CHAPTER 14

POLYPLOIDS, HAPLOIDS, AND MUTATIONS

We are concerned here with major changes in genetics of pines brought about by natural or artificial changes in chromosomes and genes. Crossing within or among species results in a new combination of genes which gives rise to distinctive types of trees, but there are no changes within the genes themselves. We are also concerned with reviewing the nature and amount of natural variants and, in addition, the methods for and results of attempts at artificially creating mutants and polyploids. These are discussed in relation to the importance of mutagenesis in creative breeding with southern pines.

Mutants are the physical manifestations of changes in genes or chromosomes. Polyloidy and haploidy relate to the number of chromosomes. Sometimes there are combinations of mutations and polyploidy or haploidy. Some specialists define mutations, polyploidy, and haploidy in terms of three distinct processes, while others group all changes in chromosomes under the general heading of mutations. Various types of mutagenic treatments create different effects, because they affect different plant parts. Haploid plants have only one set of chromosomes, in contrast to polyploids, which have more chromosome pairs than normally diploid plants. No haploid pines have been identified, but attempts have been made to produce them because they may be one step in the development of homozygous strains. Mixoploid plants have diploid cells plus cells with extra chromosomes.

In genetics, the occurrence and type of mutations are indicative of stability of the species. Low levels of mutation rate indicate strong offspring. Desirability of creating new types of polyploid trees may be indicated by characteristics of trees found in the woods. A high mutation rate may permit selection of extreme types of natural plants; in fact, several commercial varieties of fruit are mutations. If it is difficult to produce mutants and their traits are generally bad, use of mutagenesis in breeding a particular species is not promising. Marker genes resulting from mutations might be useful for some limited purposes to check inheritance, such as amount of pollen contamination in a seed orchard.

Many geneticists point to opportunities for mutation breeding in glowing terms but fail to work out actual methods of applying the techniques. Plant breeders point out that such methods and techniques are extremely difficult to devise.

Since several methods of breeding are available to the plant breeder, he has to choose among them on the basis of such factors as the traits to be improved, the genetics or inheritance of the traits, the species, and, in some instances, the geographic location. The fact that a particular breeding method can be made to work is not sufficient as a basis for recommending it over other methods.

Breeders who select plus trees in southern pines, such as those in seed orchards, may unknowingly utilize traits of mutation origin. Just what effect this will have on performance of the offspring in commercial planting is largely unknown, but it should be disclosed by progeny tests now underway.

Discussion in this chapter is limited to brief review of amount and kind of natural mutants and effects of artificial methods of inducing mutations and polyploids in southern pines. Evaluation as a breeding method is mostly theoretical because little use has been made of mutation, polyploid, and haploid breeding in the southern pines. A more detailed discussion of these methods of breeding, and particularly the advantages and disadvantages, is given in the chapter on methods of breeding. This discussion is followed by a review of methods for and results of attempts to create mutations and polyploids.

Forest tree breeders and geneticists have been cognizant of mutation and polyploid breeding for a long time. In a review of hereditary variation as the basis for selecting forest trees, the kind and amount of chromosomal abnormalities were described (Dorman 1952), and advantages and limitations of the method of breeding have been discussed (Dorman 1962; Cech 1963).

The southern pines were included in a listing of mutant forms in Pinaceae based on a literature review by Franklin (1970).

POLYPLOIDS

Characteristics of Polyploid Pines

Species

Apparently the first work on identification and characteristics of mutant southern pines was that reported on natural polyploidy in slash pine (Mergen 1958b). Abnormal seedlings were collected in the Florida State Nursery at Olustee, Florida, where some 40 million slash pine seedlings were being grown. The aberrant seedlings were located when they were about 3 months old. At this stage, their needles had a bluish hue, and the neighboring normal seedlings had not yet had time to crowd out

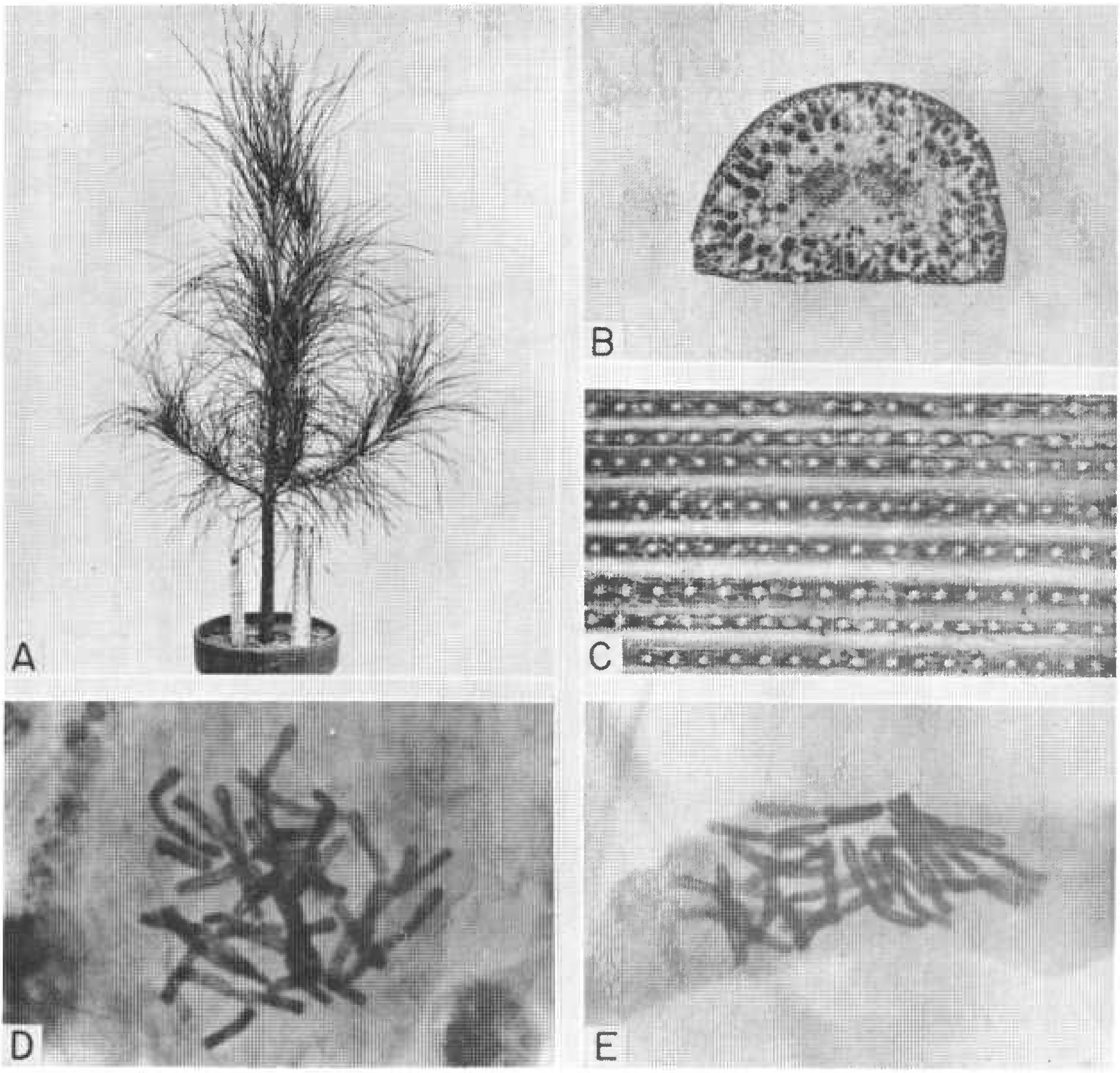

Figure 180.—Normal slash pine seedling. (A) Seedling 2 years old. (B) Cross-section through secondary needle, ×30. (C) Arrangement of the stomata on the outer face of a secondary needle, ×30. (D and E) Chromosomes in metaphase, ×1,000 (2n=24). (Mergen 1958b)

the slower growing abnormal types; the frequency of abnormal seedlings was about 0.0002 percent, and they occurred singly and at random throughout the beds. As the season progressed, the apparent percentage of abnormal seedlings decreased, presumably because they were unable to survive or were crowded out by the larger, more vigorous seedlings. Over 50 abnormal seedlings were lifted and potted in fertile soil, then moved to a greenhouse for further study. Results were reported for five of the seedlings because others did not survive or because accurate chromosome counts could not be made. All abnormal seedlings had underdeveloped root systems, and many lacked lateral roots. In those instances where lateral roots were present, they were short and club-shaped. Comparisons were made between the abnormal seedlings and normal diploid seedlings (fig. 180). When the normal seedling was photographed, it was 2 years old and bore only secondary needles. The needle stomata appeared in straight rows and were uniform in appearance. The chromosome number of mitotic figures in stomatic tissue was verified to be 24, which is normal for pines. One abnormal seedling while in the seedbed had dark bluish needles that were quite thick and long (fig. 181). The height growth was somewhat slower than that of surrounding seedlings, and the hypocotyl

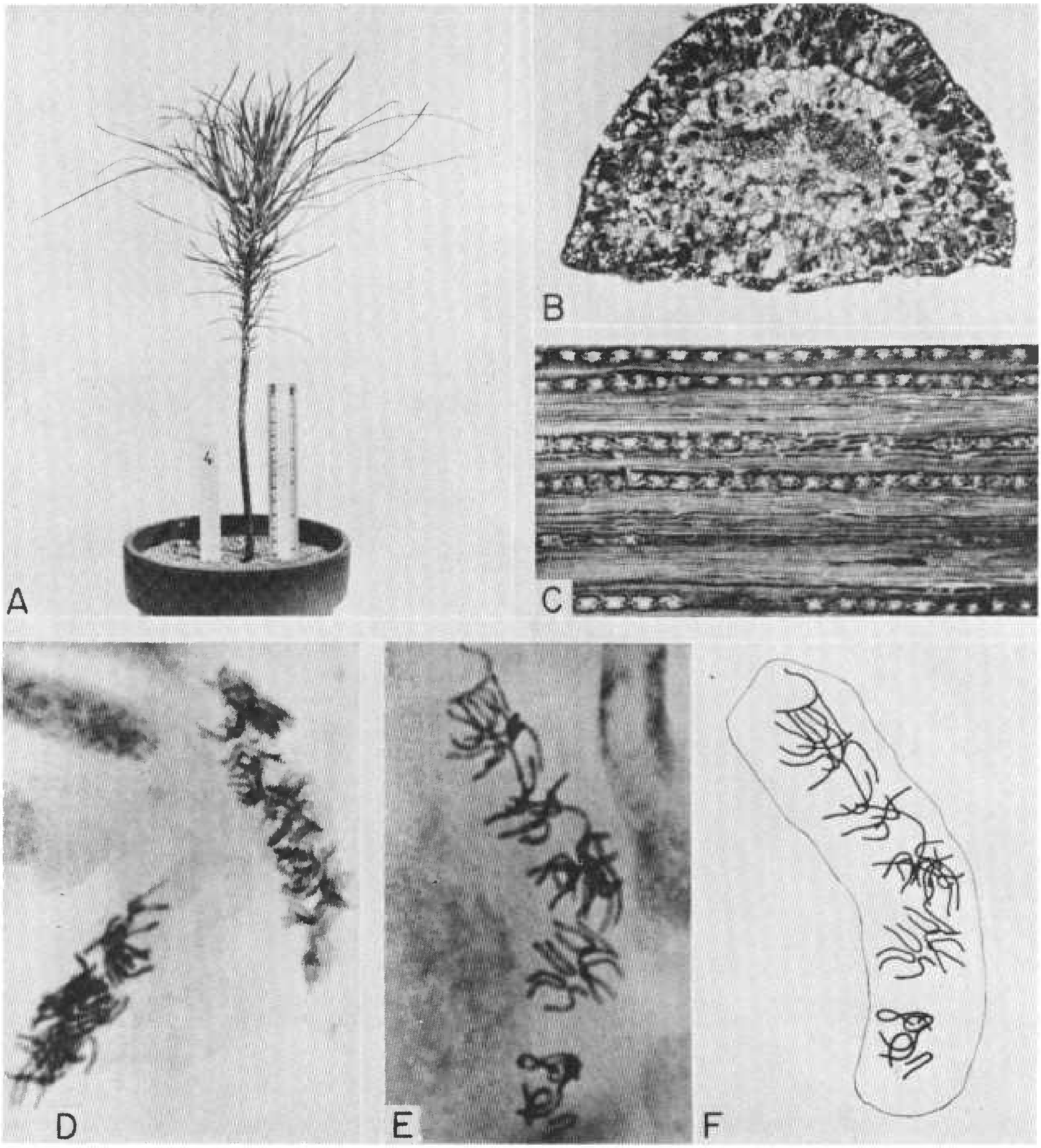

Figure 181.—Abnormal seedling No. 4. (A) Appearance of the seedling at the age of 3 years. (B) Cross-section through secondary needle, ×30. (C) Arrangement of the stomata, ×30. Note the large size. (D) Two triploid cells, ×500 (3n=36). Chromosomes in cell in upper right corner have just separated. (E) Triploid cell, ×800 (3n=36). (F) Idiogram of cell in E. (Mergen 1958b)

and the growing points were thick and succulent. After the early seedling stage, its morphology did not differ appreciably from that of normal seedlings, with the exception of the secondary needles, which were much thicker. The height growth also remained slower, and after 3 years, it had formed only one side branch, which was 3 cm long. The root system consisted of one main root with several well-branched lateral roots, the roots ending in enlarged knobs which were brittle. The needles were 1.60 mm wide, as compared to 0.99 mm in the normal seedlings. The anatomy of the needle appeared normal, with the exception of the stomata, which were arranged in uneven rows and were much larger, their diameter on the outer surface being the largest (51.8 μ) in any of the seedlings examined. Chromosome counts showed seedling No. 4 to be a mixoploid. It differed from the other plants in its relatively high frequency of polyploid cells in the bases of the needles—13 polyploid cells to 1 diploid cell. Of the polyploid cells, about three-fourths were tetraploid, and the remainder were triploid. The number of triploid cells was higher than in the other tree studied, the ratio of diploid to polyploid cells in the root tips being 1:2. Several stages of mitosis could be observed in the dividing cells. There appeared to be no tendency for the chromosomes to move toward the equatorial plate during metaphase, and during anaphase the daughter chromatids remained in an apparently random location within the cell. This partial mitosis doubled the number of chromosomes within the cell because no cell wall formed to separate the daughter chromosome.

An additional abnormal seedling described by Mergen (1958b) was 4 cm tall at 18 months of age when photographed. When 30 months old, it had not formed secondary needles (fig. 182), and, thus, all the descriptions of the needles refer to primary needles. Periodically, the seedling developed a large, succulent terminal bud, but it took considerable time for the bud to enlarge. The root system was almost nonexistent, and the growing point died back from time to time. Because of the extremely stunted growth, few slides were obtained which showed mitotic division. The needles had a well-differentiated epidermis, but the hypodermis and the sclerenchyma cells were almost lacking in most of the slides examined. As compared to primary needles of diploid plants, the palisade layer was better developed, occupying about four-fifths of the cross-sectional area; the stomata occurred in irregular rows and were small. It was possible to establish that the plant was heteroploid on the basis of slides that were prepared. Idiograms were drawn from several diploid and some tetraploid cells, but no triploid cells were located. There were, however, several aneuploid cells which had a chromosome count of 54. The ratio of diploid to heteroploid cells was approximately 1:7.

In studies of chromosome complement of cells in abnormal slash pine, none of the conventional methods tested was found to be satisfactory (Mergen and Novotny 1957). The chromosomes of the pines are very long, with a tendency to become "tangled" within the dividing somatic cells, making it difficult to obtain accurate counts. Slides for chromosome counts must contain mitotic divisions in which the metaphase chromosomes are well separated and short and are distributed throughout the cell in one plane. They also should be stained heavily so that they contrast sharply with the cytoplasm. A schedule consisting of 10 steps for the preparation of slide material for chromosome studies was used.

Interspecific Hybrids

Polyploids and mixoploids occur in hybrid progeny of southern pines. In a nursery, 0.9 percent of the slash pine seedlings and 5.2 percent of the slash × shortleaf hybrids were dwarfs. After 4 years, the percentages were 2.5 and 12.5, respectively (Schmitt 1969; Schmitt and Snyder 1971). Dwarf mixoploids occurred at a frequency of 0.0008 percent, which is higher than the rate of 0.0002 percent reported by Mergen (1958b). In plots of shortleaf × slash hybrids used in tests for tip-moth control, 16 percent of the trees were dwarf (Grano and Grigsby 1968).

Some of the variants of the backcross hybrid *Pinus rigida* × (*P. rigida* × *P. taeda*), which appeared at the forest nursery of the Institute of Forest Genetics in Korea, varied in chromosome number (Hyun *et al.* 1967). The seedlings with unusual characteristics were classified into four types as follows, based on the form of the needles: type 1, long and curled needles; type 2, short and curled needles; type 3, short and stiff needles; and type 4, short, thick, stiff, and uneven needles. From cytological investigation, the number of chromosomes of somatic cells was found to be 2n = 48 in type 4, and 2n = 24 in types 1, 2, and 3. Individuals of type 1 showed normal height growth; however, individuals of types 2, 3, and 4 were dwarf, and the length of the needles of types 2 and 3 were only 31.5 and 13.8 percent of the needle length of the control, respectively. The width and thickness on needles and central cylinder were decreased in types 2 and 3 but increased in type 4, and no difference was found in type 1 compared with the control. Types 1 and 4 showed 56 and 16 percent increase, respectively, and type 3 showed 23 percent decrease, whereas type 2 showed no difference compared to the control in the number of resin canals in the cross section of the needles. Most of the needles of types 2, 3, and 4 showed one layer at the hypodermis. Stomata length of type 3 increased 15 percent and that of type 4 decreased 24.4 percent compared with the control; types 1 and 2 were not different from the control. The length and thickness of tracheids increased 10 and 23 percent, respectively, in both types 2 and 3, but they decreased 26.4 and 10.6 percent, respectively, in type 4. The diameter of pollen grains increased 34.2 percent in type 4, and the germination and elongation of pollen tubes of the variants were retarded as in induced polyploids, but no difference was observed among various groups.

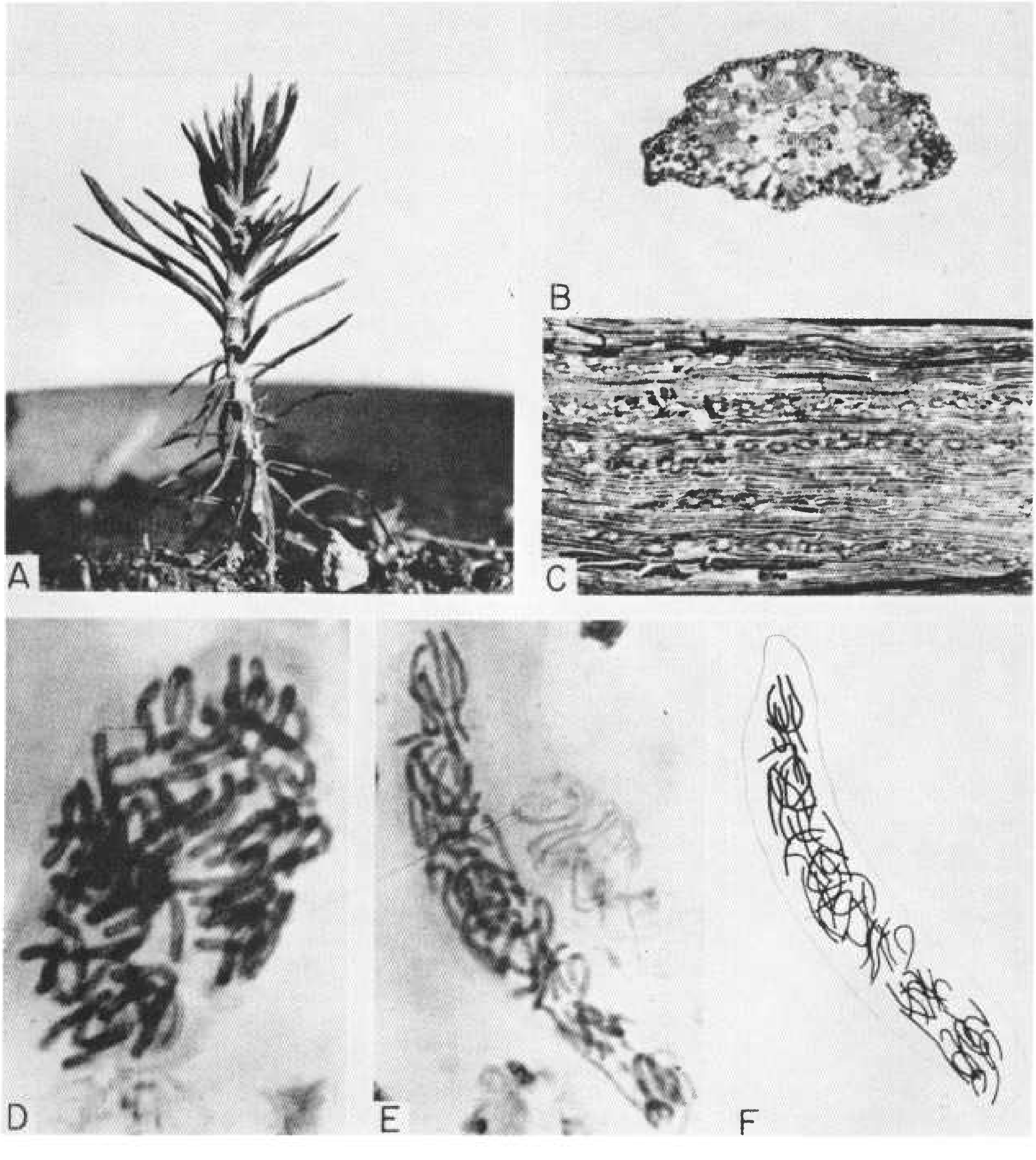

Figure 182.—Abnormal seedling No. 5. (A) Appearance of the seedling at the age of 18 months, ×1.1. (B) Cross-section through primary needle, ×40. (C) Irregular arrangement of stomata on outer face of needle, ×30. (D) Tetraploid cell in metaphase, ×1,200 (4n=48). (E) Heteroploid cell with 54 chromosomes, ×800. (F) Idiogram of chromosome complement of cell in E. (Mergen 1958b)

Induction of Polyploidy

Seed of pitch pine treated with colchicine produced tetraploid seedlings which had thick and irregular needles and poorer height and diameter growth than normal seedlings (Kim *et al.* 1967).

In tests of colchicine-induced polyploidy in pines, Mergen (1959b) found that many of the polyploid plants reverted to a diploid growth because the polyploid cells divided at a slower rate and were swamped by the faster growing diploid cells which surrounded them. By treating immature male strobili, the percentage of imperfect tetrads was increased over normal occurrence. None of the slash pine polyploids produced had any obvious features that would make them more desirable from a forestry point of view. On the contrary, their outward features make them less desirable, although nothing is known about their ability to resist insects and diseases. Significantly lower rates of photosynthesis were measured in both primary and secondary needles of polyploid slash pine seedlings, as compared to diploid plants, but polyploidy did not significantly affect rate of respiration (Bourdeau and Mergen 1959). The compensation points for both the primary and secondary needles in the polyploids were also higher. It is thought that the lower rates of photosynthesis contribute to the slower growth rate of the polyploids. The primary needles of both diploid and polyploid plants were more efficient at low light intensity than secondary needles, and they had lower compensation points.

HAPLOIDS

Haploid plants have only one set of chromosomes such as are found in gametes. In addition to having some value as botanical oddities, they deserve study from the standpoint of possibilities for using the technique as a breeding method. Mutations induced in haploid plants are readily visible, and doubling the chromosomes leads directly to homozygous individuals (Nitsch and Nitsch 1969). If homozygous diploid plants can be effectively produced, this would bypass many generations of inbreeding (Brown 1967).

Advantages and disadvantages of haploid breeding in southern pines cannot be evaluated until techniques for producing trees are perfected. Brown (1967) has pointed out that all attempts to establish pollen cultures of any southern pine and some of the hardwoods have been unsuccessful, although some of the basic work was successful with ginkgo. Tobacco has a natural tendency to produce haploid plants from pollen grains, as indicated by the appearance of two different species hybrid groups (Nitsch and Nitsch 1969). Thus, haploid breeding, as far as efficiency is concerned, may be much easier to test with certain plant groups than others. If haploid plants can be produced and chromosomes doubled, problems still remain in creation of desirable mutations and in production of plants with desirable traits or greatly improved combinations of traits. Also, there is a question about the place of genetically identical plants for monoculture in southern pine forest land management. It is important in genetics research not only to develop the biological basis for a breeding method but to perfect techniques of using it under field conditions.

MUTATIONS

Characteristics of Mutations

Based on albino frequencies and other assumptions, mutation rate in slash pine was computed to be 0.052 percent (Squillace and Kraus 1963). Inasmuch as this is much higher than mutation rates commonly found for other traits and organisms, the authors speculated that the rate in slash pine is actually much lower in that some factors, such as heterozygosis preference favoring heterozygotes over both the homozygotes, may be operating to maintain the gene at such a relatively high frequency. Of 297 wind-pollinated progenies, 11 contained one or more albinos. Albino frequencies in these progenies varied from 0.4 to 7.6 percent. All of the progenies containing one or more albinos came from parents located within the range of typical slash pine. None of the South Florida slash pine trees bore albinos. One lot of selfed seed produced 11 albinos out of a total of 34 seedlings, or 32.4 percent. This frequency was not significantly divergent from a 3:1 ratio, as determined from a chi-square test, and fits it better than other likely ratios, such as 9:7.

In breeding slash pine for high oleoresin yield, a few trees were found that produced yellow instead of transparent oleoresin. One tree was selfed and outcrossed; all of the selfed seedlings but none of the outcrossed seedlings produced yellow oleoresin. The trait was carried as a homozygous recessive, but the number of genes involved could not be determined (Kraus and Squillace 1964a). Also, in progeny tests of high gum yielders, two slash pines with virescent foliage occurred. The foliage each year showed alternating patterns of bright yellow and green, and the color was evident only during the driest summers. By late fall, both trees developed a normal green color. When the trees of virescent foliage were crossed, the seedling showed the heritable nature of the chlorophyll deficiency, but the mode of inheritance was not indicated.

Pond pine dwarf types segregated in an open-

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Figure 183.—Dwarf, left, and normal pond pine seedling, right. The dwarf abnormality segregated 1:1 in families from wind pollination.

pollinated family from seed collected from a witches'-broom on a tree in Florida (Franklin 1968b). Mutant seedlings were profusely branched, but color was normal (fig. 183). In sand pine, dwarfs occurred in ratios of 1:1 in several seedlots from witches'-brooms (Johnson *et al.* 1968). Apparently, no pollen was produced on the brooms; therefore, it was assumed that fertilization was accomplished by normal pollen.

After careful observation of seedlings in 119 selfed families from loblolly pine in a natural stand, Franklin (1969c) concluded that large numbers of mutant alleles were carried in the heterozygous condition. Twenty-two phenotypically dissimilar mutant forms were found in 30 of the families. Mutant forms were observed showing cotyledonary chlorophyll deficiencies, abnormally colored primary and secondary foliage, abnormal hypocotyl color, and morphological abnormalities (fig. 184). In many families, seedlings segregated in ratios of three normal to one abnormal, the classical Mendelian segregation pattern for a factor controlled by a single locus with two alleles, one of which is dominant. Three families segregated for two phenotypic abnormalities, and one family segregated for three.

In loblolly pine, Franklin (1972) found a sample mean of 8.5 lethal equivalents per zygote or an

Figure 184.—Abnormal loblolly pine seedlings. (Left) Fused cotyledons that were usually lethal and segregated 122 normal to 55 abnormal in families from self-pollination. (Right) A seedling with fused cotyledons which may live because the apical bud broke through the fused tissue. (Franklin 1969c)

average of 0.35 lethal equivalent per chromosome. Difference among trees was 1 to 26+ embryonic lethal equivalents per zygote among 132 trees. A lethal equivalent is a group of mutant genes of such number that, if dispersed in different individuals, they would cause on the average one death, that is, one lethal mutant, or two mutants each with 50 percent probability of causing death.

Chromosome structure and behavior were slightly more irregular in interspecific hybrids of southern pines than in parent trees, but irregularities of some types were found in pollen mother cells of all 35 trees studied (Saylor 1967). Univalents (unpaired chromosomes in meiosis) were the most common irregularity observed, with frequencies greater than 1 percent found in 15 trees for this abnormality. Other irregularities that exceeded 1 percent were as follows: lagging chromosomes, seven trees; fragments, seven trees; bridges, eight trees; and micronuclei, five trees. In a natural population, irregularity frequencies were 16.7 percent in loblolly pine, 16.7 percent in pond pine, and 31.8 percent in the hybrid.

The hybrid of longleaf × shortleaf pine is very difficult to make, 75 crosses producing only 5 seedlings. Two mutations occurred, one an albino and the other a dwarf (Campbell *et al.* 1969).

Induction of Mutations

Although there has been no large-scale attempt to induce mutations as part of forest tree breeding, there have been numerous observations on the effect of irradiation on physiological processes of a few pine species and on the effect of colchicine to induce mutations. In studying the effects of ionizing radiation on loblolly pine, researchers observed that the lethal dose was not necessarily the only dose that affects the ecology of the species (Pedigo 1963). Pine pollen disseminated by wind will be an effective mode of propagation from mutations that occur in one specific area.

Seed

Loblolly and shortleaf seedlings from pine seed receiving 1,000 roentgens of X-rays were less capable of survival and growth than untreated seed, nor was cone production stimulated during 2 to 7 years after outplanting (Snyder *et al.* 1961). A dose of 2,000 R was lethal to longleaf pine and slash pine seed. A dosage-mortality response in 1-year survival was obtained when stratified loblolly pine seeds were irradiated with 200, 400, 600, 800, and 1,000 R of X-rays. Prestratification for 3 weeks, in conjunction with dosage of 800 R and above, caused marked reductions in germination percentages. Filtering the rays by copper substantially decreased germination and green weight of loblolly and slash seedlings when seed had been stratified 3 weeks. Stratifying slash pine seed predisposed it to growth damage from high dosage of irradiation. Prestratification of shortleaf pine seed caused similar damage at high dosage.

In studying the sensitivity of pine seed to neutron, gamma, and X-ray irradiation, Yim (1963) observed that sensitivity of pitch pine seed varied with seed size, the smallest seed being the most sensitive. Radiation damage, as determined by percentage germination, increased with length of storage time of seed after irradiation count, this effect being more marked for X-rays than for gamma rays. With the 16-hour exposure period and a total exposure of 5 kR, there were significant differences in seed germination between individual control trees and 10 pitch pine trees used in the test (Mergen and Cummings 1965). Seed from the upper crown were generally more sensitive to gamma rays than those from lower positions. The variation in radiosensitivity was not related to differences in seed size. Highly significant differences were shown between and within trees in seed weight, with a highly significant interaction between trees and position. External characteristics of the seed varied considerably. Seeds of one tree were large with smooth, shiny black coats, while all the seeds of one other tree had heavily etched brown coats.

Irradiation of one stratified slash pine seedlot with Cobalt-60 gamma rays at dosages of 25,000 and 100,000 R adversely affected the rate and degree of germination (May and Posey 1958). An irradiation level of 5,000 R adversely affected the degree but not the rate of germination of unstratified slash pine seed. Irradiated doses of 500 and 5,000 R delayed initial germination of unstratified slash pine seed. After germination started, rate of germination was similar to that of nonirradiated seed.

Seedlings of loblolly, slash, and longleaf pines from seed irradiated at doses of 10,000, 20,000, 30,000, 40,000, and 50,000 R at 620 R/minute varied by species and treatment in survival (Davis 1962). None of the longleaf pine survived 4 months after planting. Survival of loblolly and slash pines receiving 10,000 R was 28 and 32 percent, respectively, after the same period, compared with percentages of 46 and 57 for controls. Eight months after outplanting, growth and survival of loblolly pine seedlings was better than that of slash pine. One year after loblolly pine seed was exposed to the six treatments between 327 and 6,530 R at 653 R/minute, surviving seedlings showed no significant difference in growth because of treatment. Dormant 1-year-old loblolly pine seedlings exposed to 500, 1,300, 1,800, 2,300, and 2,800 R at 2.70 and 6.95 R/minute showed growth to be affected by intensity of dosage as well as total dosage. Survival percentage was slightly better for the controls than for the seedlings given the 500 R treatment. A sharp decrease was noted in survival and growth for doses over 500 R, and results were about the same for slash pine seedlings.

Ionizing radiation had multiple effects on seed germination and seedling growth of pitch pine (Mergen and Johansen 1964). Seed were exposed to gamma irradiation while they were still in the cones on the trees. Rates of up to 130 R/day and a total exposure of 16,000 R radiation did not affect germination; however, at an exposure rate of 295 R/day, germination was reduced after a total exposure of 8,000 R. A temporary stimulation in root length and an increase in fresh weight of the seedlings occurred at an exposure of 6,000 to 8,000 R, and a temporary retardation in overall growth occurred at exposures above 8,000 R. The weakened seedlings were subject to damage by micro-organisms. An increase in nuclear disturbances took place as a result of exposure, but mitotic divisions were normal up to the highest level of exposure (22,700 R). The initial retardation in height growth as a result of ionizing radiation was not observed after the seedlings were 1 year old. After two growing seasons, no statistically significant differences in height were found between irradiated seedlings and controls.

Percentage of seed germination and seedling growth, physiological tolerances, and survival in both slash and longleaf pines usually varied inversely with the radiation dose (McCormick and McJunkin 1966). Germination rates, drought tolerances, and growth under low light intensities were increased by 100 to 500 R radiation doses. Slash pine seeds were more sensitive than those of longleaf pine, but in the seedlings this was reversed. In slash pine, doses of 300 R and below enhanced growth and survival under certain environmental conditions (McCormick 1967). Application of 150 mg/liter concentrations of gibberellic acid increased the radiation tolerance of seeds and seedlings.

Seedlings, Sprouts, and Trees

Chronic exposures of pitch pine seedlings to Cobalt-60 radiation caused several differences in growth (Mergen and Thielges 1966). Potted 1-year-old seedlings were exposed to 1/20 R/day from the first of June onward and were removed to a neutral environment after 156 to 485 days. Exposures as low as 3 R/day reduced the number of subterminal buds, and 15 R/day inhibited axial primordia. Shoot growth generally decreased with increasing exposures, but, at the highest exposures, basal and lateral sprouting increased. After removal of all surplus growth following the second growing season, sprouting occurred with a total exposure of up to 6,700 R, suggesting that trees may be able to survive despite high apical sensitivity. A seedling exposed to 20 R for about 300 days ending in late March developed two microsporangia on a basal sprout that had reached the first vegetative division (a state usually reached in 9 months) on April 20.

In epicormic sprouts of pitch pine trees growing at different levels of chronic Cesium-137 of 1.6 to 13.5 R per day, frequency of nuclear damage at the base of the needles was high at low levels of exposure and increased with increasing exposure (Mergen and Thielges 1966). Average diameters of resin ducts in irradiated sprouts were twice the average found in control material.

Chronic gamma radiation affects the distribution of radial increment in pitch pine stems (Woodwell and Miller 1963). High-level ionizing radiation used even in amounts considered below that accepted as lethal was found to significantly affect the xylem structure of both pitch pine and shortleaf pine (Hamilton 1963). Tracheids in pitch pine were reduced by 17.9 percent in length, 10.0 percent in lumen diameter, and 17.0 percent in wall thickness during an 8-year chronic exposure.

Doses of about 25,000 R apparently cause death of loblolly pine seedlings. Seedlings exposed during the cotyledon stage survived better than older seedlings (Pedigo 1960). Accumulative dosages at average rates as low as 5 R per day killed a high percentage of pitch pine trees after exposure for 7 years. The same dosage rate did not produce obvious effects after exposure for 8 months. Surviving trees showed various degrees of morphological deformation (Sparrow and Woodwell 1962). As a result of the effect of irradiation by a Cesium-137 source on exposed pine-oak forests, it was predicted that during 1 year 90 percent of the pitch pine population would die from exposures of 15 R per day and higher. In summarizing the cumulative radiation effects on sexual reproduction in pine and oak, Mergen and Stairs (1962) concluded that trees which had been severely damaged by chronic low-level ionizing radiation were able to differentiate floral structures and produce viable seed. However, floral phenology was retarded, and pronounced morphological aberrations were induced. During the meiotic processes in both pines and oaks, visible cytological evidence of chromosomal aberrations was obtained. Bridging and fragmentation of the chromosomes were observed at Anaphases I and II. Pollen abortion in both genera showed a significant increase with an increase in irradiation level. A decrease in pollen germination was observed in the pines, but the pollen tube length in both genera did not show a decrease. The progeny from irradiated pine trees was more variable in height than the controls after one growing season. The seedlings also showed the following abnormalities: shorter and longer needles, twisted needles, irregular growth pattern, and lack of primordia on the leaders. The effect of ionizing radiation on megasporogenesis in pitch pine was to delay fertilization for about 7 days (Mergen 1967). In addition, the chromosomal aberrations were also observed in several cells during mitosis.

DISCUSSION

Alterations of chromosomes have occurred naturally at low irradiation frequencies and have caused unusual chemical changes or malformed seedlings. Multiplication of chromosome number in the entire seedling or in parts of seedlings occurs in pines and generally results in decreased vigor and malformed structures.

No haploid plants in the natural state have been found, and attempts to create them by culturing pollen grains have been unsuccessful.

Selfing has been possible with most individual trees and has revealed that many types of mutations exist in native stands. Numerous dwarf-inducing mutations occur in species and in interspecific hybrids, as do irregularities in chromosomal structure and behavior.

A small number of attempts to create polyploid trees has produced mixoploid plants, but no polyploid strains have been developed.

Progress has been made in evaluating dosages for irradiating seed and seedlings, but techniques are not yet highly refined.

Mutations and polyploid plants are not common in natural stands, probably because of their low vigor, which makes it difficult to compete with other plants. However, only one systematic survey of a loblolly stand for mutations or polyploids has been made.

Mutant forms among the southern pines seem to be similar to those in conifers and other plants in that most of them are undesirable for commercial use. However, certain dwarf forms may be useful as specimen trees for landscaping. Evidently, no southern pine trees of proven high-value traits of mutation or polyploid origin have been created or discovered.

Southern pines are not highly self-fertile, as indicated by low yields of viable seed and low vigor in offspring. Selfing has been used in slash and loblolly pines to indicate frequencies and kinds of mutations present in natural stands.

Should mutant strains be produced in the future, modification of nursery and silvicultural methods might be required because the trees will have characteristics equivalent to separate species. If mutations yield trees which must be propagated vegetatively, stands will be highly uniform as far as inherent differences among trees are concerned and will have to be managed accordingly as a monoculture.

The most important need in the future is for ways to detect mutations and polyploid trees that occur by chance and that have desirable combinations of traits useful in forestry. Also, there is a need for methods of directing the formation of desirable mutations by artificial methods.

Before mutagenesis can be used in large-scale tree breeding, optimum treatment levels should be determined for the three major groups of mutagens: ionizing radiation, ultraviolet radiation, and chemicals. The next step should be to develop techniques for using mutagens to create preselected changes in specific traits and—what is of great importance—good combinations of traits. Also, methods are needed for using the new plants or parts of plants as foundations for improved strains. Of these, the task of learning how to direct the formation of desirable mutations is probably the most important obstacle to using the breeding method at present. With present technology, treatment effects are random, and large numbers of trees must be grown and evaluated over long periods of time. This is costly even with annual plants of small size, but with large trees that live many years, it is prohibitive. Mutation breeding should be combined with other methods such as selection and hybridization.

Development of polyploid southern pines is not considered an important method for tree improvement, because natural polyploids perform poorly. If it can be shown that certain polyploids have desirable traits, development of methods for creating them will assume higher priority. Costs of mutation breeding should be estimated before the method is chosen in preference to other methods.

The most important need in haploid breeding seems to be a method for culturing haploid tissue and producing plantlets. Until these obstacles are overcome, it will not be known whether genetically improved trees can be produced by chromosome-doubling.