Pigment Inheritance in Slash Pine Seedlings

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In tree breeding, chlorophyll defectives and other pigment variants are useful for estimating the degree of natural selfing that may occur in seed orchards (Squillace and Kraus 1963). To be most valuable the aberrations must be subjected to genetic analysis-- their variation and inheritance discerned. This paper reports studies of three groups of characters in wind- and controlled- pollinated progenies of slash pine (Pinus elliottii var. Engelm.): chlorosis in 2-1/2-month-old seedlings, cotyledon chlorophyll defectives, and hypocotyl anthocyanin variants. For the anthocyanin variants, we show how to derive com- bining ability values of wind-pollinated selections from different stands by calculating combining ability values for each stand.

SUSCEPTIBILITY TO CHLOROSIS

On April 10, 1964, self- and wind-pollinated seeds from 11 trees were sown in the nursery of the Harrison Experimental Forest in southern Mississippi., The design was a ran- domized block with 5 replicates and 22 seeds per plot. The nursery was not fertilized and by June 19 many of the seedlings were chlorotic. When nitrogen was applied the chlorotic symptoms disappeared. The self progenies were more chlorotic than their wind controls. The correlation between the two suggests an inbreeding depression. The selfs varied from 2 per- cent (parent 116) to 77 percent (parent 84) chlorotic seedlings (table 1).

In the self progeny of tree 84, all of the seedlings in two of the replications were chlorotic. A tree, all of whose self progeny were chlorotic, was noted several years ago by P. C. Wakeley (personal communication). These extreme cases were probably due to a general pologenic unbalance rather than to identifiable genes.

For the less extreme cases in table 1, we cannot distinguish between segregation and lack of penetrance. Monogenic chlorophyll mutants would be valuable in a physiological study of the metabolic nature of genetic blocks (Bell 1963).

Chlorosis is apparently unrelated to pigment characters expressing themselves shortly after germination and next discussed.

CHLOROPHYLL DEFICIENCIES OF THE COTYLEDON

Before discussing our data, it is necessary to review the color standards used in classi- fying the seedlings.

The classification of Gustafsson (1940) has been widely used for many plants including Scots pine (Fiche 1955). A slight modification by Gustafsson, Walles, and von Wettstein is printed here, and should be of general use in forestry. Their scheme, which grades from white to light green, is characterized by four one-color classes: albina, xantha, yellow viridis, and viridis. In our

Table 1. Percentage of chlorotic plants in self- and wind-pollinated progenies after 2-1/2 months in an unfertilized nursery.

Parent identity	Self-pollinated progeny		Wind-pollinated progeny	
	Population size	Plants chlorotic	Population size	Plants chlorotic
	Number	Percent	Number	Percent
84	[1/]152	77	91	32
6	89	62	86	26
87	55	49	86	15
2	110	38	86	19
9	94	36	75	16
45	46	15	86	13
85	166	13	76	4
11	244	11	74	12
161	508	8	79	10
23	37	3	89	3
116	340	2	85	5

1/ Variation below the nominal 110 seedlings was caused by poor germination of some self progeny; variation above 110 was due to extra rows of some entries.

Classification of the Chlorophyll Mutants of Higher Plants--Chlorophyll Mutants
Characterized by a Quantitative or Qualitative Alteration of the Chloroplast
Pigments of the Leaves [1]/

Group I. Mutant plants of one color

    1. Albina - white
    2. Xantha - yellow
    3a. Yellow viridis - yellow green[2]/
    3b. Viridis - light green

Group II. Mutant plants of two or more colors

    4. Alboxantha - leaf tip white, base yellow
    5. Xanthalba - leaf tip yellow, base white
    6a. Alboviridis - leaf tip white, base green
    6b. Xanthviridis - leaf tip yellow, base green
    7a. Viridoalbina - leaf tip green, base white
    7b. Viridoxantha - leaf tip green, base yellow
    8. Tigrina - chlorophyll deficiency in the form of transverse stripes
    9. Striata - chlorophyll deficiency in the form of longitudinal stripes
    10. Maculata - spotted chlorophyll deficiency
    11. Marginata - chlorophyll deficiency of the leaf margin or leaf center
    12. Costata - chlorophyll deficiency of the leaf veins or intercoastal
                 areas
    13. Transformiens - the color of the first developed leaves is different
                 from that of later leaves.

Group III. Mutant plants with color-changing leaves

    14. Albescens - bleach to a white-white yellow color
    15. Lutescens - bleach to a yellow, yellow green or light green color
    16. Virescens - chlorophyll-deficient, but turns normal green later

Note that Code de Couleurs (1908) standards originally defined colors of Group I.
However, readings by such an instrument as a "chlorophyllometer" has possibili-
ties (Inada, K. 1963. Studies on a method for determining the deepness of green
and color chlorophyll content of intact crop leaves and its practical applications.
1. Principle for estimating the deepness of green color and chlorophyll content
of whole leaves. Proc. Crop Sci. Japan 32: 157-162).

1/ Gustafsson, A., Walles, B., and von Wettstein, D. 1962. (From Records of the
Meeting of the Swedish Assoc. for Theoretical and Applied Mutation Res., Jan. 25-26,
1962 (mimeo.)) (In Swedish).

2/ Snyder-Squillace-Hamaker substitute is green xantha.

research green xantha was substituted for yellow viridis. Eiche (1955) inserted an extra
class, xanthoviridis. The viridis portion of his term referred to a particular hypocotyl
color. Aside from finding xanthoviridis confusingly similar to the established two-color
term, xanthviridis, we came upon no corresponding phenotype for slash pine.

    Our classes are based on cotyledon color. In the following descriptions, however, other
characteristics are also noted: Albina: white. In laboratory tests only one seedling with
pure white cotyledons was seen. This seedling also had a white hypocotyl. In the field,
seedlings tended toward yellow and usually had at least a spot of pink at the base of the
hypocotyl. We applied albina to only the whitest seedlings with no hypocotyl color. These
seedlings wilted and died in a couple of days' exposure to the sun.

Xantha: yellow. These seedlings had bright yellow cotyledons and vivid cherry-pink hypocotyls.
They lived several weeks if weather was favorable.

Green xantha: greenish-yellow. Similar to xantha but greener, with less vivid hypocotyls, and higher survival potential.
Viridis: light green. Hypocotyls normal pink. Some of these seedlings died; some, having cotyledons which subsequently turned normal green, lived, i.e., they were virescens (see classification).

To determine the frequency and nature of such variants, we surveyed approximately 7,380,000 slash pine seedlings from south Mississippi seed in the U.S. Forest Service's Ashe Nursery with the following results:

Variants per million seedlings

	Albina ⟶ Green (Albescens)	Xantha	Green xantha	Viridis
1963	0.0	17.2	37.9	41.7
1965	2.2	25.0	46.0	123.8

We speculate that the observed frequency of the albina class may have been more in 1965 than in 1963 because of the warmer, less cloudy conditions which existed. Under such conditions, some xanthas may have bleached to albescens. Indeed, later research may show that albescens is a preferable term to albina.

The difference between the two years in frequency of the viridis also suggests environmental influences. In both years the viridis frequency was largest even though some of the slight deviations from normal green are difficult to detect in outdoor illumination. If the rule for other plant species holds--that the viridis type mutant is the rarest--many of the viridis seen in the nursery must be non-genetic variants, i.e., non-mutants. Variability depending partially on environments is also suggested by data of Squillace and Kraus (1963), who for other nurseries and years reported the frequency of all mutants at 520 per million. Though the xantha frequencies are relatively constant, we will show later that they also are not good estimates of true occurrence.

Data on the frequency of carriers are available from two sources of wind-pollinated seed. In one, seeds were collected from 5 trees in each of 54 stands covering the natural range of slash pine, including south Florida (Squillace?/). Among 266 progenies which survived, we found that 38 (14 percent) contained one or more mutants. The carriers were in 28 of the 54 stands and were well distributed over the species range. The second source of information is from 446 trees in 8 counties of south Mississippi. Ninety-five (21 percent) of the progenies contained mutants. Thus over the entire sample there were 133 carriers among the 712 trees examined, i.e., 19 percent of them.

If the character is controlled by a single recessive gene, then self-pollinating a random group of trees would detect a high proportion of carriers. Indeed, Gustafsson (1962) has said, "We may safely presume that artificial selfing should reveal many more heterozygous trees (than wind-pollination), possibly the great majority of the trees examined." Contrary to this expectation, Pawsey (1964) failed to find mutant carriers more frequently in inbred Pinus radiata than in open-pollinated progenies. Bingham and Squillace (1955) and Barnes et al. (1962) found only 1 carrier among 28 Pinus monticola, and Fowler (1965) only 1 among 46 Pinus resinosa by selfing. Fowler (personal communication 1965) doubts that such failures apply to Scots pine, jack pine, white pine, and red pine. In no species did he find wind-pollinated progenies having mutants that were lacking in self progeny.

Forty-five slash pines whose progeny had never been examined were selfed, whereupon eight were identified as carriers in laboratory or nursery germination trials.

The deficiency of mutants after selfing may be explained by disturbed segregations. For a single red pine carrier, FOwler showed ratios deviating from the theoretical 3 normal: 1 mutant. Our data (table 2) from progenies of 29 trees, where mutants appeared either in selfs, crosses (including winds), or both, confirm Fowler (1964). We obtained segregations deviating from the 3:1 ratio (chi square, P = .05) in the self progenies of 19 of the 29 assumed carriers. Of these 19 there were 9 which produced 1 or more mutants. Six of these 9 had sufficiently large populations to show disturbed segregation with a high degree of confidence. Theoretically, only 11 seedlings are necessary to detect a mutant with 5 percent probability.

[2/] Squillace, Anthony E. Geographic variation in slash pine (Pinus elliottii Engelm.). April 1964. (Ph.D. thesis, University of Florida, 181 pp.).

The other 10 trees of the 19 had progenies which were non-segregating, i.e., produced zero mutants from selfing. Moreover, 2 of these trees, 84 and St 3-176, had 3 and 4 mutants, respectively, in their wind-pollinated progenies. The selfs of these two trees had large population sizes of 262 and 130 individuals, respectively. If a monogenic inheritance is assumed, it therefore appears that the disturbance in expected segregation can prevent phenotypic segregation entirely. Larger self populations may eliminate this difficulty for the majority of trees, but producing large self populations in slash pine is prohibitively expensive. Diluting self pollen to get norman ratios (Fowler 1964) should be tried to see if such dilution would make selfing more efficient.

In contrast to our failures with selfing, screening with wind-pollinated seed has been efficient. Only 1 of 22 xantha carriers would have been missed by using only wind progenies (table 2). Though self-pollination for detecting mutants is questionable, controlled pollinations, including selfing, are essential to further study.

It was of interest to see if crossing produced results similar to selfing and in addition, to see if trees were carrying the identical genes. Eighteen putative carriers were intercrossed in 52 combinations (table 3). Only one combination produced mutants in quantity: 32-42 X 32-58 had mutants in the ratio 6 normals: 1 mutant. These two trees may be related, as they are within a half mile of each other. More precision in classification and testing would have been desirable, but under the assumption of monogenic inheritance, our finding a quantity of mutants in only 1 combination among 18 carriers suggests that a number of genes are causing these types of chlorophyll deficiencies. Sprague and Schuler (1961) isolated 19 genes for yellow-green corn seedlings, but found 12 of them only once.

Twelve of our crosses produced a very small quantity of defectives (averaging 369:1). This quantity is too high to be reasonably attributed to current mutation in all 12 cases.

Table 2. Mutants in slash pine progenies from self- and cross-pollinations.

Parent identity and type pollination	Mutations	Normals: mutants	Fits 3:1 ratio (P + .05)	Parent identity and type pollination	Mutations	Normals: mutants	Fits 3:1 ratio (P + .05)
1/ 2S	Vir	100:30	Yes	St 3-176S	Xan	130:0	No
2C	--	99:0	--	St 3-176C	Xan	2201:2	--
2C	Xan	98:1	--	St 3-176C	Xan-G	2201:3	--
6S	Vir	174:11	No	St 3-135S	Xan	35:15	Yes
6C	--	466:0	--	St 3-135C	Xan	1236:4	--
10S	Xan	7:1	Yes	St 3-135C	Xan-G	1236:1	--
10C	--	148:0	--	St 3-60S	Xan	5:3	Yes
17S	Vir	16:1	No	St 3-60C	Xan	1744:5	--
17C	--	425:0	--	St 3-36S	Xan-G	13:5	Yes
18S	--	0:9	No	St 3-36C	Xan-G	2030:1	--
18C	Xan	240:1	--	St 3-32S	--	10:0	No
23S	Vir	42:1	No	St 3-32C	Xan	1018:2	--
23C	--	118:0	--	32-58S	Xan	46:7	Yes
84S	--	262:0	No	32-58C	Xan	1704:34	--
84C	Xan-G	288:3	--	32-42S	Xan	85:4	No
86S	--	18:0	No	32-42S	Vir	85:5	--
86C	Vir	153:1	--	32-42C	Xan	2719:36	--
116S	Xan-G	437:1	No	32-9S	Xan-G	252:61	No
116C	Xan-G	215:4	--	32-9C	Xan-G	3578:10	--
136S	--	6:0	No	31D-S	Xan	146:67	No
136C	Vir	87:1	--	31D-C	Xan	721:76	--
145S	--	4:0	No	35A-S	Xan	9:2	Yes
145C	Vir	66:1	--	35A-C	Xan	864:95	--
160S	--	12:0	No	42E-S	Xan	27:0	No
160C	Vir	158:2	--	42E-C	Xan	360:4	--
163S	--	1:0	No	G27S	Xan	23:11	Yes
163C	Xan-G	190:1	--	G27C	Xan	1122:12	--
169S	--	3:0	No	G133S	Xan-G	6:2	Yes
169C	Xan-G	96:1	--	G133C	Xan-G	1952:23	--
169C	Xan	96:1	--	G157S	Xan-G	35:5	Yes
9-2S	Xan	19:1	No	G157C	Xan-G	1122:12	--
9-2C	Xan	88:4	--				
9-2C	Vir	88:3	--				

1/ S = Progeny from self-pollination, C = Progeny from cross-pollination, including wind-pollination.

Table 3 -- Normal:mutant ratios for controlled cross- and self-pollinations and from wind-pollinations.

Parent identity	Character [1]	3-176	3-162	3-135	3-60	3-36	3-32	32-58	32-42	32-9	31-24	11-6	10-135	8-7	Winds	Selfs
3-176	Xan		--	--	11:0	514:0	--	--	295:1	471:0	426:0		--	--	484:1	130:0
	Xan-G											449:0				
3-162	Xan	--		481:0	483:0	--	267:0	476:1	437:0	482:0	192:0	--	558:0	--	550:2	--
	· Xan-G													346:1		
3-135	Xan		481:0		54:0	35:0	--	32:0	35:0		19:0	--	--	--	135:4	35:15
	Xan-G									445:1						
3-60	Xan [2][3]	11:0	483:0	54:0		160:0	8:0	177:0	505:0	54:0	14:0	69:0	--	34:0	175:5	5:3
3-36	Xan-G	514:0	--	35:0	160.0		496:0	193:0	--	15:0	14:0	77:0	--	416:0	110:1	13:5
3-32	Xan	--	267:0	--	8:0	496:0		66:0	66:0	3:0	16:0	--	--	--	96:2	10:0
32-58	Xan	--	476:1	32:0	177:0	193:0	66:0		189:30	217:0	10:0	--	--	--	344:3	46:7
32-42	Xan	295:1	437:0	35:0	505:0	--	66:0	189:30		185:0	493:0	--	--	--	514:5	4
	Vir															85: 5
32-9	Xan-G	471:0	482:0	445:1	54:0	15:0	3:0	217:0	185:0		144:0	488:1	--	--	1074:8	252:61
31-24	Xan	426:0	192:0	19:0	14:0	14:0	16:0	10:0	493:0	144:0		6:0	--	17:0	151:1	--
11-6	Xan-G	449:3	--	--	69:0	77:0	--	--	--	488:1	6:0		--	--	414:0	--
10-135	Xan	--	558:0	--	--	--	--	--	--	--	--	--		--	3	--
	Xan-G														1005: 1	
	Vir														1	
8-7	Xan-G	--	346:1	--	34:0	416:0	--	--	--	--	17:0	--	--		971:4	--
G-27 [4]	Xan [4]	--	--	--	--	--	--	--	--	--	--	--	--	--	1122:12	23:11

[1] Xan = Xantha; Xan-G = Greenish xantha; Vir = Viridis.

[2] Date are without regard to direction of cross; reciprocals are pooled.

[3] There were also no mutants of Xan-G or Vir.; i.e., the "zero" information is entered but once.

[4] Crosses of G-27 with other trees gave results as follows: 6A 42E G-133 G-157
    54:0 24:0 20:0 39:0

Possibly, multiple genes, epistasis, or unknown effects are involved. In the four cases where segregation in crosses can be compared to segregation in selfing, it appears to be no less disturbed in crosses than in selfs.

Segregation ratios can be modified. Fowler (1964) found that eliminating competitive selection at the zygotic or embryonic stage resulted in normal ratios. Eiche (1955) obtained more mutants in the greenhouse than in the nursery. We examined wind-pollinated progenies from 42 parents having mutants appearing in the laboratory, nursery, or both. There were 17 mutants per 1,000 in the laboratory in contrast to 3 per 1,000 in the nursery. For 79 percent of the parents, mutants appeared only in the laboratory. Because of the magnification of disturbance in segregation, the data indicate extreme loss of efficiency for nursery studies in comparison with laboratory studies. This disturbed segregation for cotyledon deficiencies is in contrast to the regular inheritance for hypocotyl color reported below.

HYPOCOTYL COLOR

While data for inheritance cotyledon of color were gathered under many environmental conditions, the methodology for hypocotyl color was standardized.

Seeds were given a 30-day, cold-moist stratification, exposed to 3 days of artificial light, and then germinated in the dark. After hypocotyl elongation, the hypocotyl colors were recorded as dark pink, medium pink, light pink, very light pink, or white. These five classes were compared to Munsell color standards (1960) and the colors converted to a numerical index. The change from dark pink to white involves an increase of _value_ and a decrease in _chroma_ of the hues 5.0R to 7.5Y. The hues were ignored on the expectation that color changes are due to different concentrations of a single substance, anthocyanin. However, joint action of the value and chroma was expressed as (10-value) X chroma. The resulting product is our color index. The color indices are graded according to human ability in distinguishing the colors

		Hue	Value	Chroma	Index
Dark pink	Almost red	5	7.0	8.0	24
Medium pink	Pink	10	7.0	6.0	18
Light pink	Lightest pink obvious	12.5	7.3	5.0	14
Very light pink	Trace seen only in detailed study	27.5	8.0	5.0	10
White	Separated from very light pink only by detailed study	27.5	8.0	4.0	8

A weighted mean index was calculated for each progeny from the frequencies of the several hypocotyl colors invariably found.

The wind-pollinated progenies of 605 parent trees each were examined.[3] Fifty to 100 seeds of each tree were sown. The distribution of parent trees, based on their progeny means, approached normality with a mean color index of 13.6 and a standard deviation of 1.8. Some skewness of distribution toward the lighter colors was observed. Most of the trees were from south Mississippi, but 159 were from the range-wide collection of Squillace (see footnote 3). Some of the skewness may be accounted for by geographic variation.

To examine the geographic pattern of variation, seeds were collected from trees at 48 locations over the range of slash pine--5 trees at 44 locations and 16 to 179 trees at each of four locations in south Mississippi. Mean hypocotyl color index for each location was determined and isogenes drawn. Results appear in figure 1.

Over most of the range, the indices averages 15. Along the north border, the index dropped to 14. There appeared to be an "invasion" of genes for low values northward from Cat Island, off the coast of Mississippi, with progressive indices of 12, 13, and 14. Finally, a horseshoe-like band of locations with indices of 14 penetrated northeast Florida from the Atlantic coast. Although the low values for the Cat Island trees may be a consequence of semi-isolation, no other characters or environmental conditions are known to be associated with this geographic pattern.

3/ The writers thank Dr. Francois Mergen and students for examining a portion of these during Their visit to the Institute in 1962.
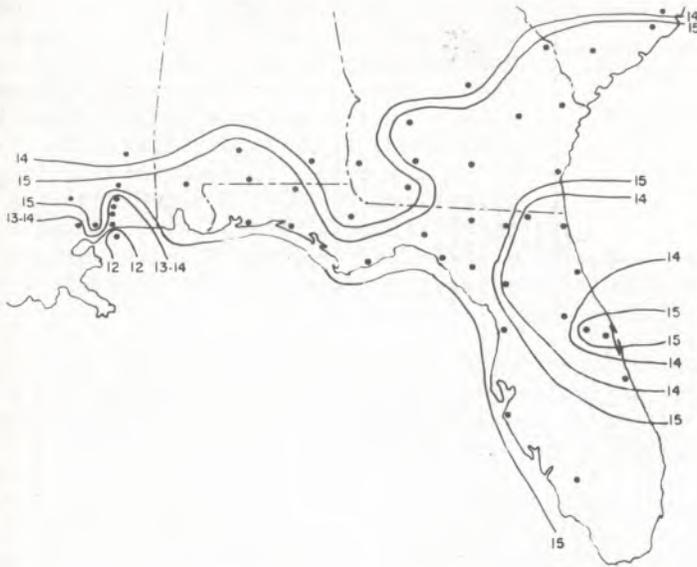
Figure 1 -- Isogenes for hypocotyl color. Indices are rounded. Larger numbers indicate pinker hypocotyls. The 48 dots generally represent 5-tree samples of stands.

In other trials of wind-pollinated progenies from 10 to 25 Mississippi parent trees, the intensity of pigmentation was not consistently associated with resistance to fusiform rust, ability to germinate without a light treatment, or seedcoat color. Trials with larger parental populations are continuing.

To determine inheritance of hypocotyl color, 14 trees whose progeny had previously exhibited a range of colors were intercrossed in 48 combinations (table 4). The observed phenotypes require no explanation, but the calculation of combining ability does. Combining ability is the value of a parent as derived from the mean value of its progeny. For a character limited to the juvenile stage, and when observation of the parents is limited to mature trees, there is no other efficient way to evaluate a parent. When there are no dominance effects and inheritance is polygenic, the observed average phenotype of the crossed progeny will be an average (mid-point) of the combining abilities of the two parents. Using this relation, the combining ability of each parent was calculated by simultaneously solving mid-point equations involving the parents.

The concept of combining ability is usually restricted to an individual parent. By enlarging the concept to include the pollen shower of a stand, an additional estimate of the value of a female parent can be made. That is, the stand is assumed to act as a single male parent having a value that is the average of the observed wind-pollinated progenies taken from the stand. Then the value for the female parent is: observed wind-pollinated progeny phenotypic mean = (female parent combining ability + stand combining ability) /2. In table 4, combining abilities from wind pollinations were calculated from stand values based on the progenies of 17 to 179 wind-pollinated trees.

Heritability estimates in table 5 indicate the precision of the combining-ability values

Table 4. Progeny hypocotyl color phenotypes observed following wind- and self-pollinations, and averaged over all controlled cross-pollinated progeny families of a parent; combining abilities.

Parent identity	Progeny phenotype observed								Combining ability determined from	
	Cross-pollination					Self-pollination			Wind-pollination	Controlled cross-pollination
	Wind			Controlled crosses						
	Trees	Reps.	Index	Number	Index	Trees	Reps.	Index	Index	Index
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
3-176	296	4	15.4	7	16.1	71	1	21.8	17.8	18.3
3-162	341	4	15.6	9	14.8	--	--	--	18.2	18.3
3-135	21	1	13.0	8	12.4	--	--	--	13.0	10.6
3-60	92	1	12.5	11	11.9	5	1	11.2	12.0	11.3
3-36	42	1	15.0	9	14.3	--	--	--	17.0	16.0
3-32	43	1	12.3	6	13.2	10	1	12.6	11.6	9.9
32-58	249	6	12.5	8	13.6	34	1	16.3	11.8	13.6
32-42	250	4	14.6	8	14.4	--	--	--	16.0	15.1
32-9	567	6	13.1	11	13.4	150	1	10.7	13.0	12.7
31-24	49	1	11.9	9	12.3	--	--	--	11.0	12.8
31-8	84	1	17.2	3	13.5	--	--	--	21.6	14.5
11-6	49	1	12.2	6	11.0	--	--	--	10.8	8.4
10-135	430	6	15.0	2	14.3	--	--	--	16.4	12.5
8-7	572	7	11.2	4	10.6	--	--	--	9.2	6.4

Table 5. Heritabilities of hypocotyl color index in slash pine.

Method	Number of families	Relation of family variance to additive variance	Heritability on family mean basis, h^2_f
Components of variance, full-sib	13	$\sigma^2_f = 0.3879\ \sigma^2_A$	.99
Components of variance, half-sib	27	$\sigma^2_f = 0.2500\ \sigma^2_A$	.90
Regression (b) of wind-pollinated half-sib families on (combining ability of female crosses + stand combining ability)/2.	5	--	.91

and illustrate that heritability can be calculated by applying the concept of a combining ability for a stand. Heritability is expressed only on a family-mean basis since selection wi] likely be for parents rather than for individual seedlings. The calculations are based on indices from 3 replications of 50-100 seedlings per entry. The full-sib coefficient 0.3879 differs from 0.5000 because some of the full sibs were related. The third method of table 5 indicates how the stand combining ability can be used to calculate heritability.

The indices of table 4 allow a comparison of wind- with controlled-pollination in obtaining parameters. First, if it is assumed that parents entering controlled crosses are a random sample of the stand, the average indices of their wind-pollinated progenies appear to approach quite well the indices of their crosses (column 4 vs. 6).

Secondly, combining abilities derived from wind pollinations agree satisfactorily with those derived from controlled cross pollination (column 10, vs. 11). It thus appears that where inheritance is polygenic with no dominance, combining abilities could be obtained more efficiently from enlarged but inexpensive tests with wind-pollinated seed than from slower and more expensive controlled pollinations. Such a possibility is discussed in the textbook of Williams (1964), and the general idea is accepted in ranking progeny means to indicate combining ability of polycrossed parents. However, calculating in a single test actual values for different unknown pollen sources (natural stands in our case) is a procedure not found in genetics literature by the authors.

Thirdly, observed values from self progeny should theoretically estimate exactly the _ value of the parent (column 9 vs. 11). Although there does appear to be a fair relation between results from crossing and selfing, the differences are too large to satisfy the theory. Some improvement might have occurred if larger self populations had been available. but small populations are characteristic of selfing. It is suspected that selfing produces subtle background changes in chlorophyll similar to inbreeding depression in growth. Change in background colors would lessen the reliability of anthocyanin readings.

The general correspondence between observed and predicted progeny mean values shown in figure 2 illustrates that the model of polygenic inheritance without dominance fits the datr In this figure the results from crossing among 14 parents (table 4) are shown. For example. tree 3-176 appears as the bottom parent in crosses 1, 2, 6, 9, 10 and 22. The progeny populations in 22 of the crosses were based on 2 to 4 replications of 50 to 100 seedlings each. The rest had only one replication and some, e.g., crosses 15 and 36, only 14 seedlings each. If the latter crosses had had more adequate populations, it is anticipated that they also would fit the model satisfactorily. Reciprocal crosses were made for combinations 1 and 38. The small differences between reciprocals are noteworthy. The figure indicates that the combining-ability concept may prove as applicable to forest trees as it has to other crops.

<u>SUMMARY AND CONCLUSIONS</u>

Three types of pigment inheritance were examined. First, in a nitrogen-deficient nurser there was inherent susceptibility to chlorosis of needles among 11 parent trees as judged by correlated performance of their wind- and self-pollinated progenies. Some of the self-progenies had two to three times as many chlorotic seedlings as their corresponding wind-pollinated progenies; one progeny had 77 percent chlorotic seedlings. This high susceptibility was attributed to an unbalanced polygenic system analogous to inbreeding depression.

Secondly, simple Mendelian inheritance--often with disturbed segretation--was found for cotyledon mutants classified <u>albina, xantha, green xantha,</u> and <u>viridis.</u> When 18'carrier• were intercrossed a common gene was found in only two neighboring trees, thus indicating, if monogenic inheritance is assumed, that the same phenotypes are produced by several nonalleles. Annual changes in the frequencies of the <u>albina</u> and <u>viridis</u> classes in a total of 7,380,000 nursery seedlings were attributed to differences in weather conditions. The generally held hypothesis that self-pollination is the most efficient method for detecting carriers of mutant genes did not hold in 29 progenies where mutant types had shown up in self-pollinations, cross-pollinations, or both. The failure of more mutants to appear in
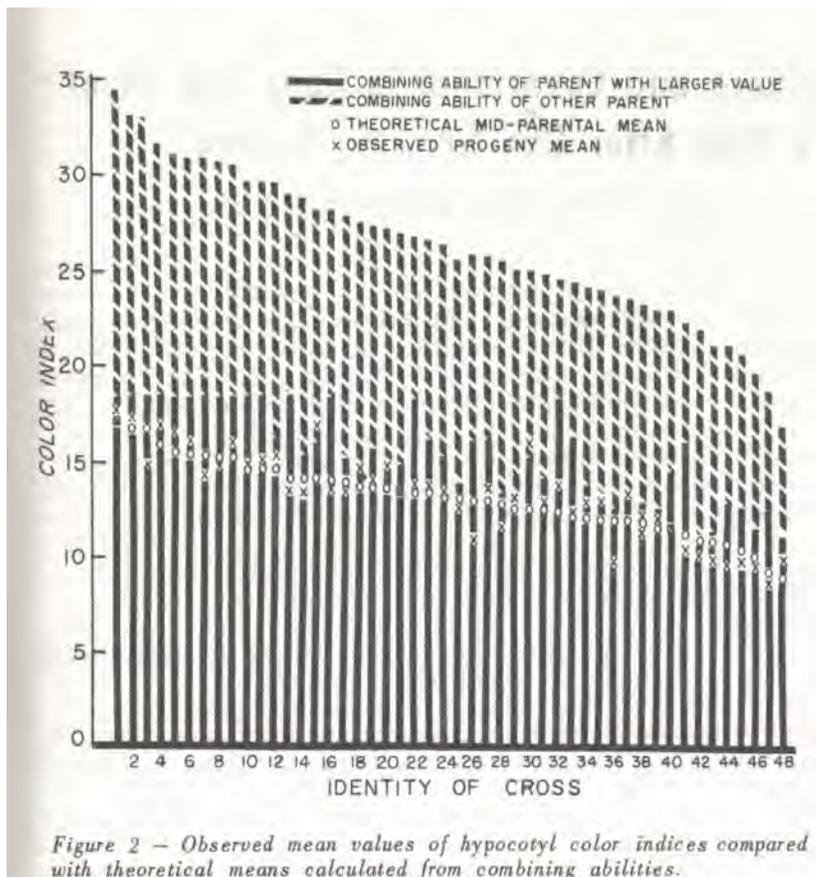
Figure 2 — *Observed mean values of hypocotyl color indices compared with theoretical means calculated from combining abilities.*

the self than in the wind progenies was attributed to disturbed segregation, in some cases so severe that no mutants appeared in the small self populations. Disturbed segregation was as severe in crosses as it was in selfs. Part of the disturbance was due to mortality during nursery germination, since six times as many mutants per unit of material were detected in the laboratory.

Finally, inheritance of hypocotyl colors, which varied from white to dark pink, was additive polygenic with no dominance. A geographic pattern of variation was evident from isogenes drawn among 48 sampled stands over the southern United States. No association of this geographic variation was found with other characters or environmental factors. Limited sampling did not reveal any relationship between pigment intensity and resistance to fusiform rust, ability to germinate without a light treatment, or seedcoat color. A new method was derived for estimating heritabilities and combining abilities when the selections in a single experiment are from one or more stands. It entailed calculating combining abilities for each stand by averaging the wind-pollinated phenotypic values of the sampled trees per stand. With hypocotyl colors converted to a numerical index', h^2_f = .90-.99. Open-pollinated progenies may be more efficient than control pollinations for estimating combining abilities.

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