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Table 1. Distribution of flower scores * in progeny rows of cytoplasmic male-sterile cottons selfed or crossed with B-lines.

Cytoplasm	Year	Cross	Number of flowers scored					Mean score for row	R X C test for similar distribution of scores χ^2†	Probability of a larger χ^2
			0	1	2	3	4			
G. arboreum	1966	A_2 ×	151	21	20	36	8	0.85	29.89	<0.005
G. arboreum		A_2 × B-line	266	71	14	23	16	0.59		
G. arboreum	1967	A_2 ×	80	28	16	4	1	0.59	6.52	0.10-0.05
G. arboreum		A_2 × B-line	93	59	14	9	--	0.65		
G. anomalum	1966	B_1 ×	81	92	36	52	34	1.55	10.87	0.05-0.025
G. anomalum		B_1 × B-line	86	147	45	46	25	1.36		
G. anomalum	1967	B_1 ×	28	42	18	10	3	1.19	5.66	0.25-0.10
G. anomalum		B_1 × B-line	71	100	24	13	2	0.93		
G. hirsutum	1967	A_2 B-line	10	22	42	96	34	2.60	86.87	<0.005
G. hirsutum		B_1 B-line	1	7	13	119	139	3.39		

* Flower scores range from 0 for no fertile anthers to 4 for 100% fertile anthers. † Chi-square tests similarity of distribution of flower scores in pair of rows, calculated by use of R X C table.

anomalum Wawra and Peyr × *G. thurberi*) × *G. hirsutum*. Reciprocal hybrids of both strains are much more fertile with *G. hirsutum* cytoplasm than that derived from either *G. anomalum* or *G. arboreum*.

The CSSA Committee on Crop Terminology has recommended that investigators who discover cytoplasmic male-sterility in other species use the established terminology in corn, sorghums, and sugar beets as a guide. In the research with cytoplasmically controlled male sterility of cotton, the terms, A, B, and R lines have been used as for sorghum. The terms are defined by Leonard, Love, and Heath[3]. An A-line is homozygous for the appropriate nuclear factors, or genes, and has a "male-sterile" cytoplasm. A lines of corn and sorghum[4], become partially fertile under some environmental conditions, but selfing is an impractical way to maintain them. For this purpose B-lines are used. B-lines are pollen-fertile lines, with homozygous male-sterility genes in a fertility-inducing cytoplasm.

Isolation of a B-line begins with finding a fertile anther from a usually male-sterile A-line. Such an anther is used to pollinate the desired Upland cotton parent variety. The F_1 plants are self-pollinated, and selection of B-lines begins with the F_2 generation. Because the B-lines are partially or entirely male-fertile, test crosses are the only reliable way to distinguish the plants homozygous for the appropriate nuclear factors when they are sought in segregating populations with "fertile" cytoplasm. For this purpose pairs of progeny rows are grown from individual plants: a test cross from using the plant's pollen on the A-line, and a selfed row. Test-cross rows with only male-sterile plants identify parents homozygous for nuclear factors for male-sterility. The selfed rows from these plants supply the B-lines, which are maintained by selfing.

During the first several years while the sterile lines with foreign cytoplasm were being developed, the data taken on B-lines usually were limited to recording which progeny rows had only sterile plants. In 1966, flower scores of test rows were compared with those of homozygous sterile rows to determine whether the B-lines transmitted male sterility as well as did selfs from male-sterile plants. Progenies in both cytoplasms were significantly *more* sterile from B-line crosses than from selfs in 1966 (Table 1). Similar selfs and crosses in 1967 did not differ significantly at the 0.05 probability level. When the B-lines themselves were compared, the *G. anomalum* B-line was very much more fertile than the *G. arboreum* B-line (Table 1).

If the apparent increased sterility from the B-lines is real, the most likely explanation would be selection for modifier genes in the most highly sterile test-cross rows. Should further development of cytoplasmic male sterility in cotton ever seem desirable, selection for accumulated modifiers of sterility genes might be worthwhile both in B-lines and in the original male-sterile stocks.

[3] Leonard, W. H., R. Merton Love, and Maurice E. Heath. 1968. Crop terminology today. Crop Sci. 8:257-261.

[4] Quinby, J. R. Personal communication.

INFERTILITY OF COTTON FLOWERS AT BOTH HIGH AND LOW RELATIVE HUMIDITIES[1]

G. J. Hoffman and S. L. Rawlins[2]

ABSTRACT

At either constantly low (25%) or high (90%) atmospheric relative humidity, cotton (*Gossypium hirsutum* L.) set very few bolls because the anthers failed to dehisce. Seed cotton yields were almost zero at both 25 and 90% relative humidity, whereas yields at 40 and 65% were 48 and 164 g/plant, respectively.

Additional index words: **Cotton yields, Cotton boll set, Environment.**

DURING an experiment designed to determine the effects of atmospheric relative humidity and salinity on the water relations and growth of cotton, Hoffman, Rawlins, and Cullen (1970)[3] observed that the anthers of cotton flowers failed to dehisce at both high and low relative humidities. As a consequence, seed cotton yields in the extreme humidity treatments were practically zero, even though plant size was not affected at low humidities and was 40% greater at 90% relative humidity. Here we show pictures of these flowers and briefly describe the conditions under which the flowers were not fertile, hoping that those working with cotton will be alerted to possible problems involved in evaluating yields under constant extreme relative humidities.

[1] Contribution from the U. S. Salinity Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, USDA, P. O. Box 672, Riverside, Calif. 92502. Received July 7, 1970.

[2] Research Agricultural Engineer and Research Soil Scientist (Physics).

[3] Hoffman, G. J., S. L. Rawlins, and E. M. Cullen. 1970. Water Relations and Growth of Cotton as Influenced by Salinity and Relative Humidity. Submitted to Agron. J.

Cotton (*Gossypium hirsutum* L. 'Acala SJ-1') was grown in four sunlit climate chambers during 1969 under controlled environmental conditions. The temperatures in all chambers were varied diurnally throughout the experiment from a maximum of 38C in the afternoon to a minimum of 26C in the early morning. The approximate daytime relative humidities in the four chambers were 25, 40, 65, and 90%. Throughout the experiment the actual average daytime temperatures and relative humidities in the four chambers were 34.0C, 26%; 34.5C, 41%; 33.3C, 69%; and 33.7C, 86%. With few exceptions, both ambient and dewpoint temperatures were maintained within ±1C. Four salinity levels were established in each chamber by adding enough NaCl to the half-strength Hoagland solution to obtain osmotic potentials of —0.4, —5.0, —10.0, and —15.0 bars in the root medium. Each salinity treatment was replicated three times.

Table 1 shows the number of squares and bolls per plant at 16 weeks of age as well as seed cotton yields at the end of the experiment for the various salinity and humidity treatments. The seed cotton yields include all mature cotton picked during the experiment as well as the contents of any immature bolls remaining at the time of harvest. The plants were 21 weeks old at harvest. Seed cotton yields from both the 25 and 90% humidity treatments are low. The yields at various salinity levels at 40% relative humidity agree closely with those for the same variety of cotton grown in field plots the same year at Riverside (Bernstein and Francois, unpublished data). Bernstein and Francois obtained seed cotton yields of 51 and 25 g/plant at average soil salinity levels of —0.7 and —11.0 bars, respectively. The average daytime relative humidity during this field plot experiment was 48%. The climate chamber yields at 65% relative humidity were at least twice those from the field plots.

The low seed cotton yields at high and low relative humidities were caused primarily by the reduced number of bolls set per plant. Table 1 shows that squares and bolls set per plant decreased as salinity increased. This result was expected, because salinity stunts growth. However, at all salinity levels, the maximum number of bolls and minimum number of squares occurred at 65% relative humidity. Almost no bolls had set on plants in the extreme humidity treatments, although they had numerous squares. At harvest there were still considerably more squares per plant at the extreme humidities than at 65%.

Photographs of representative flowers from each of the humidity treatments are presented in Fig. 1. Considerable pollen is present on the anthers of the moderate humidity treatment flowers, while almost no pollen is visible on the anthers from the extreme humidity treatments. Close examination reveals pollen grains on the stigma in the 65% humidity treatment. It appears that bolls failed to set in the extreme humidities because the anthers failed to dehisce at all. Also the stamens of flowers in the extreme humidities were shorter than those in the 40 and 65% humidity treatments.

The atmospheric relative humidity in the field plot experiment of Bernstein and Francois dropped to at least 25% on many days and rose to at least 90% on many nights. Thus, it appears that extreme humidi-

Table 1. Effect of relative humidity and salinity on the number of bolls and squares at 16 weeks of age and on seed cotton yields after 21 weeks for plants grown in climate chambers.

O. P. of nutrient solution, bar	Squares per plant	Bolls per plant	Seed cotton yield, g/plant
	25% relative humidity		
-0.4	202	2	10
-5.0	111	1	1
-10.0	69	2	7
-15.0	39	1	3
	40% relative humidity		
-0.4	193	11	48
-5.0	76	4	--*
-10.0	65	0	26
-15.0	46	3	1
	65% relative humidity		
-0.4	47	32	164
-5.0	35	8	73
-10.0	33	10	52
-15.0	23	7	22
	90% relative humidity		
-0.4	233	0	11
-5.0	170	0	5
-10.0	90	1	1
-15.0	58	1	2

* Plants died after the 16th week because of a failure in the irrigation system.

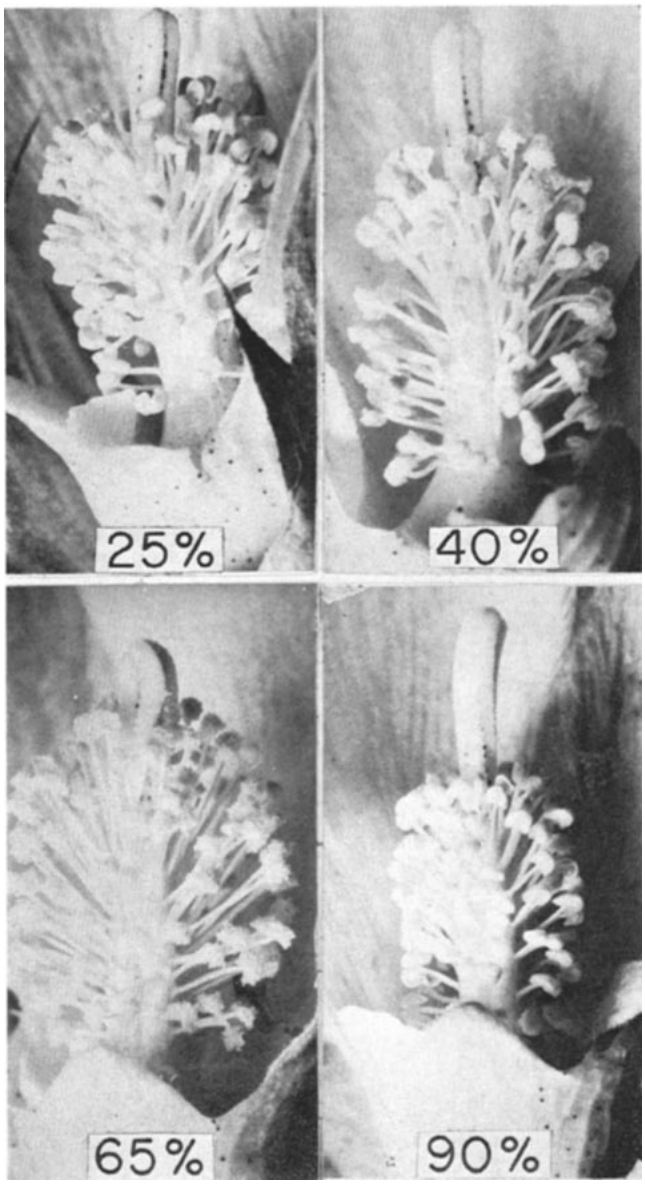

Fig. 1. Representative flowers from each of four humidity treatments.

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ties for short periods are not detrimental. It may well be the fact that humidity was constantly high or low that caused the low boll set in the climate chambers.

In the field, intermediate humidities occur at times, and pollination during these times may be sufficient to provide an adequate number of bolls.

High temperatures may cause sterility in cotton flowers. Meyer (1969)[4] reports that more and more sterile anthers appear on some cotton species as maximum temperatures increase above 38C. Thus, a possible cause of the sterility reported here may have been high temperatures. This does not seem likely, however, since the average maximum temperatures recorded throughout the experiment were 37.2, 37.9, 37.3, and 37.4C for the 25, 40, 65, and 90% relative humidity treatments, respectively.

It would be easier to speculate on a mechanism for the low fertility had it occurred only at high or at low humidity. It is difficult to imagine a single mechanism causing infertility at both extremes. Because determining the mechanisms is beyond the scope of our research, we merely present our findings here for information. This phenomenon apparently has not been reported before.

[4] Meyer, Vesta G. 1969. Some Effects of Genes, Cytoplasm, and Environment on Male Sterility of Cotton (*Gossypium*). Crop Science 9:237-242.

DEVELOPMENT OF SUBTERRANEAN CLOVER (*TRIFOLIUM SUBTERRANEUM* L.) AT VERY EARLY STAGES[1]

C. A. Raguse, F. K. Fianu, and J. W. Menke[2]

ABSTRACT

Ten unique morphological stages of plant development from emergence to full expansion of the unifoliolate leaf were described for an annual clover (*Trifolium subterraneum* L.). This system of classification provides for precise morphological description of plant development when a high degree of experimental resolution is required at very early stages.

Additional index words: **Developmental morphology, Leaf index number.**

QUANTITATIVE means for describing developmental plant morphology constitute helpful adjuncts for use with the conventional formulae of growth analysis (4). A plant's growth response to its environment during germination and early stages of vegetative growth may determine whether it persists in the plant community, e.g., an annual species introduced into a reseeding, annual vegetation type. In such a situation it is often useful to chronicle morphological development of the plant, especially since productivity may be correlated with such development. A further advantage is that the "sampling" procedure is nondestructive, so repeated observations can be made on the same plant.

We have successfully used Carlson's (2) system for defining developmental stages for 'Ladino' clover (*Trifolium repens* L.) for comparative growth studies with two morphologically similar perennial *Trifolium* species (3), and also with several varieties of two species of annual legumes, *Trifolium subterraneum* L. and *T. hirtum* L. (unpublished data).

The present study extends the developmental morphology index presented by Carlson (2) by providing for precise description of earlier stages of plant development.

Materials and Methods

Subterranean clover plants (*Trifolium subterraneum* L., subsp. *yamminicum* Katznelson and Morley, 'Yarloop') were grown in a growth chamber programmed for a 12-hr photoperiod, a constant temperature of 20C, and a light intensity of about 35,000 lux. Seeds of intermediate size were sown in sufficient number to allow thinning shortly after emergence to three uniform plants per 20-cm pot. The seeds, which were previously stored at 10C, were transferred to the growth chamber prior to seed moistening and imbibition.

Plants used to construct a photographic sequence of growth stages from emergence to unifoliolate leaf expansion were grown in the greenhouse under conditions similar to those in the growth chamber. This was done because the growth chamber pots were in fixed positions, and physical limitations (e.g., availability of appropriate camera angles) prevented satisfactory photography.

Results and Discussion

The data in Fig. 1 indicate the rate of plant development of *T. subterraneum*, a clover frequently introduced into Mediterranean-type annual rangelands. The values are based on Carlson's system of assigning numerical values to 10 morphologically distinct stages in the development of a trifoliolate leaf. For example, in this system a leaf index number of 5.8 indicates that a plant has five fully developed trifoliolate leaves plus a sixth leaf which is eight-tenths open. Annual clovers germinate with the advent of fall rains, but below-optimum temperatures at that time can markedly slow plant development. The temperature response lines in Fig. 1 indicate an interaction between temperature and rate of plant development when the

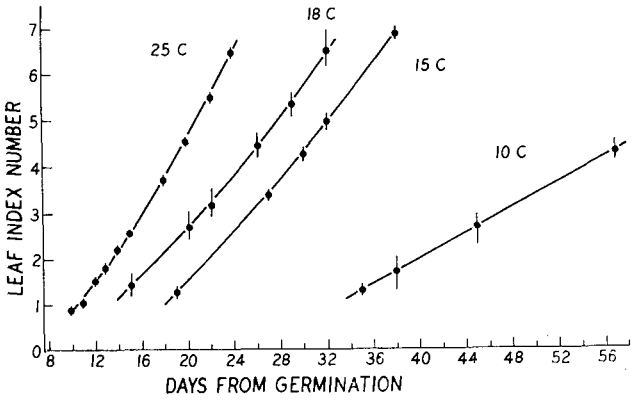

Fig. 1. Development of *Trifolium subterraneum* Yarloop plants as measured by the number of trifoliolate leaves per plant at several constant temperatures. Vertical lines indicate range of values for four plants.

[1] Contribution from the Department of Agronomy & Range Science, University of California, Davis 95616. Received June 13, 1970.

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