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EVALUATION OF SPONTANEOUS, CHEMICAL, AND RADIATION-INDUCEDMUTATIONS IN THE PINACEAE*

by

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Introduction

During meiotic and mitotic cell divisions in the Pinaceae and other plants, the chromosomes usually behave in a predictable and regular manner, and the genes reproduce themselves without error. During mitosis each duplicated chromosome is divided in such a way that the nuclei of the daughter cells are genetically identical to each other and to the parent nucleus from which they were derived. When this regulated process during division is altered a sudden heritable change takes place. Any sudden heritable change in this system is termed a mutation. These apparently spontaneous alterations may involve specific chemical changes in a single gene, or they may be changes in the chromosome quantity and quality. When at the gene level, there is a permanent genetic change into a different allelic form. When on a chromosomal level, there can be breakages, deficiencies, duplications, translocations, inversions, and other aberrations that affect the genotype and phenotypic expression.

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For this presentation the term "mutation" will be used in a collective sense, and will refer to any heritable change in the genetic system.

Natural Mutations

In the discussion of natural mutations that occur in forest trees, it should be stressed at the outset that the phenotypic expressions of trees are brought about by genetic (Mendelian) combinations, modifications caused by the interaction of the genetic system with an environment, and may be altered during the development of the tree through the occurrence of spontaneous mutations. The Mendelian combinations have as their origin spontaneous mutations that occurred during previous generations. For this discussion I will limit my comments to the spontaneous mutations that become apparent during the current generation.

In the Coniferales, and especially in the members of the Pinaceae, the chromosome number is quite uniform and stable. Polyploidy and other major chromosomal aberrations are rare. With two exceptions, all species have a gametic chromosome number of 12, with $2n = 24$. Their karyotypes, however, indicate that within a genus there are differences in overall length, position of the centromere and presence of secondary constrictions (Sax and Sax, 1933; Mehra and Khoshoo, 1956; and Saylor, 1961). In one species of Abies, satellites have also been reported on three chromosomes (Mergen and Lester, 1961b). The remarkable constancy in chromosome number would indicate that species differentiation took place as a result of gene mutations. There are, however, two species that do not have a gametic chromosome number of 12. These are Pseudotsuga with $n = 13$, and Pseudolarix amabilis with $n = 22$. Pseudolarix amabilis is a monotypic species with a restricted range in the mountainous region of eastern China. Although of importance from an arboricultural viewpoint, its

usefulness as a timber tree is limited. When Sax and Sax (1933) first described the cytology of Pseudolarix they postulated that the karyotype had arisen by a doubling of the chromosomes in an aneuploid condition with the basic number as $12-1=11$. In somatic cells, the chromosomal number was established as $2n=44$, having 40 rod-shaped chromosomes with terminal fiber attachment points, and 4 chromosomes with median or near median centromeres (Mergen, 1961). From this observation, and because polyploidy is rare in the Pinaceae, it can be assumed that this is not a polyploid condition, but that the large number is the result of the fracture at the centromere of 10 of the "V" shaped chromosomes resulting in 20 "I" or rod-shaped chromosomes.

Although polyploidy does not play a significant part in the variation pattern of the Pinaceae there are a number of reports on visible natural mutations. In the genus Picea, Andersson (1947) reported irregularities during meiotic divisions in Norway spruce trees. In these asyndetic spruces the chromosomes failed to move in a regular fashion to the poles in both the first and second division, and in some instances lagging chromosomes were eliminated in the second division, giving rise to micronuclei. The outward morphology of the asyndetic spruces was also unusual: e.g., they had larger male flowers and smaller stomates; seed-setting after selfing was inferior to controlled outcrossing and to natural wind pollination. Different results were obtained depending on the pollen source used in outcrossing, and many of the seeds possessed only a rudimentary "endosperm."

In Picea, many aberrant forms have been described; the best known one is possibly the "Snake spruce." In Figure 1 is an illustration of one

of these aberrant forms growing in a plantation at New Haven, Connecticut. This particular tree is 20 years old and does not have a central stem. Although this type of mutation has no direct value in a forest tree improvement program, it might well be of considerable importance in forest biology research. Cuttings taken from this tree rooted 100 percent, even when some of the cutting shoots were 10 to 12 years old. This certainly indicates an abnormal auxin relationship in these trees.

Spruces aberrant for chlorophyll formation can also be used for pollen dispersal studies, as was done by Langner when he evaluated the progeny from an isolated spruce tree having genes of the aurea type (Langner, 1953).

Dwarf spruces are being marketed for horticultural uses. These are either propagated asexually from "witches' broom" mutations or from mutant individuals selected out of nursery beds. Kiellander (1950) and Illies (1959) examined some dwarf spruce seedlings and found that their morphological aberrations were paralleled by gross morphological chromosome aberrations (aneuploidy and mixoploidy).

For Abies there are few reports on natural mutations, probably not because of the lack of mutations in this genus, but because this genus has received little attention. During a study of the cytology of microsporogenesis in Abies, irregularities during meiosis were observed in Abies nobilis glauca (Mergen and Lester, 1961b). Precocious movements during anaphase, formation of bridges, and the presence of acentric fragments were observed in one tree (Figure 2A). When this tree was used as a pollen parent no viable seed was obtained. In a subsequent study, further breakages were observed in two trees in the Yale Brett Pinetum, resulting in micronuclei (fragments) that were attached to the telophase nuclei.

Controlled pollinations between the two trees yielded viable seed, but the resulting seedlings exhibited a high degree of chlorophyll aberration. Only a few were pure albino, but most of the progeny were deficient in chlorophyll and contained varying amounts of xanthophylls and carotenoids (Figure 2B). The rate of germination of the seed from this cross was also slower when compared to other fir crosses.

The only report of a polyploid Abies that could be located in the literature was by Kanazawa (1949) who had examined the chromosome number in three twin seedlings of Abies firma. One of these was a tetraploid with 48 chromosomes.

Larix also appears to be stable cytologically. So far only isolated reports of mutations in larch are available. Larsen and Westergaard (1938) described the occurrence of a triploid larch in a cross between Larix decidua x Larix occidentalis. This polyploid tree was the only plant that arose from the cross. The growth of the tree was extraordinarily robust. The reduction divisions in the pollen mother cells were, as should be expected, irregular. The authors felt that this type of tree was of value in increasing the variation in the chromosomes in the two parent species by back-crossing to the diploid parents. In 1949, Christiansen (1950) located a mature (54-58 years) tetraploid Larix decidua tree on the grounds of an estate in Denmark. This autotetraploid had larger needles, larger stomates, and larger cones than the diploid. Originally this tree was recorded as Larix europaea var. pendula because of its pendulous branches. Because no viable seed was obtained from the cones, the tree was vegetatively propagated to obtain flowering material for breeding purposes. In Japan, Chiba and Watanabe (1952) found among two-year-old

transplants of Larix kaempferi 8 polyploid seedlings. Two of these had diploid roots, while the remaining six were all tetraploid. These had thicker needles, with larger, but fewer, guard cells.

The frequency of chiasmata in two species of Larix that were investigated by Mrs. Sax (1933) was remarkably similar, and ranged from 1 to 5, with an approximate average of 2.4. In the hybrid Larix eurolepis, pollen sterility was 9 percent as compared to 2 or 3 percent in the parental species. Mrs. Sax did not attribute this greater pollen sterility to irregular chromosome distribution or to differences in the frequency of chiasmata.

The genus Pinus contains some 80 to 90 species, and is the most important of the conifers. This group shows great stability in number of chromosomes, chromosome morphology, and genetic compatibility, and only a few reports of mutations are available. Tjio and Ostergren (1956) studied the occurrence of spontaneous chromosome breaks in 20 species of Pinus, and they found breaks during mitosis in 13, while in the remaining species there was no evidence of breaks. Pinus longifolia had the highest frequency (71%) and Pinus rigida, Pinus pinaster, and Pinus virginiana also exhibited strong fragmentation. In every instance the disturbances were limited to the cotyledons. The authors felt that this was of importance in the evolution of a species, namely, mutations or degeneration in the cotyledons is not harmful, as compared to the tissue of the root system which continues to grow and function for a long period of time.

Chlorophyll-deficient mutations have been reported in several species of pine and these phenotypic expressions depended on the degree of homozygosity of the progeny. These deficiencies ranged from pure albinism to light

virescence (Johnson, 1945; and Eiche, 1955). In addition to these gene mutations, spontaneous polyploids or mixoploids have been reported in four species of pine. Zinnai (1952) located five tetraploid Pinus densiflora seedlings in two-year-old transplant beds. These seedlings had shorter and thicker needles. Mergen (1958) described the anatomy and cytology of five abnormal Pinus elliottii seedlings that he had selected in nursery beds. These seedlings were mixoploids, having $2n$, $3n$, and $4n$ chromosome complements. The growth of these seedlings is still being followed, and the appearance of a seedling after seven years is given in Figure 2C. During 1957, a single seedling of Pinus pinaster with thick needles was examined cytologically; when the seedling was two years old it was mixoploid and had a high proportion of polyploid ($4n$) cells (Figure 3). Serial sections through side buds showed an almost random distribution or scattering of the polyploid cells in the meristematic tissue. This tree is growing in the Yale Forestry School greenhouse; when re-examined during the winter of 1963 when it was seven years old, no polyploid cells could be located. It appears that the tree had reverted to a diploid state. The presence of polyploidy in Pinus sylvestris in Sweden was reported by Johnsson in 1958, and Nishimura (1960) described a tetraploid seedling of Pinus thunbergii that arose from a di-embryonic seed.

Dwarf forms of pine are used in landscape work, and these are propagated vegetatively. Many of these cultivars have been given variety names, e.g., nana, pendula. These trees do not flower to any great extent, but when seed is produced on these trees, the progeny shows considerable variation in needle length, needle color, and growth rate and form (Gerling, 1958). Seedlings originating from seed collected from a "witches' broom"

of Pinus banksiana were quite variable and some were dwarfed. Three of these seedlings were grafted on a normal Jack pine by A. G. Johnson; they have remained dwarfed, but they produce female flowers in relatively large number. Figure 4A illustrates the condition of these grafts during the winter of 1963, or some nine years after grafting.

So far none of the spontaneous mutations in the Pinaceae can be considered useful in a forest tree improvement program. Most of these are scientific curiosities and are of interest in the study of abnormal cytological conditions. The majority of spontaneous mutations in forest trees have been unfavorable, and if they appear in seedlings that grow in nursery beds, these seedlings are not able to compete with normal diploid ones, and thus succumb to the unfavorable environment. These spontaneous mutations are of value, however, as specimens for botanical gardens, and as a source material for horticultural cultivars. For Pseudotsuga taxifolia (menziesii), for example, Rehder (1954) listed ten named varieties with a range of characteristics: e.g., from green to bluish-white foliage, from pyramidal to compact conical form, and from pendulous to upright branches. As forest geneticists and tree breeders we should look upon these examples from horticulture as examples of positive results that can be obtained if we learn to recognize mutations in our forest tree species, and propagate them for future use.

Induced Mutations

The mutations that were described previously were limited to those of spontaneous origin without direct influence or control by man. At present there are experimental techniques available that bring about permanent genetic changes in plants. These genetic changes, although they

are the response to treatments, cannot be made in specific genes or chromosomes. The treatments either speed up the spontaneous mutation rates, or they cause doubling of the chromosome number in dividing cells. Methods used to induce or speed up mutations can be classified under the following headings: 1) temperature, 2) chemical agents, 3) ultraviolet and other non-ionizing radiation including ultrasonic waves, and 4) ionizing radiation.

1) Temperature. From experiments with non-woody plants it was estimated that the normal mutation rate is doubled for every 10°C rise in temperature, above average. This rate increases in some instances also toward the lower extremes. Forest geneticists have attempted to bring about mutations in pines by changing the temperature surrounding the female conelets during the period of fertilization, but to my knowledge none of the treatments were successful in bringing about detectable changes in the progeny. Further work along these lines should be tried, but the timing of syngamy in the Pinaceae is difficult, and varies among the ovules within a cone.

2) Chemical agents. More than 20 chemicals of strongly mutagenic action have been recognized. Not all of these have been tried on forest trees. The plant alkaloid, colchicine, has been used on a number of tree species in the attempt to induce chromosome doubling. A number of polyploids of Pinaceae species have been produced through colchicine treatment. The mutagenic effect of colchicine is quite specific and is restricted to dividing cells. It acts on the spindle fiber mechanism and prevents the formation of spindle fibers. The chromosomes divide, but they are not pulled to the two poles and they remain scattered throughout the cell. After the nuclear membrane has formed, the result is a nucleus with double

the number of chromosomes. This action of colchicine is only temporary, and once the cell divides without the effect of colchicine, a normal bipolar spindle forms, but the resulting daughter nuclei are polyploid. If, however, a second or third division occurs in a polyploid cell while it is still affected by colchicine, further duplication of the chromosome takes place.

Because colchicine is effective only during cell division, most of the treatment techniques are similar in that attempts are made to get colchicine into the dividing cells. Although the carriers (water, glycerine, agar, lanolin) and concentrations vary, the effects are the same. Polyploidy in somatic tissue has been induced in the Pinaceae with colchicine in the following genera: Pinus (Mirov and Stockwell, 1939; Machado, 1943; Kiellander, 1950; Zinnai, 1953; Hyun, 1954, 1956; Mergen, 1959); Picea and Larix (Kiellander, 1950; Illies, 1952); and Abies (Mergen and Lester, 1961^a).

Polyploidy was successfully induced in all species attempted, and the overall changes in the seedlings or trees were similar. In general the needles were shorter and became thickened, the number of needles was reduced, the color became a darker green, the guard cells were larger, which resulted in larger stomates, branching was coarser, flowering was suppressed, and overall dwarfing of the trees was common. When polyploidy is induced in germinating seeds, the roots are often unable to grow, and are easily invaded by microorganisms. Roots are adapted to aqueous transfer and consequently absorb colchicine more readily than shoots. The effect of the damaged root has been overcome in some instances by grafting the polyploid seedlings on diploid seedlings.

Polyploid slash pines, seven years old, are illustrated in Figure 4B, C, D. These trees have been under continued observation in our greenhouse and their chromosome number has stabilized at the tetraploid stage. Some of these exhibit good growth, but none are as tall as the controls. One polyploid seedling that was grafted on diploid root stock is producing new needles each year, but the overall growth is depressed (Figure 4C). None of the treated seedlings produced reproductive organs, while some of the controls have produced female flowers.

Using an infrared gas analyzer to measure photosynthesis in polyploid Pinus elliotii seedlings, significantly lower rates of photosynthesis were measured as compared to diploid seedlings, and the polyploids also had higher compensation points (Bourdeau and Mergen, 1959). This lower photosynthetic efficiency probably contributes to the slower growth rate of the polyploids.

With most tree species, tetraploids are not considered desirable, and they are of use as an intermediate step in the production of triploids. Plans are to use the diploid pollen or flowers from the tetraploid parents in controlled pollinations with diploid trees to produce triploids. Efforts to bypass the tetraploid sporophyte stage have been made by treating microsporangiate strobili during microsporogenesis. Attempts with Larix leptolepis resulted in the production of pollen grains with both haploid and diploid chromosome numbers (Illies, 1956). Colchicine treatment of immature male flowers of Pinus nigra and Pinus mugo affected the chromosome movement in some cells during the diad stage. This resulted in the formation of pollen grains with the chromatin from two nuclei (Mergen, 1959). Unfortunately no progeny from controlled pollinations with pollen of this

type are available for members of the Pinaceae. This method is promising, however, and will undoubtedly receive further attention.

3) Ultraviolet and other non-ionizing radiations, including ultrasonic waves. Ultraviolet rays are able to raise the electrons in living cells to a higher energy level, and this excitation is thought to be the cause of the mutagenic effect of these rays. They have a limited capacity to penetrate matter and consequently, they have been used to best effect on developing microspores or on mature pollen. To my knowledge there are no reports of successful induction of mutations in members of the Pinaceae with ultraviolet rays. Sustained experiments with ultraviolet rays should be carried out on forest trees in order to evaluate the effectiveness of these radiations in inducing mutations.

After World War II, various research agencies started experimenting with ultrasonic waves hoping to induce mutations in the treated cells. Studies were done by this author on Pinus palustris and Pinus elliottii without being able to achieve permanent post-treatment effects. Seed and seedlings were treated for varying periods of time at different wave lengths and intensities (watt/cm^2) without inducing chromosomal changes that were transmissible. Chromosome fragmentation and breakage were observed, but these cells were unable to divide further. Because ultrasonic waves travel only in liquids, the pine material was treated in vessels filled with water. Regel (1954) treated Douglas-fir seed that was infested with insect larvae, with ultrasonic waves. He observed a temporary stimulation in rate of germination, but after one year's growth the seedlings grown from the treated seed showed no difference in their outward morphology. From my personal experience, and judging from

the experience of others with plant material, I do not consider ultrasonic waves of great promise as a mutagenic agent with forest trees.

4) Ionizing radiation. Included in this group are the Xrays, protons, neutrons, and the α , β , and γ rays from radioactive substances. These radiations change the charge of the atoms through which they pass, and these charged particles are called ions. This brings about a chemical change in the molecule, and if the changed molecule is part of a gene it may produce a heritable change.

Ionizing radiations affect trees in two ways: 1) somatic effects; and 2) genetic effects. Under somatic effects are included those effects that are apparent in the exposed individuals and cease with the death of that tree, while genetic effects become apparent in the offspring of the exposed trees. With gymnosperms and forest trees in general, most of the information available deals with the somatic effects of radiation on exposed trees or seed.

Sparrow and Miksche (1961) showed a good correlation between growth inhibition by ionizing radiation and the average volume of the interphase nuclei in shoot meristems. The Pinaceae have large nuclei when compared to angiosperms, and the nuclear volume of the pines is approximately five times that of oaks, although both genera have a somatic number of $2n=24$. The chromosomes of Pinus are compared with the chromosomes of Quercus in Figure 5A, B, and the greater susceptibility of the pines to radiation is probably related to their greater nuclear volume.

The first report of the effect of ionizing radiation on seed of the Pinaceae was probably the one published by Dr. Baldwin in 1936. Since this early report a considerable number of experiments have been described, many of which were carried out in Sweden.

Irradiation studies with Pinus and Picea seed yielded different tolerance values for LD₅₀, and these depended largely on the development of the embryo, water content of the seed, and method of treatment (Gustafsson and Simak, 1958; May and Posey, 1958; Vidakovic, 1960; Ohba, 1961; Ohba and Simak, 1961; Snyder, et al., 1961; Mergen and Stairs, 1963). Work in progress now by this author shows that the tolerance of Pinus rigida as measured by LD₅₀ can be doubled by cold stratification of the seed after radiation. The pine seedlings from irradiated seed are in general of poorer vigor (Figure 5C), and there is a greater number of chromosome aberrations in the developing seedlings (Figure 5D, E, F).

Although mutations have apparently occurred in the first generation after X-radiation of seed of Pinus densiflora and Pinus thunbergii (Toyama, 1954) the results obtained so far with seed irradiation indicate the limited value of this method. It may, however, be considered as a means of increasing the genetic variation in a species. Perhaps its greatest future use will be in the irradiation at low levels of large quantities of seed in the attempt to alter the genetic system so that specific disease organisms no longer find the plant a suitable host.

Since Gustafsson and Simak (1958) reported the high sensitivity of pine and spruce trees to acute radiations (Gustafsson, 1960), considerable information has been accumulated. Pedigo (1960; 1962) and Platt (1963) described the effects on Pinus taeda, and Sparrow et al. (1963) on Pinus strobus. In the latter study Pinus strobus seedlings were exposed for 16.5 hours to Co⁶⁰ radiation at dosages from 50 to 100 r during both dormant and active stages. They reported a greater sensitivity of the growing plants and related the difference to the greater nuclear volume in actively growing meristems.

During November, 1961, a Ce^{137} (9500 curies) source was placed in a natural pitch pine-oak forest at the Brookhaven National Laboratory to study the effect of radiation on a natural forest community (Sparrow and Woodwell, 1962). At an exposure level of 15 r/day Woodwell (1962) reported a severe growth inhibition of shoot elongation in pine approximately six months after irradiation had started. The effect on the reproductive capacity of the pine trees is being studied by this author; during the first season trees receiving 82 r/day and higher were unable to resume cell division in the sporogenous tissue. Microspore mother cells were found on trees receiving 75 r/day, and at 56 r/day meiosis with subsequent formation of tetrads took place. The highest level at which mature microspores were collected was 14 r/day. The percentage of microspore cells with visible chromosomal aberrations increased with an increase in exposure, e.g.: 0.36 r/day = 1%; 7 r/day = 2%; 11 r/day = 12%; 56 r/day = 76%. Pollen from the irradiated trees is being used for controlled pollinations and subsequent progeny testing.

When one-year-old Pinus rigida seedlings were exposed to various intensities of γ rays from a Co^{60} source, an interesting observation was made on the ability of the damaged seedlings to produce sprouts from dormant buds below the ground line that were partially shielded by the soil. One such seedling, receiving 20 r/day, and a total of 6000 r, produced basal sprouts after it was removed from the field and placed in a greenhouse. Within a total period of six weeks one sprout produced three microsporangiate strobili with viable pollen. In this instance flower initiation and differentiation were shortened from a normal period of six

to eight months to less than two months (Figure 6A).

Of considerable interest are the effects of low-level chronic radiation on pine trees. In the 1959 Annual Report of the Brookhaven National Laboratory is the first published report of these effects on Pinus rigida. A photograph was shown of a pine tree that had been killed after an exposure of five years at the rate of not more than 8 r/day during eight months of each year. Further observations on these effects were given by Sparrow and Woodwell (1962), Mergen and Stairs, 1962, 1963) and Woodwall and Miller (1963). Progeny tests showed the effect of cumulative radiation on seed germination, and the resulting seedlings had shorter hypocotyls (Mergen and Stairs, 1963). This damage was related to the total dose accumulated by the individual trees prior to, and during, seed formation, rather than to the total dose accumulated in the seed. One seed lot had accumulated a dose of 3500 r and had a germination capacity of 96.8 percent of the control. Flower phenology on the trees was retarded, morphological aberrations and hermaphroditic strobili were produced, and pollen abortion increased with an increase in irradiation level. During meiosis, visual cytological evidence of chromosome aberrations was obtained. Bridging and fragmentation of the chromosomes were observed at Anaphase I and Anaphase II (Figure 6 B, C). The resulting progeny was much more variable than the control, reflecting the genetic effects of mutations that occurred in the irradiated trees (Figure 7). This suggests that an appreciable degree of mutations occur in pines as a result of cumulative low-level chronic radiation. Controlled pollinations, including selfings of the irradiated trees, will be made to obtain detailed information on these mutations.

Considerable work needs to be carried out on a sustained, organized

basis before the full value of ionizing radiation for tree breeders can be appraised adequately. We should remember that the mutations, if not lethal, will increase the variation pattern, and the greater the variation pattern, the wider the range for artificial and natural selection.

General Conclusions

The chromosome morphology is quite stable in the Pinaceae, and with few exceptions, the various members have the same chromosome number. In Pinus and Picea the karyotypes are similar and cross-pollinations between species that are separated geographically are common. This reflects the stable condition in these species and indicates that the origin of different species within a genus is probably caused by gene mutations. Most of the hybrids produce fertile offspring, and in the F_1 pine hybrids that were observed cytologically by Dr. Sax (1960) meiosis was as regular as in the parent species.

Mutation breeding and especially the use of artificial polyploids at first raised the hopes for the breeder. A critical review was presented by Stebbins (1956) and one of his conclusions was that "the best guide to the successful breeding of polyploids is our knowledge of the characteristics of natural polyploids which have made them successful in evolution." Because in the Pinaceae we are dealing with cytologically stable plants, polyploidy breeding will probably be of limited application. In some angiosperms preliminary evidence indicates that certain viruses can bring about permanent genetic changes in the progeny. Because no virus has as yet been found in a member of the Pinaceae this approach will not be discussed.

Mutations increase the variation pattern to various degrees and they

supply phenotypic variations for natural and artificial selection to act upon. For the Pinaceae, the induction of mutations is possible by various techniques, and we should look upon these techniques as supplementary tools in our work, rather than methods that will substitute for the conventional tree-breeding techniques. As with most any other type of tree improvement work, selection from within a large population is the starting step. In the mutant forms we are dealing with new genotypes, and their behavioral and adaptation patterns to a variety of environments need to be studied. Some mutations that at first appear to be of little or no value might be quite useful when crossed and combined with other genotypes and also be of importance as genetic markers. The irradiation of pollen needs further work, and by this technique minor cross-incompatibilities may possibly be overcome. Pollen in which the nuclei have been severely damaged by radiation may be used to stimulate auxin formation and allow partial incompatible crosses to be made. As with every new field, as technological advances are made, valuable new information will be gathered and from this new source of knowledge a better understanding of the genetic make-up of our trees will result. A deeper understanding of the biology of the trees can only be of help.

Summary

In the Pinaceae the chromosome is quite uniform and stable. Polyploidy and other major chromosomal aberrations exist, but they are rare. The constancy of chromosome number and fertility of interspecific hybrids indicate that species differentiation has been based primarily on mutations of individual genes.

In Picea irregularities during meiosis as well as aberrant forms in seedlings and mature trees have been reported, all of which brought about

unfavorable physiological responses. The presence of bridges causing acentric fragments in the microspores of Abies nobilis glauca, resulted in seedlings with a high degree of chlorophyll aberrations. Polyploid and mixoploid Larix and Pinus seedlings and trees showed various degrees of gigantism in their needles, and their overall growth was depressed. Chlorophyll-deficient mutations caused albinism and other types of abnormalities, depending on the degree of homozygosity of the progeny. So far none of the spontaneous mutations reported can be considered of direct benefit in a forest tree improvement program. Their main value lies in providing opportunities for study of abnormal cytological conditions, as specimens for botanical gardens, and as source material for horticultural cultivars.

The rate of natural mutation can be speeded up by experimental techniques. Although mutations can be induced genetic changes cannot be made in specific genes or chromosomes. The results from the following methods were discussed: 1) temperature; 2) chemical agents; 3) ultraviolet and other non-ionizing radiations including ultrasonic waves; and 4) ionizing radiation. Although none of the temperature treatments have been successful in bringing about detectable changes in the Pinaceae, further attempts with high or low temperature should be made during syngamy or early embryo development. Of the mutagenic chemicals that are known, colchicine is the only one that has been tried on a large scale. The action of colchicine is limited to dividing cells and prevents the formation of spindle fibers. The chromosomes divide but they do not move to the poles leading to a doubling of the number of chromosomes. By this treatment polyploidy was successfully induced in all species attempted, and the results were similar. In general the polyploids had shorter and thicker needles, branching was

coarser, flowering was suppressed, and dwarfing or reduction in height is common. Tetraploids are not considered desirable and the polyploid seedlings will be used in intermediate step to produce triploids. Plans are to use diploid pollen or flowers from these trees in controlled pollinations. Moderate success in obtaining diploid pollen was achieved by bypassing the tetraploid sporophyte stage by treating microsporangiate strobili during microsporogenesis. No results from pollinations with pollen of this type are available, but this method is a promising approach and should receive further attention.

The use of ultrasonic waves was not successful and appears of little promise as a mutagenic agent with forest trees. The use of ultraviolet rays, because of their limited ability to penetrate matter, may be limited to developing microspores or mature pollen. There are no reports of successful inductions of mutations with ultraviolet light in members of the Pinaceae. Sustained experiments should be started for this appears to be a promising approach.

Ionizing radiations (Xrays, protons, neutrons, and the α , β , and γ rays from radioactive substances) change the charge of the atoms through which they pass, thereby changing the chemistry of the molecules. If the changed molecule is part of a gene it results in a mutation. Because the Pinaceae have large nuclei they are very sensitive to both somatic and genetic radiation effects. Irradiation of seed has not produced any useful mutations, and perhaps the greatest future use will be in the irradiation at low levels of large quantities of seed hoping to induce resistance to disease. Chronic radiation of entire trees at a low level over a period of several years induced mutations in pines. Progeny from such trees was more variable than the controls. Considerable work still needs to be done before the usefulness of ionization as a tool in tree breeding can be

appraised objectively.

Mutations form the basis for natural and artificial selection. We should look upon the induction of mutations as a supplementary tool that might contribute greatly to the improvement of trees, rather than a substitution for conventional approaches. Mutations bring about new genotypes, and their usefulness needs to be appraised objectively in each instance.

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Captions for Illustrations

- Figure 1. Comparison of branches from Snake spruce with those from average Picea excelsa.
- Figure 2. A. Formation of bridges during Anaphase I in Abies nobilis glauca.
B. Progeny of Abies nobilis glauca showing various degrees of chlorophyll deficiencies.
C. Mutant seven-year-old Pinus eliottii (center) with four grafts made from this tree.
- Figure 3. Comparison of diploid (A, C) and mixoploid (B, D, E, F) seedlings of Pinus pinaster.
- Figure 4. A. Progeny from a "witches' broom" of Pinus banksiana grafted on a normal Pinus banksiana. Grafts are nine years old.
B. Comparison of tetraploid (left) and diploid (right) Pinus eliottii. The diploid seedling is four years old, while the tetraploid one is seven years old.
C. Seven-year-old graft of polyploid Pinus eliottii on diploid root stock.
D. Variation in needle and branching characteristics in three seven-year-old tetraploid Pinus eliottii seedlings.
- Figure 5. Comparison of somatic chromosomes of Pinus (A) and Quercus (B) at the same magnification.
C. Pinus rigida seedlings from seed irradiated at 840 r/day. The seed was in the cones on a tree growing in the Brookhaven Gamma Forest. Left to right: Control; 2520 r; 5050 r; 7590 r; 10200 r; 16,800 r; and 22,600 r.
D.E.F. Aberrations during mitosis in root cells of Pinus rigida seedlings originating from irradiated seed.

Figure 6. A. Basal sprouting of Pinus rigida seedling after exposure to 6000r at 20 r/day. One sprout has three microsporangiate strobili.

B.C. Effect of cumulative chronic gamma radiation (4 to 5 r/day) on chromosome aberrations during meiosis in Pinus rigida.

Bridges formed at Anaphase I (B), and Telophase II (C).

Figure 7. Average height ($\pm$ one standard error) of one-year-old Pinus rigida seedlings from seed collected in a forest that was chronically irradiated for one to four years. The seedlings were kept separate by year during which the seed was formed. All seed was collected during the fall of 1961.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4.

FIGURE 5

FIGURE 6