras. The samaras and subsequent seedlings were allowed to remain in the mist bench for a total of 102 days. Germination* data were taken at three day intervals for the first 50 days and at ten day intervals for another 50 days. The observation interval was changed because the majority of the samaras germinated within the initial 50 day period; the second interval provided an opportunity to observe late germinators and mortality among the young seedlings. At 102 days the ten dominant seedlings from each treatment were removed, measured for total length of stem, and desiccated to oven-dry weight. Total percent germination as well as stem length and oven-dry weight of the ten dominants were analysed using an analysis of variance for a split-plot experimental design.

VI. RADIOGRAPHY, CUTTING TEST, AND VIABILITY DISTRIBUTION WITHIN THE GYNOECIUM

In order to obtain an extimate of the reliability of radiography in detecting filled samaras in yellow-poplar a

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*A samara was considered "germinated" when the plumule emerged through the white sand in the flats.

combination of x-ray and cutting tests were employed. This test consisted of making seed mounts of the samaras as described previously, radiographing these mounts, locating the samaras which were estimated to be filled, and cutting all samaras to find which ones were actually filled with endosperm.

A total of 1542 open-pollinated samaras on 15 mounts were examined. Samaras were obtained from the 1971 harvest from several clones from the East Tennessee Seed Orchard.

The reliability of the x-ray technique was tested by using regression analysis to obtain a sample correlation coefficient (r). This was done by comparing, for each of the 15 mounts, the number of filled and empty samaras shown by radiography with the number of filled and empty samaras found by cutting.

Many authors have noted that the lowermost (basal) samaras within the ripe gynoecium of the yellow-poplar are never viable (Kaeiser and Boyce, 1962a). Therefore, when samaras are sorted, disregarding these basal samaras will result in less wasted space in the germination apparatus. Since this definite zone of nonviability exists, it is reasonable to think that there may be other zones of low or zero viability within the yellow-poplar cone. If these zones are easily identifiable, the respective samaras could be easily found and disgarded, thus decreasing waste of space.

Five ripe, but still entire, cones were collected from each of three open-pollinated clones (clones 2, 10, 11) located in the East Tennessee Seed Orchard. Samaras were carefully picked with tweezers in order to their occurrence within the gynoecium from the top of the central spike to the bottom, including the basal samaras. Seed mounts for each cone were made up with care taken to preserve the order of the samaras. Each mount was radiographed and filled samaras noted on the resulting negatives. Ten zones were established for each seed mount: zone one consisted of the topmost ten percent of samaras from a single gynoecia and likewise down the central spike. This arrangement tended to place the great majority of basal samaras in zone ten (see Figure 1). Of course, the number of samaras per zone differed among the gynoecia due to the large variation in samaras/cone among clones, and the smaller variation within the same clone. For example, in this study, one sample for clone 2 consisted of 124 samaras taken from the same gynoecium. Therefore, each zone within the sample consisted of 12 or 13 samaras. In comparison, one sample for clone 11 consisted of 73 samaras resulting in approximately seven samaras per zone. Data from each zone was compiled and a mean computed for each zone within each clone.

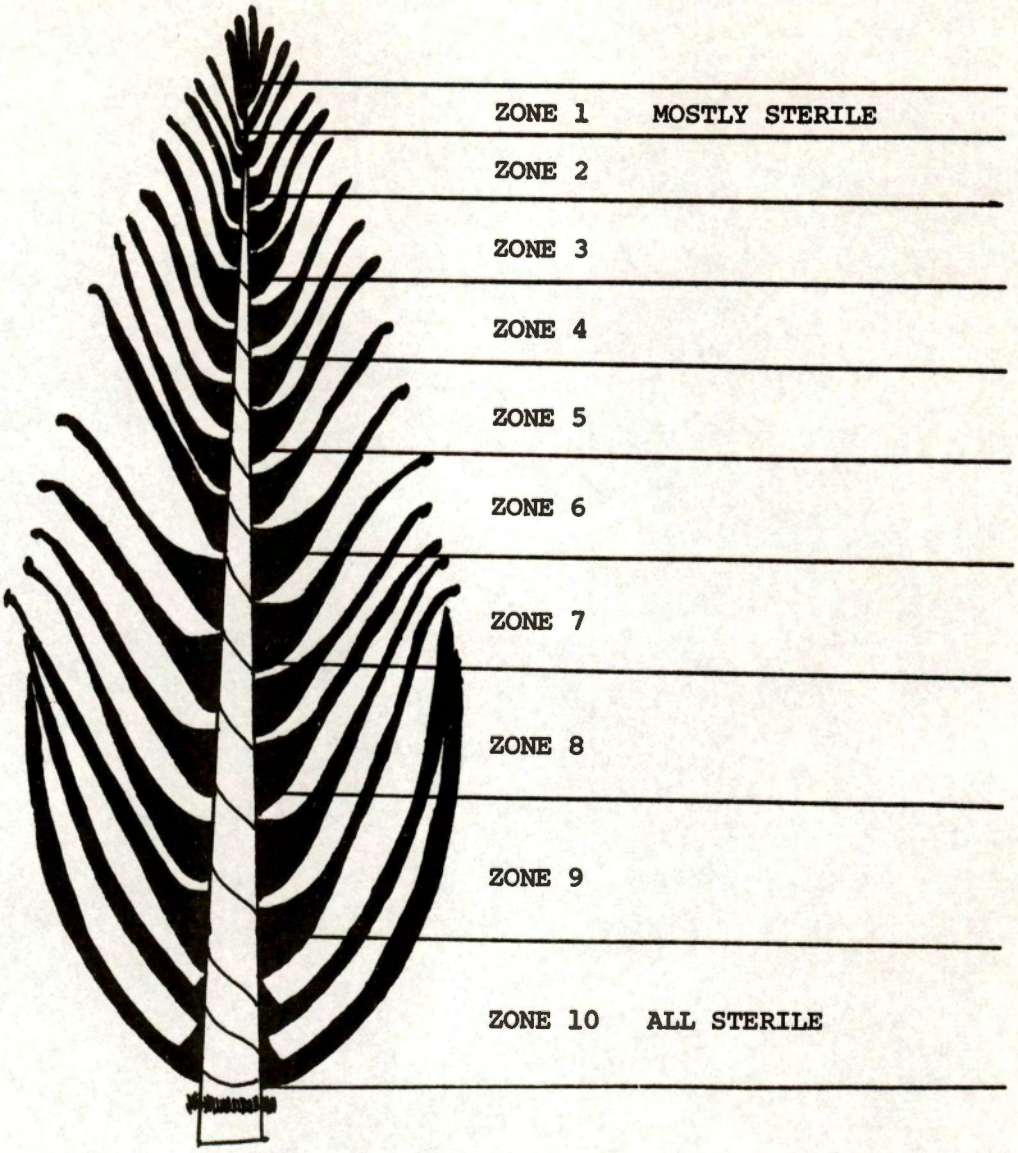


Figure 1. Schematic diagram of cross-section of ripe yellow-poplar gynoecium showing viability zones.

CHAPTER IV

RESULTS AND ANALYSES

I. RADIOGRAPHY OF CROSSES

Data from radiography analysis and subsequent mist bench germination were collected for two seasons. The first harvest (Fall 1970) consisted of 75 controlled crosses involving 13 mother parent clones and 19 pollen parent clones. The sample correlation coefficient (r) for the first season was 0.803 (r^2 equals 0.644) which indicates that 64.4 percent of the variance within the sample was dexcribed by the regression of filled samaras on samaras that germinated. The second season yielded 160 crosses which were made up of the various combinations of 19 mother parents and 25 pollen parents. The same analysis performed for the second harvest resulted in an "r" value of 0.869 (r^2 equals 0.756) indicating that 75.6 percent of the sample variation for that season was accounted for by the 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 as long as four years in forest litter (Clark and Boyce, 1964).

The harvest from the third season of the study was not germinated in the mist bench. However, these crosses were radiographed following closely the procedure used for the previous seasons' harvests. This provided the study with three years of data with respect to filled samaras. Examination of these data revealed that 15 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 appear in Table 1. Using the three years as replications and the "female" clones as treatments, a simple analysis of variance was performed. The results indicated no significant difference among years but a highly significant difference among clones used as females. These findings suggest much variation in percent filled samaras among clones within the orchards and that using years as replications was probably justified.

An analysis of variance was also performed for those clones used regularly during the three seasons as pollen parents (12 of the 25 clones were used all three seasons). The results of this analysis indicated no significant difference among years or clones. A further difference between

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

Clone	Percentage							
	1970		1971		1972		Means for 3 Seasons	
	♀	♂	♀	♂	♀	♂	♀	♂
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

clones when used as female or male parents is the range in viability among the various clones. Three year means for the female clones ranged from 4.6 percent to 34.5 percent (Table 1) while pollen parent clones ranged only from 10.3 percent to 19.3 percent.

An analysis similar to the one completed for cross-pollinated clones was performed for eleven open-pollinated clones found in the same orchards. This analysis indicated 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 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, it was noted that the clones producing samaras which exhibited high viability when cross-pollinated also tended to produce a high percentage of filled samaras when open-pollinated.

II. IRRADIATION

Data from the gamma irradiation study were analysed for germination percentage and for stem length and dryweight. All percentage data were transformed via arcsin-$\sqrt{\text{percentage}}$ to obtain a higher degree of normality.

As illustrated by Figures 2 and 3 there is little difference in percentage germination among the control,

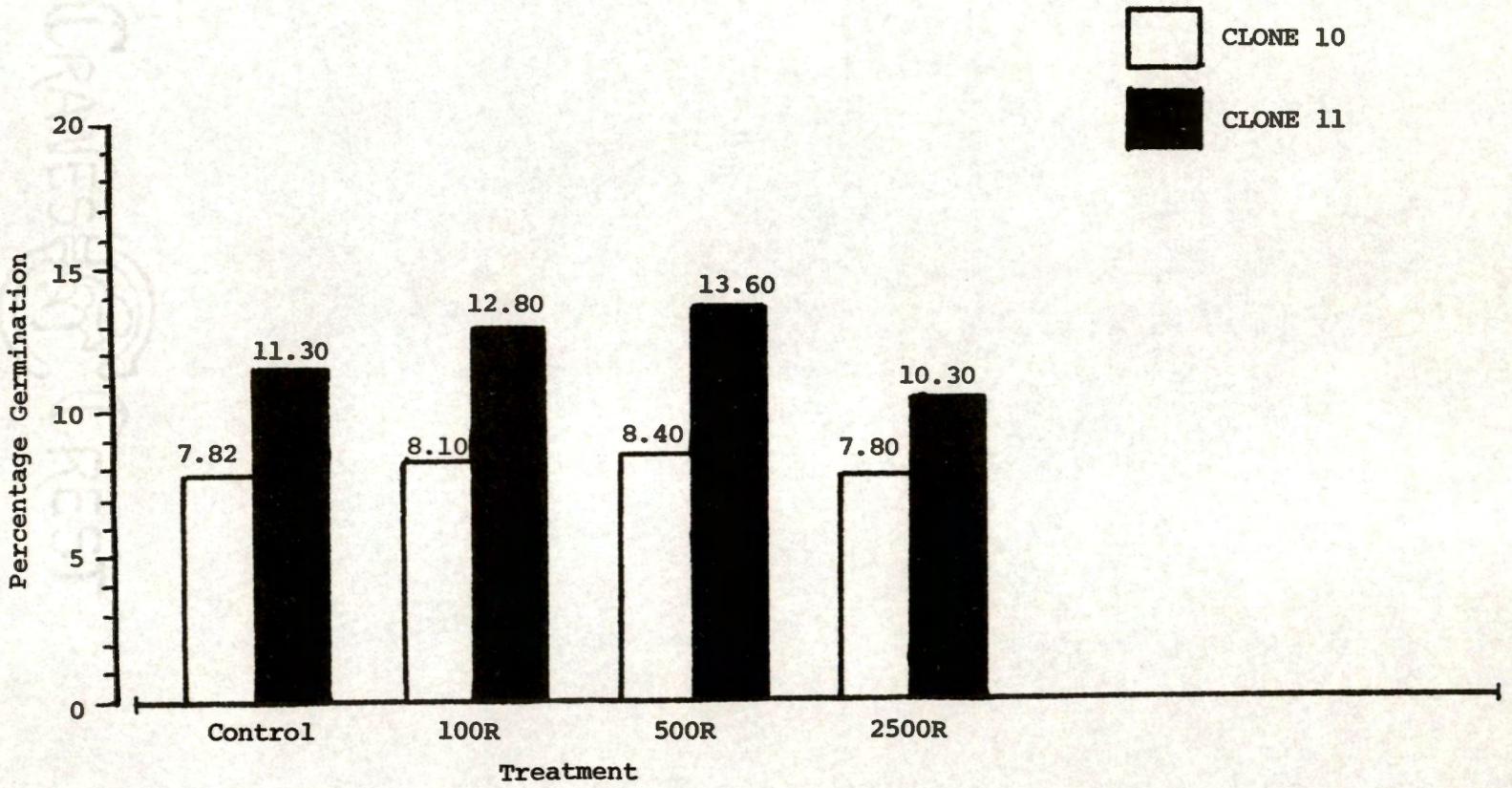


Figure 2. Percentage germination of irradiated samaras after 50 days.

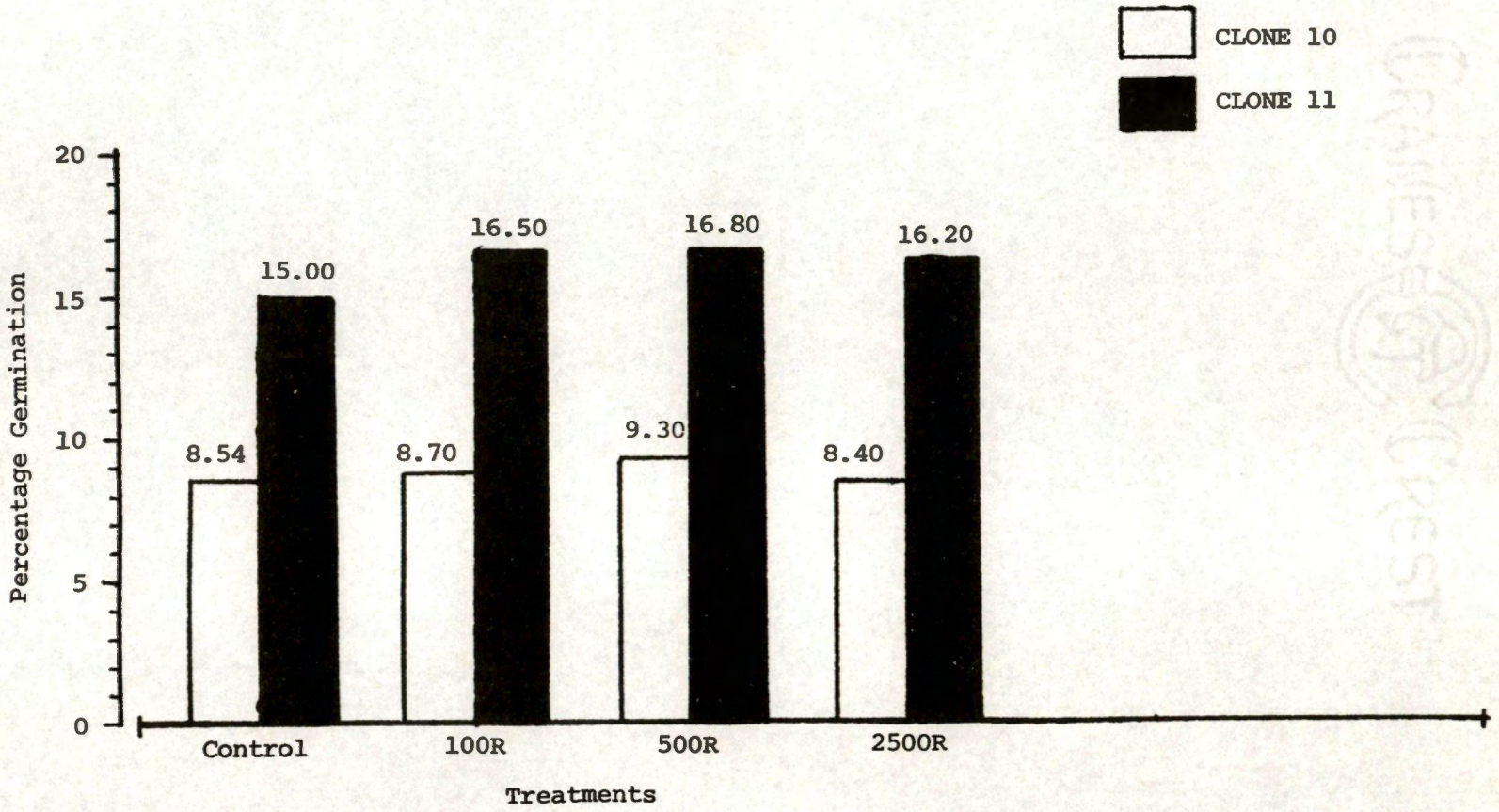


Figure 3. Percentage germination of irradiated samaras after 100 days.

the 100R, the 500R, and the 2500R treatments (nonsignificant at the 0.05 level). However, somewhere between the 2500R and the 12500R treatments there is a dosage resulting in total death of all embryos. Further investigation in this range of radiation was not warrented because exposures of this magnitude have little practical application in radiography work. No adverse effects with respect to germination were noted at exposures up to and including 2500R. In fact, a slight stimulation of germination (not statistically significant) was observed for both seed lots; this stimulation appeared to be more prominent at 100 days after planting (Figure 3) than at 50 days (Figure 2).

Similar results came from the analysis of dry weight data. No significant differences were found among the first four treatments and the only significant difference was between the two clones.

For seedling length there was also a highly significant difference between the two clones. However, there was also a significant difference (at the ten percent level of significance) among treatments. The two clones used in this experiment tend to react somewhat differently; the stimulative effect shows up in clone 10 at 100R but at 2500R stem lengths of seedlings are significantly below that of the control seedlings. Clone 11 exhibits the longest seedlings at 500R but suffers drastic and significant decrease in seedling length at 2500R (see Figure 4).

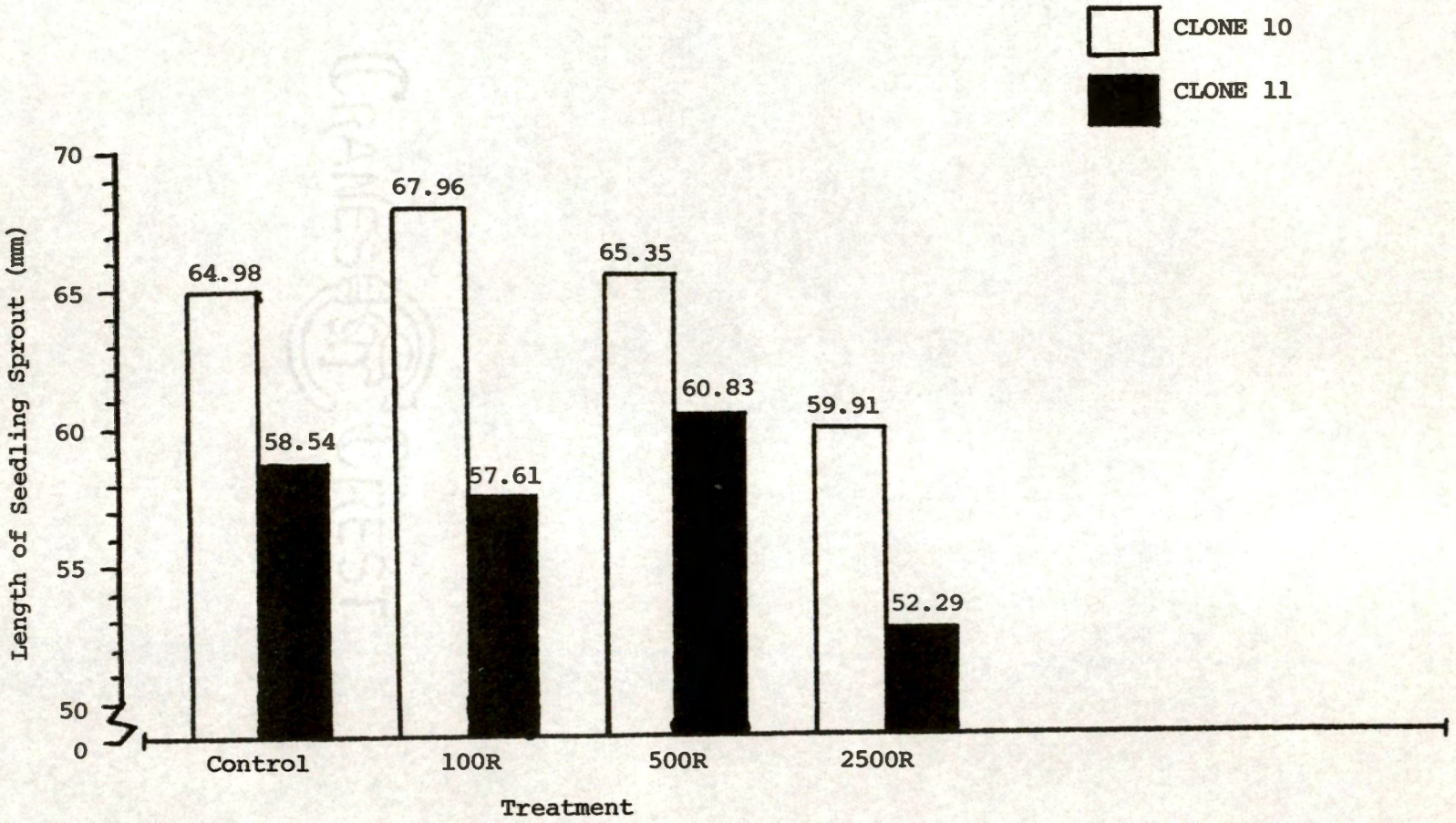
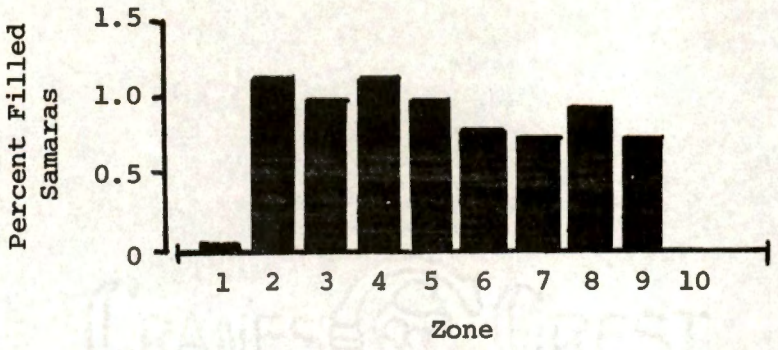


Figure 4. Length of seedlings (mm) from irradiated samaras after 102 days.

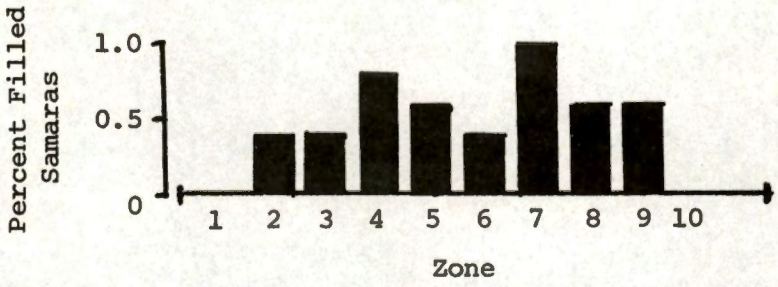
III. RADIOGRAPHY, CUTTING TEST AND VIABILITY WITHIN THE GYNOECIUM

The comparison of radiography and cutting tests involved generating a sample correlation coefficient (r) by regression analysis. This analysis produced an "r" value of 0.99 (r^2 equals 0.999) which indicates that 99.9 percent of the variation within the sample was due to the previously described regression. Considering the magnitude of this correlation coefficient, it is evident that radiography is a reliable method for detecting filled samaras in yellow-poplar.

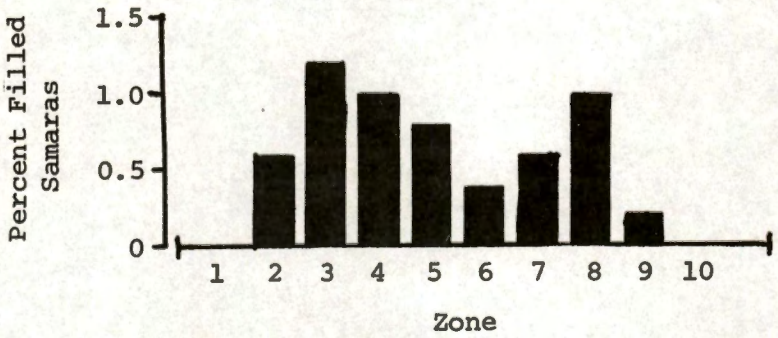
The analysis of the arbitrarily assigned "viability" zones within the ripe yellow-poplar gynoecium is graphically illustrated in Figure 5. When the means for the three clones were considered together (topmost graph) there was no significant difference among zones two through eight; i.e., it would seem that there is a tendency for samaras from these zones to have an equal chance of being filled and viable. Samaras from zone 1 tended to be small in size and have a rather "undeveloped" or aborted appearance when compared with samaras from zones of higher viability. The total number of filled samaras found in zone 1 was negligible for the three clones tested (Figure 5). Similarly, the samaras from zone 10, which consisted almost entirely of basal samaras, were found to be totally sterile (this has been noted in previous studies). Therefore, 20 percent of the samaras



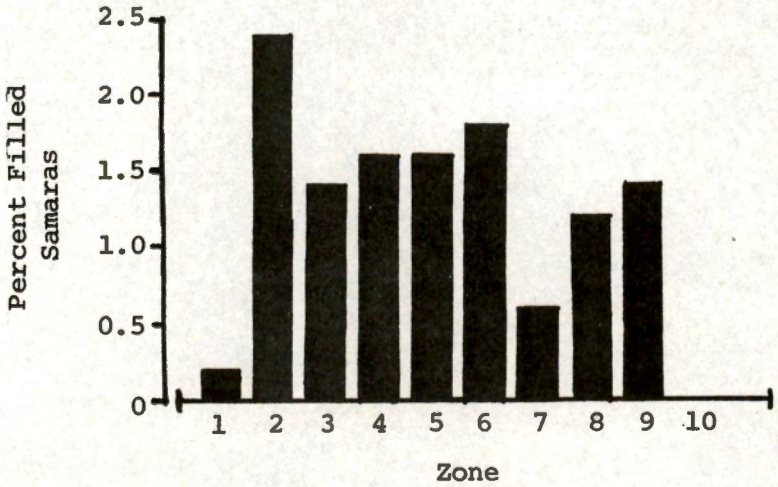
Zonal Means for All Clones



Zonal Means for Clone 2



Zonal Means for Clone 10



Zonal Means for Clone 11

Figure 5. Zonal percentage means for individual clones and average for all clones.

found on ripe yellow-poplar cones can be considered nonviable and easily identified for removal from controlled seed lots.

IV. RECIPROCAL ANALYSIS

In order to decrease the number of crosses necessary a diallel crossing scheme many researchers incorporate a partial diallel which excludes selfs and reciprocal crosses. Some authors also use a reciprocal value to fill in a diallel scheme when an actual value for a given cross is not available.

Such an approach was considered to determine whether or not a partial diallel or substitution could be applied to the diallel scheme designed for this study. A total of 62 crosses performed over the three seasons encompassed by the study had reciprocal crosses also. These data were used to determine if substitutions could be made for the character investigated. This analysis was performed by comparing crosses with their reciprocals using two statistical tests: χ^2 goodness of fit and a paired "t"-test.

Chi-square (χ^2) Test

To test the 124 crosses (62 crosses plus 62 reciprocals), a 1:1 ratio was assumed; the null hypothesis that there was no significant difference between the total percent filled samaras for the crosses and their reciprocals. Therefore,

$$\chi^2 = (\Sigma s_1 - \Sigma S_1)^2 / \Sigma S_1 + (\Sigma s_2 - \Sigma S_2)^2 / \Sigma S_2$$

where Σs_1 and Σs_2 are the total percent filled samaras for the crosses and reciprocals, respectively; and, ΣS_1 and ΣS_2 are the expected values. Hence,

$$\chi^2 = (1007.9 - 873.75)^2 / 873.75 + (739.60 - 873.75)^2 / 873.75$$
$$= 41.20$$

from the tables of cumulative distribution of χ^2;

$$P_{.01} \text{ @ } 1 \text{ d.f.} = 6.63$$

Therefore, the null hypothesis $H_0 : \mu_1 = \mu_2$ is rejected.

Paired "t"-Test

The data available from the crosses and reciprocals was well suited to a paired "t" test. Hence, such a test was employed to investigate the hypothesis:

$$H_0 : \mu_1 = \mu_2 ,$$

or that there is no significant difference between controlled crosses and their reciprocal values. The estimate for this paired "t" test is as follows:

$$t = \bar{y}_d - \mu_0 \; / \; \sqrt{S^2/n} \; = 7.98$$

where $\bar{y}_d$ is the mean of the differences between the two sets of data, μ_0 is the population mean (μ_0 is set at zero for this test), S^2 is the sample variance, and n is the total number of pairs in the sample.

For the 0.05 level of significance the critical regions for the two-tailed paired "t" test are $t > -1.671$ and $t < 1.671$. Therefore, since 7.98 lies well outside this range, the null hypothesis that the two sample means are equal is rejected.

It should be pointed out that the data used in these tests were not normally distributed. When plotted, the frequency curve for these values was badly skewed toward the origin. Transformation of the data to increase normality would be difficult. However, the chi-square test is not strictly dependent on normally distributed data; therefore the results of this test can be considered statistically valid.

Both the chi-square and the paired "t"-test rejected the null hypothesis that there was no significant difference between values for crosses and their reciprocals. Hence, one must conclude that there is indeed a difference of significant magnitude.

CHAPTER V

DISCUSSION AND CONCLUSIONS

I. RADIOGRAPHY OF CROSSES

Analysis of radiographs indicate that there is a large amount of variation among the clones used in this study with respect to seed viability. By detecting and removing the clones exhibiting exceptionally low viability, the percentage seed viability of the orchard can be improved. For instance, removing the poorest one-quarter of the clones from the orchard used in this study will increase the overall percentage filled samaras from 8.6 to 10.3 (open-pollinated percentages); an increase of nearly 20 percent.

The significant differences among clones when used as females and the lack of difference among clones when used as pollen parents point to a strong maternal effect. This maternal effect is further substantiated by the greater range of viability exhibited by the female parent clones (female: 4.6 to 34.5 percent; pollen: 10.3 to 19.3 percent). Considering these findings one is inclined to conclude that the viability level of a given cross is primarily controlled by maternal factors. This maternal effect is partially explained by reports that clonal incompatibility and

subsequent low seed viability in yellow-poplar is caused by a chemical incompatibility between the style of the mother parent and the pollen tube of the pollen parent (Kaeiser and Boyce, 1962).

Filled samara percentages were found to be generally higher for controlled crosses than for open-pollinated samples of seed. This finding substantiates the conclusions of other workers that natural pollination of yellow-poplar flowers is quite inefficient and points out that more research is needed on methods to improve insect pollination in seed orchards.

Radiography is a convenient and rapid method of estimating the viability of forest tree seeds that are naturally difficult to test by more conventional methods. An x-ray unit is a valuable tool to the tree improver, enabling him to identify clones producing low viability seed. Universities and other research centers are usually equipped with x-ray units and can use this type of technique with little additional expense. This study has shown that techniques producing reliable results can be developed for units not originally designed for seed testing.

II. IRRADIATION

From the irradiation study we learned that there is little effect of gamma-radiation on yellow-poplar seed

germination up to and including 2500R. This level is far above the small amount of radiation exposure incurred in producing a readable radiograph. Since the effects of gamma- and x-radiation are similar when applied to cellular damage, one can conclude that radiography is not harmful to yellow-poplar samaras with respect to germination and has no effect on seedling height and weight.

As far as the apparent stimulating effect on germination is concerned, many authors have noticed this effect on many types of seeds. However, since differences are small and not statistically significant further research in this field will have limited applied value.

III. RADIOGRAPHY, CUTTING TEST, AND VIABILITY WITHIN THE GYNOECIUM

The radiograph-cutting test performed as part of this project indicates that radiography is a very reliable method of detecting filled yellow-poplar samaras. Since germination of controlled crosses after radiograph testing indicated a good correlation between filled samaras and samaras that germinated, radiography can also be considered a reliable technique for estimating viability.

A comparison of ten arbitrarily placed "viability" zones within several yellow-poplar gynoecia resulted in the detection of two definite zones of "nonviability". By

disgarding these easily identifiable samaras, one can improve the viability of yellow-poplar seed lots by as much as 20 percent.

IV. RECIPROCAL ANALYSIS

The findings of the analysis performed for 62 yellow-poplar controlled crosses and their reciprocals show that a partial diallel analysis cannot be performed for the clones used in this study. Since there is a definite indication of maternal effect, a full diallel scheme (minus selfs, perhaps) should be used in analysis of seed viability. Such a scheme requires more data in the form of additional crosses and radiograph analyses; including reciprocal crosses. These results tend to cast some doubt on the validity of using a partial diallel scheme analysis in the absence of any reciprocal cross data. It is entirely conceivable that maternal effect could show up in characters manifested later in the life of the tree. The inclusion of a few reciprocal crosses in a "partial" scheme might serve as a monitor for detecting significant maternal effect.

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