Reports

Selective Gametocide Opens Way to Hybrid Cotton

As shown in a review by Loden and Richmond (*1*), significant increases in most plant characters and in the yields of cotton have been found to result from heterosis in interspecific, intervarietal, and intravarietal crosses. Peebles (*2*) obtained a 25.5 percent increase in yield over the best parent in a first-generation hybrid between two American-Egyptian cottons. When he planted a 50/50 mixture of the hybrid and the best parent seed, the gain in yield was 18.5 percent rather than the expected 12.75; Peebles attributed this to more rapid seedling development and to subsequent dominance of the hybrid plants in the field. Kearney (*3*) illustrated the high fertility and fine appearance of F_1 hybrids of Holden upland × Pima Egyptian. This cross produced large bolls and exceptionally long and uniform fibers; Kearney noted that such a cotton would have surpassing agricultural and commercial value.

First generation crosses between Sea Island and upland cottons are of similar excellence but, as in the foregoing, efforts to combine and stabilize in a new variety the many desirable characters of the F_1 hybrids have never been successful. Notable advantages in yield are commonly lacking (*4*) in hybrids of the superior upland cottons of the Southeast. On the other hand, promising results not only in yield but in fiber properties have been experienced from divergent upland crosses such as that between early and late strains and that between some southwestern and the southeastern upland varieties.

Male-sterile strains of cotton have been sought unsuccessfully for many years, for they would provide opportunity for the controlled production of hybrid planting seed. Consideration even has been given (*1*) to the practicability of planting parent varieties in alternate rows and introducing heavy populations of bees to promote a measure of cross-pollination. Breeders and geneticists recognize that, with the achievement of a good method for producing hybrid cottonseed, there would follow an intensity of interest in evaluations of the combining abilities of world cottons which would parallel or exceed the activities that occurred with the development of the double-cross procedure for hybrid corn.

In the greenhouse (*5*) in the winter, Empire cotton plants were found to produce no pollen after they had been sprayed once with a 1.2 percent solution of sodium α,β-dichloroisobutyrate (*6*). Over the 5-week period of observation, during which the plants quadrupled in size, many new branches were produced that bore flowers. When these flowers were hand-pollinated (few pollinating insects are present in greenhouses) with pollen from untreated plants, normal bolls with viable seed were developed. When not hand-pollinated, the young bolls were shed. By these observations it was indicated that the sodium salt of this chlorinated organic acid is freely absorbed by cotton plants and remains active and mobile for a long time. There was a passing toxicity from the spray in the form of patches of burned tissue followed by a chlorotic buckling of the mesophyll of expanding leaves. Some of the floral buds were shed. Terminal buds, when saturated, were killed, but new vegetative branches developed promptly.

To carry further the observations of male-sterile plants with additional cottons under field conditions, an experimental planting was made in plot S-3-L at the Citrus Experiment Station of the University of California in late May 1956. Two American-Egyptian cottons and two upland varieties were used. The four varieties were planted in 40-foot rows between alternating rows of a red-leaf cotton; in addition, there were 10-foot row segments of the red cotton at the ends of the green rows. The red-leaf character is dominant over green. When ovules of green-leaf varieties are fertilized with red-leaf pollen, the resulting seedlings have red hypocotyls and cotyledons when they are exposed to bright light.

The four green-leafed varieties were sprayed with a 1 percent solution of the sodium α,β-dichloroisobutyrate on 24 July, about 1 week before the first flowers appeared. It rained or drizzled nearly all of the next morning. Since it was not known whether a part of the material might have been washed off, the plants were again sprayed, but with a 0.5 percent solution, on the morning of 26 July.

Starting about 2 weeks after the first bolls had opened, the green-leafed cottons were picked three times at biweekly intervals. The seed from these pickings, when planted in greenhouse trays, gave seedlings with red hypocotyls in the percentages shown in Table 1.

Strain Acala 4-42 produced more green- than red-leaf seedlings, which indicated that the compound could not have effected extensive male-sterility in it. With pollen from the green-leaf Acala available in the small planting, it could not be expected that the seedlings from the remaining three varieties would all be red. The values found gave assurance, nevertheless, that a useful degree of male sterility was produced in the Empire, Amsak, and Pima cottons. As is mentioned below, the finding of varieties with high thresholds can be a distinct asset in the production of hybrid seed.

The germination of the seed picked from the inbred and weak Amsak plants was about 35 percent, but that from the other three varieties was 85 percent or better.

The cotton plants in the field were sprayed when they were 15 to 20 inches tall, while it was possible to apply the material to the desired rows without wetting the intervening male-parent rows. Such a plan would probably be followed in the production of hybrid cottonseed. Good coverage of the upper surfaces of the leaves of preflower plants with 1 percent solution used 1 oz. of the dry material per 100 feet of row. Spraying alternate rows in this manner would require about 4 lb of the material dissolved in 50 gal of water per acre.

With the foregoing promise of a practical means of producing hybrid cottonseed, the work was extended to additional greenhouse and field tests with new varieties and with salts of additional chlorinated organic acids as prepared by Rohm and Haas. Between compounds and their dosages, varietal variations have been found in the extent of the initial leaf burning and in the subsequent chlorotic buckling of the mesophyll, in

All technical papers and comments on them are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in *Science* 125, 16 (4 Jan. 1957).

Table 1. Percentages of cotton seedlings with red hypocotyls produced from seed obtained at three pickings made at biweekly intervals.

Variety	Picking		
	1	2	3
Upland cotton			
Empire	75	77	62
Acala 4-42	33	40	33
American-Egyptian cotton			
Amsak F_{18}	88	77	85
Pima S-1	75	78	66

the extent of bud shedding and the consequent delay of flowering, in the duration and regularity of pollen suppression, and in the promptness of the male-sterility response. The presence of pollen, even when the anther opening appears normal, has been found to be an uncertain criterion of pollen viability. Anthers of treated plants sometimes remain unopened in the forenoon but shed pollen in the afternoon. One of the compounds suppressed the pollen of one of the varieties into the ninth week but that of another only for a short period. A measure of response has been obtained by applying several of the materials to the soil. Wetting agents have been tried in some tests, but they appear to hasten absorption and increase burning.

In searching for means of reducing toxicity and extending the male-sterile reaction, the idea is now prominent that a first and second spraying with lower dosages would be effective. A second spraying could be carried out if the rows were spaced further apart than is practiced in commercial cotton production. Where there is a substantial difference in the threshold concentrations of the desired parents, several field-wide sprayings become possible if the most reactive variety is used as the female parent and the less reactive variety as the male parent.

FRANK M. EATON
Department of Soils and Plant Nutrition, University of California College of Agriculture, Riverside

References and Notes

1. H. D. Loden and T. R. Richmond, *Econ. Botany* 5, 387 (1951).
2. R. H. Peebles, *Am. Bee J.* 96, 51, 96 (1956).
3. T. H. Kearney, *J. Heredity* 15, 309 (1924).
4. D. M. Simpson, *J. Am. Soc. Agron.* 40, 970 (1948); P. H. Kime, and R. H. Tilley, *ibid.* 39, 308 (1947); J. H. Turner, Jr., *Agron. J.* 45, 484, 487 (1953).
5. The initial greenhouse tests reported in this paper were made by me as part of cooperative investigations between the Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture, and the Texas Agricultural Experiment Station, College Station, Tex.
6. The sodium α,β-dichloroisobutyrate was received from Rohm and Haas Company in 1955.

9 September 1957

Alkaline Denaturation of Hemoglobin of Postlarval and Adult Scorpaenichthys marmoratus

The hemoglobin of the bullfrog tadpole is different from that of the adult (*1*). Since the teleost fish *Scorpaenichthys marmoratus* undergoes an extensive postlarval metamorphosis (*2*), an investigation of the alkaline denaturation of the hemoglobin of three adult and nine postlarval specimens of this fish has been undertaken in order to find out whether an ontogenetic change in the hemoglobin is associated with the metamorphosis (*3*). The rate at which oxyhemoglobin is converted to alkaline methemochromogen through denaturation at *p*H 11 to 13 has been a standard technique in the differentiation of adult and fetal mammalian hemoglobins (*4, 5*).

Adults and postlarvae were bled from the heart into heparinized Ringer's solution (*6*); the erythrocytes were washed three times in an excess of nonheparinized Ringer's solution. Centrifugally packed erythrocytes were lysed in distilled water (9 volumes of water to 1 volume of cells). Stromata were removed by prolonged centrifugation. The preparation of the hemoglobin was done at 0° to 1°C. No bloods were pooled. The hemoglobin solutions were used immediately for alkaline denaturation in sodium phosphate buffer (*p*H 11.0 to 12.0; Γ/2, 3.0), the reaction being followed spectrophotometrically (*7*).

Some of the results obtained at *p*H 11.0 are shown in Fig. 1. Similarly to the hemoglobin in several mammals—but not in man (*4, 5*)—*Scorpaenichthys* postlarval hemoglobin has an initial alkaline labile component that denatures faster than that of the adult hemoglobin. In addition, the diphasic nature of the alkaline denaturation curve is more obvious in the case of postlarval hemoglobin. The relative proportions of fast- and slow-denaturing components vary in postlarval hemoglobin much more so than in adult hemoglobin; similar variation has been described by others for human adult hemoglobin (*5, 8*). The difference in alkaline denaturation between adult and postlarval *Scorpaenichthys* hemoglobins was consistently observed at both *p*H 11.0 and 12.0. Hence, there is a change in the biochemical nature of the hemoglobin in the development of *Scorpaenichthys* as in mammals (*5, 9*), the chicken (*10*), the terrapin (*11*), and the bullfrog (*1*).

In general, the oxygen tensions to which adult fishes and their pelagic young are subjected are approximately the same (150 mm-Hg)—a situation in contrast to that observed for most other vertebrate embryos and fetuses. Therefore, at present, a particular physiologically significant role cannot be assigned to the equivalent of a fetal hemoglobin in *Scorpaenichthys*. The occurrence of a distinct postlarval hemoglobin in *Scorpaenichthys* may represent the chance evolutionary development of a biochemical feature of little selective value. In fact, some larval and postlarval fishes—for example, the leptocephalus of the eel (*12*)—lack hemoglobin. Preliminary experiments on alkaline denaturation indicate the presence of a fetal hemoglobin in the live-bearing surf-perch *Embiotoca*

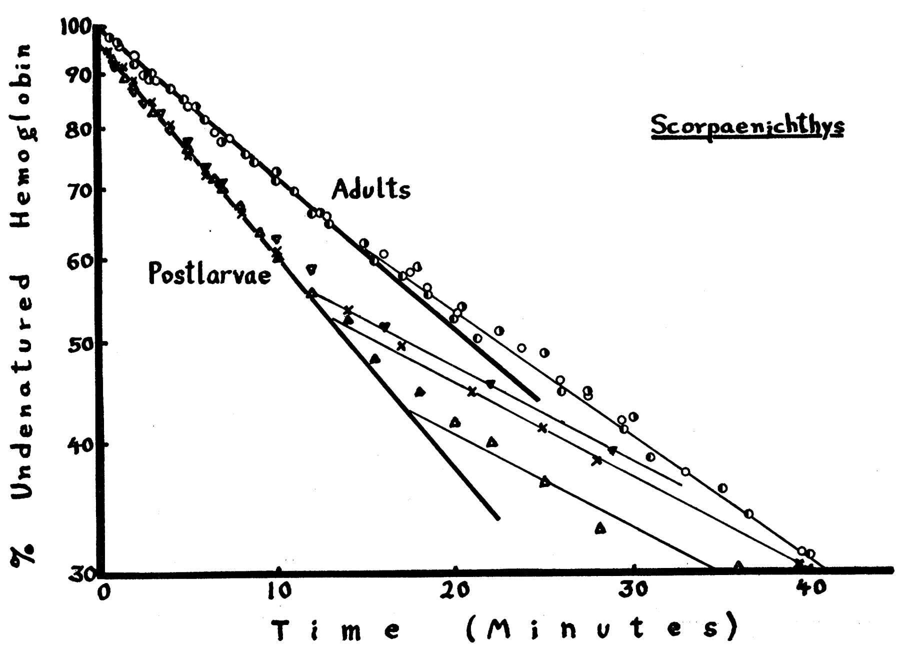

Fig. 1. Alkaline denaturation of the hemoglobin of three adult and three postlarval *Scorpaenichthys marmoratus* (*p*H 11.0; Γ/2, 3.0; 24°C).