Published July, 1971



**Table 1. Fertility and seed weight in 18 second substitution backcrosses ($SB_2$), 2 amphidiploid strains (AD), and *A. intermedium*.**

| $SB_1$ strain | % male sterility of $SB_2$ | Seeds per spike | TKW, g | Seed weight rating*, % | No. of $SB_3$ seeds harvested |
|---|---|---|---|---|---|
| $SB_2$-1-2 | 99.4 | 2.47 | 5.50 | 138.2 | 1,435 |
| $SB_2$-1-8 | 100.0 | 1.04 | 7.71 | 193.7 | 375 |
| $SB_2$-1-9 | 99.9 | 10.11 | 3.39 | 85.2 | 3,380 |
| $SB_2$-1-11 | 81.5 | 7.74 | 7.42 | 186.4 | 3,746 |
| $SB_2$-1-18 | 99.9 | 6.17 | 7.87 | 197.7 | 816 |
| $SB_2$-2-11 | 99.9 | 1.93 | 10.06 | 252.8 | 367 |
| $SB_2$-2-16 | 100.0 | 2.50 | 6.19 | 155.5 | 519 |
| $SB_2$-2-17 | 98.9 | 2.67 | 8.31 | 208.8 | 385 |
| $SB_2$-2-21 | 100.0 | 0.78 | -- | -- | 43 |
| $SB_2$-2-22 | 99.7 | 1.93 | 4.37 | 109.8 | 287 |
| $SB_2$-2-23 | 100.0 | 1.04 | 8.50 | 213.6 | 188 |
| $SB_2$-2-27 | 99.7 | 1.32 | 6.09 | 153.0 | 253 |
| $SB_2$-2-29 | 100.0 | 3.72 | 5.42 | 136.2 | 171 |
| $SB_2$-2-35 | 100.0 | 0.89 | 6.02 | 151.3 | 345 |
| $SB_2$-24-1 | 93.0 | 2.25 | 5.02 | 126.1 | 198 |
| $SB_2$-24-2 | 97.0 | 10.36 | 8.80 | 221.1 | 1,803 |
| $SB_2$-29-1 | 96.7 | 5.53 | 4.40 | 110.6 | 459 |
| $SB_2$-33-1 | 98.6 | 10.48 | 6.13 | 154.0 | 5,218 |
| Avg | 98.0 | 4.06 | 6.54 | 164.4 | 19,988 |
| Ranges | 81.5 to 100.0 | 0.78 to 10.48 | 3.39 to 10.06 | 85.2 to 252.8 | Total no. of $SB_3$ seeds |
| A. Interm. | 13.8† | 60.17† | 3.98‡ | 100.0‡ | --- |
| AD-5a-2 | 43.9 | 13.67 | 10.36 | 260.3 | 1,518 |
| AD-7-2/3 | 51.7 | 4.30 | 7.40 | 185.9 | 245 |

* Calculated as percentage of seed weight of A. intermedium. † Variety 'Oahe'. Seeds per spike calculated by Ross, 1970. ‡ Strain MSU-40b-4 (from an open pollinated single plant selection of 'Greenar'.)

The seed fertility of the $SB_2$ is compared with that of Oahe and that of 2 amphidiploid parents, AD-5a-2 and AD-7-2/3. According to Ross (2) Oahe averaged 60.17 seeds per spike. The amphidiploid parents varied in seed fertility from 4.30 to 13.67 seeds per spike. Therefore, seed fertility is not improved above the potential of the original amphidiploid parents. But further improvement should be expected during the next selection cycle in the $SB_3$ generation since meiotic irregularities have decreased substantially from the $SB_1$ to the $SB_2$ generation (5) and should further be reduced in the $SB_3$. This should be reflected in increasing egg fertility.

Seed weight was determined for only 17 of the 18 $SB_2$ strains. Only 43 seeds were harvested from strain $SB_2$-2-21, not enough to allow determination of thousand kernel weight (TKW). TKW varied from 3.39 to 10.06 g with an average of 6.54 g. Using strain MSU-40b-4 of *A. intermedium* as the 100% base, the highest seed weight rating is that for strain $SB_2$-2-11 (252.8%), which approaches that of the highest amphidiploid parent, AD-5a-2 (260.3%). The large seed size is inherited from the *T. durum* parent and may decrease with progressing SB generations because *T. durum* chromosomes are eliminated with each generation. But it is remarkable that some $SB_3$ seed still weighs 2½ times more than *A. intermedium* seed. It is easy to envision that cytoplasmic factors are involved and that large seed size could be maintained.

Maintenance of large seed size is a valuable asset in this breeding program and has been a goal from the beginning. The small seed of present dryland grasses limits the conditions under which satisfactory stands may be obtained. In a system of grassland-wheat farming there is a reluctance to plow established grass stands and to establish new stands of grass. More readily-established grasses would remove some of the hazards and provide greater flexibility in shifting from small grain to grass. This would be particularly valuable in dry years or in dry areas.

A total of 19,988 seeds of the $SB_2$ generation have been hand-thrashed at present (Table 1). More seeds have been harvested but not yet processed. Therefore, enough male sterile seed of *A. intermedium* is available to start a seed increase program.

## REFERENCES


1. Kihara, H. 1967. Cytoplasmic male sterility in relation to hybrid wheat breeding. Züchter 37:86-93.
2. Ross, J. G. 1970. Personal communication.
3. Schulz-Schaeffer, J. 1970a. The *Triticum* × *Agropyron* hybridization project at Montana State University. Wheat Inf. Serv. 30:26-29.
4. ————. 1970b. A possible source of cytoplasmic male sterility in intermediate wheatgrass, *Agropyron intermedium* (Host) Beauv. Crop Sci. 10:204-205.
5. ————, R. I. Baeva, and J. H. Kim. 1971. Genetic control of chromosome pairing in *Triticum* × *Agropyron* derivatives. Zeitschr. Pflanzenz. 65:53-57.


# COLD TOLERANCE IN A HEXAPLOID COTTON[1]

**H. Muramoto, J. D. Hesketh, and D. N. Baker[2]**


## ABSTRACT

**Plants of a recently introduced strain of hexaploid cotton, 6X-3, produced up to six true leaves after 4 months in a cold phytotron environment (average of 14 C); whereas a number of common cultivars produced only one or two true leaves and then died, apparently from a fungus. The true leaves of the hexaploid were blue green. Those of the other cottons were very chlorotic. Since seedlings of the hexaploid have been observed to survive brief periods of chilling in the field, while those of common cultivars did not, the differences observed in the phytotron would appear to be a good indicator of a form of cold tolerance.**


***Additional index words:*** **Chlorosis, Leaf spectral absorbance.**

COLD tolerance is desirable in cotton varieties because of losses due to unpredictable cold periods following planting. Seedlings exposed to cold often produce earlier plants because flowering is induced at lower nodes (3). Such plants have shorter lower internodes. If these characteristics are to be induced by planting early to produce earlier maturing crops, then more cold tolerance is necessary.

We have screened a number of varieties for cold tolerance, but no detectable differences in growth were observed. Seedlings of the recently developed strain, Hexaploid 6X-3 (4), have been observed to survive a period of chilling in a field planting while seedlings of the common cultivars have not. Therefore, we decided to repeat our survey for cold tolerance in a phytotron, including the new strain. Here we report the behavior of cotton in various light and temperature regimes to illustrate a procedure for measuring potential cold tolerance.

[1] Contribution from the Arizona Agricultural Experiment Station (Journal Paper No. 1704) and Southern Branch, Soil and Water Conservation Research Division, Agricultural Research Service, USDA, in cooperation with the Entomology Research Division and the Mississippi Agricultural Experiment Station. This project was supported in part by NSF Grant GB-7153 to H. Hellmers, Director, Duke Phytotron, Durham, North Carolina. Received Dec. 5, 1970.

[2] Associate Plant Breeder, Department of Plant Breeding, University of Arizona, Tucson, Arizona 85721; and Research Soil Scientists, USDA, Boll Weevil Research Laboratory, and Department of Agronomy, State College, Mississippi 39762.

## MATERIALS AND METHODS

Cotton plants were grown in controlled temperature greenhouses at the Duke University Phytotron and the CSIRO Phytotron in Canberra, Australia. The growth medium at both locations was 1:1 vermiculite-gravel. A modified Hoagland's solution (half strength) was applied in the morning and water in the afternoon. Day temperatures were higher than night temperatures. The daytime thermal period was 8 hours; the photoperiod was 16 hours. Varieties and strains tested in both facilities included those reported earlier (Table 1, ref. 3) as well as *G. hirsutum* L. vars. 'Super Okra,' 'Hopicala,' 'Stoneville 213,' 'Acala 44,' and an $F_1$ of Acala 44 × Deltapine Smoothleaf. *G. barbadense* L. varieties tested were 'Pima S-4,' 'Pima S-2,' and the experimental strains "14-1" and "126-1." The interspecific $F_1$'s, Pima S-2 × Deltapine Smoothleaf and Pima S-2 × Super Okra M-8, one strain of *G. herbaceum,* and Hexaploid 6X-3 (4) were also tested.

*In vivo* absorbance spectra were obtained on leaves and cotyledons of several of the varieties using a Zeiss DMR-21[3] recording spectrophotometer equipped with a modified integrating sphere attachment (1).

## RESULTS AND DISCUSSION

*Growth of common cultivars vs. temperature.* The results from the two phytotrons for 18 C and lower temperatures are combined here. Seedlings grown in a 21/16 C day/night temperature (18 C average) regime (Canberra) had green cotyledons with some bleaching, but the early leaves were chlorotic during midsummer. The plants eventually grew out of this chlorotic condition. Similar chlorosis developed in a 20/17 C day/night regime (Duke). Hesketh and Low (2) reported that squaring plants introduced into a 21/16 C regime from 24/19 C remained green and produced cotton. Plants grown during winter (low light), in partial shade, or with eight hours of fluorescent-incandescent light were not as chlorotic at 12/16 C and 20/17 C as plants grown in midsummer in greenhouses at these same temperatures. The chlorosis seemed to be enhanced by intense sunshine. An addition of iron chelate enhanced the rate of greening in chlorotic leaves.

Plants grown at 18/13 C (15 C average) were chlorotic and produced abnormal squares (see ref. 2). Plants introduced to 18/13 C from a 24/19 C regime became chlorotic but they did flower (2). Many of the plants grown at 17/11 C (14 C average) developed two true leaves in 2 months. All leaves were either yellow or white. *G. hirsutum* L. varieties developed more leaves, but *G. barbadense* L. varieties had larger cotyledons. *All of these plants were dead after 3 months,* apparently because of some fungus. All seedlings grown at 15/10 C (12 C average) failed to expand true leaves after the cotyledons unfolded.

*Growth of Hexaploid 6X-3 vs. temperature.* The cotyledons and true leaves of Hexaploid 6X-3 were bluish-green when grown at 17/11 C and above. These plants were alive and green after 4 months at 17/11 C. They produced four to six leaves and grew in height to about 15 cm. At temperatures above 18 C, however, the hexaploid strain was less vigorous than the other cotton because of slower emergence and smaller leaves. Eventually the hexaploid plants became as large as plants of other cultivars.

*Absorbance Spectra of leaves and cotyledons.* The absorbance spectra of leaves for the Hexaploid 6X-3 and for Stoneville 213 were quite different (Fig. 1). The greatest differences occurred in the spectral regions of photosynthetic activity, i.e., in the blue and green, 400-500 nm, and at 650-680 nm, orange and red orange.

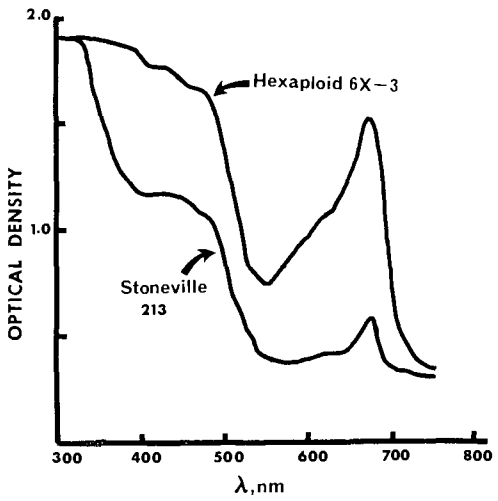


**Fig. 1. Absorbance spectra of the first true leaves of Stoneville 213 and Hexaploid 6X-3 grown in a 17/11 C temperature regime.**

**Table 1. Optical densities of Hexaploid 6X-3, Hopicala, and Stoneville 213 leaf pieces under three temperature regimes (Duke University Phytotron).**

| Variety | Temperature regimes* | Optical density at 680 nm (Two reps) | |
|---|---|---|---|
| Hexaploid 6X-3 | 17/11 C cotyledon | 1.90 | 1.55 |
| | 17/11 C 1st tru leaf | 1.50 | 1.55 |
| | 29/26 C mature leaf | 1.60 | 1.50 |
| Hopicala | 17/11 C cotyledon | 1.90 | 1.80 |
| | 17/11 C 1st true leaf | 0.70 | -- |
| Stoneville 213 | 17/11 C cotyledon | 1.75 | 1.70 |
| | 17/11 C 1st true leaf | 0.47 | 0.84 |
| | 29/26 C mature leaf | 1.60 | 1.70 |

* The ratios are day (8 hours)/night temperatures.

Optical densities of leaf pieces at 680 nm for three varieties (6X-3, Hopicala and Stoneville 213) are presented in Table 1. The average optical densities of cotyledons were not different for the three varieties at 17/11 C. However, first true leaves developed under the same temperature regime differed greatly. The first true leaves of Hopicala and Stoneville 213 revealed optical densities about half of those for Hexaploid 6X-3. Optical densities at 680 nm for mature leaves of Stoneville 213 and Hexaploid 6X-3 on plants grown at 29/26 C were the same.

## CONCLUSIONS

Seedling survival and leaf chlorosis can easily be evaluated in a greenhouse during midsummer at 17/11 C. Periods of intense sunshine tend to enhance the degree of chlorosis. More research needs to be done before other regimes or a temperature regime under artificial lights can be recommended. It is suggested that such facilities be used to test for existing cold tolerance in cotton cultivars and to develop hexaploids with more cold tolerance and better growth and yielding characteristics. Phytotron facilities quickly provide reproducible results that might be supplemented later by field tests, which are subject to vagaries in the weather.

[3] Mention of a proprietary product does not necessarily imply endorsement of this product by the University of Arizona, Mississippi State University or the United States Department of Agriculture.

## REFERENCES

1. Hennington, K. W., and J. E. Boynton. 1970. Macromolecular physiology of plastids. VIII. Pigment and membrane formation in plastids of barley greening under low light intensity. J. Cell Biol. 44:290-340.
2. Hesketh, J. D., and A. Low. 1968. Effect of temperature on components of yield and fibre quality of cotton varieties of diverse origin. Cott. Gr. Rev. 45:243-257.
3. Moraghan, B. J., J. D. Hesketh, and A. Low. 1968. Effects of temperature and photoperiod on floral initiation among strains of cotton. Cott. Gr. Rev. 45:91-100.
4. Muramoto, H. 1969. Hexaploid cotton: Some plant and fiber properties. Crop Sci. 9:27-29.


## WHITE FLAGGING OF STEM NEMATODE-INFECTED ALFALFA[1]

**D. W. Evans, J. H. Elgin, Jr., and L. R. Faulkner[2]**


### ABSTRACT

**White shoots (flags) occur sporadically on occasional plants in alfalfa stands infected with stem nematode (*Ditylenchus dipsaci,* [Kuhn] Filipjev) in central Washington State. All plants showing white flagging were found to be infected. Attempts to induce this symptom consistently under controlled conditions were unsuccessful.**


***Additional index words:*** **Chlorosis, *Medicago sativa.***

ALFALFA (*Medicago sativa,* L.) infected with stem nematode (*Ditylenchus dipsaci* [Kühn] Filipjev) in irrigated central Washington State exhibits a leaf and stem chlorosis which we have termed white flagging. This paper describes white flagging which has also been observed in western Nevada (2) and which may occur as yet undetected in other alfalfa-growing areas.

White flags consist of leaf and stem tissues lacking normal green pigmentation. The lack of color may be complete or it may be partial resulting in various shades of yellow green. There is no mottling or sectoring of the leaves as is typical of virus infection or genetic chimera, though a white leaflet may be green along the midvein or at the tip. In the extreme form, the stems and leaves are entirely white or white with a faint pink color and resemble material injured by 3-amino-*s*-triazole (amitrol). Completely white shoots usually shrivel and die early in development but occasionally survive to the flower bud stage. Less extreme forms fall into three general classes: (*1*) shoots are green part way up from the base and white at the top; (*2*) leaves are white while the stem is green (all the leaves or only alternate leaves may be affected); and (*3*) the stem and leaves are green but the axillary shoots (and often the stem apex) are white. A typical white flag is a completely white or apically white shoot (Fig. 1).

Observations of white flags marked with India ink at the edge of the affected tissues indicate that the white or pale tissue is produced by the growing point

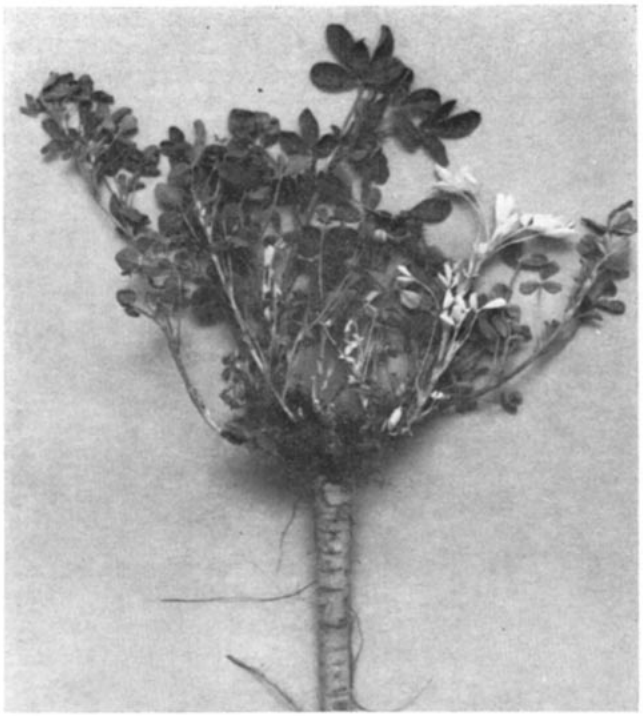

**Fig. 1. White flagging of stem nematode infected alfalfa.**

and that there is little or no progressive loss of color from previously formed tissue. The degrees of whiteness and many intergradations in pattern which can be found in different white flags are apparently related to the timing and extent of color loss in the growing apices. When loss of color is incomplete the apex may revert to the production of normal green tissue. This has not been observed in completely white apices.

Although white flagging is a strikingly visible phenomenon, only a few stems on scattered plants are actually involved at any one time. In 1970, observations were made of the incidence of white flagging in the second year of a six-replicated alfalfa variety trial infested with stem nematode. The results confirmed general observations made previously. A few white flags appeared before the first and after the third hay cuttings; more appeared after the first harvest in late May and the second in early July. Counts were made in the 10- to 14-day-old regrowth after the first and second harvests. An average of 0.6 and 0.3% of the total plant population showed white flags at the two respective times. The highest incidence recorded was 2.6 plants/m$^2$ or 2.2% of the stand for 'Ranger' after the first harvest.

While the underlying processes leading to white flag development have not been determined, stem nematode involvement is indicated. White flags were first observed in fields infested with stem nematode and have not been encountered in nematode-free stands. Examinations of 196 plants with white flags from 10 locations in central Washington during 1970 showed stem nematode to be present in the crown tissue in every case. White flags have also been observed in the greenhouse, both in seedlings and in older plants. Only plants inoculated with stem nematode or known to be naturally infected have developed

[1] Scientific paper No. 3605, College of Agriculture, Washington State University, Pullman, Project 1917. Received for publication Jan. 5, 1971.

[2] Assistant Agronomist, Washington State University; Research Agronomist, ARS, CRD, USDA; and Nematologist, Washington State University. The authors are stationed at the Irrigated Agriculture Research and Extension Center, Prosser, Washington 99350.