DEVELOPMENT OF FLORAL PRIMORDIA AND PREDICTION OF FLOWERING IN WHITE OAK (*Quercus alba* L.)

by

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INTRODUCTION

During the past 40 years, a number of theories have been advanced to explain variability in acorn yields between individual oak trees and for the same individual in different years. Most studies of acorn crop variability have focused on the development of the acorn mast after anthesis. However, a factor which could be of pivotal importance to the success or failure of the acorn crop has largely been ignored: the success or failure of the pistillate and staminate flower crops. Because of the absence of information concerning this aspect of acorn crop variability, this study of flowering in white oak (*Quercus alba* L.) had two main objectives:

1. to study the ontogeny of staminate and pistillate primordia of white oak by means of light and scanning electron microscopy (SEM);
2. to develop predictive models through which the approximate frequency distributions and densities of staminate and pistillate flower crops on a particular tree may be anticipated by comparing the established phenology of floral differentiation with that of bud samples collected up to one month prior to flowering.

LITERATURE REVIEW

Part I: Ontogeny of Floral Primordia

Although a number of studies have been completed on flowering in *Quercus*, most of these have little if anything to say about flower development before the point at which the structures emerge from the bud. The earliest study of the embryo and fruit of *Quercus* was made by Hartig (1851), who investigated the gross floral morphology of *Quercus rubra* L. Benson (1894) studied the embryo sac of *Quercus*, and was the first to describe the extension of the embryo sac known as the caecum. Conrad (1900) made the first detailed study of sexual reproduction in *Quercus*. Working with black oak (*Quercus velutina* Lam.), he described the staminate and pistillate flowers and followed gametogenesis and early embryonic development. Other significant observations of staminate flowers in *Quercus* were included in studies conducted by Busgen and Munch (1929) and Rendle (1925), who noted that the male catkins of North American oaks are colorless and that insects are not attracted to them. Langdon (1939) investigated the morphology of oak flowering and seed development in her anatomical studies of the *Fagaceae* and *Juglandaceae*. Krahl-Urban (1951) reported that exceptional oak trees occur bearing only staminate flowers.

Hjelmqvist (1953) studied the development of the embryo sac in the English oak (*Quercus robur* L.), tracing it from a mass of sporogenous tissue in the center of the nucellus, through meiosis to the eight-nucleate stage and formation of the caecum. Minina (1954) also reported on the biological basis of flowering and fruiting of European oaks.

More recently, thorough studies have been completed on microsporogenesis and embryogenesis in *Quercus alba* L. Stairs (1964) traced development in the staminate flower from meiotic division of the microsporocyte to maturation of the pollen grain. He also reported that endosperm formation is well advanced prior to the beginning of embryo growth, and that several ovules within a single acorn may contain endosperm prior to the development of the embryo. Stairs concluded that definitive ovule dominance, resulting in single seed formation within an ovary, occurs during early embryo growth, and is not a function of pollination or fertilization. Morgensen (1965) followed embryo and fruit development in *Q. alba* from fertilization to the mature acorn. He reported that usually five of the six ovules in the ovary abort early, leaving only one seed per acorn, even though fertilization takes place in the abortive ovules. Morgensen (1975) later found that contrary to previous reports, fertilization does not always occur in the abortive ovules, although they contain normal embryo sacs. He proposed that the first of the ovules to be fertilized suppresses the normal development of the others. Abortion of other ovules may be accounted for by the complete absence of embryo sacs and the occurrence of empty embryo sacs.

The most complete developmental study of flowering in *Quercus alba* to date is that of Turkel, Rebuck and Grove (1955). Their study attempted to relate the development of inflorescences and flowers to specific time periods, with the hope that future investigations at critical periods might demonstrate reasons for acorn crop failure. The authors

reported that both staminate and pistillate flowers of *Q. alba* are functionally monosporangiate and have their origin during vegetative growth of the year preceding fruit maturation. Development of the staminate inflorescence is initiated in late June (in their Pennsylvania test trees) and by October, the flowers develop to the point where stamen initials can be recognized. According to the authors, sporogenous tissue is first apparent in April of the year following initiation and pollen is shed during the final week of May.

Turkel et al. (1955) reported that the pistillate inflorescence originates during late summer, but is hardly discernible until early October because of similarities to vegetative apices. Apparently, the difficulty of distinguishing the young pistillate inflorescence from developing lateral buds is also a problem in the European pedunculate oak, *Quercus robur*. While Romasov (1957) and Lohwag (1910) recognized the formation of the young pistillate inflorescence of *Q. robur* during late summer and early fall, Kotov (1969) reported that the structure was not initiated until February or March.

Turkel et al. (1955) observed virtually no further development of the pistillate inflorescence between October and April or early May. At this time, the axis begins to elongate, bractlets appear and single flower primordia arise as dome-shaped structures. Around mid-May, the apices flatten and the carpels arise. According to the authors, by the time pollination takes place during the last week of May, the ovary is not mature and the ovules are just beginning to develop. Fertilization does not occur until a month or more following pollination. The delay

gives time for megasporogenesis and gametogenesis to be completed.

It is interesting to note that Turkel et al. (1955) and Hjelmqvist (1953) do not agree on the origin of the embryo sac in *Quercus*. Hjelmqvist and Stairs (1964) contended that the embryo sac is monosporic, while Turkel et al. indicated that it is of the tetrasporic Adoxa type.

Part II: Prediction of Floral Density

As noted earlier, relatively few studies have attempted to relate variability in acorn crops to flower crop variability in white oak. Indeed, a few studies have actually reported that flower crop variability is not a factor contributing to acorn crop variability (Sharp and Chisman, 1961; Olson and Boyce, 1971; Romasov, 1957). Most studies have concentrated on the development of the acorn following pollination and have attempted to relate acorn production to variables having their effects during this period.

The variability of acorn crops found between individual trees has been attributed to and correlated with a number of variables. Toumey and Korstian (1937) listed such variables as available water, soil conditions, tree nutrition, crowding of roots and crowns by adjacent trees and climate during the growing season as determinants of fruit yields in forest trees. They also included heredity as a component. Ledig et al. (1971) found innate differences in reproductive ability between white oaks upon which controlled pollinations were performed. Gysel (1956) and Tryon and Carvell (1962) reported that there was little relationship between acorn crop size and site factors or site quality.

Burns et al. (1954) reported that in their study, of several tree

characteristics measured, only crown diameter had a strong correlation with acorn production. Only weak correlations were found between acorn production and diameter at breast height (dbh), crown length and crown surface. Christisen and Korschgen (1955) also observed that crown diameter had the highest correlation with acorn yield when they correlated acorn yield with dbh, crown length, crown diameter, crown surface and radial increment. Similarly, Goodrum et al. (1971) found that acorn yields increased with increasing crown radius. They also noticed that acorn yields generally increased as trees matured and expanded with enlargening bole size. Chalupa (1973), working with *Quercus robur*, reported a significant correlation between acorn crop and dbh in some years of his study, but found that variation within dbh classes made it unreliable as a criterion for prediction. Downs and McQuilkin (1944) observed that in white oak total acorn production actually declined with increasing diameter of trees above 27 inches dbh.

Gysel (1956) reported that most acorns were produced in the sunny part of an oak's crown. Similarly, Sharp and Sprague (1967) observed that open-grown white oak trees produced acorns uniformly over the tree crown, whereas trees in closed canopies produced acorns only on branches exposed to sunlight, indicating that shade reduced production. Thus, according to Goodrum et al. (1971), acorn production could be limited even on trees with large crowns by shading or by the inherent inability to produce, and the relationship of acorn yields to crown size or bole diameter might not always have a high correlation.

The contention that acorn crop periodicity is not related to flower

crop periodicity is supported by observations of consistent flowering from year to year while acorn crops undergo large fluctuations. This observation has led to the belief that flowering in white oak is innate and non-cyclical while fruiting is environmentally induced and cyclical.

Baldwin (1942) attributed perodicity in all tree seed crops to environmental influences and not to innate hereditary or physiological functions. Gysel (1956) believed that climate influenced acorn production, but did not have evidence that climate was responsible for cyclical production. Wachter (1953) also found that in Quercus, climate had a great influence on mast production. Besides late frosts, rain and dry periods, however, he also listed insect damage as a factor detracting from the mast once flowers are present.

Romasov (1957) reported that periodicity of acorn crops in the USSR was not caused by inherent traits in oak, because flowering occurred every year. Failure of an acorn crop, said the author, is generally due to injuries by external factors, of which the most important are: destruction of flowers and leaves by insects; late frost; hail; rain and low temperatures during flowering, preventing full pollination; drought or drying winds in May or June, adversely affecting ovule development and causing pistillate flowers to drop before fertilization.

Sharp and Chisman (1961) reported on the emergence, early growth and maturation of the staminate inflorescence (catkin) and the environmental factors favorable or unfavorable to pollen development and shedding. In their 12-year study, all of their white oak test trees produced a good to heavy crop of staminate flowers each year. The authors

concluded that the periodic occurrence of a white oak acorn crop failure is not due to periodic flowering. Their study did not show a correlation between catkin abundance, pistillate flowers and acorn production in any season. Like Romasov, Sharp and Chisman reported that factors detrimental to male flowering are dry, dessicating winds and killing freezes. Turkel et al. (1955) supported the view that any failure of staminate flowering caused by environmental conditions would occur during the growing season when the catkins emerge. The overwintering condition of the staminate flowers, claimed the authors, would seem to preclude any low temperature damage, except as might occur as a result of late frosts in the spring.

Sharp and Sprague (1967) investigated pistillate flowering and fruiting in _Q. alba_. They found that spring air temperatures strongly affect acorn yields, the best crops occurring when a warm period in late April was followed by a cooler period in early May. The authors expanded their discussion from this point to differentiate between innate, noncyclic flowering and induced, cyclic fruiting in _Q. alba_. They considered white oak, which produced a good to heavy crop of acorns in five of the fourteen years of their study, to be cyclic in fruit production. But among their test trees, an abundance of staminate flowers was produced in each of the 14 years. Pistillate flowers also occurred, although their numbers varied from year to year. Therefore, according to the authors, flower production and consequently fruiting potential in white oak is innate--a hereditary, noncyclical characteristic. Actual fruiting is cyclical and induced by exogenous environmental influences.

Not all the evidence, however, indicates that the number of flowers breaking bud each spring is as dependable as Sharp and Chisman (1961) and Sharp and Sprague (1967) contend. Romasov (1957) believed that rain and low temperatures in late summer, at the time of initiation and early growth of the staminate inflorescences, could be detrimental to their development. Bonnet-Masimbert (1973) noted that flowering sometimes passed almost unnoticed in _Quercus petraea_ L. because the flowers had been destroyed by _Operophtera brumata_ larvae. He suggested that insects were a major cause of the irregularity of acorn crops. Wachter (1953) argued that climate not only has its effect on the acorn crop once flowers are present, but also has a great influence on the success of the flower crop. He found that warm, dry summers were associated with good flowering 53 percent of the time, while cool, wet summers were associated with a good flower crop only 25 percent of the time. It should also be noted that Sharp and Chisman (1961) admitted that random observations of white oaks outside their selected sites revealed an occasional tree that produced no flowers. The authors, however, did not say whether or not these trees were flowerless every year.

Closely associated with the problem of solving the reasons behind variability in acorn crops is that of accurately predicting acorn mast size. Most authors seem to agree that a heavy flower crop is no guarantee of a heavy acorn crop (Sharp and Chisman, 1961; Wachter, 1953; Olson and Boyce, 1971; Romasov, 1957). Grisez (1975), in predicting acorn crops for white oak, did not bother to rate flower crops on his test

trees, because he did not believe that flower crops varied from year to year, and because pistillate flowers were difficult to see from the ground. Conner et al. (1976) found that flower production could not be used as an accurate indicator of final acorn yield.

Opinions differ, however, as to the predictability of acorn crops once pollination has occurred. Williamson (1966) reported that most premature abscissions of acorns occurred during early development. He believed that the high abscission rate during pollination and fertilization periods was probably common for wind-pollinated species, such as white oak. Minina (1954) also noted that trees that abscised only a small portion of their acorns soon after pollination produced the largest number of mature acorns. Since as Olson and Boyce (1971) stated, acorns never develop by apomixis, the pistillate flowers that do not receive pollen or are not fertilized abscise during the early development period. Kossuth (1975) supported this explanation for early abortion by demonstrating that early abortion of white oak acorns is not caused by competition with leaves for assimilates. The studies conducted by Williamson (1966) and Minina (1954) imply that relatively accurate predictions of acorn crop size may be obtained by sampling subsequent to the period of abortions that occurs early in acorn development. Gysel (1958), however, concluded that there is no infallible method of making an early prediction of the size of mature acorn crops.

Although prediction of white oak acorn crops has received attention in the literature, prediction of flower crops has not--again

probably because so many authors have reported consistent flowering from year to year and no correlations between the sizes of flower crops and acorn crops. However, at least one author has reported a positive correlation between the number of staminate flowers and acorn crop size in *Quercus robur* (Chalupa, 1973). As noted previously, variations in flower crops do occur. Therefore, the prediction of staminate and pistillate flower crops in *Quercus alba* is potentially useful in solving the problem of acorn crop variability.

MATERIALS AND METHODS

Part I: Ontogeny of Floral Primordia

Collection of Material

Beginning in mid-April, 1977, buds were collected from each of four white oak test trees at the Reynolds Homestead Research Center. Table 1 characterizes each of the test trees. At least 20 buds, including terminal and lateral samples, were collected from each tree and fixed in FAA (formalin-acetic alcohol, Berlyn and Miksche, 1976), using an aspirator to ensure infiltration. Collections were made at two-week intervals throughout the summer and fall until early October. Additional material was collected just prior to bud-swell in mid-March and immediately following bud-break in early April. Table 2 lists collection dates for the four trees.

Examination of Material

The first attempts to examine the floral primordia were by light microscopy. This necessitated dehydration of the buds through a graded ethanol series into TBA (tert-butyl alcohol), following the schedule presented in Appendix A. Buds were embedded in paraffin and sectioned. Because of a hardening of the plant material, possibly the result of prolonged storage in FAA, satisfactory paraffin infiltration of buds could not be accomplished, and usable sections of the floral primordia could not be obtained. A number of techniques were attempted in an effort to soften the material and to increase infiltration, but none were successful (see Appendix A).

After exhausting the available techniques to improve paraffin

infiltration, it was discovered that useful information could be obtained by examination of peeled buds through the dissecting scope (maximum magnification 310x). Buds in FAA were peeled under the dissecting scope, taking care to keep the material covered with fixative. Bud scales, leaf primordia and other extraneous parts were removed to expose floral primordia. In cases where the pistillate structures were the main object of study, the staminate structures were also removed.

The peeled buds were dehydrated through a graded ethanol series and transferred to 100 percent acetone. The tissue was critical point dried in a Polaron Critical Point Dryer using liquid CO_2. Table 3 contains a dehydration schedule and critical point drying schedule.

The dried material was examined under the dissecting scope and additional micromanipulation of bud parts was done to further expose floral parts.

Any floral material which could be adequately resolved under the dissecting scope was photographed in black and white and in color. Representative material for each collection date was further trimmed of excess tissue, mounted on stubs and coated to a thickness of 200 Å with 60 percent gold - 40 percent palladium using a Denton Vacuum Evaporator (DV-515). Tissue was then examined in an AMR 900 Scanning Electron Microscope operated at 20 kV.

Part II: Prediction of Floral Density

Bud collections made in mid-March and early April in Part I served in the predictive study as well as in the developmental study. Collections (Table 4) were made in the following manner: On each of the four

test trees, 20 branch-whorls in the upper crown were labeled with numbered aluminum tags. Ten were labeled in March and ten were labeled in April. Within each whorl, two branches of approximately equal gross morphology and bud density were chosen for paired sampling. These branch pairs were considered as the experimental treatments. One of the two branches from each labeled whorl was pruned and the tag was attached to the remaining branch. From each pruned branch the terminal cluster of buds and the top two distinct lateral buds were clipped and fixed in FAA.

The fixed buds were later peeled under the dissecting scope. The numbers of staminate inflorescences (catkins) and pistillate inflorescences (stalks) found in each bud were recorded. However, this procedure was modified according to the time of bud collection. On buds collected in mid-March (just prior to bud-swell) both staminate and pistillate counts were recorded. On buds collected in early April (just after bud-break) the catkins had already elongated beyond the bud and therefore were not counted. Also, since individual pistillate flowers were visible on the April material, their numbers were recorded in addition to those of the pistillate inflorescences.

In early April, counts of catkins were completed on the branches tagged in mid-March. As with the fixed material, the numbers of catkins arising from the terminal cluster of buds and from the top two distinct laterals were recorded. At this date, the pistillate inflorescences were not visible because the leaves had not yet expanded from the bud conformation. In mid-April, tallies of stalks and pistillate flowers were recorded for the branches labeled in mid-March and for

those labeled in early April. It was again unnecessary to record the number of catkins on the branches tagged in early April, since the catkins were already free from the buds when the branches were labeled.

In order to collect data which would indicate the flowering variation to be expected between branches within a single whorl at anthesis, eight to ten branch-whorls were chosen for observation in the upper crown of each tree. Within each whorl two branches, referred to as experimental controls, were selected for paired sampling using the same criteria as were applied earlier in the choice of the treatment (tagged) branches. For each of these branch pairs, the numbers of staminate inflorescences, pistillate inflorescences and pistillate flowers were recorded in mid-April. As with the treatment branches, the counts were made on growth arising from the terminal cluster of buds and the top two distinct laterals on each branch.

RESULTS

Part I: Ontogeny of Floral Primordia

Staminate Structures

Initial development of staminate primordia may be observed in material collected in late May. The apex of the structure which subsequently develops into a staminate inflorescence (catkin) arises between the developing leaf primordia and the innermost bud scales. Even at this early stage, the half-cylindrical shape of the mature catkin is being molded by pressure from the leaf primordia beneath the structure, resulting in a flattening of that side of the catkin (Figures 1, 2). By early June, the staminate primordium has lengthened and appears closer to its mature shape, with a clearly defined ridge-like tunica (Figure 3). At this time, the staminate inflorescence is easily distinguished from vegetative primordia (which actually do not arise until a month later) by its shape and the complete absence of appendages (Figure 3).

In late June and early July, rounded meristematic areas arise all over the surface of the lengthening primordium, making their earliest appearance near the base of the structure (Figures 4, 5, 6). Each of these bulges subsequently develops into a staminate flower. Very soon after the individual flowers can be distinguished, ridges of cells arise to encircle the top of each dome. It is believed that these ridges indicate the location of the arising perianth (Figure 4). During July, the axis primordium continues to elongate and new flower primordia continue to arise acropetally (Figure 7).

By early August, the catkin primordia are easily distinguishable macroscropically (Figures 19, 20). The most mature flowers at the base of each male inflorescence show development of a perianth ring, soon followed by initiation of the anthers just inside the perianth (Figures 8, 9). Eight anthers is the most common number observed in a single staminate flower.

Within two weeks after the appearance of anthers in the lower flowers on the axis, the perianths of these flowers have extended over the anthers (Figure 10). Also by late August, the axis has essentially completed its pre-dormancy elongation (Figure 21) and the flowers nearest the apex begin to differentiate perianth members (Figure 11).

Few major structural changes occur before dormancy begins in the fall. By early September, each of the flowers on the axis has expanded so that all spaces between adjacent flowers are closed (Figure 12). Macroscopically, the surface of the catkin primordium appears to be composed of polygons as the growing flowers press against each other on all sides (Figure 22).

In late September, the anthers, now almost totally covered by tepals, begin to take on the lobed appearance characteristics of their mature stage (Figure 13). Also at this time, in the center of some of the staminate flowers, there is development of a small mass of rounded structures that is clearly not an anther (Figure 13), and which fails to continue to develop in later stages.

Very little change in the configuration of the catkin or the anthers occurs from early October until mid-March. Around the time of

bud-swell in late March, the perianths of the individual flowers seem to form a continuous covering over the entire staminate inflorescence. As a result, the individual flowers become indistinguishable and the anthers invisible (Figure 23). Meanwhile, the anthers have resumed development. They enlarge and continue to become more obviously lobed (Figure 14).

By the end of the first week in April, the axis of the staminate inflorescence emerges from the bud, usually from between the lower bud scales, preceding the appearance of the leaves. Over the following days, the axis continues to elongate to a length of one to three inches, with the individual staminate flowers spaced along most of its length.

At maturity, the tepals of the staminate flower are reflexed, completely exposing the now conspicuously lobed anthers (Figure 24). Anther insertion can now clearly be seen to be terminal (Figure 15). A cross section of the mature anther a few days prior to dehiscence shows that the four locules are filled with pollen grains that are no longer in tetrads (Figure 16). The pollen grains are three-lobed and show no conspicuous surface appendages (Figures 17, 18).

Pistillate Structures

The pistillate inflorescence (stalk) is first identifiable in early August. Prior to that time, it may be visible, but it is indistinguishable from the developing lateral buds. Both pistillate primordia and vegetative buds make their appearance in July, and since both arise in leaf axils and both initially appear as rounded, meristematic domes of cells, they cannot be differentiated. Later in the season, however, the

lateral buds take on a different appearance from the pistillate primordia. Pistillate inflorescences are usually located in the axils of only the top three or four leaves of the bud axis. By early August, a vegetative bud growing in one of these leaf axils has developed appendages (leaf primordia or bud scales) which flank the apex of the young bud axis and begin to arc over it. By contrast, a pistillate inflorescence in one of these leaf axils has developed no appendages by August and maintains its rounded, naked appearance for weeks after the lateral bud has well-developed foliar primordia (Figure 25). In fact, at any point during development, a lateral bud primordium will always possess a greater number of appendages than a pistillate inflorescence at the same level of the shoot axis.

Approximately two months after pistillate primordia can be recognized, appendages make their appearance on the surfaces of some of the primordia. In late September or early October, the most mature inflorescences produce one or two bracts near the inflorescence base. These bracts elongate slightly before the onset of dormancy (Figure 26).

The pistillate inflorescence resumes development by late March, expanding the size of its bracts, adding more bracts and enlarging (Figures 27, 28, 29). The most dramatic changes take place during the last week of March and the first week of April: The inflorescence elongates rapidly, with the apex taking on a pointed appearance. As the axis elongates, it produces a number of additional bracts in a spiral arrangement. In the axil of each bract develops a

single pistillate flower. Usually, there are two or three functional flowers on each inflorescence, although the number may range from one to five. Development is acropetal, with the lower bracts almost always concealing functional flowers (Figures 30, 31). The uppermost bracts on the stalk apex (Figure 32) often subtend rudimentary flowers which never reach maturity.

Upon removal of the subtending bract, the pistillate flower primordium can be seen to be composed of three concentric rings. The first ring to develop is the perianth. Usually six primordia arise on the periphery of the flattened floral apex, eventually fusing to form a continuous perianth (Figures 32, 33, 34). Subsequent to the appearance of tepals, three carpels originate from the flattened apex inside the perianth. The perianth ring continues to elongate, curling in over the developing carpels (Figure 34).

Also by the time of carpel initiation, the outermost ring of structures, the involucre, has begun its development below the perianth. The involucre begins as a few bracts (Figures 32, 34). Additional bracts are added throughout the development of the flower and fruit (Figure 35) until eventually the cluster of bracts fuses to form the familiar cup of the mature acorn.

The leaves emerge from the bud during the second week of April, carrying the female inflorescences, now more stalk-like, in their axils. As the vegetative shoot elongates over the next few days, so does the stalk, growing to the length of about a centimeter in most cases before the pistillate flowers are receptive. The mature pistillate flower can

be recognized as being receptive when its three stigmas extend beyond the perianth (Figure 36).

Part II: Prediction of Floral Density

The following statistical tests and procedures were completed on the experimental data collected in the predictive study. Where pertinent, the same procedures were performed on the data collected from control branches.

The Kolmogorov-Smirnov test conducted by KSLtest on SAS (Helwig, 1977) indicated that none of the distributions--staminate inflorescences (catkins), pistillate inflorescences (stalks), pistillate flowers, total inflorescences (catkins plus stalks)--were normal at any of the sampling dates. The hypothesis of normality was rejected at the .05 level (Table 5). Control distributions showed a similar deviation from normality in most cases (Table 6).

The reason for rejection of normality became apparent when histograms of the frequency distributions were examined. It was seen that for staminate inflorescences (Figures 37, 38), the observation with the highest frequency was for branches bearing no catkins at all, on both sampling dates. Elimination of the zero class from the distributions did little to help them appear more normal. In fact, when the zero counts were eliminated from the data, the results of the Kolmogorov-Smirnov test still called for rejection of normality for all the distributions (Table 5).

The histograms of distributions of stalks (Figures 39, 40, 41) and pistillate flowers (Figures 42, 43) reveal a different reason for the

rejection of normality in their cases: They were all skewed heavily to the right, except for the distribution of stalk counts at date 1. The most frequently observed numbers of stalks on a branch were three and four at two sampling dates, although the distributions ranged up to 16 and 19 stalks on a single branch. The most frequently observed count of pistillate flowers at both sampling dates was seven, although the distributions ranged as high as 40 and 56 flowers per branch. The distributions for total numbers of inflorescences per branch showed similarly skewed distributions (Figures 44, 45).

On the basis of the results of the Kolmogorov-Smirnov tests and the histograms, nonparametric procedures were chosen for paired-data tests and the generation of correlation coefficients.

The Wilcoxon Matched-Pairs Ranked-Signs test conducted using SPSS (Nie et al., 1977) indicated that there was no significant difference at the .05 level between the means of the distributions of catkins at date 1 and date 2. The same results were obtained for the distributions of stalks at date 2 and date 3, the distributions of pistillate flowers at date 2 and date 3 and the distributions of total inflorescences at date 1 and date 2. The only distribution pair for which there was a significant difference between the means was the one involving stalk counts at date 1 and date 3 (Table 7).

The Wilcoxon Matched-Pairs Ranked-Signs analysis of the control data produced quite different results from the treatment data. Significant differences were found at the .05 level between the means of the distributions of paired stalk counts, paired pistillate flower

counts and paired total inflorescence counts. As with the experimental data, however, no significant difference was found between the means of the paired catkin distributions (Table 7).

Kendall Tau-b correlation coefficients, calculated using the CORR procedure on SAS (Barr et al., 1976), pointed to a strong linear relationship between catkin counts at date 1 and date 2, between stalk counts at date 1 and date 3 and between total inflorescence counts at date 1 and date 2. Much weaker linear relationships were found between stalk counts at date 2 and date 3 and between pistillate flower counts at date 2 and date 3. There was no evidence of correlation between stalks and catkins (Table 8).

Correlation coefficients generated for the control data proved to be quite comparable to those computed for the treatment data. Strong linear relationships were found for the paired catkin counts and paired total inflorescence counts, while weaker linear relationships were found between paired stalk counts and paired pistillate flower counts (Table 8).

Scatter diagrams reveal the most obvious linear relationships are between catkin counts at date 1 and date 2, between stalk counts at date 1 and date 3 and between total inflorescence counts at date 1 and date 2 (Figures 46, 47, 50). The relationships illustrated by the plot of pistillate flower counts at date 2 versus those at date 3 were not so clearly defined (Figures 48, 49).

Similarly, all of the paired variable scatter diagrams for control counts, except for the paired pistillate flower counts, indicated well-

defined linear relationships (Figures 51-54).

Linear models for the paired variables were created by the least squares method, using the GLM procedure on SAS (Barr et al., 1976). F values associated with the relationships between catkin counts at date 1 and date 2, between stalk counts at date 1 and date 2 and between total inflorescence counts at date 1 and date 2 indicated strong regressions in these cases. F values associated with the model relating stalk counts at date 2 to those at date 3 and the model relating flower counts at date 2 to those at date 3 suggested much weaker regressions (Table 9).

R-square values indicated that the regressions accounted for considerable amounts of the variation in the cases of the catkin model, the model relating stalk counts at date 1 to those at date 3 and the total inflorescence model. The regressions accounted for little of the variation in the model relating stalk counts at date 2 and date 3 and in the pistillate flower model (Table 9).

Linear models generated for the control paired variables showed trends similar to those found for the treatment models. However, the control regressions were generally weaker than those for the treatment data and accounted for less of the total variation (Table 9).

DISCUSSION

Part I: Ontogeny of Floral Primordia

Scanning electron microscopy (SEM) has made it possible to gain a new perspective on the development of floral primordia in white oak. The most significant advantages of the technique in creating a record of ontogeny are the clear views of surface phenomena accompanying initiation of new floral structures.

SEM observations of staminate primordia parallel those obtained by Turkel et al. (1955) using light microscopy. Initiation of the staminate inflorescences (catkins) in the current study preceded initiation of catkins in Turkel et al.'s Pennsylvania test trees by approximately one month. However, catkins in both studies suspended development at the same stage with the onset of winter dormancy. In both cases, perianths were fully developed and anthers were structurally mature with slight lobing in the overwintering condition. Resumption of anther development in the Virginia trees preceded that in the Pennsylvania trees by approximately one month, as did anther dehiscence.

Even with the SEM, identification of the pistillate inflorescence (stalk) during the late summer and fall was extremely uncertain. The problem is created by the fact that lateral bud primordia are initiated at the same time as pistillate inflorescence primordia and both arise in the axils of developing leaves. By August, it is usually possible to distinguish the two structures, because by this time most of the vegetative primordia have at least two lateral appendages beginning to cover the apex, while pistillate inflorescences remain naked and simply

grow larger. Eventually, however, the pistillate inflorescences also develop appendages--the bracts in the axils of which individual pistillate flowers arise. Once again it becomes difficult to differentiate a pistillate inflorescence from a slowly developing lateral bud, until the floral axis begins to elongate prior to anthesis.

Turkel et al. (1955) did not observe formation of bracts on the stalk axis until a few weeks before flowering in the spring. In this study, by contrast, bract formation was repeatedly noted on pistillate inflorescence primordia in material collected in late September and early October. It could be that pistillate development reaches a more advanced stage before the onset of dormancy in the Virginia test trees than in the Pennsylvania test trees samples by Turkel et al. due to a longer growing season in Virginia.

Figure 21, which shows staminate and pistillate primordia side by side in a late August bud, illustrates the striking contrast between the developmental schedules of the two structures from initiation to structural maturity. Staminate inflorescences are initiated in May and reach almost full pre-emergence length by the onset of dormancy in October. Individual staminate flowers develop throughout the summer and are structurally mature by October, with well-developed perianths and lobed anthers. The pistillate inflorescence, by contrast, arises in July and develops little before dormancy. Individual pistillate flowers do not originate until approximately a week before budbreak. However, by the end of that week, the pistillate inflorescence has increased in size ten-fold, and individual pistillate flowers look strikingly similar to individual staminate flowers in early August of the preceding growing

season (compare Figures 9 and 33).

Within three weeks after they originate, the pistillate flowers are structurally mature and stigmas are receptive--usually less than a week after the beginning of anthesis of the staminate flowers on the same tree. Thus, in less than one month, pistillate flowers reach a stage of structural maturity comparable to the stage reached by staminate flowers after three months of development.

Part II: Prediction of Floral Density

The statistical analyses and plotting procedures performed on the flowering data suggest some general conclusions concerning the predictability of white oak flower crops by examination of bud samples collected as early as one month prior to flowering.

An idea of the predictability of frequency distributions for counts of staminate inflorescences (catkins), pistillate inflorescences (stalks), pistillate flowers and total inflorescences (catkins plus stalks) may be obtained by reviewing the histograms of these distributions and the results of the Wilcoxon Matched-Pairs Ranked-Signs test. The frequency distributions of catkin counts at date 1 and date 2 have a very similar overall shape, suggesting that a mid-March sampling supplies a useful impression of the distribution of catkins on branches when actual flowering occurs in April. The results of the Wilcoxon test support this conclusion, indicating that the means of the two distributions could have come from the same population.

Similar conclusions may be reached regarding the predictability of distributions of pistillate flowers and total inflorescences at

anthesis from the results of the Wilcoxon test and by comparing the histograms. Again, the pairs of histograms show similarly skewed distributions. However, the results of the Wilcoxon test indicate that the pairs of distributions are not as strongly linked as were the catkin distributions, suggesting that predictability is not as strong. It should also be noted that the pistillate flower distribution cannot be predicted earlier than two or three weeks before actual flowering, because pistillate flowers are simply not present prior to that time.

The histograms and Wilcoxon test results indicate that the most difficult distribution to predict one month in advance of flowering is that of the pistillate inflorescence. The Wilcoxon test shows a significant difference at the .05 level between the means of the distributions of stalks at date 1 and date 3. Inspection of the histogram of the distribution of stalks at date 1 reveals that the distribution lacks the general skewed shape of other distributions in the study. The most likely explanation for this anomaly lies in the fact that in mid-March, pistillate inflorescences are still extremely difficult to distinguish visually from developing lateral buds. This leads to a large amount of error in the counting of stalks at date 1.

The distributions of stalks at date 2 and date 3 display a great deal more similarity. The similarity is supported by the Wilcoxon test results, leading to the conclusion that a sample taken two weeks before anthesis is a much more reliable indicator of mature pistillate flower distribution.

The discrepancy between the Wilcoxon test results for the treatment

data and those for the control data are also believed to be due to the error created by the difficulty of counting pistillate inflorescences and flowers.

The Kendall Tau-b correlation coefficients, scatter diagrams and linear models suggest that on an individual branch, the densities of staminate inflorescences, pistillate inflorescences and total inflorescences are predictable enough to make practical the use of linear models. The same procedures indicate that individual branch densities of pistillate flowers are relatively unpredictable, even when estimated from samples taken only two weeks before anthesis. Furthermore, comparison of the ranges of the distributions of pistillate flower counts at date 2 and date 3 shows that the largest number of flowers counted on a branch just after budbreak was only 40 while the largest number of flowers counted at pollination was 56. Again, the speed with which individual pistillate flowers appear is a problem. Two weeks before anthesis, it is difficult to estimate even how many pistillate flowers will be on a single stalk. At this point in the development of the inflorescence, usually only one or two flowers have appeared. By pollination, however, the stalk may display as many as five or six flowers. The rapidity of their appearance makes prediction of their final number extremely difficult.

The lack of relationship between staminate and pistillate inflorescence densities implied by the low correlation coefficients is consistent with Sharp and Chisman's (1961) finding that there was no correlation between catkin abundance and pistillate flower abundance among

white oaks.

As with the Wilcoxon test results, the discrepancy between the strength of the treatment data regressions and the regressions performed on control data is believed to be due to an inadequate sample size of control branches.

The close similarity of correlation coefficients generated for the control data to those generated for the treatment data suggests the following point: A mid-March sample of catkins and stalks on a particular branch is as good an estimator of the density of inflorescences on a paired branch as is an April sample. However, in the case of catkins, the control correlation coefficient is noticeably larger than that generated for the treatment data. Also, comparison of the ranges of the distributions of catkin counts at date 1 and date 2 reveals another dichotomy. The largest number of catkins counted on a branch in the buds was 35, while the largest number counted at anthesis was only 20. Since catkins are very easy to count in March, we can be reasonably sure that the error in their tallies is low. The large difference in ranges suggests that a number of catkins present in the buds in March did not subsequently emerge from the buds in April. Thus it is possible that even if a consistent number of catkins is initiated each year, the number breaking bud may not be as consistent, depending on environmental conditions at or preceding budbreak. The results of this study suggest that while flowering in white oak is predictable enough to be characterized by linear models, environmental influences may induce variability in the number of flowers present at the time of pollination.

Contrary to Sharp and Chisman's (1961) contention, our study indicates that although the number of flowers initiated each year may be under strong genetic control, making this an "innate" characteristic, all primordia formed may not develop to maturity and it is possible that there is environmentally induced year-to-year variability in the numbers of maturing primordia.

CONCLUSIONS

Part I: Ontogeny of Floral Primordia

While the results of our study have contributed to a new perspective on the development of floral primordia in white oak, they have also suggested new areas to be investigated. One of the problems in the study was the failure to consistently distinguish pistillate inflorescence primordia from developing lateral buds. Perhaps a more thorough study of the two structures would reveal differences, possibly in phyllotaxy, that would make it possible to more positively differentiate them earlier than two weeks before flowering. Another area of exploration is suggested by the interesting contrast between the staminate and pistillate developmental schedules. Investigation of the possible selective advantages to this arrangement and the physiology behind the contrasting sequences seems warranted. Finally, the differences between the development of white oak floral primordia in Virginia and Pennsylvania (Turkel et al., 1955) point to an aspect of clinal variation that may be worth describing in more detail.

Part II: Prediction of Floral Density

As in the ontogeny study, a major difficulty in predicting pistillate flower density was created by the structural similarity of the young pistillate inflorescence to the developing lateral bud. Again, a dependable method of distinguishing these two structures must be discovered before an accurate predictive model for pistillate flower density can be formulated. It is also possible that more detailed investigation will reveal a means of predicting approximately how many mature

pistillate flowers will ultimately be present on an inflorescence, even before the first flower is initiated. Perhaps a correlation may be discovered between the mid-March size or shape of pistillate inflorescence primordia and the number of pistillate flowers they will initiate. Staminate inflorescences are relatively easy to count in the bud using the dissecting scope as early as eight months prior to flowering. It is likely that a useful predictive model for mature catkin density could be derived based on the density of catkin primordia observed in buds collected in September.

The question of whether or not flowering in white oak is innate can only be completely settled by a study in which year to year variation in flower crop density is correlated with environmental conditions during initiation of floral primordia and throughout their development. It is possible that only extreme environmental variation will have a significant enough effect on flower crops to convince investigators that there is indeed an environmental component. Since staminate inflorescences are easy to count throughout most of their development, a logical starting point would be to determine at what stages during their development they are aborted. Our data, for example, indicates that something happened to a measurable percentage of catkins just prior to or during budbreak, so that they failed to emerge from the buds. Turkel et al. (1955) suggested a similar investigation of critical periods, which to our knowledge has not been undertaken.

The models derived by this study for prediction of floral density will become meaningful only when they are applied in related studies.

It is hoped that they will be tested for accuracy and improved upon by future investigators of oak flowering and fruiting.

Figure 1. Top view of a staminate inflorescence primordium in late May (200X).

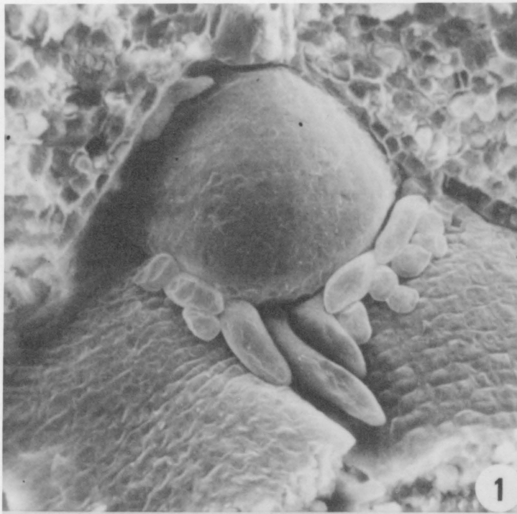
Figure 2. A staminate inflorescence primordium in late May (400X).

Figure 3. A staminate inflorescence primordium in early June, showing a ridge-like tunica (200X).

Figure 4. Staminate flowers in late June. Arrow indicates the position of the arising perianth (400X).

Figure 5. An early July bud with scales removed, showing a staminate inflorescence (catkin) primordium (c), a leaf primordium (lp) and the bud apex (*) (200X).

Figure 6. An early July catkin primordium lying on its side, showing arising staminate flowers (400X).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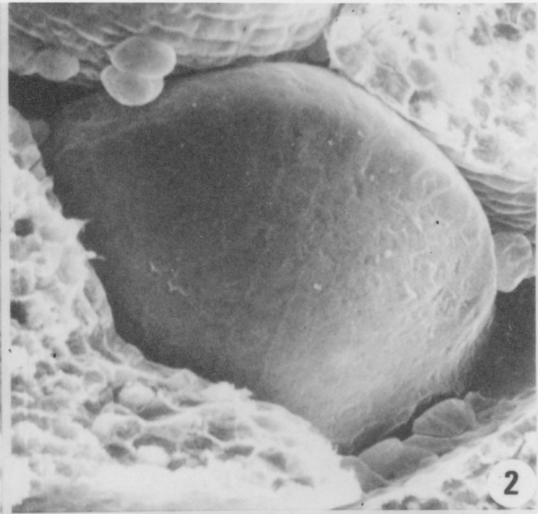
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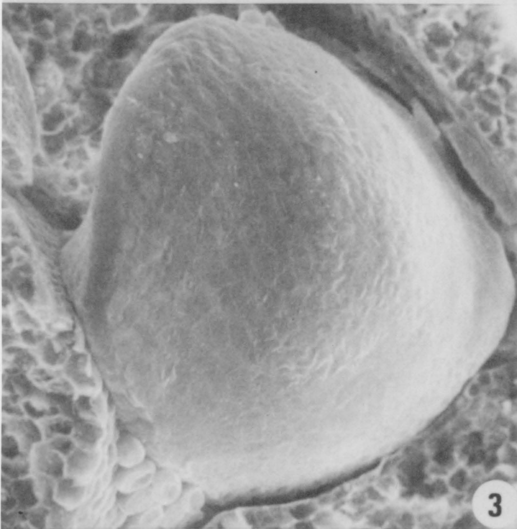
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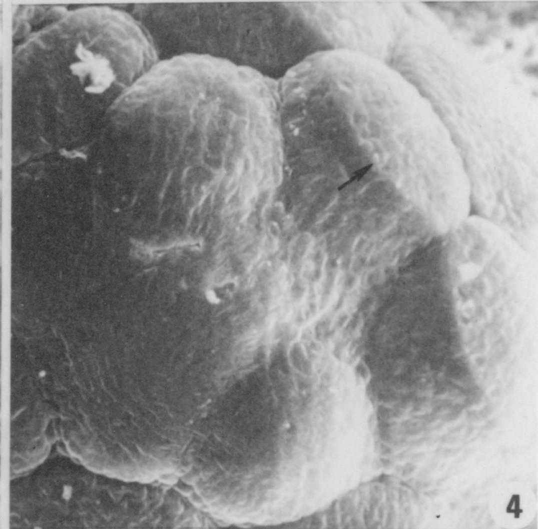
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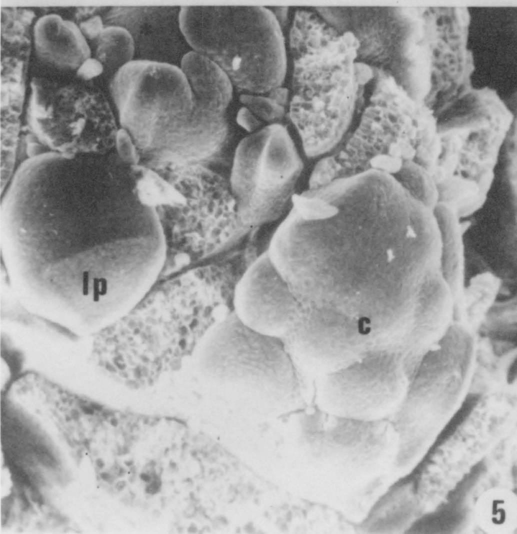
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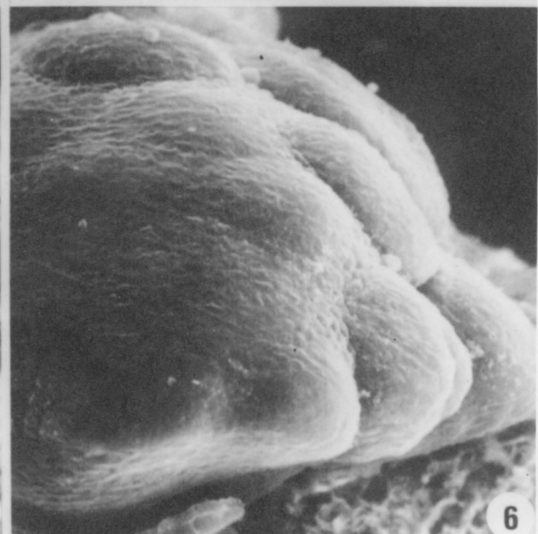
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Figure 7. Top view of a late July catkin primordium (190X).

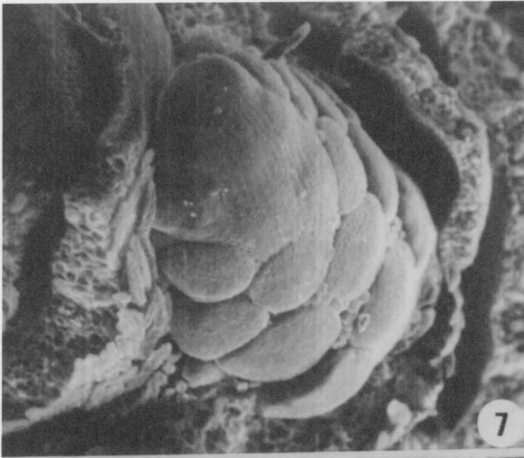
Figure 8. An early August catkin primordium. Box encloses a single staminate flower (190X).

Figure 9. A single staminate flower in early August, showing stamen primordia (s) and perianth parts (p) (850X).

Figure 10. Staminate flowers in late August, with perianths covering stamen primordia (500X).

Figure 11. Top half of a late August catkin primordium, covered with staminate flowers in various stages of development (200X).

Figure 12. Surface of an early September catkin, showing that the staminate flowers have closed the spaces between them (200X).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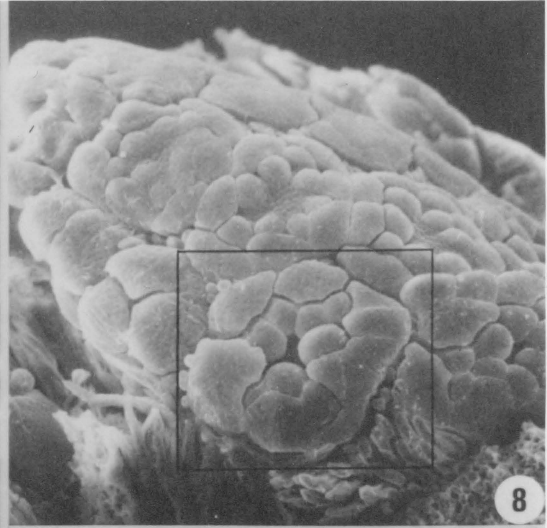
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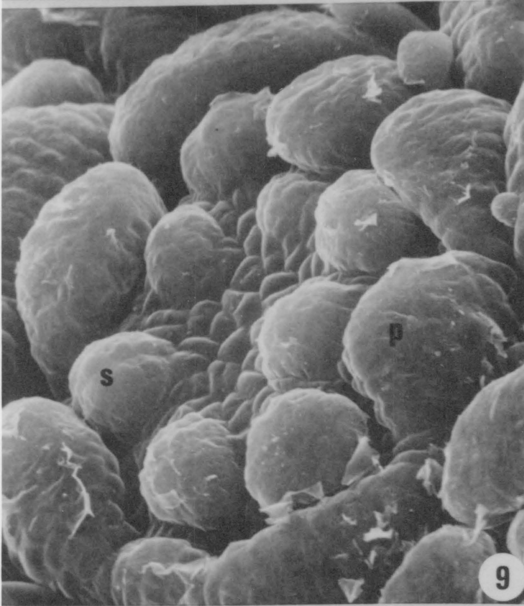
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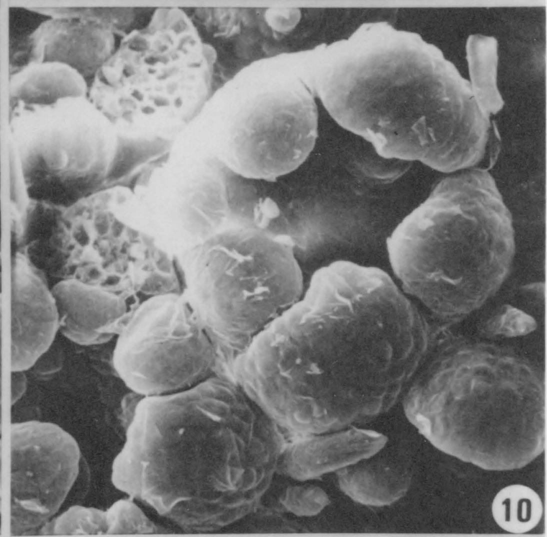
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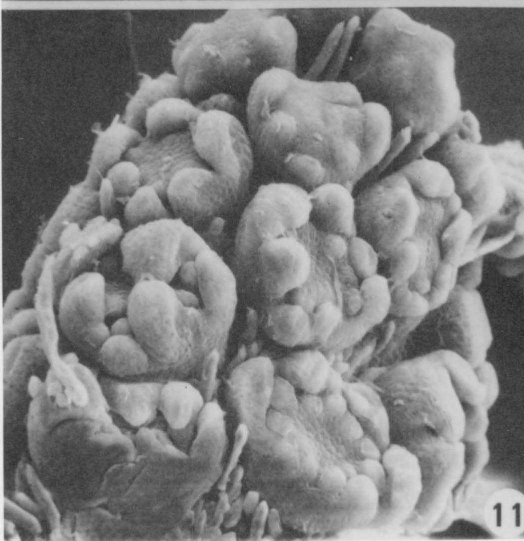
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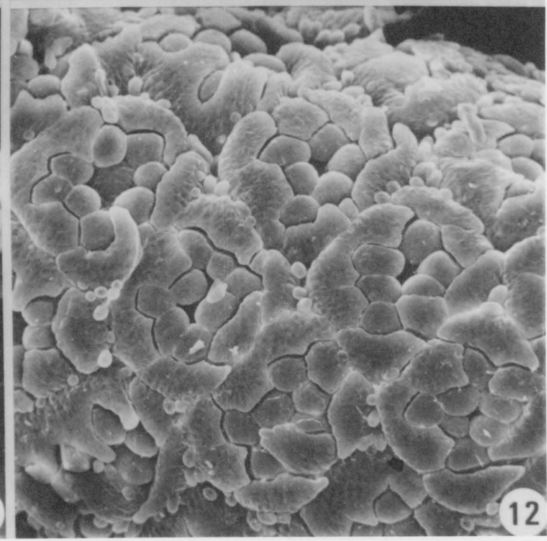
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Figure 13. A late September staminate flower, with the perianth cut away to expose slightly lobed anthers (a) (400X).

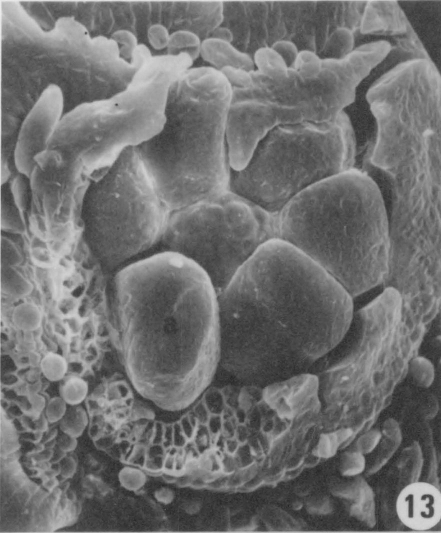
Figure 14. A mid-March staminate flower with the perianth removed to expose lobed anthers (a) (500X).

Figure 15. A mature (early April) anther (100X).

Figure 16. Cross-section of a mature anther (160X).

Figure 17. Mature pollen (1000X).

Figure 18. A mature pollen grain (2000X).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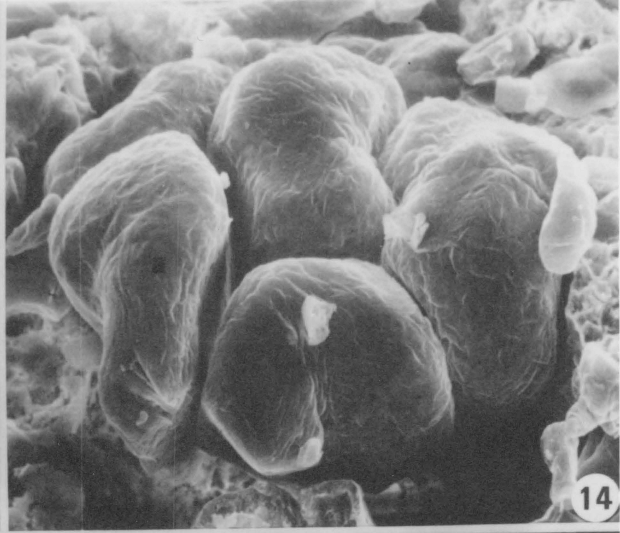
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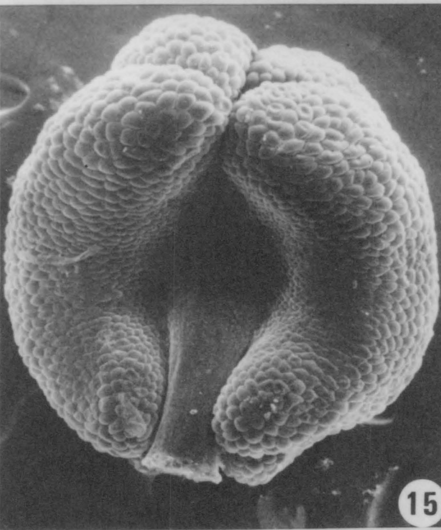
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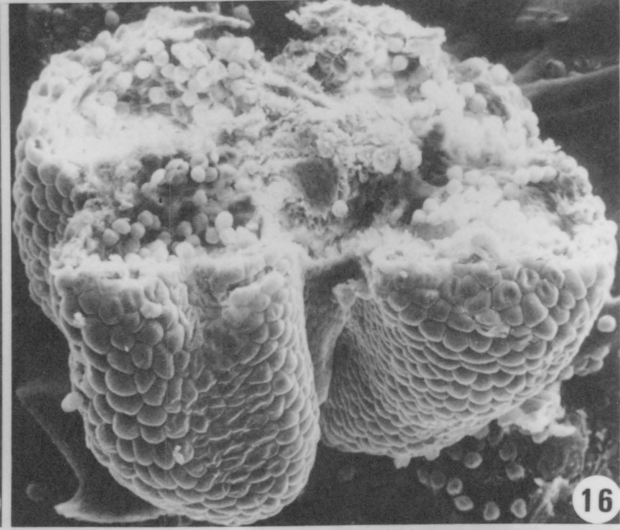
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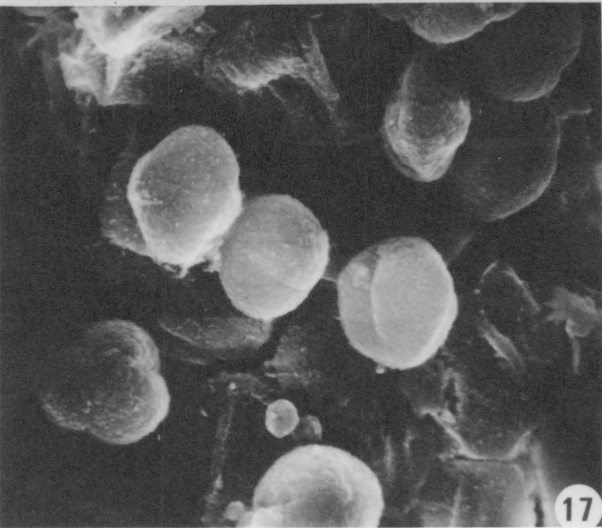
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Figure 19. An early August bud with scales removed, showing three catkin primordia (310X).

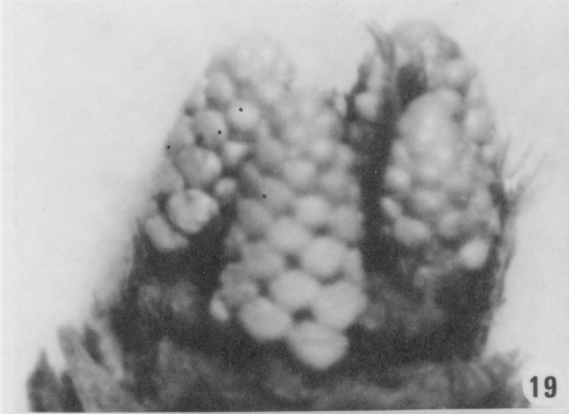
Figure 20. An early August bud with scales removed, showing a top view of four catkin primordia (310X).

Figure 21. A late August bud with scales removed, showing catkin primordia and a pistillate inflorescence primordium (arrow) (310X).

Figure 22. An early September bud with scales removed, containing catkin primordia (310X).

Figure 23. A mid-March bud with scales removed, containing catkin primordia (310X).

Figure 24. A mature (early April) staminate flower (200X).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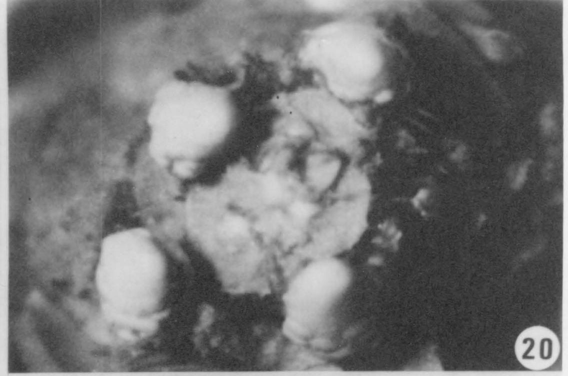
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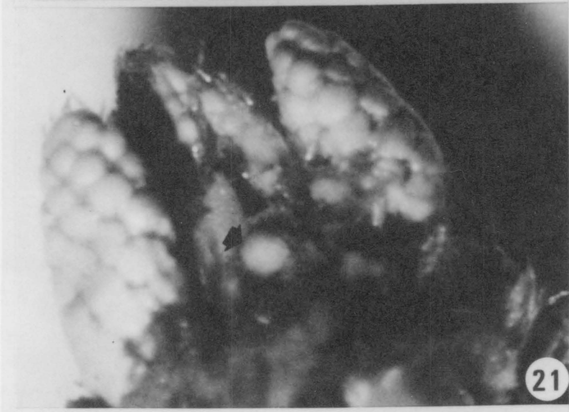
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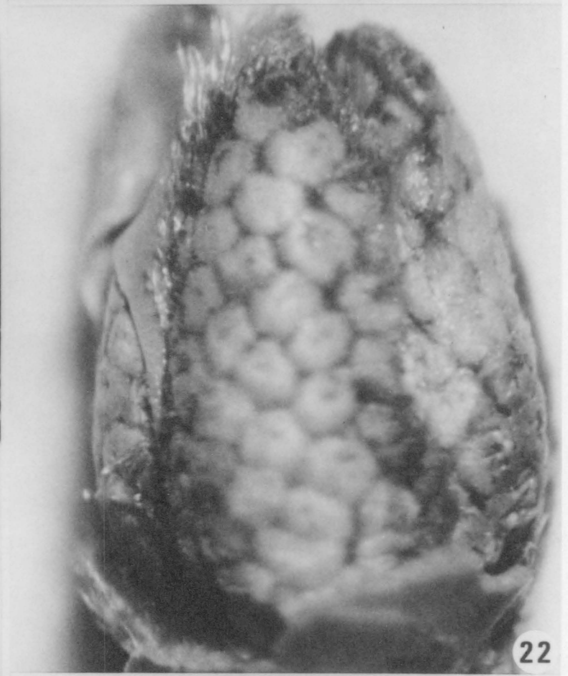
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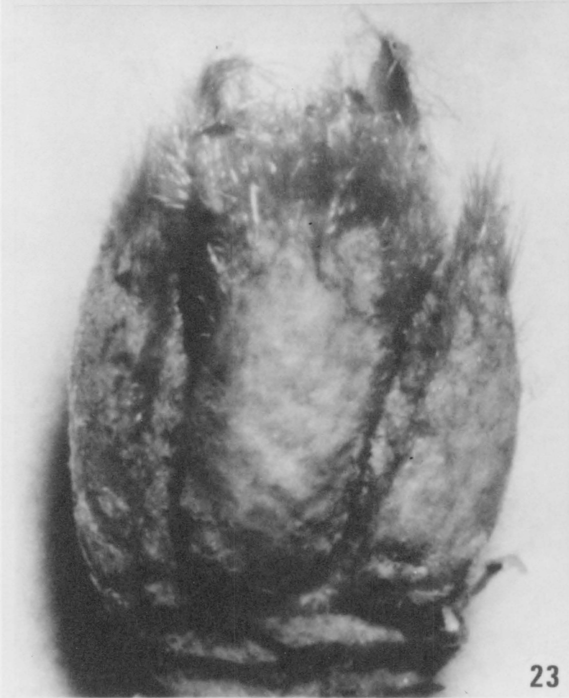
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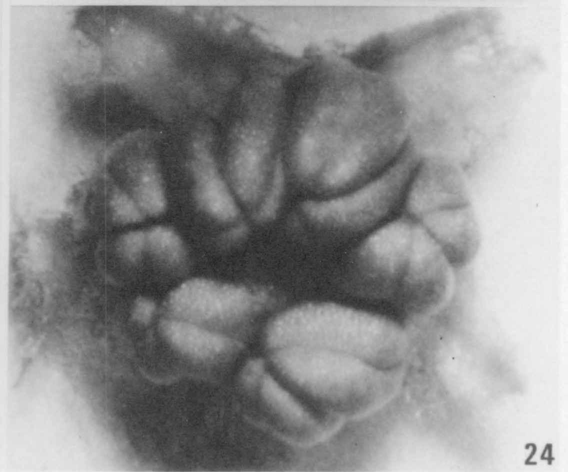
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Figure 25. An early August pistillate inflorescence primordium (400X).

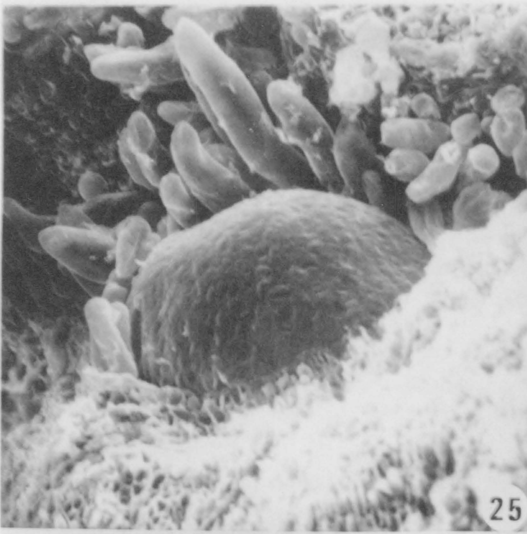
Figure 26. A late September pistillate inflorescence primordium (500X).

Figure 27. A mid-March pistillate inflorescence primordium (310X).

Figure 28. Top view of a mid-March pistillate inflorescence primordium with bracts (b) (400X).

Figure 29. Side view of a mid-March pistillate inflorescence primordium with bracts (b) (200X).

Figure 30. An early April pistillate inflorescence, with bracts (b) covering individual pistillate flowers (310X).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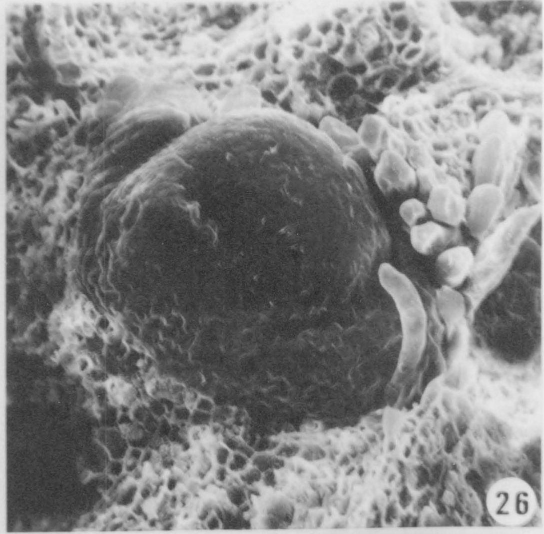
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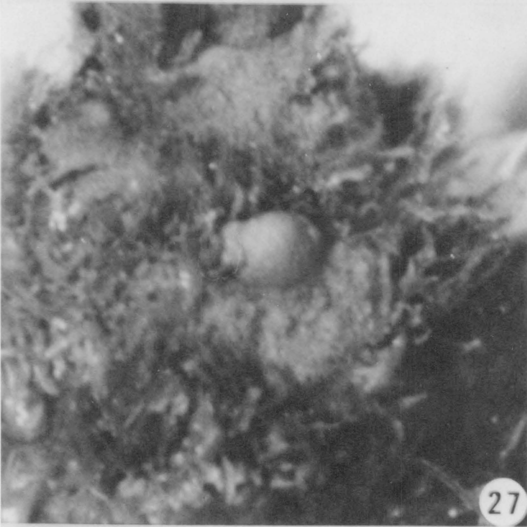
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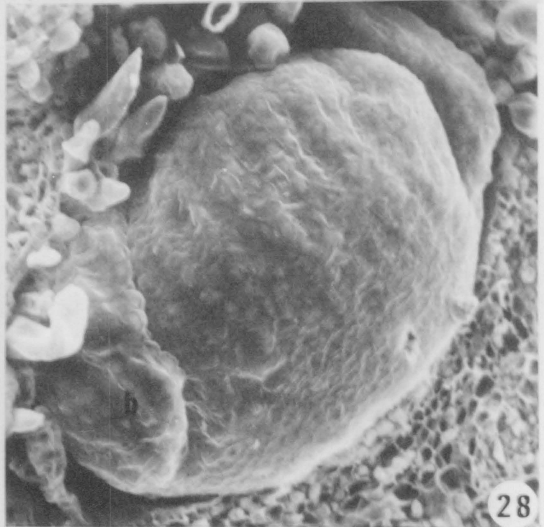

b
28

b
29

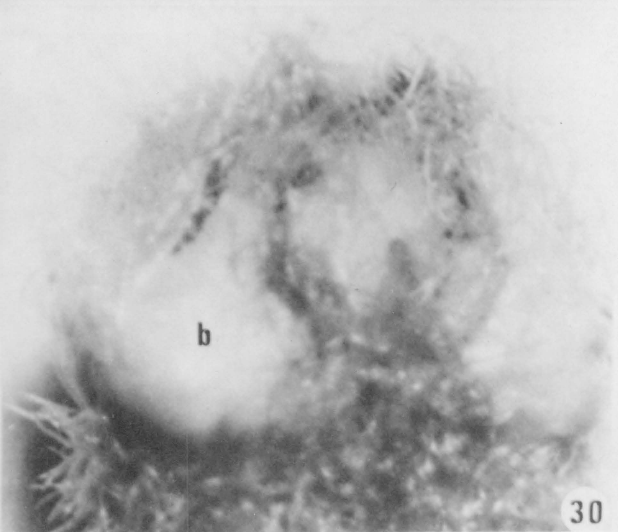

b
30

Figure 31. An early April pistillate inflorescence with bracts removed to expose a pistillate flower (f) and the inflorescence apex (arrow) (310X).

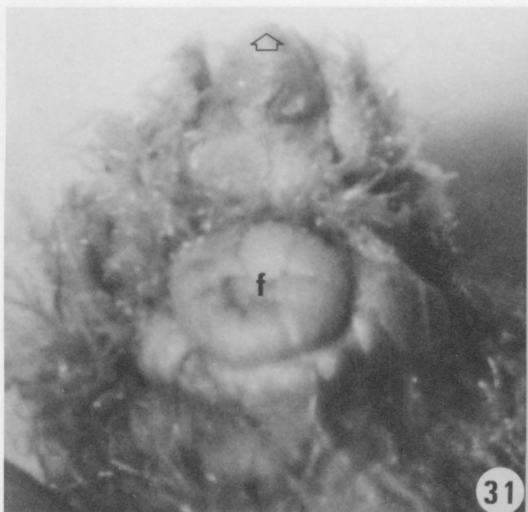
Figure 32. An early April pistillate inflorescence showing the inflorescence apex (arrow), a bractlet (b) which conceals a rudimentary pistillate flower, and a normal pistillate flower with perianth parts (p) (190X).

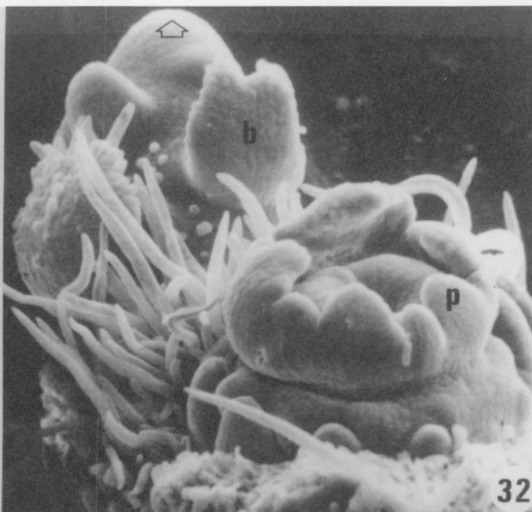
Figure 33. Top view of an early April pistillate flower with the perianth just beginning to arise (p) (375X).

Figure 34. A more mature early April pistillate flower, showing the perianth (p), an arising carpel (arrow) and bracts of the developing involucre (i) (190X).

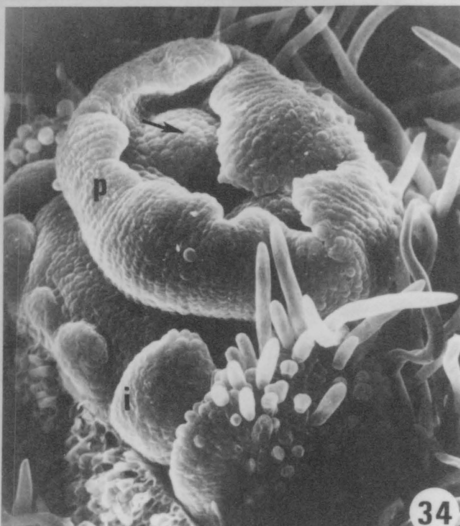
Figure 35. An early April pistillate flower with a well-developed involucre (i) (310X).

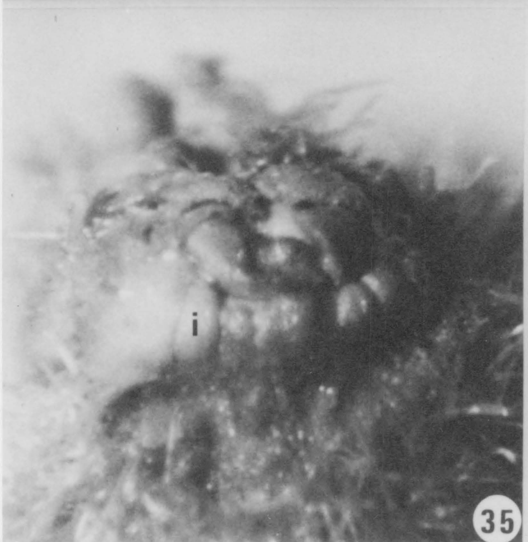
Figure 36. A receptive (mid-April) pistillate flower with stigmas (s) extended (200X).

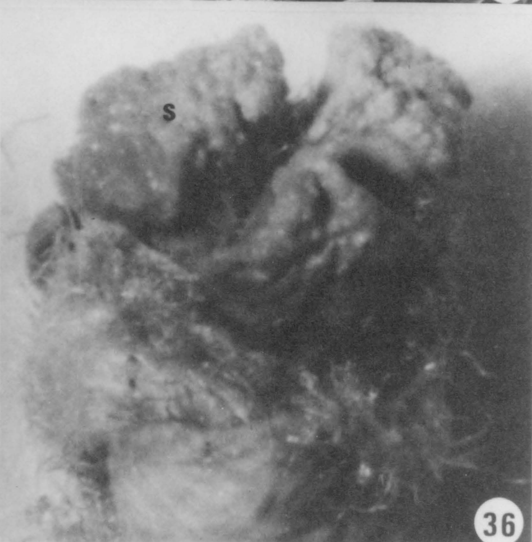

f
31


b
p
32

p
33


p
i
34


i
35


s
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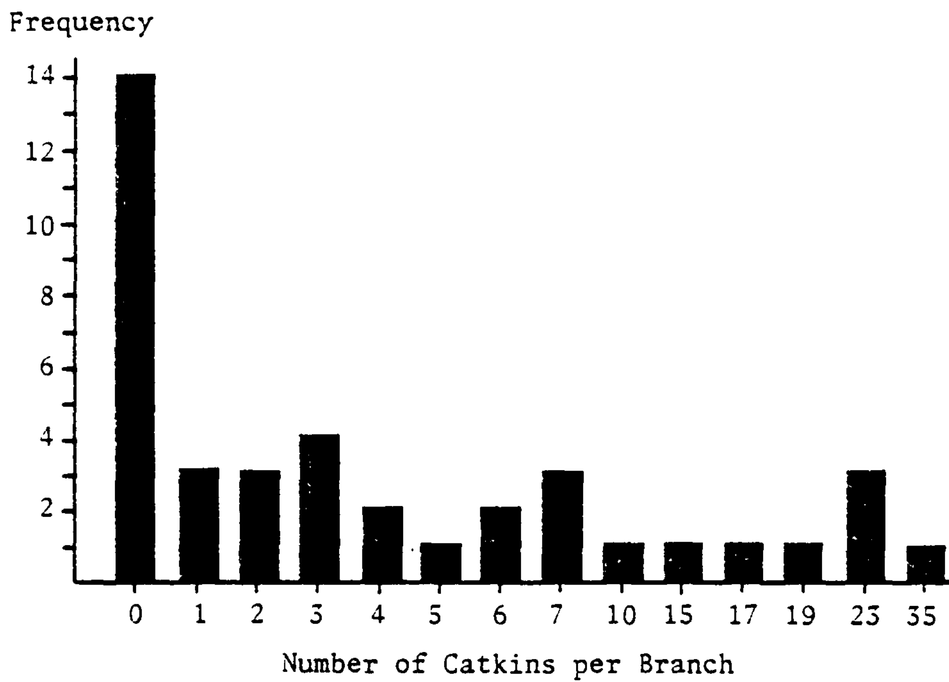

Figure 37. Frequency histogram for number of catkins (staminate inflorescences) per branch, date 1.

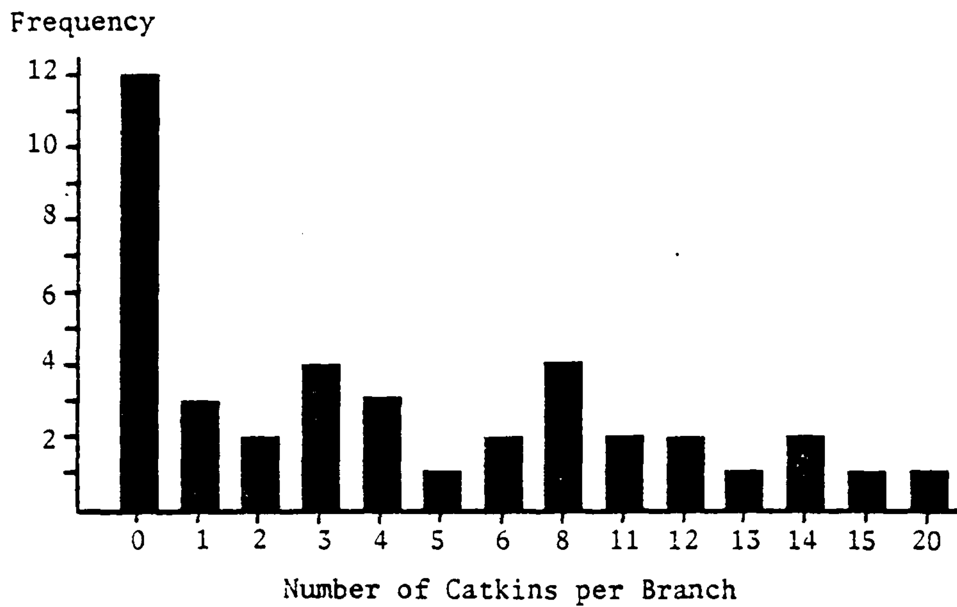

Figure 38. Frequency histogram for number of catkins (staminate inflorescences) per branch, date 2.

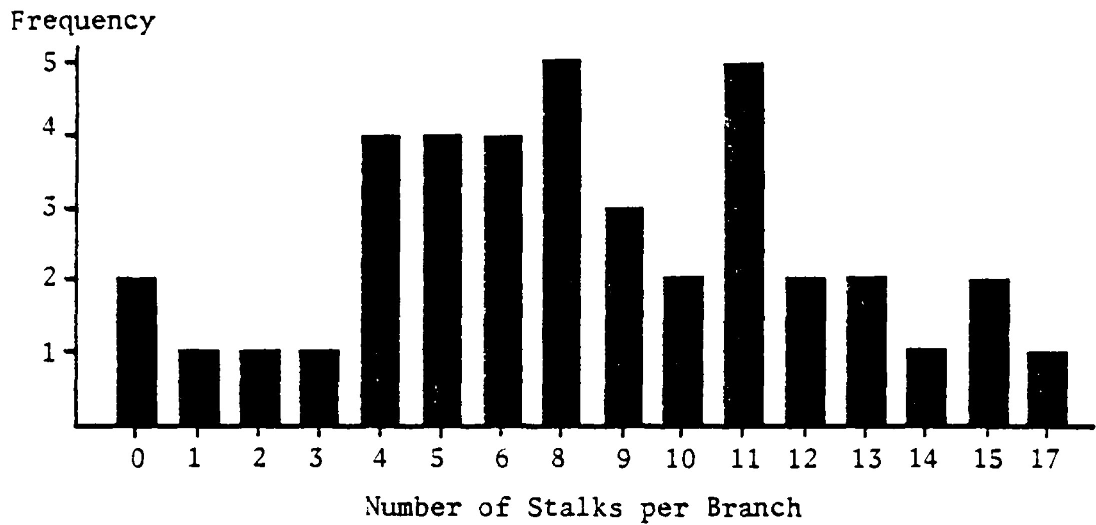

Figure 39. Frequency histogram for number of stalks (pistillate inflorescences) per branch, date 1.

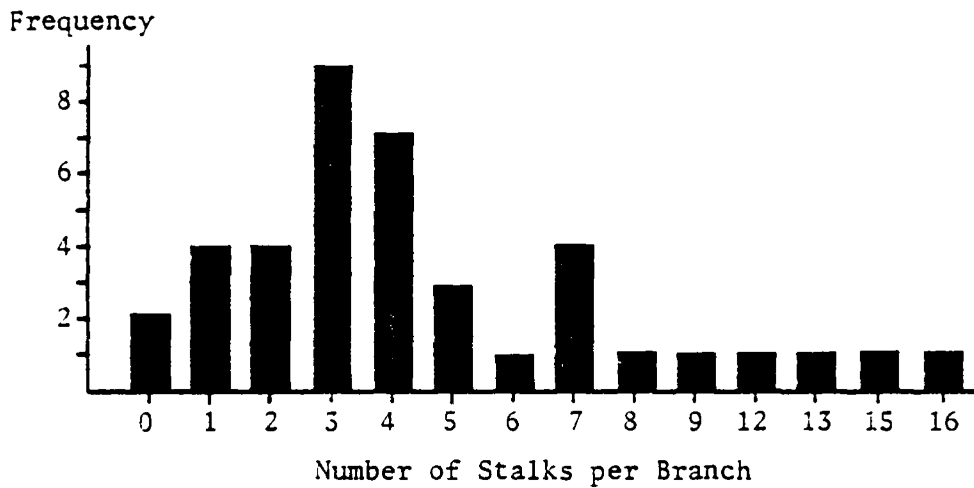

Figure 40. Frequency histogram for number of stalks (pistillate inflorescences) per branch, date 2.

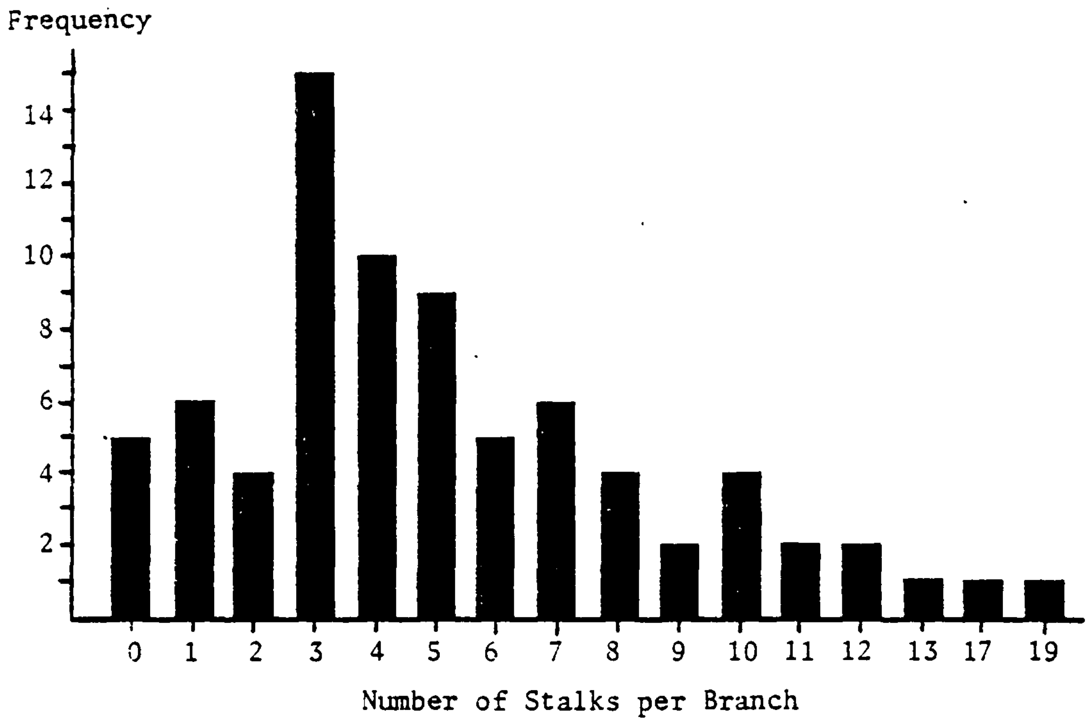

Figure 41. Frequency histogram for number of stalks (pistillate inflorescences) per branch, date 3.

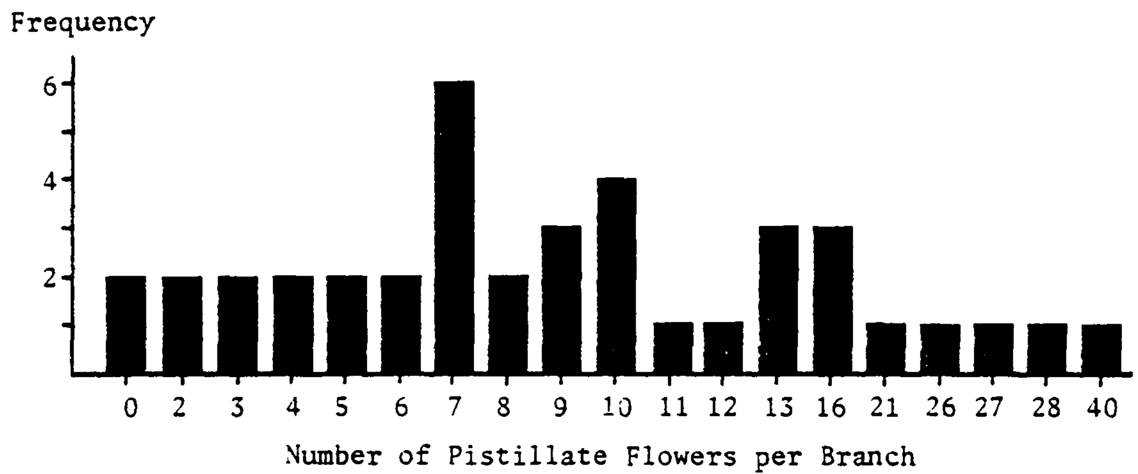

Figure 42. Frequency histogram for number of pistillate flowers per branch, date 2.

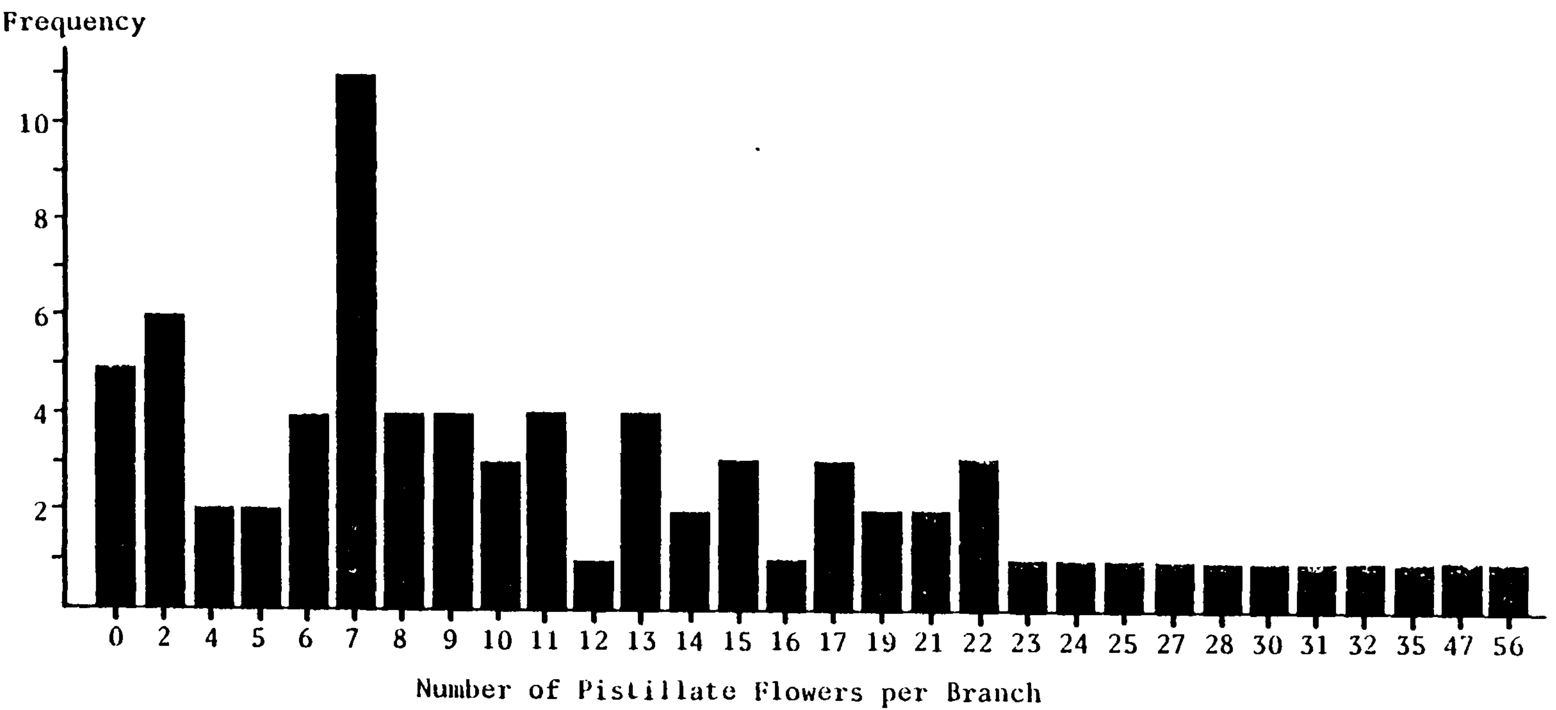

Figure 43. Frequency histogram for number of pistillate flowers per branch, date 3.

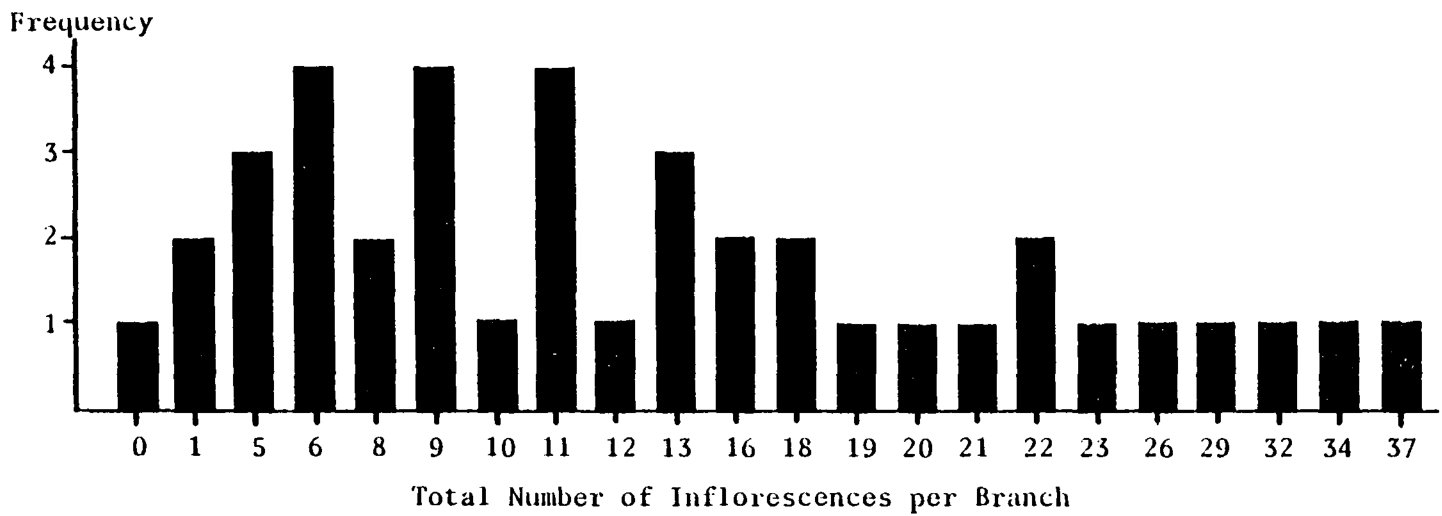

Figure 44. Frequency histogram for total number of inflorescences per branch, date 1.

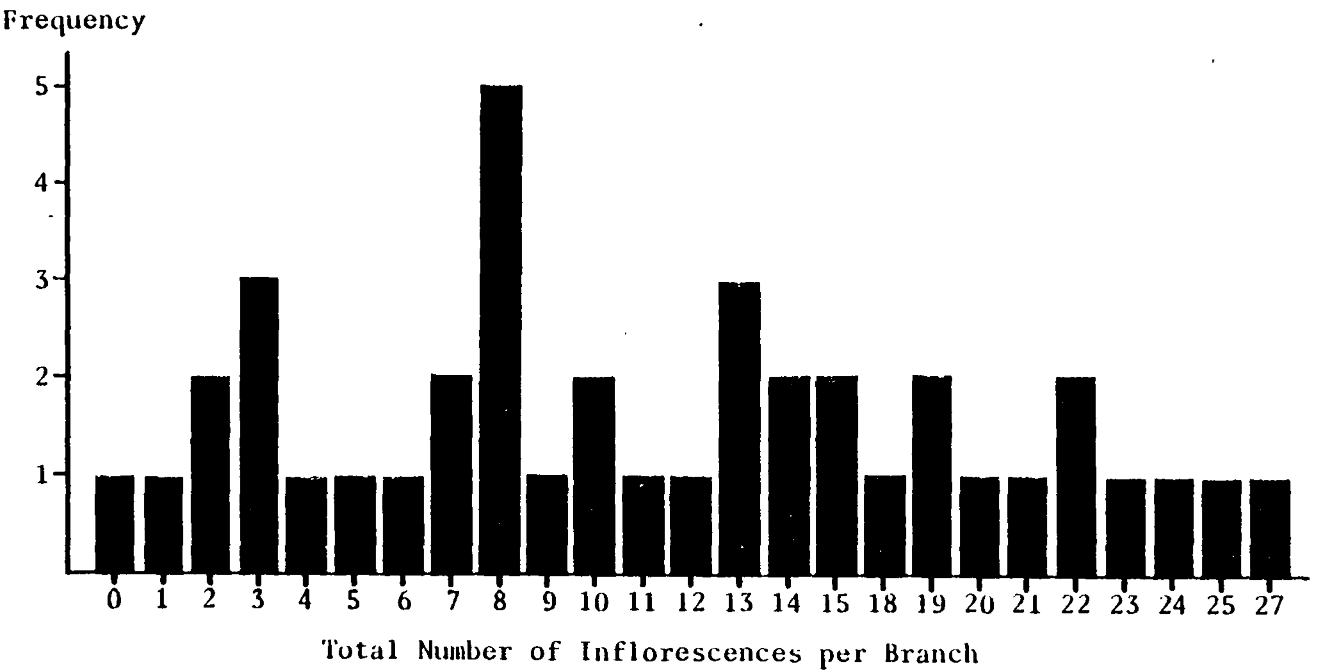

Figure 45. Frequency histogram for total number of inflorescences per branch, date 2.

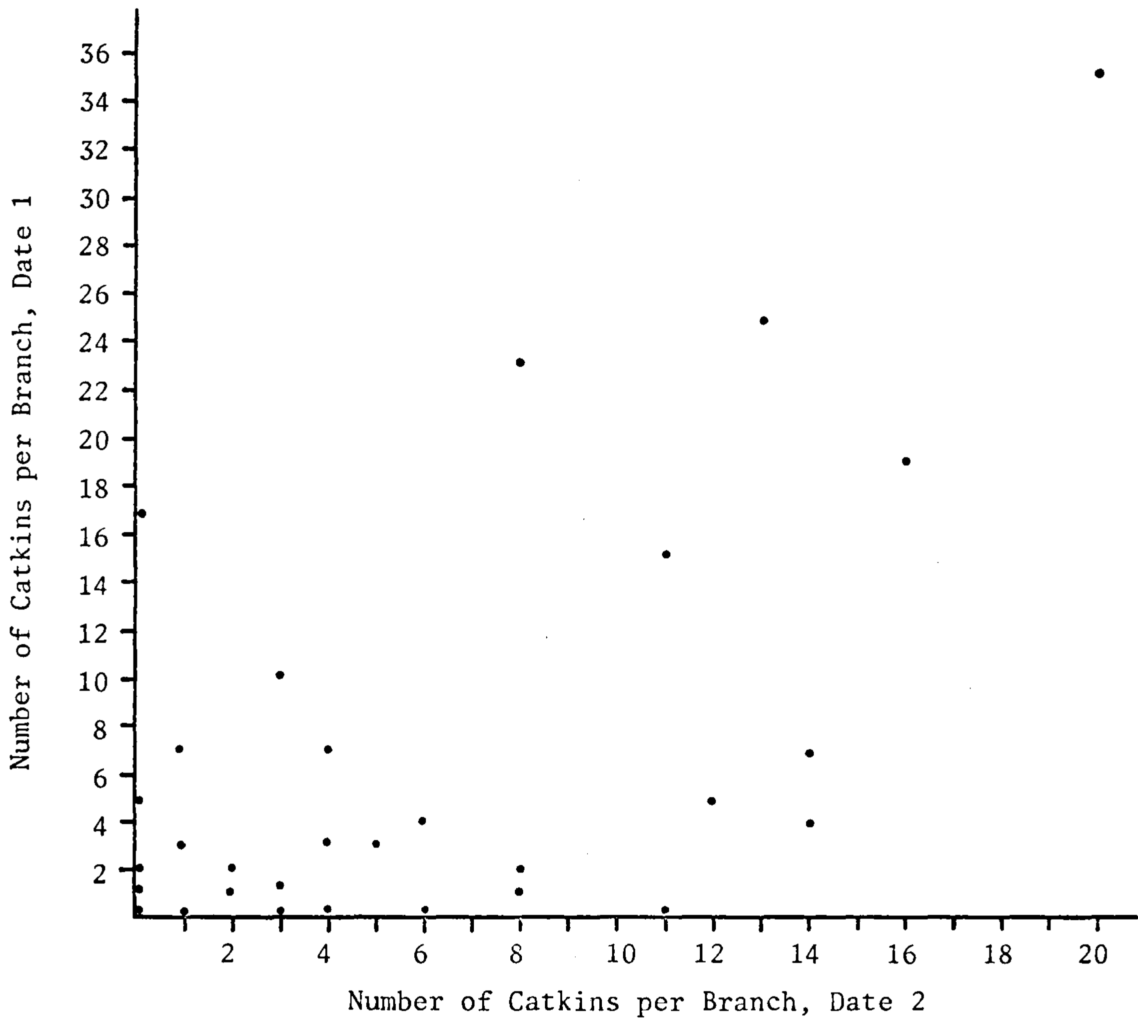

Figure 46. Plot of number of catkins (staminate inflorescences) per branch, date 1, by number of catkins per branch, date 2.

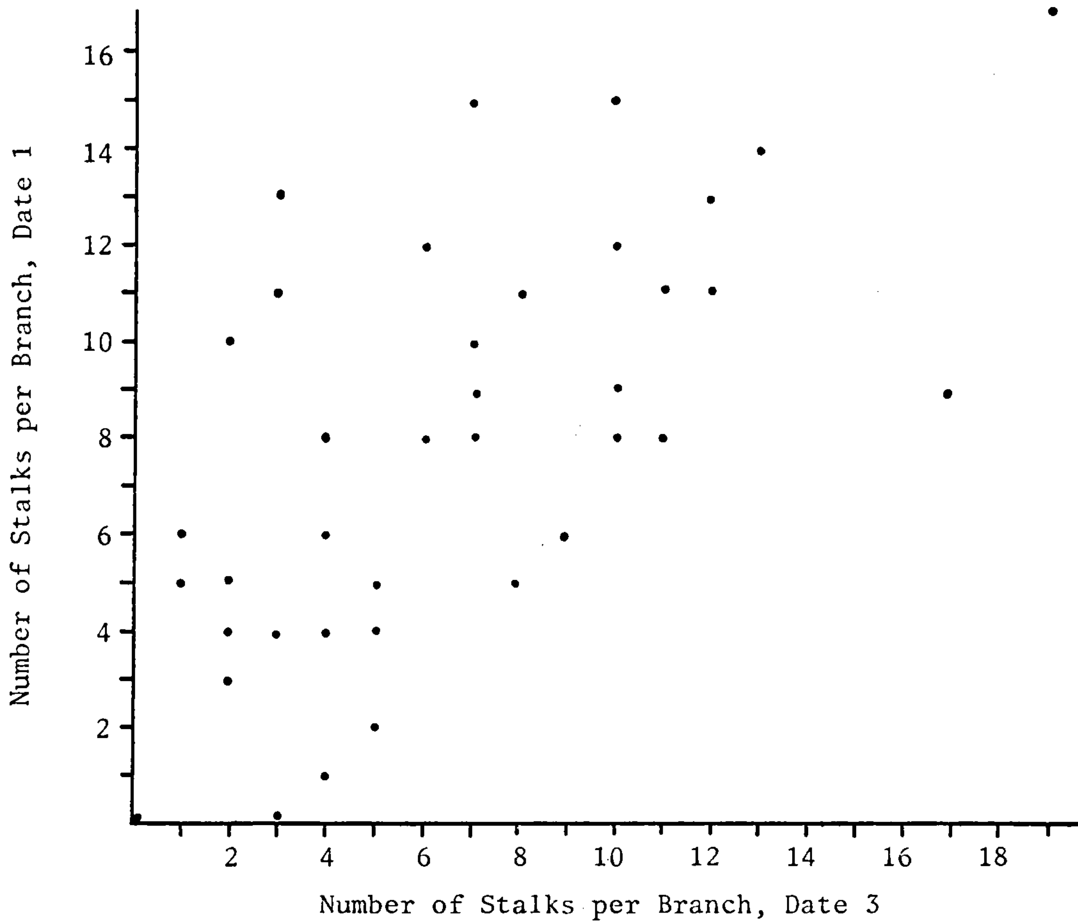

Figure 47. Plot of number of stalks (pistillate inflorescences) per branch, date 1, by number of stalks per branch, date 3.

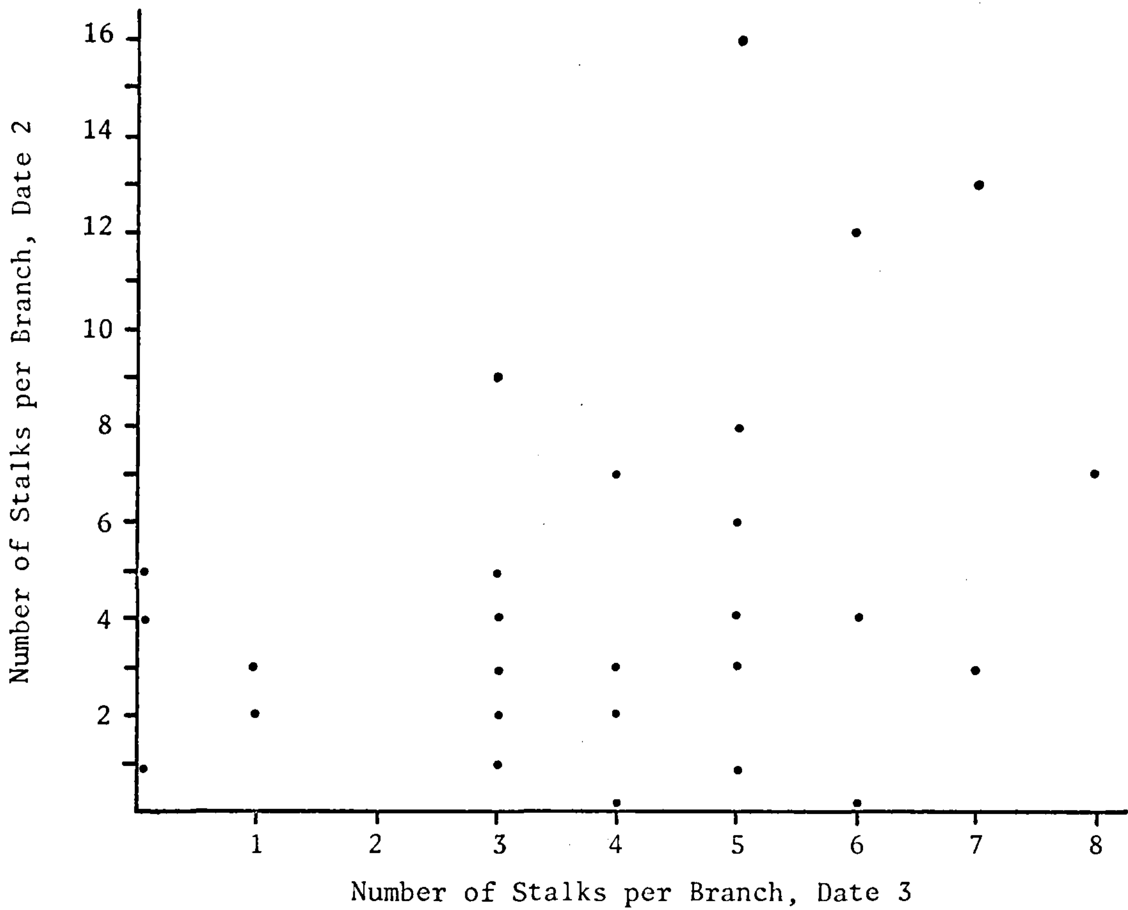

Figure 48. Plot of number of stalks (pistillate inflorescences) per branch, date 2, by number of stalks per branch, date 3.

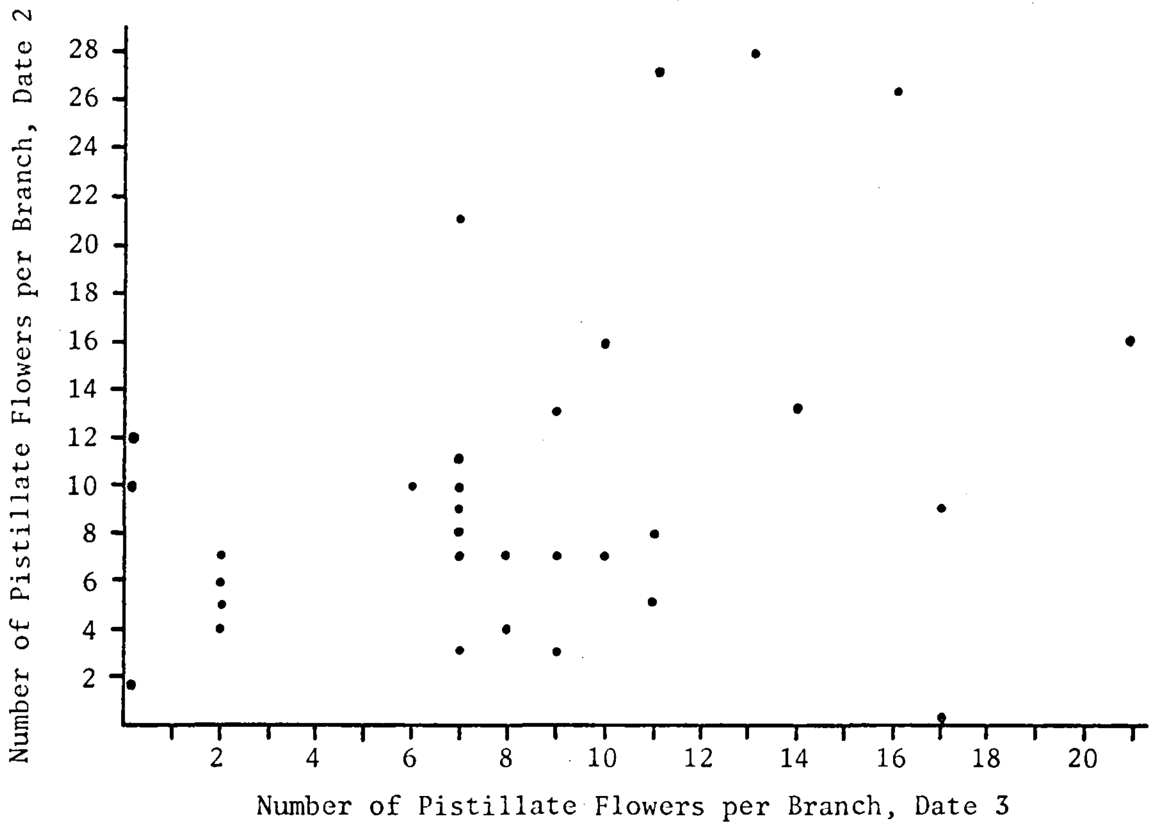

Figure 49. Plot of number of pistillate flowers per branch, date 2, by number of pistillate flowers per branch, date 3.

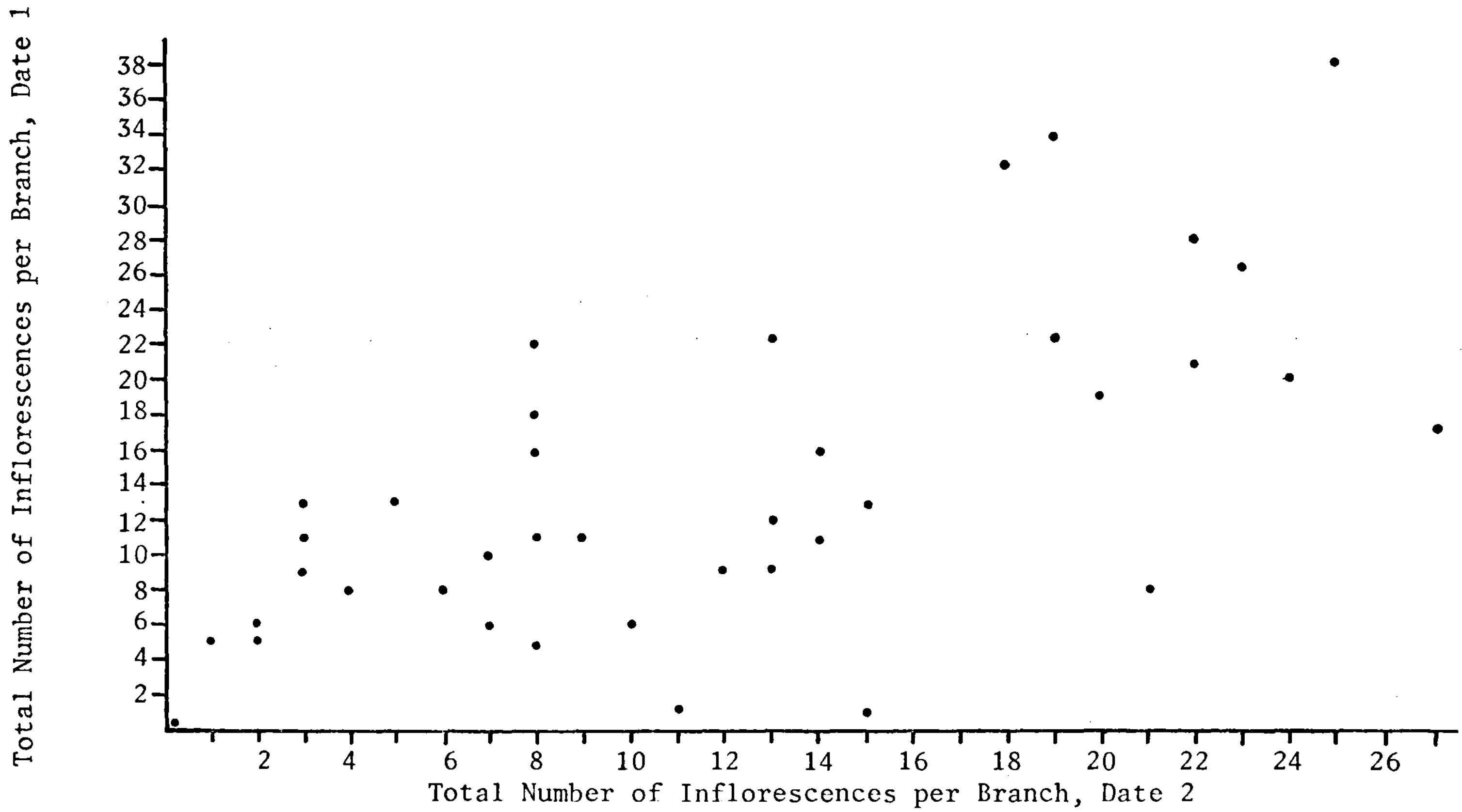

Figure 50. Plot of total number of inflorescences per branch, date 1, by total number of inflorescences per branch, date 2.

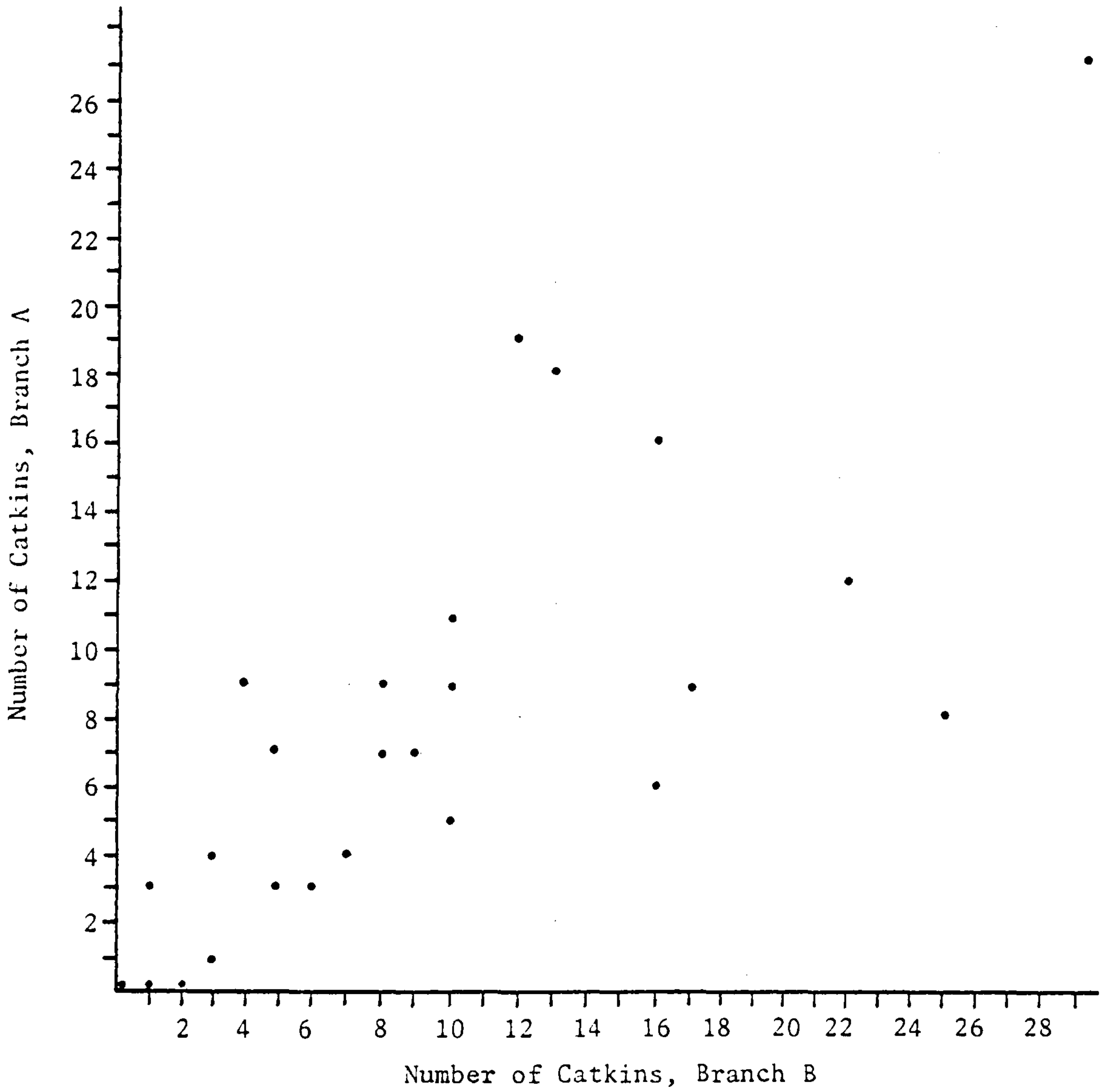

Figure 51. Plot of number of catkins (staminate inflorescences), branch A, by number of catkins, branch B.

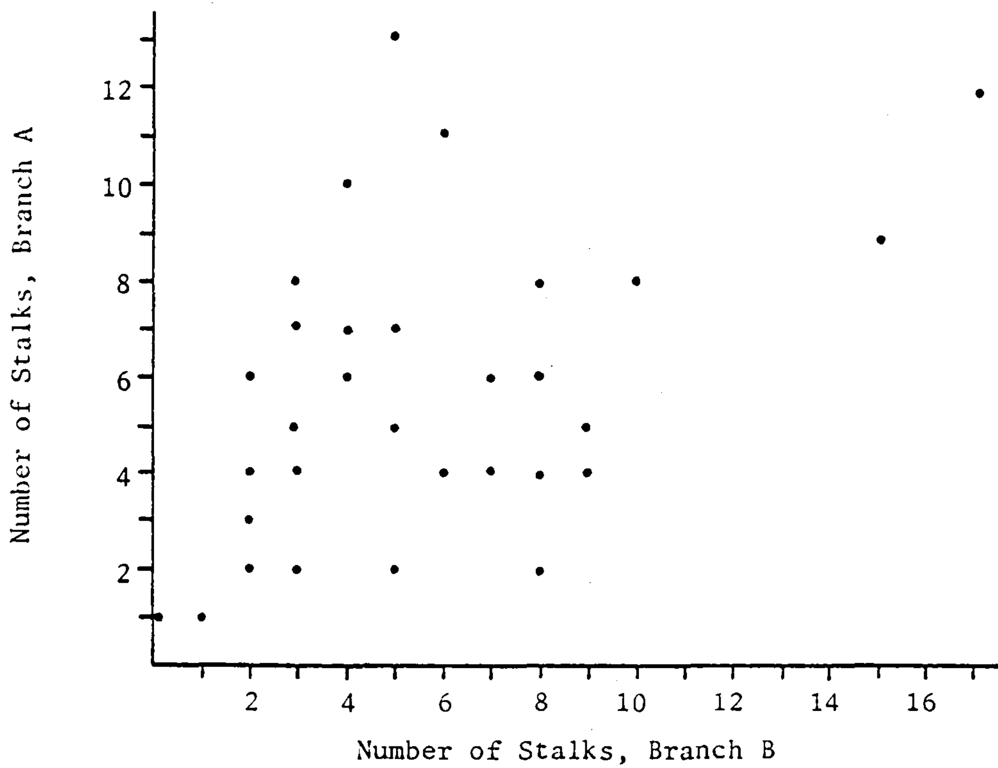

Figure 52. Plot of number of stalks (pistillate inflorescences), branch A, by number of stalks, branch B.

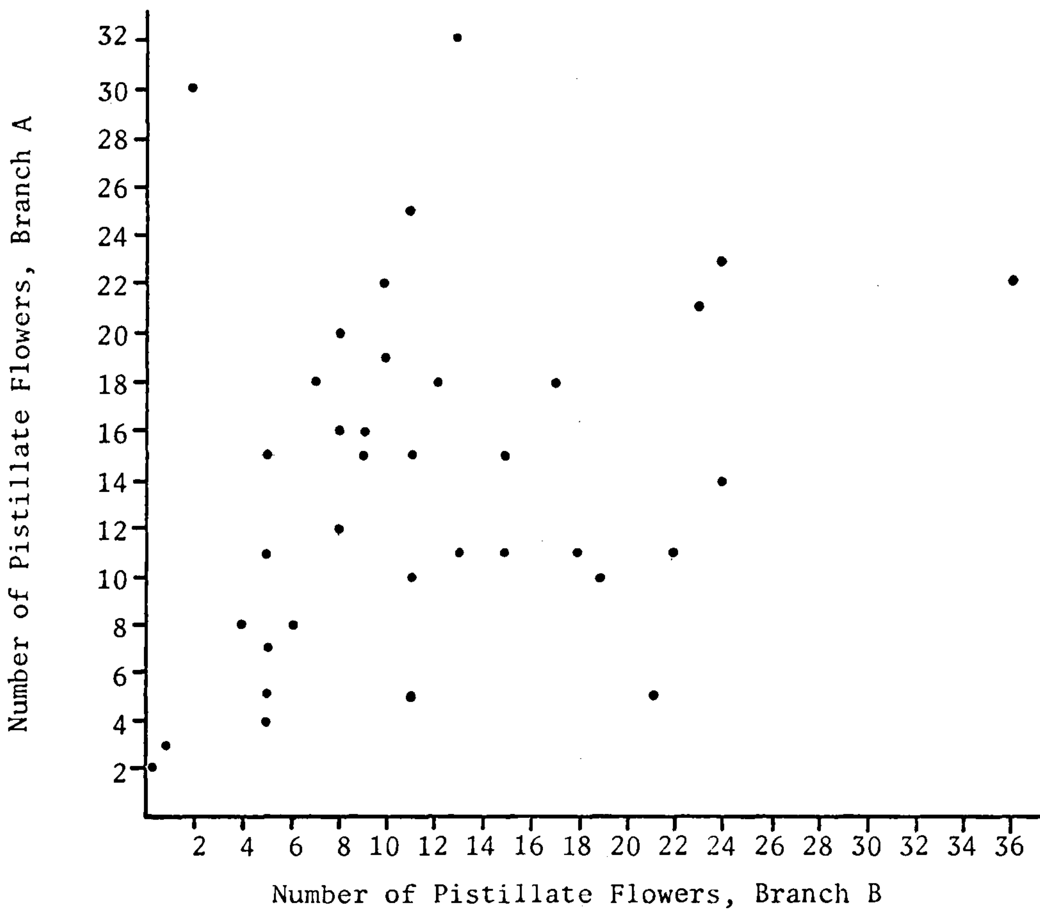

Figure 53. Plot of number of pistillate flowers, branch A, by number of pistillate flowers, branch B.

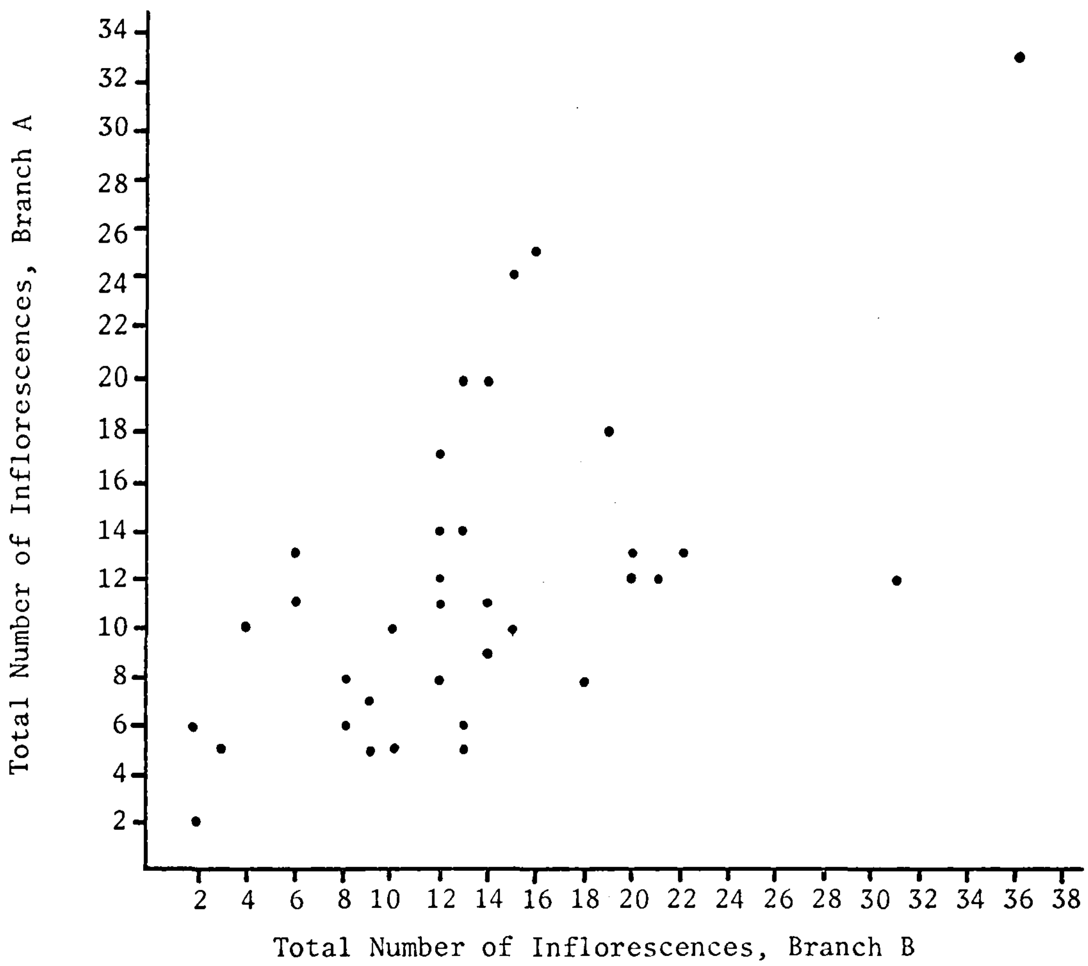

Figure 54. Plot of total number of inflorescences, branch A, by total number of inflorescences, branch B.

Table 1. Test tree dimensions and site characteristics.

Tree	DBH	Height	Site	
			Aspect	Slope Position
1	32.3 cm	18 m	SW	midslope
2	69.0 cm	18 m	SE	topslope
3	62.7 cm	21 m	SW	topslope
4	37.2 cm	18 m	NE	topslope

Table 2. Collection Dates for Study of Ontogeny of Floral Primordia.

1977 Collections	1978 Collections
April 18	March 21
May 4	April 6
May 27	
June 16	
June 29	
July 14	
July 28	
August 11	
August 24	
September 7	
September 21	
October 5	

Table 3. Dehydration and critical point drying schedules.

Dehydration Schedule	Critical Point Drying Schedule*
FAA	1st flushing, 3 minutes
50% ethanol, 1 hour	infiltration, 12 minutes
70% ethanol, 1 hour	2nd flushing, 3 minutes
85% ethanol, 1 hour	
95% ethanol, 1 hour	
100% ethanol, 1 hour	
100% ethanol, 1 hour	
100% ethanol, 1 hour	
100% acetone, 4 hours	
100% acetone, 4 hours	

*The transitional fluid used was liquid carbon dioxide (CO_2).

Table 4. Collection schedules for treatment branches and control branches of predictive study.

Date Designation	Calendar Date	Condition of Bud	Stage of Development of Inflorescence or Flower
Treatment Branch Counts:			
Staminate Inflorescences (Catkins):			
Date 1	March 20, 1978	Dormant	Catkins structurally mature, in bud conformation
Date 2	April 6, 1978	Budbreak	Catkins fully extended prior to anthesis
Pistillate Inflorescences (Stalks):			
Date 1	March 20, 1978	Dormant	Stalks structurally immature with few or no appendages
Date 2	April 6, 1978	Budbreak	Stalks elongating, producing individual pistillate flower primordia
Date 3	April 18, 1978	2 weeks subsequent to budbreak	Pistillate flowers receptive
Pistillate Flowers:			
Date 2	April 6, 1978	Budbreak	Pistillate flower primordia structurally immature
Date 3	April 18, 1978	2 weeks subsequent to budbreak	Pistillate flowers receptive

Table 4. Collection schedule for treatment branches and control branches of predictive study (continued).

Date Designation	Calendar Date	Condition of Bud	Stage of Development of Inflorescence or Flower
Total Inflorescences (Catkins + Stalks):			
Date 1	March 20, 1978 (both)	Dormant	Catkins structurally mature, in bud conformation; stalks structurally immature with few or no appendages
Date 2	April 6, 1978 (catkins)	Budbreak	Catkins fully extended prior to anthesis
Date 3	April 18, 1978 (stalks)	2 weeks subsequent to budbreak	Pistillate flowers receptive
Control Branch Counts:			
Date 3	April 18, 1978	2 weeks subsequent to budbreak	Catkins shedding pollen; pistillate flowers receptive

Table 5. Kolmogorov-Smirnov test for normality--Results for data collected on treatment branches for catkins (staminate inflorescences), stalks (pistillate inflorescences), pistillate flowers and total inflorescences (catkins + stalks).

Variable	Number of Observations	D-Max	Probability of Normality
Observations of 0 Included:			
Catkins, date 1	77	0.4471	.01
Catkins, date 2	77	0.1950	.01
Stalks, date 1	77	0.1808	.01
Stalks, date 2	77	0.3549	.01
Stalks, date 3	77	0.1574	.01
Pistillate flowers, date 2	77	0.2645	.01
Pistillate flowers, date 3	77	0.1483	.01
Total inflorescences, date 1	40	0.2243	.01
Total inflorescences, date 2	40	0.2011	.01
Observations of 0 Deleted:			
Catkins, date 1	52	0.4897	.01
Catkins, date 2	52	0.4637	.01
Stalks, date 1	52	0.4724	.01
Stalks, date 2	52	0.3568	.01
Stalks, date 3	52	0.1764	.01
Pistillate flowers, date 2	52	0.3292	.01
Pistillate flowers, date 3	52	0.1529	.01
Total inflorescences, date 1	39	0.2448	.01
Total inflorescences, date 2	39	0.2204	.01

Table 6. Kolmogorov-Smirnov test for normality--Results for data collected on control branches for catkins (staminate inflorescences), stalks (pistillate inflorescences), pistillate flowers, and total inflorescences (catkins + stalks).

Variable	Number of Observations	D-Max	Probability of Normality
Catkins, branch A	36	.1530	.05
Catkins, branch B	36	.2312	.01
Stalks, branch A	36	.1043	.20
Stalks, branch B	36	.1534	.05
Pistillate flowers, branch A	36	.1085	.20
Pistillate flowers, branch B	36	.1506	.05
Total inflorescences, branch A	36	.1728	.01
Total inflorescences, branch A	36	.2569	.01

Table 7. Wilcoxon Matched-Pairs Ranked-Signs test--Results for data collected on treatment branches and control branches for catkins (staminate inflorescences), stalks (pistillate inflorescences), pistillate flowers, and total inflorescences (catkins + stalks).

Variable Pair	Cases	Z Value	2-Tailed Probability*
Treatment Branches:			
Catkins, date 1 Catkins, date 2	22	-0.672	0.502
Stalks, date 1 Stalks, date 3	38	-1.998	0.046
Stalks, date 2 Stalks, date 3	32	-1.471	0.141
Pistillate flowers, date 2 Pistillate flowers, date 3	32	-1.275	0.202
Total inflorescences, date 1 Total inflorescences, date 2	39	-1.765	0.078
Control Branches:			
Catkins, branch A Catkins, branch B	30	-0.357	0.721
Stalks, branch A Stalks, branch B	35	-3.356	0.001
Pistillate flowers, branch A Pistillate flowers, branch B	35	-3.851	0.000
Total inflorescences, branch A Total inflorescences, branch B	36	-2.573	0.010

* Probability that means of distributions came from same population.

Table 8. Kendall Tau-B Correlation Coefficients for data collected from treatment branches and control branches for catkins (staminate inflorescences), stalks (pistillate inflorescences), pistillate flowers, and total inflorescences (catkins + stalks).

Variable Pair	Correlation
Treatment Branches:	
Catkins, date 1 Catkins, date 2	.4515
Catkins, date 1 Stalks, date 1	.0199
Catkins, date 2 Stalks, date 3	.2267
Stalks, date 1 Stalks, date 3	.4603
Stalks, date 2 Stalks, date 3	.2230
Pistillate flowers, date 2 Pistillate flowers, date 3	.1907
Total inflorescences, date 1 Total inflorescences, date 2	.4602
Control Branches:	
Catkins, branch A Catkins, branch B	.6411
Catkins, branch A Stalks, branch A	-.1590
Catkins, branch B Stalks, branch B	-.1350
Stalks, branch A Stalks, branch B	.3246
Pistillate flowers, branch A Pistillate flowers, branch B	.2512
Total inflorescences, branch A Total inflorescences, branch B	.4498

Table 9. Linear models for prediction of mature catkin (staminate inflorescence), stalk (pistillate inflorescence), pistillate flower, and total inflorescence (catkins + stalks) crops from treatment and control branch data.

Variable Pair($\binom{X}{Y}$)	Model	F Value	PR>F	R-Square
Treatment Branches:				
Catkins, date 1 Catkins, date 2	Y = .40X + 2.74	23.59	.0001	.3831
Stalks, date 1 Stalks, date 2	Y = .64X + 1.68	23.87	.0001	.3858
Stalks, date 2 Stalks, date 3	Y = .23X + 2.55	6.15	.0181	.1495
Pistillate flowers, date 2 Pistillate flowers, date 3	Y = .25X + 5.63	4.82	.0348	.1211
Total inflorescences, date 1 Total inflorescences, date 2	Y = .54X + 4.32	29.82	.0001	.4397
Control Branches:				
Catkins, branch A Catkins, branch B	Y = 1.07X + 3.25	10.67	.0025	.2389
Stalks, branch A Stalks, branch B	Y = .56X + 2.18	9.11	.0048	.2114
Pistillate flowers, branch A Pistillate flowers branch B	Y = .30X + 7.50	2.93	.0958	.0794
Total inflorescences, branch A Total inflorescences, branch B	Y = 1.20X + 1.03	12.26	.0013	.2650

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APPENDIX A

Development of Floral Primordia in White Oak (Quercus alba L.)

Progress Report--Methods Attempted to Date (May 1, 1978)

(Buds collected throughout the spring, summer and early fall of 1978 were fixed in FAA. Each collection was aspirated in the fixative for at least five minutes or until bubbling activity had for the most part subsided.)

1. Dehydrated whole buds in ethanol series (30%, 50%, 70%, 85%, 95%, 100%) for half-hour intervals, making three changes of absolute ethanol. The buds then went into 100% tert-butyl alcohol (TBA) overnight and were embedded in paraffin by making four changes of paraffin at four-hour intervals, until the TBA smell was gone, and were cast into a mold.
   Result--The buds crumbled and fell out of the paraffin when microtomed and made a scratching noise when they hit the blade, indicating that they were too hard.

2. Soaked whole buds in 3%, 5%, and 10% KOH solutions at room temperature for 3, 10 and 15 days. Then the buds were washed in running water overnight, and dehydrated and embedded as in #1.
   Result--Same as in #1.

3. Peeled bud scales off of buds while in FAA and proceeded with dehydration as in #1. After embedding, the blocks were microtomed down to the plant material and the blocks were allowed to soak in 5% and 10% KOH solutions for 2, 4, and 10 days before proceeding with microtoming.
   Result--Material separated from paraffin when microtomed. The problem at this point seemed to no longer be hardness but infiltration.

4. Peeled bud scales off of buds while in FAA and placed in 5% and 10% KOH solutions in 35°C oven for 2, 4, and 10 days. Then, the 2-day and 4-day soaked buds were rinsed, dehydrated and embedded as in #2.
   Result--Similar to #3, except some structures did not separate from the paraffin upon microtoming. However, these parts had been disorganized beyond recognition by the KOH treatment.

5. Dehydrating agent was changed to 2,2-dimethoxypropane (DMP). Peeled buds were rinsed with distilled water (3 changes in 30-minute period), dehydrated with acidified DMP (3 changes in 30-minute period), flooded with 100% TBA and embedded as in #1. The blocks were microtomed down to the plant material and soaked in 10% KOH solution as in #3 before proceeding with microtoming.
   Result--Material separated from paraffin when microtomed.

6. Peeled buds were soaked in 10% KOH solution for 7 days, rinsed in

distilled water, dehydrated in DMP-acetone-TBA, and embedded as in #5.

Result--Some sections stayed whole, but as in #4, the structures were mostly disintegrated by the KOH treatment.

7. Dehydrating procedure was changed again to sequence recommended in Berlyn and Miksche's Botanical Microtechnique and Cytochemistry (1976). From FAA, the peeled buds were flooded with 50% ethanol, run through a five-step gradient of mixtures of water, ethanol and TBA (4 hours per step), flooded with 100% TBA (3 changes of 4 hours each), and embedded as in #1. Blocks were microtomed down to the plant material and soaked for 24 hours in 10% KOH solution in a 35°C oven before microtoming.

   Result--Material separated from paraffin, even though in a few sections it seemed to slice cleanly, indicating as believed earlier, that the problem was no longer one of hardness of the material, but of infiltration.

8. Same dehydrating sequence was used as in #7, but buds in the first and last change of 100% TBA were placed under 20 inches of vacuum in a 58° vacuum oven for about 10 minutes. During paraffin infiltration, 20 inches of vacuum was applied for one hour at the beginning of each of four paraffin changes. The blocks were microtomed down to the plant material and soaked in 5% KOH solution for 24 and 48 hours in a 35°C oven before proceeding with microtoming.

   Result--Material separated from paraffin on microtoming, indicating that it still had not been properly infiltrated.

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DEVELOPMENT OF PRIMORDIA AND PREDICTION OF FLOWERING IN WHITE OAK (Quercus alba L.)

by

Scott A. Merkle

(ABSTRACT)

A two-part study was undertaken to gain a better understanding of oak floral development and flower crop variability as they relate to acorn crop variability. The first part traced the ontogeny of staminate and pistillate primordia of white oak (Quercus alba L.). It was found that staminate inflorescences are initiated in late spring and staminate flowers are structurally mature before the onset of dormancy in October. Pistillate inflorescences are not initiated until late summer, and pistillate flowers complete almost all of their development during the three weeks prior to anthesis. The second part of the study attempted to formulate predictive models for mature staminate and pistillate flower crop frequency distributions and densities by comparing the established phenology of floral differentiation with that of bud samples collected up to one month prior to flowering. It was found that the densities of staminate inflorescences (catkins), pistillate inflorescences (stalks) and total inflorescences are sufficiently predictable to make practical the use of linear models. However, because the pistillate inflorescence is so difficult to distinguish from the developing lateral bud, a large amount of error is generated in stalk counts completed one month before flowering. Also, the rapidity with which individual pistillate flowers are initiated just prior to pollination makes anticipation of their final number very difficult. The data suggests

that although the year to year production of mature flower crops by a particular white oak may be consistent, it is not necessarily innate.