Published July, 1966

Environmental Influences on Sterility in Cytoplasmic Male-Sterile Cottons[1]

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ABSTRACT

The influence of different environmental conditions on male sterility was investigated in interspecific cytoplasmic male-sterile cottons. In *Gossypium arboreum* cytoplasm, the sterility scores in male-sterile stocks of cotton showed positive correlations with wind velocity, pan evaporation, and heliometer in the period 2 to 3 weeks before anthesis while maximum and minimum temperatures showed negative correlations. The male-sterile stocks with *Gossypium anomalum* cytoplasm had negative correlations with the temperature and heliometer during the same period. Multiple correlations showed interactions between the environmental conditions. Different locations were affected by different conditions.

IN AN EARLIER study of plant age, sterility differences were observed between the partially male-sterile cotton plants at different times of the season and at two different locations (9 and Sarvella, unpublished). Environmental influences on male sterility were noted in several plants (5). In corn, higher humidity and lower temperatures were associated with increases in fertility (2,3); whereas, long photoperiods at high temperatures probably reduced the fertility in various corn inbreds crossed with a Texas source of cytoplasmic male sterility (1). Peterson (8) found that cytoplasmic male-sterile onions, like corn, had more mature pollen at low temperatures probably due to a delay in, or absence of pollen aborting conditions. Viable potato pollen was also reduced by a rise in temperature (6). In Sudangrass, fertility restoration was complicated by environmental variances — "Unfavorable weather, mainly high temperture and excessive drought, caused stress on the plants which could have increased fertility" (4). Environment, therefore, appeared to influence sterility patterns in many plants and provided complications in analyses. Thus, the influence of different environmental factors on sterility needed to be investigated in cytoplasmic male-sterile cottons to determine which environments might cause sterility or fertility.

MATERIALS AND METHODS

The same stocks were used for this study as in a previous study (Sarvella, unpublished). The A_2 stock resulted from backcrosses and selfs on *(G. arboreum* × *G. thurberi)* × *G. hirsutum* and the B_1 stock from *(G. anomalum* × *G. thurberi)* × *G. hirsutum*. Several generations of both the A_2 and B_1 male-sterile stocks were studied in preliminary experiments at State College, Miss., in 1961 through 1963. In 1961 and 1962 sterility scores for comparison purposes were obtained from Stoneville, Miss., (Courtesy of Mrs. J. R. Meyer). In the summer of 1964, plants from the following generations of the A_2 stocks were investigated: for line 741-1 — BC_4+S_1, BC_5+S_1, BC_5+S_2, BC_6+S_1; for line 907 — BC_4+S_1, BC_4+S_2, and BC_4+S_3, and in the B_1 stocks: line 913-1 — BC_5+S_1, BC_6, and BC_7; and for line 999 — BC_3+S_3, BC_3+S_4, and BC_3+S_5.

Every flower was scored every day during the blooming season until the boll weevil population became too large to continue accurate scoring. Flowers were scored on the basis of a flowering scale 0-4 developed by Dr. Norman Justus: where Class 0 flowers are completely sterile, Class 1 has 1 anther open to 1/3 of the anthers open, Class 2 has 1/3 to 2/3 of the anthers open, Class 3 has 2/3 anthers open, and Class 4 has all anthers open. Flowers from the sterile plants in each generation were separated from the ones on the partially sterile plants.

Correlations in 1961, 1962, and 1963 compared the environmental conditions with the average weekly sterility scores of the A_2 and B_1 male-sterile stocks. Environmental conditions were obtained from 3 to 4 weeks before flowering until the end of the scoring period. Since the pollen breaks down about 10 days before anthesis (normally the buds take 12 to 15 days from meiosis to anthesis; Sarvella, unpublished), the environmental conditions were studied before flowering started.

In 1964, measurements were taken on wind velocity (mph), maximum and minimum air temperatures (°F), maximum and minimum soil temperatures (°F), heliometer (total solar radiation g. cal./cm²/day), and accumulative pan evaporation (inches per day). Maximum and minimum thermometers were placed in the field and read daily. Soil temperatures were recorded at a two-inch depth. An anemometer was placed in a field although the values used were from the State College weather station located about 1 mile from the field. Heliometer and pan evaporation values were taken from the State College weather station data. Environmental data for Stoneville was from the Stoneville Weather Station. The environmental conditions were obtained for the period of scoring and for the previous 3 to 4 weeks.

To select the days to be tested for correlations, graphs were prepared for the average daily sterility scores and for each weather condition from 4 weeks preceding and throughout

[1] Contribution No. 1378, Agronomy Department, Mississippi State University, State College, Miss. A summary of this paper was presented at the 1964 Annual Meetings of the American Society of Agronomy, Kansas City, Mo. (Agron. Ahstr. 1964). Received March 11, 1966.

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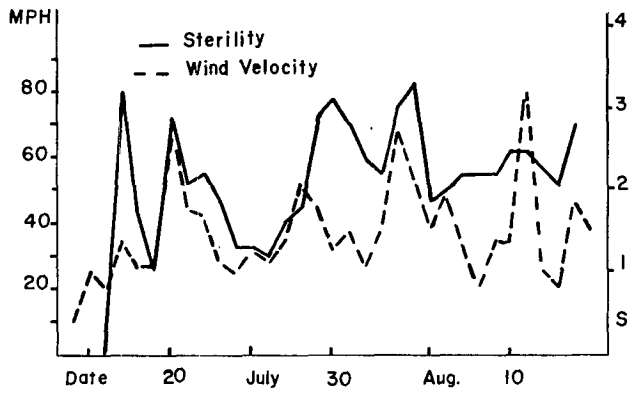

Figure 1. Daily mean sterility scores during July and August in young A_2 cytoplasmic partially male-sterile cotton compared with the daily wind movement (mph). Line 741-1, BC_6+S_1. State College. 1964.

the scoring period. The graphs for the environmental conditions were superimposed over the ones for the sterility scores and the graphs were moved until the curves appeared to be associated or else negatively related (Figure 1). These days were tested for simple correlations (10). When a pattern for a day appeared to occur regularly, all the generations were tested. Multiple correlations were determined. The computer program determined all the regression coefficients in the primary model, tested each for significance, dropped the least significant term from the model, and recomputed the coefficients on the new model until it reduced the equation to one term.

A moisture stress condition was imposed on three A_2 plants and two B_1 plants in 1964. These plants were placed in crocks and buried in the field on the North Agronomy Farm. A plastic sheet was placed over them and drawn whenever it looked like rain. The soil water content in each crock was measured and when equivalent to about 3 bars suction, water was added. The main field was irrigated when needed. In 1965 moisture was limited by growing plants in 2 different soils. A few sandy pockets were interspersed between the black loam areas. Four plants of the A_2, B_1 and the doubled haploid (M8) stocks were scored for sterility patterns in each soil type.

RESULTS

The weekly sterility scores in 1963 showed as had the data in 1962 that there was a cytoplasm-environment interaction (Sarvella, unpublished). Significant correlations in 1963 were obtained for several environmental factors for the B_1 stocks but not for the A_2 stocks (Table 1). The most sensitive period appeared to be about 3 weeks before meiosis. Correlations were run between the 1964 weather conditions and the average daily sterility scores. Many of the environmental conditions were correlated with each other (Table 2). Since the air and soil temperatures were correlated, correlations were run only for air temperatures.

The number of generations in the partially sterile A_2 cytoplasm stocks which were significantly correlated with the weather conditions are shown in Figure 2. Positive correlations are above the line and negative below. Thus, positive correlations would show an increase in fertility; whereas, negative correlations would show an increase in sterility. The wind velocity showed the highest positive correlation the day preceding anthesis and 22 days before anthesis (5 days before meiosis). Thus, the higher the wind is, the higher the fertility of the flowers. Maximum and minimum temperatures showed similar correlations. Positive correlations were observed 10 to 14 days before anthesis. Negative correlations were observed from

Table 1. Correlations between environmental conditions and average weekly sterility scores in B_1 cytoplasmic male-sterile cottons. State College. 1963.

	Generation	Weeks before anthesis showing signif. correl.
Avg heliometer		None
Maximum air temperature	BC_3+S_3	-3*
	BC_6+S_1	-2, 4
Maximum soil temperature (2")	BC_6+S_1	4
Wind velocity	BC_3+S_3	-3
Rainfall		None
Pan evaporation	BC_6+S_1	4
Relative humidity		None

* A minus sign preceding weeks means a negative correlation of average weekly sterility with the environmental conditions.

Table 2. Correlations between 1964 weather conditions.

Wind--Maximum air temperature	ns	Relative Humidity--Wind	ns
Wind--Minimum air temperature	**	Heliometer--Wind	-ns
Maximum air--Minimum air	**	Heliometer-Maximum air temp.	**
Maximum soil--Minimum soil	P_{05}-P_{10}	Heliometer-Minimum air temp.	ns
Maximum air--Maximum soil	**	Heliometer-Relative Humidity	-**
Maximum air--Minimum soil	ns	Pan evaporation--Heliometer	**
Minimum air--Minimum soil	**	Pan evaporation--Wind velocity	*
Relative humidity--Maximum air	-ns	Pan evaporation--Maximum air temp.	**
Relative humidity--Minimum air	ns	Pan evaporation--Minimum air temp.	ns

* Significant at the 5% level. ** Significant at the 1% level. ns Non-significant

about 15 days to 23 days before anthesis. For the heliometer and pan evaporation data, positive correlations occurred 13 days before anthesis preceded by a period of negative correlation.

In the B_1 partially male-sterile cottons 6 generations were studied, and the average daily sterility scores were correlated with the different environmental conditions (Figure 3). More generations were significant in the A_2 plants than in the B_1 plants, perhaps because the partially sterile B_1 plants were more sterile than the A_2 partially sterile stocks. However, some correlations were observed. Certain periods showed negative correlations in most of the weather conditions; i.e., from 16 to 22 days before anthesis. From 10 to 15 days, there was a positive correlation period. Just before anthesis there were both positive and negative correlations. Higher wind velocity just preceding anthesis appeared to increase fertility — probably causing more desiccation of the anthers, the result being easier dehiscing.

Sterile young plants occasionally had a few fertile flowers. Five environmental conditions were correlated with the average sterility. The only consistent trend in both the A_2 and B_1 stocks was a negative correlation from 18-20 days before anthesis with both the maximum and minimum temperatures.

In addition to the young partially male-sterile cotton, several old A_2 and B_1 partially and completely male-steriles were also studied. Plant ages ranged from 1 to 4 years. The same five environmental conditions were studied. Wind velocity showed a positively correlated period at 1 and 23 days before anthesis in both stocks. The period 17 to 20 days before anthesis showed a negative correlation for both the minimum and maximum temperatures, and a positive correlation at 13 days. Heliometer readings showed correlations at the same time as the young plants. Pan evaporation showed positive correlations from 12 to 19 days before anthesis and one negative at 19 days. These periods were similar to the young plants. Thus, plant age did not seem to affect the environmental influence, although sterility is generally influenced by plant age (Sarvella, unpublished).

In another experiment in 1964 plants were placed under a water stress condition. The three A_2 cyto-

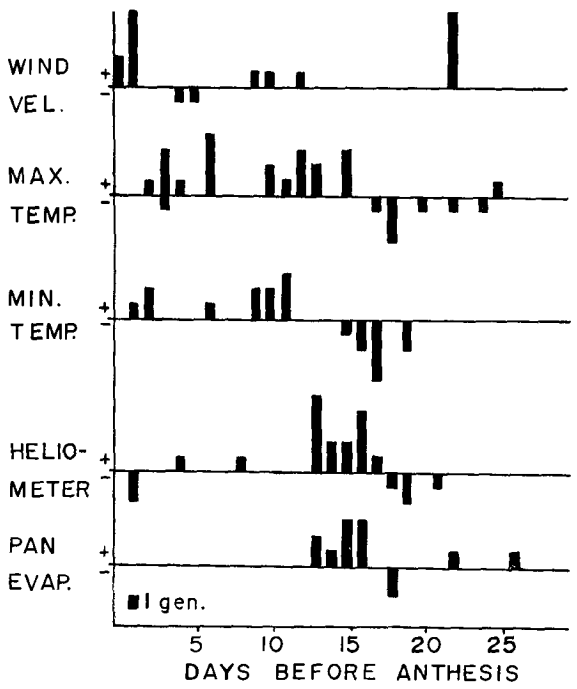

Figure 2. Number of generations (7 partially male-sterile generations) of young A_2 cytoplasmic male-sterile cottons with positive and/or negative correlations with the environmental conditions. State College. 1964.

plasmic plants had an average sterility score of 2.7. Control plants of this generation had a high fertility rating of about 3.5. Two B_1 cytoplasmic plants were also placed in the water stress test. The 913-1 BC_6 plant had only 4 completely sterile flowers. This plant in the greenhouse in the winter 1964-5 scored 3.5. The 999-2 BC_3+S_4 plant was more fertile in the greenhouse than in the field (1.0 vs. 0.7, respectively). The plants in the moisture stress test were about the same as the plants in the field at the same time.

The 1965 results showed differences between the B_1 sterility scores in plants grown in the loam and sandy soils. B_1 plants scored 0.35 and 0.30, respectively. A_2 plants averaged 0.51 on the loam soils and 1.17 on the sandy soils. The control (M8) plants scored 3.8 and 3.7 on the loam and sandy soils. Since the plants in the sandy areas were often wilted, they were definitely under a moisture stress; yet no differences were observed in 1965 between the 2 sets of plants in the A_2 stocks, but the B_1 plants were more sterile in the moisture stress.

Since the environmental effects may not result from purely simple correlations, multiple correlations were also run in the A_2 and B_1 partially male-sterile cottons. Four different stocks (two A_2 and two B_1 generations) were selected from the 1964 plants for testing at selected days. Four main environmental effects were tested: wind, maximum temperature, heliometer, pan evaporation and the 6 interactions of these factors (Table 3). From 34 to 70% of the variability in the sterility scores was accounted for by removing these 10 factors. On the day preceding anthesis, heliometer readings were the most significant factor. Wind velocity also was a fairly important factor along with the wind $\times$ temperature interaction. At 7 days preceding anthesis, these 3 conditions were not as important. Two other interactions, temperature $\times$ heliometer and temperature $\times$ pan evaporation, were much more significant. The period from 9 to 15 days before anthesis was a period which accounted for a low amount of variability. In 2 cases no values were significant. Wind velocity was important in 3 lines, but not so important in line 999. The heliometer reading was the most important factor in this line. From 18 to 22 days before anthesis, 44 to 55% of the variability in sterility at anthesis was accounted for. Both wind velocity and heliometer readings appeared fairly important. In 1 generation maximum temperature appeared very important, yet in the other tests had a relatively minor effect. Pan evaporation was one of the last variables to be removed, and the interaction of temperature $\times$ pan evaporation appeared to be important. Wind $\times$ pan evaporation interaction was important in 2 of the A_2 lines at 22 days before anthesis. Wind and heliometer were the 2 most important simple correlations. The degree of importance depends upon the line and also depends on the time before anthesis. Temperature and heliometer $\times$ pan evaporation interactions also appeared as fairly important effects.

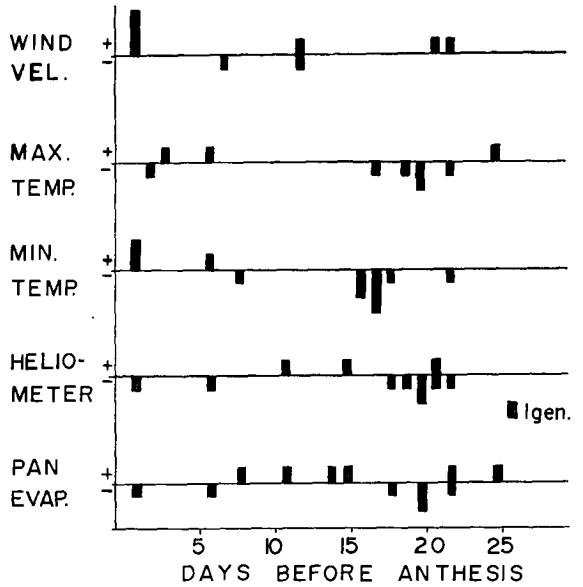

Figure 3. Number of generations of young B_1 cytoplasmic partially male-steriles (max. 6 generations) with positive and/or negative correlations with environmental conditions. State College. 1964.

Comparisons between environmental conditions in 1962 at State College and Stoneville, Mississippi, showed 2 conditions between the locations which could explain the 25% increase in fertility at State College (Sarvella, unpublished). There were both higher rainfall and heliometer readings at Stoneville than at State College the week preceding the difference in fertility. In 1961 sterility differences were also observed between State College and Stoneville. Soil temperature 4 weeks before anthesis was the only condition to be correlated with the sterility at State College. However, at Stoneville, soil and air temperatures 2 and 3 weeks prior to anthesis, and wind velocity 2 weeks prior to anthesis were significant.

DISCUSSION AND CONCLUSIONS

There were similar correlation patterns in the A_2 partially male-sterile plants between the various en-

Table 3. Stepwise importance of environmental influences on the sterility scores of young A_2 and B_1 cytoplasmic partially male-sterile cottons. State College. 1964.

Cytoplasm	A_2	A_2	B_1	A_2	A_2	B_1	A_2	B_1	A_2	B_1	B_1	A_2	A_2
Line	741-1	907	913-1	741-1	907	913-1	741-1	913-1	907	913-1	999	741-1	907
Generation	BC_4+S_1	BC_4+S_2	BC_5+S_1	BC_4+S_1	BC_4+S_2	BC_5+S_1	BC_4+S_1	BC_5+S_1	BC_4+S_2	BC_5+S_3	BC_5+S_3	BC_4+S_1	BC_4+S_2
Days preceding anthesis	1 da.	1 da.	1 da.	7 da.	9 da.	12 da.	15 da.	15 da.	18 da.	20 da.	20 da.	22 da.	22 da.
† ‡	2**	7	2	7*	8	5	6	7	2	9	1	5	2
	8**	2	8	6*	2	2	3	9	6	4	2*	1	6
	10**	8*	10	10**	7	10	10	4	10*	8	7*	6*	10
	6**	6*	6	1**	4	3	2	1	7*	2	5*	9*	8*
	9**	4*	9	3**	6	4	8	10	5**	6*	6*	4*	9*
	4**	1*	1	4**	5	9	9*	2	8**	7*	10*	3**	3*
	7**	5*	5	2**	3	7	4*	8	3**	5*	8*	10**	4**
	1**	9*	7	5*	10	6	7*	5	1**	3*	4*	8*	1**
	5**	10	4	9	9	1	1*	6	4**	1*	3**	7**	5**
	3**	3	3	8	1	8*	5	3*	9**	10*	9**	2	7**
Amt. of variability removed i. e. R^2	77%	52%	45%	62%	35%	35%	38%	34%	55%	44%	54%	55%	50%

* Significant at the 5% level. ** Significant at the 1% level. † Stepwise importance of environmental effects: least important at the top of the column to most important at the bottom. ‡ Code numbers stand for the following environmental effects: 1-wind, 2-maximum temperature, 3-heliometer, 4-pan evaporation, 5-wind × temperature, 6-wind × heliometer, 7-wind × pan evaporation, 8-temperature × heliometer, 9-temperature × pan evaporation, 10-heliometer × pan evaporation.

vironmental factors and sterility. In all environmental conditions studied, except wind velocity (Figure 2), there was a change from positive to negative correlations about 15 days before anthesis (about meiosis). Since pollen breakdown occurs about 10 days before anthesis, the environmental condition or interaction among the various factors must precede this period. The days for multiple correlations for the two A_2 lines were selected because they had several simple correlations which were significant. No one factor seemed to be consistently significant. However, wind and its interactions seemed important at 15 to 22 days before anthesis. It was surprising since no simple correlations were observed between 15 to 20 days preceding anthesis. Pan evaporation and its interactions were also important. Heliometer readings and temperature were usually not as influential as the other conditions.

The simple correlations showed that temperature and heliometer values influenced the sterility scores of the B_1 cytoplasmic partially male-steriles more than the other factors. Multiple correlations showed heliometer values were more influential than temperature. In 1963 temperature and wind velocity were important in inducing sterility. Both partially male-steriles, therefore, appeared to be influenced by several environmental conditions and the interactions were also important.

The 1961 data for Stoneville, which were confirmed by Meyer and Meyer (7) showed that the temperature was the most important factor influencing sterility; while at State College, temperature in all 4 years in the A_2 cytoplasm did not appear to be the critical factor. The B_1 cytoplasm revealed that temperature as well as other conditions were critical. Since Stoneville and State College are on the same latitude, separated by 130 miles and 300 feet of elevation, it is interesting that the sterility should be affected and that there is a difference between the A_2 and B_1 stocks at the 2 locations. Of course, Stoneville is in the Mississippi delta region while State College is in the hill section of the state.

These experiments were conducted in the field since these conditions are the ones that a plant breeder would encounter. If cytoplasmic male-sterility is to be of value to the plant breeder, the factors which limit the sterility must be determined in the field where the interactions of the different environmental factors are encountered. Interactions in the State College environment are very important. Since there is considerable variation in the expression of sterility, the environment must act on some system which either promotes or inhibits a substance such as an auxin which in turn influences pollen formation or affects the breakdown of the tapetum.

ACKNOWLEDGEMENTS

The author would like to thank Mr. J. C. McWhorter, Mr. Oliver Newton, Dr. Walter Drapala, Dr. Donald Baker, and Dr. R. R. Bruce for their advice and help and Mrs. Sandra Guice for her technical assistance.

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