CHAPTER 3

SEXUAL REPRODUCTION

The tree breeder must know typical or normal processes of flower and seed production so that he can understand variations in them. Exceptions to general rules may be serious limiting factors, or, conversely, the means whereby new approaches to solution of certain problems can be developed. For example, the southern pines in a natural forest are usually cross-pollinated and they are generally good seed producers. Certain trees, however, may be self-fertile, and the families of progeny may show either reduced vigor, no change in vigor, or increased vigor. Thus, in certain seed orchard clones the factor of self-ability might be a serious defect if low-vigor offspring were produced. On the other hand, if the selfed offspring were highly vigorous, the clone would be very desirable for seed orchards, and furthermore, it might prove valuable as breeding stock because a mating scheme involving selfing may be the most efficient method for solving certain breeding problems.

The southern pines in general are good seed producers, but individual trees may be very poor seed bearers, or they may produce very small numbers of one sex or the other. These latter trees may be very poor candidates for use in seed orchards or other types of breeding programs.

Controlled pollination is a highly specialized process requiring the utmost care throughout all the many stages from flower induction to the harvest of mature seed if viable seed in sufficient quantity are to be produced. Three growing seasons or nearly 3 years are needed to produce a seedling for progeny tests or commercial planting. One mistake at nearly any point will cause failure, which means another start, with a considerable loss in time.

Botanists classify the reproductive structures of the pines as *strobili* rather than flowers because of a difference in structure. However, the function of each is to reproduce the plant, and throughout this book the term *flower* will be used because it is more familiar.

Most of the principal characteristics of flowering in the southern pines are common to all species and apply universally in pollinating and seed production. Also, the insect and disease enemies seem widely distributed and attack species indiscriminately. However, certain exceptions to these general statements may become apparent when more is learned about the reproductive processes in the minor species of southern pines.

In addition to their good seed-producing characteristics the southern pines reach sexual maturity at an early age, but flowering is delayed in well-stocked stands. Male and female flowers are borne in separate structures on the same tree. Open-grown trees may have flowers of both sexes throughout the crown, although there is a tendency toward production of females in the upper crown and males in the lower part. Male cones may ripen before the female cones are receptive on the same tree but at the same time as female flowers on another tree. The result is more crossing among trees than within trees. Male flowers are produced at the base of twig buds, and the female flowers near the tip. Mature cones vary widely in size among species; those of large size, such as longleaf, slash, and loblolly pines, may contain more than a hundred seed, while the small cones of shortleaf, spruce, and sand pines usually have many less. Pollen is produced in very large amounts, each grain having two air sacs to facilitate wind transportation. The pollen is so durable it can with proper handling be stored several years or shipped long distances without loss in viability. Pollen grains have many similarities in structure among coniferous species (Hanover 1973).

LIFE HISTORY OF FLOWERS AND SEED

Because the pines are such a valuable and productive group of forest trees, their reproductive processes have attracted the attention of botanists for a long time. Some investigators carried out extensive studies of flower and seed production throughout the entire cycle by using material from one or a few species. Other researchers have concentrated on certain stages of the reproductive cycle.

Some outstanding work was done with pines by Ferguson (1904), who reported in detail on the sporogenesis, development of the gametophytes, and fertilization in white pine; in addition, nearly every observation was confirmed for five other species, one of which was pitch pine. Shortleaf pine and three other pine species were used by Buchholz (1918) in a study of the development of the embryo. Summaries of the work on pine reproductive processes were given by Coulter and Chamberlain (1917), Chamberlain (1935), and Konar and Oberoi (1969).

The most comprehensive work in the South was on longleaf pine (Mathews 1932); based on study specimens collected in North Carolina, 29 illustrations show microsporogenesis and development of the mature pollen grain, 7 show the germination of the pollen grain and development of the male gametophyte, 9 show development of the cellular female gametophyte, and 33 show development through fertilization. Later, reproduction in Virginia pine was studied intensively and found to be similar to that reported for other pines (Thomas 1951).

More recently, studies of the initiation and development of flower primordia in slash pine in Florida (Mergen and Koerting 1957) and pitch pine in Korea (Hong and Lee 1970) have given more insight in the various stages of flower development. In northern Florida male flowers become visible to the naked eye about the middle of October and the female flowers during December. Primordial tissue is formed much earlier.

Formation of the Staminate Cone

Slash pine twig buds collected in northern Florida during May and early June showed no evidence of primordia of staminate or male flowers (Mergen and Koerting 1957). However, in one of the buds collected on June 25 a swelling could be seen in the axil of the scalelike leaf that precedes the foliage leaves. Development of the staminate cone could be seen in the microscope in twigs collected after late June, and by mid-December the pollen sacs were filled with well-defined microspore mother-cells. Growth was slow during the coolest part of the winter, but in late January growth had been resumed and mature pollen grains were ready for release in February. After the pollen is shed, the catkin dries rapidly, soon falling from the twig. Development of the staminate cone in slash pine from summer through fall and winter is shown in figures 16 and 17, and development of pollen grain in figure 18.

In southern Mississippi, micro-climatic conditions and inherent differences among trees caused wide variation in development of shortleaf and loblolly pine pollen grains (Mergen *et al.* 1963). Normal pollen shedding during a 9-year period ranged from February 11 to 21 in 1957, and from March 19 to April 10 in 1958 for loblolly pine. For shortleaf pine, the range was March 19 to April 5 in 1957, and April 13 to 20 in 1958. Time of flowering is, of course, earlier in southern latitudes than farther north, with strong and consistent differences among species (Dorman and Barber 1956). Additional information on time of blooming is given later in this chapter in the section on age and time of flowering.

The presence of male flower buds in slash pine twig shoots may be indicated by a higher electrical potential than is found in vegetative shoots (Asher 1964).

When mature, the staminate or male catkins are smaller in shortleaf, pond, and spruce pines than in slash, longleaf, or loblolly pines (fig. 19). In all southern pines the cones are large enough to produce adequate amounts of pollen for controlled pollinations. The staminate cones range in color from yellow or greenish yellow in pond and loblolly pines to reddish purple in slash and longleaf pines.

Formation of the Pistillate Cone

The primordia of the female strobili or cone of slash pine in Florida were not observed until August, so this tissue is not formed as early as the male cone (Mergen and Koerting 1957). They were first noticeable as slight protuberances in the axis of scalelike leaf tissue near the tips of vegetative buds. Growth is continuous from this time on, the female flower becoming recognizable in December, or about 2 months after the male cones. Unlike the male cone, the female cone does not rest during the winter but continues growth until it is ready for pollination in late January or early February. Growth of the pistillate cone from September through November is shown in figures 17 and 20. Figure 21 shows December and January stages of development important in breeding.

The southern pines all flower in the spring season but on different dates. Although the date of blooming may vary from year to year, the various species always follow the same time sequence. Because the pines adapt themselves to weather conditions, male and female flowers mature early or late in accordance with spring temperatures. Low temperatures slow development of female flowers, while male flowers continue to develop. Also, the span of time during which female cones are initiated may be 2 months or more, as indicated by "debudding" studies (Eggler 1960). A predominance of female cones on the east-facing part of the crown were observed on open-growth slash pine trees in three stands in northern Florida (Smith and Stanley 1969).

Mechanism of Pollination

After the male flowers have matured and released pollen grains for wind dissemination, and after female flowers or cones are fully developed and the scales separated, the next step in the reproductive cycle is pollination. For our purposes *pollination* spans the time during which pollen enters the opening between the cone scales and comes to rest in a position suitable for pollen tube growth.

Details of the pollination process were observed by Doyle and O'Leary (1935) using Scots pine (*P. sylvestris*), and by McWilliam (1958) using slash pine and two additional species. The latter investigation concentrated on the role of the micropyle.

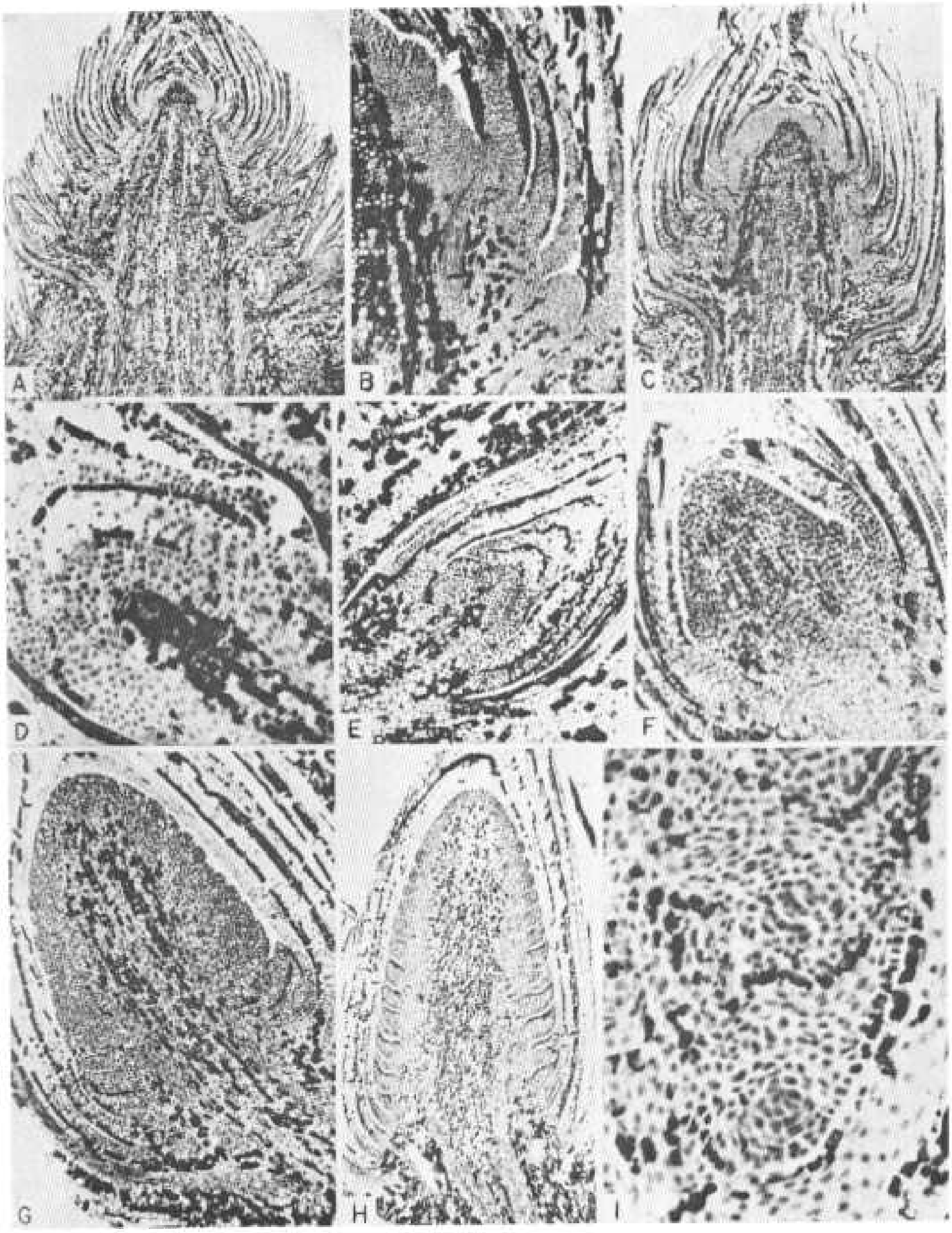

Figure 16.—Development of the staminate cone in slash pine.

A. Section through bud collected May 25 showing sterile series (7×).

B. Primordia (♂) in the axil of a cataphyll; July 12 (18×).

C. Section through bud collected July 19. Four ♂ flower primordia in various stages of development are visible (30×).

D. Vascular differentiation in ♂ primordia; August 9 (84×).

E. Protective hood formed by the scales over the developing ♂ primordia; September 13 (38×).

F. Hood scale primordia forming at base of developing ♂ ament; September 13 (53×).

G. Initiation of rudimentary microsporophylls on ♂ ament; October 11 (31×).

H. Differentiation of microsporophylls; October 25 (34×).

I. Sporogenous tissue in basal part of microsporophyll; November 1 (120×).

(Mergen and Koerting 1957)

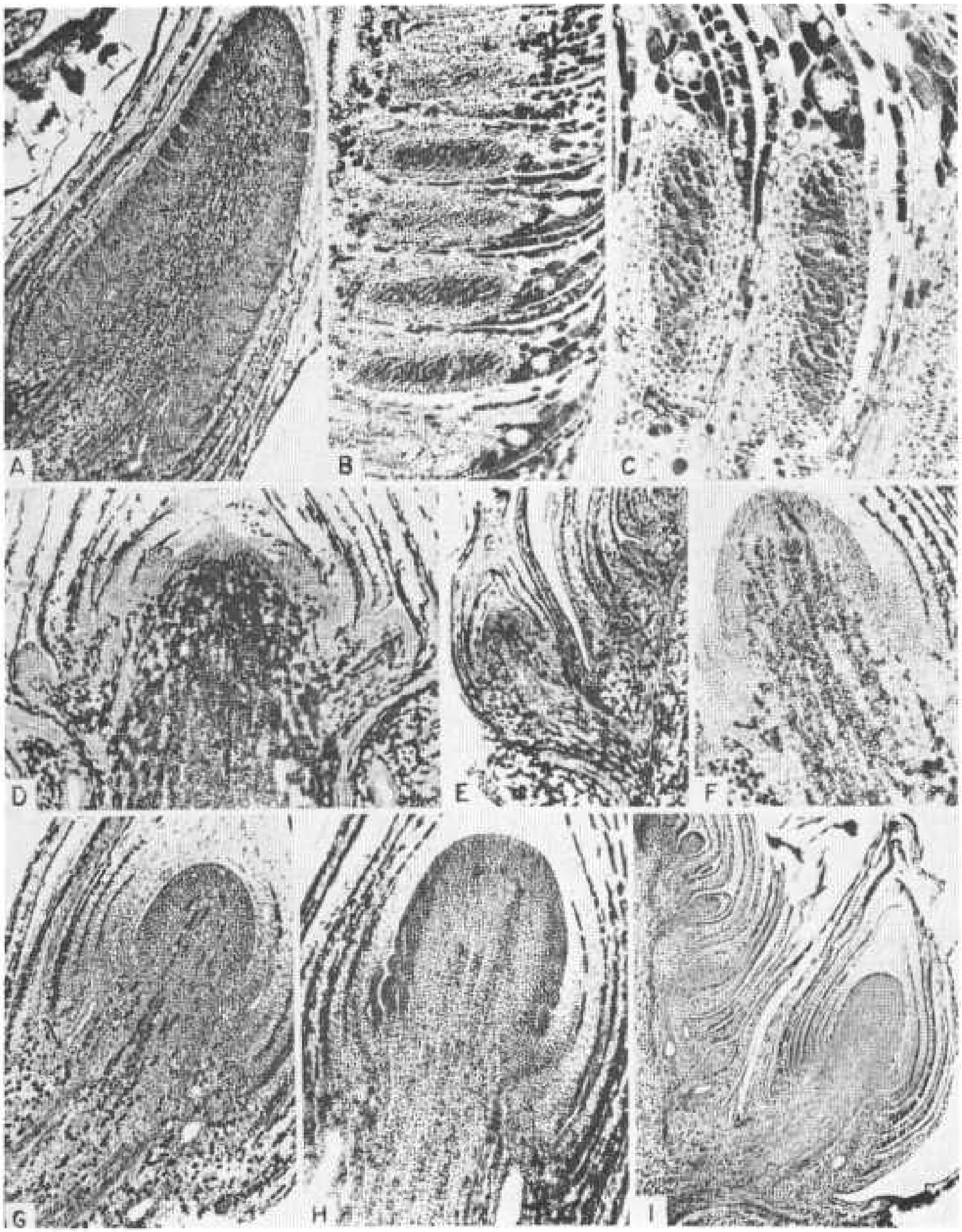

Figure 17.—Development of staminate and pistillate cone in slash pine.

A. Male ament collected November 1 (24×).

B. Microsporophylls in ament collected December 14 (41×).

C. Microsporophylls containing microspore mother-cells; December 14 (45×).

D. Primordia of ♀ flower on bud collected September 17 (26×).

E. Pistillate strobilus enveloped with hood scales; October 4 (15×).

F. Differentiation of vascular tissue in midrib area of ♀ strobilus; October 18 (32×).

G. Vegetative stalk on ♀ flower; November 1 (32×).

H. Initiation of bract scale primordia; November 15 (35×).

I. Condition of ♀ flower collected November 22. Details of vegetative bud are plainly visible (10×).

(Mergen and Koerting 1957)

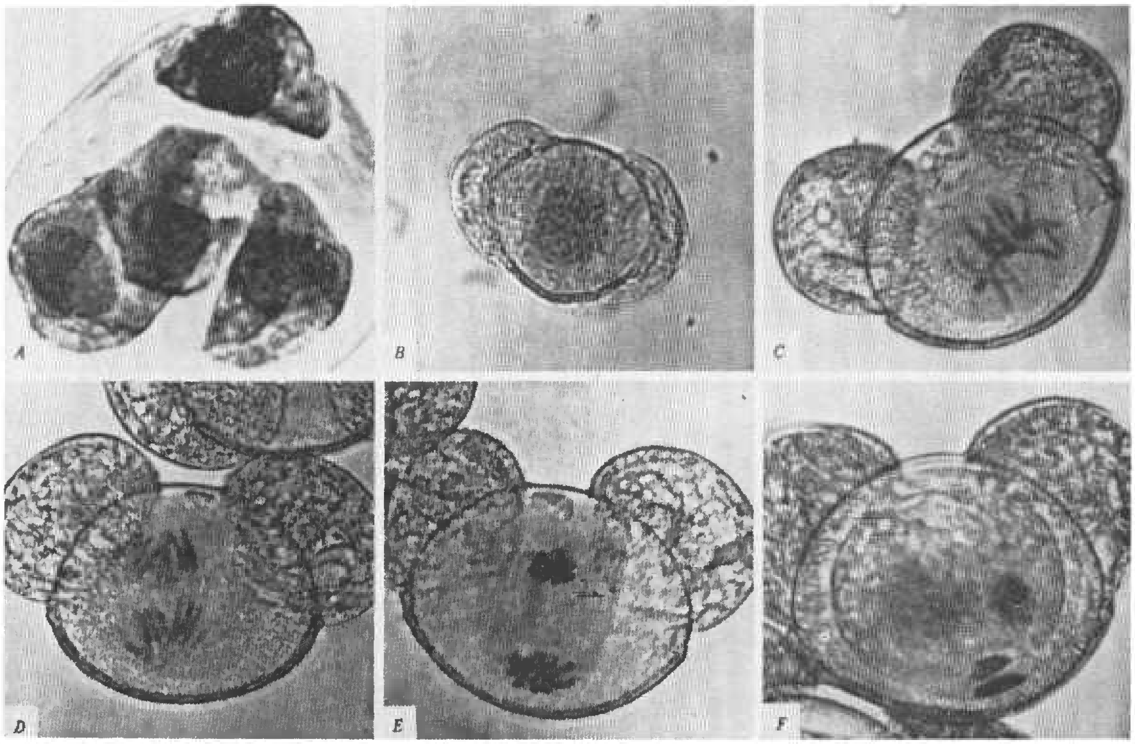

Figure 18.—Cytology of microsporogenesis in shortleaf pine.
A. Quartet of pollen grains contained within the walls of microspore mother-cell (MMC).
B. Microspore after release from MMC.
C, D, E. Metaphase, anaphase, and telophase of first vegetative division.
F. Mature pollen grain following second vegetative division.
(Mergen *et al.* 1963)

The ovule in pines is located on the upper surface of a cone scale, with the opening or micropyle facing the cone axis in a downward position. The surface of the tissue near the opening of the micropyle is covered with a sticky film to which pollen grains adhere (Doyle and O'Leary 1935). When mature, the ovules secrete a fluid that fills the opening of the micropyle, and after pollen grains are enclosed, the fluid is withdrawn until the grains rest against the nucellus. Next, the opening in the micropyle closes and seals in the pollen grains, thus completing the pollination process.

Inasmuch as pollination of unbagged flowers with pollen of a different species and with pollen of the same tree (selfing) have both been successful, it is evident that the process is not discriminatory as far as type of pine pollen is concerned (Wakeley *et al.* 1966; Squillace and Kraus 1963).

Development of the Seed

This stage in the life history of southern pines begins with the germination of the pollen grain on the nucellus, continuing through fertilization of the egg and development of the ovule into a seed. These stages are illustrated and discussed in detail in such papers as those by Ferguson (1904), Chamberlain (1935), and Mathews (1932). During the year following pollination the pollen tube grows slowly downward through the tissue of the nucellus, and the egg cell is also developing so that fertilization can take place. The zygote formed by the union of sperm and egg then completes its development and the ovule becomes a seed. The seed has a hard outer layer, a wing, and contains the storage tissue. The seed and the mature cone in which it is produced ripen during the second fall after pollination takes place. Seed properly dried, sealed in containers, and stored at cool temperatures may remain viable for many years—germination of slash pine seed was 70 percent and shortleaf pine seed 23 percent after 40 years (Barnett 1972). Seed for controls may be stored and used for successive generations in progeny tests.

Pine seed usually has one embryo and produces one seedling, although more than one embryo in the early stages of seed development is common since if

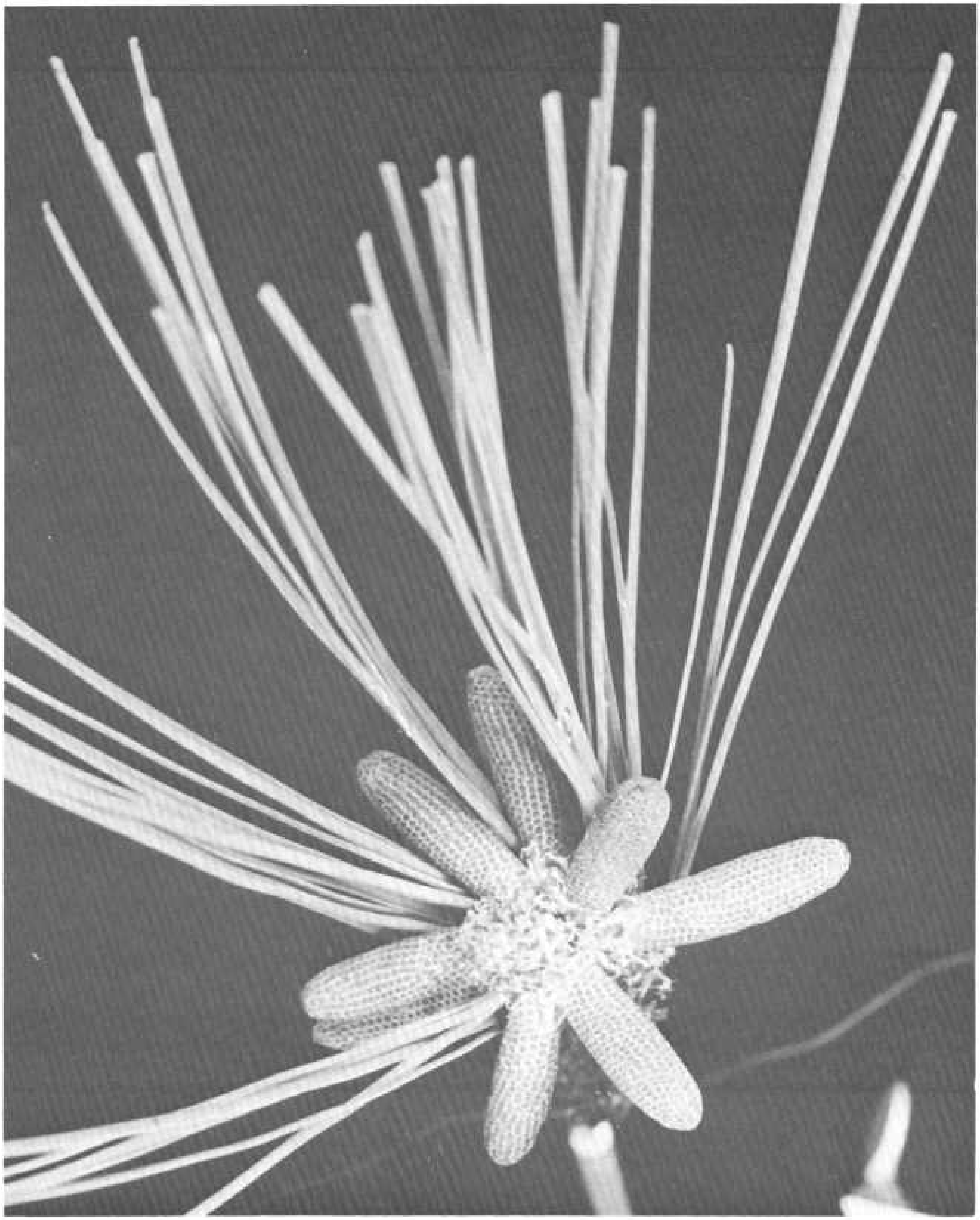

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Figure 19.—Normal staminate or male flowers of southern pines are borne in clusters at the base of vegetative buds, as shown for slash pine. In the spring new growth of the twig takes place above the flowers.

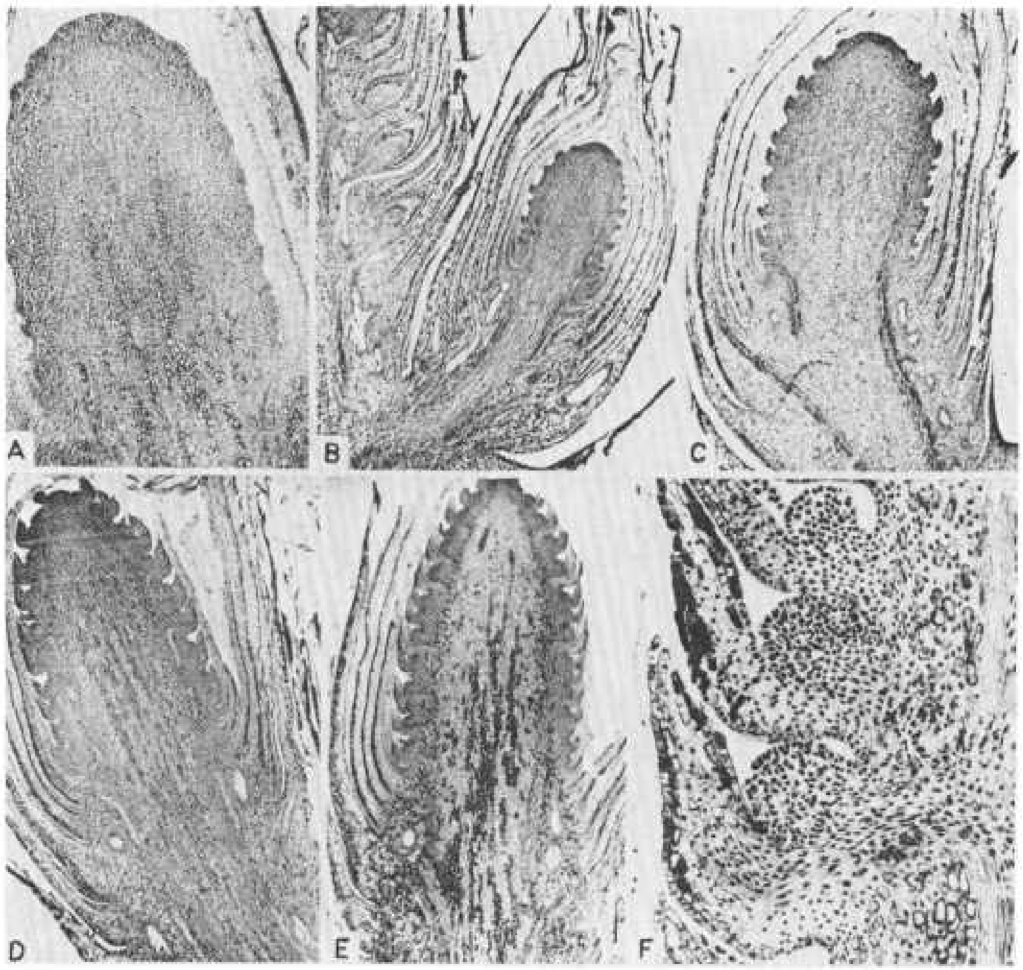

Figure 20.—Initiation and development of ovuliferous scale in slash pine.
A. Rudimentary ♀ strobilus covered with bract scale primordia; November 29 (25×).
B. Development of the bract scales; December 22 (9×).
C. Initiation of ovuliferous scale in axil of bracts; December 30 (11×).
D and E. Further differentiation and growth of ♀ strobili; December 30 (11×).
F. Well-formed bract scales subtending the development of ovuliferous scales which contain the ovular initials; January 6 (72×).
(Mergen and Koerting 1957)

more than one egg in a gametophyte is fertilized, one embryo may develop from each. This is called simple polyembryony (Chamberlain 1935). Another type, cleavage polyembryony, refers to those embryos that come from a single egg by splitting after a single fertilization. The characteristics of seedlings from polyembryonic seed will be discussed later in this chapter in the section on eccentric flowering.

The seed of various southern pine species show minor differences but the basic structure is similar. Seed of shortleaf and loblolly pines usually have a high degree of dormancy, while those of slash and longleaf pines do not. Seed of longleaf pine has a rather soft coat that is easily cracked during handling, in contrast with a very hard seedcoat in other species. Also, the wing of longleaf pine seed does not naturally dehisce as do the wings on seed of other species.

Southern pines reproduce themselves by an interesting but intricate process. It is remarkable how the highly specialized flower parts are initiated and developed on separate parts of the same tree, or on different trees, and brought to maturity. Wind, a prime agent in transporting pollen, is, of course, not controlled by the researcher. On the advantage side, wind pollination of pines is a vast and dependable natural force, much more dependable than the insect pollination or water-borne pollination of certain other plants. It insures pollination in a seed orchard. On the disadvantage side, it also brings contamination.

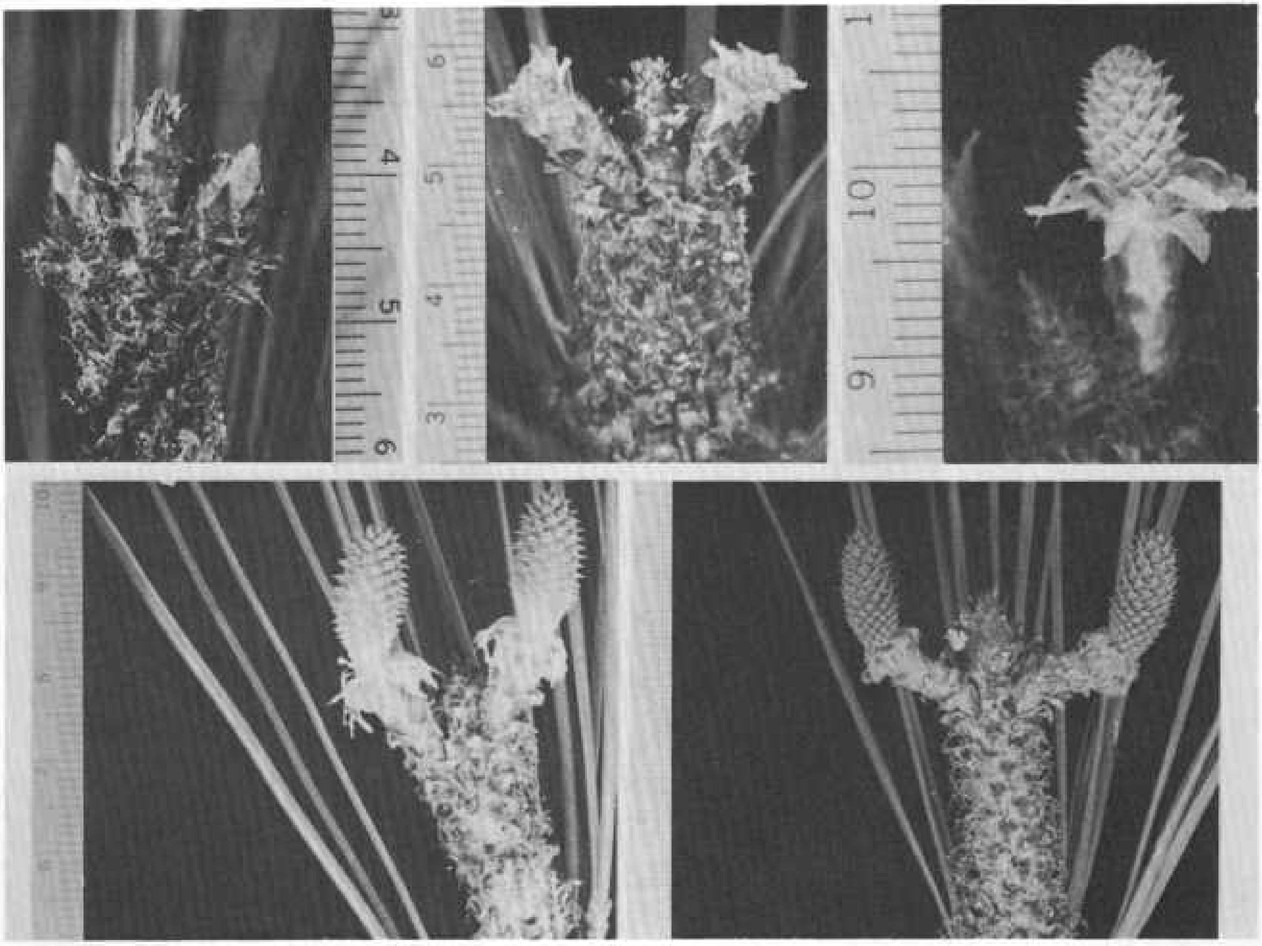

Figure 21.—Stages of development of ♀ strobili on slash pine used as criteria in breeding.
Stage I, good time to bag for controlled breeding.
Stage II, latest stage at which bagging should be done.
Stage III, about the optimum development for pollination.
Stage IV, bags may safely be removed (lower photos).

Many additional details of the flowering process are given in the references cited in this chapter. The process has been carefully studied and there are no gaps in our knowledge to severely limit tree breeding activities, but worrisome questions do exist, such as why is some loblolly and shortleaf seed highly dormant though some is not.

Differences in flowering or seed characteristics in different parts of the natural range, and thus in different environments and among trees in stands or in the same environment, are summarized in *Part III, Variation Among Geographic Locations,* and *Part IV, Variation Among Trees.*

SELECTIVE FERTILIZATION

Selective fertilization might be defined as the preference indicated in flowering parts for pollen from a specific source. The mechanics of the processes involved in the reception or rejection of pollen have been the subject of study for some time. In his monograph on selective fertilization, Jones (1928) pointed out that self-fertility, dioecism, dichogomy, hetero-styly, and other floral devices have evolved in favor of cross-fertilization to bring about germinal mixing. The reverse tendency, that of self-potency, occurs in a few tree species and is also insured in various ways.

The mature pine embryo is the result of six or more natural selections made during the course of development (Stockwell 1939). Some are chance selections, others are competitive and reflect inherent vigor or genetic superiority. By this series of selections, species hybrids and inherently weak or partly compatible forms are eliminated, thus maintaining the vigor and uniformity of pine stands.

In pine species hybrids, incompatibility has been attributed to eccentric growth of pollen tubes, which results in failure to reach the egg in time to affect fertilization (Stockwell 1939; Buchholz 1944). In several pine crosses, among which were slash × Austrian pine, red pine × pitch pine, and Austrian pine × pitch pine, the causes of incompatibility appeared to be related to the inability of the pollen tubes to function normally in the nucellar tissue of a foreign species (McWilliam 1959a, 1959b).

In Korea, pollen tube growth varied among interspecies crosses but in certain crosses such as pitch × loblolly pine it was normal (Hyun and Yim 1963).

On the basis of studies of the carbohydrate contents of the pollen of 20 pine species, Chira and Berta (1965) suggested that interspecific differences in the type of sugar present, and the effect of these sugars in either inhibiting or stimulating penetration of the pollen tube during germination, may be one of the reasons for the incompatibility of some species. The flavonoid fraction and the carbonate soluble material from ether extractives but not the phenolic acids or coumarate esters varied in the pollen of loblolly, slash, shortleaf, longleaf, eastern white, red (*P. resinosa*), jack (*P. banksiana*), and ponderosa (*P. ponderosa*) pines (Strohl and Seikel 1965).

The part that selective fertilization plays in promoting outcrossing among trees within species of southern pines is not known. Self-fertility occurs, but it varies among trees, ranging in one study among seven trees from 4 to 36 percent when expressed as percentage yield of seedlings from self-pollinations versus outcrosses (Kraus and Squillace 1964b). Furthermore, the vigor of progeny from selfing varies among parent trees in both loblolly and slash pine (Greene *et al.* 1964; Nikles 1966; and Franklin 1969b).

The possibility for using selective fertilization to promote crosses among seed orchard clones while excluding crosses from foreign pollen has not been investigated. From a review of the literature on selective fertilization in plants, Mirjuta (1967) concluded that it should be possible to select lines that are almost exclusively fertilized by pollen from other varieties or lines even when self pollen is present.

Mass pollination with slash pine pollen of unbagged female flowers on 10 shortleaf pine resulted in an average of 10.7 percent hybrid seedlings, but the range among the individual trees involved was from 1.2 percent to 21.5 percent hybrids (Wakeley *et al.* 1966). The mass-pollinated cones yielded an average of 13.2 full seeds, while control cones pollinated with the same pollen mix averaged only 6.6. No reason was discovered why some shortleaf pine trees produce hybrids with slash pine and some do not.

ECCENTRIC FLOWERING

Vitally concerned with flower production and seed development in pines, the tree breeder and forest geneticist note any deviation from the normal. They may wish either to take advantage of an aberrant condition or to avoid it in genetic studies or seed production.

In general, the southern pines have both male and female flowers on the same tree. The male flowers are borne in a cluster at the base of the twig bud, and the female flowers in a group of one or a few cones at the apex of the bud. Individual twigs may have flowers of both sexes but usually they occur on separate branches. As stated, a seed usually produces only one seedling. There are, as in most genetic phenomena, exceptions to these patterns.

One of the earliest reports of abnormal flowers described androgynous or bisexual conelets on slash pine (Mohr 1897). In Texas, bisexual flowers were observed on loblolly and longleaf pines. The flowers were staminate at the base and pistillate at the end (Zobel and Goddard 1954). On certain twigs all catkins were abnormal. A cluster of flowers of this type is shown in figure 22 (Ruark and Jones 1966).

Pollen catkins of a longleaf pine that were normal as far as numbers and position on the twig are concerned all had swellings and stubby divisions near the tips (fig. 23).

A cluster of sand pine cones was found on two trees in an 8-year-old plantation (Burns and McReynolds 1966). One occurred on the main stem 3 feet below the terminal bud and the other on a lateral branch. One cluster had 58 cones, the other 28 cones. The few seed that could be obtained from the large cluster were nearly all empty, but of 125 seed from the small cluster 51 were sound and 44 of those germinated. It is not known whether these clusters, which were abnormal in number of cones, originated as normal pistillate flowers or as bisexual flowers. On slash pine the author has observed a cluster of pistillate flowers, abnormally large in number, at the base of the twig instead of at the tip.

In Texas, on loblolly pine, numerous twigs were observed which produced normal conelets and then after normal vegetative growth an additional set of conelets (Doak 1935). Production of conelets at intervals during the growing period in one season has also been observed by the author on sand and South Florida slash pines. A twig of *P. caribaea* with three whorls of normal year-old cones and female cones at pollination were photographed by Little and Dorman (1954).

Figure 22.—Bisexual shortleaf pine flowers with male at base and female at top. (Ruark and Jones 1966)

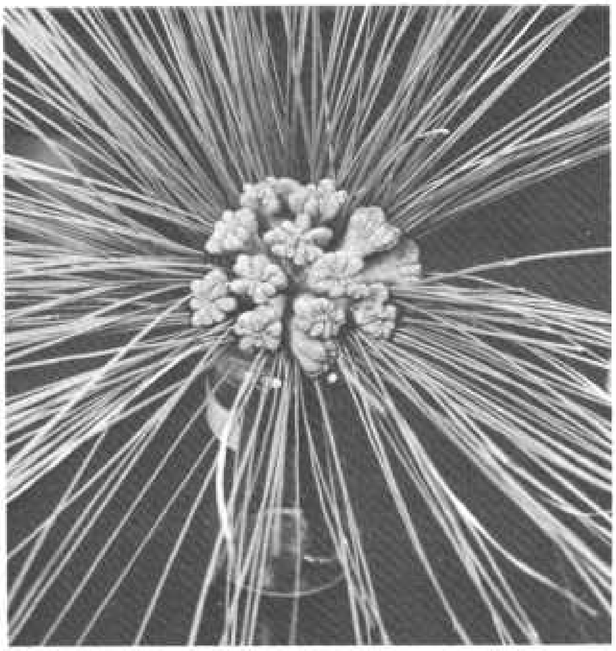

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Figure 23.—Longleaf pine staminate cones with deformed tips.

Abnormalities have been observed in seed and cones. Polyembryony, often characterized by two or more embryos in a single seed, was noted in loblolly, slash, and shortleaf pines (Nelson 1941). One seedling would often be large and apparently normal, the other quite small and imperfectly developed. Twin embryos are usually oriented in the same direction in the seed, and the root tip or radicles emerge from the seed at the same point. However, at rare intervals the embryos are inverted (fig. 24). The two types of polyembryony in conifers (Chamberlain 1935) were described in the section of this chapter on development of the seed.

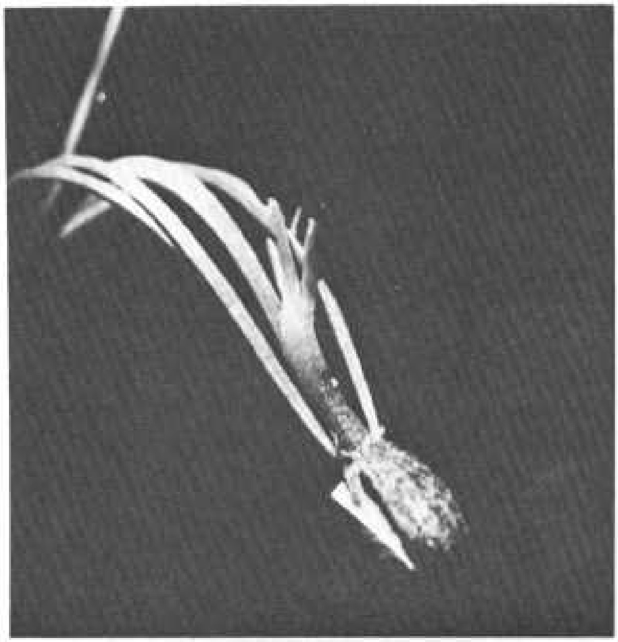

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Figure 24.—Twin inverted embryo in loblolly pine seed. Multiple embryos are usually oriented in the same direction.

Several types of albino seedlings have been found in slash pine (Squillace and Kraus 1963), and seedlings with multiple sets of chromosomes or polyploids (Mergen 1958b). Mutant forms of loblolly pine seedlings have been disclosed by self-pollination (Franklin 1969c).

In Louisiana, a longleaf pine cone produced after artificial pollination contained no seed, either filled or empty (Wakeley and Campbell 1960). Sometimes pistillate cones proliferate and a normal branch grows from the tip (fig. 25). This type of abnormality occurs in other conifer genera, with branches capable of producing normal flowers (Chamberlain 1935).

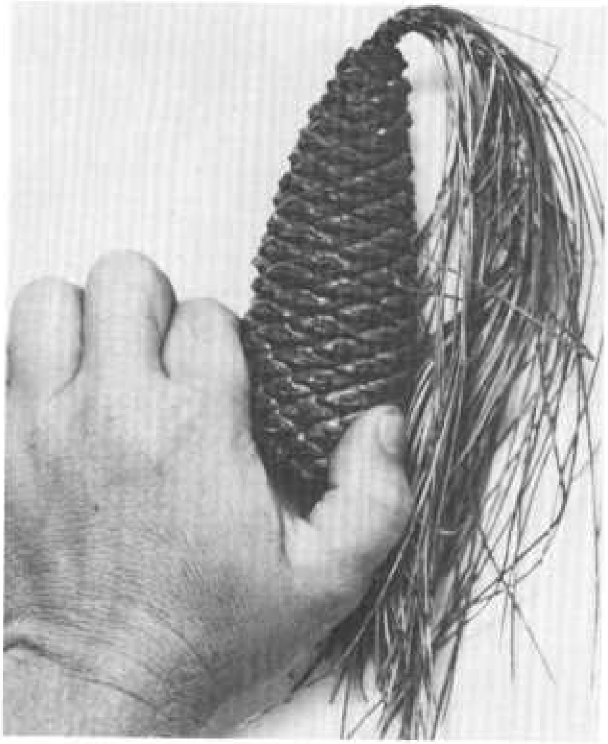


Photo courtesy of Georgia Forestry Commission

Figure 25.—Proliferated slash pine cone with normal twig growing from the tip.

The occurrence of abnormal flowers such as the bisexual cone was believed by Thompson (1940) to indicate the pathway by which present flowering parts evolved. This is pertinent if the female parts are considered to be more advanced than the male and separate male and female flowers more advanced than a single flower. Thompson (1940, p. 83) explained it this way:

> In the male cone where the requisite intermediate degree of specialization is more often found than in the female, the trend is of more general occurrence and more clearly expressed than in the female cone. This trend is exceptionally clear in the common type of bisexual cone in which the more primitive (male) sporophylls are borne at the base and the more specialized (female) above, an explanation which is equally applicable to the angiosperm flower. In the basal region of the female part of such cones the sporophylls may bear ovules on the lower surface, a reversion to their original position as indicated by the normal location of the pollen sacs of the more primitive male sporophylls. In other cases the ovules in this region may be laterally located, as in cycads, and so show partial attainment of their normal location in the upper part of the cone. A similar primitive location of ovules may occur in the case of sporophylls from the base of wholly female cones.

The position of ovules in bisexual flowers of southern pines does not appear to have been observed.

Abnormalities occur in the development of flowers and seed of the southern pines but the occurrences are rare and present no obstacles in breeding work. The atypical conditions were largely unknown until controlled pollinations and seed research programs were begun and close attention paid to plant material. They occur also in seedling characteristics, tree form, and oleoresin color. Additional inheritance studies will be needed to show whether these are mutations or arise from some other cause such as environmental factors.

AGE AT FLOWER PRODUCTION

Inheritance studies of traits such as oleoresin yield and resistance to drought and pests that can be evaluated in seedlings are important in the southern pines. Thus, length of time needed to produce and test each generation, which is controlled by the age at sexual maturity, strongly influences progress. Age at which flowering production begins in seedlings and grafted plants is vital in seed orchard design and culture.

In New Jersey, among 400 pitch pine seedlings, one produced 33 staminate strobili 22 months after the seed was planted, and two seedlings produced one or two ovulate strobili each at 24 months after planting (Andresen 1957). Mature cones were fully formed at the end of the fourth growing season. Germinative energy for the seed of two cones was 52 percent at the end of 9 days. In Mississippi, Namkoong (1960) observed a total of four ovulate strobili on 3 out of 20 potted seedlings 12 months from seed. The seed was from a controlled cross of pitch pine trees of New Jersey origin.

In Georgia, near the northern extension of the range of loblolly pine, Greene and Porterfield (1962) observed wildling seedlings 3 and 4 years old bearing ovulate strobili. Greene (1966a) found loblolly cones produced at 4 years from seed on trees in 12 different progenies. Most trees had one to three cones, although one had seven. Number of seed was 24 to 109 per cone from wind-pollination. Some of these early-flowering loblolly seedlings were in open-pollinated progeny and others in control-pollinated progeny. Schmidtling (1971) reports sexually mature trees 3 to 4 years in the field bore both male or female flowers in a study of flower stimulation.

Greene (1966a) reported also that ovulate strobili produced by shortleaf pine seedlings 4 years from seed developed into normal cones. Seedlings from five different progenies produced 1 to 7 cones each and seed yield varied from 9 to 47 per cone.

Slash and loblolly pine clones from grafting, air layering, or rooted cuttings produce cones at earlier ages than trees from seed. Grafted clones in seed orchards may produce sufficient conelets for controlled pollinations at 2 to 3 years after planting and the number of conelets increases thereafter. Darby (1966) reported collection of significant amounts of cones in 1962 from grafted trees in seed orchards planted in 1955, or 7 years earlier. Trees from seedlings in seed orchards are not expected to begin flowering until 10 to 15 years of age, especially in slash pine (Barber and Dorman 1964). In Florida, slash pine grafts and air layers produced a light crop of both male and female flowers 7 to 9 years after planting (Squillace 1967b). Conelets were observed on two slash pine seedlings 2 months old while in the cotyledon stage of growth (Smith and Konar 1969).

In sand pine, cones often occur on 5-year-old trees (Cooper *et al.* 1959).

Longleaf pine is capable of flowering at an early age, although time in the grass stage and factors such as racial origin, site, spacing, and perhaps others are important. In southwest Alabama, plots of longleaf pine in a racial variation study had 71 percent of the flowers on trees of Richmond County, North Carolina, origin after 6 years in the field. At 10 years, trees from 6 of the 11 geographic locations represented in the outplanting produced from 1 to 67 flowers but no regional pattern seemed apparent.

In a cultivated longleaf pine plantation in Mississippi, planted at a spacing of 10 by 10 feet, 2.1 percent of the trees bore male flowers at 5 years of age, 13.2 percent bore female flowers, while an additional 2.1 percent bore both sexes. In the following year these respective percentages were 12.4, 7.7, and 5.2 for a total of 25.3 percent (Smith 1966). The pattern of flower production seemed to follow that observed for other species, in that the first flowers were female on individual clones. Following this, male flowers appeared on other clones, and eventually clones bore both male and female flowers. In an adjoining area, longleaf pine planted at a spacing of only 6 by 6 feet and uncultivated did not produce cones until age 12, and these appeared on border trees, where exposure to light was greater. From trees in the cultivated plantation, ovulate flowers appeared on branches that originated within 1 foot of the ground, but flowering did not appear closely related to tree size or branching habit. No flowers were found on terminal shoots. In longleaf pine, where height growth is characteristically delayed, flowering seems to occur only after the seedlings are several years old. In Louisiana, longleaf pine seedlings that were only about 1 foot high produced cones the 16th growing season (Pessin 1936).

In planted Virginia pine, production of female flowers began at age 2 and male flowers at age 5 (Bramlett 1971); at age 6, 94 percent of the trees produced female flowers and 92 percent male flowers. The flowering trees were generally taller than nonflowering trees. Virginia pine grafter clones produced many conelets at 4 years of age in an Alabama seed orchard (North Carolina State University 1966).

The southern pines become sexually mature at a very early age. Thereafter, age plus crown size and other environmental factors as well as genetic factors strongly influence total production.

TIME OF FLOWERING AND SEED RIPENING

In a Southwide survey of pines, Dorman and Barber (1956) found a consistent pattern in the sequence of bloom. Slash pine, first, was followed by longleaf, loblolly, and shortleaf. The relationship with latitude was strong (fig. 26), time of blooming occurring roughly 10 to 15 days later in the spring for each 2 degrees of latitude in a northerly direction.

Time of cone ripening is somewhat difficult to estimate accurately, but in general it follows the same pattern as blooming. Slash pine seed ripened about September 15; longleaf pine October 1 through 20; and loblolly pine October 5 through 30. Both time of blooming and seed ripening vary rather widely among individual trees of each species and from year to year.

In Texas, slash pine is pollinated from the end of January to early in February; loblolly pine and longleaf pine from the end of February to the middle of March; and shortleaf from the end of March to early in April (Zobel and Goddard 1954). In loblolly pine, a great deal of variability exists in flowering. In 1952, there was considerable overlap in flowering date of shortleaf and loblolly pines, which usually differ by about 3 weeks. The majority of flowers may be in bud, while a few are in the receptive stage. This possibly accounts for nontypical, intermediate types of trees observed throughout the range. Late flowers are sometimes produced on loblolly twigs arising below insect-killed twigs or buds. These can be pollinated by a species blooming later than loblolly—like shortleaf pine or pond pine. Variation in the development of flowers on a single tree was least for slash and greatest for shortleaf and loblolly. Tree-to-tree variation is great—one tree may have strobili in the bud stage while those on another tree will be receptive.

In shortleaf and loblolly pine seed orchards, time of pollen shedding may vary widely among clones (Wasser 1967). In certain clones the period of maximum pollen shed was passed before it started for other clones. The period during which conelets were fully receptive varied about the same amount as pollen shedding. The period when flowers were fully receptive and when maximum pollen was shed

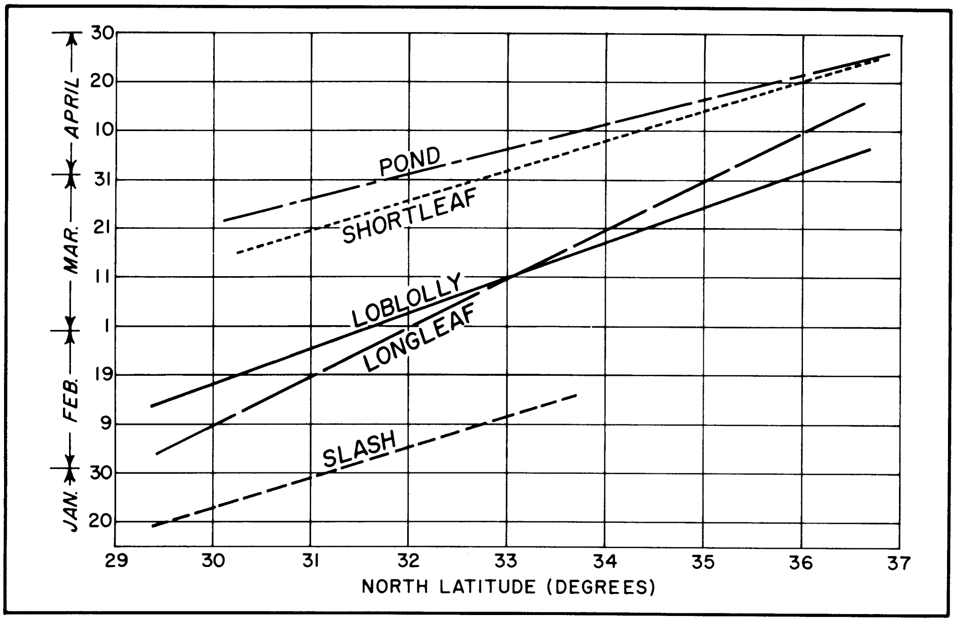


Figure 26.—Pollen ripening of principal yellow pines in relation to latitude. (USDA Forest Service 1957)

varied in length among clones of both species. Generally, pollen was available for all clones when flowers were receptive, but the peaks of pollen production and receptivity did not coincide. Initiation of the period of active growth varied widely in both female and male flowers among clones also. These conclusions were the result of observations made in Virginia.

In longleaf pine McLemore and Derr (1965) found the period of seed development was constant for individuals but varied between trees, and that time of cone maturity and ripening was independent of pollination date. The order of cone ripening among individual trees is nearly identical from year to year.

An individual tree flowering early in the spring for the species may ripen seed early, also, so that seed collection has to be carefully planned.

POLLEN FLIGHT

The pollen grains carrying out wind pollination of southern pines have remarkable attributes. The gross structure with the two air sacs gives the grains great buoyancy that permits air transportation for extremely long distances and also fluid transportation for the short but critical distance in the micropyle. The pollen grains are generated in staggering numbers, their walls composed of such a remarkable material that the pollen grain contents are not only extremely well protected in flight but endure for centuries in geological deposits; pollen grain characteristics and frequency of occurrence in buried soils near Spartanburg County, S.C., were used to trace ecological changes (Cain 1944).

Pollen grain size varies among southern pine species, although the structure is similar. Measurements of 10 grains provide the following data for length in microns as given by Cain (1940): Virginia 72, shortleaf 69, loblolly 69, pond 65, longleaf 62, pitch 62, sand 59, and spruce 55. Slash pine pollen is about 60 microns long (Wang *et al.* 1960). There seems to be no strong difference in pollen size between the major and the minor southern pines.

In shortleaf pine, pollen size varied between geographic locations, increasing from south to north (Cain and Cain 1948). In pitch pine, size variability among pollen grains from different trees can be as great as that among species (Deevy 1939).

From a study of polyphenols of pine pollen, Strohl and Seikel (1965) concluded that phenolic acids and coumarate esters varied little with the eight species studied but the flavonoid fraction and the carbonate-soluble material from the ether extractives were promising for taxonomic diagnosis. Pollen of four southern pines was used as well as that from northern and western species of different taxonomic groups. Pollen of loblolly pine contained 7.5 to 9 percent lipids, mostly triglycerides of oleic, linoleic, and palmitic acids (Scott and Strohl 1962).

The relationships between chemical composition of pollen grains and ovulate flower parts, with consequent selection or rejection of material in selective fertilization among or within species, are not known.

Pollen dispersal is an important factor in seed orchard size, planting design, and location. Contamination by foreign pollen is generally recognized as an important obstacle to realization of maximum genetic gain, not only in seed orchards but also in seed production areas. Many recent studies of pollen flight or dispersal have been made to shed light on the problem of contamination.

Early studies of pollen flight, such as that by Buell (1947), utilized traps made by coating microscope slides with a sticky material. Recently, stationary traps have been developed with materials like cellulose tape (Grano 1958, 1966; Hoekstra 1965). Grano (1966) took continuous samples with tape plus thermograph clock mechanisms.

Pollen deposition in open areas or seed orchards is extremely variable from year to year, and also varies with length of blooming period, temperature, vapor pressure deficit, amount produced, and wind characteristics. Buell (1947) found shortleaf pollen was carried the greatest distance when temperature and other conditions were suitable, rather than when the maximum volume was produced. The volume of pollen at distances 10 times the height of the source was low. In Australia, pollen falling in a seed orchard 2½ miles from the pollen source and surrounded by a screen of hardwoods was considered to be well below the frequency that would appreciably reduce seed quality (Florence 1956).

In Florida, pollen measurements in several seed orchard areas showed slash pine pollen strikes the ground within about 400 feet of the source when bare land is used as an isolation barrier. At distances of 400 to 500 feet the volume of pollen was 2 to 5 percent of that at the source. There was a distinct correlation between pollen output and rising temperature (Wang *et al.* 1960).

Using an isolated longleaf pine tree as a pollen source, Boyer (1966) found wide variation in deposition from year to year, with no clear-cut relation to distance from the tree or prevailing winds. Pollen drift could be much higher than deposition, inasmuch as vertical traps at certain times caught nine times more pollen than horizontal traps.

In Florida, when male catkins were removed in a young slash pine seed orchard, volume of pollen from outside was such that a bushel of cones produced about 1.1 pounds of seed, which is about average for cones from natural stands. There were about 50 full seed per cone (Squillace 1967a).

Measurements of pollen production, as well as pollen flight and deposition in relation to seed orchard design and management, are certainly among the more difficult problems in tree breeding research. It is to be expected that contamination by foreign pollen will occur in seed orchards, but what effect this will have on genetic gains is unknown. As the genetic superiority of seed orchard clones increases, the problem of contamination will become more serious. In that case, pollen production or flowering characteristics of the clones as well as seed orchard cultural practices and silvicultural treatment of stands from seed orchard seed may require intensive study to reduce effects of contamination by foreign pollen as much as possible.

POLLEN HANDLING AND TESTING

Pollen handling is an important part of controlled pollination, but there are no limiting factors or highly involved techniques required for work with southern pines. Produced in large amounts, the pollen is fairly easy to collect, and, as described previously, can be stored for years under refrigeration. However, it is not indestructible and the procedures developed have to be followed to insure that viable pollen is injected in pollination bags. Such procedures are summarized in this chapter, but collection and extraction for large-scale breeding projects will be covered in the section on controlled pollinating techniques.

Moisture content of pollen is a very important factor in successful storage. Snyder (1961a) found that desiccation for 15 minutes at 5 mm of vacuum will quickly reduce moisture content of pollen to a safe level. Silica gel is a convenient desiccant.

While in storage, pollen keeps best in containers no larger than 2-ounce pill bottles and filled no more than half full. The bottles should be plugged loosely with cotton. Temperature during storage should be freezing or slightly above.

In the South, freezing weather about the time of meiosis has rendered many lots of pollen useless (Dillon and Zobel 1957). These authors suggest a simple method of testing pollen viability: 2 or 3 cm^3 of distilled water are placed in a screwcap vial (8 by 15 mm), then a small amount of pollen is dropped into the solution, thoroughly shaken, capped, and set in the constant-temperature oven for 72 hours at 26° to 27° C. Inasmuch as this temperature is approximately room temperature, the method can be used without a constant-temperature oven if one is not available. Several times during the 72-hour period the vials are agitated slightly to redistribute the grains, which usually rise to the top of the liquid. At the end of the 72-hour period the vial is thoroughly shaken and a drop or two of the dispersed grains placed on a slide and covered with a cover glass. Pollen grains producing a tube as long as the pollen grain or longer are counted as germinated (fig. 27). The method described is a simplification of the hanging-drop method described by Righter (1939). Distilled water was superior to 3-, 5-, or 10-percent sucrose medium. A small amount of sugar or honey, less than 0.1 percent, is often used.

However, other tests show that certain media are superior to distilled water for increasing germination (Echols and Mergen 1956). Germination tests were conducted with pollen from slash pine

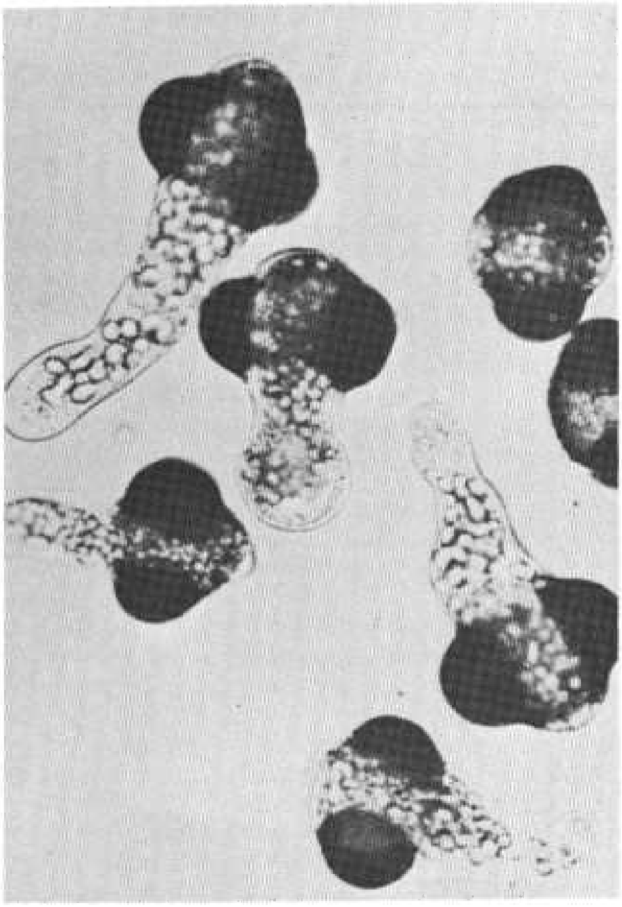


Figure 27.—Pollen that has been germinated for 48 hours at room temperature, highly magnified. This loblolly pollen will produce much longer tubes by 72 hours. Note the tubes and grainy inclusions. (Texas Forest Service 1955b)

using 38 different media containing various concentrations of organic and inorganic compounds. Ethylenediamine disodium acetate-iron complex diluted to contain 5 to 10 p/m of iron produced the highest germination (96 percent); 10-percent sucrose resulted in 90-percent germination, whereas only 76 percent of the pollen grains germinated in distilled water. Plant growth regulators gave varying results, ranging from complete inhibition to slight stimulation of germination, depending upon the concentration in the medium. Four distinct forms of pollen tubes were found associated with certain media. Aqueous extracts from female flowers inhibited germination of pollen. Effect of the extract solution varied among the four trees tested (fig. 28).

During a test of germination media, viability of slash pine pollen dropped 16 percent over a period of 52 days while stored at 4° C (40° F) and 30 percent relative humidity (fig. 29) (Echols and Mergen 1956).

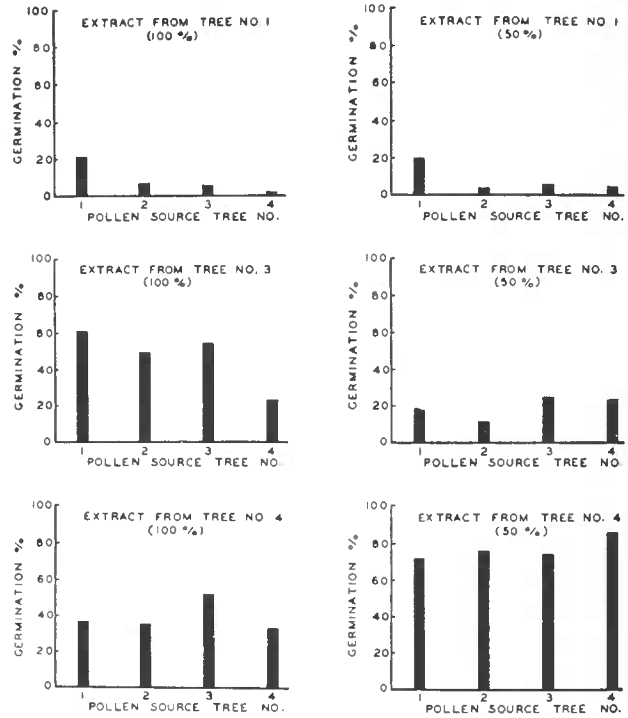


Figure 28.—Germination of slash pine pollen in distilled water containing extracts from young female cones. (Echols and Mergen 1956)

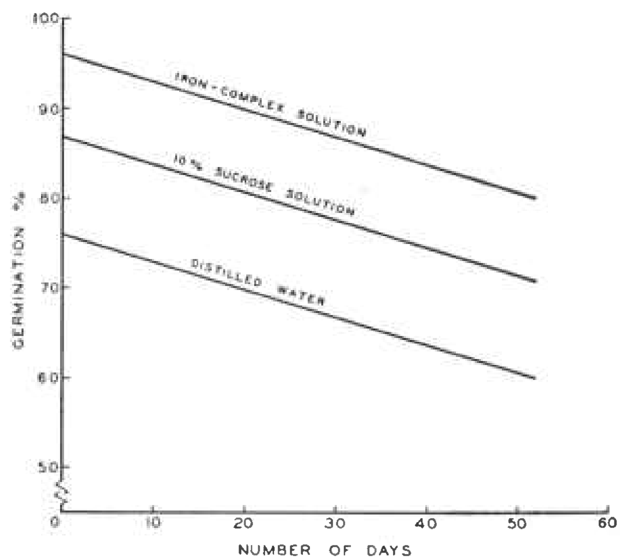


Figure 29.—Decrease in viability of slash pine pollen over a period of 52 days while stored at 4° C and 30 percent relative humidity. (Echols and Mergen 1956)

During hot, sunny weather in northern Florida, high temperatures lethal to germinating pollen (above 46° C or 115° F) do occur in strobili on exposed portions of crowns enclosed in sausage casing bags. By insulating these bags, particularly with some type of reflecting paint, the temperature can be kept almost as low as that of uncovered strobili (McWilliam 1959a). It was observed also that pollen is sensitive to heat during dehydration. Control of temperature within pollination bags may be more important in species that bloom late in the spring than in those blooming earlier.

Temperatures of about 115° F are required for sterilization of pollen (McWilliam 1959a), but Hodgkins (1952) suggested 176° to 180° F for 15 hours. Hodgkins found that there was some variation among trees in the tolerance of pollen to heat: one loblolly pine tree had pollen that germinated 28 percent after 24 hours at 152° F to 156° F, and zero germination after 12 and 24 hours at 170° F to 176° F; pollen of another tree showed 8 and 4 percent germination after 12 and 15 hours, respectively, at 170° F to 176° F; loblolly pine pollen did not differ from that of eight other pine species in resistance to heat injury.

Pine pollen is quite durable, indeed more resistant to heat-killing than vegetative tissue, and possibly as heat resistant as most tree seeds (Hodgkins 1952). Stanley *et al.* (1960) found that pollen of shortleaf and loblolly pines germinated fairly well after 15 years of storage. Germination was zero in pollen stored under 25-, 50-, or 75-percent relative humidity. At 10-percent relative humidity and 32° F temperature, however, pollen of shortleaf and loblolly pines germinated 66 and 33 percent, respectively. Under the same relative humidity condition but at 122° F, germination was 33 and 36 percent, respectively, for shortleaf and loblolly pines.

One of the earliest attempts to freeze-dry longleaf pine pollen for prolonged storage was by Hesseltine and Snyder (1958). Results were unsatisfactory when bovine serum was used with various combinations of dextran and sucrose plus different rehydration environments.

Duffield and Callaham (1959) reported on effects of deep-freezing pollen of pitch pine. Pollen in cotton-stoppered glass bottles desiccated over anhydrous calcium chloride for 1 week was placed on the quick-freezing shelf of a domestic deep freezer at about −23° C. In April of the following year the pollen was transferred to a commercial reach-in refrigerator at about 5° C. The pollen germinated 70 percent when fresh, and 40 percent after storage. Stored pollen gave about as good seed set on trees of the same species as fresh pollen.

King (1959) also reported on methods for freeze-drying pollen. He concluded that each species of grass, hardwood tree species, or softwood tree species responds within the limits of its own range of exposures to auto-freezing. Loblolly pine pollen exposed to auto-freezing for 30 minutes or 1 hour germinated well after recovery. A sample of loblolly pine pollen that had been treated was shipped to New Zealand and returned, and another sent to Brazil and returned—with no apparent loss of pollen viability in any of the samples, although they had been shipped in ordinary packaging.

Pollen can be stored by several methods, but additional work is needed to accurately relate methods: for example, freeze-drying as related to seed set, not just to germination percent.

CONTROLLED POLLINATION TECHNIQUES

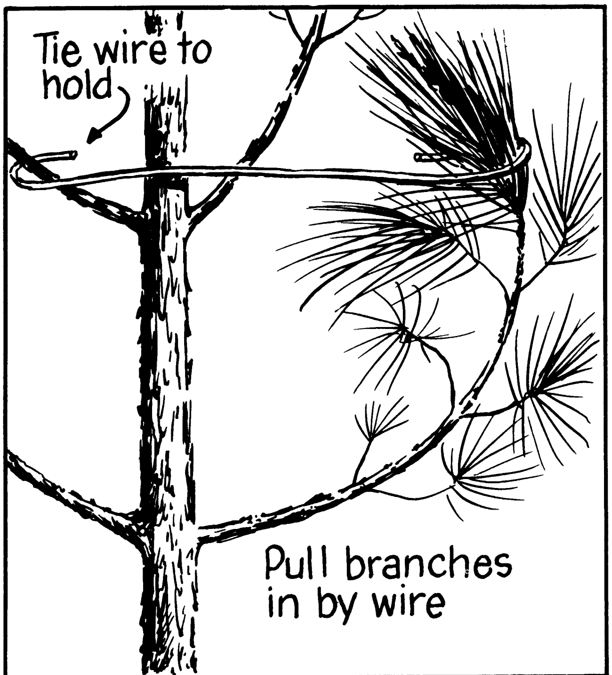
Four stages of female strobili development (fig. 21) are recognized by Snow *et al.* (1943) and five by Campbell and Wakeley (1961) as important in controlling pollination in breeding southern pines. Pollination bags may be placed any time up to stage II, the period when viable pollen will germinate and bring about fertilization. Pollen should be applied to conelets when the scales are open, as in stage III. Stage IV is reached when the conelet scales are grown and thickened so that pollen cannot enter; pollination bags can be removed at this time. In Florida the elapsed time between stages I and IV averages about 15 days for slash pine. If not pollinated, there is a tendency for the strobili to remain in stage III for a longer period.

Pollen germinating as little as 65 percent may be well worth using in attempts to make certain desired crosses (Campbell and Wakeley 1961), or even less than 65 percent in special situations.

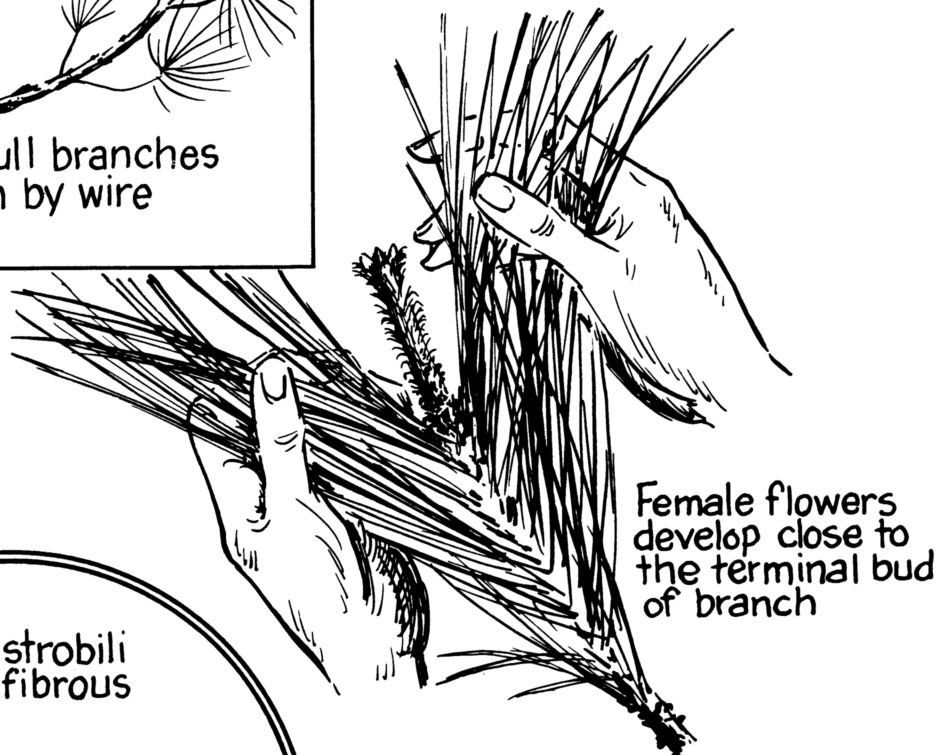
Simple plastic pollinators with a ¾-inch No. 16- or 20-gauge needle can be used, or an ear syringe. The syringe is cheap, simple to use, and can be discarded after each use.

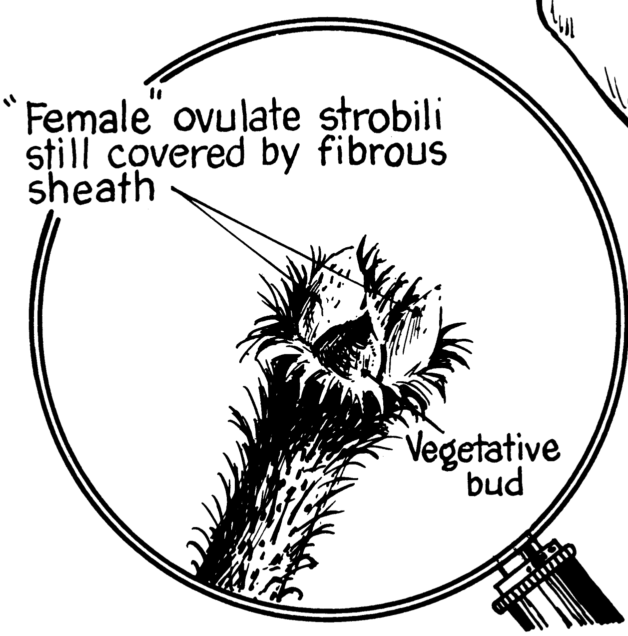
Efficient methods of performing controlled pollination in pine developed for use with various species are described by Mergen *et al.* (1955), and their drawings (fig. 30a-d) are easy to follow. The important steps in controlled pollinations in pine from the standpoint of the tree breeder were described by Goddard and Allen (1955), Perry (1954), and some variations were proposed by Wakeley and Campbell (1954).

In addition to the widely used plastic sausage casing, small plastic tubes 6 to 12 inches long and similar to those used for packaging toothbrushes have been successful, particularly on longleaf and shortleaf pines (Schoenike 1956). Vigorous loblolly pine shoots elongate more than those of other species, thus requiring longer tubes. Longleaf pine twigs and those of other species with more than one conelet require cylinders as large as 1½ inches in diameter. Higher air temperatures around the conelets caused by bagging will advance flowering date (Boyer and Woods 1973).



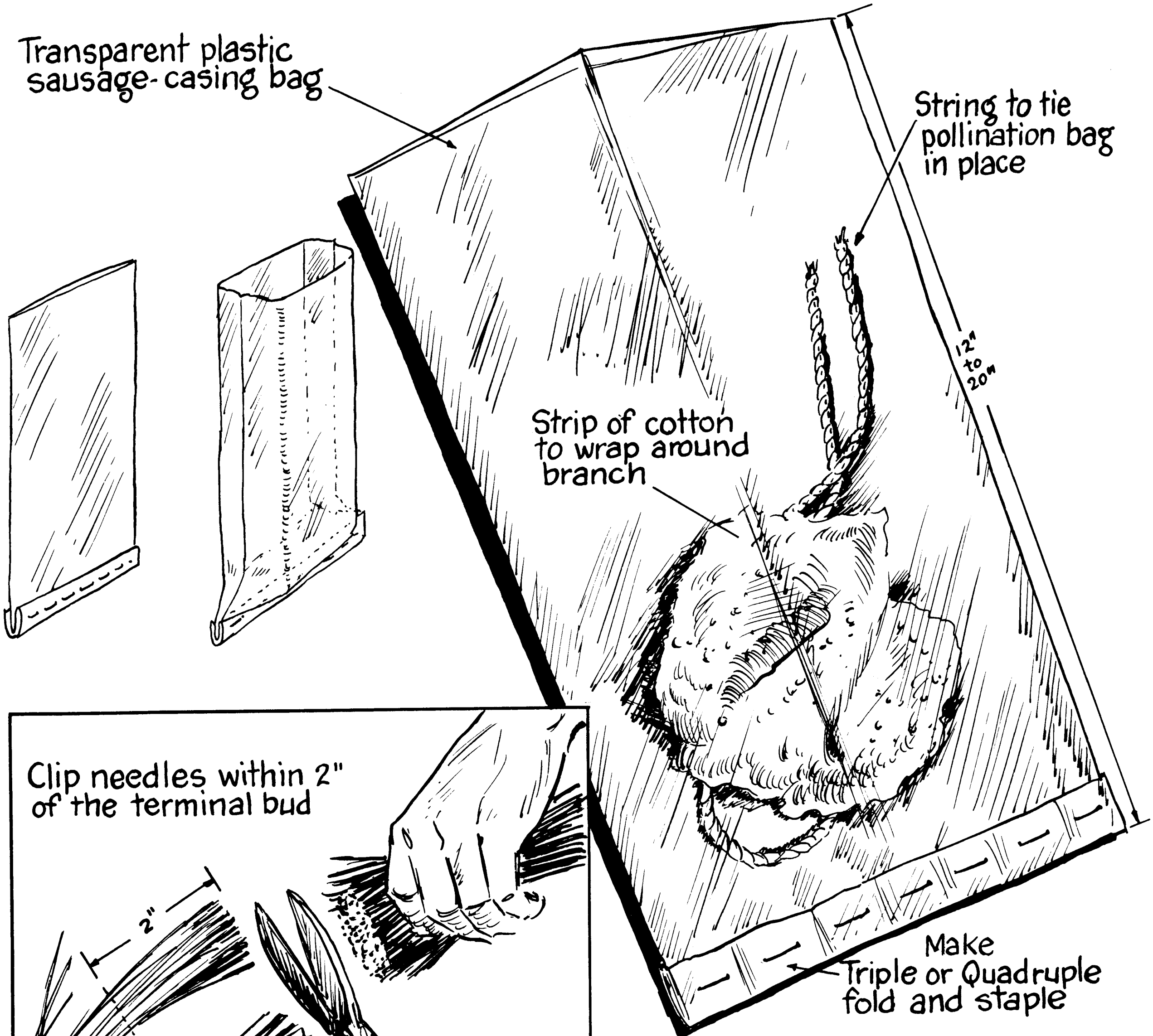
The female flowers or ovulate strobili are born right next to the terminal buds in the upper part of the crown. Slash pine flowers become visible early in January, while those of longleaf pines appear about 2 weeks later. A wire hook is an essential tool for pulling branch tips within "comfortable" working distance.

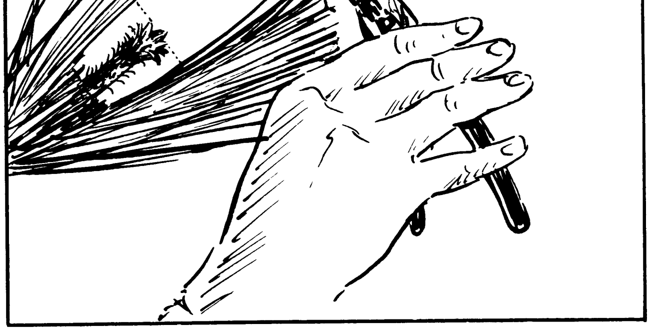




A white fibrous covering encloses the female flowers at the time they push through the thin scales of the "dormant" bud. The flowers appear either singly or in clusters of up to 20 per branch tip.

Figure 30a.—Isolation of female flowers. (Mergen *et al.* 1955)

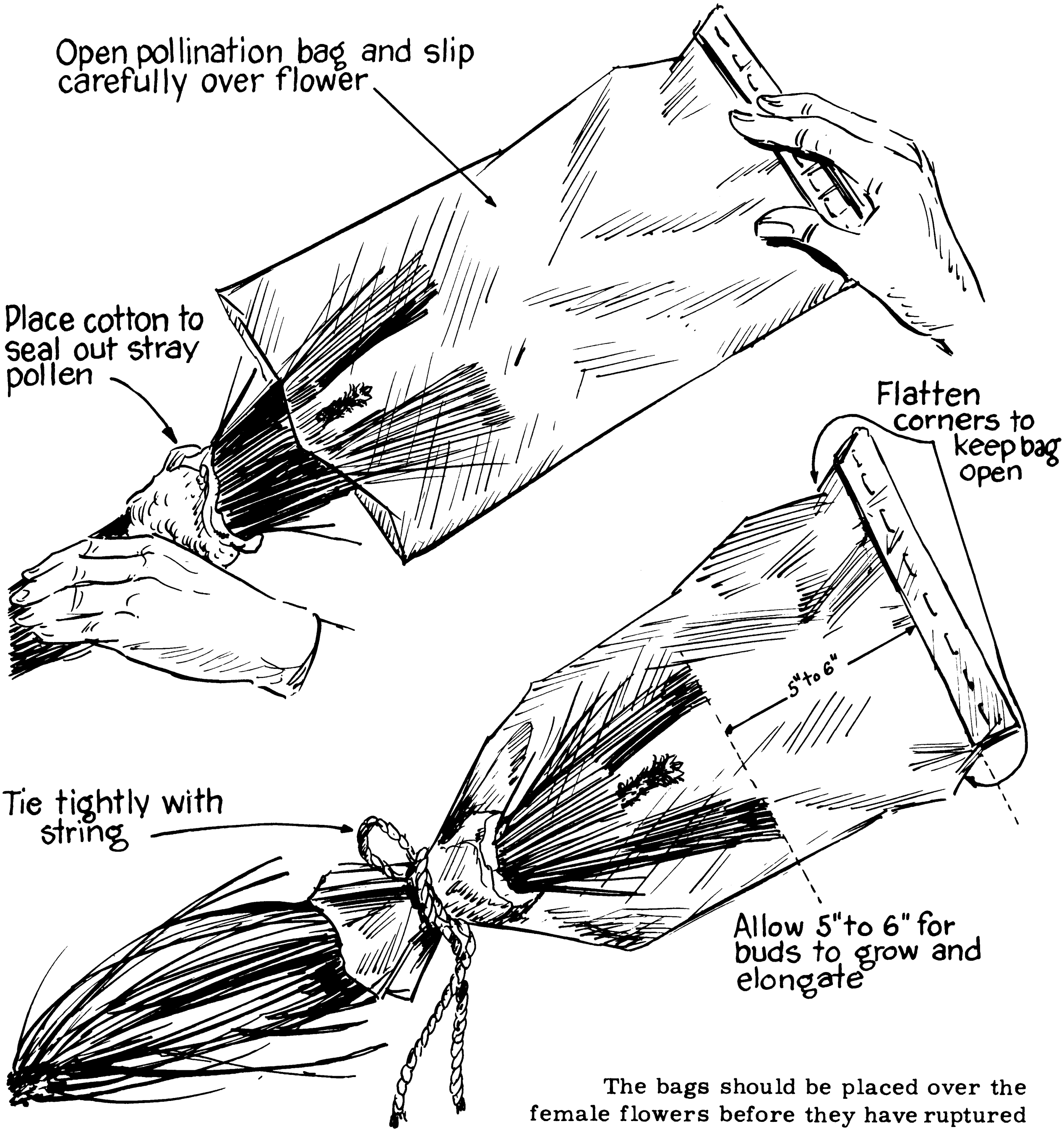




Plastic sausage casing is satisfactory for bagging slash and longleaf pine conelets. This type of bag is permeable to water and light, is strong, and inexpensive. The bags should be assembled on the ground; a strip of nonabsorbent cotton, and a piece of string are placed inside the bag to facilitate handling while in tree crowns.

When clipping the needles, a "brush" of needles should be left on each side of the flowers to protect them in case the bag collapses.

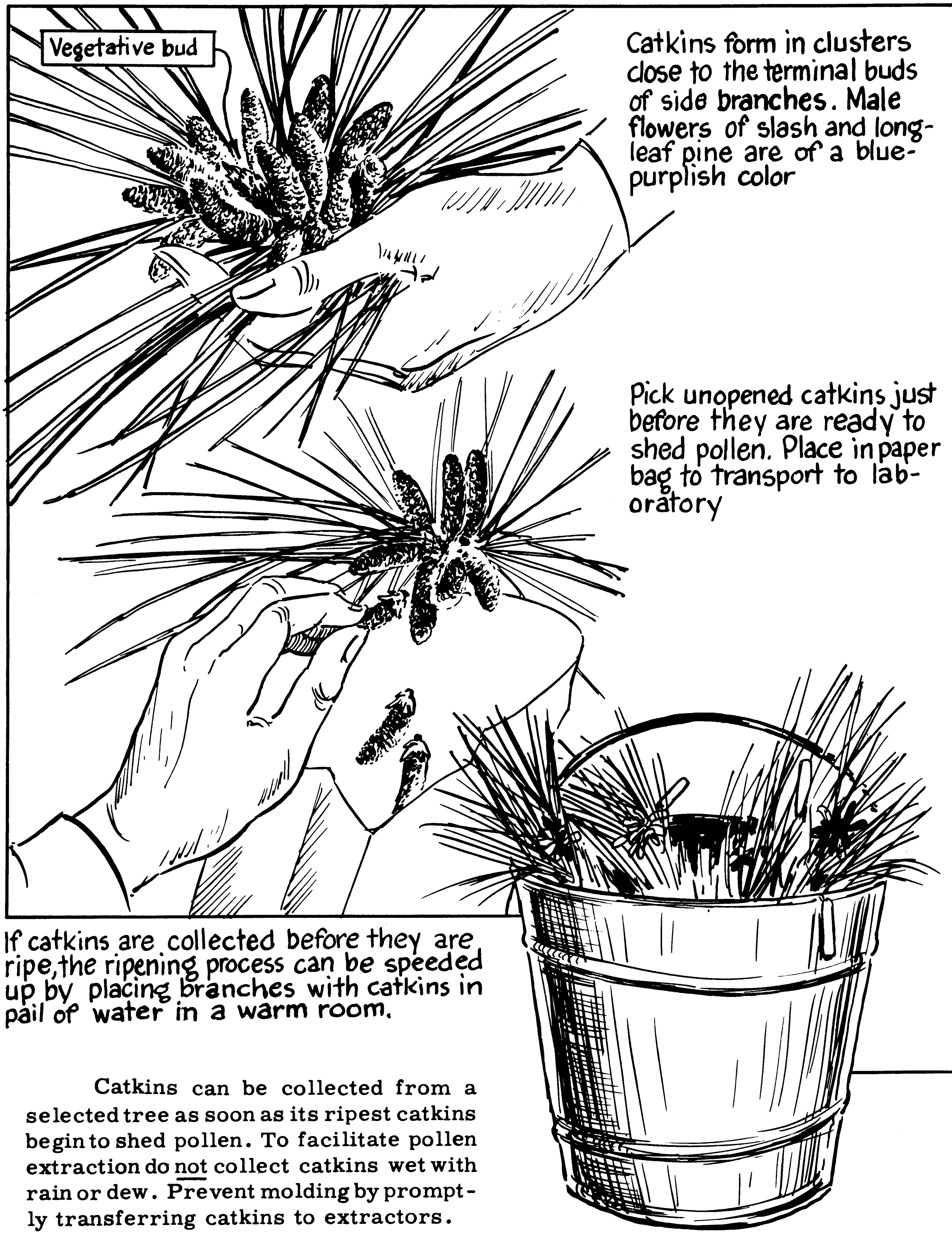
Figure 30a.—Isolation of female flowers (continued).



The bags should be placed over the female flowers before they have ruptured the protective sheath and the scales of the conelet (pinkish-white in color) become visible. Before placing bag, check bud for male catkins. These should be twisted off unless selfing is desired. Place bag so that there is adequate space for the elongation of the vegetative bud during spring growth.

The bags should be tied firmly to the branches with a cotton string, or a combination paper-wire plant "twistem."

Figure 30a.—Isolation of female flowers (continued).



Catkins can be collected from a selected tree as soon as its ripest catkins begin to shed pollen. To facilitate pollen extraction do not collect catkins wet with rain or dew. Prevent molding by promptly transferring catkins to extractors.

Figure 30b.—Collection of male catkins. (Mergen *et al.* 1955)

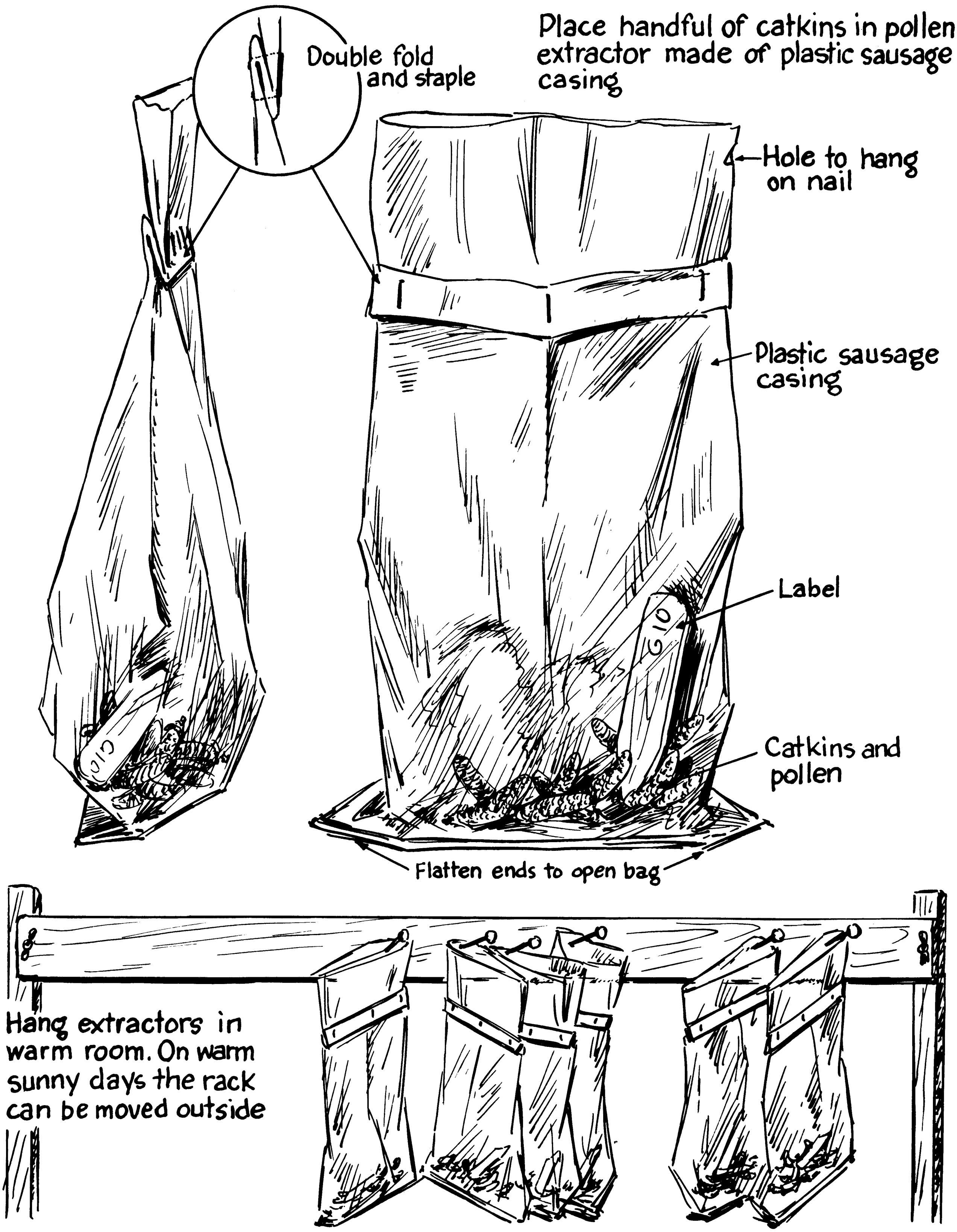
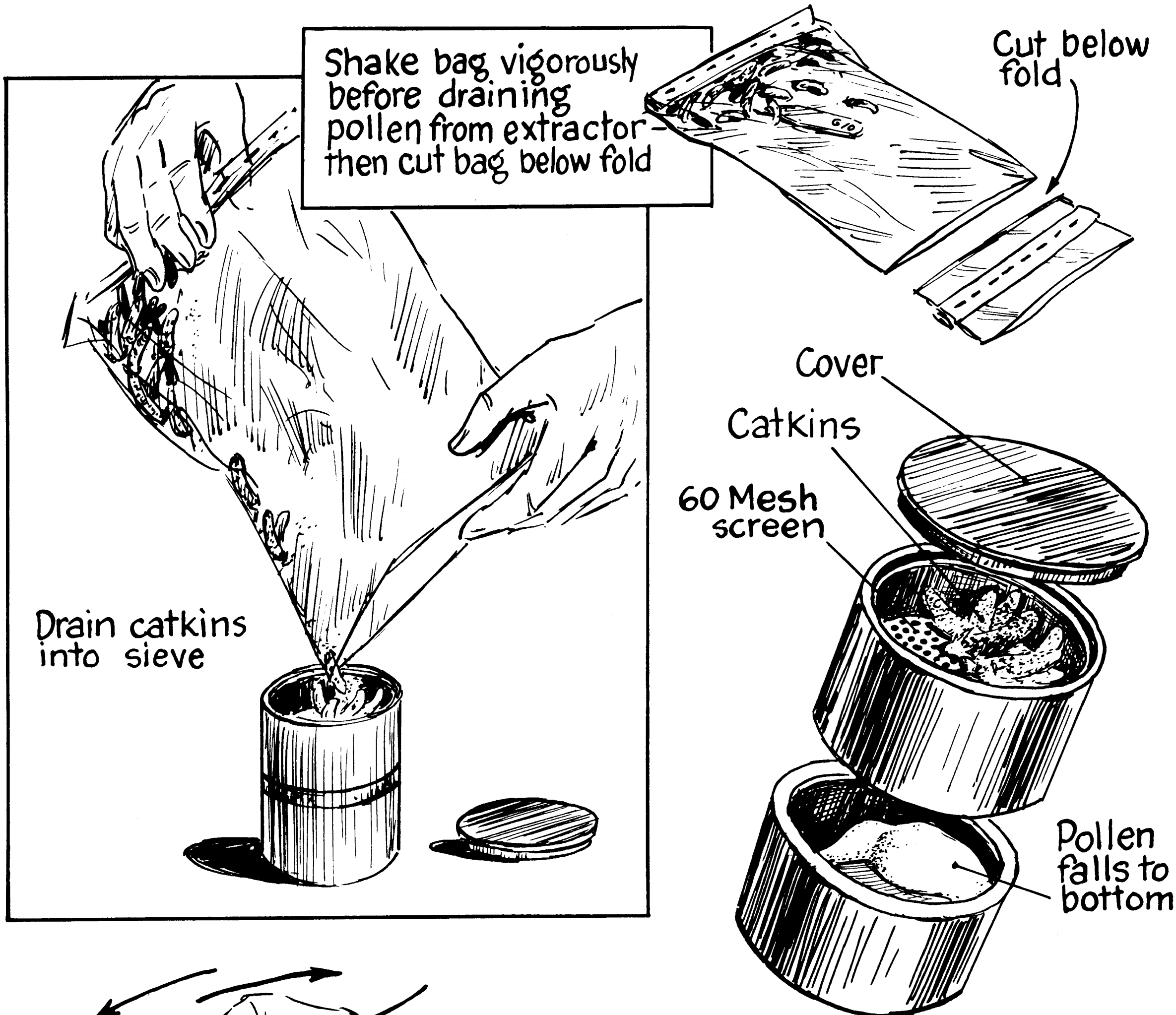


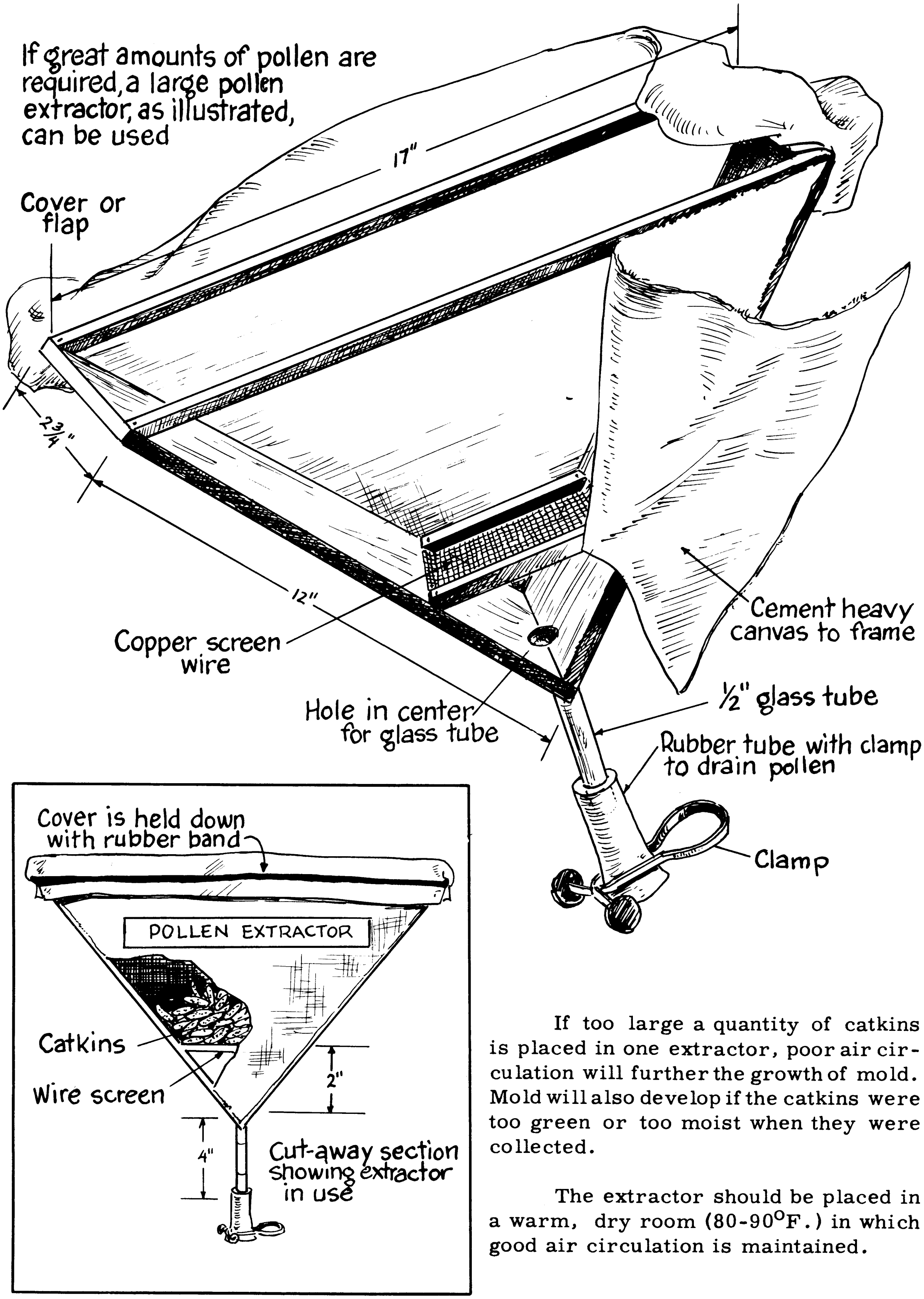
Figure 30c.—Extraction of pollen. (Mergen *et al.* 1955)



If only a small amount of pollen is desired, a hole can be punched in the wall of the bag and the pollen strained through it.

A regular soil sieve (60 mesh) is used when a large quantity of pollen is needed. To prevent contamination, the sieves should be sterilized after each use by either washing them in 95 percent alcohol, or by placing them in a hot oven for several minutes. A combination of these two methods is very satisfactory.

Figure 30c.—Extraction of pollen (continued).



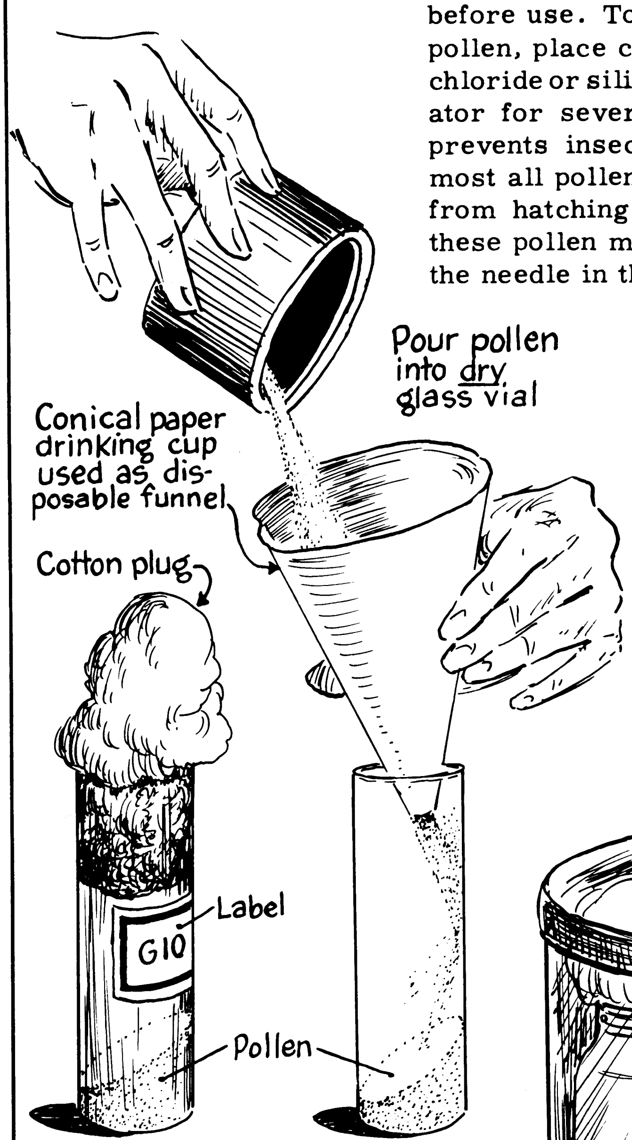
If too large a quantity of catkins is placed in one extractor, poor air circulation will further the growth of mold. Mold will also develop if the catkins were too green or too moist when they were collected.

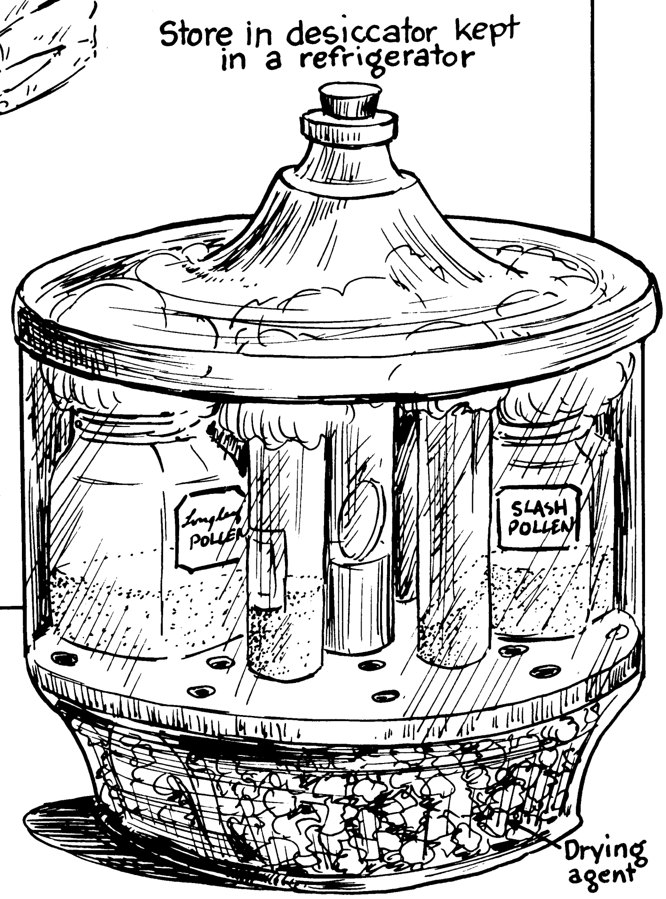
The extractor should be placed in a warm, dry room (80-90°F.) in which good air circulation is maintained.

Figure 30c.—Extraction of pollen (continued).

The pollen should be handled in a draft-free room. All equipment should be sterilized before use. To prevent molding of extracted pollen, place cotton-stoppered vials in calcium chloride or silica gel desiccator in a refrigerator for several days. This treatment also prevents insect eggs, which are found in almost all pollen lots of slash and longleaf pine, from hatching. Besides injuring the pollen, these pollen maggots have a tendency to clog the needle in the pollinator.

After pollen has been stored at a low temperature, do not open vial until the glass of the container has reached room temperature. This prevents moisture from condensing on inside walls.

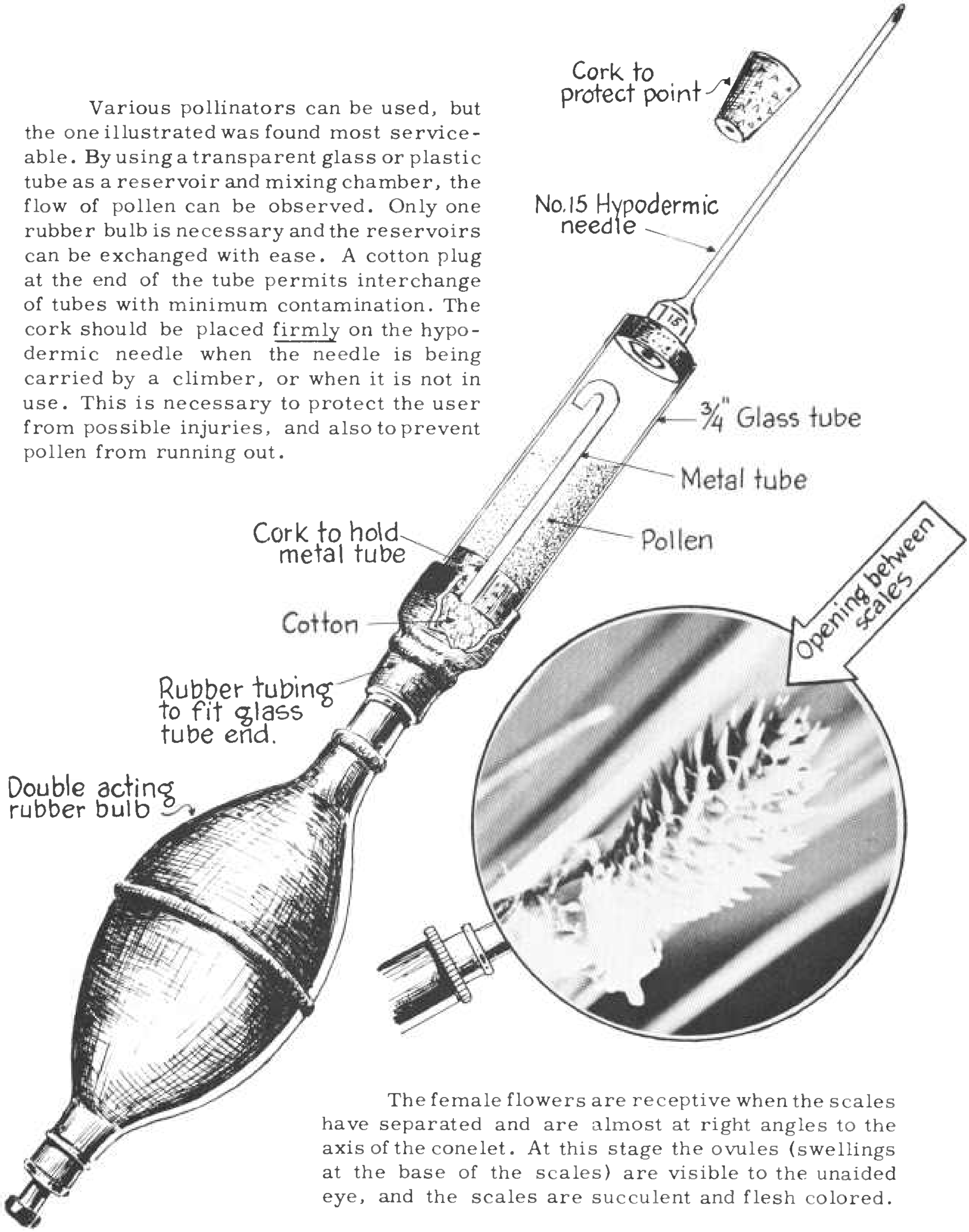




Pollen may stay viable for a year when stored at 39°F. at a relative humidity of 30 percent. A relative humidity of approximately 30 percent is obtained by placing an excess of a sulfuric acid solution with a density of 1.45 in the well of a desiccator.

Figure 30c.—Extraction of pollen (continued).

Various pollinators can be used, but the one illustrated was found most serviceable. By using a transparent glass or plastic tube as a reservoir and mixing chamber, the flow of pollen can be observed. Only one rubber bulb is necessary and the reservoirs can be exchanged with ease. A cotton plug at the end of the tube permits interchange of tubes with minimum contamination. The cork should be placed firmly on the hypodermic needle when the needle is being carried by a climber, or when it is not in use. This is necessary to protect the user from possible injuries, and also to prevent pollen from running out.



The female flowers are receptive when the scales have separated and are almost at right angles to the axis of the conelet. At this stage the ovules (swellings at the base of the scales) are visible to the unaided eye, and the scales are succulent and flesh colored.

Figure 30d.—Pollination of female flowers. (Mergen *et al.* 1955)

To pollinate the flowers, the needle is pushed into the bag and pointed directly at the flowers. The best distribution of pollen is obtained if several small puffs are directed at the conelets. The scales should be covered adequately but not coated heavily with pollen. Too much pollen dries the liquid at openings to the egg cells, thus hindering pollen germination.

Permanent labels should be tied near each bag. A plastic label, code marked by a conductor's punch, is satisfactory.

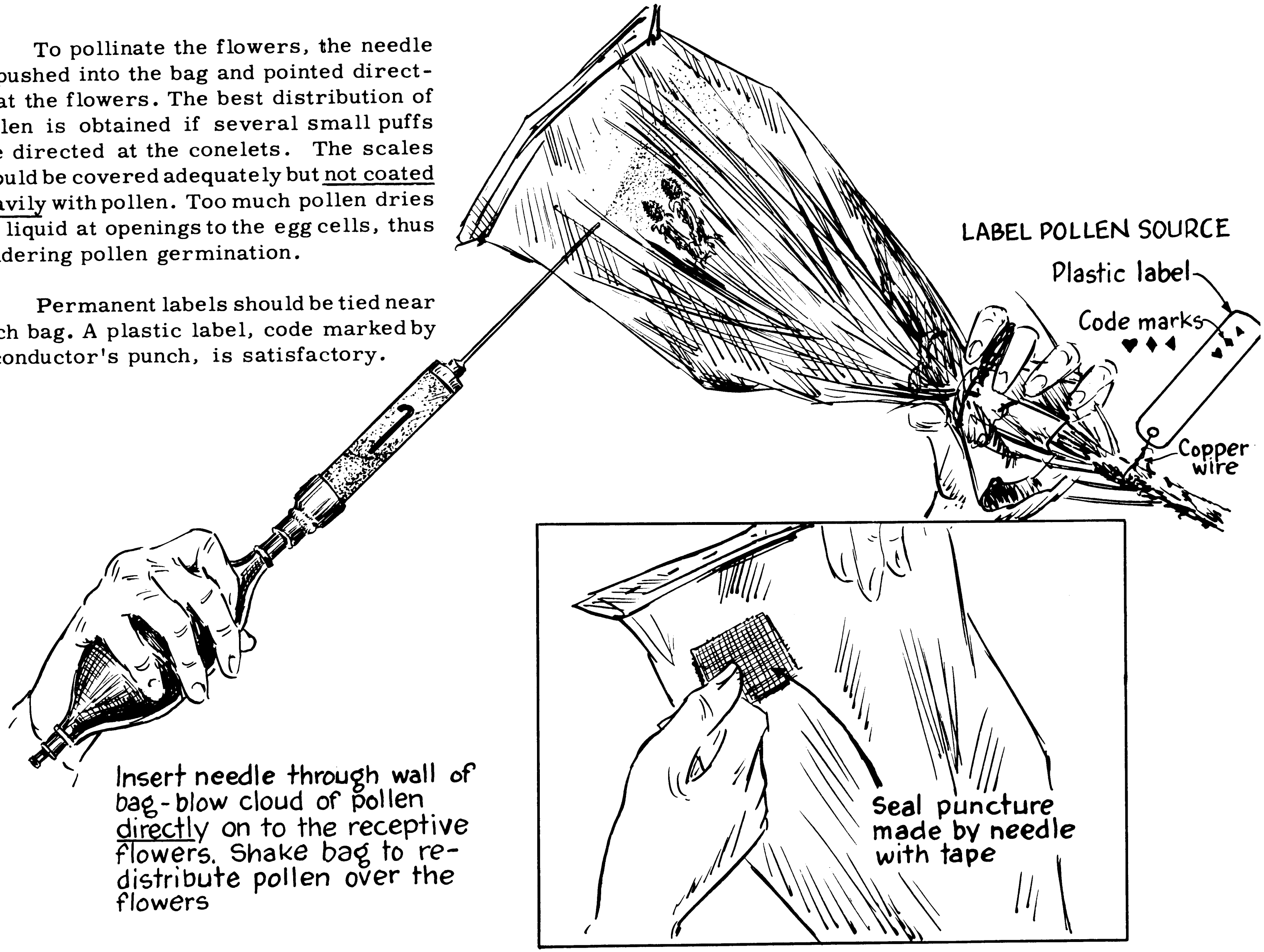
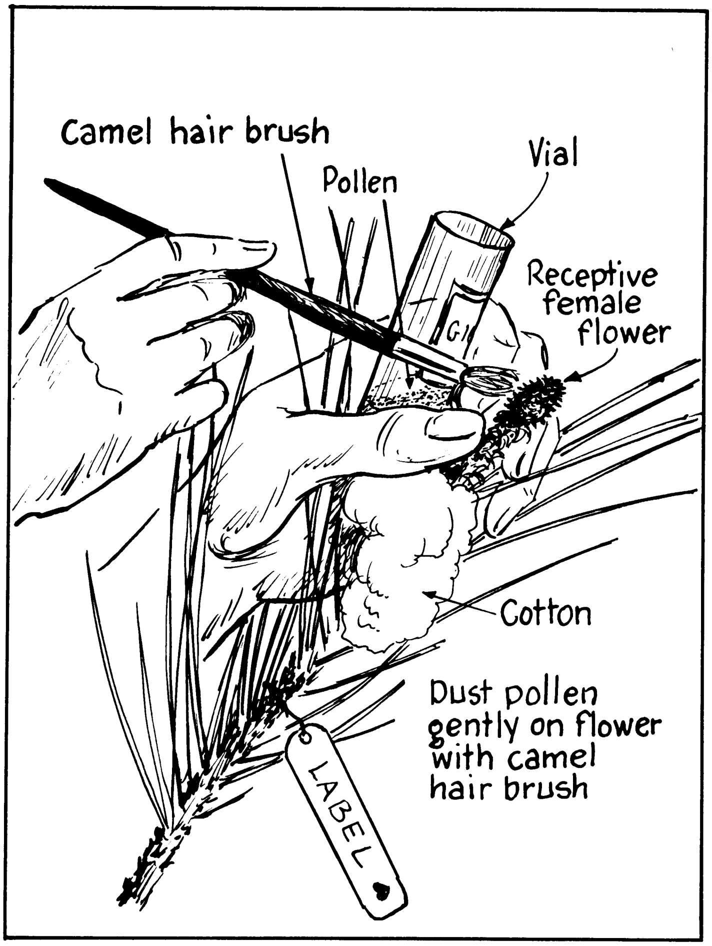


Figure 30d.—Pollination of female flowers (continued).



If only a small amount of pollen is available for a particular cross, it can be brushed on the receptive flower with a camel hair brush as illustrated. To minimize the danger of contamination by stray pollen, pollination should be done during the early morning hours when the relative humidity of the air is very high and when there is no wind movement. To pollinate the flower, the bag is removed temporarily and the pollen is dusted on the conelet. The bag is replaced right after pollination. Several hundred flowers can be pollinated with 1 cc. of pollen if this method is used. This technique results in a good seed set. It is recommended for making crosses in a grafted seed orchard where flower handling does not present a problem.

To remove the bag, one can use either of the two following methods: (1) Loosen string and slip bag carefully over flowers, or (2) with a pair of scissors or a razor blade cut the bag just above tie, and slip bag over flowers. When the second method is used, the remaining collar of cotton is plainly visible for some distance and helps to relocate the labels.

The bags should be removed as soon as the scales have closed, to minimize injury to the flowers.

Figure 30d.—Pollination of female flowers (continued).

Studying the variable yields, Snyder and Squillace (1966) concluded from a survey of 10-year records that survival of cones from controlled cross-pollinations were less than 40 percent, and seed yields per cone averaged about half those from wind-pollinations. Self-pollinations produced about 15 percent as much seed as cross-pollination. Interspecific pollinations were generally less productive than intraspecific pollinations.

Several devices for transporting equipment and for tree climbing (figures 31 and 32) have each shown certain advantages as well as disadvantages (Cech 1961; Easley 1962). Swedish sectional ladders are best where individual trees accessible to motor vehicles are being climbed. In seed orchards or other areas, they have disadvantages because erecting and lowering the one ladder needed for each climber is slow, tedious, and demanding physically. Grimpettes or Angel Shoes have been used, but the job of sawing off all limbs to the live crown is onerous; to be sure, grimpettes do not injure the tree, but they are also difficult to use on stems smaller than 6 inches in diameter (Harkin 1956).

Men using pole climbers (Allen 1960) find that it is hard work to insert the spurs into the tree trunk and that considerable oleoresin bleeding follows. In this case, trees should be sprayed with BHC (benzene hexachloride) to prevent insect attack. Such climbing spurs are rarely used because of the fear of encouraging bark beetle attack.

Pickup-mounted extension ladders are used extensively (Cech 1961). The Lake City model (Johansen and Arline 1958) is good (fig. 32), and modifications have been developed by the tree breeding projects of the Texas Forest Service, International Paper Company, West Virginia Pulp and Paper Company, Union-Camp, and Buckeye Cellulose. In the Lake City model the mount is used on a ½-ton pickup truck and supports a 40- or 50-foot, heavy-duty ladder made of aluminum or an alloy. In traveling position, the ladder rests across the top of the removable mount. Hydraulic lifts such as those used in telephone line maintenance can raise workers to tree crowns.

Another system for hoisting workers into trees from the ground was developed by Strickland and Peters (1961). A weight with a 40-pound-test trolling line is shot with a slingshot over several branches of a tree. A heavier rayon cord, ½-inch manila climbing rope, and a block and tackle are then pulled into position. A climber can be pulled up into the tree crown by one or two men or a 12-volt electric capstan on the ground. The climber later lowers himself via his climbing rope.

To overcome the tendency for the Swedish ladder chain, which fastens around the tree, to slip out of its lockhook, Miles and Hoekstra (1954) suggest that a moderately stiff coil spring (0.105, 3¼ to 2 inches) be inserted between the U-bolt on the side of the ladder section and one end of the chain. The spring makes it possible to keep the chain under tension when the tree sways in the wind, when bark flakes off, or when the weight of the climber causes the ladder to shift.

In special cases when it is necessary to work in the tops of trees with very weak stems, such as pines grown at close spacing, the terminal shoot can be splinted with 2- by 2-inch wood strips of appropriate length. Also, guy wires can be used to make treetops more stable where splints are or are not used (Dorman *et al.* 1944).

Rope knots and the use of rope in climbing have been described by Thompson (1955) and Eversole (1954).

Safety rules for tree climbers have been developed by Snyder and Rossoll (1958) and Thompson (1956).

FLOWERING CONTROLS IN MAJOR SPECIES

The large investment in seed orchards of southern pine species has stimulated many studies in seed production. Heavy seed production in young orchards is particularly needed for getting genetically improved seedlings into the hands of tree planters. In later years when grafted trees are much larger, maximum seed production will be desirable but not as critical. However, this relationship will not always hold true, since young flowering loblolly pine (Schmidtling 1969) and Virginia pine (Bramlett 1971) have been found to be taller than nonflowering trees.

Inasmuch as seed orchards vary in regard to the tree species and its particular requirements as well as to soils on which they are placed and the climate of the geographic region, no single set of cultural methods will apply. Variation among trees or clones in cone and seed characteristics and resistance to pests will influence seed production, also (fig. 33). Therefore, in the following review of studies of seed production, the species and the location at which the work was done are stressed.

Loblolly Pine

Cone production in southeast Virginia was substantially increased by fertilizer application (Wenger 1953). Following application of either 25 or 50 pounds of a 7–7–7 mix per tree, cone production of 25-year-old trees increased about three times over that of check trees in the third season after treatment. But in a 40-year-old stand where trees averaged 14.7 inches, cone production did not increase significantly. For the younger trees, light or

Bottom photo courtesy of N.C. State University

Figure 31.—Portable tree-climbing equipment is essential. (Top) Trailer-mounted tree ladder to be used in seed production areas and seed orchards. The prototype was developed by Dr. Franklin Cech, working at International Paper Company, Southlands Experiment Forest (Texas Forest Service 1961). (Bottom) Hydraulic hoist and scaffolding.

F-494198

Figure 32.—Control pollinating in a unique plantation at Lake City, Florida, which contains 550 slash pines 15 years old, bred from superior selections. Several of these trees yield three times as much gum as average slash pine, others grow nearly twice as much wood as average, and still others have outstanding characteristics such as high or low specific gravity, long or short fibers. (USDA Forest Service 1961)

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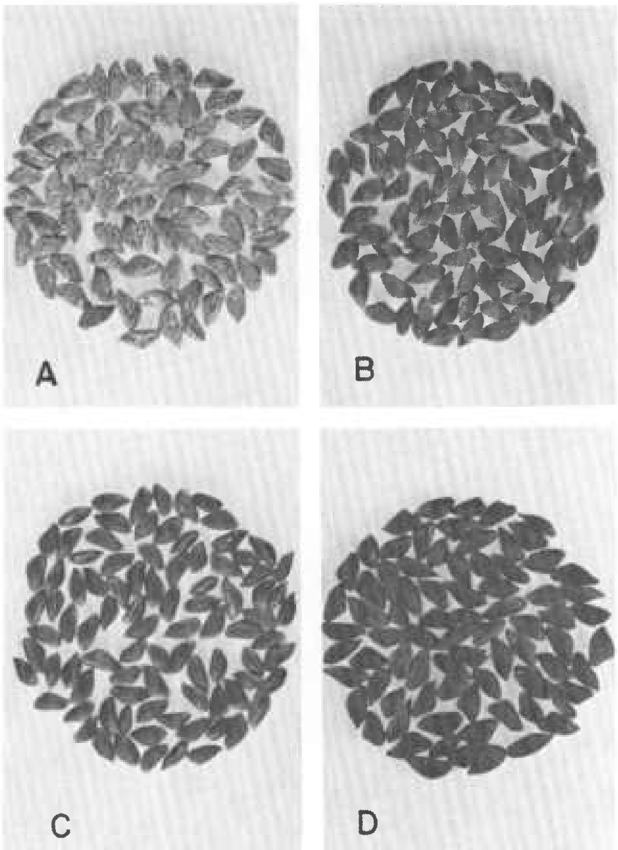

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Figure 33.—Color of sand pine seed varies considerably from tree to tree. Lots A, B, and C are typical of the Choctawhatchee race; D is Ocala race. (Barnett and McLemore 1965)

heavy fertilizer treatment gave no significant difference in number of cones produced. Cone production after release was directly related to fruitfulness before release. In the same study a knife-cut wound halfway around the stem healed quickly and did not stimulate flowering. However, a ¼-inch-wide cut halfway around the stem stayed open through a growing season, bringing a significant increase in flowering the following year.

Poor seed-producing loblolly pine trees in Arkansas did not increase production after girdling and banding (Grano 1960). Trees 3.1 to 14.9 inches in diameter were observed during an 8-year period. The treatments had no effect on diameter and height growth. However seed production in stands varied by tree diameter class, crown-volume class, and past production class (fig. 34) (Grano 1957).

Full crown release was the most successful method of stimulating cone and seed production of loblolly pine in North Carolina (Bilan 1960). It resulted in a sevenfold increase in cone production the third year after release and in a fourfold increase the fourth year. Crown release almost doubled the number of viable seeds per cone and increased diameter growth and crown development. Partial girdling (two semicircular incisions) and wiring of stems (two double strands approximately 1.5 inches apart) did not contribute significantly to cone and seed production of released or unreleased trees, but such treatments decreased diameter growth and reduced the live crown ratio of unreleased trees. Banding (metal band 1.5 inches wide) suppressed cone production of released and unreleased trees. The number of viable seeds per cone borne on the third whorl from the tip or above was consistently at least 30 percent higher than the number of viable seeds per cone borne on the branches below the fourth whorl from the tip. Cone production of released trees was positively correlated with the amount of nitrogen but not the total amount of carbohydrate reserve or the carbon-nitrogen ratio in the bark and the wood of the 1- to 2-year-old twigs in the upper part of the crown.

The effect of crown pruning on strobili production of mature and young loblolly pine trees in Texas was summarized by van Buijtenen and Brown (1962, p. 92) as follows:

> The results of all the experiments indicate that pruning, in general, reduces the number of flowers in the part of the crown which is being pruned. This effect is persistent for several years. In the case of pruning from below, however, the decrease in the production of male strobili, which are normally produced predominately in the lower part of the crown, is compensated for by the increase in production of female strobili in the remaining part of the crown. The decrease in the pollen production appears serious enough to cause some concern. When applied in an orchard, this type of treatment might cause a lowering of seed set. At present none of the pruning treatments applied in this study can be safely recommended for use in seed orchards. Further experiments might go in the direction of studying the effect of moderate trimming from below and slight trimming of the main leaders only.

In Texas, van Buijtenen (1966a) observed that various factors influenced flower production in 8-to 10-year-old loblolly pine seed orchard grafts. Disking stimulated both seed and flower production but mowing did not. Fertilization with a complete fertilizer stimulated female flower production but had a depressing effect on male flower production—total seed production was increased. A spacing of 30 by 30 feet was preferable to 20 by 20 both in flower production and seed production on a per-acre basis. Physiologically juvenile grafts (scions from open-pollinated seedlings of plus trees) produced more male flowers than physiologically older material (scions from the plus trees), but production of female flowers did not differ.

Because rainfall may be low in Texas, it has been

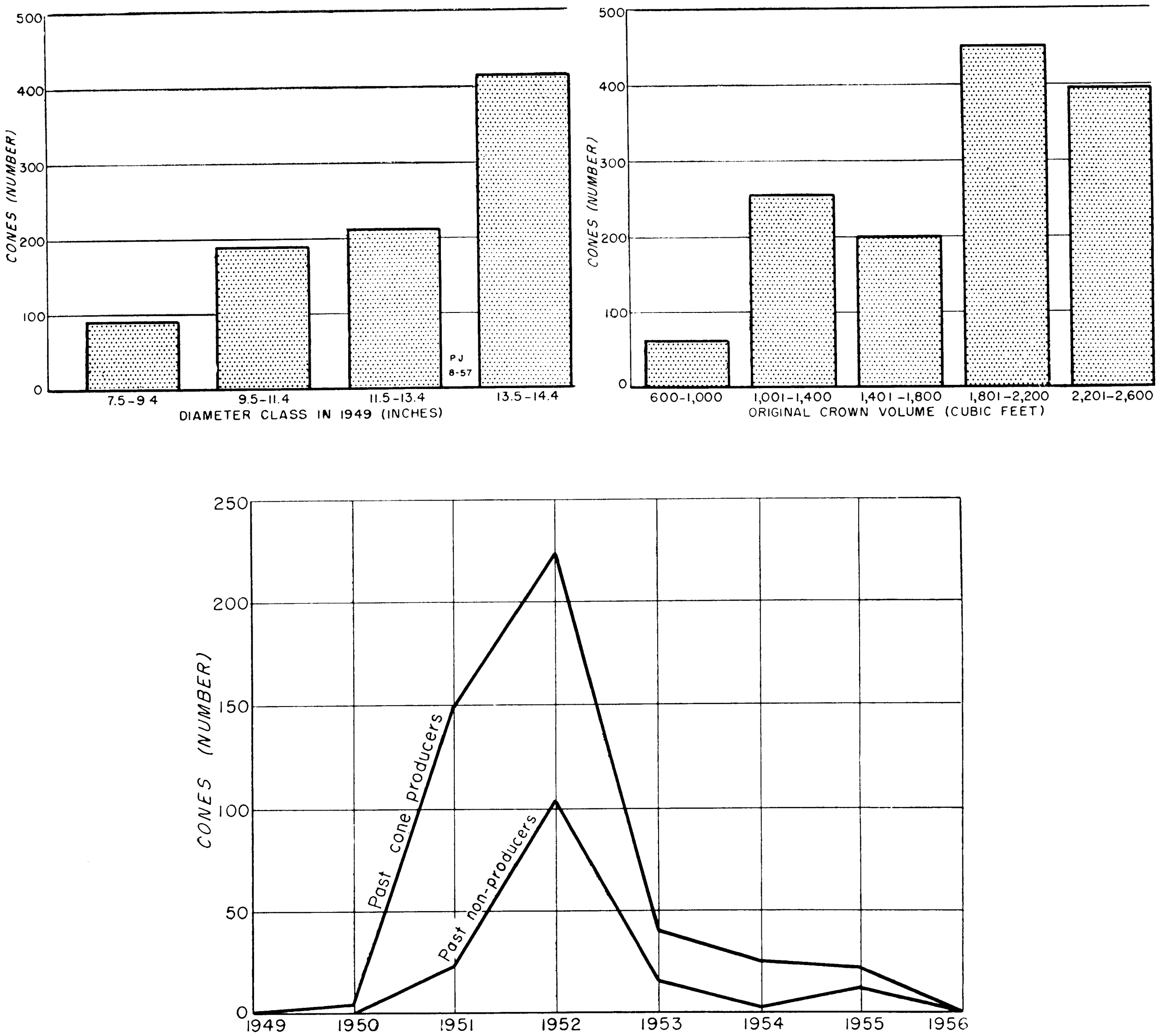

Figure 34.—Cone production relationships in loblolly pine. Average number of cones produced per tree in 8 years, by diameter class (top left), crown-volume class (top right), and average yearly cone production per tree by past production class (bottom). (Grano 1957)

observed that irrigation of loblolly pine seed orchard clones from April through June, although followed by drought from July to September, improved conelet production. Conelet production was not increased by irrigation from April through September or drought from April through June followed by irrigation from July through September (Dewers and Moehring 1970).

Fertilization and cultivation substantially increased the likelihood of both male and female flowering by 4- to 7-year-old loblolly pine in Mississippi (Schmidtling 1971). Application of more than 100 lb per acre of N, 50 lb of P_2O_5 and 50 lb of K_2O did not increase the likelihood of flowering among individual trees, but it did increase the number of cones produced on those trees which were flowering (figures 35 and 36).

In Australia, Florence and McWilliam (1956) found that the maximum production on single trees of both loblolly and slash pines between 13 and 16 years of age can be expected at a spacing of 30 by 30 feet (50 per acre) or wider, and the seed yields will be in the region of 0.78 and 0.44 pounds, respectively, of viable seed per tree. It was observed also that pollen production followed the relationship between cone production and spacing, and that trees on better sites produced more seed than those on poorer sites.

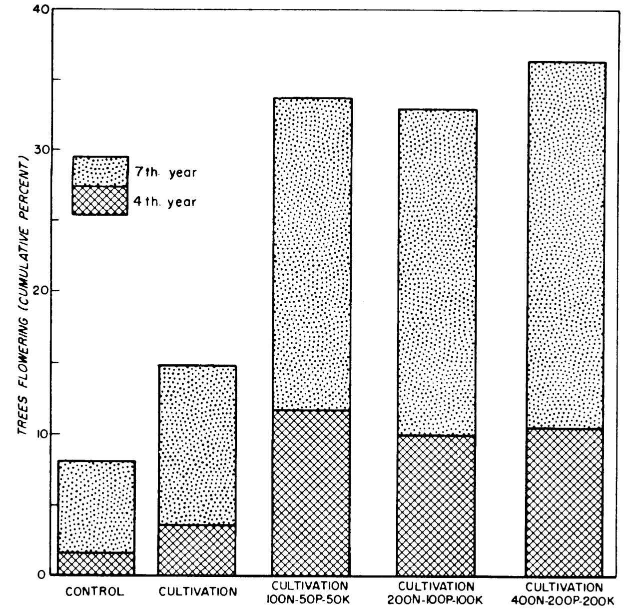

Figure 35.—Female flowering in loblolly pines, by treatment, 4 and 7 years after planting. (Schmidtling 1971)

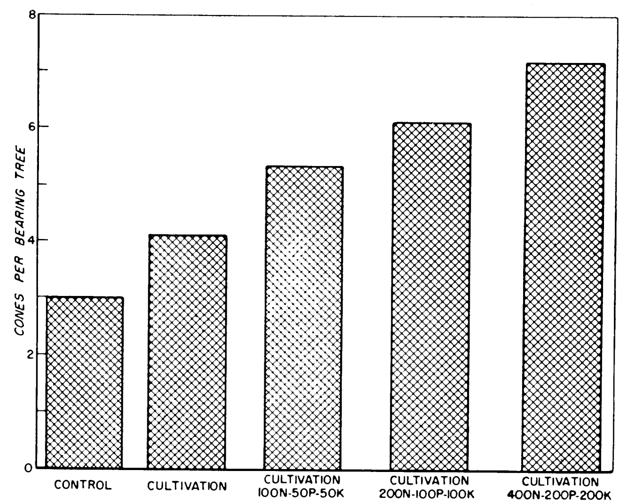

Figure 36.—Cone production of cone-bearing loblolly pines, by treatment, 9 years after planting. (Schmidtling 1971)

In studies of the effectiveness of the Seed Tree Law in Virginia, Stewart (1965) observed that a combination of unfavorable weather can greatly reduce the crop of loblolly pine cones. He concluded that it was safest to complete preharvest release cuttings in the dormant season, or not later than the first of May, to get increased seedfall within three calendar years.

In controlled pollinations and to a somewhat lesser extent in clonal seed orchards, the type of mating scheme used will influence seed yields. Inbreeding depression was found for seed yield, seed quality, and seedling performance in 75 selfed families in comparison to 75 cross-pollinated families in loblolly pine (Franklin 1968a). The trait most severely affected by selfing was filled seed, which averaged 14.3 percent in selfed families in contrast to 74.2 percent for crossed families. Frequency of natural self-fertilization in natural stands was estimated as 2 percent in the upper crown and 31 percent in the lower crown of trees.

In attempts to induce male sterility by spraying pines with growth regulators, Brown and Greene (1961) found that none tested was very effective. Those tested were 2,3-dichloroisobutyrate (FW-450), naphthaleneacetic acid (NAA), methyl ester of naphthaleneacetic acid (MNAA), maleic hydrazide (MH), and 2,4-dichlorophenoxyacetic acid (2,4-D). The most promising results were obtained with high concentrations of synthetic growth substances, especially 2,4-D, but additional work will be required to work out appropriate concentrations and timing of application to insure complete sterility without injury to treated trees. Later, Hong (1963) tested some of the same materials on pitch pine in Korea. Promising results were obtained with concentrations of 5,000 to 10,000 p/m of 2, 4-D (which were best), MH-30, and FW-450. The higher concentrations were more effective, but caused some damage such as foliage burning, chlorosis, and shoot-killing. Phosphon was only partially effective and indoleacetic acid (IAA), indolebutyric acid (IBA), NAA, and gibberellin were wholly ineffective.

In tests of the effect of low-level chronic gamma irradiation of a pitch pine-oak forest, there was a decrease in cone length with an increase in the gamma radiation accumulated by the trees (Mergen and Stairs 1962b). A decrease in seed germination and seedling height also occurred in plants grown from irradiated cones. Flower phenology was retarded by 7 to 14 days in both pine and oak.

Inherent differences among trees, indicated by performance of seed orchard clones, in time of flower maturity and number of flowers produced are sufficiently large to be a factor in selection (Wasser 1967; Zoerb 1969). Observations are summarized in the chapter on variation among trees.

Longleaf Pine

The effect of fertilizing and releasing longleaf pine in south Mississippi was observed by Allen (1953). Eight-inch trees were given a dosage of 19 pounds of a 5-15-5 mix, 10-inch trees 30 pounds, and 12-inch trees 44 pounds of the same mix. Three years after treatment the treated trees produced 12 times more cones than the untreated ones (check trees bearing 1.8 cones per tree) and in the fourth year 4 times more cones than the check, which averaged only 0.9 cone per tree. The 2-year average cone production was 1 to 3 cones for the control treatment, 6.9 per release, 13.5 per fertilized, and 16.7 per fertilized and release. Size of tree appeared to be important: 8-inch trees yielding only 4.6 cones, but 10-inch trees bearing 10.3 cones, and 12-inch trees bearing 13.9 cones. Of perhaps greater significance than the treatment effects was the observation that 3 years after treatment those trees bearing seed in a good seed year were more than three times as fruitful as those trees bearing no cones at the time of treatment. The actual values for both groups was 14.7 cones per tree versus 4.5 cones, respectively. Thus, fruitful trees increased production of cones, but few unfruitful trees started cone production.

Croker (1964) found a highly significant positive correlation between the yield of cones of individual longleaf pine before and after stimulation treatments in Alabama. It was concluded that inherent cone-producing ability is more important in cone production than the cultural treatments used. In this study, 60-year-old trees at a stocking of 30 square feet of basal area per acre were treated for 5 years with 1,900 pounds per acre of 8-13-5 NPK fertilizer and irrigated with 13 inches of water from April through October. Cone production annually per tree was 33 for controls and 43 for treatments, but the differences were not statistically significant.

Collecting a large number of cones of one species in seed orchards or seed production areas is complicated by the shortness of the period between cone ripening and when they start to open. McLemore (1959) recommends that early harvesting be limited to trees on which the cones have specific gravities of 0.88 or less. The fact that cones on any one tree tend to ripen simultaneously lessens the amount of testing that is necessary if ripe cones are to be collected from individual trees. These study results were based on data for 6 cones taken from each of 27 open-grown, 40-year-old longleaf pine on each of four dates in Louisiana. Collection could have begun on September 24, when five trees had ripe cones, and could have continued at least to November 1, when four trees still had cones that had not opened.

Longleaf pine cone production can be stimulated by certain measures. After 3 years, trees that had been girdled more than doubled cone and seed production, but there was no response from strangulation (Mann and Russell 1957). Partial girdling of primary branches in the upper portion of the crown did not affect production of female flowers but it did increase the proportion of branches bearing male flowers (Varnell 1970a).

Environmental conditions beyond the natural range of longleaf do not always limit seed production. At Athens, Georgia, Greene and Reines (1959) found viable seed in cones produced by planted 20-year-old longleaf of North Carolina origin. Naturally regenerated seedlings were found also. This location is about 60 miles northwest of the nearest natural stand of longleaf pine.

Variation in time of flowering in young longleaf pine was observed among trees from different geographic locations (Boyer and Evans 1967), and this subject will be discussed in the chapter on racial variation.

In Louisiana, it was concluded by Shoulders (1968) that annual fertilization of seed production areas was practical if mortality of female strobili could be minimized. Annual fertilization of a heavily thinned, cultivated longleaf pine stand with 1,000 pounds per acre of 15-25-10 fertilizer markedly increased female flower and cone production. Flowering was also related to March-through-July rainfall in the previous year and to the size of the flower crop 2 years earlier. In a given year, the numbers of female flowers of longleaf pine, in descending order of importance by inherent ability to flower, were related to spring and early summer rainfall in the year of flower bud formation, annual rate of fertilizer application, and flowering 2 years earlier (Shoulders 1967). Collectively, these factors and their interactions accounted for 66 percent of the variation in flowering in central Louisiana from 1961 to 1965.

Longleaf pine has large-diameter shoots and the largest cones of all the southern pines. Pessin (1934) and Eggler (1961) made some observations on the effect of cone production on growth of the terminal shoot. At about the end of the growing season the stems produced by the shoots bearing the pistillate strobili were twice the length of the stems produced by those bearing the staminate strobili, while the stems produced by the vegetative shoots were about one and one-half times as long as those produced by the male-bearing shoots. Furthermore, it was evident that the shoots bearing the smaller number of staminate strobili were larger than those bearing the larger number of male flowers. Pessin speculated about the relationship between flower production and twig growth, noting that pistillate strobili are borne in the upper crown area, where branches are fast growing, and staminate strobili in the lower crown, where branches make relatively slow growth.

Shortleaf Pine

Seed yield per cone in shortleaf pine is usually much lower than for other southern pines. Some of the factors and combinations of factors involved in defining seed production problems of the species were listed by Bramlett (1965). The 10-year records of seedfall throughout the Piedmont region showed that three good seed years occurred. In the Georgia Piedmont, seed crops were more frequent than in the northern portions. Climate as well as the physical soil-site environment influenced seed production of trees. Destructive agents, by reducing a good flower crop to a poor seed crop, can obscure the weather effects. In good seed years, sound seed averaged 57 percent, and in the seven poor years it averaged 41 percent.

Shortleaf pine cone production can be influenced by altering certain environmental factors. Phares (1960) and Phares and Rogers (1962) found that in Missouri the optimum stocking level for maximum seed production per acre was 25 to 35 square feet of basal area in 30-year-old stands. Stands thinned selectively and from below produced more than twice as much seed as stands thinned from above, apparently because the larger trees are better seed producers. Removal of the understory hardwoods also significantly increased seed production. The increase in seed production was attributed mainly to the availability of additional soil moisture for the seed trees. None of the test treatments eliminated annual variation in seed crop size. Also in Missouri, Brinkman (1962) found that trees receiving fertilizers with large amounts of phosphorus and potassium produced about twice as many sound seed as control trees.

Applying large amounts of nitrogen resulted in a smaller increase in seed production. The stand in which the treatments were applied was 37 years old and was treated as a seed production area in 1958. The trees ranged from 8 to 16 inches in a stand with basal area of about 90 square feet. The stand had been thinned in 1950 and 1956 and there was an understory of 2,000 to 5,000 hardwood trees per acre.

Partial girdling of shortleaf pine in the Ouachita Mountains in Arkansas resulted in tripled cone production the third year following treatment (Bower and Smith 1961). Girdling, done during the winter, consisted of two half-circles (each 1 inch wide) through the cambium on opposite sides of the stem. The lower cut was about 18 inches above ground, the other about 22 inches. The girdles overlapped about 1 inch. The trees, which had been good seed producers, were 50 to 80 years old with well-formed crowns.

Shortleaf pine clones grafted on loblolly pine rootstocks showed a greater tendency to produce cones than those on shortleaf or slash pine rootstocks (Schmidtling 1969).

Slash Pine

For trees 7 years old in Florida, root pruning, stem injury, and application of 5 pounds of 3–12–6 fertilizer per tree increased the number of trees which bore female flowers as compared to untreated trees (Hoekstra and Mergen 1957). In the stem injury treatments, partial girdling was more effective than strangling by wire. On 21-year-old trees, partial girdling increased the number of female flowers. Of two types of fertilizers, 20 pounds per tree of 7–7–7 mixture stimulated flowering, whereas the same dosage of a 3–18–6 mixture had no effect. Doubling the application to 40 pounds per tree showed no additional effect with the 7–7–7 fertilizer, but increased the flower crop with the 3–18–6 mixture.

In an intensively cultured clonal plantation of slash pine in Florida, there were strong relationships between environmental and inherent factors and flowering plus seed production (Varnell *et al.* 1967; Barnes and Bengtson 1968; Bengtson 1969). Fertilizing with ammonium nitrate increased free arginine, total free amino acids, total nitrogen of the twigs, and total nitrogen of the needles. Fertilizing also doubled female flower production. Irrigation decreased total foliar nitrogen and increased male flowering as an interaction with clones. Cover-cropping with a legume increased arginine content and decreased soluble sugar content of the twigs; it also interacted with irrigation in affecting female flowering. There were highly significant differences among clones in female and male flowering, all measures of nitrogen content, and soluble sugar content. There were also several significant interactions between clones and treatments in affecting flowering and nitrogen composition. Certain clones began flowering earlier than others, but some of the late starters showed signs of greater ultimate productivity.

Also in Florida, flower production was influenced by rate of fertilization and flowering potential of the clones (Goddard and Strickland 1965). In a seed orchard unfertilized for 4 years, ammonium nitrate was applied for 2 years to trees in each of four flowering classes at rates of 0, 1½, and 6 pounds per tree. The general response to fertilization in all flowering classes was similar, but, because of their greater potential, the greatest numerical increase was in the high-flowering category (fig. 37). In this class, flower production of unfertilized clones was 76.8 conelets, while those heavily fertilized averaged 155.9 conelets per tree.

In Louisiana, annual fertilization of a heavily thinned and cultivated slash pine stand with 1,000 pounds per acre of 15-25-10 fertilizer increased flower and cone production. Also, flowering was related to March-through-July rainfall in the previous year and to the size of the flower crop 2 years earlier (Shoulders 1968).

Increased cone production was immediate follow-

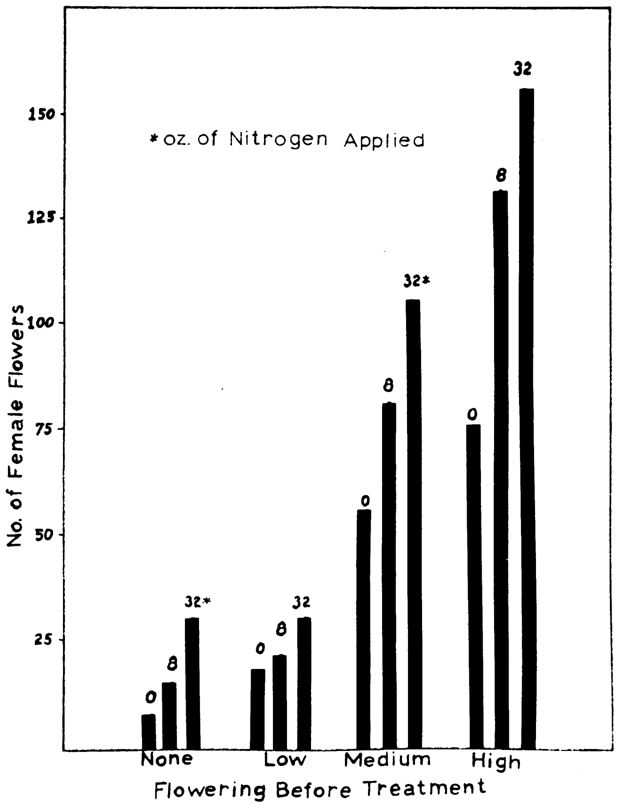

Figure 37.—Ammonium nitrate application is most effective for female flower production of slash pine if applied to trees with a history of medium to high flower production. (Goddard and Strickland 1965)

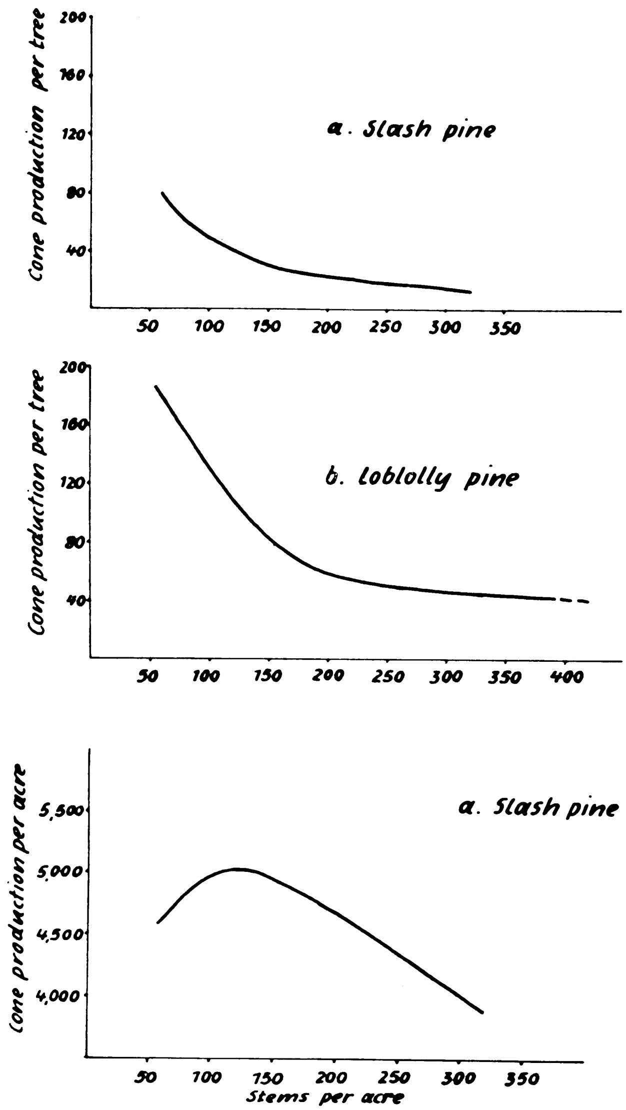

Figure 38.—Cone production per tree and per acre is strongly influenced by number of trees or spacing. (Florence and McWilliam 1956)

ing release of slash pine seed trees in Georgia (Halls and Hawley 1954). There was a substantial increase in cones the third year after cutting, the effect of release being carried over to the fourth year. Released trees (five or six left per acre) averaged 11 inches. The uncut stand averaged 50 square feet of basal area per acre of comparable dominant trees. Both areas were fertilized with moderate applications of phosphate and potash for cattle-grazing experiments.

In Australia, Florence and McWilliam (1956) concluded from a study in slash pine plantations that stands between 13 and 16 years of age and approximately 50 feet in height, if grown initially at wide spacing, can be expected to produce, on the average, 27 pounds of viable seed per acre when grown at optimum spacing of 19 by 19 feet, or 120 stems per acre (fig. 38). Maximum seed production on single trees can be expected at a spacing of 30 by 30 feet or wider (about 50 trees per acre). Pollen production in relation to tree spacing followed about the same pattern as did cone production. The better sites had higher cone production per tree than did the poorer sites. There was no effect of tree spacing on viability of the seed.

In a young slash pine seed orchard, Goddard (1964) observed that grafts planted at 15- by 15-foot spacing had lower flowering per tree and a smaller proportion of trees flowering, but production per acre was higher than with wider spacings because of the greater number of trees. Also, flowering varied among clones from different geographic locations. Clones from the central part of the range produced the most flowers. Trees from northern locations produced cones earlier than trees from southern locations in two racial variation studies (Gansel 1973).

Branch pruning in slash pine clonal seed orchards is of questionable value because it mechanically reduces for at least 1 year the number of branches which subsequently bear conelets (Varnell 1969).

In slash pine, type of cross, whether self-pollinated or cross-pollinated, has a strong effect on seed yield per cone, seed germination, seedling yield per cone, and relative yield of seedlings from self-pollination (Kraus and Squillace 1964b). Selfing lowered the values for each factor, but there were wide differences among the seven maternal parents used.

On branches of slash pine that had produced 44 female conelets and no male strobili, fixing the branches in a horizontal position (90° from vertical) or downward (120°) produced no change in number of conelets produced, but 16 male strobili were produced. A reduction in growth of the treated branches was observed (VanHaverbeke and Barber 1961). Vigorous secondary branches of slash pine, the first-order lateral branches on primary branches emerging from the tree trunk, produced more conelets than slow-growing branches, but conelet production on lateral branches was found not to be related to growth (Varnell 1970b).

In this review of environmental effects on seed production, hereditary factors have not been stressed, but this important subject will be discussed further in the chapters on variation and inheritance. Generally, the southern pines begin seed production while in the seedling stage of growth. Thereafter, production may increase and become consistent and abundant in grafted trees at about age 7 and saplings at age 10. Genetic factors are most important in controlling flower production if young trees have adequate growing space. Thereafter, protection from insects and diseases is necessary; fertilization and irrigation may be helpful in certain locations, and treatments such as girdling are sometimes effective. Refinement of intensive cultural practices to increase flowering is needed.

PROTECTION OF FLOWERS, SEED, AND CONES

Mature cones of the southern pines have admirable characteristics for protecting seed, in that they are resistant to many environmental factors and provide a good storage place. In serotinous species the seed stays viable for many years while still on the tree. Immature conelets or strobili are quite the reverse in that they are soft, succulent, and tempting food. As newly formed growing tissue, they are highly susceptible to low temperatures. Moderately low temperatures of 15° to 20° F, or higher with wind, delay growth or kill the organ.

The southern pines are inherently good flower producers, but environmental factors plus insects and diseases can cause great loss during pollination and cone ripening. To keep losses down in the early stages of seed orchard or production area development, when seed yield should be high and dependable, intensive control measures are necessary.

Weather

In late March of 1955 in northwestern Louisiana, a freeze killed shortleaf conelets that had emerged from the protection of bud scales (Campbell 1955). Low temperatures ranged from 25° to 28° F over a 3-day period. At this time of year certain shortleaf flowers were still in the flower bud, while others were fully developed and receptive.

In southern Arkansas on the same dates, all female conelets of shortleaf were killed when the temperatures dropped to 21° to 24° F over a 3-day period (Schoenike 1955). Male catkins produced very small amounts of pollen during this period. Male and female conelets that opened after the freeze appeared normal and pollen was abundant. However, pollen viability was low. Germination tests of 17 pollen lots from individual trees showed only one with as much as 3 percent germination.

In 1955, in southern Arkansas, Schoenike (1955) observed that loblolly pine produced an abundance of both male and female conelets. Heavy rains over a period of 5 days during the peak of pollen shedding washed much pollen from the air. Actual pollination was only one-fourth to one-third of that expected in favorable weather.

While studying pollen flight from longleaf pine in south Alabama, Boyer (1966) observed that during rainstorms pollen in the air dropped to a very low amount.

Insects

At the Institute of Forest Genetics at Gulfport, Mississippi, 24 different species of destructive insects were collected from the four major southern pines (Coyne 1957a). They were of three main orders—the moths, beetles, and flies. The most troublesome insects in the South are the larvae of caterpillars that feed on the bracts or scales of cones. Flies and midges are the most common pests, occurring in young conelets as white or pink maggots. Yearian and Warren (1964) listed 39 species of insects reared or collected from cones or conelets of loblolly and shortleaf pines. Ebel (1963) collected and photographed insects in northern Florida and prepared a key to common insect damage in flowers and cones of slash and longleaf pines. Insects and diseases of seed orchards have been described, together with recommended controls by Goolsby *et al.* (1972). A bibliography on insects destructive to flowers, cones, and seeds of North American conifers was prepared by Barcia and Merkel (1972).

Coyne (1957b) found that four sprayings, beginning in March on a bimonthly schedule, or 0.5-

percent benzene hexachloride in a water emulsion resulted in a threefold increase in seed yield. There were no bad effects. Usual losses of about 75 percent were reduced to only 30 percent. (These pesticides are not being recommended because of rapid changes in registration and technology. Current registration must be determined prior to selecting a pesticide for actual control of insects and diseases.)

In south Mississippi, a ¼-percent wettable benzene hexachloride or ½-percent emulsifiable benzene hexachloride spray applied in April, June, August, and October resulted in a loss of only 26 percent of first-year longleaf pine cones as compared with a loss of 68 percent on unsprayed trees (Allen and Coyne 1955).

A blister beetle, *Pomphopoea polita* (Say), was observed to have consumed all the pollen catkins on certain shortleaf pine trees in south Mississippi (Allen and Coyne 1956).

While testing a fixed-pipe system for insecticide application on loblolly pine in Arkansas, Grigsby (1964) found that trees sprayed three times with a 0.5-percent benzene hexachloride water emulsion with a spreader-sticker added had only 15 percent of the cones damaged, while an unsprayed tree had 55 percent damaged.

Periodic observations on flower, seed, and cone losses in slash pine in north Florida were made by Merkel (1961). Twenty branches were tagged on each of seven unsprayed check trees in late February. The branches had a total of 332 female flowers. The flowers were observed over a 2-year period. As high as 20-percent mortality of female slash pine flowers killed by thrips was observed on individual trees. There were pronounced differences between loss of seed caused by *Dioryctria* spp. (coneworms) and the seedworm, *Laspeyresia* spp. Losses to *Cronartium strobilinum* (cone rust) were large and may in certain localities approach 100 percent. The life history of *Laspeyresia anaranjada* Miller has been described by Merkel (1967b). In north Florida in late April, 46 percent of 442 flowers examined had been lost and an additional 28 percent of those remaining had injury on cone scales by thrips (DeBarr 1969).

Ebel (1961) observed that thrips (*Gnophotrips piniphilus*) fed on both scales and bracts of slash pine flowers and conelets, leaving tiny, barely visible punctures and abrasions marked by beads of resin. Severe injury caused death of scales and bracts, the entire flower or conelet often shriveling and dying (fig. 39). The insect was found in early January on vegetative buds, was common during February on female flowers or flower buds and on vegetative buds, and continued to appear on young cones and vegetative buds or shoots until mid-May. Thrips damage was found to range from negligible to as high as 20-percent mortality.

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Figure 39.—After pollination is completed, slash pine conelets killed by *Gnophothrips piniphilus* show characteristic resinosis and small size, in contrast to resin-free and large-size, healthy cones. (Ebel 1961)

Thrips injury to female strobili of slash pine was effectively minimized by 0.1-percent spray of heptachlor applied to individual strobili clusters and also by 0.05-percent spray applied periodically to individual trees in combination with the fungicide ferbam (Ebel 1965a).

Details of the biologies of the three *Dioryctria* coneworms as they occur in cones and vegetative parts of slash and longleaf pines have been given by Ebel (1965b).

Merkel and Yandle (1965) conducted a field experiment to compare the effectiveness of hydraulic sprays with mist blower applications of insecticides for cone insect control. They found that cone infestation by *Dioryctria* spp. was significantly lower on all sprayed trees than on unsprayed trees at midseason and at cone harvest. The mist blower application of a 1.0-percent Guthion® water emulsion gave significant control of the seedworm, *Laspeyresia anaranjada*. Initial tests of a systemic insecticide induced into splash pine tree trunks resulted in a 72-percent reduction in coneworm infestation and a 92-percent reduction in seedworm infestation on second-year cones (Merkel 1969).

In Virginia during the spring of 1963, a survey of shortleaf pine female flowers revealed that 9.6 percent of the flowers were killed by pine sawfly, *Neodiprion pratti pratti* (Dyar), larvae. The following spring, mortality from sawfly larvae was 3.8 percent. In 1964, the percentage of flower mortality attributable to sawfly was low. Male strobili were also damaged by sawfly larvae (Bramlett and Hutchinson 1965).

Control of pine tip moth, which causes losses of potential flowering twigs in loblolly and shortleaf pines, may be possible with systemic insecticides (Cade and Heikkenen 1965). In nursery studies with 10-percent granules of three systemic insecticides raked into the soil at 5, 10, and 15 pounds per acre, Thimet® was 88 to 89 percent effective in reducing second-generation attacks, Shell 1836 was variable, and Di-Syston® ineffective. None caused seedling mortality. In a seed orchard where the insecticides were applied within an 8-foot radius of 4-year trees at 25 and 50 pounds per acre, both Thimet® and Di-Syston® at 50 pounds gave 96- to 100-percent control of second- and third-generation attacks. In planting-out trials with 1-year seedlings dipped in a clay root-dip containing 1- or 5-percent dosages, Thimet® and Di-Syston® at both concentrations prevented injury during one growing season, but 5-percent Thimet® was phytotoxic. There were no increases in seedling height growth.

Diseases

Merkel (1958) suggests that cone rust (*Cronartium strobilinum*) may be indirectly responsible for heavy losses in second-year cones by providing a favorable breeding place for cone moth larvae. Field observations in north Florida disclosed that rust-infected cones invariably supported populations of larvae of two cone moths, *Dioryctria abietella* (D. & S.) and *D. amatella* (Hulst.) as early as April. Two months later, in mid-June, attacks on second-year cones were most likely to occur on branchlets where there were first-year cones that had been hit by rust earlier the same season. Apparently, the insect population had built up in the rusted first-year cones and had then moved down the branch to attack the maturing second-year cones.

Observation of 318 wind-pollinated cones of slash pine in southern Mississippi showed 27-percent infection, while none of 229 cones in pollination bags was infected (Jewell 1957). Infection of cones by *Cronartium strobilinum* no doubt occurs during the pollinating period, so standard bagging practices protect them from infection. Healthy and infected cones are shown in figure 40.

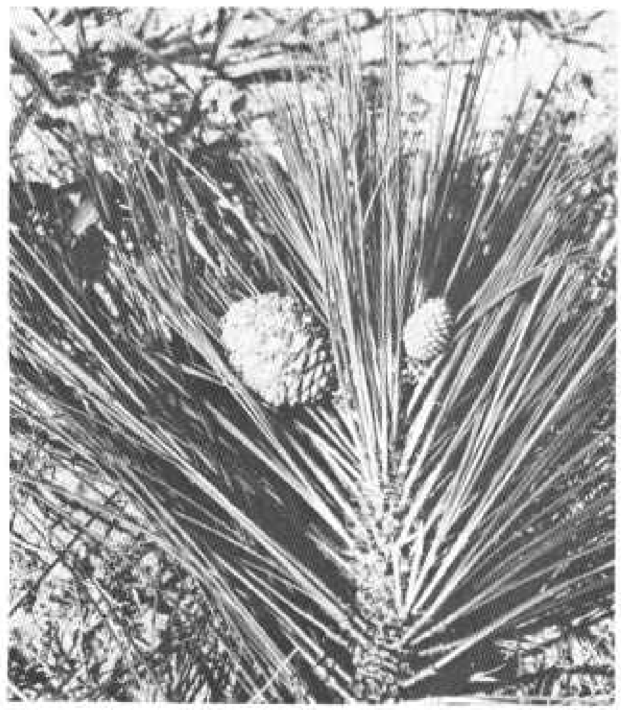

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Figure 40.—In midsummer of the first year, slash pine conelet (left) infected with cone rust caused by *Cronartium strobilinum* is abnormally large in size and decayed on the surface, in contrast to smaller-sized but healthy conelet. (USDA Forest Service 1958)

Cone rust on slash pine can be controlled by ferbam (Lightle 1959). A single spray application of ferbam (2 lb per 100 gal water) was applied to slash pine conelets in four development stages: (1) strobilus tip just discernible in the bud scales; (2) strobilus fully exposed but the scales not open; (3)

strobilus scales open and horizontal, receptive to pollen; and (4) strobilus scales closed, now a conelet. About 10 weeks after treatment, at the time when the rust was fruiting, infection was determined to have been reduced from 70 percent for unsprayed controls interspersed among the sprayed trees to 53 percent for flowers treated in stage 1, and to 39 percent for flowers sprayed in stage 4. It is suggested that at least two sprays might be necessary for adequate protection because flowers on the same tree mature at different times. No phytotoxicity was apparent, but the number and viability of seeds should be examined before this can be verified.

In Florida, Matthews and McLintock (1958) tested various fungicides on germination of slash and longleaf pine pollen. Ferbam, at 2 pounds or 0.2 pound per 100 gallons of water, did not retard pollen germination and may increase it when used as a conelet spray. It was not shown whether ferbam is effective against *Cronartium strobilinum*. A high concentration of the ferbam solution gave highest germination for longleaf pollen and lowest for slash pine. With low concentrations the effect was reversed. Puratized Agricultural Spray, Captan 50-W, and Basi-cop should not be used for control of cone rust regardless of their fungicidal effectiveness because they injure conelets.

Control Equipment

In Texas, cones on trees in seed production areas were protected from squirrels by placing wide aluminum bands around the tree trunks (Texas Forest Service 1957). Generally, squirrels are not considered serious pests over most of the South.

In trials in south Mississippi, a turbine mist blower mounted on and powered by a Jeep successfully and economically sprayed 70-foot pines with benzene hexachloride (Coyne 1957b). Spraying time averaged 3 minutes per tree. A 6-hole whirl plate in the spray nozzle gave the best spray pattern.

Some success has been achieved by the use of fixed-pipe insecticide sprayers in tall trees (Grigsby 1964). A ¼-inch galvanized pipe is strapped to the tree trunk from the base to 2 feet above the crown. A power sprayer forces the chemical into the crowns. The Rain Bird irrigation nozzle No. 20–A, a rotating nozzle with a 7° angle and ⅛-inch orifice, was best because its radial coverage is about 25 feet and the droplet is large enough to prevent undue deflection by wind. Material to outfit a 100-foot tree costs about $12.50 and requires about 2 man-hours to install. The cost of the fixed-pipe system is considered reasonable when breeding work with certain trees is to be carried out for 4 or 5 years.

Summing up insect losses to flowers, seed, and cones, Merkel *et al.* (1959) list the more important problems as biology of the insect, statistical design, reliability of pilots in aerial spraying, safety hazards, assessment of application, and evaluation of results.

The high value of seed orchard trees and the seed produced by them requires much more intensive control measures than are common in forestry. It is to be expected that pest-control research in seed orchards will continue to require much research effort apart from that devoted to breeding traits for resistance.

Observations by tree breeders, entomologists, and pathologists have shown that environmental factors and pests can cause great losses in pine flowers and seed. The losses are important to both research and seed orchard or other seed production programs. Control of environmental factors is difficult except that areas managed for seed production might be placed where probability of losses is low if this is administratively feasible. Considerable success has been attained with control programs for insects and diseases. Development of cheaper and more effective chemicals and equipment to apply them should lead to lower costs.

Research on pest control is continuing, such as with systemics, and new methods should be used as recommended when they are fully tested.

DISCUSSION OF SEXUAL REPRODUCTION

The process of flowering or seed production is well known, but there are striking exceptions to the general process or relationship in almost every stage of seed production, and these may signal trouble ahead in highly specific studies, or, on the other hand, provide the opportunity for solving certain problems. People working with southern pines should be alert to the possibility of these exceptions and not be surprised when they occur.

Age of flower production and time of flower maturity and seed ripening are important to the silviculturist. He may be especially interested in the review of factors influencing seed production, particularly that part dealing with the failure of poor seed producers to respond to fertilizer treatment or other measures to stimulate seed production. The strong evidence for inherent differences among trees in seed production means that attention must be given this trait in any forest treatment where seed production is a factor. The section on protection of flowers and cones has information on the amount and kind of predators that can cause year-to-year variation in seed crops over and above those caused by weather or other environmental factors.

Of particular interest to tree breeders is the evidence for selective fertilization, the variation in seed production as a result of selfing or inbreeding, and inherent variation among trees in seed production areas. Selective fertilization could be an important factor in seed orchard programs if inbreeding is detrimental to production of high-quality seed. Tree-to-tree variation in seed production as a result of selfing could have an important bearing on choice of mating schemes in creative breeding. If it can be done successfully, one cycle of selfing might be a very effective way of increasing gene frequencies for a large number of quantitatively inherited traits. This possibility has not been fully explored. Variation among trees in seed-producing ability is an important factor in selection of seed orchard clones. The fact that inherently poor seed producers do not respond to changes in environmental factors such as fertilization, irrigation, or cultivation means a loss of the investment in these practices in seed orchards, lower total yield of seed, and nonrandom pollination among clones. The smaller the number of clones in a seed orchard, the more important it is that they be good seed producers. Seed-producing ability should be one of the traits given major emphasis in progeny testing seed orchard clones.

Geneticists will be interested in the evidence for tree-to-tree differences in many traits involved in seed production including mutations, age at sexual maturity, selective fertilization, and response to selfing. The inheritance and heritability of these traits are largely unknown.

Much research has been done on flowering in southern pines; these pines are good seed producers, controlled pollinations are now carried out successfully, and seed in volume is being produced in seed orchards and seed production areas.

Although seed orchards are producing seeds, improved techniques might bring them into production at an earlier date or improve production of young grafts during the period when seed of improved quality is urgently needed. It is generally assumed that selfing in pines is detrimental to mass production of seed, in contrast possibly to creative breeding, and that selfing occurs in seed orchards, but we are not sure about the effects of natural selfing on the overall quality of seed orchard seed. This should be investigated. Protection of seed crops will be a continuing problem, and refinements are needed. The costs of breeding for resistance to seed pests in comparison to the costs of environmental control should be investigated. This will require basic-type studies in variation and inheritance of resistance to each pest, and basic studies of plant breeding methods to combine resistance with economically important traits that merge to increase growth, yield and quality of wood products. Basic data such as these are essential if tree breeders are to create the required clones in sufficient quantity for seed orchard use.