Variability in Needle Characteristics of Soft Pine Species and Hybrids

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The production of pine hybrids by controlled pollination has led to a renewed interest in needle anatomy, because of difficulties in verifying hybridity in young trees. Pine breeders have made studies in the course of their work and in some cases published data on one or a few taxa. In at least one case a comprehensive summary has been published of the entire genus Pinus (KENG and LITTLE 1961).

Similar studies have been made over a period of years in connection with white pine breeding programs at the Ohio Agricultural Experiment Station and the Southern Research Station, Maple, Ontario. These examinations have included a fairly large number of white pine species and hybrids of both clonal and seedling origin. In most cases we examined a variety of selections with prdbable genetic diversity. By coincidence, a few of the hybrids examined are of the same progenies on which KENG and LITTLE based their descriptions.

Needle characters studied included length, relative cross-sectional area, number of marginal serrulations, position and number of rows of stomata, position of resin canals, and number of resin canals. In the Ohio studies, internal characters were examined in over 150 permanently-mounted and replicated needle sections. These sections were taken from the middle part of the needle (1 cm. from the base), killed, fixed, embedded in Tissuemat, sectioned on a microtome, and stained by the safranin-fast green technique (JOHANSEN 1940). Ontario examinations were made by cutting hand sections from the middle of the needle, mounting in water, and recording internal characters.

The distal portion of the needle was used for serrulation counts because earlier work by FOWLER and HEIMBURGER (1958) had shown that this portion gave the greatest difference between P. peuce and P. strobus in number of serrulations. Neodle samples in the Ohio work were taken about 2 centimeters below the top of last year's growth, on the main shoot. Needle samples in the Ontario work were taken at random.

Most of the results of these studies agreed with those of KENG and LITTLE, but we found differences which show that considerably more varlation exists than their descriptions indicate. These results are presented in the following discussion and tables.

Number of Needle Serrulations

The number of needle serrulations on the axial margin is frequently quite different from the number on the abaxial margins. It is necessary to examine both axial and abaxial margins before concluding that a species has "entire" or "serrulate" needles. For example, although Pinus peuce commonly has serrulations on abaxial margins, FOWLER and HEIMBURGER (1958) noted that in P. peuce most needles were entire on *the* axial edge. This is an especially useful diagnostic character, because the hybrid P. peuce × strobus was found by FOWLER and HEIMBURGER to be intermediate in the number of serrulations on the axial needle margin. It also appears to be a good character to help identify the hybrid P. *lambertiana* X koraiensis. In fact, in these studies it was the only needle character that clearly separated this hybrid from P. koraiensis.

Evidence from this and other studies (e.g. MERGEN 1958. WRIGHT 1959) indicates that descriptions of needle characteristics of pine hybrid should take into account the diagnostic value of measurements of number of serrulations per unit of needle length. Our observations on several species and hybrids indicate that the number of serrulations on needles from young trees is higher than on needles from older trees (cf. also FOWLER and HEIMBURGER 1958). Tables *1* and 2 summarize our counts of the number of serrulations on the axial needle margin for old and young trees respectively. Extremes are presented, and also the range in tree means. The tree mean is the arithmetic rnean for five needles (usually from a single fascicle).

Table 1. — Observed range in number of serrulations in the dista. 2 centimeters of the axial needle margin of some white pinc species and hybrids (grafted trees from ortets more than 10 years old).

Species or Hybrid	Total number of			Range in number of serrulations	
	Clones measured	Trees measured	Needles measured	Individual needle	Tree mean
P. koraiensis	5	5	25	22—59	33—51
P. *cembra*	7	8	40	3—58	7—48
P. flexilis	8	8	40	0	0
P. armandii	7	7	35	10—74	21—64
P. lambertiana X koraiensis	1	1	5	5—12	8
P. *lambertiana*	3	3	15	0—21	1—13
P. *parviflora*	8	8	40	0—36	1—29
P. ayacahuite	3	3	15	16—56	22—45
P. monticola X ayacahuite	3	4	20	3—15	4— 9
P. monticola	9	9	45	0—26	3—23
P. monticola X peuce	2	3	15	0— 3	0— 1
P. peuce	7	8	45	0—12	0— 8
P. monticola X (peuce X *strobus)*	2	2	10	4—18	6—15
P. monticola X strobus	1	1	5	17—25	21
P. peuce X strobus	5	5	25	6—33	12—26
P. strobus	14	14	70	11—61	16—48
P. monticola X *griffithii*	2	3	15	0—10	2— 8
P. griff*ithii*	9	9	60	30—67	33—53

Table 2 illustrates the effect of tree age on the number of serrulations.

Position and Number of Rows of Stomata

The presence or absence and number of rows of abaxial stomata is partly dependent on the location of the sampling point in the needle. We found 1 to 2 rows of abaxial stomata on needles from two mature clones of P. koraiensis. These stomata in P. koraiensis were more frequent near the distal tip of the needle than in the middle. They extended to the needle mid-section in only two cases. Obser-

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Table 2. — Observed range in number of serrulations in the distal 2 centimeters of the axial needle margin of some white pine species and hybrids (seedlings 2 to 10 years of age).

Species or Hybrid	Age	Number of		Range in Number of serrulations	
		Trees measured	Needles measured	Individual needle	Tree mean
P. flexilis	2	10	30	0	0
P. monticola × *peuce*	5	5	25	0—21	3—17
P. strobus × *flexilis*	2	5	15	22—50	26—48
P. strobus	2	10	50	42—73	46—68
P. strobus	4	10	50	31—56	34—53
P. strobus × *griffithii*	2	10	30	20—60	18—44
P. griffithii × *strobus*	10	5	25	28—64	31—40
P. griffithii	4	5	25	34—62	41—58
P. griffithii	10	2	20	28—57	33—50

vations of *P. lambertiana*, in combination with earlier work, indicate a similar situation in this species, with as many as 5 rows of abaxial stomata in the distal portion and a usual maximum of 2 rows in the basal portion. There is a wide range of variation in abaxial stomata number in *P. flexilis*. We observed 0—7 rows of stomata on the abaxial surface of needles of this species; one clone averaged 5 rows. *Figures 1A and 1B* indicate the diversity found between half-sibs and between needles of the same fascicle in *P. flexilis*.

Number of Resin Canals Adjacent to the Axial Needle Surfaces

Axial or "ventral" resin canals were found in numerous species and hybrids of soft pines, rarely in some taxa, fairly commonly in others. *Table 3* summarizes the range of variation in 22 taxa, based on this study and on previous investigations as indicated.

Table 3. — Current status of knowledge of the number and position of resin canals near the axial leaf margin of some white pine species and hybrids.

Species or hybrid	Number and position of canals
P. koraiensis	1 (rarely 2) medial
P. cembra	0—1 medial
P. flexilis	0—1 medial or external[3])
P. armandii	1 medial
P. lambertiana × *koraiensis*[1])	1 medial or subexternal
P. lambertiana	1 external, subexternal or medial
P. parviflora	0—1 external, subexternal or medial
P. ayacahuite[2])	0—4 external
P. monticola × *ayacahuite*[2])	0—1 external
P. monticola	0—1 external
P. monticola × *peuce*[1])	0—1 external[4])
P. peuce	None
P. monticola × (*peuce* × *strobus*)[1])	None
P. monticola × *strobus*	None
P. peuce × *strobus*	Rarely 1 external
P. strobus	0—1 external[3])
P. flexilis × *griffithii*[1])	None
P. monticola × *griffithii*[1])	0—1 external, rarely medial
P. strobus × *flexilis*[2])	0—1 external
P. strobus × *griffithii*	0—1 external[3])
P. griffithii × *strobus*	0—1 external rarely medial
P. griffithii	0—1 external or medial

[1]) Same progeny described by KENG and LITTLE (1961).
[2]) Not described by KENG and LITTLE (1961).
[3]) None found by KENG and LITTLE (1961).
[4]) 0-1 observed by KENG and LITTLE (1961), none by the authors.

Position of the Resin Canals within the Needles

Several soft pine species and hybrids must be added to the previously published list (KENG and LITTLE, 1961) of

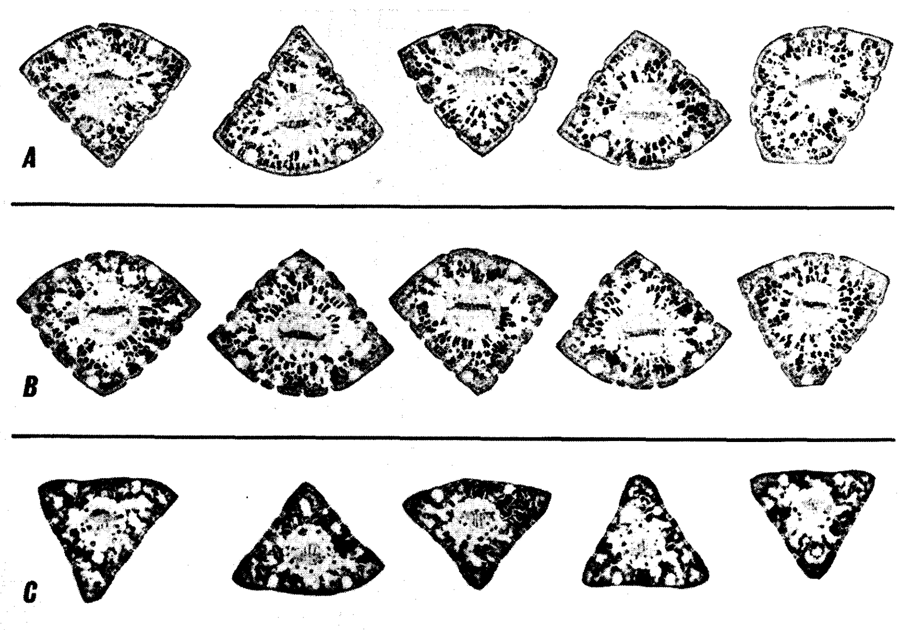

Figure 1. — Needle sections from three fascicles, taken 1 cm. from the needle base. (A) *P. flexilis* OAES 1043-8 (Morton Arboretum, Lisle, Ill.), with 0, 1, and 2 rows of stomata on the abaxial surface of different needles, and one abnormal needle; (B) *P. flexilis* OAES 1042-2 (Morton Arboretum, Lisle, Ill.) with 2, 3, and 4 rows of stomata; (C) *P. monticola* × *griffithii* OAES 656-3 (Inst. of For. Gen., Placerville, Calif.) showing one needle with an axial medial resin canal (extreme right).

those which sometimes have a medial resin canal in the axial part of the needle. A revision of the list is in *Table 3*. *Figure 1 C* shows a medial resin canal in a section from a needle of the hybrid *P. monticola* × *griffithii*. Age of the tree may have a relationship to the position of the resin canals. In *P. koraiensis,* such canals in needles taken from mature trees were all medial. However, "subexternal" or even external abaxial resin canals were common in young greenhouse-grown seedlings. Axial resin canals in these seedlings were all medial in position.

Thickness of Cell Walls in the Hypodermis

In our studies we have found thick-walled cells in the hypodermis of all *P. peuce* samples examined. Thirty needles were examined from six trees. *(Figure 2,* ortet a tree in the Morris Arboretum, Philadelphia, Pa.) Our results do not agree with those of Keng and Little (1961), who describe *P. peuce* as having thin-walled cells in the hypodermis.

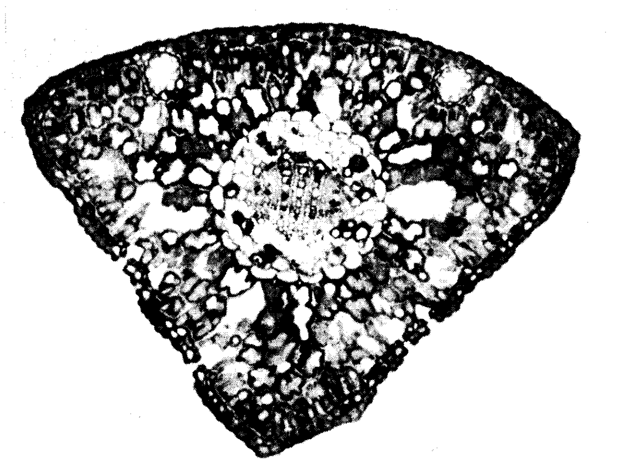

Figure 2. — A needle section of *P. peuce* OAES 476-1 (Morris Arboretum, Phila., Pa.) showing thick-walled cells in the hypoderm.

Discussion

It is apparent that there is a considerable amount of variability in needle characters of soft pines within any particular species or hybrid. There is also great variability within a small number of trees of a single F_1 hybrid family, and even between needles of the same fascicle, as illustrated in *Figure 1.* There is no reason to doubt that this variability is also present in the hard pines. More studies are needed of individual pine hybrids, with consideration given to both genotype and environment, before a systematic treatment of the entire genus is possible. The papers by Zobel (1951), Fowler and Heimburger (1958), Wright (1955) and Mergen (1963) are examples. The first two applied the hybrid index concept (Anderson 1936, 1949). Wright applied frequency distribution analysis to a series of cone characters. Some of the characters were intermediate in the hybrids. Mergen reported a relation between geographic origin and needle characteristics of *P. strobus.*

Frequency distribution analysis has been used in studies of the suitability of individual characters for hybrid verification (Mergen 1958, 1959; Schütt and Hattemer 1959). Some other biometrical techniques which appear to be useful for description or verification of pine hybrids include discriminant analysis (Mergen and Furnival 1960), probit analysis and partial regression (Kriebel 1962).

It is clear that special care is necessary for identification of soft pine hybrids, especially young trees. The problem is accentuated in the F_2 generation, particularly in crosses involving more than two species, of which we already have several. The old system of taxonomic description will certainly not be applicable to many of these new genetic combinations. Description must be quantitative and must take into account the natural variability that exists both within and between populations. Although our measurements may seem to indicate that variability is so great that some of these needle characters may be of less diagnostic value than previously believed, this is not necessarily the case. The utility of a character varies with the species combination. Further, the relationship of environment and genotype to needle morphology will later be clarified. It is quite possible that such clarification may enhance the usefulness of quantitative measures which now seem to be of limited value.

Summary

Some needle characters of species and hybrids of soft pines are described, including number of serrulations on the axial margin, position and number of rows of stomata, position of resin canals, number of resin canals, and thickness of hypodermal cell walls. The studies show that there is more genetic variability within the various taxa than was indicated by earlier investigations. Moreover, certain characters vary with the age of the tree or with the part of the needle that is sampled. More information is needed on quantitative variation in these characters, as related to heredity and environment, rather than formal botanical descriptions which would not now be accurate because of the definite, restrictive nature of such statements.

Résumé

Titre de l'article: *Variabilité dans les caractères des aiguilles des pins à cinq feuilles et de leurs hybrides.*

Les auteurs ont étudié certains caractères des aiguilles des pins à cinq feuilles et de leurs hybrides: nombre de denticulations sur l'axe de l'aiguille, position et nombre de rangées de stomates, position et nombre de canaux résinifères, épaisseur des parois des cellules de l'hypoderme. Ces études montrent que la variabilité génétique dans les différent taxa est plus élevée qu'on ne le pensait d'après les recherches antérieures. En outre, certains caractères varient avec l'âge de l'arbre ou la partie de l'aiguille qui est examinée. De nouvelles recherches sont nécessaires sur la variation quantitative de ces caractères. Elles doivent être basées sur des considérations d'hérédité et d'influence du milieu plus que sur une description botanique formelle insuffisamment précise et trop rigide.

Zusammenfassung

Titel der Arbeit: *Über die Variabilität bei Nadelmerkmalen von Weich-Kiefernarten und Bastarden.*

Einige Nadelmerkmale solcher Kiefernarten und Bastarde werden beschrieben. — Es zeigte sich, daß es mehr genetische Variabilität innerhalb mancher Taxa gibt, als früher mitgeteilt worden war. Mehr noch, gewisse Merkmale variieren mit dem Alter des Baumes oder je nach untersuchtem Nadelabschnitt. Es sind mehr Kenntnisse von der quantitativen Variation dieser Merkmale erforderlich, und zwar im Zusammenhang mit Erblichkeit und Umwelt, und es genügen nicht die formalen botanischen Beschreibungen,

die hier infolge der bestimmten und einschränkenden Art und Weise solcher Feststellungen nicht genau genug wären.

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Effects of Inbreeding in Red Pine, Pinus resinosa Ait.[1])

IV. Comparison with other Northeastern Pinus Species

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Introduction

In the three preceding sections of this study, natural variation, self-pollination, and factors affecting natural selfing were studied in red pine (FOWLER, 1964, 1965 a, b). It was concluded that red pine, unlike other species of the genus *Pinus* which have been studied, is homozygous for a large number of alleles, self-fertile and that self-fertilized seeds produce normal seedlings. It was postulated that one possible reason for the high degree of self-fertility of this species is its mode of reproduction. Red pine often reproduces after stand disturbances, such as fire or logging, from a few surviving trees.

Jack pine, *Pinus banksiana* LAMB., and white pine, *Pinus strobus* L. occupy, at least in part, a similar geographic range and have been subjected to similar recent climatic and geological disturbances as red pine. Unlike red pine, the breeding population of these species is often relatively large.

No self-pollination studies have been reported in jack pine, but published studies indicate that the species is quite variable (SCHANTZ-HANSEN and JENSEN, 1952; RUDOLF, LIBBY and PAULEY, 1957; VAARTAJA, 1959; BATZER, 1961; AREND *et al*, 1961; GIERTYCH and FARRAR, 1961, 1962). From a comparison of the variation observed in jack pine and red pine for similar differences in latitude and longitude, it is evident that the variation found in jack pine is many times greater than that observed in red pine.

Two self-pollination studies have been reported in white pine. JOHNSON (1945) reported that, while there was no appreciable difference in seed set or germination from open-, cross- or self-pollinated cones, the four-year-old seedlings from self-pollination were clearly slower growing than those from open- or cross-pollination. The progeny resulting from selfing exhibited a definite chlorophyll deficiency in a ratio of approximately 1 chlorotic : 3 normal. PATTON and RIKER (1958) reported obtaining small stunted seedlings from self-pollinated seeds of white pine. Provenance tests of this species have uncovered genetic variation between trees of different origins and between trees of the same origin (PAULEY, SPURR, WHITMORE, 1955; SANTAMOUR, 1960). Unlike red pine, both jack pine and white pine can be crossed quite easily with other closely related species.

On the basis of provenance and pollination studies, and considering the method of natural regeneration of the three species, one would expect jack pine to be the most variable and red pine the least variable. On this same basis, jack pine should be heterozygous in respect to a larger number of alleles, be more self-sterile, suffer greater inbreeding depression, and exhibit more numerous phenodeviants than red pine. To test this hypothesis, a pollination study with jack pine was begun in 1961.

Material and Methods

Jack pine

Five jack pine trees, of unknown origin, growing at the Southern Research Station, Maple, Ontario were selected for pollination work. The basis of selection was availability of ovulate strobili in adequate numbers for comparisons between self- and cross-pollination. The pollination techniques used in this study were the same as used in red pine (FOWLER, 1965a).

The pollen for cross-pollination was obtained by collecting approximately equal volumes of male catkins (microsporangiate strobili) from ten trees. No pollens from any of the five trees used as female parents were included in this mixture.

The cones were collected on October 18th, 1962. The seeds were extracted from up to ten cones from each pollination and were kept separate by cones. Full and empty seeds were separated with absolute ethyl alcohol and the empty (floating) seeds cut open to determine if any gametophyte tissue was present.

On December 8th, 200 seeds from each pollination, except Tree 1 × Self and Tree 3 × Self, which had only 173 and 53 seeds respectively, were placed on moist sand in Petri dishes, 25 seeds being placed on each dish. The dishes containing the seed were stored for two days at temperatures just above freezing, after which they were placed in a random design on a laboratory table. The laboratory temperature fluctuated between 70° and 80° F. Seed germinated was recorded daily. A seed was considered germinated

[1]) Contribution 64 - 8, Ontario Department of Lands and Forests. This paper is the fourth of a series based on a dissertation submitted to the Graduate School of Yale University as partial fulfillment of the requirements of the PhD degree.

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