COMPARISONS AMONG STRAINS OF THE TOBACCO CULTIVAR HICKS ILLUSTRATING VARIABILITY WITHIN A SINGLE CULTIVAR

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SUMMARY

Random effect models were used to compare seven strains of Hicks tobacco at two sites for 3 years in north Queensland. Broadleaf Hicks (U.S.A.) was found to be commercially and significantly superior to the local strain. There were no differences in quality, but the saleable yield of the introduction was 129% of that of the Queensland strain, and the former was not inferior in any other important characteristic. The new strain has been renamed "Hicks Q46" for local use. Commercial and statistical significances of the results were contrasted. The most divergent strains were identified, Coley Special being the strain most different from the average Hicks phenotype in the test environments.

I. INTRODUCTION

Despite the release of new cultivars which may be improvements over some or all predecessors, a few older cultivars continue to find favour among farmers. The reasons for this persistence may include particular desirable properties, good average performance and conservatism, which may be soundly based. If warranted, refinement of these cultivars may be attempted through re-selection and stricter seed maintenance.

The flue-cured tobacco cultivar Hicks may be such a cultivar. Although it is susceptible to several diseases and yields relatively poorly, it has had wide popularity throughout the world, and it is still a major cultivar in Australia. Its popularity stems from generally good ripening and curing properties and good quality. The cultivar is variable in several important characters, and several strains have been selected in various countries. Morphological variations among and within some strains may be conspicuous.

The Queensland strain of Hicks has been criticised mainly for variability in yield and appearance, and improvement of the cultivar was warranted because the local importance of Hicks seemed assured for some time. Therefore, the local strain was compared with other commercial strains, with the object of promoting the best strain for local use. This paper reports on these comparisons in north Queensland environments, and also illustrates the variation which may be found in a "single" cultivar.

II. MATERIALS AND METHODS

Strains and cultural details.—Seven putative strains of Hicks were compared. They were Hicks (Queensland), Iwata 2 (Japan), Broadleaf Hicks (U.S.A.), Coley Special (U.S.A.), Hicks Broadleaf 62-975 (Canada), Certified Hicks (Rhodesia) and Warner's Hicks (Queensland). These were considered to represent well the commercial Hicks strains at the commencement of the work and to be a random sample from all such strains. The one generation of selfed seed was used for each genotype throughout these experiments.

Six experiments were conducted, one at each of two sites in each of 3 years. Each experiment consisted of four randomized complete blocks. The sites were at Parada Research Station, Mutchilba, and at Tobacco Research Institute, Mareeba. The seasons at both sites were 1964-65, 1965-66 and 1966-67. Environmental effects were considered to be random.

Two-row plots of 40 plants were used, with 4 ft (1·22 m) between rows and spacings of 21 in. (53·3 cm) between plants. No interplot guards were used, but buffer plants were grown at the ends of each plot and there were peripheral guard rows for each experiment.

Normal cultural practices were used (Chippendale *et al.* 1961). Seedings were raised at Parada and transplanting was done by hand in order to minimize stand variability and plant losses. Replanting was done 1 week later where required. Final plot stands were excellent in all experiments. Each experiment was planted in a single day. The two sites were planted as close to each other in time as was practicable.

Inflorescences were removed at 3-day or 4-day intervals, following the opening of at least five corollas per plant. The inflorescence was removed below the uppermost true leaf. Cumulative topping totals per plot were recorded. Cured leaf was bulked down for several weeks prior to sorting into trash (nondescript leaf) and into 1-3 other sub-bundles to facilitate quality assessment. The sorting room was maintained at approximately 75°F (23·75°C) and 70% relative humidity, and only standard neon light was provided. Leaf was weighed after sorting.

Characters measured.—The measured characters, their units of measurement and the abbreviations used subsequently are listed as follows:

Total cured yield (g per plot). (Y).
Overall quality index (points). (Qual.).
Yield of saleable leaf (g per plot). (S).
Proportion of total leaf which was saleable ($\arcsin\sqrt{S/Y}$). (S/Y).
Quality index of saleable leaf (points). (Qual. S).
Ratio of nondescript yield to total yield ($\log_{10}$ (100ND/Y)). (ND/Y).
Area index of 15th leaf from top of the topped plant (sq. in.). (Area, 1f 15).
Area index of 10th leaf (sq. in.). (Area, 1f 10).
Area index of 5th leaf (sq in.). (Area, 1f 5).
Length-width ratio of 15th leaf (10W/L). (L/W, 15).
Length-width ratio of leaf 10 (10W/L). (L/W, 10).
Length-width ratio of 5th leaf (10W/L). (L/W, 5).
Number of commercial leaves. (Lf No.).
Mean cured weight per assessed leaf (g per leaf). (Wt/Lf).
Period to median harvest (days). (Hvst).
Period to median flowering (days). (Flwr).
Period between flowering and harvest (days). (Flwr→Hvst).
Final commercial height (in.). (Ht).
Mean internode length (in.). (Int.).
Mean number of suckers per count per plant. (Skrs).
Mean number of sucker nodes per plant. (Skr Nodes).

Total yield included all cured leaf, excluding only the dark trash (nondescript). Saleable yield was the yield of cured leaf which exceeded the estimated minimum saleability level of quality in the respective auction season. Minimum saleability level was estimated from random samples of bales on the

Mareeba auction floor which had reserve prices equal to, or less than, 80 cents per lb. Several sampling days were used in each season. Minimum saleability level was estimated as the 5% upper confidence limit of the mean quality rating of those bales which sold only after arbitration. The levels adopted were 34, 22 and 34 points on the quality index for the years 1965, 1966 and 1967, respectively.

Quality (Garner 1946; Barnard 1960; Green 1966) was judged subjectively following traditional principles (French 1964). The schedule of properties used, including definitions and points, is given in Table 1. The quality index of the leaf bundle (qy) was the total of these points over all properties. Plot quality indices were calculated from weighted averages of the appropriate qy's, the weighting coefficient being the masses of the bundles. Quality of saleable leaf was estimated in the same manner using only those bundles which equalled or exceeded the minimum saleability level.

TABLE 1

Schedule of Properties, Definitions, and Points Used in Assessing Tobacco Quality

Property	Definition		Points
Colour	Lustrous	Orange; lemon	50
		Bright; mahogany; deep orange	45
	Semi-lustrous	Orange; lemon	40
		Light orange; bright	35
		Deep orange; mahogany	30
	Drab	Orange	25
		Light orange; bright	20
		Mahogany; deep orange	15
	Dark	Mahogany	10
	Off-type Colours:		
	Pale	— — —	30
	↓	Red — Two-faced	25
	↓	↓ Slatey ↓	20
	↓	↓ ↓ ↓	15
	↓	↓ ↓ ↓	10
	↓	↓ ↓ ↓	5
	↓	↓ ↓ ↓	0
Elasticity	Fully elastic		10
	Semi-elastic→elastic		8
	Soft, pliable		6
	Semi-pliable		4
	Semi-stiff; slightly shattery		2
	Stiff; shattery		0
Grain	Open	"Corky" surface	10
	Medium→open	"Corky" surface	8
	Medium	"Corky" surface	6
	Medium→close	Smooth surface	4
	Close	Smooth surface	2
	Slick	Smooth surface	0
Maturity	Fully mature; slightly overripe		30
	↕		24
	↕		18
	↕		12
	↕		6
	Grossly overripe; grossly immature		0

Maximum = 50 + 10 + 10 + 30 = 100.

Leaf lengths and maximum widths were measured on adaxial surfaces at early senescence. Mean values of 10 random plants per plot were used to estimate leaf shape indices (L/W ratios), and leaf area indices ($A = 0 \cdot 66$ LW). Commercial leaf number was counted between the top of the soil-hill and the uppermost node after topping. Period to median harvest was estimated using the number of leaves actually harvested, and mean weight per cured leaf was based on the number of leaves actually cured. Commercial height and mean internode length were estimated over the same portion of the stem as was used for commercial leaf number, using the same plants. Desuckering was done so that suckers did not exceed 6 in. in length, nor did they exceed two-thirds of the aboveground nodes in number. Sucker counts at each desuckering were made on the same 10 marked random plants in each plot.

Data processing.—Original data were tested for normality using a class interval of $\frac{1}{4}\hat{\rho}$. Phenotypic variances of the strains over all environments were tested for homogeneity using Bartlett's chi-square procedure. These two criteria were the principal ones used in determining transformations (Bartlett 1947; Steel and Torrie 1960). The scales given previously were those found to be suitable for these data. The usual random effects model for randomized complete blocks experiments was used, as were the usual procedures for the analysis of variance of the individual experiments (Steel and Torrie 1960; LeClerg, Leonard and Clark 1962). Error variances of the individual experiments were tested for homogeneity prior to pooling. The model used subsequently depended upon whether the set of experiments with homogeneous errors was balanced with respect to sites and years.

The random effects models used for the pooled analyses assumed that both sites and years were sampled. Model 1 classified environments with respect to these two elements, but model 2 did not. Both models were the common ones used in such analyses (Comstock and Moll 1963), with the usual assumptions about the effects. Model 1 was used where the experiments formed a balanced set with respect to sites and years, and model 2 was applied where they did not. The usual expectations of the mean squares for these models were used (Jones, Matzinger and Collins 1960; Comstock and Moll 1963), and the usual procedures for the pooled analyses were followed (LeClerg, Leonard and Clark 1962). Significances of mean squares were tested at 0·05 and 0·01 levels of probability with the F-test or with the F′-test where no exact F-test was available (Cochran 1951; LeClerg, Leonard and Clark 1962), using the appropriate degrees of freedom (f′) for these tests (Satterthwaite 1946).

The variance of the pooled phenotypic mean was estimated in the usual way from:

$$\sigma^2_{\overline{X}P} = \sigma^2_{GS}/s + \sigma^2_{GY}/y + \sigma^2_{GSY}/sy + \sigma^2/rsy \text{ for model 1, and}$$

$$\sigma^2_{\overline{X}P} = \sigma^2_{GE}/e + \sigma^2/re \text{ for model 2,}$$

where (r, s, y) or (r, e) define the total pooled experiment for the character and other symbols have their usual meanings (Jones, Matzinger and Collins 1960; Liang, Heyne and Walter 1966; Tyson and Bradner 1967; Schutz and Bernard 1967). Estimation of least significant differences (L.S.D.) was done in the usual way using the appropriate $\sigma^2_{\overline{X}P}$ as the error variance. Least significant differences were assumed to be satisfactory for multiple comparisons among means (Balaam 1963).

III. RESULTS

Error homogeneity.—The maximum numbers of experiments constituting a set with homogeneous error variances are given in Table 2, together with listings of omitted experiments. These reduced sets of experiments generally were not balanced with respect to sites and years, in which case the pooled analysis followed model 2. Only 9 of the 21 characters had all error variances homogeneous.

TABLE 2

NUMBER OF EXPERIMENTS WITH HOMOGENEOUS ERRORS, OMITTED EXPERIMENTS AND COEFFICIENTS OF VARIATION FOR ALL CHARACTERS

Character	No. of Experiments	Omitted Experiments†	Coefficient of Variation (%)
Y	6	Nil	7·88
Qual.	5	P2	5·95
S	6	Nil	12·23
S/Y	4	P1, C1	7·19
Qual. S	4	C1, P3	4·63
ND/Y	6	Nil	13·91
Area, lf 15	5	C3	10·67
Area, lf 10	4	C1, C2	7·34
Area, lf 5	6	Nil	11·99
L/W, 15	6	Nil	1·98
L/W, 10	6	Nil	4·00
L/W, 5	5	C2	3·98
Lf No.	4	C2, C3	3·66
Wt / Lf	3	C1, P2, C2	12·94
	3	P1, P3, C3	6·50
Hvst	6	Nil	2·30
Flwr	5	C2	3·10
Flwr—>Hvst	6	Nil	8·23
Ht	4	P2, C2	3·94
Int.	5	C3	4·43
Skrs	5	C3	10·37
Skr Nodes	6	Nil	10·36

† P=Parada, C=CSIRO, 1=1964–65, 2=1965–66, 3=1966–67.

Coefficients of variation rarely exceeded 12% (Table 2), indicating that all characters were evaluated with acceptable levels of precision.

Significance tests.—The genotype mean square was significant at the 0·01 level for 14 of the 21 characters, and at the 0·05 level for a further 1 character (Table 3). Both quality indices had non-significant genotype mean squares, as may be expected among strains of Hicks where high quality was a common characteristic. Although the genotype mean square estimates from the two sets of experiments for the character weight per leaf were of similar dimension, the significances for these mean squares were very different. This resulted from a larger error variance in the second set of environments.

It was apparent that considerable genetic variability existed for most agronomic characters investigated, in spite of the apparent restriction of the genetic base in this group of genotypes. Least significant differences are given in Table 3, and the practical utility of these differences will be discussed subsequently.

Genotype x environment interactions were non-significant in all characters except length-width ratio for leaf 15. This indicated that these genotypes were similarly adapted to the test environments. Consequently, estimates of the

TABLE 3

SIGNIFICANCE OF GENOTYPE MEAN SQUARES, GENERAL MEANS, AND LEAST SIGNIFICANT DIFFERENCES AT P = 0·05 AND P = 0·01 FOR ALL CHARACTERS†

Character	Significance of Genotype Mean Squares‡	General Mean	L.S.D.	
			0·05	0·01
Y	**	5,848§	453	601
Qual.	N.S.	48·20	1·80	2·38
S	**	4,871‖	341	452
S/Y	N.S.	64·71¶	3·29	4·36
Qual. S	N.S.	53·78	1·76	2·33
ND/Y	**	1·0261[a]	0·0816	0·1083
Area, lf 15	N.S.	203·7	13·7	18·1
Area, lf 10	*	190·6	9·9	13·1
Area, lf 5	**	160·0	11·0	14·6
L/W, 15 (10W/L) ..	**	7·210[b]	0·144	0·192
L/W, 10 (10W/L) ..	**	4·346[c]	0·099	0·132
L/W, 5 (10W/L) ..	**	3·883[d]	0·097	0·128
Lf No.	**	23·4	0·6	0·8
Wt / Lf	N.S.	9·12	0·97	1·29
	**	8·62	0·97	1·29
Hvst	N.S.	102·3	1·4	1·8
Flwr	**	62·3	1·6	2·1
Flwr—>Hvst ..	N.S.	40·0	1·9	2·5
Ht	**	48·1	1·34	1·77
Int.	**	2·14	0·08	0·10
Skrs	**	8·54	0·56	0·74
Skr Nodes	**	5·19	0·31	0·41

† Units of measurement were given in Methods.
‡ N.S. = not significant; * = significant at P=0·05; ** = significant at P=0·01.
§ Equivalent to 2,248 kg/ha, or 2,012 lb/ac.
‖ Equivalent to 1,873 kg/ha, or 1,676 lb/ac.
¶ Equivalent to 82% in original units.
[a] Equivalent to 0·11 in original units.
[b] Actual L/W ratio was 1·39.
[c] Actual L/W ratio was 2·30.
[d] Actual L/W ratio was 2·58.

variance of a phenotypic mean included only error variance in all characters except the length-width ratio for leaf 15, for which genotype x year interaction variance was included. The coefficient of variation of this test variance was 2·22%.

Comparisons among phenotypic means.—The mean performance of each strain over all pooled environments is given in Table 4 for all characters. The most important commercial characters in this study were total and saleable yields, because neither quality index had significant genotype variance. From the least significant differences in Table 3 and the means in Table 4, Broadleaf Hicks (U.S.A.) and Coley Special were found to be the highest yielding. They were 129% and 128% respectively of the Queensland strain for saleable yield, and 124% and 123% respectively for total yield. For these two high-yielding strains, the proportion of leaf that was saleable was 106% of that of the local strain, but this difference was not significant. The ratio of nondescript weight to total yield of Broadleaf Hicks was 20% lower than that of Queensland Hicks (significant at P=0·01), while that of Coley Special was not different from that of the local strain. Cured weight per leaf was the same for these three genotypes in the first set of environments for this character, but Broadleaf Hicks had heavier cured leaves in the second set (115% that of Queensland Hicks). This was not the result of larger leaf areas in Broadleaf Hicks, because there were no significant differences for leaf area of any leaf position among these two genotypes. However, areas of leaves 10 and 5 of Coley Special were only 92% and 83% respectively of those of Queensland Hicks (significant at P=0·01).

TABLE 4

MEAN PERFORMANCE OF INDIVIDUAL STRAINS OVER ALL ENVIRONMENTS FOR ALL CHARACTERS†

Character	Genotype‡						
	1	2	3	4	5	6	7
Y	5,098	5,947	6,283	6,219	5,633	5,853	5,937
Y (kg/ha) ..	1,960	2,286	2,416	2,391	2,166	2,250	2,283
Y (lb/ac) ..	1,754	2,046	2,161	2,139	1,938	2,013	2,042
Qual. ..	46·85	48·51	48·55	48·94	49·55	47·93	47·06
S	4,120	4,956	5,305	5,275	4,830	4,787	4,819
S (kg/ha) ..	1,584	1,905	2,040	2,028	1,857	1,840	1,853
S (lb/ac) ..	1,417	1,705	1,825	1,815	1,662	1,647	1,658
S/Y	62·49	64·62	66·27	66·08	67·15	62·38	63·97
S/Y (%) ..	79	82	84	84	85	79	81
Qual. S ..	53·12	54·95	53·72	54·10	54·03	54·04	52·51
ND/Y ..	1·1909	0·9144	0·9498	1·1053	1·0349	1·0265	0·9608
ND/Y (%) ..	15	8	9	13	11	10	9
Area, lf 15 ..	200·0	198·9	211·0	207·0	200·0	206·9	201·9
Area, lf 10 ..	193·8	193·9	196·6	177·9	186·4	189·2	196·6
Area, lf 5 ..	170·0	158·5	164·5	141·6	165·4	158·7	161·6
10W/L, 15 ..	7·396	7·334	7·161	6·941	7·214	7·245	7·183
10W/L, 10 ..	4·531	4·462	4·240	4·030	4·399	4·455	4·304
10W/L, 5 ..	4·046	3·930	3·829	3·614	3·938	3·922	3·906
Lf No. ..	22·5	22·3	23·0	26·0	23·4	23·4	23·6
Wt / Lf ..	8·61	9·87	9·15	8·46	9·93	9·54	8·28
	7·89	9·19	9·06	7·96	8·80	8·80	8·63
Hvst	101·6	102·0	102·9	103·7	102·2	101·7	101·7
Flwr	61·3	60·9	62·6	65·9	61·6	61·5	62·7
Flwr—>Hvst	40·7	40·7	40·9	38·2	40·5	40·0	39·0
Ht	46·73	45·62	48·03	54·98	46·42	47·36	47·48
Int.	2·16	2·12	2·18	2·21	2·07	2·14	2·07
Skrs	8·72	9·01	8·40	7·85	8·72	8·66	8·44
Skr Nodes ..	5·48	5·55	5·02	4·49	5·35	5·31	5·10

† Units of measurement have been given in Methods.
‡—
1 = Queensland Hicks, 2 = Iwata 2, 3 = Broadleaf Hicks (U.S.A.),
4 = Coley Special, 5 = Canadian Hicks, 6 = Rhodesian Hicks,
7 = Warner's Hicks.

The two introductions had significantly (at P=0·01) narrower leaves than Queensland Hicks at all leaf positions, those of Coley Special being particularly narrow in the top of the plant. Coley Special had significantly more commercial leaves than the other genotypes (P=0·01), and was taller. Internode length was the same among these three strains. No significant differences existed among the strains for days to median harvest. Coley Special and Broadleaf Hicks (U.S.A.) had significantly fewer suckers than Queensland Hicks (90% of the latter).

These data revealed that the two highest yielding strains differed substantially for several characters. Broadleaf Hicks had heavier leaves, less trash and less narrowness in the top leaves, and conformed more closely to the familiar morphology of Queensland Hicks than did Coley Special. For these environments, Broadleaf Hicks had considerably higher saleable yield than Queensland Hicks, but the two were similar in saleable quality. It has been renamed "Hicks Q46" for local commercial use, and the Queensland strain has been renamed "Hicks Q34".

IV. DISCUSSION

(a) Commercial Importance of the Genotypic Differences

Statistically significant differences existed among strains for many of the characters investigated (Tables 3 and 4) and it is necessary to examine the practical significance of these differences. The phenotypic means of each strain for each character are presented as percentages of their respective general means in Table 5. The range between the greatest and least mean for each character in percentage units is given also.

TABLE 5

Overall Phenotype Means Expressed as Percentages of Their Respective General Means, and the Ranges of the Phenotype Means in Percentage Units

Character	Genotype†							Range
	1	2	3	4	5	6	7	
Y	87	102	107	106	96	100	102	20
Qual.	97	100	100	101	103	99	97	6
S	85	102	109	108	99	98	99	24
S/Y	97	100	102	102	104	96	99	8
Qual.S	99	102	100	101	100	100	98	4
ND/Y	116	89	93	108	101	100	94	27
Area, lf 15	98	98	104	102	98	102	99	6
Area, lf 10	102	102	103	93	98	99	103	10
Area, lf 5	106	99	103	89	103	99	101	17
L/W, 15	103	102	99	96	100	100	100	7
L/W, 10	104	103	98	93	101	103	99	11
L/W, 5	104	101	99	93	101	101	101	11
Lf No.	96	95	98	111	100	100	101	16
Wt / Lf	94	108	100	93	109	105	91	17
	92	107	105	92	102	102	100	13
Hvst	99	100	101	101	100	99	99	2
Flwr	98	98	100	106	99	99	101	8
Flwr→Hvst	102	102	102	96	101	100	98	6
Ht	97	95	100	114	97	98	99	19
Int.	101	99	102	103	97	100	97	6
Skrs.	102	106	98	92	102	101	99	14
Skr Nodes	106	107	97	87	103	102	98	20

†— 1 = Queensland Hicks, 2 = Iwata 2, 3 = Broadleaf Hicks (U.S.A.), 4 = Coley Special, 5 = Canadian Hicks, 6 = Rhodesian Hicks, 7 = Warner's Hicks.

With few exceptions, significance of the genotype mean square at $P=0\cdot05$ was paralleled by the range being equal to, or greater than, 10% of the general mean. Differences of this magnitude were regarded as being meaningful in practice. The 29% superiority of Broadleaf Hicks over Queensland Hicks for saleable yield is clearly of commercial significance, as these two strains occupy the two extremes of this character for which the range is of practical importance. Where genotype mean squares were not significant at $P=0\cdot05$, the characters, without exception, had ranges of phenotypic means which were less than 10% of their respective general means, and thus were of no practical consequence.

The definition of what constitutes a difference of practical significance is somewhat arbitrary. The standard adopted here intuitively appears to be reasonable. Using this criterion, the data of this study suggested that statistical and commercial significance can be closely associated.

(b) Heterogeneity of Hicks

It was apparent that large differences existed among the tested strains for many of the characters measured. The data indicated that some genotypes were more divergent than others, which suggested that these genotypes included distinctly different strains of Hicks, or that some genotypes may not have been of Hicks origin. In order to identify the divergent genotypes, phenotypic mean was regarded as being substantially different from the population mean if the genotype mean square was significant at $P=0 \cdot 05$, and if the mean was outside a range of $\pm 5\%$ of the general mean for the character in question.

On these bases, Coley Special was a diverse line for 12 characters, Queensland Hicks for 6 characters, Iwata 2 for 4 characters, Broadleaf Hicks for 2 characters, and Warner's Hicks for 1 character, while Canadian and Rhodesian Hicks approximated the general mean in all characters measured. Thus Coley Special was found to be widely divergent from the average Hicks phenotype. Despite this, it could be of Hicks origin. It has been observed that Hicks is a variable cultivar (Jones and Mann 1958), and other conspicuous variants have been described (Gordon 1969).

Several possible sources of such genetic variability exist for a predominantly self-fertilized cultivar. They include the following: (1) the origin of Hicks may have been heterogeneous; (2) a small level of cross-fertilization occurs (McMurtrey, Wilson and Pointer 1960 reported 2–11%) which may maintain a characteristic degree of heterozygosity (Bennett and Binet 1956; Allard and Workman 1963); (3) mutation may account for some variation, especially of simply inherited variations; (4) differential selection pressures among linked genes may cause gene shifts when new crossovers occur, or when new selection pressures arise (Mather 1942; Dobzhansky *et al.* 1959).

The actual origin of the variability in this cultivar is not known, but the present level of variability has probably been enhanced by differences in natural and artificial selection in the diverse environments under which the various strains have been derived. Regardless of the origin of the variability, the data have indicated that substantial advances should be possible from selection among strains of the Hicks cultivar.

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