HERITABLE RELATION OF WAX CONTENT AND GREEN PIGMENTATION OF LINT IN UPLAND COTTON [1]

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INTRODUCTION

The wax content of cotton lint has been the subject of a number of investigations. Lecomber and Probert (*8*)[3] studied cottons originating in different parts of the world in order to determine the extent to which their origins could be identified by the content and properties of wax. It was found that cottons could not be identified in this way. Fargher and Higginbotham (*6*) studied scoured cottons with reference to the percentage losses sustained and the amount of certain noncellulose constituents, including waxes, retained after various kiering treatments. Ahmad and Sen (*1*) were interested in the possible influence of wax content on the classer's judgment of "silkiness" or "harshness." Fargher and Probert (*4*), Clifford and Probert (*2*), and Fargher and Higginbotham (*5*) have reported that the different components of the wax from the lint in American and Egyptian cottons are highly complex mixtures of various classes of waxy substances.

As early as 1911, Knecht (*7*) reported that Egyptian raw cotton from which the wax had been removed was difficult to handle in the drawing and spinning processes. Excessive amounts of waste, irregular results, and a tendency for the fiber to adhere to the rollers were encountered. In spinning finer count yarns, excessive breakage, which was also observed in the weaving processes, occurred. Knecht suggested that benefits in the manufacturing process and in the strength of the material might result from supplementing the low natural wax content of ordinary cotton fiber with additional oily or waxy substances. This has been done occasionally by spraying mineral oils and other substances on the fiber at different stages in the progress of manufacture.

Conrad (*3*) has shown that whereas the wax content of most cotton lint varies within the range of from 0.4 to 0.7 percent, that of Arkansas Green Lint cotton, based upon the dry weight, reached the high value of 17 percent. This finding suggested the possibility of studying the influence of a high natural wax content on the spinning quality of cotton.

The Arkansas Green Lint cotton is not suitable for commercial production because of its small yield and low ginning outturn. Neely (*9*) has shown that the green pigmentation and low ginning outturn are "spurious pleiotropic" effects of the same gene and that it is very unlikely that green-lint strains with lint percentages approximating

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[3] Italic numbers in parentheses refer to Literature Cited, p. 312.

the commercial white cottons can be developed through breeding. In many cases the green-lint cotton would not be suitable for commercial production because of its color and fiber shortness. Since no other cotton is known that has the high wax content of the green-lint strains, interest has been manifested in the possibility of combining, through hybridization and subsequent selection, the high wax content of the green-lint strains with the lint color and other desirable characteristics of the commercial white-lint strains. Thus, information regarding the type of heritable relation that exists between the wax content and the green-lint character is of considerable importance. It was the purpose of this investigation to study these relations.

MATERIAL AND METHODS

The samples employed in these studies were collected at Stoneville, Miss., during the 1938 and 1939 crop season and were used in studies to determine the relation of green lint to lint index in upland cotton. Two strains of cotton were used in these studies: Arkansas Green Lint (high wax content) and Half and Half white lint (low wax content). These strains were self-pollinated for several generations prior to the beginning of the study.

The methods employed for selecting parent plants and for self- and cross-pollination have been described by Ware (*10*). The procedure followed in obtaining samples from parental lines and of F_1, backcross, F_2, and F_3 segregations and in classifying the samples, as well as the mode of inheritance of the green-lint characteristics, have been outlined by Neely (*9*).

Lint samples of the 1938 growth from all plants of a given lint-color classification and parental line or progeny were composited and thoroughly mixed for wax-determination samples.[4] Because of the large number of plants in the 1939 growth, only about half of the plants of a given lint-color classification and progeny were composited for each determination.

After compositing and thoroughly mixing, duplicate $10 \pm .01$-gm. samples of lint were weighed out and placed, without grinding, in the extraction compartments of 50 by 250 mm. Soxhlet extractors, provided with 300-ml. distillation flasks. Thimbles were not used. The material was compacted so that all fiber would be covered with the condensed liquid before the latter siphoned over. Then, 250 ml. of 95-percent alcohol was poured into the cotton; a portion siphoned over and collected in the flask below. The condenser was attached, the water turned on, and the extraction continued for 6 hours from the time of boiling. At the end of this period the flame under each flask was turned out at the moment (within 1 to 5 minutes) that the alcoholic extract remaining in the flask amounted to about 100 ml.

The amount of wax contained in the alcoholic solution was determined by a method to be described elsewhere by Conrad.[5] Briefly, it consists of the transfer of the hot alcoholic solution to 100 ml. of chloroform in a 1,000-ml. separatory funnel, the addition of sufficient distilled water to cause separation, and the removal of the chloroform layer from the bottom. The aqueous layer is then washed three times

[4] Although, from the genetical standpoint, it would be desirable to determine, separately, the wax content of the lint from each plant of the progenies, the amount of work involved was prohibitive for the amount of time that could be allotted.

[5] CONRAD, C. M. A NEW EXTRACTION METHOD FOR THE DETERMINATION OF TOTAL WAX IN COTTON FIBER. (In preparation.)

with 50-ml. portions of chloroform, after which the combined chloroform extracts are washed once with distilled water. The sugars and other water and alcohol-soluble substances pass more or less completely to the water layer, while the waxy substances are retained in the chloroform layer. The chloroform-soluble fraction is then evaporated and dried in a tared weighing bottle at 105° C., weighings being made at 30-minute intervals until the loss is not more than 1 mg.

Separate determinations of moisture were made, and the wax content was computed to the oven-dried weight.

RESULTS

The percentages of wax of the lint-color phenotypes of the F_1, backcross, F_2, and F_3 populations and of the corresponding parental lines from the 1938 and 1939 growths are given in tables 1 and 2, respectively. The percentage of wax in each case represents the mean of two or more closely agreeing values.

TABLE 1.—*Wax content of lint-color phenotypes of F_1 and first-generation backcross progenies of a cross between Half and Half white lint and Arkansas Green Lint cotton and of the corresponding parental lines; 1938 growth, families 1, 2, and 3*

Generation	Family	Progenies of—	Wax content of lint-color phenotypes		
			White	Intermediate green	Green
			Percent	*Percent*	*Percent*
P_2	1	P_1 parents	0.63		15.04
BC	1	$F_1 \times$ green-lint parent		3.42	13.92
BC	1	$F_1 \times$ white-lint parent	.60	2.11	
F_2	1	F_1 (white $\times$ green)	.54	3.96	12.32
P_1	2 and 3	Parents of F_1	[1] .56		13.12
F_1	2 and 3	Green- $\times$ white-lint parents		[2] 2.17	

[1] Lint from only 1 plant represented.
[2] Lint from 2 families, 2 and 3, combined.

TABLE 2.—*Wax content of lint-color phenotypes of first-generation backcross, second-generation backcross, F_2, and F_3 progenies of a cross between Half and Half white lint and Arkansas Green Lint cotton and of the corresponding parental lines; 1939 growth, families 1, 2, and 3*

Generation	Family	Progenies of—	Wax content of lint-color phenotypes		
			White	Intermediate green	Green
			Percent	*Percent*	*Percent*
P_3	1	Family 1 parents	0.48		12.64
BC_2	1	Intermediate BC on green	.53	2.84	12.53
BC_2	1	Intermediate BC on white	.58	2.36	12.13
F_3	1	Green F_2 phenotype			12.90
F_3	1	Intermediate F_2 phenotype	.56	2.26	11.36
F_3	1	White F_2 phenotype	.66		
P_2	2	Family 2 parents	.63		12.82
BC	2	$F_1 \times$ white-lint parent	.59	1.95	
F_2	2	F_1 (white $\times$ green)	.51	2.40	12.42
P_2	3	Family 3 parents	.61		12.82
BC	3	$F_1 \times$ white-lint parent	.55	1.92	
F_2	3	F_1 (white $\times$ green)	.54	2.52	12.12

PARENTAL LINES

The original cross was made between white-lint and green-lint stocks with wax percentages of 0.63 and 15.04, respectively. Inbred progenies of the parental lines were grown each season, and very little variation in the wax content was found. The range was from 0.48 to 0.63 percent for the white-lint parental line and from 12.64 to 15.04 percent for the green-lint line. The difference in the wax content of the two stocks is striking.

THE F_1 POPULATION

The F_1 plants were classified as intermediate green, and the wax content was found to be 2.17 percent, as shown in table 1. Thus, there is not complete dominance in regard to the wax content, but the F_1 value is nearer that of the low-wax-content parent.

THE BACKCROSS POPULATION

Backcross populations consisted of the progenies of the $F_1 \times$green-lint parent and of the $F_1 \times$white-lint parent crosses. Information regarding the relationship of the lint-color and wax-content characteristics is afforded by the comparison of the wax percentages of the intermediate-green phenotypic classes of the two backcrosses. Theoretically, the two classes should be homologous for the green-lint color basic-factor pair. The $F_1 \times$green genotypes should have a preponderance of wax-content genes from the green-lint parent and the $F_1 \times$ white genotypes should have a preponderance of wax-content genes from the white-lint parent, if the wax content is controlled by multiple factors. It is shown in table 1 that the mean wax content is 3.42 percent for the intermediate-green class of the $F_1 \times$green backcross and 2.11 percent for the intermediate-green class of the $F_1 \times$white backcross, or a difference of 1.31 percent.

The $F_1 \times$green intermediate-green progenies were considerably more intensely green than were the $F_1 \times$white intermediate-green progenies. It is indicated that the secondary genes that modify the expression of the green-lint factor also modify the wax content.

The wax content of the intermediate-green segregates of the backcross $F_1 \times$white of families 2 and 3 is 1.95 and 1.92 percent, respectively. The white-lint segregates of the two families have average wax percentages of 0.59 and 0.54 percent.

The progeny of the intermediate-green phenotypes of the backcrosses segregated into 1 white: 2 intermediate green: 1 green. The wax content of these classes was 0.53, 2.84, and 12.53, percent, respectively, for the backcross to the green parent and 0.58, 2.36, and 12.13 percent, respectively, for the backcross to the white parent. The wax content of the comparable white-lint and green-lint parental lines is 0.48 and 12.64 percent, respectively. The difference between the wax content of the white and green segregates and the corresponding parental type is apparently of no consequence. The wax-percentage differences between the three phenotypes of the second backcross generations verify the association between the wax-content character and the gene affecting the green-lint character.

THE F_2 POPULATIONS

The F_2 populations, consisting of one family in 1938 and two families in 1939, were grown. Segregation for the lint-color character in each family corresponded satisfactorily to the expected 1:2:1 ratio of white, intermediate green, and green phenotypes. The association of high wax content and green lint is shown. The wax percentages for the white, intermediate-green, and green phenotypes, as shown in tables 1 and 2 for family 1, were 0.54, 3.96, and 12.32, respectively; for family 2, 0.51, 2.40, and 12.42; and for family 3, 0.54, 2.52, and 12.12. Comparable white-lint parental lines for the three families had wax percentages of 0.63, 0.63, and 0.61, respectively; and comparable green-lint parental lines for the three families had wax percentages of 15.04, 12.82, and 12.82, respectively. It is possible that the wax content of the F_2 green-lint segregates was significantly lower than that of the green-lint parental lines. Again it is indicated that the secondary genes that modify the expression of the green-lint factor pair also modify the wax content, since the F_2 green-lint segregates were less intensely green than the green-lint parental lines.

THE F_3 POPULATIONS

The progeny of the intermediate green F_2 plants segregated into three classes: green, intermediate green, and white, with mean wax percentages of 0.56, 2.26, and 11.36, respectively. The association between the wax content and green-lint pigmentation is again demonstrated.

The parental lines were propagated in order that comparisons between the wax percentages of these lines and the respective F_3 phenotypes could be made. The difference between the parental white and the F_3 white from the F_2 heterozygote is 0.08 percent and is probably of no consequence. Similarly, the difference between the parental green and the F_3 green from the F_2 intermediate green is rather small in comparison with the difference between the two phenotypes.

The F_3 white progeny from the F_2 white phenotypes had a wax content of 0.66 percent as compared with a wax content of 0.48 percent for the parental white. There is some indication that small differences in wax content which are not associated with differences in lint pigmentation may occur. The difference between the wax content of the F_3 green progeny from th F_2 green phenotypes and the green-lint parental line is small and probably of no significance.

SUMMARY AND CONCLUSIONS

The total wax content of lint from Half and Half white lint and Arkansas Green Lint upland cottons and of the F_1, backcross, F_2, and F_3 populations of crosses between the two strains was determined. The samples represent two consecutive crop years and three sets of families representing three different crosses. The wax content of the white-lint parent varied within the low limits of 0.48 to 0.63 percent and that of the green-lint parent between the much higher limits of 12.64 to 15.04 percent.

The F_1 was intermediate green with a mean wax content between that of the two parents but closer to that of the white parent.

In the analyses of samples from the backcross, F_2, and F_3 phenotypes, it was shown that the green lint and high wax content were closely associated.

While there is no evidence against the relationship being one of genetical linkage, it is indicated that the relationship is probably a physiological one and that the genetic factor that affects the green pigmentation also affects the wax content. Previous work has shown that the presence of green pigmentation in the fiber cell wall is associated with a suppressed development of fiber initials into mature fibers. Just what is the interrelationship of cause and effect between high wax content, green pigmentation, and suppressed development of fiber wall remains to be clarified.

The results are not conclusive, but it is indicated that there is little likelihood of combining the high wax content of the green-lint strain with the white lint of commercial cotton. It is possible that the genetic behavior in crosses between other green-lint strains and the same or other white-lint varieties would be different. These studies are being extended to include such crosses.

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