

Fig. 2. Schematic diagram of electrical control circuit for darkbox.

run type can be changed by reversing the current in either the starting or running windings with respect to the other. This principle is utilized in the controls for the darkbox shown schematically in Figure 2. Electrical leads marked X and Y go to the motor running and starting windings, respectively.

The action of the control circuit is triggered by the opening or closing of the contacts on a clock switch (S-1). As shown in Figure 2, the box opens when S-1 closes. As S-1 closes, R-1 is switched to the normally open contact which simultaneously switches the contacts of the DPDT relay (R-2) to the normally open (lower contacts in drawing) position. This completes a circuit through S-2 to the motor to open the box. S-2 is opened by the box lid when it reaches the full open position, breaking the motor circuit.

When S-1 is opened by the clock, R-1 and R-2 switch back to the normally closed positions, the motor circuit is completed through S-3, and the starting winding current is reversed by R-2. The motor then runs backward to close the chamber.

The motor and all materials used in the construction of the darkbox cost less than \$150. A list of the materials used in the construction will be supplied on request. The darkbox has given us two years of excellent service and should be good for many more. Since the darkbox is only 107 cm high, large species such as sorghum and pearl millet cannot be left in the box until anthesis. In practice we usually plant the millet seeds in 20-cm (8-inch) pots of soil on a greenhouse bench and leave the plants there through their juvenile period (for about 3 weeks) while they are insensitive to photoperiod (1). These plants are moved into the darkbox for about 4 weeks to permit the nonreversible induction of floral primordia (2). We then move them out into the greenhouse and arrange them to facilitate hybridization or selfing. This procedure allows us to initiate flowering in up to 140 different plants each month and gives us the capacity and flexibility needed in our grass breeding program.

## REFERENCES

- Burton, Glenn W. 1965. Photoperiodism in pearl millet, *Pennisetum typhoides*. *Crop Sci.* 5:333-335.  
 ———. 1966. Photoperiodism in pearl millet, *Pennisetum typhoides*, its inheritance and use in forage improvement. *Proc. Xth Int. Grassland Congress*, 720-723.

## ANALYSIS OF A DOMINANT GENE FOR MALE-STERILITY IN UPLAND COTTON, *GOSSYPIUM HIRSUTUM* L.<sup>1</sup>

J. B. Weaver, Jr. and Terry Ashley<sup>2</sup>

### ABSTRACT

Two male-sterile plants were found in the cotton (*Gossypium hirsutum* L.) variety 'Acala 1517D' in 1968. F<sub>1</sub> hybrids produced from these plants produced a 1:1 ratio of male-sterile to fertile plants. Self-pollinated seed from fertile plants produced all fertile offspring. Additional crosses made onto male-sterile plants continued to produce approximately a 1:1 ratio of male-sterile to fertile plants. A double haploid technique is proposed for producing homozygous dominant male-sterile plants. The gene symbol *Ms<sub>7</sub>* is proposed for this new dominant male-sterile gene.

**Additional index words:** Hybrid, Homozygous dominant male-sterile.

**B**ECAUSE of the potential practical value of hybrid cotton, geneticists have made an extensive search for male-sterile strains. Two independent recessive genes, *ms<sub>1</sub>* and *ms<sub>3</sub>*, were shown to induce partial male-sterility. Richmond and Kohel (4) discovered the *ms<sub>2</sub>* recessive gene, which results in complete male-sterility in the homozygous condition. A dominant gene, *Ms<sub>4</sub>*, that causes complete male-sterility was reported by Allison and Fisher (1). Weaver (5) reported a male-sterile that was conditioned by two pairs of homozygous alleles, *ms<sub>5</sub>* and *ms<sub>6</sub>*. Meyer and Meyer (2) showed that a type of male-sterility was influenced by cytoplasmic factors. However, their sources of cytoplasmically controlled male-sterility have not proven satisfactory because environmental factors affect the degree of male-sterility. This paper reports another completely male-sterile type that is controlled by a dominant gene.

In the spring of 1968 a packet of seed was obtained from Dekalb Ag Research, Inc. labeled "Acala 1517D *ms<sub>2</sub>* backcross 8." From this packet two male-sterile plants were found in a group of 150 plants. These two plants did not show the leaf abnormality that Quisenberry and Kohel (3) reported to be associated with the *ms<sub>2</sub>* gene. Hundreds of other male-sterile plants in our breeding nursery known to have the *ms<sub>2</sub>* gene did show the leaf abnormality.

Since these two plants did not show the typical leaf abnormality, and only 2 plants out of 150 were male-sterile, they were dug and placed in the greenhouse in the fall of 1968. Seven different pollinator parents were used in making crosses with these male-sterile plants during the winter of 1968-69. The seven F<sub>1</sub> hybrids were grown in the field during the summer of 1969. They produced a total of 49 fertile plants and 43 completely male-sterile plants. When it was discovered, in the 1969 growing season, that some of the F<sub>1</sub> hybrids were male-sterile, open-pollinated seed from the male-sterile plants were sent to the winter nursery facilities at Iguala, Mexico. Open-pollinated

<sup>1</sup> Journal Series Paper No. 896 of the University of Georgia, College of Agriculture Experiment Stations, College Station, Athens, Ga. Received Feb. 1, 1971.

<sup>2</sup> Assistant Professor of Agronomy and Assistant Professor of Botany, University of Georgia, Athens, Ga. 30601.

seed from six individual plants produced a total of 42 male-sterile plants and 48 fertile plants. Self-pollinated seed from two fertile  $F_1$  plants produced 40 fertile plants and no male-sterile plants.

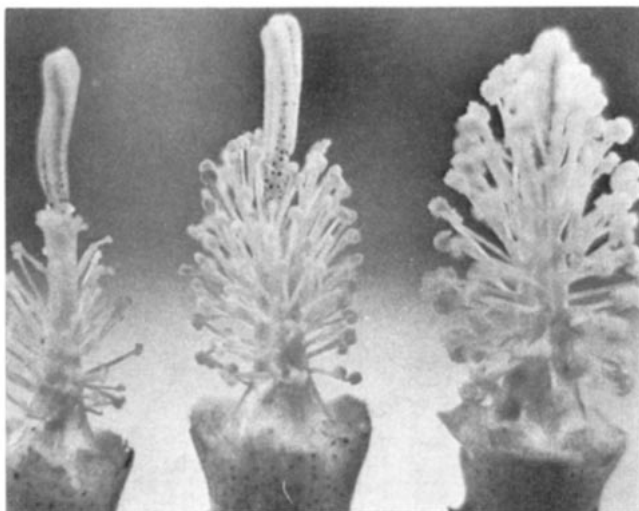
An effort was made at Iguala during the winter of 1969-70 to cross each fertile plant with each male-sterile within the six progeny rows. The sib crosses were grown during the summer of 1970. Table 1 shows the ratio of male-sterile to fertile plants obtained in the summer of 1970. The percentage of male-steriles for the entire population was 45.64%. Allison and Fisher (1) obtained 46.9% male-steriles in a field population of  $Ms_4$ . Weaver (5, 6) reported a deficiency of expected male-steriles with  $ms_2$  and  $ms_5$  and  $ms_6$ . Thus, the male-sterile gene reported in this paper follows the same pattern as previously reported genes by producing a deficiency of male-sterile plants.

Figure 1 shows a comparison of flowers taken from plants carrying  $Ms_4$ , the new male-sterile gene, and normal fertility genes. All were taken from plants of Acala 1517D. The new gene produces anthers of reduced size with a small amount of nonviable pollen. The  $Ms_4$  flowers have very few extremely small anthers with no pollen. The author has observed the  $Ms_4$  gene in many genotypes for several years, and it can be stated beyond a reasonable doubt that the two types of flowers are different in gross morphology. Since both are dominant genes, there is no possibility for making the appropriate cross to prove genetically that they are different. Such proof could occur through linkage groups or by use of monosomics, provided they are on different chromosomes.

**Table 1.** Analyses of ratios observed in populations of sib-crosses between fertile and male-sterile plants in Upland cotton.

Family no.	No. of sib crosses	Sterile	Fertile	Total	$\chi^2$	P (1:1)	$\chi^{2*}$
1	12	69	93	162	3.55	.05 - .1	1
2	23	208	217	425	0.19	.5 - .75	1
3	14	160	185	345	1.81	.1 - .25	2
4	14	148	197	345	6.96	.005 - .01	0
5	30	368	413	781	3.09	.05 - .10	2
6	57	488	611	1,099	13.76	.005	7

\* Number of individual sib crosses with significant  $\chi^2$ .



**Fig. 1.** Three flowers from Acala 1517D variety, showing  $Ms_4$  on left,  $Ms_7$  in middle, and normal fertile on right.

During the winter of 1970-71, acetocarmine squashes were made from Carnoy fixed anthers of various ages collected from plants of  $Ms_4$  and plants carrying the new gene. Anthers from  $Ms_4$  contained no recognizable pollen, tetrads, or even pollen mother cells. There was, therefore, no evidence that  $Ms_4$  initiated meiosis. This supports the observations of Allison and Fisher (1), who also reported no evidence of development of sporogenous tissue. The anthers from plants carrying the new gene, however, undergo an apparently normal meiosis, form tetrads with four equal-sized spores, and develop some pollen grains whose gross external morphology is indistinguishable from that of fertile grains. The cause of sterility of the new gene is not immediately apparent, but the present cytological evidence suggests that the two genes are distinct and most likely nonallelic.

Additional gross morphological differences were observed in the greenhouse during the winter of 1970-71.  $Ms_4$  flowers were smaller and their petals tended to only partially open, in contrast to a more normal behavior of flowers of the new gene. In  $F_1$  hybrids between  $Ms_4$  and 'Pima double haploid 57-4' the male-sterile flowers had white anthers. In contrast, the male-sterile flowers of the new gene  $\times$  Pima double haploid 57-4 had from 20 to 100% yellow anthers. More than 100 male sterile interspecific flowers of each gene were observed, with no exceptions in anther color differences noted. Haploid plants obtained from 57-4 had yellow anthers.

The question remains as to the origin of the two male-sterile plants found in 1968. The probability that two independent mutations occurred is too remote to be feasible. The seed that produced the two male-sterile plants probably were open-pollinated and harvested from several plants in a DeKalb Nursery row, one of which was male-sterile. The two male-sterile plants were indistinguishable morphologically to their fertile sibs and were typical Acala 1517D plants.

The  $Ms_4$  gene and the new dominant male-sterile strain could possibly have some economic value. Efforts are being made to produce haploid plants that have the  $Ms_4$  or the new dominant gene. The haploid plant could be treated with colchicine to produce a diploid homozygous for the dominant gene. The homozygous dominant male-sterile plant could be increased by root cuttings. Crosses onto the homozygous dominant male-sterile plant would produce all male-sterile offspring. A three-way hybrid could then be made that would segregate into approximately a 1:1 ratio. Such a three-way hybrid could be grown only in areas of moderate to high bee activity.

The results reported in this paper, plus numerous other crosses made in the greenhouse, support a genetic hypothesis that the observed male-sterility is due to one dominant gene. No indication of a genetic-cytoplasmic interaction has been found.

It is proposed that this male-sterile character be assigned the gene symbol  $Ms_7$ . L. H. Harvey (Linkage of male-sterility in Upland cotton, *Gossypium hirsutum* L. 1969. Ph.D. dissertation, Univ. of Ga.) used the gene symbol  $ms_7$ . However, his data indicated that the male-sterile strain designated as  $ms_7$  was actually  $ms_2$ . Test crosses observed by the senior author in

1970 produced evidence that the recessive *ms<sub>7</sub>* was a misnomer for *ms<sub>2</sub>*.

## REFERENCES

1. Allison, D. C., and W. D. Fisher. 1964. A dominant gene for male-sterility in Upland cotton. *Crop Sci.* 4:548-549.
2. Meyer, Vesta G., and James R. Meyer. 1965. Cytoplasmically controlled male-sterility in cotton. *Crop Sci.* 5:444-448.
3. Quisenberry, J. E., and R. J. Kohel. 1968. A leaf abnormality for the identification of a genetic male-sterile in *Gossypium hirsutum* L. *Crop Sci.* 8:369-370.
4. Richmond, T. R., and R. J. Kohel. 1961. Analysis of a completely male-sterile character in American Upland cotton. *Crop Sci.* 1:397-401.
5. Weaver, J. B. 1968. Analysis of a genetic double recessive completely male-sterile cotton. *Crop Sci.* 8:597-600.
6. ———. 1969. The present status of male-sterile cotton. *Proc. 1969 Beltwide Cotton Prod. Res. Conf.* P. 79-80.

## AGE VS. NET CO<sub>2</sub> EXCHANGE RATE OF LEAVES OF COASTAL BERMUDAGRASS<sup>1</sup>

Richard H. Hart and David R. Lee<sup>2</sup>

### ABSTRACT

Net CO<sub>2</sub> exchange (NCE) of 'Coastal' bermudagrass (*Cynodon dactylon* (L.) Pers.) leaves was highest at collar emergence and decreased with age. It decreased more rapidly at high light intensities, although initial NCE was higher. Leaves died in about 18 (natural sunlight) to 35 days (22 klux artificial light) after collar emergence. The much higher photosynthetic rate of younger leaves may explain the high production of frequently cut Coastal stands.

**Additional index words:** Photosynthesis, Respiration.

**P**HOTOSYNTHETIC efficiency of leaves of several forage species has been shown to decline with age (1, 5, 9, 10, 12, 13), and with prolonged shading (4, 5, 6, 7, 11, 13). The rate of decline has important implications in devising models of assimilation and growth of stands, and in the management of stands.

### PROCEDURE

In 1966, we grew Coastal bermudagrass (*Cynodon dactylon* (L.) Pers.) plants in 15-cm pots, in a growth chamber with a temperature of 30±2C, a light intensity of 22 klux, provided by VHO, cool, white fluorescent tubes, and a 16-hr photoperiod. We tagged leaves with the date of collar emergence, and measured net CO<sub>2</sub> exchange (NCE) 1, 14, or 29 days after collar emergence. We measured 4 to 8 leaves at each age, using the IR gas analyser and the system described by Alexander and McCloud (2), at a leaf temperature of 30C and light intensities of 0, 11, 22, 43, and 86 klux, provided by a bank of 300-w incandescent photoflood lamps, with light filtered through 10 cm of water.

In 1968, we grew Coastal bermudagrass plants in 15-cm pots in a growth chamber with a temperature of 30±1C, a light intensity of 33 klux, and a 16-hr photoperiod. We also grew plants in 15-cm pots outdoors where mean daily high and low temperatures were 29.2 and 16.8C, respectively; photoperiod was 15 to 16 hr, and mean daily solar radiation was 577 cal cm<sup>-2</sup>min<sup>-1</sup>, with peak sunlight intensity of 115 klux. We tagged leaves as before and measured NCE, with the system described by Wolf et al. (14), at a leaf temperature of 30C and light intensities of 0, 5, 11, 22, 43, and 65 klux.

We could measure NCE of the same leaf repeatedly with this open system, so we started with 20 leaves from each environment, and measured NCE each week until all leaves were dead. The gas analyser broke down after measuring only four leaves from the chamber-grown plants at 15 days after collar emergence; the remaining leaves were measured when the analyser was repaired, 18 days after emergence.

## RESULTS AND DISCUSSION

NCE and respiration declined as the leaves aged in 1966 (Fig. 1a). By 29 days after collar emergence, NCE at 86 klux was 55% lower than at emergence and was not significantly higher than NCE at 22 klux. By 42 days after collar emergence all leaves were dead. Respiration rate was much higher in 1966 than in 1968. This may have been caused by damage to the leaf when the chamber was sealed around the leaf base in 1966; no seal was required with the chamber used in 1968.

In 1968, leaves from chamber-grown plants behaved much as they had in 1966, for the first 15 days after collar emergence (Fig. 1b). NCE appeared to decrease abruptly between 15 and 18 days after emergence, but this may be an artifact, because only four leaves were measured at 15 days. At 18 days, NCE at 65 klux was 69% lower than at emergence and was not significantly higher than at 22 klux. By 30 days after emergence, one leaf was dead, and mean NCE of the 19 remaining leaves at 65 klux was 84% lower than at emergence. NCE did not increase significantly when light intensity was increased above 11 klux.

Leaves from plants grown outdoors had a much higher initial NCE rate (47% higher at 65 klux) than leaves from chamber-grown plants, but also showed a much more rapid decline with age (Fig. 1c). There was no significant difference between NCE of leaves from the two environments 7 days after collar emergence. By 14 days after emergence, four leaves were dead, and NCE of the 16 survivors at 65 klux was 69% lower than at emergence, and 52% lower than NCE of chamber-grown leaves of the same age. However, NCE of the surviving leaves still increased significantly with each increase in light intensity. By 21 days after emergence, all leaves were dead.

We concluded that the more rapid decline in NCE of leaves grown outdoors was caused by higher light intensities, rather than cooler night temperatures. NCE of leaves from chamber-grown plants decreased more rapidly in 1968, when light intensity was 33 klux, than in 1966, when light intensity was 22 klux; temperatures were the same in both years. Woledge (13) reported that leaves of tall fescue plants grown in bright light showed a higher initial rate of photosynthesis, but a more rapid decline in photosynthesis with age, than leaves from plants grown in dim light. On the other hand, Treharne, Cooper, and Taylor (12) found no interaction between aging and temperature when they studied photosynthesis of orchardgrass leaves.

The CO<sub>2</sub> uptake of Coastal bermudagrass stands, as measured by Alexander and McCloud (2), is consistent with our results. They reported CO<sub>2</sub> uptake of stands cut to 2.5 cm daily was over 50% of the uptake of stands cut to 20 cm daily, even though the LAI of the shorter stands was only 25% that of the taller

<sup>1</sup> Contribution from the Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705. Received Dec. 19, 1970.

<sup>2</sup> Research Agronomist and Agricultural Research Technician.