

## BREEDING AND GENETICS

### Variation in Marginal Bract Trichome Density in Upland Cotton

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#### ABSTRACT

**Elimination or reduction of trichomes around the margins of bracts should improve the cleaning efficiency of cotton (*Gossypium hirsutum* L.) lint. The objective of the study was to establish sampling protocols for measuring marginal bract trichome density by examining variation over fruiting positions of the cotton plant, sampling time, cultivars, and environments. To determine variation of bract and leaf trichome density, plants of six contrasting cultivars in two environments were evaluated. To determine variation in bract trichome density over time, bracts of three contrasting cultivars were sampled from three canopy sites on three dates from two years. To determine variation in bract trichome density over cultivars and environments, bracts were evaluated for multiple cultivars at multiple sites over four years. Bract trichome density tended to increase as leaf trichome density increased and to decline with canopy age. Significant interactions involving sympodial position indicated that bracts should be sampled from the same position. The first-position is suggested. Interactions involving nodes became non-significant by dropping the highest and lowest nodes. Bract trichome density declined with older canopy sites and later sample dates, but variation among cultivars was relatively consistent over locations and years. Although cultivars varied significantly, none of the cultivars had glabrous bracts. A significant cultivar by location interaction in one year became non-significant by dropping a highly stressed location. These data indicate that bract samples should be collected from full-sized, mid-canopy, first-position bolls soon after flowering ceases and that bract trichome density can be adequately characterized by sampling bracts at one test site.**

Like many other plant species, cotton (*Gossypium hirsutum* L.) plants have hair-like protrusions or trichomes on the surfaces of several plant parts (Bradlow and Wartelle, 1998; Lee, 1984; Oosterhuis and Jernstedt, 1999). Most research on cotton pubescence has focused on abaxial leaf and stem trichomes. Cotton cultivars have been developed that exhibit leaf and stem pubescence levels ranging from glabrous to very hairy or pilose. As summarized by Jenkins and Wilson (1996), increased cotton plant pubescence on leaves and stems may be associated with increased resistance to some cotton insect pests, but increased susceptibility to others. The glabrous trait generally had neutral or positive effects on yield and fiber quality, while the pilose characteristic often had negative effects. In some studies, relationships with yield and fiber traits were different with specific genes affecting pubescence. Also, low pubescence levels on cotton have been associated with improved seedcotton cleaning efficiency and low foreign matter content in harvested lint, which resulted in higher leaf grades in ginned cotton (Novick et al., 1991).

Pubescence on cotton bracts has received little attention. Bracts are modified leaves surrounding the flower buds and bolls of the cotton plant. Morey et al. (1976) found that bracts are a major contributor to leaf trash in harvested cotton. This seems reasonable, since bracts are in closer proximity than leaves to the cotton fibers on the plant, and most leaves are removed from the plant prior to harvest if defoliation is successful. Bract tissue has also been implicated as a causative agent in byssinosis, a lung disease of cotton mill workers (Ayer, 1971).

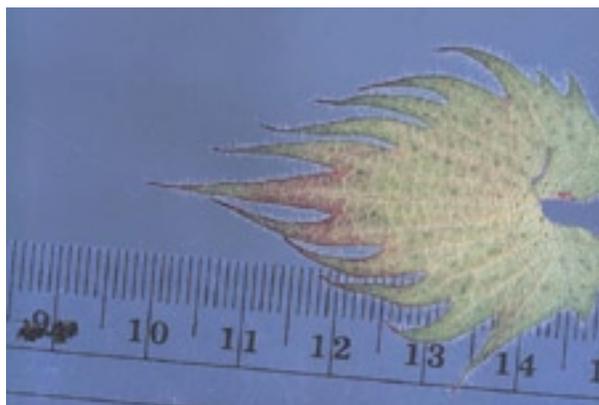
Methods with the potential to reduce lint contamination by bract tissue have included the development of cotton lines having smaller bracts (Bowman and Jones, 1982, 1983; McDaniel, 1996; Milam et al., 1975), deciduous or caducous bracts (Muramoto et al., 1987), and withering bracts (Knight, 1952). Reducing bract size or lessening their persistence may have negative effects on the physiology of the plant, since the role of bracts relative to leaves increases under stress conditions, such as drought (Wullschelger et al., 1990; Zhao and Oosterhuis, 2000).

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Among Upland cotton genotypes, both glabrous and pubescent genotypes, as well as glabrous and pubescent stem genotypes, have trichomes subtending from the margin of bracts (Fig. 1). Intuitively, these marginal bract trichomes might play an important role in the cleaning efficiency of ginned cotton, since they would likely increase the adherence of bract tissue to cotton lint. Only one report on bract trichomes was found, but it addressed trichomes on adaxial bract surfaces rather than marginal bract trichomes (Dimitropoulou et al., 1980).



**Figure 1.** Trichomes subtending from margin of cotton bracts.

The objective of this study was to establish a sampling protocol for measuring marginal bract trichome density by examining variation over fruiting positions of the cotton plant, time, cultivars and environments.

## MATERIALS AND METHODS

Three separate studies were conducted to address the objective. A conventional cotton production system with furrow-irrigation was used in each test, except those designed as non-irrigated sites, and cultural practices were standard for each individual region. To facilitate counting of abaxial leaf trichomes and marginal bract trichomes, a hole-punch (6-mm diameter) was made in an index card and placed over the plant tissue. Using a viewing microscope, trichomes exposed through the hole were counted. Marginal bract trichome density was determined by counting marginal trichomes on two representative marginal areas of the center tooth of each bract, then converting to number per centimeter. Abaxial leaf trichome density was determined by counting trichomes for a representative abaxial, mid-vein area of each sampled leaf, then converting to number per

square centimeter. Each branch of stellate trichomes was counted as an individual trichome. Maximum bract length was measured from base of the bract to the tip of the center tooth.

**Whole plant study.** A study was established in 1996 to survey the variation in bract trichome density over the surface of the plant. Six cultivars, selected for their variation in leaf pubescence, were planted at two contrasting locations. Plots were four rows by 12 m long on 1-m centers and arranged in a RCB design with two replications. The two locations were Fayetteville in the Boston Mountains of Northwest Arkansas and Keiser in Mississippi River Delta of Northeast Arkansas. Soils were a Captina silt loam (fine-silty, siliceous, active, mesic Typic Fragiudults) at Fayetteville and a Sharkey clay (very-fine, smectitic, thermic Chromic Epiaquerts) at Keiser. The six cultivars and their expected leaf pubescence (based on rating scale described by Bourland et al., 2003a) included Stoneville 474 (very hairy) (Stoneville Pedigreed Seed Co.; Memphis, TN), Paymaster H1330 (hairy), Paymaster H1244 (light hair) (Paymaster Cottonseed; Stuttgart, AR), SureGrow 501 (light hair) (Sure-Grow Seed, Inc.; Leland, MS), Paymaster HS200 (glabrous leaf) (Paymaster Cottonseed), and Deltapine 50 (glabrous leaf) (Delta Pine and Land Co.; Scott, MS). In addition to having glabrous leaves, HS200 also displays glabrous stems, while the other cultivars have hairy stems.

In late August, after termination of flowering but prior to defoliation, leaf pubescence of plants was examined and six representative plants were taken from each plot and transported to the laboratory. Abaxial trichomes were counted on all leaves associated with first, second, and third sympodial positions on two plants per plot. To increase the probability of sampling bolls at every position, bracts from each retained boll on all six plants per plot were sampled. Marginal bract trichomes were counted and bract length was determined for one bract per boll for each first, second, and third sympodial position boll. Measurements were recorded by main-stem node number from the base of the plant and by sympodial position from the main-stem. Data were analyzed by SAS using PROC GLM (SAS Institute, Inc.; Cary, NC).

**Temporal bract trichome study.** The effect of sampling time was evaluated in a study conducted at the Lon Mann Cotton Research Station near Marianna, AR, on a Calloway silt loam soil (fine-silty, mixed, active, thermic Fraglossudalfs). The test included

selected cultivars in the 2001 and 2002 Arkansas Cotton Variety Test at the Marianna irrigated location. Plots were two rows, 12 to 15 m long, on 1-m centers and were arranged in RCB designs with four replications. Cultivars (and expected leaf pubescence) examined included SureGrow 215BR (glabrous leaf) (Delta Pine and Land Co.), PayMaster 1218BR (light hair) (Delta Pine and Land Co.), and Stoneville 4892BR (very hairy) (Stoneville Pedigreed Seed). Each sampled plant (one plant per plot) occurred in uniform spacing between plants (approximately 9 plants per meter of row), exhibited representative leaf pubescence and morphological traits for each cultivar, displayed normal plant structure (i.e., no terminal or other obvious damage or distortions), and possessed a first position boll on a bottom (first fruiting node), middle (approximately fruiting node 10 from bottom), and top (approximately node six down from plant apex) main-stem nodes.

In mid-August, plants were selected and string tags were placed around the peduncle of a first-position boll occurring at the designated bottom, middle and top nodes. One bract from each tagged boll was removed and labeled by plot and canopy site (bottom, middle, or top). Bracts were then taken to the laboratory and marginal trichome counts were made. A second and third bract from the tagged bolls was removed on two later dates (pre-defoliation and pre-harvest), and trichome counts were repeated. Data were analyzed by SAS using the general linear models procedure (PROC GLM) (SAS Institute, Inc.).

**Cultivar study.** Cotton cultivars in the Arkansas Cotton Variety Test at multiple locations in 1999 (Bourland et al., 2000), 2000 (Benson et al., 2001), 2001 (Benson et al., 2002), and 2002 (Bourland et al., 2003b) were selected for evaluated for leaf pubescence and marginal bract trichomes. Leaves and bracts were sampled from the same first-position site at a mid-canopy position after termination of flowering but before defoliation each year. Soil types and production practices for tests are listed in the variety test publications cited above. Bract samples were taken from the Keiser irrigated test in 2000, 2001, and 2002; the Keiser non-irrigated test in 2001 and 2002; the Clarkedale irrigated and non-irrigated tests in 1999; the Marianna irrigated test in all four years; the Marianna non-irrigated test in 1999, 2001 and 2002; and the Rohwer irrigated test in 1999 and 2000. Plots were two rows, 12 to 15 m long, on 1-m centers and planted in RCB design with four replications.

In 1999 and 2000, all cultivars in the tests were sampled. In 2001 and 2002, only cultivars common to the 2000 test were examined. Samples were taken from 10 random plants per plot in two replications. On each plant, a full-size, mid-canopy leaf was visually rated for pubescence (as described by Bourland et al., 2003a) by observing the mid-veinal area on the abaxial surface of the leaf. Then, a bract from a full-sized, mid-canopy (approximately fruiting node 10 from bottom), first-position boll was sampled and the leaf pubescence rating for that plant was written on the abaxial bract surface with a marking pen. The bracts were taken to the lab, where the leaf ratings were recorded and marginal bract trichomes were counted. Data (means of 10 plants per plot) were analyzed by SAS using the general linear models procedure (PROC GLM) (SAS Institute, Inc.).

Analyses of variance using the general linear models procedure was performed across locations for each year. The objective of the analysis of variance across locations was to determine if any significant location by cultivar interactions existed. If significant interactions were found, specific locations were dropped within each year and the analysis was repeated. Additionally, an analysis of variance (using PROC GLM) of four cultivars (having contrasting leaf pubescence characteristics) was performed across 4 yr at the Marianna irrigated test site. The objective of the analysis across years was to determine if any significant year by cultivar interactions existed.

## RESULTS AND DISCUSSION

**Whole plant study.** Location, cultivar, node, and position (the main effects), significantly affected abaxial leaf trichome density, marginal bract trichome density, and maximum bract length (Table 1). Significant location by cultivar interactions for leaf trichome density and bract length were primarily because of the expression on the two most pubescent cultivars, ST 474 and PM H1330, at the two locations (data not shown). Although rankings of the cultivars were the same at each location, average leaf trichome density of ST474 was relatively higher and bract length was relatively shorter at Fayetteville than at Keiser. The opposite case was observed for PM H1330. The non-significant cultivar by location interaction for bract trichome density indicates that bract trichome density of cultivars can be characterized by evaluation at one location.

**Table 1. Probabilities associated with sources of variation for leaf and bract parameters of six cultivars at Fayetteville and Keiser, AR, in 1996**

| Source of variation <sup>z</sup> | Leaf trichomes | Marginal bract trichomes | Bract length |
|----------------------------------|----------------|--------------------------|--------------|
| Location (L)                     | 0.0625         | <0.0001                  | <0.0001      |
| Cultivar (C)                     | <0.0001        | 0.0055                   | 0.0010       |
| L x C                            | 0.0314         | 0.6034                   | 0.0441       |
| Node (N)                         | <0.0001        | <0.0001                  | <0.0001      |
| L x N                            | 0.0082         | 0.0024                   | 0.0026       |
| C x N                            | 0.0023         | 0.0027                   | 0.3538       |
| L x C x N                        | 0.2932         | 0.0598                   | 0.5191       |
| Position (P)                     | <0.0001        | <0.0001                  | <0.0001      |
| L x P                            | 0.0999         | 0.7482                   | 0.4072       |
| C x P                            | 0.0723         | 0.0209                   | 0.4783       |
| L x C x P                        | 0.5364         | 0.3361                   | 0.4776       |
| N x P                            | 0.0712         | 0.0111                   | 0.0113       |
| L x N x P                        | 0.6712         | 0.7352                   | 0.7918       |
| C x N x P                        | 0.9553         | 0.1511                   | 0.5669       |
| L x C x N x P                    | 0.9791         | 0.7369                   | 0.9552       |

<sup>z</sup> Locations are Fayetteville and Keiser; cultivars are Stonewall 474, Paymaster H1330, Paymaster H1244, Paymaster HS200, and Deltapine 50.

Significant location by node interactions for leaf and bract trichome density and bract length, and significant cultivar by node interactions for leaf and bract trichome density indicate that main stem node should be standardized for measuring these traits (Table 1). Similarly, significant cultivar by position and node by position for bract trichome density indicate that sympodial position should be standardized for sampling bract trichomes.

Plants at Fayetteville were relatively larger and had more second and third position bolls than plants at Keiser (data not shown). Bracts at the Fayetteville location had higher bract trichome density and longer bract length than bracts at Keiser (Table 2). Leaf trichome density was the same at both locations. Trichome density on both leaves and bracts were significantly different between each of the six cultivars. Except for the order of the most glabrous cultivars (HS 200 and DP 50), cultivar rankings for trichome density on bracts were the same as for leaves. This indicates that trichome density on bracts and leaves are positively related. Both leaf and bract trichomes tended to be most sparse on positions closest to the main-stem and for nodes lower on the plant. Therefore, trichome density tended to become less dense on older leaves and bracts. Lower density may result from thinning of

trichomes as the leaf or bract becomes larger or from abscission of trichomes over time. Except for shorter bracts in the top two nodes, there was only slight variation in bract length among positions and nodes. Therefore, the decline in bract trichome density from middle to lower nodes was most likely associated with abscission rather than increase in bract size.

**Table 2. Location, cultivar, node and position effects on leaf and bract trichome density, and bract length at Fayetteville and Keiser, AR, in 1996**

| Factor             | Level              | Leaf trichomes/cm | Marginal bract trichomes/cm | Bract length (mm) |
|--------------------|--------------------|-------------------|-----------------------------|-------------------|
| Location           | Fayetteville       | 240               | 48                          | 48                |
|                    | Keiser             | 242               | 37                          | 44                |
|                    | LSD ( $P = 0.05$ ) | ns                | 2                           | 1                 |
| Cultivar           | ST 474             | 491               | 53                          | 45                |
|                    | PM H1330           | 295               | 51                          | 46                |
|                    | SG 501             | 206               | 44                          | 47                |
|                    | PM H1244           | 153               | 41                          | 45                |
|                    | PM HS 200          | 114               | 36                          | 50                |
|                    | DP 50              | 49                | 38                          | 46                |
|                    | LSD ( $P = 0.05$ ) | 32                | 2                           | 1                 |
| Sympodial position | First              | 203               | 42                          | 47                |
|                    | Second             | 216               | 45                          | 46                |
|                    | Third              | 225               | 45                          | 47                |
|                    | LSD ( $P = 0.05$ ) | 23                | 2                           | 1                 |
| Node (from bottom) | 22                 | 295               | 60                          | 43                |
|                    | 21                 | 377               | 62                          | 41                |
|                    | 20                 | 414               | 63                          | 45                |
|                    | 19                 | 321               | 54                          | 44                |
|                    | 18                 | 362               | 50                          | 46                |
|                    | 17                 | 282               | 53                          | 45                |
|                    | 16                 | 266               | 48                          | 46                |
|                    | 15                 | 199               | 45                          | 46                |
|                    | 14                 | 255               | 46                          | 46                |
|                    | 13                 | 222               | 45                          | 47                |
|                    | 12                 | 167               | 45                          | 48                |
|                    | 11                 | 165               | 43                          | 47                |
|                    | 10                 | 169               | 41                          | 48                |
|                    | 9                  | 155               | 40                          | 47                |
|                    | 8                  | 160               | 40                          | 47                |
|                    | 7                  | 124               | 37                          | 46                |
|                    | 6                  | 114               | 32                          | 46                |
| 5                  | 152                | 42                | 46                          |                   |
| LSD ( $P = 0.05$ ) | 92                 | 5                 | 2                           |                   |
| $R^2 * 100$        |                    | 90.3              | 87.9                        | 81.6              |
| C.V. (%)           |                    | 56.5              | 18.1                        | 7.8               |

Significant interactions associated with position for bract trichome density indicated that the position for sampling should be standardized. Since first-position bolls are the most frequently occurring bolls and are not affected by competing younger fruit on the same sympodia, it is logical to standardize sampling of bracts to first-position bolls. A second analysis was performed that included only first-position bracts and leaves. Cultivar and node effects were significant for leaf and bract trichome density and bract length of first-position leaves and bracts (Table 3). Location effects were significant for the bract trichome density and length, but not for leaf trichome density. Variation in the three traits over locations, cultivars, and nodes for first-position leaves and bracts (Table 4) follow similar trends as variation for these traits for all positions (Table 2). No interactions were significant for leaf trichome density or first-position bract length (Table 3). The location by node and location by cultivar by node interactions were significant for bract trichome density. Less than one-half of the plots had a plant with a representative first-position boll on node 5 (lowest) or on nodes 19 and above (highest four nodes) (Table 4). When data for these nodes were deleted (i.e., considered only nodes 6-18), none of the interaction effects were significant for first-position bract trichome density (Table 3).

**Table 3. Probabilities associated with sources of variation for first-position abaxial leaf trichomes and first-position marginal bract trichomes and bract length for six cultivars at Fayetteville and Keiser, AR, in 1996**

| Source of variation <sup>z</sup> | All nodes      |              |                          | Nodes 6-18               |
|----------------------------------|----------------|--------------|--------------------------|--------------------------|
|                                  | Leaf trichomes | Bract length | Marginal bract trichomes | Marginal bract trichomes |
| Location (L)                     | 0.2089         | 0.0105       | 0.0002                   | 0.0038                   |
| Cultivar (C)                     | 0.0002         | 0.0065       | 0.0229                   | 0.0443                   |
| L x C                            | 0.0710         | 0.2325       | 0.8364                   | 0.9536                   |
| Node (N)                         | <0.0001        | <0.0001      | <0.0001                  | <0.0001                  |
| L x N                            | 0.6274         | 0.0028       | 0.0028                   | 0.1627                   |
| C x N                            | 0.5735         | 0.2142       | 0.2142                   | 0.9969                   |
| L x C x N                        | 0.9256         | 0.0340       | 0.0340                   | 0.2980                   |

<sup>z</sup> Locations are Fayetteville and Keiser; cultivars are Stonerville 474, Paymaster H1330, Paymaster H1244, Paymaster HS200, and Deltapine 50.

With the exclusion of lowest node and highest four nodes with bolls, rankings of cultivars for trichome densities on first-position leaves and bracts were identical (data not shown). Over nodes, leaf

and bract trichome density tended to be less dense on lower nodes, i.e. older bracts (Table 4). Considering bracts on nodes 6-18, length of first-position bracts was relatively constant over nodes. A proper sampling strategy for bract trichomes would be to take bracts from full-sized, first-position bolls at approximately the same mid-canopy node.

**Temporal bract trichome study.** Bract trichome density varied significantly among years, cultivars, canopy sites (top, middle, or bottom), and sample dates (Table 5). In addition to these main effects, the interactions for cultivar by canopy site, cultivar by sample date, and year by sample date were significant. As indicated by the relative probability levels, cultivar and sample date were the primary factors affecting bract trichome density.

Bract trichome density was different among the three cultivars (Table 6); however, this variation was not uniformly consistent over canopy sites or sample dates. Trichome density decreased, as expected, from the top to the middle bolls for all three cultivars, and also declined from the middle to the bottom bolls on SG 215BR. These decreases agree with the findings in the whole plant study and are consistent with the observations of Webber (1938) and Smith (1964) of decreasing trichome numbers as tissues age. Trichome density remained the same for bracts from middle and bottom bolls on PM 1218BR, and increased from middle to bottom on ST 4892BR. Although the rate of change in trichome density was different for each cultivar over canopy sites, the relative differences among cultivars were similar within each canopy site.

Similar results were obtained for the interaction of cultivar with sample date. Bract trichome density declined over successive sample dates for each cultivar (Table 6). The cultivar by sample date interaction appeared to be associated with a slightly higher rate of trichome loss after cutout for ST 4892BR and after defoliation for PM 1218BR. Although the rates of loss in bract trichomes was different, the order of the cultivars (from most to least pubescent) remained the same within each sample date. Examination of means for year by sample date indicated that bract trichome density on date 1 (near cutout) was the most consistent over the two years, and that variation in each sample date increased with later dates. The increased variation between years for the later sampling dates may be because of the effects of prolonged weathering on the bracts.

**Cultivar study.** Leaf pubescence ratings and bract trichome density was highly significant among cultivars each year (Table 7). Variation among locations was significant in two of four years for each trait. The location by cultivar interaction for bract trichome density was significant only in 1999. Two non-irrigated sites were included in the 1999, 2001, and 2002 tests. The 1999 data were re-analyzed with each non-irrigated site excluded alone and with both non-irrigated sites excluded. The location by cultivar interaction for bract

trichome density became non-significant only when the Marianna non-irrigated site was excluded. As indicated by relative yields reported for the variety tests, the 1999 non-irrigated sites suffered a higher level of drought stress relative to non-irrigated sites than in other years. Cultivars in the two non-irrigated sites in 1999, 2001, and 2002 yielded approximately 44, 79, and 75%, respectively, as much as cultivars in adjacent irrigated sites (Benson et al, 2002, Bourland et al. 2000, Bourland et al., 2003b).

**Table 4.** Means for first position abaxial leaf trichome density and first position marginal bract trichome density (MBT) and bract length (BL) at Fayetteville and Keiser, AR, in 1996

| Factor                      | Level                    | All nodes                 |                                       | All nodes                 |         | Nodes 6-18                  |                             |
|-----------------------------|--------------------------|---------------------------|---------------------------------------|---------------------------|---------|-----------------------------|-----------------------------|
|                             |                          | Sample (no.) <sup>z</sup> | Leaf trichome (no. cm <sup>-2</sup> ) | Sample (no.) <sup>z</sup> | BL (mm) | MBT (no. cm <sup>-1</sup> ) | MBT (no. cm <sup>-1</sup> ) |
| Location                    | Fayetteville             | 149                       | 222                                   | 175                       | 49      | 47                          | 46                          |
|                             | Keiser                   | 75                        | 166                                   | 148                       | 45      | 36                          | 35                          |
|                             | LSD ( <i>P</i> = 0.05)   |                           | Ns                                    |                           | 2       | 3                           | 3                           |
| Cultivar                    | ST 474                   | 37                        | 463                                   | 52                        | 45      | 50                          | 48                          |
|                             | PM H1330                 | 37                        | 264                                   | 55                        | 46      | 47                          | 46                          |
|                             | SG 501                   | 42                        | 194                                   | 55                        | 48      | 43                          | 41                          |
|                             | PM H1244                 | 35                        | 154                                   | 57                        | 45      | 40                          | 37                          |
|                             | PM HS 200                | 35                        | 92                                    | 50                        | 51      | 35                          | 36                          |
|                             | DP 50                    | 38                        | 50                                    | 54                        | 47      | 36                          | 33                          |
|                             | LSD ( <i>P</i> = 0.05)   |                           | 53                                    |                           | 1       | 3                           | 3                           |
|                             | Node<br>(from<br>bottom) | 22                        | 2                                     | 295                       | 4       | 43                          | 60                          |
| 21                          |                          | 4                         | 376                                   | 4                         | 42      | 58                          |                             |
| 20                          |                          | 10                        | 299                                   | 6                         | 45      | 61                          |                             |
| 19                          |                          | 15                        | 307                                   | 11                        | 44      | 53                          |                             |
| 18                          |                          | 17                        | 302                                   | 19                        | 46      | 46                          | 46                          |
| 17                          |                          | 19                        | 248                                   | 18                        | 47      | 47                          | 47                          |
| 16                          |                          | 20                        | 245                                   | 20                        | 47      | 46                          | 46                          |
| 15                          |                          | 21                        | 191                                   | 23                        | 47      | 44                          | 44                          |
| 14                          |                          | 18                        | 183                                   | 24                        | 48      | 42                          | 42                          |
| 13                          |                          | 19                        | 152                                   | 23                        | 48      | 42                          | 42                          |
| 12                          |                          | 19                        | 140                                   | 24                        | 49      | 41                          | 41                          |
| 11                          |                          | 15                        | 156                                   | 24                        | 49      | 40                          | 40                          |
| 10                          |                          | 11                        | 112                                   | 24                        | 49      | 37                          | 37                          |
| 9                           |                          | 12                        | 138                                   | 24                        | 48      | 37                          | 37                          |
| 8                           |                          | 11                        | 140                                   | 23                        | 47      | 37                          | 37                          |
| 7                           |                          | 6                         | 88                                    | 24                        | 45      | 35                          | 35                          |
| 6                           |                          | 4                         | 162                                   | 20                        | 44      | 32                          | 32                          |
| 5                           |                          | 1                         | 320                                   | 8                         | 44      | 44                          |                             |
| LSD ( <i>P</i> = 0.05)      |                          |                           | 134                                   |                           | 3       | 6                           | 4                           |
| <i>R</i> <sup>2</sup> * 100 |                          |                           |                                       | 91.2                      |         | 79.4                        | 88.1                        |
| C.V. (%)                    |                          |                           | 56.5                                  |                           | 8.0     | 17.2                        | 18.0                        |

<sup>z</sup> Total of 24 samples (2 locations by 6 cultivars by 2 replications) possible for each node. First-position data from all nodes of multiple plants in a plot were combined to form a composite plant.

**Table 5. Probabilities associated with sources of variation for marginal bract trichomes in canopy site and sample date study at Marianna, AR, in 2001 and 2002**

| Source of variation <sup>z</sup> | Marginal bract trichomes |
|----------------------------------|--------------------------|
| Year (Y)                         | <0.0001                  |
| Cultivar (C)                     | <0.0001                  |
| Canopy site (CS)                 | 0.0015                   |
| Sample date (SD)                 | <0.0001                  |
| C x CS                           | 0.0390                   |
| C x SD                           | 0.0139                   |
| CS x SD                          | 0.2589                   |
| C x CS x SD                      | 0.8008                   |
| Y x CS                           | 0.0881                   |
| Y x C                            | 0.6149                   |
| Y x C x CS                       | 0.0990                   |
| Y x SD                           | 0.0164                   |
| Y x CS x SD                      | 0.8592                   |
| Y x C x SD                       | 0.1843                   |
| Y x C x CS x SD                  | 0.7750                   |

<sup>z</sup> Years are 2001 and 2002; cultivars are SureGrow 214BR, Paymaster 1218BR, and Stoneville 4892BR; canopy sites are the first fruiting node, fruiting node 10 from the bottom, and node six down from the plant apex; sampling dates are after termination of flowering, prior to defoliation, and pre-harvest.

These results indicate that bract trichome density was affected by genetics (cultivar effect) and also by environment (location effect), but that relative differences among cultivars remained consistent over locations, excluding the highly drought-stressed, non-irrigated environment in 1999 (Table 8). Irrigation did not have a consistent effect on bract trichome density. Irrigated and non-irrigated sites did not vary significantly at Clarkedale in 1999, Keiser in 2001, or Marianna in 2001; however, irrigated sites had lower

bract trichome density at Marianna in 1999 and 2002, but higher density at Keiser in 2002. Except for the Rohwer site in 1999, location means among irrigated sites did not vary significantly within any year.

High coefficient of determination ( $R^2$ ) values for leaf pubescence ratings and bract trichome density indicated that non-error sources of variation accounted for most of the variability (Table 9). Coefficients of variation were higher for the subjective visual ratings of leaf pubescence than for bract trichome density. Variation among cultivars was relatively consistent over years. Cultivars with the highest leaf pubescence ratings (most leaf trichomes) tended to have the densest bract trichomes; however, variation in bract trichome density was significant among the three most hairy-leaf cultivars, as well as among the three most glabrous leaf cultivars within each year.

The Arkansas Cotton Variety Tests of 1999 through 2002 had only four cultivars that were common to all 4 yr. Fortunately, these cultivars represented a wide range of leaf pubescence levels (PSC 355 - a very hairy-leaf type; PM 1218BR and SG 105 - varying levels of intermediate pubescence; and DP 436R - a glabrous-leaf type). Year and cultivar effects for leaf pubescence rating and bract trichome density were significant when these four cultivars were evaluated over years at the Marianna irrigated location (data not shown). The year by cultivar interaction was not significant for either; therefore, leaf pubescence ratings and bract trichome density for the cultivars remained relatively stable over years, indicating that relative variation among cultivars was consistent from year to year. Although significant cultivar effects are typically observed, all Upland cultivars and breeding lines examined over the past 10 yr possess bract trichomes, including genotypes with glabrous leaves and stems.

**Table 6. Number of marginal bract trichomes associated with bolls of three cultivars at three canopy sites and three sample dates at Marianna, AR, in 2001 and 2002**

| Factor <sup>z</sup> | Level              | Marginal bract trichomes ( no. cm <sup>-1</sup> ) |             |        |        |             |        |         |
|---------------------|--------------------|---|-------------|--------|--------|-------------|--------|---------|
|                     |                    | All   | Canopy site |        |        | Sample date |        |         |
|                     |                    |   | Top         | Middle | Bottom | Cutout      | Defol. | Harvest |
| Cultivar            | ST 4892BR          | 46  | 47          | 42     | 50     | 58          | 43     | 32      |
|                     | PM 1218BR          | 30  | 35          | 28     | 28     | 35          | 29     | 18      |
|                     | SG 215BR           | 20  | 23          | 21     | 15     | 24          | 19     | 14      |
|                     | LSD ( $P = 0.05$ ) | 6   |             | 6      |        |             | 6      |         |
| Year                | 2001               | 34  | 40          | 35     | 30     | 40          | 34     | 28      |
|                     | 2002               | 28  | 32          | 26     | 39     | 40          | 26     | 17      |
|                     | LSD ( $P = 0.05$ ) | 2   |             | ns     |        |             | 5      |         |

<sup>z</sup> Across all factors for marginal bract trichomes,  $R^2 * 100 = 88.5$  and C. V. = 29.5%.

**Table 7. Probabilities associated with sources of variation for leaf pubescence ratings and marginal bract trichomes of cotton cultivars evaluated in Arkansas Cotton Variety Tests from 1999 through 2002**

| Year              | Source of variation | Leaf pubescence rating <sup>y</sup> | Marginal bract trichomes |
|-------------------|---------------------|-------------------------------------|--------------------------|
| 1999              | Location (L)        | 0.0328                              | 0.0171                   |
|                   | Cultivar (C)        | <0.0001                             | <0.0001                  |
|                   | L X C               | 0.1019                              | 0.0174                   |
| 1999 <sup>z</sup> | Location (L)        | 0.0152                              | 0.1234                   |
|                   | Cultivar (C)        | <0.0001                             | <0.0001                  |
|                   | L X C               | 0.1607                              | 0.1191                   |
| 2000              | Location (L)        | 0.0796                              | 0.3338                   |
|                   | Cultivar (C)        | <0.0001                             | <0.0001                  |
|                   | L X C               | 0.0852                              | 0.1167                   |
| 2001              | Location (L)        | 0.2000                              | 0.5651                   |
|                   | Cultivar (C)        | <0.0001                             | <0.0001                  |
|                   | L X C               | 0.4840                              | 0.7283                   |
| 2002              | Location (L)        | 0.0395                              | 0.0093                   |
|                   | Cultivar (C)        | <0.0001                             | <0.0001                  |
|                   | L X C               | 0.9979                              | 0.7849                   |

<sup>y</sup> Leaf pubescence based on rating scale of 1 (glabrous) to 9 (pilose) established by Bourland et al. (2003a).

<sup>z</sup> ANOVA excluding Marianna non-irrigated location.

## SUMMARY

Variation in bract trichome density over the plant was evaluated by examining plants of six cultivars (ranging from glabrous to very hairy leaf pubescence) from two contrasting environments (Keiser and Fayetteville, AR) in 1996. Leaves and bracts associated with first, second, and third positions at all main-stem nodes were evaluated. Bract trichome density appeared to be related to leaf trichome density, and both declined with age. Significant interactions associated with sympodial position indicated that sampling for bract trichomes should be restricted to one position. Sampling from first-position bolls is suggested, since they are the most frequently occurring bolls and are not affected by bolls nearer to the main stem. Interactions associated with bract trichome density on first-position bolls became non-significant when samples from the lowest node and highest four nodes were dropped. Sampling should be restricted to full-sized, first-position bolls in the middle of the plant canopy.

Variation in bract trichome density over time was evaluated at Marianna, AR, in 2001 and 2002. Bolls from three canopy sites (upper, middle and bottom)

**Table 8. Test site means for leaf pubescence ratings and marginal bract trichomes for cultivars evaluated in Arkansas Cotton Variety Tests from 1999 through 2002**

| Year               | Location           | Irrigated | Leaf pubescence rating <sup>z</sup> | Bract trichomes (no. cm <sup>-1</sup> ) |    |
|--------------------|--------------------|-----------|-------------------------------------|---|----|
| 1999               | Clarkedale         | Yes       | 3.1                                 | 36                                      |    |
|                    |                    | No        | 3.2                                 | 34                                      |    |
|                    | Marianna           | Yes       | 3.8                                 | 36                                      |    |
|                    |                    | No        | 3.7                                 | 43                                      |    |
|                    | Rohwer             | Yes       | 2.8                                 | 28                                      |    |
|                    | LSD ( $P = 0.05$ ) |           |                                     | 0.2                                     | 2  |
|                    | 2000               | Keiser    | Yes                                 | 2.8                                     | 34 |
| Marianna           |                    | Yes       | 2.3                                 | 36                                      |    |
| Rohwer             |                    | Yes       | 3.2                                 | 36                                      |    |
| LSD ( $P = 0.05$ ) |                    |           | ns                                  | ns                                      |    |
| 2001               | Keiser             | Yes       | 3.8                                 | 41                                      |    |
|                    |                    | No        | 3.7                                 | 40                                      |    |
|                    | Marianna           | Yes       | 4.4                                 | 41                                      |    |
|                    |                    | No        | 4.0                                 | 37                                      |    |
|                    | LSD ( $P = 0.05$ ) |           |                                     | ns                                      | ns |
|                    | 2002               | Keiser    | Yes                                 | 3.8                                     | 30 |
| Keiser             |                    | No        | 3.1                                 | 24                                      |    |
| Marianna           |                    | Yes       | 4.2                                 | 30                                      |    |
| Marianna           |                    | No        | 4.2                                 | 44                                      |    |
| LSD ( $P = 0.05$ ) |                    |           | 0.7                                 | 5                                       |    |

<sup>z</sup> Leaf pubescence based on rating scale of 1 (glabrous) to 9 (pilose) established by Bourland et al. (2003a).

of three contrasting cultivars were tagged, and bract trichome density on one bract from each tagged boll was determined on three dates (after termination of flowering, prior to defoliation, and prior to machine harvest). Bract trichomes also tended to decline with older plant canopy sites and with later sample dates. Because of these losses, bracts should be sampled no later than when plant flowering ceases. Variation in bract age might confound measurements if samples were taken earlier in the season.

Variation in bract trichome density over cultivars and environments was evaluated by sampling bracts from 10 to 32 cultivars at three to five sites of the 1999 through 2002 Arkansas Cotton Variety Tests. Bract trichome density on cultivars was relatively consistent over locations and years. Although variation among cultivars was always significant, none of the cultivars had glabrous bracts. A significant cultivar by location interaction was significant in only one year, and that interaction

became non-significant when one highly stressed location was dropped. Although variation in years was significant, the year by cultivar interaction was not significant. Therefore, samples of bracts from only one test site per year are required to characterize bract trichome density of cotton cultivars, as long as highly stressed sites are avoided.

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**Table 9. Cultivar means over locations for leaf pubescence ratings and marginal bract trichomes in Arkansas Cotton Variety Tests from 1999 through 2002**

| Cultivar <sup>y</sup>       | 1999 <sup>z</sup>                   |   | 2000                   |   | 2001                   |   | 2002                   |   |
|-----------------------------|-------------------------------------|---|------------------------|---|------------------------|---|------------------------|---|
|                             | Leaf pubescence rating <sup>x</sup> | Bract trichomes (no. cm <sup>-1</sup> ) | Leaf pubescence rating | Bract trichomes (no. cm <sup>-1</sup> ) | Leaf pubescence rating | Bract trichomes (no. cm <sup>-1</sup> ) | Leaf pubescence rating | Bract trichomes (no. cm <sup>-1</sup> ) |
| ST 4691B                    |                                     |   | 4.7                    | 52                                      | 6.6                    | 51                                      |                        |   |
| ST 4793R                    |                                     |   |                        |   | 6.7                    | 50                                      | 6.3                    | 42                                      |
| ST 4892BR                   |                                     |   | 5.0                    | 46                                      | 5.5                    | 43                                      | 6.2                    | 47                                      |
| ST 474                      | 6.0                                 | 39                                      | 4.2                    | 49                                      |                        |   |                        |   |
| PSC 355                     | 6.1                                 | 38                                      | 3.9                    | 44                                      | 5.8                    | 51                                      | 5.4                    | 33                                      |
| DP 388                      | 4.8                                 | 38                                      | 3.7                    | 41                                      |                        |   |                        |   |
| ST BXN47                    | 5.4                                 | 36                                      | 4.2                    | 39                                      | 5.7                    | 43                                      |                        |   |
| SG 747                      | 2.5                                 | 35                                      | 2.0                    | 36                                      | 3.7                    | 41                                      |                        |   |
| Arkot 8712                  |                                     |   | 2.6                    | 36                                      | 3.1                    | 41                                      |                        |   |
| FM 966                      |                                     |   | 2.0                    | 29                                      | 2.9                    | 40                                      | 2.4                    | 39                                      |
| FM 958                      |                                     |   | 2.4                    | 37                                      | 3.6                    | 41                                      | 3.1                    | 30                                      |
| DP 451BR                    |                                     |   |                        | 33                                      | 2.3                    | 41                                      | 2.0                    | 31                                      |
| FM 819                      | 2.7                                 | 32                                      | 2.0                    | 32                                      |                        |   |                        |   |
| FM 832                      | 2.2                                 | 29                                      | 2.4                    | 33                                      |                        |   |                        |   |
| PM 1218BR                   | 3.1                                 | 30                                      | 2.6                    | 29                                      | 3.5                    | 32                                      | 5.3                    | 34                                      |
| DP 436R                     | 2.3                                 | 32                                      | 1.7                    | 32                                      | 2.2                    | 30                                      | 2.1                    | 25                                      |
| SG 501BR                    |                                     |   | 2.0                    | 28                                      | 2.7                    | 36                                      |                        |   |
| SG 105                      | 2.4                                 | 30                                      | 2.1                    | 29                                      | 2.8                    | 33                                      | 2.6                    | 24                                      |
| FM 989                      | 1.8                                 | 30                                      | 1.6                    | 22                                      |                        |   |                        |   |
| SG 215BR                    |                                     |   | 1.4                    | 24                                      | 1.8                    | 28                                      | 2.8                    | 24                                      |
| Test mean                   | 3.2                                 | 33                                      | 2.8                    | 35                                      | 4.0                    | 40                                      | 3.8                    | 32                                      |
| LSD ( <i>P</i> = 0.05)      | 0.8                                 | 5                                       | 0.7                    | 5                                       | 0.9                    | 6                                       | 1.1                    | 7                                       |
| CV (%)                      | 20.8                                | 12.8                                    | 22.3                   | 13.4                                    | 23.5                   | 15.7                                    | 28.9                   | 21.9                                    |
| <i>R</i> <sup>2</sup> x 100 | 91.7                                | 83.1                                    | 89.4                   | 89.0                                    | 87.8                   | 77.6                                    | 84.9                   | 84.7                                    |

<sup>x</sup> Leaf pubescence based on rating scale of 1 (glabrous) to 9 (pilose) established by Bourland et al. (2003a).

<sup>y</sup> Only cultivars evaluated in two or more years are listed. Test means are for all cultivars evaluated, which included 21, 6, and 2 additional ones in 1999, 2000, and 2001, respectively. Cultivars sorted by MBT divided by test mean averaged over years.

<sup>z</sup> Means for 1999 do not include the Marianna non-irrigated test site.

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