

## Chapter 10

# *Fixing Transgressive Vigor in Nicotiana Rustica\**

Hybrid vigor has been observed to varying degrees among certain inter-varietal hybrids of the self-pollinated cultivated species *Nicotiana rustica* L. (Bolsunow, 1944; East, 1921). In experiments undertaken to obtain a larger *N. rustica* plant giving increased yield of nicotine, it was reported (Smith and Bacon, 1941) that inbred lines derived as selections from hybrids among three varieties exceeded the parents and  $F_1$ 's in plant height, number of leaves, or size of the largest leaf.

The general experience of breeders of self-pollinated plants has been that improved varieties can be developed through hybridization followed by selection and inbreeding, to fix desirable transgressive characteristics. Yet it is difficult to find data from which quantitative relationships of parents,  $F_1$ , and transgressive inbred can be adequately evaluated; as from replicated and randomized experiments in which the generations have been grown at the same time under comparable conditions. In view of the increasing number of reports on hybrid vigor in self-pollinated crop plants and its suggested utilization (Ashton, 1946), it was considered opportune to present relevant data accumulated on *N. rustica*.

Since methods of partitioning phenotypic variance have become generally available there was additional interest in making further study of the *N. rustica* material. Breeding results obtained in advanced selections could be related to the heritability estimated from data on early generations.

### MATERIALS AND METHODS

Four varieties of *Nicotiana rustica* were used in these experiments. Three of them—*brasilia* strain 34753, Olson 68, and tall type have been described

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in Smith and Bacon (1941). The fourth was received originally from the director of the Tabak-Forschungsinstitut, Baden, Germany, under the name of texana, a designation which we have retained. It is a small, early-maturing type. The four parental varieties were of highly inbred stocks maintained by the Division of Tobacco, Medicinal and Special Crops of the United States Department of Agriculture. The earlier part of the breeding program was carried out while the writer was associated with this organization.

The advanced selection, designated A1, used in these experiments has a complex genetic history of crossing, backcrossing, and inbreeding. This can be briefly summarized by stating that its ultimate composition was, on an average, 60 per cent 34753, 22 per cent Olson, 12 per cent tall type, and 6 per cent texana. About 82 per cent of the A1 genotype was, on chance alone, contributed by the two most vigorous parents, 34753 and Olson 68. This calculation does not take into account any differential effect of selection on changing the frequency of genes introduced from diverse parental origins. Observation of the A1 phenotype led us to believe that selection had further increased the proportion of genes from the two most vigorous parents.

In 1947 the four parents, the six possible  $F_1$ 's, the three double crosses, and the  $F_4$  generation (preceded by three generations of inbreeding) of line A1 were grown in a randomized complete block design with fifteen plants in each plot and replicated six times. In 1949 the two most vigorous varieties (Olson 68 and 34753), the  $F_1$ ,  $F_2$ , backcrosses of the  $F_1$  to each of its parents, and the  $F_6$  generation of line A1 were grown in a randomized complete block design with twenty plants in each plot and replicated eight times.

Measurements were made on plant height, number of leaves or nodes, and length of the largest leaf. In addition, data were taken on the width of the largest leaf, number of days from planting to appearance of the first flower, and total green weight of individual plants.

Typical plants of Olson 68, 34753, the  $F_1$  between these two varieties, and selection A1 are illustrated in Figure 10.1.

## EXPERIMENTAL RESULTS

Data obtained from the 1947 and 1949 plantings are summarized in Tables 10.1 and 10.2, respectively.

### Phenotype-Genotype Relations

Preceding further biometrical analysis of the data, tests for evidence of differential environmental effects and genetic interactions were made. For the former, the relation between genotype mean and non-heritable variability was determined by comparing means and variances of the parents and  $F_1$  (1949 data, Table 10.2). For the characters plant height and leaf length, the variances were unrelated to the means and the parental variances were not significantly different from each other. For node number, however, the

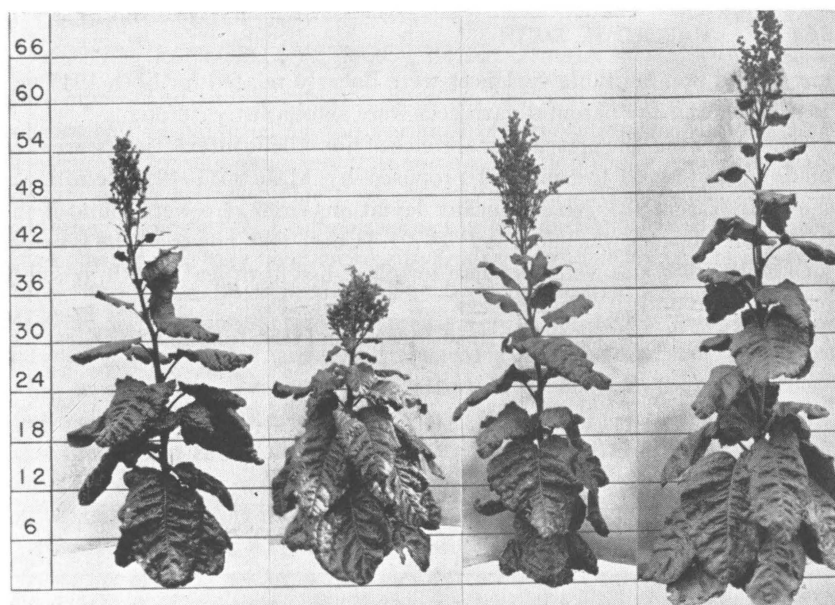


FIG. 10.1—Typical field-grown (1949) plants of *Nicotiana rustica*. Left to right: Olson 68, *brasilia* strain 34753, F<sub>1</sub> Olson 68 × 34753, and selection A1(F<sub>6</sub>). The scale shown at the left is in inches.

TABLE 10.1  
PLANT CHARACTERISTICS IN PARENTAL VARIETIES, HYBRIDS,  
AND AN INBRED SELECTION OF *NICOTIANA RUSTICA*\*

GEN- ERA- TION	TYPE	PLANT HEIGHT				LEAF NUMBER				LEAF LENGTH				MEAN LEAF WIDTH	MEAN DAYS TO MA- TURE	MEAN GREEN WGT. PER PLANT
		Mean		Total within Plot		Mean		Total within Plot		Mean		Total within Plot				
			d.f.	Var.		d.f.	Var.		d.f.	Var.		d.f.	Var.			
P <sub>1</sub>	Olson 68 ( <i>A</i> ).....	in.							in.			in.				lbs.
	34753 ( <i>B</i> ).....	49.9	73	17.8	18.8	72	4.68	11.7	72	1.07	8.7	70.1	1.51			
	Tall type ( <i>C</i> ).....	29.0	84	20.0	15.8	82	7.96	8.7	82	1.43	7.5	66.6	0.89			
	Texana ( <i>D</i> ).....	46.7	83	30.3	16.0	83	1.36	6.2	83	1.24	5.5	48.0	0.51			
		33.6	83	11.1	12.7	83	1.04	6.6	83	1.01	5.4	40.9	0.48			
	Average.....	39.8	...	19.8	15.8	...	3.76	8.3	...	1.19	6.8	56.4	0.85			
F <sub>1</sub>	Olson×34753 ( <i>A</i> × <i>B</i> ) ..	48.5	74	58.3	16.8	74	7.18	10.8	74	1.92	8.7	75.0	1.47			
	Olson×tall ( <i>A</i> × <i>C</i> ).....	42.9	80	25.4	13.0	80	7.74	10.1	80	1.82	9.6	65.1	1.16			
	Olson×texana ( <i>A</i> × <i>D</i> ).....	40.1	81	20.8	11.2	81	4.99	10.6	81	1.49	9.7	70.6	1.13			
	34753×tall ( <i>B</i> × <i>C</i> ).....	47.1	84	45.2	16.6	84	1.77	7.8	84	1.37	6.7	50.5	0.76			
	34753×texana ( <i>B</i> × <i>D</i> ).....	40.3	83	28.1	14.4	83	6.34	8.7	83	2.30	7.4	60.0	0.93			
	Tall×texana ( <i>C</i> × <i>D</i> ) ..	44.2	84	29.3	15.5	83	1.49	7.7	83	1.88	7.0	51.8	0.95			
	Average.....	43.8	...	34.5	14.6	...	4.92	9.3	...	1.80	8.2	62.2	1.07			
F <sub>1</sub> ×F <sub>1</sub>	( <i>A</i> × <i>B</i> )×( <i>C</i> × <i>D</i> ).....	41.9	75	25.1	14.0	74	10.60	8.8	74	2.36	7.7	61.9	0.86			
	( <i>A</i> × <i>C</i> )×( <i>B</i> × <i>D</i> ).....	39.6	82	61.1	12.4	79	9.29	9.8	79	3.11	8.8	66.0	0.99			
	( <i>A</i> × <i>D</i> )×( <i>B</i> × <i>C</i> ).....	42.5	81	39.9	13.9	80	10.80	9.7	80	2.50	8.7	60.4	1.06			
	Average.....	41.3	...	42.0	13.4	...	10.23	9.4	...	2.66	8.4	62.8	0.97			
F <sub>4</sub>	Selection A1.....	54.9	78	78.8	19.9	77	5.28	10.4	77	1.51	8.0	79.6	1.83			
Least significant diff. at 5% level..... 1% level.....		2.68	...	...	1.22	...	...	0.89	...	...	0.76	4.11	0.25			
		3.56	...	...	1.62	...	...	1.19	...	...	1.00	5.46	0.34			

\* Summary of 1947 data.

means and non-heritable variances were linearly related for both 1947 and 1949 data, and the parental variances were significantly different.

Tests to reveal the presence or absence of non-allelic interactions were then made according to the method proposed by Mather (1949). Results are shown in Table 10.3. No significant deviations from zero were found if the level of significance was taken as  $P \geq .01$ . In each test, however, the P values for number of nodes were less than for plant height or leaf length, possibly owing to non-additive gene effects.

It was concluded, on the basis of these tests, that for the two characters

TABLE 10.2  
PLANT CHARACTERS IN THE TWO MOST VIGOROUS VARIETIES OF  
*N. RUSTICA*, THEIR  $F_1$ ,  $F_2$ , AND FIRST BACKCROSS PROGENY  
AND IN SELECTION A1( $F_6$ )\*

GEN- ERA- TION	TYPE	PLANT HEIGHT			NO. OF NODES			LEAF LENGTH		
		Mean	Total with- in Plot		Mean	Total with- in Plot		Mean	Total with- in Plot	
			d.f.	Var.		d.f.	Var.		d.f.	Var.
		in.						in.		
P <sub>1</sub>	Olson 68.....	47.8	141	15.46	24.5	136	3.45	11.6	142	0.68
P <sub>2</sub>	34753.....	28.7	143	22.63	21.8	106	10.10	10.5	127	0.81
F <sub>1</sub>	Olson 68×34753.....	43.2	140	39.18	22.5	110	8.60	11.1	131	0.63
F <sub>2</sub>	(Olson×34753) self...	40.6	149	99.19	23.7	119	10.52	11.2	142	1.08
B <sub>1</sub>	F <sub>1</sub> ×Olson 68.....	47.3	149	40.28	24.9	138	10.49	11.8	144	1.10
B <sub>2</sub>	F <sub>1</sub> ×34753.....	36.2	148	101.50	21.6	117	9.45	10.8	135	0.95
F <sub>6</sub>	Selection A1.....	55.6	133	29.77	31.0	126	6.44	12.0	141	0.69

Least significant diff. at

5% level.....	2.55	1.37	0.49
1% level.....	3.42	1.83	0.66

\* Summary of 1949 data.

TABLE 10.3  
SCALING TESTS FOR AVERAGE ADDITIVENESS OF GENE EFFECTS\*

CHARACTER	TEST A				TEST B				TEST C			
	Dev.	Var.	Dev./ S.E.	P	Dev.	Var.	Dev./ S.E.	P	Dev.	Var.	Dev./ S.E.	P
Plant height..	3.6	2.86	2.13	.03-.04	0.54	1.13	0.25	.80-.81	-0.5	15.48	0.13	.89-.90
No. nodes...	2.8	1.50	2.30	.02-.03	-1.11	1.71	0.84	.40-.41	3.5	6.18	1.40	.16-.17
Leaf length..	0.9	0.47	1.32	.18-.19	0.00	.47	0.00	1.00	0.5	1.77	0.38	.70-.71

\* Based on 1949 means.

plant height and leaf length, the data, as taken, could be used without serious error in partitioning the variance of segregating generations. For node number it was indicated that some correction should be made with the data before further biometrical analysis was undertaken.

Mather suggested that difficulties of the sort encountered in these data with node number may be overcome by finding a transformation of scale on which they would be minimized. The transformations  $\sqrt{X}$ ,  $X^2$ ,  $X^3$ , and  $\sqrt{a + bx}$  on the individual measurements were made. In the latter transformation  $b$  is the linear regression coefficient and  $a$  the intercept. Also, for  $\sqrt{a + bx} = K$ ,  $\sqrt{-K}$  was taken as  $-\sqrt{K}$ . In some cases the transformations reduced the departure from the preferred relationship in one test, only to make the transformed data less preferable by another test. No transformation tried resulted in a consistent improvement over the original scale, and consequently none was used.

It is evident that the significantly different variances in node number of the two parental types were due mainly to different interactions between genotype and environment. From previous experience we know that under ideal conditions of growth, Olson 68 and strain 34753 show approximately the same variability. The adverse weather conditions of the 1949 season were observed to have a more deleterious effect on leaf number in strain 34753. Consequently it was considered that the greater variability of this variety, compared to Olson 68, could be attributed to a greater phenotypic interaction between genotype and environment. In view of these relationships, the analysis of the data on node number was approached in another way, as mentioned below under "Partitioning Phenotypic Variance."

### First Generation Hybrids

Deviations of the  $F_1$  means from mid-parent values (arithmetical average between parental means) can be used to estimate the preponderance of dominant gene effects, acting in one direction, at loci by which the parental complements differ. Mid-parent values were calculated from the 1947 data on the four original varieties. The results for each line are summarized in Table 10.4. The data shown were obtained by first calculating the difference between the  $F_1$  mean and the mid-parent ( $\bar{F}_1 - \bar{MP}$ ) for each cross, then taking the average of the differences for each group of three  $F_1$ 's involving the parent variety under consideration. The ratio of the deviation of the  $F_1$  from the mid-parent to half the parental difference,  $\bar{F}_1 - \bar{MP} / \frac{1}{2}(\bar{P}_2 - \bar{P}_1)$ , is a measure of the relative potency (Mather, 1949; Wigan, 1944) of the gene sets. Potence ratios, calculated from averages, are shown in Table 10.4. For plant height and leaf length the  $F_1$  means fall, on an average, about .6 of the distance from the mid-parent toward the larger parent. For leaf number the  $F_1$  means fall, on an average, about .7 of the distance from the mid-parent toward the smaller parent.

The  $F_1$ 's were taller and had larger leaves, on an average, than the mid-parent. It was concluded, therefore, that a preponderance of dominant+genes was involved in determining differences in plant height and leaf length. In the development of the parent varieties, selection resulted in the accumulation of dominant+modifiers, as is usually the case in naturally cross-pollinated plants.

The result with the character leaf number was different in that the  $F_1$  had fewer leaves, on an average, than the mid-parent. Evidently, in the evolution of the varietal gene sets, there had been accumulated a preponderance of recessive+modifiers (or dominant genes for *fewer* leaves) at the loci by which

TABLE 10.4

DIFFERENCE BETWEEN THE  $F_1$  AND MID-PARENT ( $\bar{F}_1 - \overline{MP}$ ) AND THE POTENCE† RATIO IN INTERVARIETAL HYBRIDS. BOTH VALUES ARE EXPRESSED AS THE AVERAGE FOR EACH VARIETY IN CROSSES WITH THE OTHER THREE VARIETIES\*

VARIETY	PLANT HEIGHT		NO. LEAVES		LEAF LENGTH	
	$F_1 - MP$	Potence†	$F_1 - MP$	Potence	$F_1 - MP$	Potence
	in.				in.	
Olson 68.....	+0.7	+0.10	-3.2	-1.62	+0.9	+0.43
34753.....	+9.1	+1.26	+0.1	+0.09	+0.3	+0.33
Tall.....	+2.6	+0.46	-0.9	-0.87	+0.8	+0.63
Texana.....	+3.8	+0.68	-1.1	-0.53	+1.1	+0.97
Average....	+4.0	+0.62	-1.2	-0.73	+0.8	+0.59

\* 1947 data.

† Potence =  $\bar{F}_1 - \overline{MP} / \frac{1}{2}(\bar{P}_2 - \bar{P}_1)$ .

the parents differed. There can be little doubt that selection for *many* leaves was practiced in producing the parent types. This is especially true for Olson 68 which was developed from hybrid origin by the late Mr. Otto Olson (Smith and Bacon, 1941) by selection for plants yielding large amounts of nicotine. In crosses with Olson 68, the  $F_1$  was consistently below the mid-parent. This result, interpretable as due to an accumulation of a preponderance of recessive genes for the character favored by selection, might be expected occasionally in naturally self-pollinated plants. Dominance is of less importance here than in cross-pollinated organisms, since selection is largely a matter of sorting out superior homozygous combinations.

The 1949 results (Table 10.2) on Olson 68  $\times$  34753 were consistent with those of 1947 discussed above.

### Double Crosses

The three possible double crosses involving all six  $F_1$  hybrids of four varieties were grown in 1947 in order to obtain evidence on genic interactions by comparing experimental results with predicted values. The latter were made

in the manner employed in corn breeding, namely Jenkins' method, in which the average of the four  $F_1$ 's not contributing to the double cross was used. These comparisons are shown in Table 10.5 for the three plant characters studied. The differences between observed means and predicted values in the nine comparisons made were all within the limits required for odds of 19:1. It was concluded that the double cross means for plant height, number of leaves, and leaf length in *N. rustica* could be predicted with a high degree of precision by Jenkins' method. The results indicated that there were no

TABLE 10.5

COMPARISON BETWEEN PREDICTED AND OBSERVED VALUES FOR PLANT HEIGHT, NUMBER OF LEAVES, AND LEAF LENGTH IN THREE DOUBLE CROSSES INVOLVING FOUR VARIETIES OF *N. RUSTICA*

Double Cross	Observed	Predicted	Difference, Obs.-Pred.
<i>Plant height (in.):</i>			
$(A \times B) \times (C \times D)$ .....	41.9 $\pm$ 2.68	42.6 $\pm$ 1.34	-0.7 $\pm$ 3.00
$(A \times C) \times (B \times D)$ .....	39.6 $\pm$ 2.68	45.0 $\pm$ 1.34	-5.4 $\pm$ 3.00
$(A \times D) \times (B \times C)$ .....	42.5 $\pm$ 2.68	44.0 $\pm$ 1.34	-1.5 $\pm$ 3.00
Average.....	41.3	43.8	-2.5
<i>No. leaves:</i>			
$(A \times B) \times (C \times D)$ .....	14.0 $\pm$ 1.22	13.8 $\pm$ 0.61	+0.2 $\pm$ 1.36
$(A \times C) \times (B \times D)$ .....	12.4 $\pm$ 1.22	15.0 $\pm$ 0.61	-2.6 $\pm$ 1.36
$(A \times D) \times (B \times C)$ .....	13.9 $\pm$ 1.22	14.9 $\pm$ 0.61	-1.0 $\pm$ 1.36
Average.....	13.4	14.6	-1.2
<i>Leaf length (in.):</i>			
$(A \times B) \times (C \times D)$ .....	8.8 $\pm$ 0.89	9.3 $\pm$ 0.44	-0.5 $\pm$ 0.99
$(A \times C) \times (B \times D)$ .....	9.8 $\pm$ 0.89	9.2 $\pm$ 0.44	+0.6 $\pm$ 0.99
$(A \times D) \times (B \times C)$ .....	9.7 $\pm$ 0.89	9.3 $\pm$ 0.44	+0.4 $\pm$ 0.99
Average.....	9.4	9.3	+0.1

*A, B, C, D* represent the parent varieties as shown in Table 10.1.

marked interactions between the genes or gene sets from the four varieties when combined in a variety of associations.

To illustrate this point, let us assume that each parent is homozygous for a different allele at each of two independent loci so that  $A = XXYY$ ,  $B = X^1X^1Y^1Y^1$ ,  $C = X^2X^2Y^2Y^2$ , and  $D = X^3X^3Y^3Y^3$ . The  $F_1$ 's represent six different combinations of these alleles. Each double cross contains all four alleles of each locus in four particular combinations. For example, the population  $(A \times B) \times (C \times D)$  is  $1/4XX^2 + 1/4XX^3 + 1/4X^1X^2 + 1/4X^1X^3$  for the  $X$  locus and  $1/4YY^2 + 1/4YY^3 + 1/4Y^1Y^2 + 1/4Y^1Y^3$  for the  $Y$  locus. Sixteen different combinations of alleles at the two loci are possible in this double cross. Accurate prediction of the double cross value on the basis of only four of these combinations, namely:  $F_1$ 's  $A \times C$ ,  $A \times D$ ,  $B \times C$ , and  $B \times D$ ,

indicates that the other 12 possible combinations do not introduce any significant non-additive effects.

Another indication that epistatic effects were unimportant in the inheritance of plant height, leaf number, and leaf length was afforded by the evidence that the means of the double crosses did not differ significantly from each other (Table 10.1).

The average variance of the double crosses was greater than that of the parents or  $F_1$ 's (Table 10.1), as would be expected from segregation.

### Partitioning Phenotypic Variance, Heritability, and Number of Effective Factors

Estimates of the magnitude of the non-heritable variation ( $\sigma_E^2$ ), in populations involving Olson 68 and 34753 (1949 data), were obtained by taking

TABLE 10.6

ESTIMATES OF COMPONENTS OF VARIABILITY, NUMBER OF EFFECTIVE FACTORS ( $K_1$ ), HERITABILITY, AND GAIN FOR PLANT HEIGHT, LEAF LENGTH, AND NUMBER OF NODES IN THE *N. RUSTICA* CROSS OLSON 68  $\times$  BRASILIA, STRAIN 34753\*

Character	$\sigma_E^2$	$\sigma_D^2$	$\sigma_G^2$	$K_1$	Heritability Per Cent	Gain
Plant height . . . .	25.76 $\pm$ 15.3	67.32 $\pm$ 53.5	113.20 $\pm$ 71.3	0.81	54.9	1.74
Leaf length . . . . .	0.71 $\pm$ 0.45	1.04 $\pm$ 1.05	0.22 $\pm$ 0.69	1.38	11.2	0.91
Node number . . . .	7.38 $\pm$ 4.38	8.16 $\pm$ 13.00	2.20 $\pm$ 8.11	0.83	12.4	2.42

\* 1949 data.

an average of the total within plot variance of the non-segregating families— $P_1$ ,  $P_2$ , and  $F_1$ . As shown in Table 10.6, the values obtained were 25.76 for plant height, 0.71 for leaf length, and 7.38 for number of nodes.

The following symbols are used for the components of heritable variance (total phenotypic minus environmental):  $\sigma_G^2$  = variance depending on additive gene effects,  $\sigma_D^2$  = variance depending on dominance. The heritable variance of the  $F_2$  was calculated and equated to:  $1/2\sigma_G^2 + 1/4\sigma_D^2$ . The pooled heritable variance of the two first backcrosses was equated to  $1/2\sigma_G^2 + 1/2\sigma_D^2$ . Solving for  $\sigma_D^2$ , the values obtained were 67.32 for plant height, 1.04 for leaf length, and 8.16 for number of nodes. Values for  $\sigma_G^2$ , as calculated by substitution, were 113.20 for plant height, 0.22 for leaf length, and 2.20 for number of nodes.

In view of the influence on node number of a differential interaction of the two parental genotypes with environment, an additional way of approaching an analysis of the data on this character was tried. If a simple relation



between the environmental variances of the  $P_1$ ,  $P_2$ , and  $F_1$  is assumed, so that  $\sigma_E^2$  of the  $F_1 = 1/2(\sigma_E^2$  of  $P_1 + \sigma_E^2$  of  $P_2)$ , then  $\sigma_E^2$  of the  $F_1 = 6.78$ . The environmental variance of  $B_1$  may then be equated to  $1/2$  (variance of  $P_1 + \text{variance of } F_1$ ), which is 5.12. By a similar relation, the environmental variance of  $B_2$  is equal to 8.44. The pooled heritable variance of  $B_1 + B_2$ , i.e.,  $1/2\sigma_G^2 + 1/2\sigma_D^2$ , may be equated to:  $(10.49 - 5.12) + (9.45 - 8.44)$ . This gave 6.38. The heritable variance of the  $F_2$ , i.e.,  $1/2\sigma_G^2 + 1/4\sigma_D^2$ , may be equated to  $(10.52 - 6.78)$ . This gave 3.74. Solving:  $\sigma_D^2 = 10.56$  and  $\sigma_G^2 = 2.20$ . The former,  $\sigma_D^2$ , has a somewhat larger value than that obtained by the original analysis (8.16, Table 10.6); the latter,  $\sigma_G^2$ , is the same.

Heritability of a character was estimated as the ratio, expressed in per cent, of the variance component due to additive, fixable gene effects ( $\sigma_G^2$ ) to the sum,  $\sigma_G^2 + \sigma_D^2 + \sigma_E^2$ . Heritability of plant height was calculated to be 54.9 per cent, of leaf length 11.5 per cent, and of node number 12.4 per cent.

Estimates of the number of effective factors ( $K_1$ ) were made on the assumptions inherent in the equation  $K_1 = (\bar{P}_1 - \bar{P}_2)^2 / 4\sigma_G^2$ . The values obtained (Table 10.6) were 0.81 for plant height, 1.38 for leaf length, and 0.83 for number of nodes. These estimates were undoubtedly too low, due in part to non-isodirectional distributions of  $+$  and  $-$  genes in the parents. Experimental evidence of non-isodirectional distribution was afforded by the fixing of transgressive characteristics in inbred selections following hybridization between varieties. Some  $+$  genes were contributed by each parent, and consequently could not have been concentrated in one. Linkage in coupling phase and/or differences in magnitude of effect of the individual genes or gene blocks might also have contributed to the low estimates of the number of effective factors.

In the absence of data on  $F_3$ 's, biparental progenies, and double backcrosses (Mather, 1949), the errors of the estimates of  $\sigma_E^2$ ,  $\sigma_D^2$ , and  $\sigma_G^2$  for each character were computed as follows. From the eight replications, four means were calculated by grouping replications 1 and 2, 3 and 4, 5 and 6, and 7 and 8. The standard error of the four independent means was then obtained (Table 10.6). These errors are maximum estimates since there was a pronounced gradient of environmental effects from replication 1 to replication 8.

Mather (1949) is in the process of making an extensive biometrical genetic analysis of plant height in a *Nicotiana rustica* cross, and it was of interest to compare his published results with corresponding statistics presented in this study. From his data so far reported, the average values (mean of 1946 and 1947) for components of variance for plant height are: 9.30 for  $\sigma_E^2$ , 9.25 for  $\sigma_D^2$ , and 18.05 for  $\sigma_G^2$ . The heritability calculated from these estimates is 44.1 per cent. The results reported in this discussion are similar in that heritability is high and  $\sigma_G^2$  has about twice the value of  $\sigma_D^2$ .

### Results of Selection

The result of selection for tall plants with many, large leaves can be seen by comparing the means of A1 with those of the parental and hybrid generations in Tables 10.1 and 10.2.

From the 1947 data it is evident that in the  $F_4$  generation of selection A1 a significant increase had been obtained over the parents and  $F_1$ 's in plant height and green weight. This was accompanied by a lengthening in time required to reach maturity. With regard to this latter character, it was noted that the average time for reaching maturity in five of the six  $F_1$ 's was later than the average of their respective parents. This is contrary to the usual result in first generation hybrids of certain other plants, as maize and tomatoes; and, where early maturity is an important economic character, would generally not be considered a manifestation of hybrid vigor, at least in a "beneficial" sense.

The number of leaves in selection A1 was significantly higher ( $P < .05$ ) than in any of the  $F_1$ 's, and all but the most vigorous parent, Olson 68. Leaves of the selection were shorter than the parent with the longest leaves (Olson 68), not significantly different from the three  $F_1$ 's that involved this parent, and longer than in the other three parents and three  $F_1$ 's.

The 1949 data (Table 10.2) corroborated the 1947 results. There was a significant increase ( $P \leq .01$ ) in plant height and in number of nodes over the two main parents and their  $F_1$ . Number of nodes, rather than of leaves, was used since it is a more reliable criterion of the same character. As in 1947, there was a less marked effect of selection on leaf length, though there appeared to be an increase in A1 from the  $F_4$  to the  $F_6$ . For this character the selection was superior to 34753 and the  $F_1$ , but not significantly different from Olson 68, although a close approach to significance at the 5 per cent level of probability was reached.

The total within plot variances of selection A1( $F_6$ ) for plant height, number of nodes, and leaf length were in no case significantly higher than for the more variable parent. It was deduced, therefore, that the inbred selection had reached relative homozygosity.

The general conclusions were that an inbred selection had been produced which had increased plant height, more nodes, heavier green weight, and a longer growth period than any parent or  $F_1$ . Length of leaf had been maintained at least at the level of the best parent variety.

It was also noted, though no quantitative data were taken, that selection A1 had markedly less vigorous *sucker* growth at *topping* time than any of the other varieties or hybrids. This is an important agronomic character.

### Heritability and Gain

One of the objectives in conducting these experiments was to attempt to determine to what extent the progress realized in actual selection experi-

ments could be related to the heritability of a character as determined from  $F_2$  and first backcross data.

Results on the three main characters studied were similar in that there was no indication of complex genic interactions, and that estimates of the number of effective factors were low and of the same order of magnitude in each. If we wish to assume that the "reach" or selection differential (in terms of standard deviations) was the same for each character, and this is approximately correct though exact records on this point are lacking, then the gain (in terms of standard deviations) due to selection should be roughly proportional to the heritability. The gain was calculated as the difference between the mean of selection A1 and the mid-parent value, divided by the standard deviation of the  $F_2$  (1949 data, Table 10.2).

The relationships between heritabilities and gains can be observed by comparing the last two columns in Table 10.6. With regard to plant height and leaf length, both heritability and gain are higher in the former character; though the gain is less in plant height than would have been anticipated from the relative heritabilities. Some possible explanations for this latter result could be that the selection differential for plant height was lower than for leaf length, that there was a relatively more rapid reduction in heritability, or that an approach to a physiological limit for tallness was made.

The gain in node number is disproportionately high in relation to its heritability. Some possible explanations for this result could be that the selection differential was higher, that there was a genetic correlation with plant height, or that the selected character was determined by a preponderance of recessive genes (see  $F_1$  result), and individual plants selected for high node number were largely homozygous for recessive+ genes.

## DISCUSSION

The experimental results have shown that first generation crosses among different varieties of *Nicotiana rustica* exhibit different degrees of character expression ranging from the smaller parent value to above the larger parent. By selection and inbreeding it was possible to develop an essentially true-breeding improved line which exceeded the best  $P_1$  or  $F_1$  in most characteristics measured.

This same type of result has also been obtained in our experience with the commercial species, *N. tabacum*, and it may be generally characteristic of self-fertilized plants, as, e.g., *Phaseolus vulgaris* (Malinowski, 1928), soybeans (Veatch, 1930), and *Galeopsis* (Müntzing, 1930).

## Crossbreeding

There have been relatively few fundamental changes in the standard domestic varieties of *N. tabacum* over a long period of years, except for recent development of types resistant to destructive diseases (Garner, 1946).

Houser (1911) originally suggested the use of first generation intervarietal tobacco hybrids on a commercial scale to increase yields. He presented breeding results on cigar filler types, dating back to 1903, in which the hybrids outyielded the parent types by as much as 57 per cent. Plant breeders in various tobacco-growing areas of the world have observed hybrid vigor among first generation hybrids of commercial varieties (Ashton, 1946), and have suggested its use in practice to increase production. Currently, consideration is being given to improving the yield of flue-cured varieties by this method (Patel *et al.*, 1949).

The results of Hayes (1912), Hayes, East, and Beinhart (1913), and East and Hayes (1912) showed that by intervarietal hybridization, selection, and inbreeding the number of leaves, an important factor in yield of tobacco, could be fixed at a level exceeding the parents or  $F_1$ . Regarding the use of  $F_1$  hybrids on a commercial scale, they stated (Hayes, East, and Beinhart, 1913),

While it is doubtless true that by this method the yield could be somewhat increased, the yield factor, for cigar wrapper types at least, is only of secondary importance compared with quality. Because of the great importance of quality it seems much more reasonable to suppose that further advance can be made by the production of fixed types which in themselves contain desirable growth factors, such as size, shape, position, uniformity, venation, and number of leaves, together with that complex of conditions which goes to make up quality, than by any other method.

The problem of producing higher yielding varieties of *N. tabacum* with acceptable quality characteristics of the cured leaf remains today. Kosmodemjanskii (1941) bred four families from the cross Dubec 44×Trebizond 1272, two Russian varieties of *N. tabacum*, which, he reported, were uniform for morphological characters and flavor and maintained transgression in plant height and number of leaves to the  $F_7$  generation.

While first generation hybrids between selected parents may be of use as a temporary measure to improve self-fertilizing crop plants, it would appear, in so far as can be generalized from the results on *Nicotiana*, that production of fixed types with favorable transgressive characteristics offers a better long-time solution. Within any one type of tobacco, such as flue-cured, there are currently available a number of high quality inbred varieties which, though of similar phenotype, may be expected to differ by genes of a multifactorial system affecting size characteristics (Emerson and Smith, 1950). Selections from intervarietal crosses may be expected, therefore, to yield fixed types of increased size without presenting undue difficulties to the breeder attempting to maintain quality.

In order to discuss the hereditary basis for experimental results on heterosis and inbreeding, current concepts of the genetic and evolutionary mechanisms involved are briefly presented. In the evolution of naturally crossbred organisms, mutation and selection result in the accumulation of dominant favorable genes, hidden deleterious recessives, and alleles or complexes of

linked polygenes which give heterotic effects as heterozygotes. Heterosis is explained genetically as due to the accumulated effect of the favorable dominants and/or coadapted heterozygous combinations. It is an adaptive evolutionary phenomenon (Dobzhansky, 1950).

### Selfing

In naturally selfed populations there are accumulated, for the most part, favorable genes that are either dominant, recessive, or lacking in strong allelic interactions. Dominance is of little evolutionary significance, and hence a preponderance of favorable dominant genes is not to be expected. Furthermore, there would ordinarily be no adaptive significance to favorable heterozygous combinations. One possible exception is suggested by Brieger's (1950) demonstration that "if survival values for both homozygotes should be below 0.5 (compared to the heterozygote value of 1.0) in selfed populations, a final equilibrium is reached with all three genotypes remaining in the population." Such a condition might have adaptive value in maintaining variability in selfed organisms. Hybrid vigor in self-pollinated plants, in view of the above considerations, is a chance manifestation, an "evolutionary accident" causing luxuriant growth (Dobzhansky, 1950), and not an adaptive product of mutation and selection.

However, from published data on crosses within selfed species of cultivated plants, it appears that hybrid vigor is of frequent rather than chance occurrence. Reported results with flax (Carnahan, 1947), wheat (Harrington, 1944), barley (Immer, 1941), tomatoes (Larson and Curran, 1944), eggplants (Odland and Noll, 1948), and soybeans (Weiss, Weber, and Kalton, 1947) all demonstrated that hybrid vigor is characteristic of  $F_1$ 's. If these data constitute a representative sample, then, although hybrid vigor is an *evolutionary accident* in naturally selfed species, it is not a *genetical accident*.

The result may be interpreted genetically as follows: Selfed species are purged of deleterious genes by selection. Different varieties within the species have accumulated different alleles all of which control "non-defective," slightly different physiological reactions. The combination of divergent alleles in heterozygous condition may, more frequently than not, act as East has suggested in a complementary manner to produce a more efficient physiological condition. This is expressed phenotypically by the hybrid manifesting more vigorous growth than midway between the homozygotes. Subsequent selection and inbreeding, however, would permit an accumulation of the most favorable alleles or gene complexes in the homozygous condition.

As a simplified schematic example, let us assume that two varieties,  $P_1$  and  $P_2$ , differ by three alleles or linked polygene complexes:  $X^1$  is dominant and favorable for vigorous growth,  $Y^1$  is a favorable recessive, and at the  $Z$  locus the product of the heterozygous condition is above the mean of the

homozygotes. The composition of parents,  $F_1$ , and selected inbred is shown below with arbitrary "size" values assigned to each.

$$P_1 = X^1X^1(4) + Y^1Y^1(4) + Z^1Z^1(2) = 10$$

$$P_2 = X^2X^2(2) + Y^2Y^2(2) + Z^2Z^2(6) = 10$$

$$F_1 = X^1X^2(4) + Y^1Y^2(2) + Z^1Z^2(5) = 11$$

$$\text{sel.} = X^1X^1(4) + Y^1Y^1(4) + Z^2Z^2(6) = 14$$

Although the difficulty in selecting superior inbreds would become greater with increasing numbers of effective segregating units, the following advantages of selfed over crossbred systems would enhance the opportunity for success: (1) lack of deleterious recessives, (2) less preponderance of dominant favorable alleles, (3) homozygous pairs of alleles are superior, as a result of an adaptive evolutionary process, to heterozygous combinations. Naturally inbred organisms are products of historical evolutionary processes in which harmonious systems of homozygous loci have been selected to attain optimum adaptation. These considerations favor the expectancy and practicability of obtaining maximum advance through selection and inbreeding with self-fertilized organisms.

#### SUMMARY

There were two general purposes in conducting these experiments: First, to demonstrate that by selection following intervarietal hybridization in a self-fertilized organism, inbreds could be produced which transgressed the character expression in parents and  $F_1$ ; secondly, to investigate the relation between estimated heritability and the actual results of selection.

An inbred selection of *Nicotiana rustica* which transgressed the  $P_1$  and  $F_1$  characteristics in plant height, node number, and leaf length was obtained. The heritabilities for these three characters were calculated to be 54.9 per cent, 12.4 per cent, and 11.2 per cent, respectively. The gains (in terms of standard deviations) due to selection were 1.74, 2.42, and 0.91, respectively. Some possible explanations for the lack of direct proportionality between heritability and gain were discussed.

The number of effective segregating factors for each of the three characters studied was estimated to be of the same order of magnitude and relatively few. Non-isodirectional distribution of + and - genes in the parent varieties contributed to an underestimation of this number.

Non-allelic interactions were apparently not an important source of variation, as indicated by scaling tests and evidence from double cross means.

Reasons for expecting greater advances by selection and inbreeding, as contrasted to the use of first generation hybrids, in naturally self-fertilizing genetic systems were reviewed.