# ABAXIAL SURFACE AND EMULSIFIED LEAF pH OF COTTON, *GOSSYPIUM* SPP.

W.J. Smith, C. Wayne Smith, and Robert L. Meagher, Jr.

Graduate Research Assistant and Professor, Department of Soil & Crop Sciences, Heep Center, Texas A&M University, College Station, TX 77843-2474; Research Associate Entomologist, USDA-ARS, CVAVE, 1700 S.W. 23rd Dr., Gainesville, FL.

## ABSTRACT

The abaxial surface pH of the uppermost main stem leaf measuring at least 3 cm and the oldest fully expanded main stem leaf from the lower canopy of seventeen genotypes were determined when plants, grown under greenhouse conditions, were typically at the first square stage of growth and four weeks later. Emulsified leaf pH was determined on the crude extract after grinding leaf tissue in deionized water. Only minor differences were found in abaxial leaf surface or emulsified leaf pH relative to plant age or leaf position. Abaxial leaf surface pH ranged from 8.24 to 9.63 and emulsified leaf pH ranged from 5.75 to 6.56 among the seventeen genotypes evaluated.

## INTRODUCTION

Insects caused an estimated loss of over a million bales of cotton annually in the U.S. during 1989-1994 (Carter 1996). Losses in 1995 more than doubled to 2.3 million bales. Unrealized income plus cost of control in 1995 were valued at 889 million dollars. The major insect pests of cotton include the boll weevil, *Anthonomus grandis grandis* (Boheman), the bollworm/tobacco budworm complex, *Helicoverpa zea* (Boddie)/*Heliothis virescens* (F.), the plant bug complex, *Lygus* spp., *Neurocolpus nubilus* (Say), and *Pseudatomoscelis seriatus* (Reuter), aphids, *Aphis gossypii* (Glover), pink bollworm, *Pectinophora gossypiella* (Saunders), the whitefly complex, *Bemisia argentifolii* (Bellows and Perring) and *B. Tabaci* (Gennadius), and the most recent addition to the major pest list, beet armyworm, *Spodoptera exigua* (Hubner) (Metcalf and Metcalf 1993).

Insecticides have been the mainstay of insect control since the USDA recommended calcium arsenate for the control of boll weevil about 1920. Synthetic chlorinated hydrocarbon insecticides were introduced during the 1940's, followed by the organophosphates in the 1950's. Boll weevil resistance to the chlorinated hydrocarbon compounds was evident by the 1950's (Brazzel 1961, Roussel and Clower 1955), and by 1965 resistant bollworm and budworm populations were evident (Adkisson 1964, Adkisson and Nemec 1966, Brazzel 1963, 1964). Carter (1996) recently reported that the cotton producer does not have the insecticide chemistry to deal with even moderately resistant populations of tobacco budworm. Similar scenarios can be documented for many of the insect pests of cotton relative to the development of resistance to classes of insecticides.

A number of alternative control strategies have been reported. Integrated pest management has been effective as a concept for reducing dependency on chemical insecticides (Carruth and Moore 1973, Collins et al. 1979, Frisbie et al.1976, Larson et al.1975, Sterling and Haney 1973). Biological agents and insecticides based on naturally produced metabolites may provide effective control of some insect pests in the immediate future (Dimock 1996, Thompson 1996). Transgenic Bt cotton cultivars were available commercially for the first time in 1996. These unique cotton cultivars genetically produce the same protein, CryIA(c), as produced by the bacteria, *Bacillus thuringiensis*, that is toxic to some lepidopterous insect pests of cotton. This biological insecticide has been sold in the U.S. for over 35 years (Navon 1993). Resistance potential in *Heliothis virescens* to this method of insecticide delivery has been documented already (Gould et al. 1995).

Several plant morphological or allelochemical traits have been studied for their host plant resistance properties. Frego bracts (Jones et al. 1987, Pieters and Bird 1976), varying levels of plant pubescence (Butler et al. 1991, Butler and Henneberry 1984, Meredith and Schuster 1979, Stephens and Lee 1961, Walker and Niles 1973, Wannamaker 1957), absence of leaf, bract, and/or floral nectaries (Benedict and Leigh 1976, Meredith 1976, Schuster and Maxwell 1974, Wilson 1982), plant pigmentation (Bailey 1981, Jones et al. 1987), and gossypol content (Lukefahr and Martin 1966, Singh and Weaver 1971, Zummo et al. 1984) have been implicated as possible host plant resistant traits to one or more insect pests of cotton. Others include okra leaf shaped leaves, earliness of maturity, other allelochemicals, and genetic background ( Butler et al. 1988, Butter et al. 1992, Ozgur and Sekeroglu 1986, Sippell et al. 1987, Smith 1992). One other possible source of resistance that has received little attention is leaf pH (Berlinger et al. 1983, Berlinger 1986).

Cotton has an unusual and little understood characteristic of having a very high leaf surface pH, averaging about 10, compared with most plant species, that average around 7 (Harr et al. 1980). This value reportedly varies by leaf age and by species. This high surface pH is due to epidermal glands similar to hydathodes that secrete cations, mostly Ca, Mg, and K onto the leaf surface (Elleman and Entwhistle 1982, Harr et al. 1980). Harr et al. (1980) reported that this high surface pH can be detrimental to pesticide longevity and fungal pathogens.

Many insects may prefer plant tissue of a specific age or during specific portions of the growing season. Some of this preference may be explained by the fact that the pH of cell sap changes with leaf age (Harr et al. 1980, Husain et al. 1936). Berlinger et al. (1983) determined that *B. tabaci* could distinguish pH of artificial diets in increments as low as 0.25 units, with a preference for diets buffered to pH's from 6.0 to 7.25.

The objective of this study was to determine the genetic variability for abaxial leaf surface and emulsified leaf pH among seventeen cotton genotypes.

## MATERIALS AND METHODS

Four replications, consisting of one plant each, of seventeen diverse cotton genotypes were grown under greenhouse culture in 1992 and 1993. The selection of the seventeen genotypes evaluated for pH in this study was based on several criteria, including leaf pubescence, foliage color, leaf shape, species, earliness of maturity, and yield potential (Table 1). Several of these genotypes had been observed by the junior authors to be especially susceptible or apparently resistant to *B. argentifolii* in 1991 in the Lower Rio Grande Valley of Texas. Deltapine 50 is considered one of the most field resistant current cultivars available to producers while Stoneville 453 is considered one of the most whitefly susceptible current cultivars. Pima S6 was included because it represents a different species, *Gossypium barbadense*, than the other sixteen that are *G. hirsutum*, the species normally grown in Texas.

Strain 86L²14 L has okra leaf shaped leaves as does strain MACAOS that also has red foliar pigmentation. Tamcot CAB-CS and MACAOS are from the Texas Agricultural Experiment Station, Stoneville 453 and Deltapine 50 are current cultivars from the those companies, Lone Star is an obsolete cultivar released for production in Texas in the early 1900's, and the numbered strains were developed by the corresponding author at Texas A&M. As noted earlier, these seventeen genotypes represented a range in leaf trichome density, from the obsolete cultivar Lone Star with three trichomes $cm^{-2}$ of leaf surface to strain 89E51 and Stoneville 453 that averaged 119 trichomes $cm^{-2}$ (Smith 1994).

TABLE 1. Phenotypic Characteristics of Cotton Genotypes Evaluated for Abaxial Leaf Surface and Emulsified Leaf pH.

| Genotype | Leaf shape | Visible leaf pubescence | Foliage color |
|---|---|---|---|
| Deltapine 50[a] | normal | smooth | green |
| Stoneville 453[a] | normal | very hairy | green |
| Tamcot CAB-CS[a] | normal | smooth | green |
| Lone Star[b] | normal | smooth | green |
| Pima S6[a,c] | normal | very hairy | green |
| 86E20 | normal | smooth | green |
| 86L²9 | normal | moderate | green |
| 86L²14L | okra | smooth | green |
| 88G104 | normal | smooth | green |
| 89E51 | normal | very hairy | green |
| 89E62 | normal | smooth | green |
| 89F46H | normal | hairy | green |
| 89F46S | normal | moderate | green |
| 90C19H | normal | moderate | green |
| 90C19S | normal | smooth | green |
| 90J57 | normal | smooth | green |
| MACAOS | okra | moderate | red |

[a] Current commercial cultivar.

[b] Obsolete commercial cultivar.

[c] *Gossypium barbadense*; all other genotypes are *G. hirsutum*.

Seeds were placed into moistened peat pellets for germination on 17 September and 8 December 1992. Seedlings were transplanted about ten days later to 7.6 liter pots filled with a commercial potting mixture. Plants were fertilized once with 16 g/pot of a commercial fertilizer with a formulation of 21-7-11 (N-P-K). Soil tests of the potting mixture used indicated that all other plant nutrients were sufficient. Plants were placed in a large, walk-in, organdy cage to exclude insects. Plants were not treated with insecticides to avoid chemically influencing leaf surface pH but were periodically hand sprayed with deionized water to dislodge and kill insect pests (*Trialeurodes* sp. and aphids). Efforts were made to avoid any of these immatures when recording leaf surface pH but some insects were present on some leaves when emulsified.

The pH of the abaxial leaf surfaces and emulsified leaf pH were determined for the youngest main stem leaf measuring at least 3 cm in diameter, and on the oldest, normal shaped main stem leaf from the lower canopy. Measurements were taken when plants across the seventeen genotypes typically had reached first square, 27 and 28 October 1992 for the first planting and 22 to 26 February 1993 for the second planting. A second measurement was taken

4-weeks post first square stage. Plants were typically blooming at 4-weeks post first square for the first planting but not for the second planting. This was probably caused by reduced incoming radiant energy and reduced temperatures encountered during the winter months.

Abaxial pH was measured with an Orion flat surface pH electrode. One droplet, 55 $\mu$l, of deionized water was placed between major veins on the undersurface of the leaf. The electrode was placed on the water droplet and pH recorded from an Orion pH meter after 90 sec. Emulsified leaf pH was determined as outlined by Berlinger et al. (1983). One gram of upper, expanding main stem leaf tissue from each plant and three grams of lower, fully expanded main stem leaf tissue were collected from each plant. Eight ml of deionized water were added to the 1 g sample and 24 ml were added to the older plant leaf tissue. The mixtures were ground until smooth, approximately 45 sec., with a tissue homogenizer. The pH of the crude extract was taken with an Orion pH probe and meter. Three readings of each sample were taken after stirring and averaged to provide a final pH value.

Pots were arranged in the greenhouse in a randomized complete block design. Data were analyzed as a split plot with sampling dates split to genotypes and genotypes split to leaf sampled. Means were separated by the Waller-Duncan LSD at k=100 which approximates the 5% probability level.

## RESULTS AND DISCUSSION

Plant age, first square and 4-weeks post first square, genotype, and leaf position significantly affected abaxial leaf surface pH during 1992 and 1993, while emulsified leaf pH was affected by genotype and leaf sampled in both years (Table 2). Genotype x plant age interaction was significant during 1992 for abaxial leaf surface pH while leaf sampled x plant age interactions were significant for leaf surface pH during 1993 and for emulsified leaf pH during both years.

TABLE 2. Mean Squares for Abaxial Leaf Surface and Emulsified Leaf pH of Upper and Lower Main Stem Leaves of Seventeen Cotton Genotypes Greenhouse Grown at College Station, Texas during 1992 and 1993.

| | | Abaxial surface | | Emulsified leaf | |
|---|---|---|---|---|---|
| Source | Df | 1992 | 1993 | 1992 | 1993 |
| PA | 1 | 5.42[a] | 36.01[a] | 0.83 | 0.20 |
| Error a | 3 | 0.30 | 1.71 | 0.00 | 0.08 |
| G | 16 | 1.82[b] | 1.11[b] | 0.23[b] | 0.11[a] |
| G x PA | 16 | 0.86[b] | 0.18 | 0.04 | 0.02 |
| Error b | 93 | 0.27 | 0.36 | 0.07 | 0.01 |
| L | 1 | 23.92[b] | 1.77[b] | 0.46[b] | 0.52[b] |
| L x PA | 1 | 0.17 | 8.95[b] | 3.81[b] | 0.29[b] |
| L x G | 16 | 0.18 | 0.29 | 0.07 | 0.01 |
| L x PA x G | 16 | 0.25 | 0.17 | 0.06 | 0.01 |
| Error c | 93 | 0.23 | 0.25 | 0.06 | 0.01 |

PA=Plant age (first square and first square plus 4 weeks); G=genotype; L=leaf sampled (top most expanding leaf and oldest fully expanded leaf).
[a,b] Significant at P=0.05 and 0.01, respectively.

Abaxial leaf surface pH averaged 9.06 at the first square stage of growth during 1992 and increased to 9.37 four weeks later when the plants were beginning to flower. Average pH

during 1993 at first square was 9.31, higher than in the first planting, but dropped 0.75 units to 8.56 four weeks later. Emulsified leaf pH of the seventeen genotypes averaged 6.16 units at first square during 1992, lower than 1993 when the average pH was 6.38. There was not a difference in the emulsified leaf pH between sampling dates during 1993 with values of 5.98 and 5.91 for first square and first square plus four weeks, respectively.

Abaxial leaf surface and emulsified leaf pH varied by location of the leaf sampled in both years (Table 2). The significant first order interactions of leaf position with plant age were caused by differences in direction of response across plant ages. In all cases, pH varied by less than 0.5 units (data not shown). Across these seventeen genotypes, the surface pH of the upper-most main stem leaf measuring 3 cm in diameter averaged 8.91 during 1992 and 8.85 during 1993, significantly less than that of the lower main stem leaves that averaged 9.53 and 9.03 respectively for 1992 and 1993. Emulsified upper main stem leaf pH of the seventeen genotypes averaged 6.18 at first square during 1992, while lower leaves averaged 6.44 units. The opposite trend was observed during 1993 with the pH of emulsified upper leaves having a higher pH at 5.99 than lower main stem leaves at 5.90.

Leaf surface pH ranged from 8.41 for Pima S6 during 1992 to 9.63 for Stoneville 453 (Table 3). Pima S6 and 90J57 were among the lowest in abaxial leaf surface pH while Stoneville 453, Deltapine 50, 89F46H, 86E20 and several others were significantly higher during both years. Somewhat of the same trend was observed for emulsified leaf pH with Pima S6 and 90J57 having the lowest or near the lowest pH during both 1992 and 1993. Even so, the ranges in pH observed were not greatly encouraging relative to genetic modification of leaf pH in cotton.

TABLE 3. Genotypic Means[a] of Abaxial Leaf Surface and Emulsified Leaf pH for Seventeen Cotton Genotypes.

| | Abaxial surface | | Emulsified leaf | |
|---|---|---|---|---|
| Genotypes | 1992 | 1993 | 1992 | 1993 |
| Stoneville 453 | 9.63 a | 8.98 a-c | 6.56 a | 6.02 bc |
| Deltapine 50 | 9.59 ab | 9.07 a-c | 6.37 a-e | 6.01 bc |
| 89F46H | 9.50 ab | 9.02 a-c | 6.50 a-c | 5.94 c |
| 86E20 | 9.44 a-c | 9.21 ab | 6.46 a-d | 6.06 ab |
| 89E51 | 9.39 a-c | 8.77 cd | 6.51 a-c | 6.00 bc |
| Lone Star | 9.39 a-c | 8.77 cd | 6.32 a-e | 5.86 ef |
| 86L$^2$14L | 9.39 a-c | 8.85 bc | 6.54 ab | 5.95 cd |
| 89E62 | 9.37 a-c | 9.20 ab | 6.43 a-d | 6.11 a |
| 90C19S | 9.34 a-d | 9.26 a | 6.12 ef | 5.94 c-e |
| 89F46S | 9.33 a-d | 9.07 a-c | 6.28 b-e | 5.97 c |
| MACAOS | 9.31 a-d | 9.00 a-c | 6.26 c-e | 5.82 fg |
| 86L$^2$9 | 9.27 b-d | 8.76 cd | 6.22 d-f | 6.01 bc |
| Tam. CAB-CS | 9.13 c-e | 9.00 a-c | 6.23 d-f | 5.87 d-f |
| 90C19H | 9.05 de | 9.00 a-c | 6.23 d-f | 5.86 ef |
| 88G104 | 8.94 e | 9.26 a | 6.34 a-e | 5.98 bc |
| 90J57 | 8.46 f | 8.49 de | 6.12 ef | 5.87 d-f |
| Pima S6 | 8.41 f | 8.24 e | 5.97 f | 5.75 g |
| Test mean | 9.23 | 8.94 | 6.31 | 5.95 |
| CV (%) | 5.2 | 5.6 | 3.8 | 1.7 |

[a] Means followed by the same letter within columns are not different according to Waller-Duncan LSD at k=100.

While the objectives of this research did not include direct correlation of pH with any specific insect activity, observations of *B. argentifolii* colonization of these 17 genotypes under greenhouse and field conditions provided no clear distinction in susceptibility between those genotypes with the highest and lowest pH (Smith 1994). Deltapine 50 is one of the most resistant cultivars to *B. argentifolii* under field conditions in the Lower Rio Grande Valley of Texas while Stoneville 453 is one of the most susceptible. These two cultivars were near identical in abaxial surface and emulsified leaf pH in this study, suggesting that these characteristics are not useful selection criteria for *B. argentifolii* resistance. On the other hand, the number of genotypes evaluated in this study might be too small to conclude that pH could not be manipulated as a host plant resistance trait in cotton for *B. argentifolii* or other cotton insect pests.

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