To the Graduate Council:

I am submitting herewith a thesis written by John Hartman Yandell entitled "Movement of Pollen by Honeybees in a Yellow-Poplar Seed Orchard." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

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MOVEMENT OF POLLEN BY HONEYBEES IN A YELLOW-POPLAR SEED ORCHARD

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee

John Hartman Yandell

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ABSTRACT

The large percentage of seed of low viability produced by yellow-poplar (Liriodendron tulipifera L.) is perhaps the most important problem in seed orchards of this species. Low viability is related to the effects of honeybees (Apis mellifera L.) on cross pollination. Since honeybees can also be managed by man, it seems reasonable to use them to increase seed set in yellow-poplar. The objective of this study is to determine the movement of pollen by honeybees in a yellow-poplar orchard. This information may be useful in determining management procedures, such as number and distribution of hives in the orchard.

In a yellow-poplar seed orchard at the University of Tennessee Plant Science Farm, seven grafted yellow-poplars were selected. A yellow-poplar in the middle of the orchard was used as the source tree. Three holes were drilled in its trunk, and these holes were filled with a radioactive solution (P^{32}), which was used as labeling material, about a week before the yellow-poplar flowers bloomed. At each distance of 40, 160, and 355 feet east and southwest of the source tree, a sample tree was selected. When the trees started flowering a full strength beehive was placed near the source tree.

Twelve days after the last day of injection of P^{32} (May 7), five flowers were picked from each selected tree; flowers were also picked on May 8, 9, 11, and 16. Each flower sample was broken into three parts (petals, top gynoecium, and bottom gynoecium), and each part was placed in a vial and measured in a liquid scintillation system. Eighty-four out of 450 flower samples had a CPM (count per minute) significantly higher than background ($P < 0.05$).

There were significant ($P = .05$) differences among the high CPM flower sample numbers for petals, top gynoecia, and bottom gynoecia; petal samples more often had a high CPM. No significant ($P = .05$) difference was observed between the total numbers of significant ($P < 0.05$) flower samples east and southwest of the source tree. There was no significant difference between number of high CPM samples of two sample trees located the same distance from the source tree.

The equation of the regression line for the association between the significant ($P < 0.10$) numbers of samples and the distance from the source tree is:

$$Y = 24.65 - .0366X$$

where X is distance in feet from the source tree. The correlation coefficient $\underline{r}$ was .765, slightly smaller than that required for significance at the 5 percent level.

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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. Its straight, excurrent branching and the excellent workability of its wood make it prime for lumber and veneer. Also the length of its fibers are acceptable for hardwood pulp. Yellow-poplar is appreciated as a specimen plant for home planting.

The large percentage of seed of low viability produced by yellow-poplar is perhaps the most important problem faced in management of seed orchards. Under natural conditions only about 10 percent of yellow-poplar samaras contain a filled seed, and they rarely contain two. Based on two potential seeds per samara, this is only 5 percent viability (Wilcox and Taft, 1963).

In a natural forest stand, a forester may have no problem with yellow-poplar reproduction because its large number of samaras usually possess an adequate number of viable seeds to replace cut trees. However, the orchard manager faces an economic problem because his profit depends on the number of bushels of viable seed that he produces.

Three factors seem to be responsible for the low seed viability of yellow-poplar:

1. Earliness of the yellow-poplar flowering date and the consequent weakness of most wild honeybees (Apis mellifera L.) at that time.

2. Movement of insects from flower to flower within the crown of a single tree, resulting in much self-pollination.

3. The short receptivity period of each individual flower.

Low viability is related to the effects of honeybees on cross pollination; no other insect excels the honeybee in pollination activities. Since honeybees can also be managed by man, it seems only reasonable to use them to attempt to increase seed set in yellow-poplar.

Since low seed viability is a critical problem in the artificial regeneration of this species, the objective of the study reported here was to determine the movement of pollen by honeybees in a yellow-poplar orchard.

CHAPTER II

REVIEW OF LITERATURE

I. FLOWERING

Morphology

The flower of yellow-poplar is perfect, pedunculate, and cup shaped. It is surrounded by a sheath of imbricated, early-deciduous sepals. The corolla is comprised of six petals, light green in color, each about two inches long, and with an orange band at its base in which the nectary is located. Adaxial to the petals are the stamens, numbering 20 to 40. The gynoecium is located within the ring of stamens and consists of 60 to 100 pistils (carpels) each with a one-celled ovary, a narrow, laterally flattened style, and a short recurved stigma (Sargent, 1947).

Phenology

Flowers usually begin to appear at about age 15, but grafts may begin flower production at two to three years (Thor, 1966). They are large and showy and begin to open early in the spring after leaves are produced.

The flowering period varies from two to six weeks, depending on the size and age of the tree and the number of flowers per tree. Trees ten to twelve inches in diameter

and larger, with well-developed crowns, usually have abundant flowers. Variation in flower development within a single tree, as well as among different trees, is great. This variation contrasts with flowering in certain other species which bloom uniformly and mature within a short period of time (Taft, 1962).

II. SEED PRODUCTION

Fruit and Seed

The fruit of yellow-poplar is an erect, conelike aggregate of samaras which ripens in the early autumn (Thor, 1966). When ripe, the samaras dehisce from the receptacle 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 seeds are empty (Boyce and Kaeiser, 1961). Although the total viability of a crop of seeds tends to be low, seed is produced in such great abundance that there is usually ample viable seed to produce a crop of natural seedlings (Thor, 1966).

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 and it is doubtful that wind dissemination occurs.

This lack of pollen movement 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).

Viability and Incompatibility

Yellow-poplar produces seed of low viability with respect to total seed production because of inefficient cross-pollination, which results in a small number of zygotes. Pollen and stigma of the same tree have been found to be generally incompatible, but some cross-pollinations are apparently no more effective than selfs (Boyce and Kaeiser, 1962a). There appears to be relatively little cross-pollination 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). Another cause for low viability includes the short receptivity period of the stigmas (12-24 hours) (Kaeiser and Boyce, 1962a).

Incompatability and subsequent low seed viability may be caused by a chemical incompatability between the style of the mother parent and the pollen tube of the pollen parent (Cox, 1973).

III. HONEY BEES AS POLLINATORS

Insects are the principal pollinating agents of yellow-poplar, in contrast to other tree species where wind is the

main factor. Flies, beetles, honeybees and bumblebees, in decreasing order of abundance, have been observed on opened yellow-poplar flowers (Wright, 1953).

The honeybee represents the highest point that has been reached in the insect world as a flower pollinator. Its own existence in both the larval and adult stages are dependent on both pollen and nectar. The habit that it possesses of working one species at a time make it a more effective pollinator than an insect that visits flowers of several species promiscuously (Dietz, 1925).

The pollinating potential of a single honeybee colony becomes evident when it is realized that its bees make up to four million trips per year, and that during each trip an average of about 100 flowers are visited (Free, 1970).

The efficiency of the honeybee is very strongly related to whether domestic or wild hives are present, and if domestic, to whether they have been managed to take full advantage of the early, profuse yellow-poplar nectar flow (Wilcox and Taft, 1969).

Although a bee species may be polytropic, most individuals keep to one flower species during a single trip. According to Taft (1969) honeybees tend to move from flower to flower within the crown of a single tree, which would result in selfing. But Free and Williams (1972) point out that pollen is transferred between bees as they brush against

each other inside the hive, so that much of the pollen on the bodies of bees leaving to forage comes from species other than the crop on which they are working. The pollen being collected most abundantly by a colony occurs on most of its bees.

Feeding the Beehive

Yellow-poplar usually blooms during the early hive building period, and thus the full effect of the honeybee is not obtained. Under proper management, domestic hives can be given an early pollen supplement with which they can raise young, and then be at full strength or maximum amount of brood at flowering time (Taft, 1962; Wolfe, 1950). A pollen supplement may be either pollen substitutes (4-9 parts soybean flour to 1 part brewer's yeast to which dry skim milk, commercial casein and dried egg yolk is sometimes added), or pollen (Haydak, 1945, 1958; Spencer-Booth, 1960).

Discussing the Hive Parts

Starting at the bottom (Figure 1) and working up, the hive has an alighting board (hive stand). Although not essential, it keeps the hive off the ground. Resting on the hive stand is the bottom board or floor of the hive. The bees enter the hive through the opening between this board and the hive body above it.

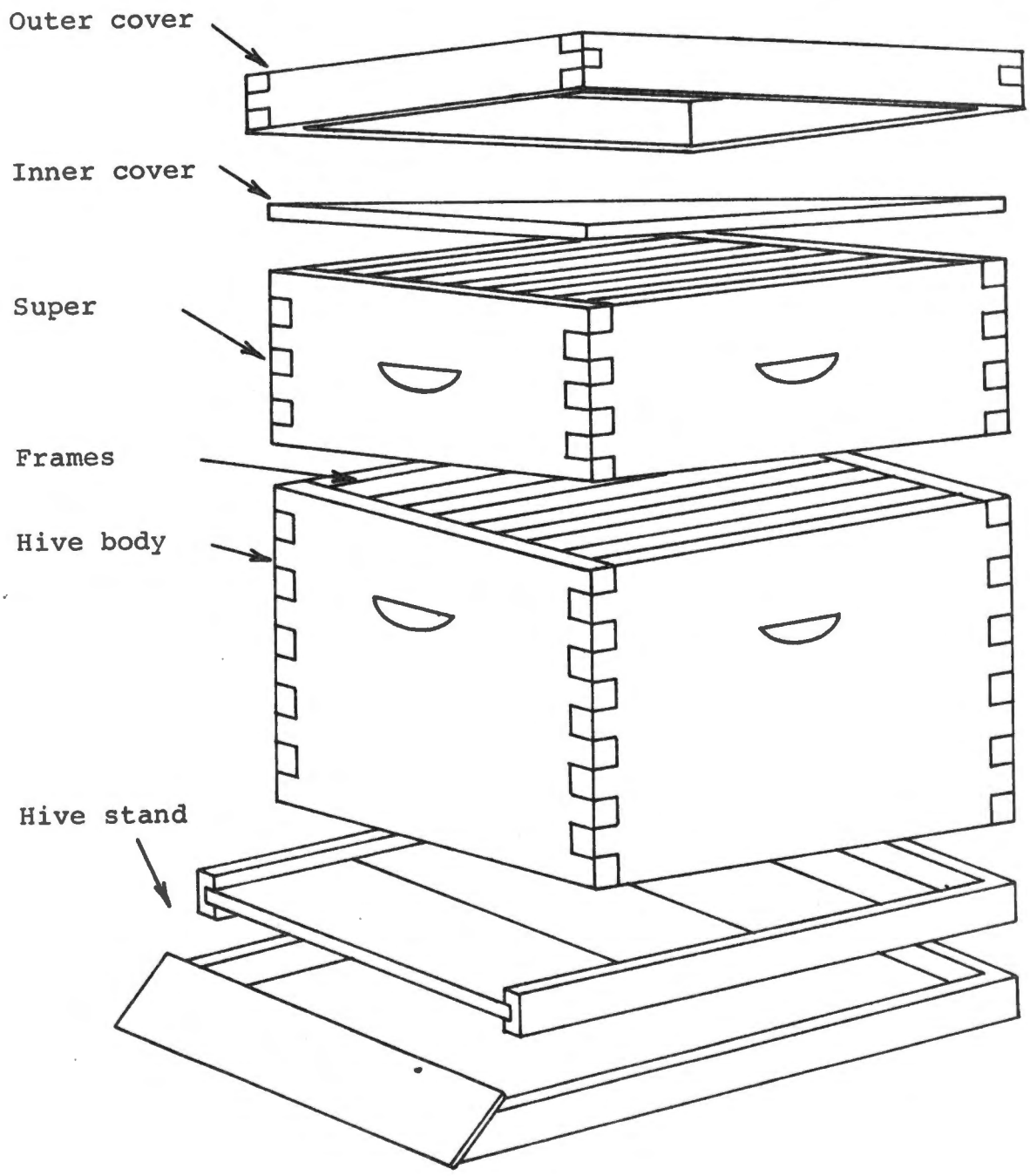


Figure 1. Diagram of parts used in beehive.

Next comes the hive body or brood chamber, sometimes called deep super. This is simple a box that holds the brood frames. Most beekeepers (apiarists) use hive bodies containing ten brood frames. Each frame has a comb foundation made of pure beeswax that is embossed on both sides with six-sided cells. The queen lays her eggs in these cells. There is also ample cell space for honey and pollen.

Every hive has a super or a "warehouse," a place for bees to store their honey. Finally, the outer and inner covers lap over all four edges of the top super to protect the hive (Little and Wallace, 1969).

Measuring the Brood Population

Pollen-gatherers are generally more valuable pollinators, and work faster than nectar-gatherers so it is beneficial to increase their numbers. Because the proportion of foragers that collect pollen increases with the amount of brood in their colonies, it is important that colonies for pollination should contain plenty of brood (Free, 1970).

Maximum pollen yields are obtained from colonies with approximately 800 square inches of brood. Colonies with more than this level of brood gather no more pollen, while those below this level gather significantly less. Brood area is a good index to the potential pollinating value of a colony and is a sound basis for grading colonies for use in pollination services (Todd and Reed, 1970).

Spacing Beehives for Pollination

Recent research indicates that the most effective pollination activity occurs within a radius of 100 yards from the hive. A radius of 100 yards covers six and one-half acres; therefore, groups of hives should be located at intervals which will overlap the 100 yard radius. The number of colonies needed per acre will dictate the number of hives to be placed in a group (Wyndham, 1970).

Provided the number of colonies per acre is constant, the smaller the groups the closer they should be together and the greater the overlap of their foraging areas. When there are up to four or five colonies per group, the overlap between the adjacent groups suffices to prevent the numbers of foragers decreasing midway between them. With twice as many colonies per group, the distance between them is too great for any overlap of the larger foraging populations to compensate for the decrease in the number of bees with distance from the colonies. Therefore, to ensure an even distribution of bees among the trees, colonies should be put in small equidistant groups throughout an orchard (Free and Spencer-Booth, 1963).

Placing the Beehive

Bees should be placed in sunny situations in early spring. They can be placed in groups so the orchardist can move equipment, if necessary, through the orchard.

Placing beehives in the orchard, just before the flowers bloom, could be necessary to attract bees away from other plants, which may be more attractive to bees (Eckert and Shaw, 1960).

If bees are to be moved a considerable distance, they should be moved at least two or three miles beyond the flight range of their former place. When this is done few bees will return to the old spot. It is preferable also to do the moving when the field bees are all in the hive, either late in the day or very early in the morning (Grout, 1973).

Whenever possible colonies are moved at night. When daytime moves are necessary the colonies are likely to be exposed to hot dry conditions and the colonies and hives should be sprayed with water. Bees can cool their colony by evaporating the water on the combs and on their mouthparts (Dunham, 1931; Lindauer, 1954).

Using a Pollen Trap on a Colony of Honeybees

For collecting pollen, traps of various designs are available; all of these are equipped with grids that function as pollen scrapers. When a bee lands on the surface of the trap and walks through the grid, the pollen is scraped from her legs and falls into a tray beneath the grid.

The first traps constructed were placed at the bottom of the hive, but in that position they were cumbersome to

handle and service. Lavie and Fresnaye (1963) then presented the idea of placing the pollen trap at a higher level in the colony. Makar (1964) improved on this concept, but his pollen traps were complex in design. Their construction is difficult and time consuming, and their use requires considerable extra handling of colonies.

For these reasons Harp designed a new type of trap to eliminate such problems (Figure 2). This trap is easy to build and install. It does not retard brood rearing, since it is in place in the hive for such a short time. The tray can be removed without disturbing the bees and the bees are not disturbed drastically when the trap is installed (Harp, 1966).

IV. RADIOACTIVE ISOTOPES

A radioactive isotope may either occur naturally or be made artifically. A radioactive isotope, in contrast to a stable isotope, contains atoms that undergo change and emit radiation of one or more types such as alpha (α), beta (β^+ or β^-), or gamma (γ).

An example of a radioactive isotope is P^{32} which has "too many" neutrons. Stable phosphorus has 15 neutrons, while radioactive P^{32} has 16 neutrons; therefore, P^{32} has one additional neutron. The most favored route by which a high N/Z ratio (neutron-rich) nuclide decays is by the

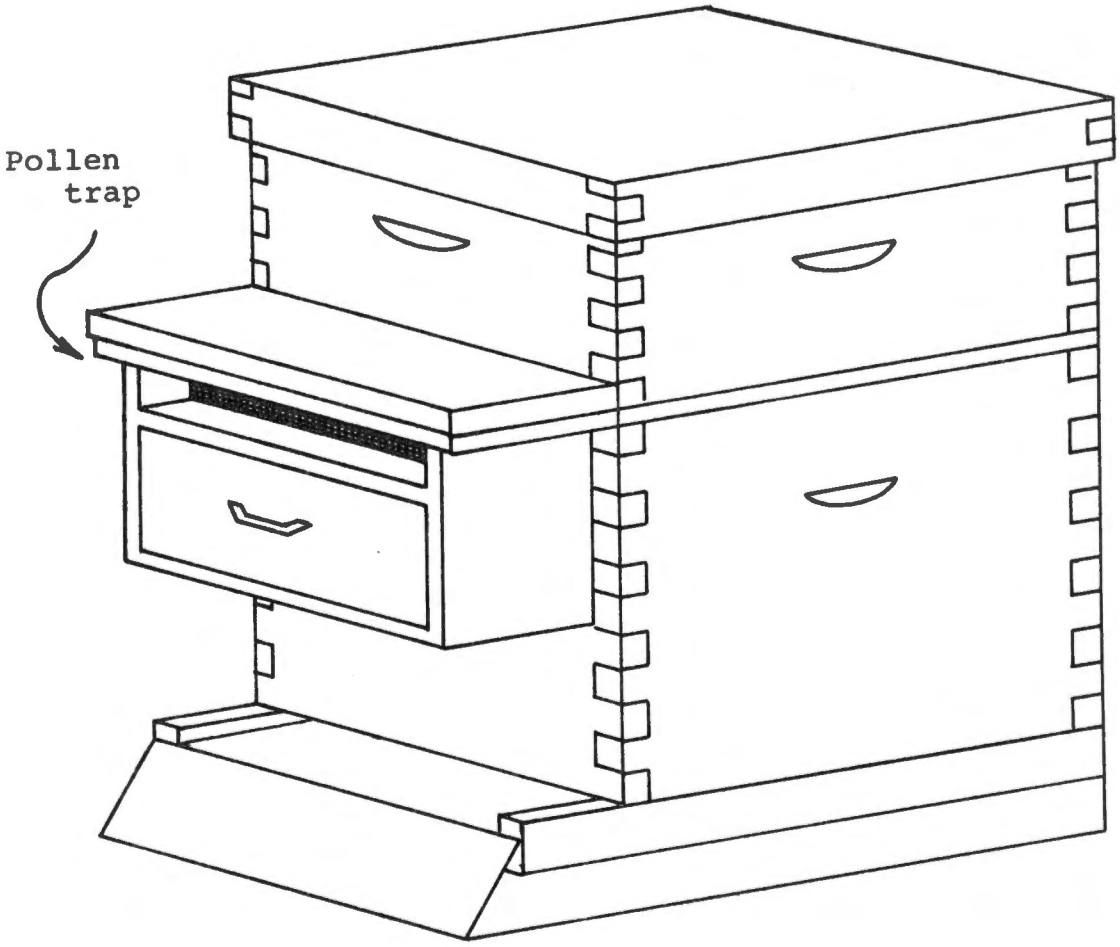


Figure 2. Diagram of beehive with a pollen trap.

conversion of a neutron into a proton with the expulsion of a beta particle and an antineutrino (Wang and Willis, 1965).

P^{32} has an energy of 1.7 Mev (million electron volts) with a half-life of 14.3 days. Hazard classification for P^{32} is "dangerous," the most critical danger of beta emissions being to the bone marrow. Effective shielding is attained with plastic or glass.

Use of Radioisotopes as Tracers

The use of radioactive isotopes as tracers in the study of biological, medical and agricultural problems is of the greatest significance. It is made possible because a radioactive atom has the same chemical behavior as the corresponding stable atom.

Sensitivity of detection using radiotracers far exceeds that with most other chemical or physical methods. This high sensitivity is equivalent to the magnification of biological processes to an extraordinary power to allow ready detection (Wang and Willis, 1965).

The short half-life, low cost, and relative safety in handling P^{32} led to the use of this radioactive isotope of phosphorus as a labeling material. P^{32} is readily available as H_3PO_4 in HCL solution.

Types of Radioactivity Measurements

Two systems were used to detect radiation in this study, the Geiger-Mueller (G-M) system and the liquid scintillation system.

The G-M detector tube is filled with a gas mixture, usually at a reduced pressure. A readily ionizable, inert gas (argon, helium, neon) makes up the bulk of the gas filling; only a small amount of organic or halogen gas is introduced as the quenching agent.

When an ionizing radiation enters the chamber and produces ionization, it throws the entire chamber into saturation ionization. This event is registered on a meter which displays the rate at which these events take place (Wang and Willis, 1965).

The liquid scintillation system is a beta counting spectrometer that is primarily designed to count H^3, C^{14}, and P^{32}. The basic detectors used in this system are two 12-stage headon type of photomultiplier tubes. When an emission from a radioactive sample strikes a scintillating substance (fluor) in which it is immersed or dissolved, a flash of light is given off. These light flashes are very short, usually being less than 0.1 μsec in duration.

This incident light impinges on the curved photocathode surface of each photomultiplier. When the light is absorbed, photoelectrons are given off. Electrons given off in the

direction of the back of a tube can come free of the surface and are accelerated in the electrical field gradient toward the first dynode. The high secondary emission ratio of the dynodes are such that a single electron can give rise to two or more secondary electrons which in turn are accelerated toward the next dynode. This cascading process causes an avalanche of electrons to arrive at the anode thus producing a signal. When a signal from each photomultiplier reaches an appropriate gate at the same time, the event is recorded as a "count" (Wang and Willis, 1965).

CHAPTER III

METHODS

P^{32} was used as labeling material as H_3PO_4 in HCL solution. Phosphorus is one of the minerals taken up from the soil by the roots and transported to the pollen via the vessels in yellow-poplar. Most of the water and minerals is transported from the root to the crown by transpiration via the xylam; thus the cambium layer may only account for a very small amount of the transportation (McElwee, 1970).

To determine whether this assumption is correct, a dye water was used as a tracer. In late February, at the Cherokee Woodlot which is located near the University of Tennessee Agricultural Campus, a loblolly pine and a yellow-poplar with diameter at breast height of about six inches were chosen as experimental trees.

A half inch hole was drilled slightly downward into each trunk toward the pith. This hole was two feet above the ground and was plugged with a cork containing a small hole. Cement was applied around the cork to seal the small cracks. Using a hypodermic needle, this large hole was filled with dye water through the hole in the cork. A small plastic tube, three feet long, was inserted into the cork hole, and the other end was placed into the stopper of a

one-liter bottle. Both the tube and the bottle were full of dye water before using, so that air bubbles could not develop. This bottle was suspended about two feet above the drilled hole (Figure 3).

Four days later, each tree was cut down and a straight yellow line painted from the drilled hole to the crown. Sections five feet long were cut above the drilled hole; then, discs two inches thick were sawed off the bottom of each section and numbered.

The first disc of the loblolly pine, at the drilled hole, contained a circle of dye at least four inches in diameter between the pith and the bark directly behind the yellow-painted bark. The same type of dye circle appeared in the next four discs, but they were smaller. The fifth disc, taken 20 feet above the drilled hole, contained only a very small amount of dye. Since all the dye spots were located behind the yellow strip the dye water went straight up from the hole. The sixth disc and the branches contained no dye. This tree absorbed four liters of dye water in four days through one drilled hole.

This technique did not work on yellow-poplar because the top of the first disc contained no dye. Either a larger yellow-poplar nearby could take the dye water away from this tree by root grafting or, since there was no foliage on the tree, the transpiration pull may have been inadequate to move the dye up in the trunk.

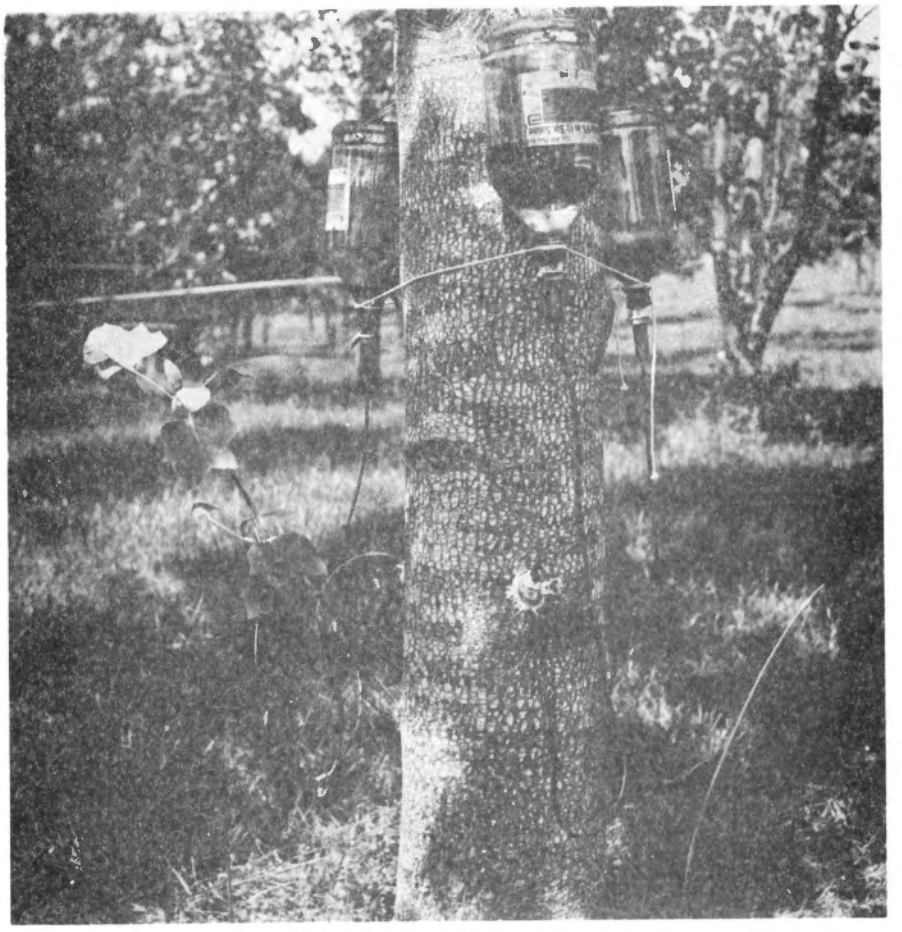


Figure 3. A yellow-poplar source tree with the three one-liter bottles, corks, and tubes.

The next test of this technique was to determine how many holes were needed to obtain distribution of dye all around the pith at five feet above the drilled hole. With one drilled hole on the loblolly pine, dye covered about a third of the area of the second disc. Thus, three drilled holes on one tree was estimated to be sufficient for obtaining dye in the entire cross section of the tree before the dye reached the first branch.

Another yellow-poplar, which had no other yellow-poplar growing nearby, was chosen and dye water applied using the same method as before, but with three drilled holes and three bottles. Four days later the results were negative; no dye had moved up the stem.

On April 23, about four to seven days before the yellow-poplar flowers bloomed, three holes were drilled into the stem of an orchard tree two feet above the ground. These holes were evenly spaced around the stem (Figure 3). The cork, tube, and liter bottle were installed by each drilled hole of the tree.

Phosphorus liquid solution (H_3PO_4 in HCL solution) of 2.5 ml was available for the experiment. This liquid solution was equivalent to 100 millicuries. To dilute 2.5 ml of liquid solution (P^{32}) to 18 liters with H_2O (pH of 5.0), .14 ml of liquid solution (P^{32}) was added to one liter of H_2O in each bottle. A long pipette with a rubber bulb was

used to draw liquid solution (P^{32}) from the plastic vial and to empty it into a liter bottle.

It was estimated that each drilled hole of this tree would absorb two liters of liquid per day; therefore, six liters of liquid per day would be needed for three drilled holes. After three days the drilled tree was expected to absorb all 18 liters of available solution. A check of the foliage with a Geiger-Mueller counter showed that this technique of applying P^{32} into the yellow-poplar was successful.

For all studies in which transportation of radioisotopes was required, the liquid solution (P^{32}) was stoppered in a plastic vial and transported in a 1/4 inch sheet lead-lined (screw-cap) bottle. Protective clothing, plastic boots, and plastic gloves were used in each instance where isotopes were handled in the field.

I. MANAGING BEES

Management of the beehives was started in February to strengthen them for the early spring yellow-poplar flowers (Taft, 1962; Wolfe, 1950).

In early February, pollen substitute and sugar solution (Spencer-Booth, 1960; Grout, 1973) were prepared for the colonies of bees. Water was added to the pollen substitute to make paste. This paste was formed into a disc one inch

thick which weighed about six pounds; a plastic sheet covered both top and bottom of this paste to maintain moisture longer. The paste was divided into two parts to feed two hives and many small holes were punched in the plastic covers to enable the bees to eat pollen substitute without difficulty.

The sugar solution was poured into two quart bottles, and then caps with holes were screwed onto these bottles.

After removing the outer and inner covers from the hive, the shallow super was taken off and the pollen substitute was placed on top of the brood chamber. The shallow super was then replaced. A deep super without frames was placed on top of the shallow super, and a full quart bottle of sugar solution was placed carefully upside down in the deep super. The hives were checked once a week to keep the bottles full of sugar solution and to replace pollen substitute when needed.

Three weeks before the yellow-poplar flowers bloomed, these two hives were moved to the University of Tennessee Agricultural Campus which is located about four and one-half miles away from the seed orchard. According to Dr. Pless (Agricultural Biology Department, the University of Tennessee) during a personal interview, the hives should be moved at least two or three miles beyond the flight range of the seed orchard and placed in a temporary location until the yellow-poplar flowers bloom. The purpose of moving the hives beyond

the flight range of the seed orchard was that the bees would not have developed a foraging habit to a more distant (but perhaps richer) source at the time of flowering in the orchard. Dr. Pless was of the opinion that the bees in the previous year showed preference for nectar trees in a natural stand of yellow-poplar near the seed orchard.

The better of the two hives, having a larger brood, was moved back to the seed orchard when the yellow-poplar flowers started blooming in early April.

II. SELECTION OF YELLOW-POPLAR SAMPLE TREES

The yellow-poplar seed orchard is located about four and one-half miles southwest of the University of Tennessee Agricultural Campus. This orchard is part of the Plant Sciences Farm and has a total of about four acres (Figure 4).

The sample trees had to be in good health and with a sufficient numbers of flower buds which would bloom four to seven days later. About 100 flower buds on each yellow-poplar were considered to be sufficient.

Seven yellow-poplars were selected as sample trees. One of these was the radioactive source tree which was located near the center of the yellow-poplar seed orchard. Three sample trees were located east of the source tree 40, 160, and 437 feet, respectively. Three trees were located

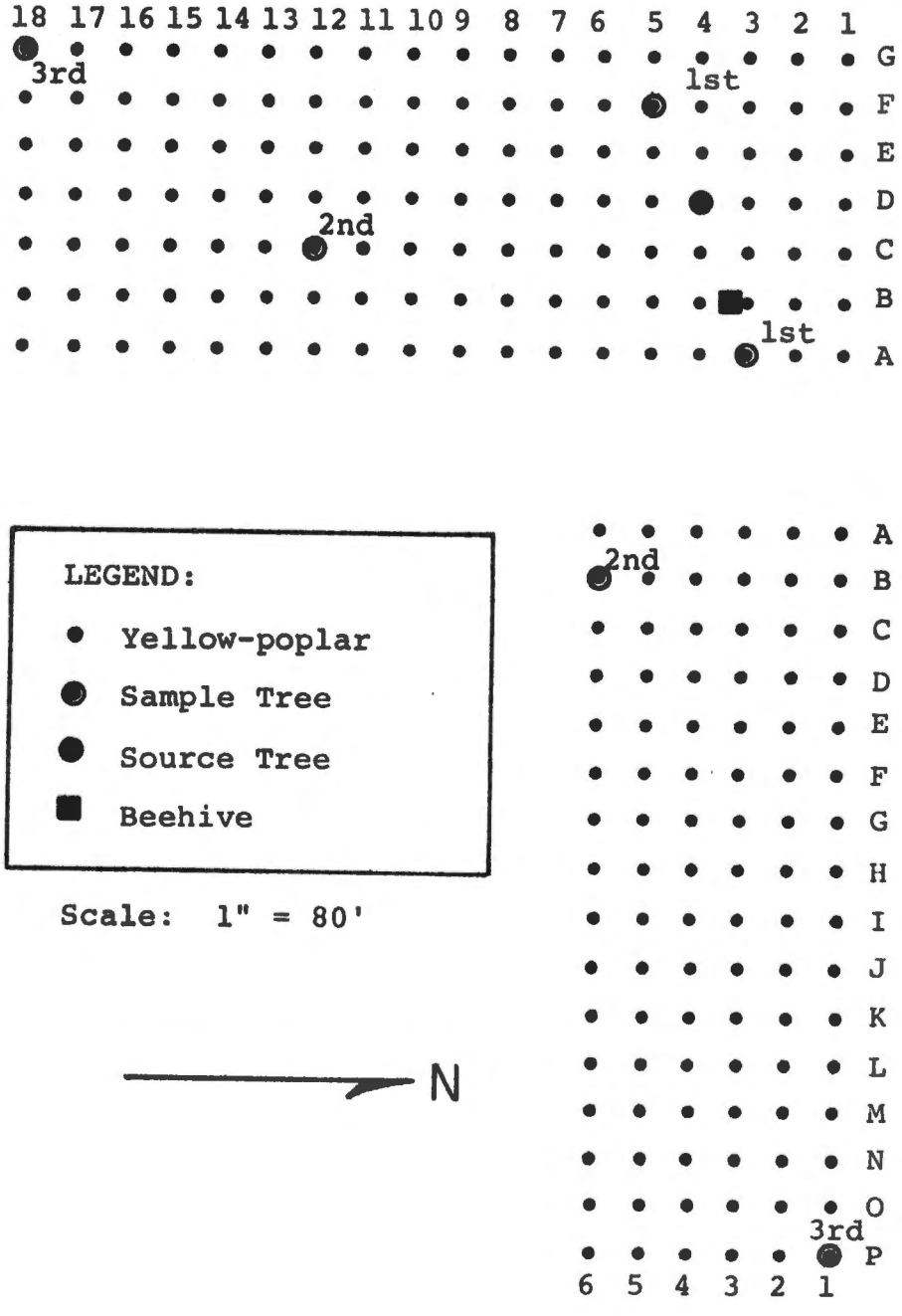


Figure 4. Diagram of the location of the beehive, sample trees, and the source tree in the yellow-poplar seed orchard.

southwest of the source tree 40, 160, and 280 feet, respectively (Figure 4).

The distance of the first, second, and third trees east and southwest of the source tree should be equal, but the selection of qualified yellow-poplars made it impossible to have exactly equal distances. For purposes of analysis, the first, second, and third trees will be assumed 40, 160, and 355 feet away from the source tree.

On May 7, twelve days after the last day of injection of p^{32} five flowers were picked from each sample tree. These flowers had been in bloom about 24 hours; five yellow-poplar flowers were also picked from each tree on May 8, 9, 11, and 16.

III. MEASUREMENT OF RADIOACTIVITY

Each picked flower was handled carefully with a paper towel to prevent contact between the anthers and the gynoecium. The corolla of the flower was broken off, and the bottom parts of two petals were placed inside a plastic vial. The gynoecium was cut into two parts in which the top part contained most of the pistils. The top and bottom parts were placed in separate plastic liquid scintillation vials. Each vial was numbered with a marker.

After each collection day the yellow-poplar flower parts were transported to the Biology Department, where the

liquid scintillation system was located. The liquid scintillation system was located. The liquid scintillation system is a soft-beta counting spectrometer that is primarily designed to count H^3, C^{14}, and P^{32}. The two basic units are an automatic two-channel instrument and an automatic three channel instrument, both of which have a manual capability. Its printout device is a modified Model 33 Teletypewriter.

The vials were filled halfway with scintillating materials (PPO or 2,5- diphenyloxazole in toluene) which is known as fluor. These vials were placed in the conveyor which can hold 200 vials.

With the samples inside the conveyor and the control panel ready, the AUTO-START/REJECT pushbutton was depressed. Then the automatic counting cycle which was programmed into the instrument was started.

On May 6, three honeybees were caught with an insect net just after they had visited the flowers of the source tree. With a tweezer, two hind legs of the first bee, which had full pollen sacs, were placed inside the vial; the rest of the body was placed inside the other vial. Each hind leg, head, and the part left over of a second bee were placed in the vials separably. The whole body of a third bee was put in a vial. These vials were filled halfway with

fluor and counted as the vials which contained yellow-poplar flowers.

On May 7, the pollen trap (Harp, 1966) was used on the hive located near the source tree. Six hours later, the pollen trap drawer was pulled out, and the pollen sacs were collected with a tweezer. Each of the orange, yellow, and gray pollen sacs were placed in a separate vial. A mixture of orange, yellow, and gray pollen sacs was placed in the fourth vial. These four vials were also filled halfway with fluor and counted in the liquid scintillation system.

IV. STATISTICAL METHODS USED IN THE MEASUREMENT OF RADIOACTIVITY

The statistical aspects of radioactive counting are limited to estimation of the accuracy of counting rate determinations. The formula used was as follows:

$$D = K \left[\frac{C_s}{t_s} + \frac{C_b}{t_b} \right]^{1/2}$$

where D = difference between C_s and C_b

C_s = count rate of sample

C_b = count rate of background

t_s = time of sample count

t_b = time of background count

K = 1.64 at 10 percent probability

K = 1.96 at 5 percent probability

K = 2.58 at 1 percent probability

Blank vials which contain only fluor were placed among every 20 vials in the liquid scintillation system. These blank vials were used for background count rates.

When the difference in count between the background and sample was greater than D then the count was regarded as significant at the selected level of probability (Jarrett, 1946).

CHAPTER IV

RESULTS

Four hundred sixty-two samples were counted in the liquid scintillation system. Jarrett's (1946) statistical formula was used to determine which samples had a count significantly higher than the background count (Table 1).

I. BEE AND POLLEN SAMPLES

All counts of the three bees which were initially caught to provide a check on the technique were significant ($P < 0.01$). This included the whole bee as well as legs, head, and bodies (Table 2).

Five pollen samples collected from the pollen trap on May 7 had CPMs which were not significant ($P < 0.05$).

II. FLOWER SAMPLES

About 19 percent of the yellow-poplar flower samples had a CPM significantly higher than background ($P < 0.05$). While about 14 percent of the flower samples had a higher CPM ($P < 0.01$)(Table 3 and 4) nearly half of the high CPM ($P < 0.05$) and ($P < 0.01$) flower samples were petals.

Nearly 90 percent of the radioactive ($P < 0.05$) and ($P < 0.01$) flower samples were collected on the first three

Table 1. Number of samples with a CPM significantly higher than background.

Samples	Number of Samples	Statistical Significance 95%	99%
Bee's structures	7	7	7
Pollen	5	0	0
Flowers' parts	450	84	61
Total	462	91	68

Table 2. Count per minute of structures from three bees.

Bee Number	Bee's Structure	CPM*
1	Two hind legs	2,579
	Body with no hind legs	2,627
2	Whole body	10,350
3	One hind leg	326
	One hind leg	380
	Head	2,780
	Remaining body	9,282

*All counts significant at 0.01.

Table 3. Number of flower samples with a significantly (at the 95 percent probability level) higher CPM than background count by dates, parts of flowers, and designated trees.

Tree Number and Location	Date	Petals	Gynoecium		Total
			Bottom	Top	
	May				
A-3 40 Feet East of Source Tree	7	3	2	2	7
	8	4	3	4	11
	9	2	1	1	4
	11	1	0	0	1
	16	0	0	0	0
Total		10	6	7	23
B-6 160 Feet East of Source Tree	7	2	2	0	4
	8	2	2	3	7
	9	0	0	0	0
	11	1	0	0	1
	16	1	0	0	1
Total		6	4	3	13
P-1 437 Feet East of Source Tree	7	3	4	0	7
	8	2	2	1	5
	9	0	0	0	0
	11	0	0	0	0
	16	0	0	0	0
Total		5	6	1	12
F-5 40 Feet Southwest of Source Tree	7	3	0	3	6
	8	2	3	0	5
	9	3	1	2	6
	11	2	0	0	2
	16	2	0	1	3
Total		12	4	6	22
C-12 160 Feet Southwest of Source Tree	7	0	1	0	1
	8	0	0	0	0
	9	3	2	2	7
	11	0	0	0	0
	16	0	0	0	0
Total		3	3	2	8
G-18 280 Feet Southwest of Source Tree	7	2	0	1	3
	8	0	0	1	1
	9	0	0	0	0
	11	1	0	0	1
	16	1	0	0	1
Total		4	0	2	6
Grand Total		40	23	21	84

Table 4. Number of flower samples with a significantly (at the 99 percent probability level) higher CPM than background count by dates, parts of flowers, and designated trees.

Tree Number and Location	Date	Petals	Gynoecium		Total
			Bottom	Top	
	May				
A-3 40 Feet East of Source Tree	7	2	1	1	4
	8	3	2	4	9
	9	2	0	0	2
	11	0	0	0	0
	16	0	0	0	0
Total		7	3	5	15
B-6 160 Feet East of Source Tree	7	1	1	0	2
	8	2	2	3	7
	9	0	0	0	0
	11	0	0	0	0
	16	1	0	0	1
Total		4	3	3	10
P-1 437 Feet East of Source Tree	7	3	3	0	6
	8	2	2	1	5
	9	0	0	0	0
	11	0	0	0	0
	16	0	0	0	0
Total		5	5	1	11
F-5 40 Feet Southwest of Source Tree	7	1	0	3	4
	8	2	3	0	5
	9	3	0	1	4
	11	1	0	0	1
	16	0	0	1	1
Total		7	3	5	15
C-12 160 Feet Southwest of Source Tree	7	0	0	0	0
	8	0	0	0	0
	9	3	2	2	7
	11	0	0	0	0
	16	0	0	0	0
Total		3	2	2	7
G-18 280 Feet Southwest of Source Tree	7	1	0	0	1
	8	0	0	1	1
	9	0	0	0	0
	11	1	0	0	1
	16	0	0	0	0
Total		2	0	1	3
Grand Total		28	16	17	61

dates (Table 5 and 6). A chi-square goodness of fit test (Table 7) was used to test the hypothesis that there was no significant difference among the high CPM sample numbers for petals, top gynoecia, and bottom gynoecia. For samples significant ($P<0.05$), the calculated x^2 value was 7.78. Because the calculated x^2 exceeds the table x^2 value for the appropriate degrees of freedom, the null hypothesis is rejected ($P=.05$). For samples significant ($P<0.01$), the calculated x^2 is 4.35 and the null hypothesis $Ho:u_1=u_2=u_3$ can not be rejected ($P=.05$).

Effect of Direction

The second null hypothesis was tested to determine whether there was a significant difference between the total number of significant samples east and southwest of the source tree (Table 8). With significant samples ($P<0.05$), the calculated x^2 is 1.72. This calculated x^2 does not exceed the table x^2 at ($P<0.05$) for the appropriate degree of freedom; therefore, the null hypothesis $Ho:u_1=u_2$ is not rejected. For highly significant samples ($P<0.01$), the calculated x^2 is 1.98 and the null hypothesis can not be rejected at the 5 percent level of significance. Hence, there was no significant difference between the total number of high CPM samples east and southwest of the source tree.

Table 5. Number of flower samples with a significantly ($P < 0.05$) higher CPM than background level by dates and designated trees.

	Designated Trees						
	East			Southwest			
Date	1	2	3	1	2	3	Total
May 7	7	4	7	6	1	3	28
8	11	7	5	5	0	1	29
9	4	0	0	6	7	0	17
11	1	1	0	2	0	1	5
16	0	1	0	3	0	1	5
Total	23	13	12	22	8	6	84

Table 6. Number of flower samples with a significantly ($P < 0.01$) higher CPM than background level by dates and designated trees.

	Designated Trees						
	East			Southwest			
Date	1	2	3	1	2	3	Total
May 7	4	2	6	4	0	1	17
8	9	7	5	5	0	1	27
9	2	0	0	4	7	0	13
11	0	0	0	1	0	1	2
16	0	1	0	1	0	0	2
Total	15	10	11	15	7	3	61

Table 7. The test of a null hypothesis among numbers of significant samples of petals, top gynoecium, and bottom gynoecium.

Sample	a Actual Number	e Expected Number	a-e	$(a-e)^2$	$\frac{(a-e)^2}{e}$	Total x^2	P
			Significant Samples ($P < 0.05$)				
Petals	40	28	12	144	5.14		
Gynoecium (bottom)	23	28	-5	25	.89	7.78	.01<p<.025
Gynoecium (top)	21	28	-7	49	1.75		
			Significant Samples ($P < 0.01$)				
Petals	28	20.33	7.67	58.83	2.89		
Gynoecium (bottom)	16	20.33	-4.33	18.75	.92	4.35	.1<p<.25
Gynoecium (top)	17	20.33	-3.33	11.09	.54		

Table 8. The test of a null hypothesis between the total numbers of significant flower samples east and southwest of the source tree.

Direction	a Actual Number	e Expected Number	a-e	$(a-e)^2$	$\frac{(a-e)^2}{e}$	Total	P
			Significant Samples ($P < 0.05$)				
East	48	42	6	36	.86	1.72	.1<p<.24
Southwest	36	42	-6	36	.86		
			Significant Samples ($P < 0.01$)				
East	36	30.5	5.5	30.25	.99	1.98	.1<p<.25
Southwest	25	30.5	-5.5	30.25	.99		

Effect of Distance

Since the two sample trees farthest away from the source tree were at distances of 280 and 437 feet, respectively, the average of these two distances (355 feet) was used in the statistical analysis.

The third null hypothesis was tested to determine whether there was any significant difference among the total numbers of high CPM samples from three trees at different distances east of the source tree (Table 9). With samples ($P < 0.05$), the calculated x^2 is 4.62. Therefore, the null hypothesis $Ho: u_1=u_2=u_3$ can not be rejected ($P = 0.05$). With significant samples ($P < 0.01$), the calculated x^2 is 1.16 and the null hypothesis $Ho: u_1=u_2=u_3$ again can not be rejected. Hence, there was no significant difference among the significant numbers of samples of three trees located at different distances east of the source tree.

The fourth null hypothesis was tested to determine whether there was a significant difference among the total number of high CPM samples of three trees located southwest of the source tree (Table 10). Using significant samples ($P < 0.05$), the calculated x^2 is 12.66. Therefore, the null hypothesis $Ho: u_1=u_2=u_3$ is rejected ($P = .05$); a significant difference in total number of high CPM samples was observed among the three trees located at different distances southwest of the source tree. With highly

Table 9. The test of a null hypothesis among number of significant flower samples from three trees at different distances east of the source tree.

Distance (feet)	a Actual Number	e Expected Number	a-e	$(a-e)^2$	$\frac{(a-e)^2}{e}$	Total	P
		Significant Samples ($P < 0.05$)					
40	23	16	7	49	3.06		
160	13	16	-3	9	.56	4.62	.05<p<.10
437	12	16	-4	16	1.00		
		Significant Samples ($P < 0.01$)					
40	15	12	3	9	.75		
160	10	12	-2	4	.33	1.16	.50<p<.75
437	11	12	-1	1	.08		

Table 10. The test of a null hypothesis among number of significant flower samples from three trees at different distances southwest of the source tree.

Distance (feet)	a Actual Number	e Expected Number	a-e	$(a-e)^2$	$\frac{(a-e)^2}{e}$	Total	P
		Significant Samples ($P < 0.05$)					
40	22	12	10	100	8.33	12.66	P<.005
160	8	12	-4	16	1.33		
280	6	12	-6	36	3.00		
		Significant Samples ($P < 0.01$)					
40	15	8.33	6.77	44.49	5.34		
160	7	8.33	-1.33	1.77	.21	8.96	.01<p<.025
280	3	8.33	-5.33	28.41	3.41		

significant samples ($P < 0.01$), the calculated x^2 is 8.96. Therefore, this null hypothesis $Ho: u_1 = u_2 = u_3$ is also rejected ($P = .05$).

The fifth null hypothesis was tested to determine whether there was a significant difference between number of high CPM samples of two trees located the same distance from the source tree (Table 11). At a distance of 40 feet from the source tree, the calculated x^2 is .02 for samples significant ($P < 0.05$). Also, the calculated x^2 is 0 for the highly significant samples ($P < 0.01$). Thus both null hypotheses $Ho: u_1 = u_2$ are accepted ($P = .05$). At a distance of 160 feet from the source tree, the calculated x^2 is 1.18 for the significant samples and .52 for the highly significant samples. Therefore, the null hypotheses $Ho: u_1 = u_2$ are not rejected at the 5 percent level of significance. At a distance of 355 feet from the source tree, the calculated x^2 is 2.00 ($P < 0.05$), and 4.58 for the highly significant samples. Then the null hypothesis is accepted ($P = .05$) for the samples significant at the 95 percent level of probability but is rejected for the highly significant samples.

The regression line for the association between the significant numbers of samples and the distance from the source tree is shown in Figure 5. With samples significant ($P < 0.10$), the equation of the regression line of best fit is:

Table 11. The test of a null hypothesis between number of significant samples of two trees located the same distance from the source tree.

Distance (feet)	Direction	a Actual Number	e Expected Number	a-e	$(a-e)^2$	$\frac{(a-e)^2}{e}$	Total	P
			Significant Samples ($P < 0.05$)					
40	East	23	22.5	.5	.25	.01	.02	P=.90
	Southwest	22	22.5	-.5	.25	.01		
160	East	13	10.5	2.5	6.25	.59	1.18	.25<p<.50
	Southwest	8	10.5	-2.5	6.25	.59		
355	East	12	9	3	9	1	2.00	.10<p<.25
	Southwest	6	9	-3	9	1		
			Significant Samples ($P < 0.01$)					
40	East	15	15	0	0	0	0	P=1.0
	Southwest	15	15	0	0	0		
160	East	10	8.5	1.5	2.25	.26	.52	.025<p<.05
	Southwest	7	8.5	-1.5	2.25	.26		
355	East	11	7	4	16	2.29	4.58	.025<p<.05
	Southwest	3	7	-4	16	2.29		

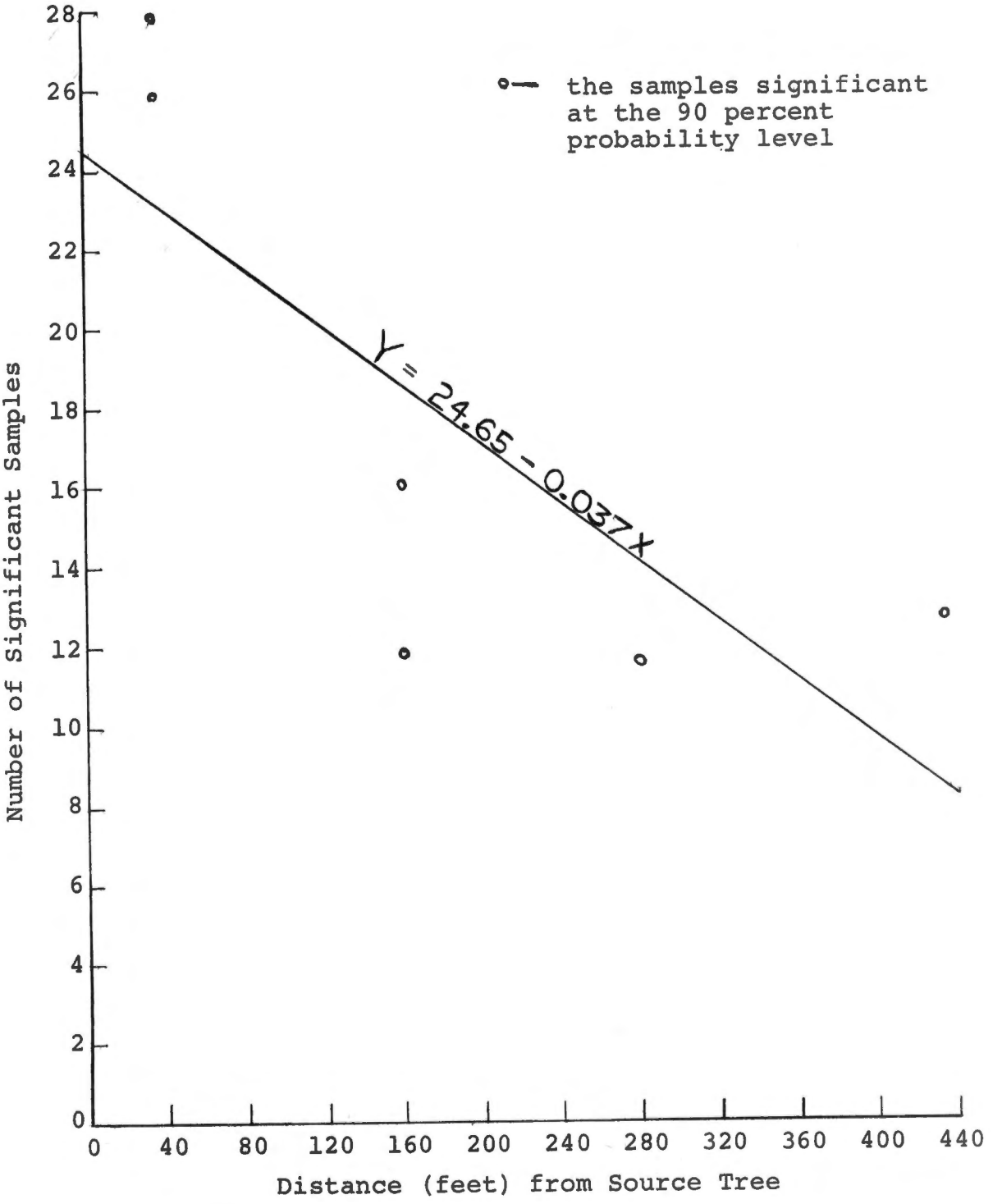


Figure 5. Regression line for the association between the number of significant samples and the distance from source tree.

$$Y = 24.65 - 0.037X$$

Where X is distance in feet from the source tree and the 95 percent confidence limits of the regression coefficient (b) are:

$$-.037 \pm .043$$

The null hypothesis was tested to determine if the b value may be equal to zero (no population relationship). The test statistic for that null hypothesis is:

$t = b/s_b$ (Student's t with n-2 degrees of freedom)

With samples significant ($P < 0.10$), t equals 2.56, and the null hypothesis is accepted ($P = .05$).

With samples significant ($P < 0.10$), r equals to 0.765. This correlation coefficient is almost significant ($P_{.05} = .811$).

With samples significant at the 5 percent level, the equation of the regression line of best fit is:

$$Y = 19.713 - 0.031X$$

and 95 percent confidence limits of b are:

$$-.031 \pm .042$$

With samples significant ($P < 0.05$), t equals 2.07, and the null hypothesis cannot be rejected; therefore, -0.031 is not significantly different from zero. The value of $\underline{r}$ equals to 0.660. This correlation coefficient is not significant ($P_{.05} = .811$).

With highly significant samples ($P < 0.01$), the equation of the regression line of best fit is:

$$Y = 13.043 - 0.015X$$

and 95 percent confidence limits of b are:

$$-.015 \pm .037$$

With highly significant samples ($P < 0.01$), t equals 1.11, and the null hypothesis is not rejected; -.015 is not significantly different from zero. The value of $\underline{r}$ equals to 0.504. This correlation coefficient is not significant ($P_{.05} = .811$).

CHAPTER V

DISCUSSION AND CONCLUSION

To determine whether honeybees contained radioactive pollen on their hairs, three honeybees that had just visited the flowers of the source tree were caught with an insect net. All counts of these honeybees were significantly ($P < 0.01$) higher than the background (Table 2, p. 30). The third bee's head and remaining body had a greater CPM than its hind legs; this honeybee may have collected only nectar. The first bee may have collected pollen and nectar because high CPM were obtained in both the hind legs and remaining body. A possible factor causing such a wide range of CPMs among the three honeybees could have been the different number of the source tree's flowers visited by each of the three honeybees.

On May 7, five pollen samples collected from the pollen trap of the hive near the source tree had CPMs which were not significantly ($P < 0.05$) higher than the background (Table 1, P. 30). The reasons for these non-significant ($P < .05$) samples was the small amount of radioactive pollen and few (five) number of pollen samples. One reason for the small amount of radioactive pollen could have been that the honeybees concentrated on the collection of nectar, and not

pollen from the yellow-poplar source tree. Also, many of the honeybees that visited the flowers of the source tree probably came from other hives located outside the orchard.

Nearly 90 percent of the flower samples with a CPM significantly ($P < 0.05$) and ($P < 0.01$) higher than the background level, were collected during the first three dates (May 7, 8, 9) (Table 5, p. 34). Most of the flowers were probably open during the first three dates and then a much smaller number opened on May 11 and 16 (last two dates). After the third date (May 9), most of the honeybees probably disregarded the yellow-poplar flowers in the seed orchard in favor of sources outside the orchard.

Two reasons may explain the rejection of the null hypothesis of no significant difference for number of samples of petals, top gynoecia, and bottom gynoecia which had a CPM significantly ($P < 0.05$) higher than the background were petals. When the honeybee entered the yellow-poplar flower, the sticky nectar at the basal part of the petals probably removed more pollen from the honeybee hairs than the gynoecia. Another reason for the small number of gynoecia that had a CPM significantly ($P < 0.05$) higher than the background, was the partial or full opening of the flowers at different periods of time. The honeybees were forced to go between the gynoecium and the anthers to obtain nectar only during the first eight hours after flower

opening. After the flowers had been open about eight hours, the honeybees could visit the flowers for a couple of days going between the anthers and the petals to collect nectar. While the honeybees collect nectar between the petals and the anthers, the gynoecia will probably rub less pollen off the honeybee hairs than that removed by the petals.

There was no significant ($P < 0.05$) samples east and southwest of the source tree (Table 8, p. 36). The honeybees with the radioactive pollen on their hairs probably traveled irrespective of the direction from the source tree to the other yellow-poplars in the orchard. Environmental factors, i.e. wind, apparently did not affect the direction of the honeybees' flight. Since prevailing winds came out of the West a larger number of significant samples would have been expected east of the source tree if wind were a significant pollen vector for yellow-poplar. The number of significant ($P < 0.05$) samples of two sample trees located the same distance from the source tree were not significantly different ($P = .05$) (Table 11, p. 41), offering evidence that the honeybees probably traveled irrespective of the direction from the source tree to the other yellow-poplar sample trees.

The null hypothesis of no significant difference ($P = .05$) among the total number of significant ($P < 0.05$) flower samples of three sample trees east of the source tree is accepted (Table 9, p. 38).

Most of the yellow-poplar flowers east of the source tree had bloomed about one week before the sample flowers bloomed. This difference in flower stages probably caused the large number of significant flower samples from the farthest sample tree east of the source tree. Honeybees may have lost less radioactive pollen since they visited a smaller number of flowers east of the source tree. Also, by chance, there may have been an unusually high number of significant samples from one or two sample trees east of the source tree; the number of sample trees was too small to compensate for such variation in samples.

The null hypothesis of no significant difference among the total numbers of significant ($P < 0.05$) flower samples of three sample trees southwest of the source tree, was rejected (Table 10, p. 39). Honeybees probably have fewer radioactive pollen on their hairs when they visited the next sample tree which was located at greater distance from the source tree. This is probably true because the number of significant samples was lower at each successive distance farther from the source tree.

Three nonsignificant ($P = .05$) $\underline{r}$ values for the association between the number of significant ($P < 0.10$, $P < 0.05$, and $P < 0.01$) samples and the distance from source tree were .765, .660, and .504, respectively. The $\underline{r}$ value of .765 is just below the significant $\underline{r}$ value of .811

(P = .05). The larger the number of significant flowers in the sample, the greater the r value can be expected if a real relationship is present for distance and flowers with high counts. The low number of sample trees and significant samples were the main reasons for correlation coefficients to be nonsignificant (P = .05).

Every time honeybees visited the flowers of another yellow-poplar, their hairs lost more radioactive pollen. Some honeybees after visiting the flowers of the source tree and getting radioactive pollen on their hairs, probably flew directly back to the hive to unload their nectar or pollen. While these honeybees were in the hive, they transferred radioactive pollen to other honeybees as they brushed against each other inside the hive. Therefore, a large number of honeybees may had some radioactive pollen on their hairs when they visited the flowers in the orchard. This pollen would be redistributed in a random pattern.

Pollen in the yellow-poplar seed orchard was probably distributed evenly in all directions from the source tree, and the pollen movement probably decreased gradually at a greater distance from the source tree.

The distance of the third sample trees east and southwest from the source tree were 280 feet and 437 feet, respectively. However, these distances were changed to 355

feet for the purpose of one analysis; this change from actual distances may have caused some error in the reported interpretation of results.

Even though it was determined that movement of pollen in a yellow-poplar seed orchard was carried out by honeybees, the author was unable to prove that cross-pollination of yellow-poplar occurred. Additional research is needed to determine the percentage of filled seeds which may be obtained with honeybee management.

Only 19 percent of the yellow-poplar flower samples had a CPM significantly higher than background ($P < 0.05$). The technique of counting the beta of the radioactive pollen with the large amount of flower tissue inside the vials was the main reason for some low counts. The flash which is produced by beta emission in the fluor was blocked by the flower tissue before the flash could reach the photomultiplier tube of the liquid scintillation system. The technique of counting can be improved by washing with fluor the pollen from the flower tissue into the vials.

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