POLLEN TUBE GROWTH AND DEVELOPMENT IN RELATION TO CHEMICAL THINNING OF PECAN NUTS

By

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CHAPTER I

INTRODUCTION

The pecan is the predominate nut crop of the southern states and comprises 39.7 percent of the total tree nut crop produced in the United States, based on 1960-70 average yields. Oklahoma ranks fifth among the states in pecan production, producing a yearly average of 21,745,000 pounds with an average value of $5,786,344 (26).

One of the most important problems in pecan production is irregular bearing (11). A heavy nut set results in poorly filled nuts and lessens the food reserves for pistillate flower bud formation and nut set the following year (3, 10). Consequently, irregular bearing results causing a relative unstable market, due to nut quality and price variation.

Irregular bearing is not limited to pecans, and many fruits tend to bear lightly after a heavy crop year. Chemical thinning of apples and other deciduous fruits is effective in securing annual production and is used commercially in the United States (8). It has been shown that chemicals can effectively thin pecans without phytotoxicity to the trees (12, 13).

The critical time of application of chemical sprays for fruit thinning is an important factor in achieving the desired amount of thinning (5).

The objectives of this study were:

(a) to determine pollen tube growth and development;

(b) to determine the time of fertilization;

(c) to determine pollen tube development in relation to nut size and chemical thinning.

CHAPTER II

REVIEW OF LITERATURE

The pecan (Carya illinoensis, K. Koch.) is monoecious, bearing pistillate and staminate flowers separately on the same plant. Very little work had been done prior to the 1920's concerning the floral development.

The catkins or staminate flowers are differentiated during the summer to early fall in lateral axillary, composite buds situated on the previous season's growth (22, 23, 28). The pistillate flowers are differentiated from lateral buds near the terminal when growth is initiated in the spring (14, 21, 23, 29). The development of the pistillate flower from time of pollination to fertilization is of major concern.

The pistillate flowers first occur as a cluster of blister like blunt protuberances which form on the meristem. This protuberance becomes cup shaped approximately two weeks after formation and will later become the husk. Four points on the cup develop to form bracts of the calyx. They enclose the stigma which forms around a second depression (21).

Approximately two months after differentiation starts, the stigma encloses the ovarian cavity and an ovule begins to develop (29). The nucellus of the ovule initiates as a relatively narrow, bluntly conical primordium which terminates the short columnar placenta (22). Accord-

ing to N. C. Woodroof (32), a megaspore mother cell is formed prior to the initiation of the single integument. Shuhart (22) reports that a well differentiated megaspore mother cell was formed three days after the nucellus was initiated, and the integument is initiated three days after nucellus primordia developed. The ovule and nucellus are arranged in such a manner to form what is termed an orthotropous ovule. In this type the ovule develops in a straight structure, and the integument encloses the nucellus in such a way that the micropyle is in a straight line with the chalaza and hilum.

The ovule continues to develop and about three days after differentiation of the megaspore mother cell, the four megaspores were present in the linear tetrad. In approximately three more days, three of the megaspores were disintegrating, while the fourth, or chalazal megaspore was considerably enlarged. Six days later the eight nucleate megagametophytes were present. Fusion of the polar nuclei occurred soon after formation of the eight nuclei. The mature embryo sac is rounded to oval at the top, containing two synergid nuclei near the apex. The three antipodal cells, having been forced into a point at the base are nearly cut off from the rest of the embryo sac (21, 32). Before fertilization the egg nucleus moves to the micropylar end of the embryo sac.

The time of pollination and ovule development varies greatly with seasonal conditions, and to some extent with cultivars. Billings (6) reported that the nucellus is tightly enclosed by the integument at the time of pollination. N. C. Woodroof (32) found that the nucellus is only about one half enclosed by the integument and the four megaspore stage is reached by pollination. Shuhart (22) observed that

pollination usually coincides with the differentiation of the eight nuclei of the megagametophyte. The integument was one half to two thirds the length of the nucellus and did not entirely enclose it until after fertilization.

The course of the pollen tube in the pecan has been studied by Billings (6). He reported that the pollen tube passes down the axial tissue of the style until near the cavity of the ovary, where it turns and passes down the ovary wall close to the margin of the cavity. The tissue through which it passes after leaving the style has nothing by which it can be designated conducting tissue but consists of nearly isodiametric cells. When a point is reached a little below the funiculus, the pollen tube curves, passes through the region of darkly stained cells, and when under the ovule turns upward toward the embryo sac. He also states that a micropylar canal is present at the apex of the ovule, but it is not penetrated by the pollen tube. Rather, the pollen tube penetrates the chalazal region.

Adriance (1) seems to agree with this to some extent, although he does not give a detailed description of the course of the pollen tube. His observations were that the pollen tube grows inward through the stigma tissue toward the central region of the style. It then grows downward along the general course of its vascular tissue, not in the stylar canal. The pollen tube grows down the ovary wall or even in the integument to the base of the ovule and returns through the chalaza and nucellus to the embryo sac.

N. C. Woodroof (32) describes the pollen tube as growing inward from the sides of the stigma toward the micropyle. The pollen tubes never enter the stylar canal but grow downward in the tissue on each

side of the micropyle. The place of entrance into the ovarian cavity is usually near the micropyle, although a few enter at a point nearly opposite the chalaza. The pollen tube does not enter the nucellus until the embryo sac is mature but continues growing in the cavity and may branch profusely. This branching was also noticed by Billings (6). In all cases pollination was promptly followed by germination of the pollen grains and growth of the pollen tubes down to the ovarian cavity within twelve hours (1, 6, 27, 31, 32).

N. C. Woodroof (32) reports the pollen tube entering the embryo sac by way of the micropylar end. This disagrees with Billings' report that no pollen tubes were found entering the micropyle. The time of fertilization also varies greatly among the reports. Shuhart (22) reports fertilization two weeks after maturation of the megagametophyte. Long delayed fertilization was not observed. N. C. Woodroof (32) gives the time of fertilization as two weeks after pollination but in some cases may be even later. Adriance (1) also reported a delay between time of pollination and fertilization and places it around four weeks. Woodroof and Woodroof (30) give an even longer delay of five to seven weeks. These variations are best summarized by Wolstenholme and Storey (27). They reported that the time it takes between pollination and ovule fertilization by the male gametes is not known, but estimates range from four days to seven weeks.

The general development of the ovule, pollen tube growth, and time of fertilization seems to follow the following pattern. The pistillate flowers differentiate in early spring. Approximately two months later the stigmas enclose the ovarian cavity and the nucellus is initiated. One week later a well differentiated megaspore mother cell

is present, and the integument is initiated. At pollination, four megaspores to eight megagametophytes are present with the ovule partially enclosed by the integument. The pollen tube grows down through tissue near the center of the style but not in the stylar canal. It reaches the ovarian cavity within twelve hours where it may continue to grow and branch. The pollen tube may enter the embryo sac when it is mature through the chalaza or micropylar end. The exact time of fertilization is not known but estimated to be three to five weeks after pollination.

It has been reported that a large number of nuts drop or abort about the same time fertilization usually occurs (1, 23, 27, 31). This drop may account for about 75 percent of the seasonal drop and is thought to be a lack of pollination or fertilization. Adriance (1) found that unfertilized, bagged flowers dropped at this same time. This seems to confirm the fact that this drop is due to lack of pollination and/or subsequent failure of fertilization.

The physiological and genetical implications of this drop is of importance. If it can be controlled by reducing drop in 'off' years or by increasing drop in years of high nut set, the final crop size could be more consistently controlled. This would reduce the phenomenon of biennual bearing which is the condition which causes trees to bear heavy and light crops in alternate years.

Thinning fruit of trees has been recognized as a method to improve regular bearing. In recent years chemical thinning of apple and other deciduous tree fruits has become an increasing commercial practice in the United States. The correct timing of spray to achieve satisfactory and consistent chemical thinning is essential. Cultivars

also differ in thinning responses (24).

Lombard and Mitchell (18) reported thinning in apples was correlated with embryo development. Favorable thinning resulted when naphthalene acetic acid (NAA) was applied as a spray to apple trees when the small fruit was in the 8 to 16 cell stage, fourteen days after bloom. At this stage cytokinesis in the endosperm had ended.

Timing of chemical thinning in peaches has been correlated with number of days after full bloom, fruit size and stage of fruit development. Leuty and Bukovac (17) reported maximum abscission of fruit when NAA was applied during endosperm cytokinesis. The pericarp length was a consistent index of endosperm development. This was a more consistent index for timing of thinning sprays than the number of days after full bloom.

Stembridge and Gambrell (24) agree, stating the consistent thinning results with 3-chlorophenoxy-a-propionamide (3-CPA) were obtained by basing spray applications on ovary development. Cytokinesis began in the Halehaven peach when the ovule was 8.5 mm long and this apparently coincides with the critical stage in many varieties. Sprays based on number of days after full bloom did not give consistent results.

Bausher et al. (5) reported ovule length and water volume displacement of 100 fruits as an effective basis for timing of 2-3 chlorophenoxy propionamide (CPA) sprays for peaches. Optimum thinning varied for cultivars but was most effective when the ovule length was 7.0 - 10.0 mm. This corresponded to 280 ml water displacement for 100 fruits which was an easier means of measurement.

Tests have shown that pecans can be thinned with chemical sprays (2, 10, 12, 13, 20). Thinning of pecan nuts to control irregular bear-

ing has been studied, but chemical thinning of pecans has not become a commercial practice. This may be due to inadequate information for a basis of timing of spray application to achieve optimum consistent results.

Sharpe (20) reported a 65 percent reduction in nut set when the cultivar Moneymaker was sprayed with 20 ppm 2,4-D April 27 at Gainsville, Florida. The same treatment had no effect when applied June 24 and August 11 or on the cultivar Moore. He also found that a 20 ppm spray of 2,4,5-T applied June 11 to the cultivars Randall, Curtis, Success and Moneymaker reduced nut set by an average of 47 percent. When applied at 100 ppm to the above cultivars, nut set was reduced an average of 95 percent. For the cultivars Stuart and Kennedy, 20 ppm of 2,4,5-T applied June 11 had no effect, while the rate of 100 ppm reduced nut set by an average of 60 percent. When 2,4,5-T was applied to Moneymaker and Stuart at 10, 40, and 100 ppm July 25 and August 6, no significant thinning resulted. Maleic hydrazide applied June 16 at 330 and 660 ppm to the cultivars Moore and Moneymaker had no effect. From these tests Sharpe concluded that early June applications were most effective in thinning heavy pecan nut crops.

Harris and Smith (10) at Shreveport, Louisiana found that maleic hydrazide caused more dropping of Moore and Success nuts when applied in May than in June and thinning increased with chemical concentration. May applications had no effect on Stuart and Mahan nut set. Applications of 20 ppm 2,4,5-T on the cultivars Moore, Success and Schley May 24 reduced nut set by 20 percent. This was 21 days after the approximate date of stigma receptivity which was May 3.

Amling and Dozier (2) applied 3-CPA to the cultivar Stuart in

four concentrations (50, 100, 150, 200 ppm) June 1 at Auburn, Alabama. Nut set was 52, 26, 12, and 2 percent respectively as compared to 57 percent nut set on the check.

Hinrichs (12) made preliminary tests to determine if 3-CPA would thin nuts of the cultivars San Saba Improved and Western at Sparks, Oklahoma. Applications of 100, 150, and 200 ppm were applied two, four, and six weeks after pollination (May 23, June 6, June 20 respectively). The treatment May 23 caused all the nuts to drop while the June 20 treatment had the least effect on the cultivar Western. This last application of 100 ppm for Western was considered the best response, resulting in 24 percent reduction in nut set over the check treatment.

Hopfer (13) applied three concentrations (40, 80, 160 ppm) 3-CPA, two concentrations (200, 400 ppm) isopropyl N-3-chlorophenyl carbamate (CIPC) and two concentrations (50, 100 ppm) 2-chloroethanephosphonic acid (ethephon) to the cultivar Western three, five, and seven weeks after pollination (June 3, June 17, July 1 respectively) at Sparks, Oklahoma. He reported that significant thinning occurred only from the June 3 treatment. The 3-CPA and ethephon treatments thinned nuts effectively while CIPC caused all the nuts to drop. On June 3 the average nut length ranged from 7.8 to 8.5 mm, and the average diameter ranged from 2.8 to 3.1 mm. The volume of water displaced for 10 nuts ranged from .68 to .79 ml. On June 17 the average nut length ranged from 12.5 to 13.0 mm, and the average diameter was 4.2 mm. The volume displaced was 1.59 to 1.74 ml. Nut diameter was the least variable of the measured items, since 2.8 to 3.1 mm appeared to be the optimum size for thinning Western nuts with 3-CPA.

Woodroof and Woodroof (29) reported external characteristics of 28 varieties of pecans at the time of pollination which was April 29. The average length ranged from 5.5 to 8.0 mm.

Studies indicate that chemicals are effective in thinning pecans (2, 10, 12, 13, 20). It appears that the time and concentration of spray applications is critical and if correlated to the proper stage of development, may have potential commercial use.

CHAPTER III

METHODS AND MATERIALS

Excise Method of Determining Pollen Tube Location

This technique was used as a quick and easy method to approximate the time it took the pollen tubes to grow past a point just below the bracts in the style of the pecan.

To control the time of pollination it was necessary to cover the pistillate flowers in order to prevent foreign pollen from coming in contact with the stigma. Bags 3.9 cm in diameter and 12.5 cm long were made from Nojax[1] sausage casing. The pistillate clusters of the cultivar Stuart were bagged April 30 and May 3, 1971 prior to stigma receptivity and pollen shedding. The bags were placed over clusters containing at least three pistillate flowers and were tied upon a ball of cotton placed around the young shoot for padding.

The leaves near the base of the cluster were removed to facilitate covering. Leaves that developed in the bag were removed as they expanded to prevent abscission of the cluster. This was done without removing the bags by pinching off the new leaves and shoot buds through the bags. The clusters were tagged with numbered metal rim

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[1]Union Carbide Corporation trademark for cellulose sausage casing. Furnished courtesy of Animal Science Department, Oklahoma State University.

paper tags for identification purposes at the time the flowers were bagged.

A large amount of pollen from the cultivar Dooley was available before the time of pollination. Dooley also has good shelling and kernel characteristics which would be desirable in a hybrid, and it was chosen as a pollen source for these two reasons. Mature catkins in which the anthers had started to turn yellow were collected May 7 and spread on paper in the laboratory to dry. Three days later the pollen was separated from the catkins and stored in five dram plastic vials at 7° C.

Pollinations were made the morning of May 11 without removing the bags by inserting a hypodermic needle through the cotton pad. The attached syringe consisted of a single hole, heavy 50 ml rubber bulb with an 18 gage, 1½ inch long hypodermic needle attached by means of rubber tubing to a glass tube. A loop in the glass tube was used to control the quantity of pollen applied.

The stigmas are sessile and completely covers the style above the bracts. To estimate how fast the pollen tubes were growing, twenty stigmas per treatment were excised at various time intervals following pollination. Excisions were made at hourly intervals the first day and at least daily for four days following pollination. Only one cut could be made to remove the stigma without severely injuring the nutlet, and this was at a point just above the bracts (Figure 1).

The first observation of nut drop as relating to pollen tube growth was made May 17 and continued at three day intervals until May 26 when the time was extended to seven day intervals. The last observation was made July 7.

Figure 1. The point just above the bracts of the calyx at which the stigmas were excised

Microscopical Examination of Pollen Tubes in the Style

The methods used for covering, pollinating, and identification of pistillate clusters were the same as those used for the excise technique. One cluster, containing at least three nutlets, was collected at various time intervals after pollination. Hourly collections were made the first day, and then daily collections were made until June 24. Observation of kernel development was continued until September 20.

Immediately after collection, the clusters were placed in individually coded test tubes and fixed in 50 percent FPA (19). The clusters were fixed for at least 24 hours and then stored in 70 percent ethyl alcohol. Individual pistillate flowers were then dehydrated by passing them through a tertiary butyl alcohol (TBA) dehydration series (19). The last change of TBA was aspirated and left overnight. The flowers were then infiltrated and embeded in Paraplast.[2]

Longitudinal sections were cut 10 microns in thickness on a rotary microtome and affixed to slides with Haupt's adhesive (16). Due to the hardness of the flowers after dehydration and infiltration, the paraffin blocks were softened in Gifford's softening solution II (9) to facilitate sectioning.

Safranin and aniline blue; Heidenhain's hematoxylin (19) and aniline blue; lacmoid and tannic acid-ferric chloride combination (7) stains were tried but failed to adequately differentiate the pollen tubes for observation with the microscope using white incandescent

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[2]Trademark for paraffin embedding material with a melting point of 56 - 57° C.

light as an illumination source.

Callose[3] forms a component of pollen tube walls but occurs primarily in the form known as callose plugs within the pollen tubes (25). Aniline blue in dilute concentrations is a vital histochemical test for callose. A 0.005 percent solution aniline blue in 0.15M K_2HPO_4 at pH 8.65 (15) was used to stain sections and observations were made with a microscope using a fluorescent light source. Ultraviolet exciter filter Schott BG12 and Schott OG1 barrier filter were used. The callose plugs fluoresced bright yellow green, the pollen tube wall fluoresced yellow green, and the cell walls and nuclei fluoresced brown. This was a very satisfactory technique for quick and easy location and observation of the pollen tubes in the style.

Permanent slides were not produced by this method. Pertinent slides were selected after observation with the above technique and stained with lacmoid[4] and safranin[5], then made into permanent slides for further observations.

The diameter and length of the pistillate flowers were measured in millimeters from flowers collected for sectioning and microscopical examination.

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[3]A polysaccharide composed mainly of chains of 1:3-linked β-D-glucopyranose residues.

[4]0.25 gm lacmoid (resorcin blue) in 100 ml 30 percent ethyl alcohol with few ml 1 percent sodium bicarbonate solution. Stained 12 to 18 hours.

[5]0.125 percent safranin O in 50 percent ethyl alcohol. Stained five seconds.

Chemical Thinning of Pecan Nuts

Nineteen year old trees of the cultivar Stuart were selected for the study. The trees were located at the Oklahoma Agriculture Pecan Research Station near Sparks, Oklahoma.

Three concentrations (30, 40, 50 ppm) of ethephon[6] were applied per treatment June 1, June 8, June 15, 1971 (3, 4, 5 weeks after pollination). Each treatment consisted of three replications, using one tree per replication, and 300 nuts were sprayed per replication. A check was included in each replication. The treatments were tagged, and the nuts counted the day of application.

Applications were made using a hand sprayer to apply the chemical to individual branches to the drip stage. Tween-20 (polyoxyethlene-20-sorbitan monolaurate) was added to the mixture as a surfactant at the rate of four drops per quart.

The nuts were counted at weekly intervals following treatment to determine the amount of thinning.

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[6]Amchem 68-240 furnished by Amchem Products, Inc., Ambler, Pa.

CHAPTER IV

RESULTS AND DISCUSSION

Excising Stigmas to Determine Pollen Tube Location

The cumulative number of nuts abscised from each of the treatments is shown in Table I. The greatest amount of abscission occurred between June 16 and 29.

The pollen tube appears to have passed the point just below the bracts of the pecan nut 6 to 9 hours after pollination. A greater percent of nuts had abscised from those excised 0 to 4 hours after pollination than those excised later than six hours after pollination. These determinations were made 36 to 49 days after treatments were made. The range of nuts abscised by June 16 for stigmas excised before five hours following pollination was 30 to 60 percent (Figure 2). This compared with 5 to 30 percent of the nuts abscised for stigmas excised six or more hours following pollination. The results were also similar for the percent of nuts abscised June 23. The number of nuts abscised from treatments 0 to 6 hours after pollination was 55 to 90 percent, compared with 10 to 55 percent for treatments applied seven or more hours later (Figure 3).

Another indicator of the time the pollen tube had passed the point of excision was the rate of abscission. Treatments applied from 0 to 4 hours after pollination caused a steady increase in percent of

TABLE I

EFFECT OF EXCISING STIGMAS ON NUT ABSCISSION 1971

Treatment (Hrs. after pollination) (May 11)	Number of Nuts Excised	Cumulative Number of Nuts Abscised									
		Days Readings Taken									
		5-17	5-20	5-23	5-26	6-2	6-9	6-16	6-23	6-29	7-7
Not Pollinated	0	1	1	1	1	2	7	10	13	20	20
0	20	1	1	2	2	6	8	9	14	20	20
1	20		1	3	3	5	7	12	16	20	20
2	20					3	4	9	11	16	20
3	20		2	2	4	4	5	6	13	20	20
4	20					2	5	10	18	20	20
6	20		2	2	2	3	4	4	14	20	20
7	20					1	2	3	10	19	19
8	20		2	2	2	3	4	4	8	20	20
9	20							1	2	17	20
22	20					1	1	3	4	19	20
25	20							1	8	19	20
28	20			1	1	1	1	2	9	17	20
32	20						1	2	3	18	20
46	20		2	2	2	3	3	4	8	19	20
52	20	1	1	1	1	1	1	2	7	19	19
72	20			1	1	1	1	3	7	14	16
96	20		2	3	3	3	3	6	11	15	15
Pollinated	0	1	3	3	4	4	5	6	8	12	12

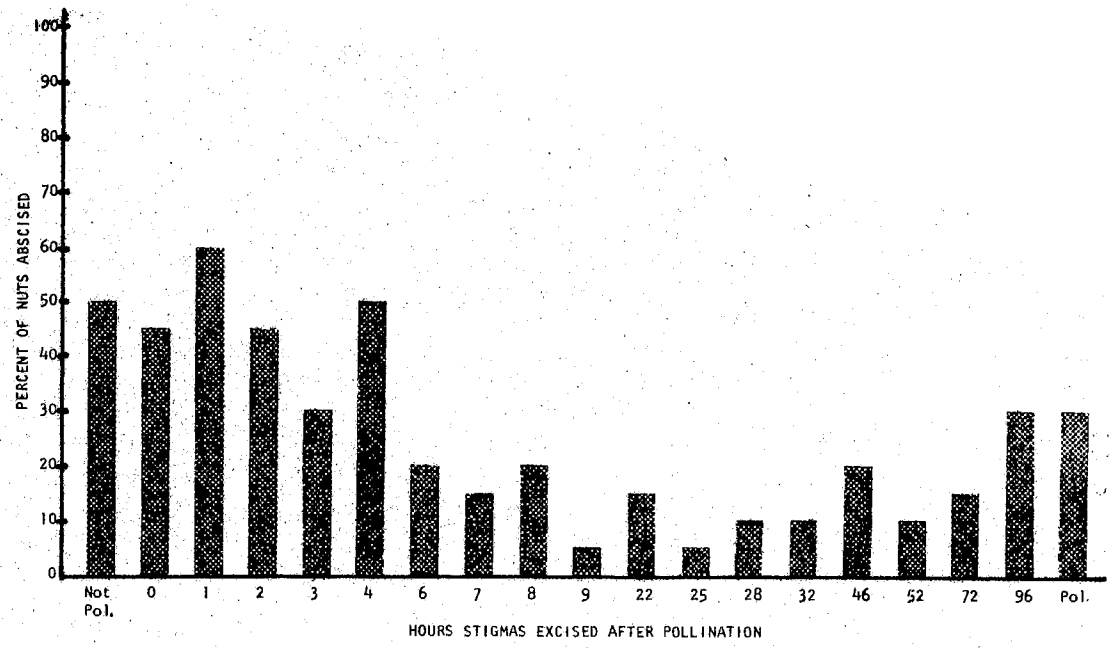

Figure 2. Effect of excision stigmas on nut abscission June 16

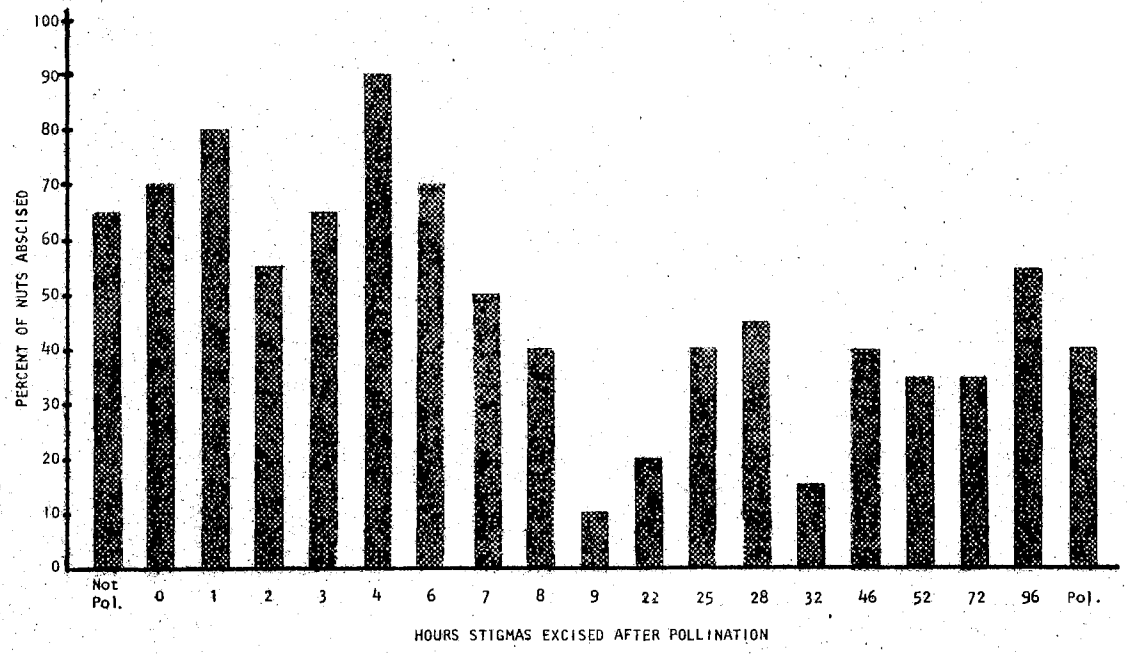

Figure 3. Effect of excision stigmas on nut abscission June 23

nuts abscised (Figure 4 and 5). This compares closely with the abscission rate for nuts that were not pollinated or excised. The rate of nut drop for treatments applied six hours and later was fairly stable until June 23 when a very rapid increase of abscission occurred (Figure 5, 6, and 7).

The number of nuts remaining June 29 and July 7 was also an indication of the time the pollen tubes had grown past the excision point. The treatments applied nine or more hours later had few nuts remaining June 29. This compared with the abscission of all the nuts for treatments made until seven hours after pollination with the exception of one treatment. Abscission of all the nuts in the treatments had occurred by July 7 except for treatments 7, 52, 72, and 96 hours after pollination with one, one, four, and five nuts remaining respectively, compared with eight nuts remaining for the check pollinated treatment. Pollen tubes had grown below the point of excision by seven hours after pollination.

Microscopical Examination of the Pollen Tubes

At the time of pollination, May 11, the embryo sac contained four to eight nuclei. Those that were in the four nuclei stage divided shortly after pollination to form the eight nuclei stage. The nucellus was one half to two thirds enclosed by the integument at pollination (Figure 8).

Within two hours after pollination, the pollen had germinated, and the pollen tubes had grown into the stigma. The pollen tubes grew inward toward the center of the stigma following the general

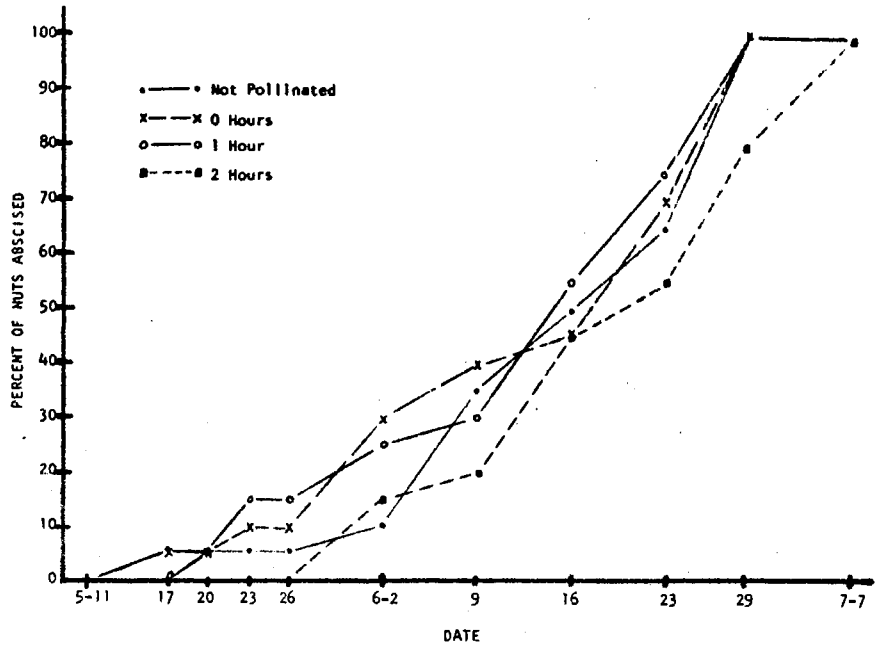

Figure 4. Cumulative nut abscission as affected by non-pollinated check and by excising stigmas 0 to 2 hours after pollination

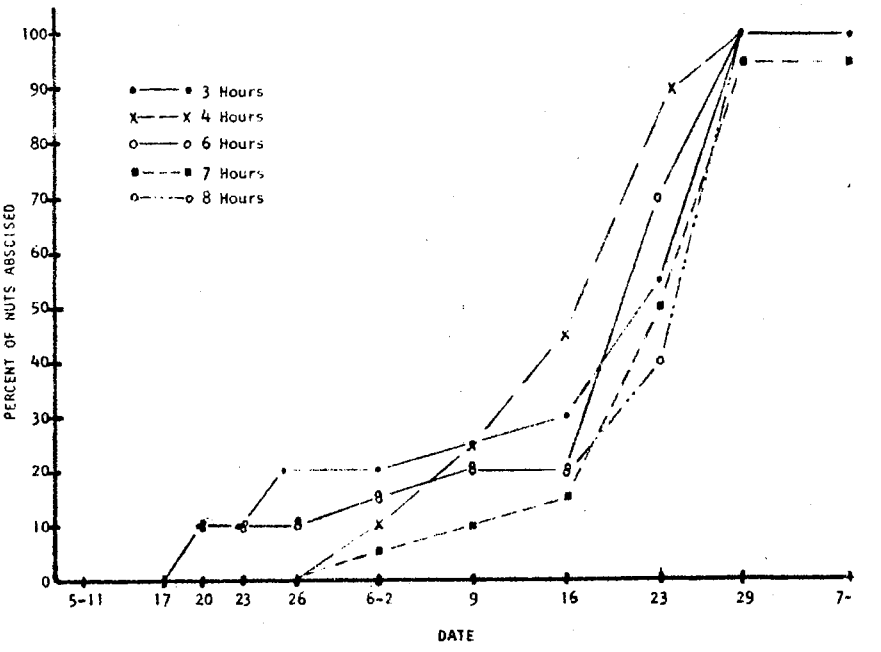

Figure 5. Cumulative nut abscission as affected by excising stigmas 3 to 8 hours after pollination

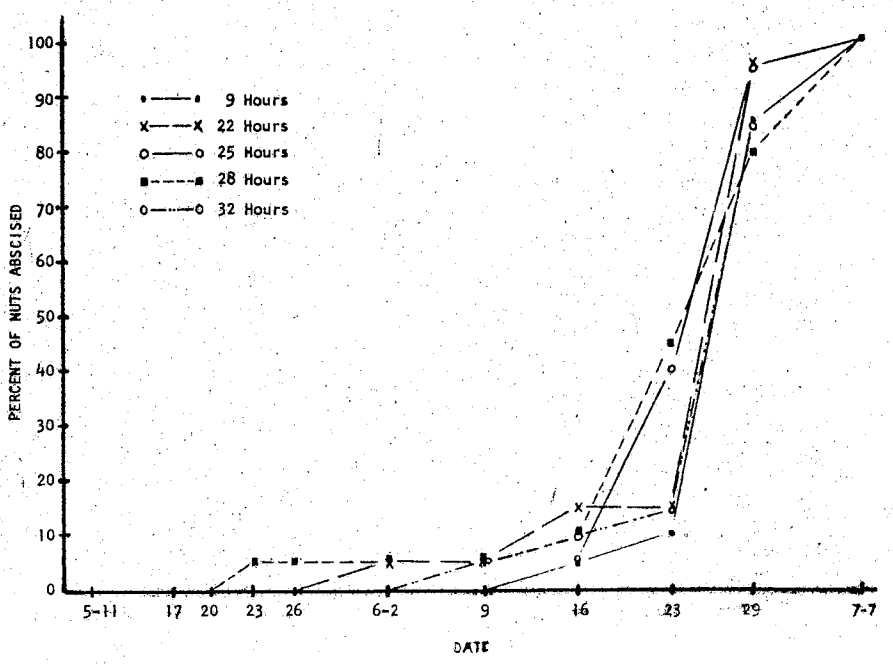

Figure 6. Cumulative nut abscission as affected by excising stigmas 9 to 22 hours after pollination

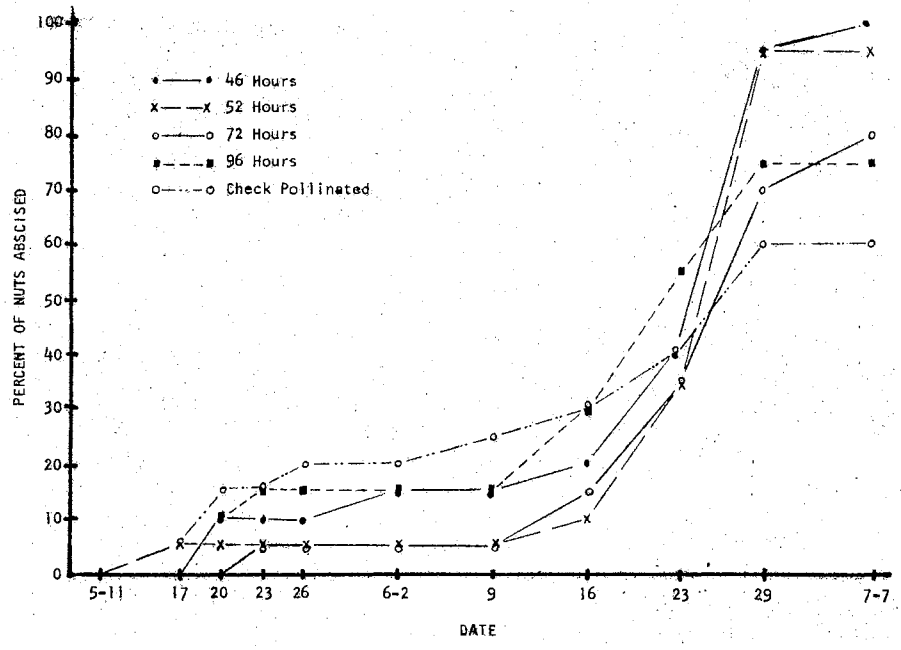

Figure 7. Cumulative nut abscission as affected by excising stigmas 46 to 96 hours after pollination and by pollinated, not excised check

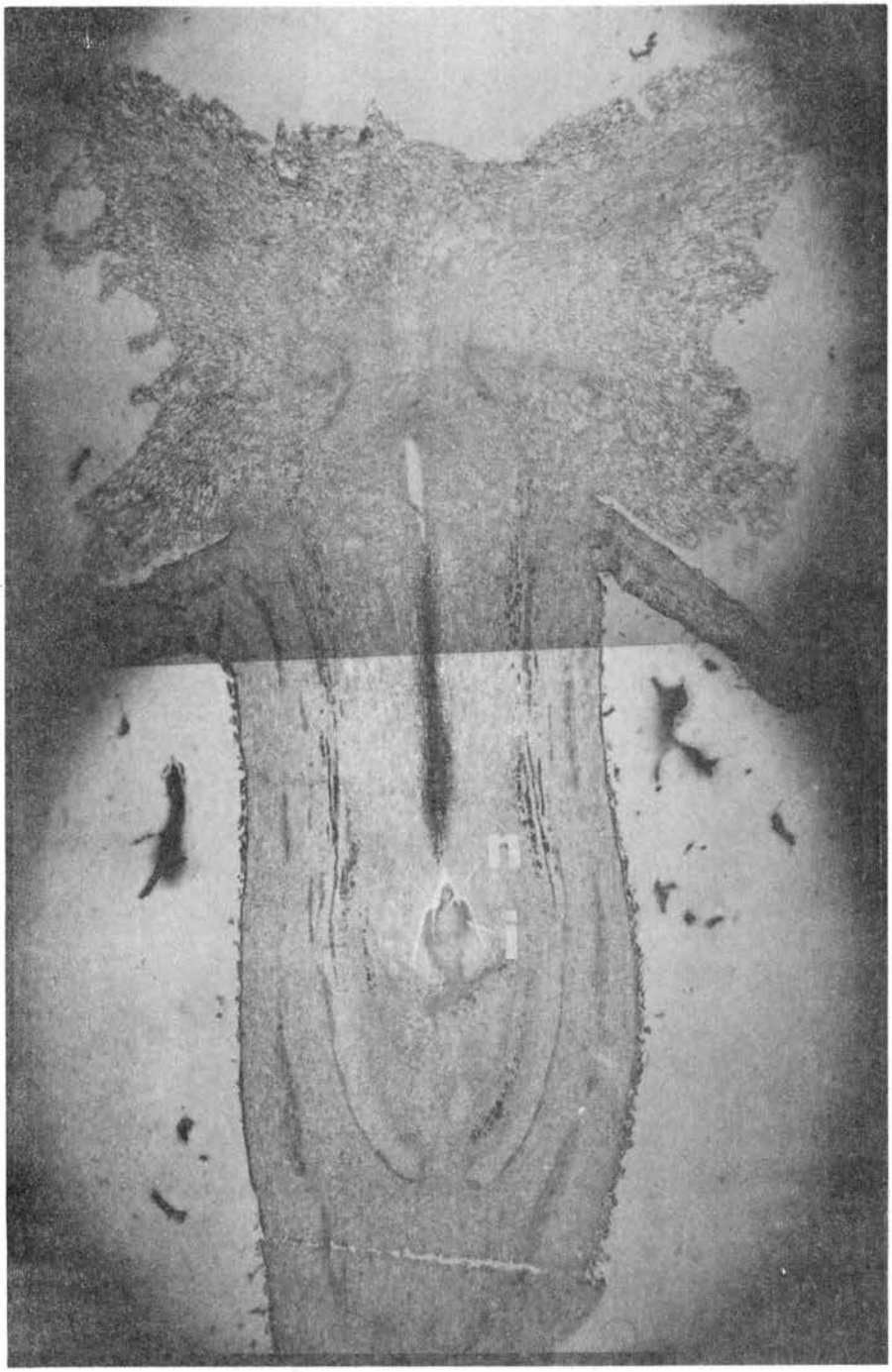

Figure 8. Longitudinal section of a pecan flower at the time of pollination, May 11, showing the nucellus (n) and integument (i)

contour of the cells (Figure 9a). It then turned and grew down the center of the style, between the inner vascular bundles (Figure 9b). No pollen tubes were found growing in the dark staining connecting tissue in the very center of the style, but rather in the tissue adjacent to it.

The pollen tubes had not extended below the bracts of the calyx nine hours after pollination, but they were observed below the bracts 22 hours after pollination. The approach to the ovarian cavity by the pollen tubes was directly above the nucellus (Figure 10a) and entry was at the apex of the cavity (Figure 10b). Forty-six hours after pollination the pollen tubes had entered the ovarian cavity. The pollen tubes grew to the base of the nucellus either in the cavity wall or more commonly along the side of the cavity wall (Figure 10c, d).

The pollen tubes entered the nucellus at the base of the integuments and grew to the embryo sac through the chalaza and nucellus. The nucellus was completely enclosed by the integument nine days after pollination. Eighteen days after pollination, pollen tubes were observed above and below the embryo sac (Figure 11a).

Only one branched pollen tube was observed, which occurred five days after pollination. It had grown below the nucellus where it branched (Figure 11b).

The egg was fertilized 10 to 15 days after pollination (May 21 to 26). In some cases the embryo sacs appeared to be disintegrating as though no fertilization took place.

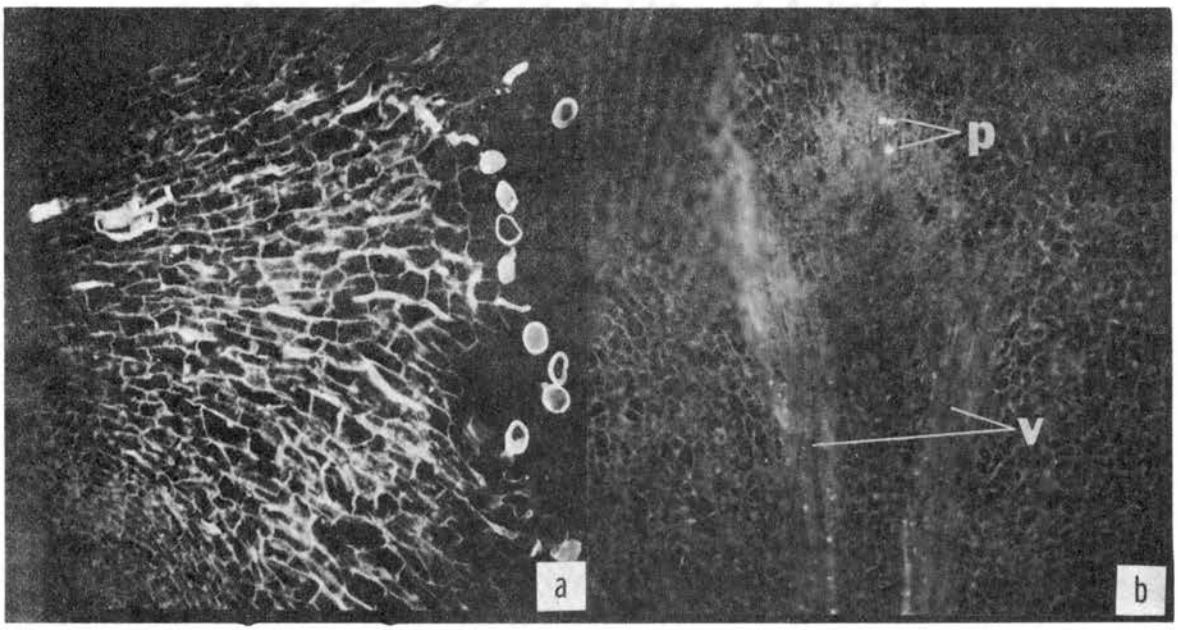

Figure 9. Longitudinal sections of pistillate flowers of pecan made through the stigma and style. a. Pollen tubes growing inward toward the center of the stigma following the general cellular arrangement. b. The inner vascular bundles (v) with pollen tubes (p) growing between them, in the center of the style

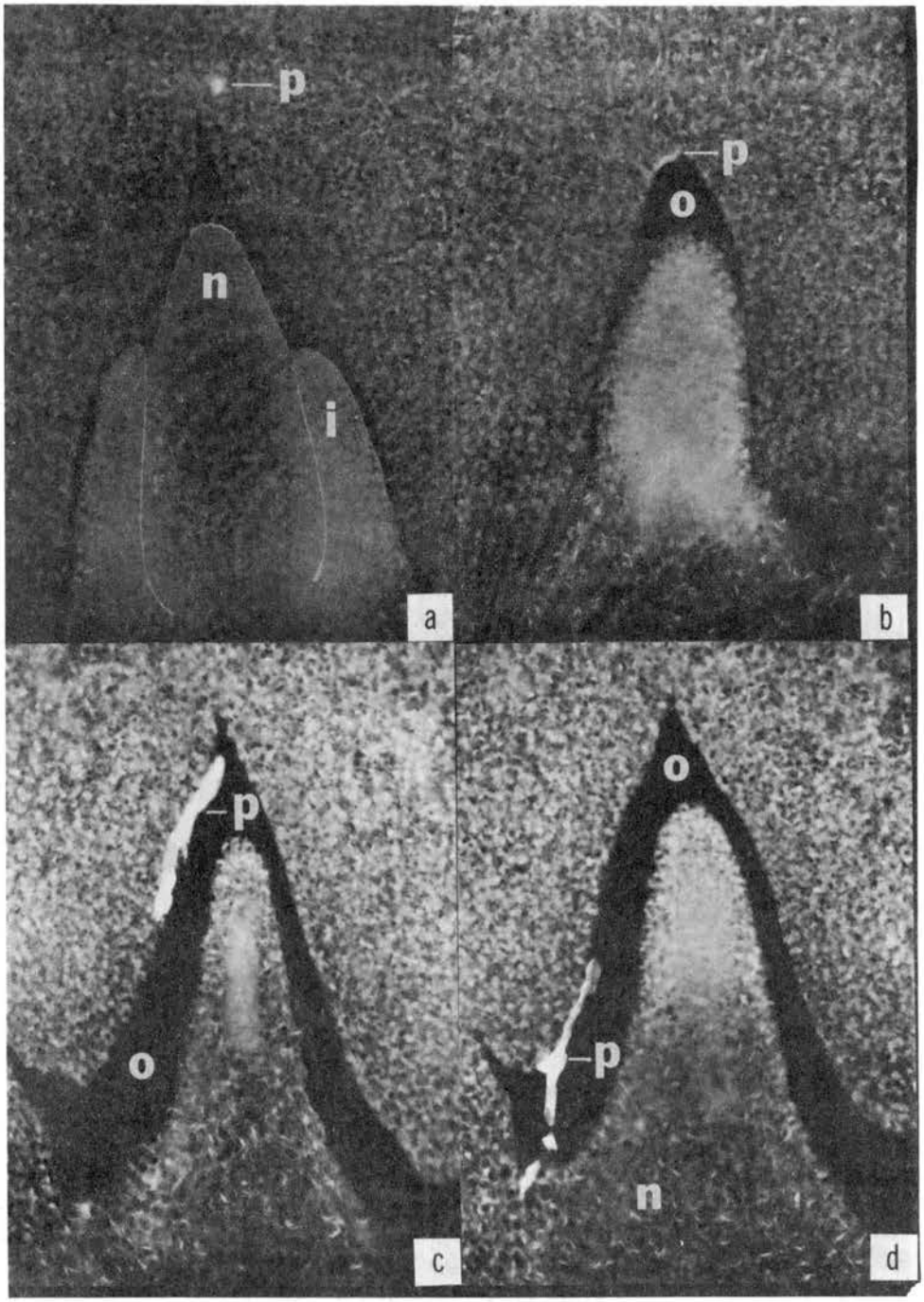

Figure 10. Longitudinal sections of the ovarian cavity (o), 46 to 80 hours after pollination. a. Pollen tube (p) approaching the nucellus (n) from above. b. Pollen tube entering ovarian cavity at the apex. c. Growth of the pollen tube along the ovarian wall. d. Pollen tube growing to the base of the nucellus

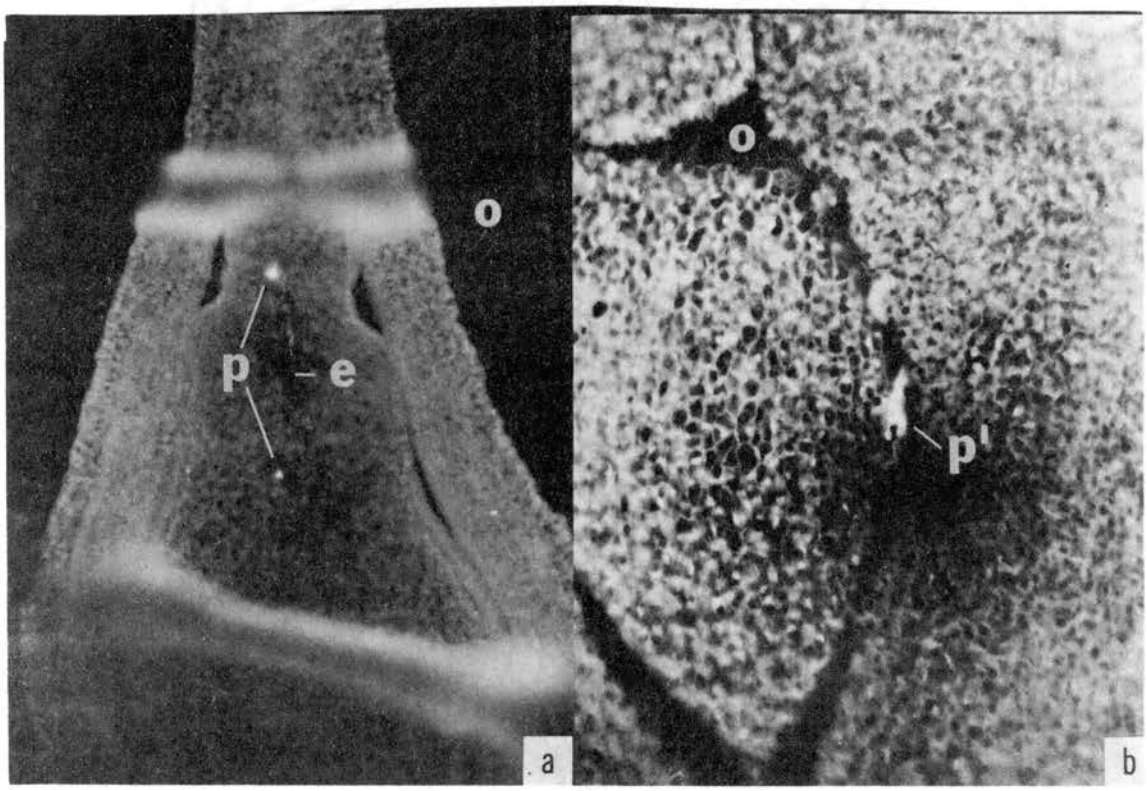

Figure 11. Longitudinal sections through the ovarian cavity (o).
a. Pollen tube (p) above and below embryo sac (e).
b. Branched pollen tube (p') which had grown below the ovarian cavity

Determination of Nut Size

The average diameter and length of nuts collected from May 11 to July 7, 1971 are shown in Table II. On May 11, the date of pollination, the average diameter was 1.93 mm, and the average length was 6.10 mm. On May 29, the average diameter was 3.00 mm, and the average length was 9.37 mm while by June 15, the average diameter was 4.50 mm, and the average length was 13.75 mm. The diameter of the nuts increased slowly for the first month, but increased more rapidly thereafter.

Chemical Thinning

The percent of nuts abscised from the ethephon treatments at each date of application is shown in Table III. The ethephon treatments caused the greatest amount of thinning the first week following application and only a small amount two and three weeks following application.

The 30 ppm treatment applied June 1 caused 78 percent of the nuts to drop one week after application (Figure 12). Treatments consisting of 40 and 50 ppm sprays were equally effective in thinning response. One week after application nut set was reduced 89 and 92 percent respectively when compared with the check. Excessive thinning occurred from the three spray applications.

The treatments applied June 8 also caused excessive thinning. The 30 ppm treatment reduced nut set 49 percent one week after application, compared with the check (Figure 13). Nut set, one week after treatment with 40 and 50 ppm sprays, was reduced 64 and 73 percent respectively. The second week there was a decrease of only 7 to 9

TABLE II

EFFECT OF TIME ON AVERAGE DIAMETER AND LENGTH OF STUART PECAN NUTS

Date 1971	Average Nut Size		Date 1971	Average Nut Size	
	Diameter (mm)	Length (mm)		Diameter (mm)	Length (mm)
5-11	1.93	6.10	6-8	3.50	10.12
5-12	1.85	6.05	6-9	3.95	11.06
5-13	2.00	7.17	6-10	3.83	11.33
5-14	1.87	6.42	6-11	3.83	11.67
5-15	2.00	6.00	6-13	4.08	12.00
5-16	1.87	6.88	6-14	4.00	12.00
5-17	1.94	6.94	6-15	4.50	13.75
5-18	2.12	6.38	6-16	4.75	14.00
5-19	1.62	6.62	6-17	4.75	13.67
5-20	2.08	7.50	6-18	5.75	16.00
5-21	2.00	6.50	6-20	6.17	16.41
5-23	2.25	7.62	6-21	6.00	16.50
5-24	2.37	7.12	6-22	6.50	17.00
5-25	2.00	6.12	6-23	6.17	17.17
5-26	2.87	8.08	6-24	6.50	17.50
5-27	2.50	7.78	6-26	6.75	16.67
5-28	3.00	9.25	6-28	6.75	17.00
5-29	3.00	9.37	6-30	7.75	20.00
5-30	2.55	8.35	7-2	9.00	21.00
6-1	2.58	8.17	7-5	8.75	21.00
6-2	3.50	10.00	7-7	9.50	21.40
6-3	3.00	10.00	7-9	10.00	23.15
6-4	3.00	10.00	7-12	11.12	24.00
6-7	3.37	10.00	7-14	12.20	26.00

TABLE III

EFFECT OF ETHEPHON ON THINNING STUART PECAN NUTS

Treatment (ppm)	Date Applied 1971	Percent of Nuts Abscised — Days After Treatment		
		7	14	21
Check (00)	6-1	3.56	10.67	22.67
Ethephon (30)	6-1	81.78	84.89	85.78
Ethephon (40)	6-1	92.44	94.67	96.44
Ethephon (50)	6-1	95.11	96.44	96.44
Check (00)	6-8	4.44	15.11	24.89
Ethephon (30)	6-8	53.33	60.89	62.67
Ethephon (40)	6-8	68.89	77.78	77.78
Ethephon (50)	6-8	77.78	86.22	87.11
Check (00)	6-15	15.11	28.44	28.44
Ethephon (30)	6-15	57.33	67.56	70.67
Ethephon (40)	6-15	66.22	70.67	72.44
Ethephon (50)	6-15	58.22	66.22	66.22

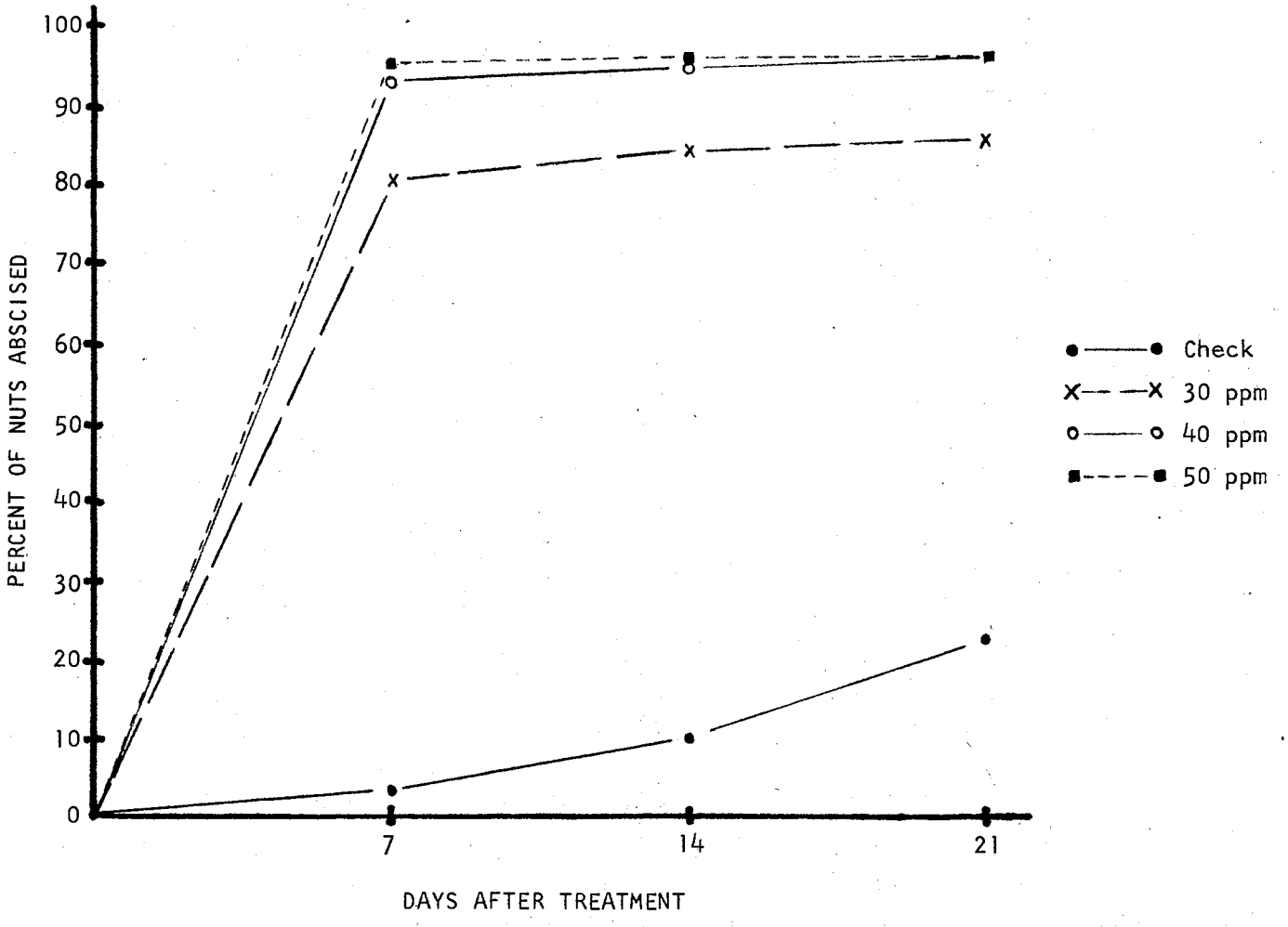

Figure 12. Effect of ethephon sprays applied June 1, 1971 on thinning Stuart pecan nuts

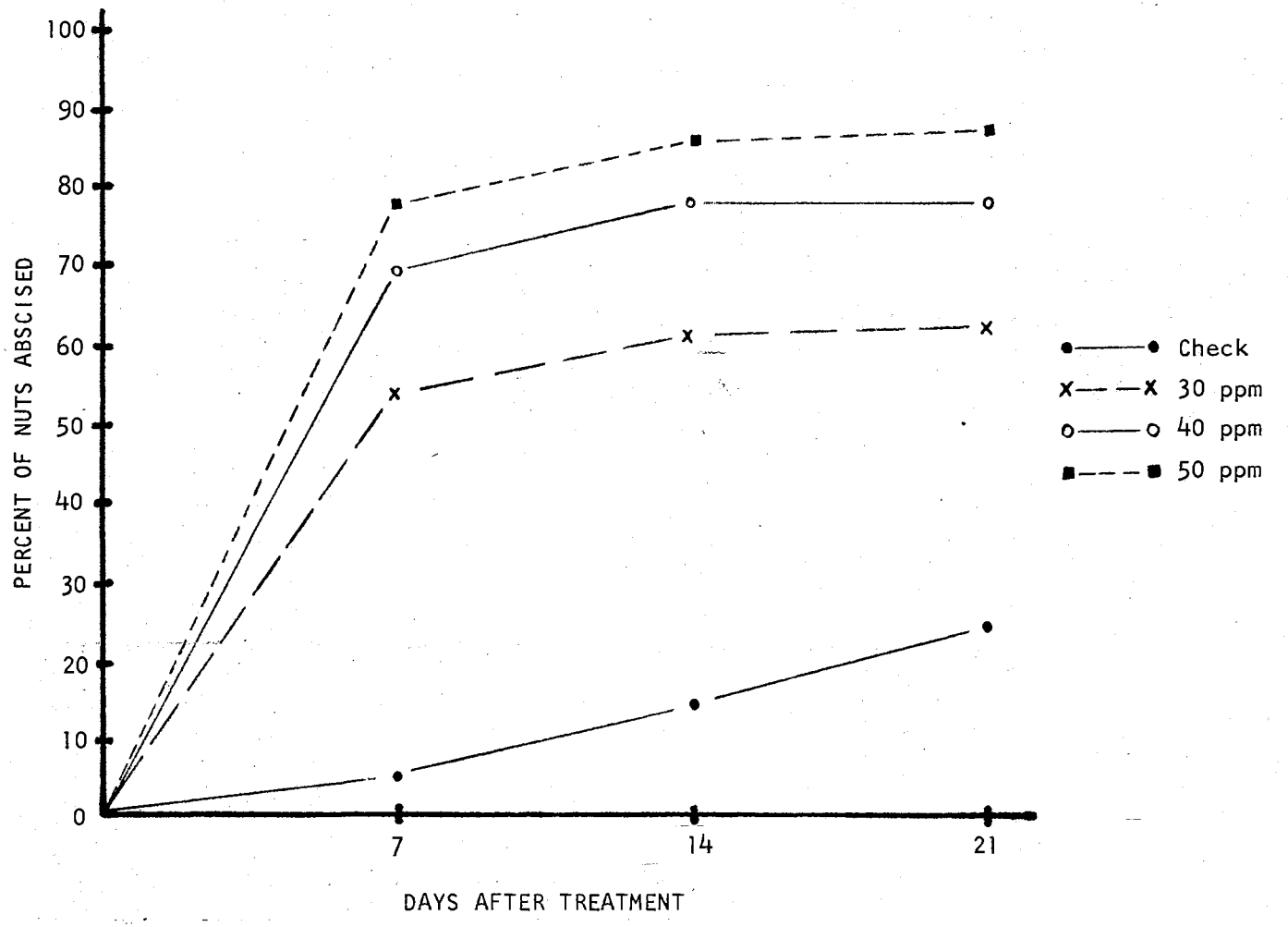

Figure 13. Effect of ethephon sprays applied June 8, 1971 on thinning Stuart pecan nuts

percent in nuts set. Thinning response the third week was negligible.

Nut set was reduced the least by treatments applied June 15 when compared to those applied June 1 and 8. The 30 and 50 ppm treatments were equally effective one week after application (Figure 14). Nut set was reduced 42 percent when compared with the check.

All of the ethephon treatments applied June 1, 8, and 15 were effective in reducing nut set. The 30 ppm treatment applied June 8 gave the best thinning response without excessive nut drop. The applications made June 1 resulted in excessive thinning. In nearly all instances an increase in the concentration of ethephon applied resulted in an increase in nut thinning.

The average nut was 3.50 mm in diameter and 10.12 mm in length when optimum chemical thinning occurred June 8 at the 30 ppm treatment. This optimum thinning occurred four weeks after pollination and approximately two weeks after fertilization.

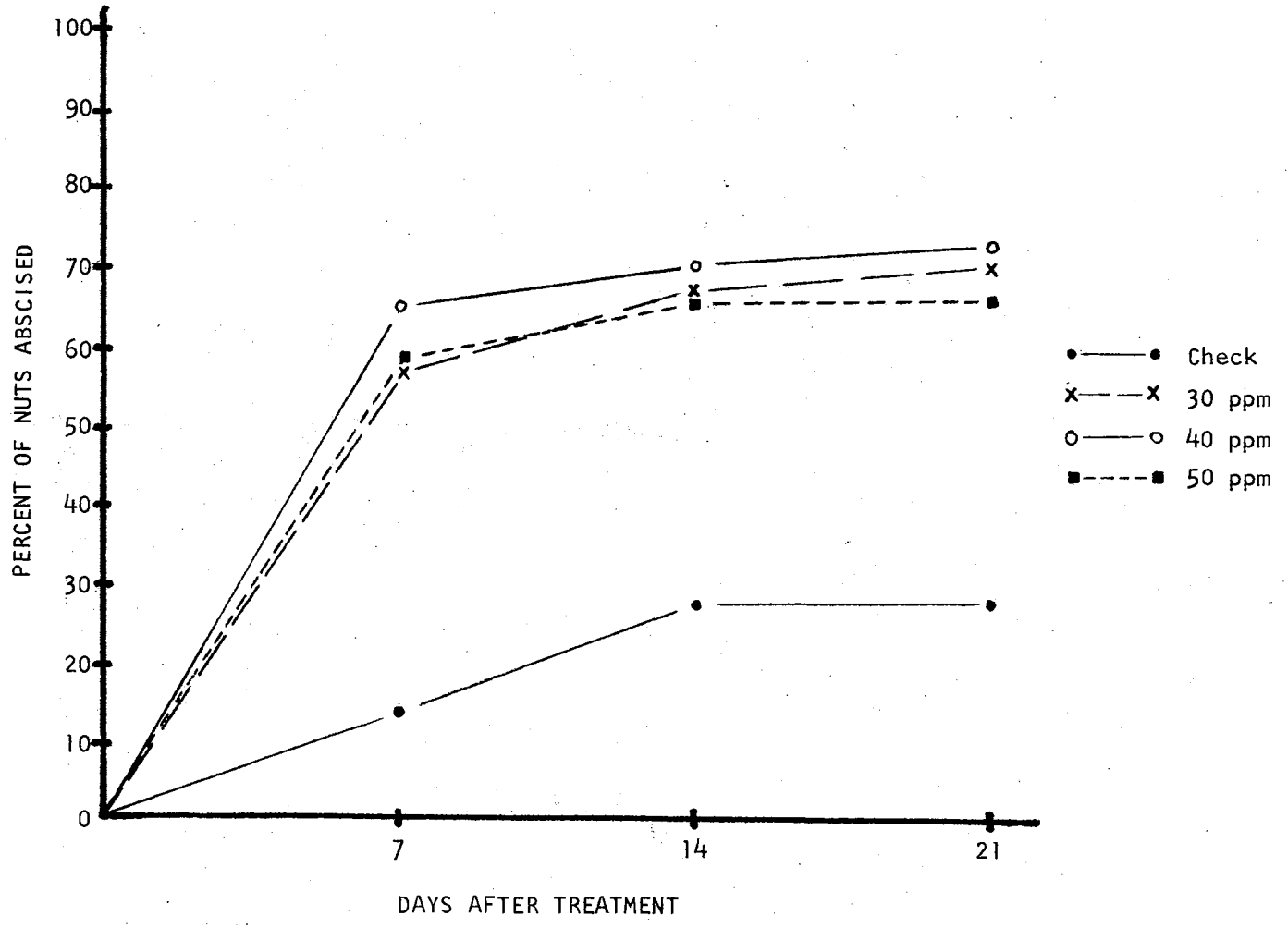

Figure 14. Effect of ethephon sprays applied June 15, 1971 on thinning Stuart pecan nuts

CHAPTER V

SUMMARY AND CONCLUSIONS

One of the most important problems in pecan production is irregular bearing. To reduce irregular bearing by chemical thinning, an effective method to determine the time of spray applications for optimum thinning results is needed.

The objectives of this study were to determine: (1) pollen tube growth and development; (2) the time fertilization occurred; (3) the relation of pollen tube development to nut size and chemical thinning.

Excising the stigmas of pistillate pecan flowers just above the bracts of the calyx was used to determine the growth rate of the pollen tube. All the nuts had abscised by July 7 from treatments in which the stigmas had been excised within six hours following pollination. One nut remained for the treatment excised seven hours following pollination (Table I). The treatments excised 52 hours following pollination and later had a few nuts remaining July 7.

Microscopical examinations of sections of pistillate pecan flowers were used to make actual observations of the pollen tube. The pollen had germinated within two hours after pollination. The pollen tube was found to follow the cellular pattern to the center of the stigma where it followed the general course of the inner vascular bundle down the middle of the style to the ovarian cavity. Pollen tubes were observed below the bracts of the calyx 22 hours after pollination. The

pollen tube was not found in the dark staining connecting tissue but in the tissue adjacent to it. Entry of the pollen tube into the ovarian cavity was near the apex, and it then grew down inside the cavity to the base of the nucellus where it grew through the chalaza and nucellus to the embryo sac.

On May 11, the time of pollination, the average nut size was 1.93 mm in diameter and 6.10 mm in length (Table II). At the time of fertilization (May 21 to 26) the average nut size ranged from 2.00 to 2.87 mm in diameter and 6.50 to 8.08 mm in length.

Chemical thinning of pecan nuts was obtained from all concentrations of ethephon sprays applied June 1, 8, and 15 (Table III). The treatments applied June 1 caused the greatest amount of thinning. Seven days after application the nut set was reduced by 49, 64, and 73 percent for the 30, 40, and 50 ppm sprays respectively when compared with the check. The 30 ppm spray applied June 8 gave the best thinning results without excessive nut drop. Nut set was reduced by 38 percent the third week after application when compared to the check.

Results of this study for the cultivar Stuart indicate:

(1) Pollen tubes had grown below the bracts of the calyx seven hours after pollination as determined by the excise method.

(2) Pollen tubes had entered the ovarian cavity 46 hours after pollination.

(3) No significant change in nut diameter occurred between pollination and the time the pollen tube reached the ovarian cavity.

(4) Fertilization of the embryo sac occurred 10 to 15 days after pollination.

(5) Optimum thinning was obtained with 30 ppm ethephon sprays applied June 8.

(6) The average nut was 3.50 mm in diameter and 10.12 mm in length when optimum chemical thinning with ethephon occurred.

Additional applications of ethephon after June 15 are needed to determine how long the chemical will effectively reduce nut set. Investigations are needed to determine:

(1) The stage of embryo development at the time chemical thinning is most effective.

(2) The relation between chemical thinning and embryo abortion.

(3) Environmental and cultivar differences that affect nut development in relation to optimum chemical thinning.

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