

Frequency of Pigment Glands and Capitate and Covering Trichomes in Nascent Leaves of Selected Cottons¹

Charles T. Bryson, Jack C. McCarty, Jr., Johnie N. Jenkins, and W. L. Parrott²

ABSTRACT

Twenty-nine cotton strains, *Gossypium hirsutum* L., were evaluated to assess potential physical and chemical barriers confronting *Heliothis* oviposition and feeding in relationship to the frequency of pigment glands and covering and capitate trichomes on the laminae of nascent terminal leaves. Significant differences were found among strains for each of the structures, with a two-fold difference in the number of pigment glands among glanded strains, a two-fold difference in capitate trichomes, and a range of 0 to >1500 covering trichomes per cm². Frequencies of covering trichomes between adaxial and abaxial surfaces were significantly different among strains. The frequencies of glands and of the two types of trichomes seemed to be independent of each other. Of the structures studied, only the density of glands had any influence on *Heliothis* larval growth. The number of pigment glands per cm² of leaf tissue was negatively correlated with larval weight after five days of feeding.

Additional index words: *Gossypium hirsutum* L., *Heliothis virescens* (F.)

THE foliar structure of cotton (*Gossypium*) includes internal glands and epidermal outgrowths commonly referred to as hairs, or trichomes. Glands that contain pigments and gossypol are commonly called "pigment glands" or "gossypol glands." They are located at least two layers deep in most organs and tissues except in the seed coat and xylem of cotton. Anatomically, pigment glands are composed of a lysigenous intercellular space, in the form of a cavity, enveloped by one or more layers of cells. In leaves, glands are found just below the palisade parenchyma. At the site of the gland, each gland is surrounded laterally and below by spongy parenchyma (Reeves and Beasley, 1935; Stanford and Viehoveer, 1918). McMichael (1954, 1960) described the inheritance of glands and produced cotton plants and seed without pigment glands. Kohel (1973) lists two alleles responsible for glandless bolls, *gl*₁ and *gl*₆, and four alleles responsible for glandless plants, *gl*₂, *gl*₃, *gl*₄ and *gl*₅. The double recessive *gl*₂*gl*₃*gl*₄*gl*₅ conditions glandlessness in most cotton strains. Many insects that normally do not attack glanded cotton caused considerable damage to glandless cotton (Maxwell et al., 1965, 1972; Bell and Stipanovic, 1977), thus generating an interest in the field of host plant resistance and in the chemical nature of these pigment glands.

Like the pigment glands, epidermal outgrowths have also been reported to influence certain insect populations. The cotton leaf surface is comprised of two primary types of epidermal outgrowths, capitate trichomes and covering trichomes (Webber, 1938). The capitate trichomes are small, multicellular structures comprised of one or two stalk cells and a two- or three-tiered head of from 10 to 20 cells (Wall, 1970). Capitate trichomes were considered to be secretory in other species of plants as early as 1745 by Guettard. The

capitate trichomes are thought to elicit a protective function by the production of chemical substances, phenolic in nature (Beckman et al. 1972), which are olfactory and gustatory repellents to insects (Levin, 1973). In addition, small insects are sometimes trapped by sticky exudates from glandular (capitate) trichomes. Tingey and Laubengayer (1981) reported that glandular trichomes protect wild potato, *Solanum berthaultii* Hawkes, from the green peach aphid, *Myzus persicae* (Sulzer), and the potato leafhopper, *Empoasca fabae* Harris.

The covering trichomes in higher plants can be single, tufted, or stellate (Cutter, 1978). In cotton, density and pattern of pubescence can be controlled by alleles at a number of loci, *H*₁, *H*₂, *sm*₁, *sm*₂, and *sm*₃ (Kohel, 1973; Lee, 1968). Dense pubescence has been reported as a factor in reducing boll weevil, *Anthonomus grandis* Boheman (Stephens and Lee, 1961; Hunter et al., 1965); and jassids, *Empoasca* (Parrell et al., 1949; Reed, 1974) populations; and glabrousness was reported as reducing number of eggs, larvae, and damage by *Heliothis* (Lukefahr et al., 1971, 1975). Lukefahr (1977) suggests that 200 or fewer trichomes per square inch could produce a 50% reduction in oviposition and larval populations of the tobacco budworm, *Heliothis virescens* (F.).

Much oviposition and larvae feeding of first instar *Heliothis* is in cotton terminals. This study was conducted to examine and characterize the nascent leaf surfaces in terminals for the density of pigment glands, covering trichomes, and capitate trichomes of several cotton strains and to assess these as potential physical and chemical barriers confronting *Heliothis* oviposition and feeding.

MATERIALS AND METHODS

Twenty-nine cotton strains, mostly those in a 1981 regional *Heliothis* resistant strains test plus a few selected additional entries, were planted in the field at Mississippi State, Miss., on 30 Apr. 1981 (see Table 1). Six nascent leaves (four on 5 to 6 July and two on 13 July) with a mean leaf area of 7.87 cm² were collected from the terminal area of randomly selected plants of each strain. The leaves were measured with an area meter and the number of pigment glands, covering trichome bases, and capitate trichomes were counted in five grid areas (0.36 mm²) on both abaxial and adaxial surfaces using a grid ocular micrometer at 35×. The presence or absence of single, double, and triple, or more, lobed covering trichomes was recorded for each grid. Counts were made on both surfaces of the leaf blade, adjacent to the midvein but not including secondary or tertiary veins (Fig. 1). Our study was conducted on nascent leaves rather than fully expanded leaves, as studied by Smith (1964), because this is one of the primary regions used as food by newly hatched *H. virescens* larvae. Each of the five grid areas was summed and data were expressed as number of pigment glands, covering trichome bases, and capitate trichomes per cm².

RESULTS AND DISCUSSION

Pigment Glands

The pigment glands are visible from both leaf surfaces (lateral to the leaf veins). Thus, no differences in gland

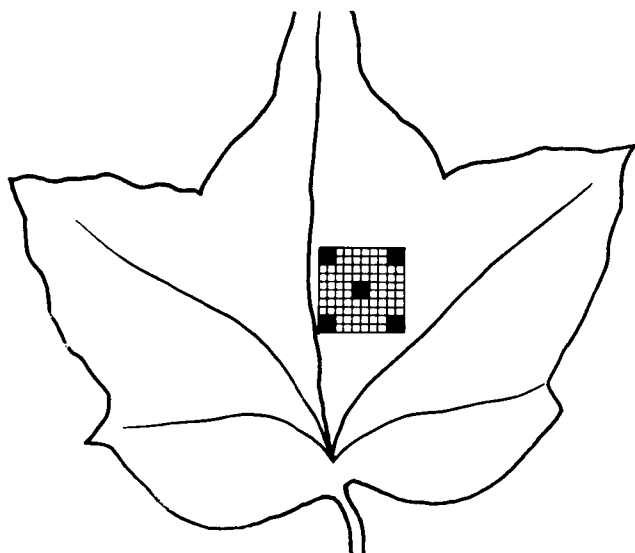
¹ Contribution of USDA, ARS in Cooperation with Mississippi Agric. and Forestry Exp. Stn. Journal article No. 5195 of the Mississippi Agric. and Forestry Exp. Stn. Received 21 May 1982.

² Agricultural research technician (presently research botanist, Southern Weed Science Laboratory, Stoneville, MS 38776), research agronomist, research geneticist, and research entomologist, respectively, Crop Science Research Laboratory, P.O. Box 5367, Mississippi State, MS 39762.

Table 1. Leaf size and frequency of pigment glands, covering trichomes and capitate trichomes on nascent cotton leaves of 29 cotton strains.

Strain	\bar{X} Leaf area cm ²	Pigment glands No./cm ²	Covering trichomes									Capitate trichomes	
			Adaxial surface†			Abaxial surface†						Adaxial surface No./cm ²	Abaxial surface No./cm ²
			1	2	≥ 3	1	2	≥ 3					
DH 66	7.42	0	0	—	—	—	454	+	+	+	3426	3398	
DH 121	7.60	1356	0	—	—	—	1102	+	+	+	3435	3240	
DH 36	8.31	0	0	—	—	—	592	—	+	+	3046	2555	
DH 40	7.58	0	0	—	—	—	518	+	+	+	3694	4601	
DH 126	8.20	1171	0	—	—	—	759	+	+	+	2805	2620	
ST 213	7.88	1500	37	+	—	—	1213	+	+	+	4268	3111	
ST 857	8.10	1602	0	—	—	—	870	+	+	+	6212	6740	
ST 731N	7.84	1356	92	+	+	—	> 1500	+	+	+	3962	3314	
ST 1019	7.79	1143	18	+	—	+	> 1500	+	+	+	4398	3787	
ST 7AGN	7.68	0	65	+	—	+	1185	+	+	+	5499	5768	
HG 469	8.00	1935	0	—	—	—	176	+	+	+	4740	4851	
LAHG 1488	8.96	1273	0	—	—	—	55	—	+	+	4944	4407	
HG-10	7.63	1217	0	—	—	—	0	—	—	—	3935	3888	
MOHG	7.17	1282	0	—	—	—	898	—	+	+	3879	3370	
MHR-1	8.10	1092	18	—	+	—	1176	+	+	+	3546	2972	
BW-76-31 DH	6.99	1278	18	+	—	+	1102	+	+	+	3490	2981	
DES-24	8.87	1417	37	+	+	—	1037	+	+	+	4222	4111	
PD 875	7.62	1227	0	—	—	—	139	+	+	+	4925	4888	
PD 8619	7.26	1074	157	+	+	—	> 1500	+	+	+	3490	3601	
DPL-61	8.04	1014	0	—	—	—	194	+	+	+	3824	4694	
La-T27-1740YP	7.53	1245	9	—	+	—	222	+	+	+	4842	4342	
CMS × PIMA RES	8.76	1231	28	—	+	—	> 1500	+	+	+	5536	5027	
Laxmi	7.02	1393	1842	+	+	+	> 1500	+	+	+	3537	4648	
T-1055	7.98	1194	0	—	—	—	0	—	—	—	5851	5768	
SATU-65	9.08	972	435	+	+	+	1435	+	+	+	3638	4259	
BJA-592	9.12	944	916	+	+	+	> 1500	+	+	+	3787	3185	
1073-IC	7.66	844	0	—	—	—	18	—	—	+	3102	3027	
AET-5	6.92	805	92	+	+	+	> 1500	+	+	+	3694	3657	
TX-LY-18-72	7.02	0	9	—	—	+	518	—	—	+	3018	3398	
\bar{X}	7.87	1019	127				657				4095	4005	
LSD 0.05		273	235				270				1081	1042	
CV		33	161				36				24	23	

† Present = +, absent = — for 1, 2, > 3 lobes per covering trichome.

**Fig. 1.** Location on upper or lower surface of nascent leaves of selected cotton strains at which counts of pigment glands, covering trichomes, and capitate trichomes were made.

numbers were detected between the adaxial and abaxial surfaces. The numbers of pigment glands were significantly ($P=0.05$) different among glanded strains (Table 1). Gland numbers on glanded cottons ranged from 1935 per cm² in HG-469 to 805 per cm² in AET-5. Five strains (TX-LY-18-72, 'Stoneville 7AGN', D.H.36, D.H.40, and D.H.66)

were glandless (Table 1). In glanded strains, the 2.4-fold difference in pigment glands supports the findings of McMichael (1954, 1960), Lee (1973, 1977), and Kohel (1973) that gland frequency, as well as their presence or absence, can be manipulated genetically.

Covering Trichomes

The strains used could be classed as glabrous, intermediate, or pubescent; however, differences in types of frequency of trichome structure can be best detected with magnification. The size of the count area made it impossible to count each branch (lobe) from the basal structure in many of the strains. Thus only the basal epidermal attachments were counted. The type of covering trichome was then categorized as either single, double, equal to, or greater than, three branched (lobed) (Table 1). Epidermal attachments were significantly ($P=0.05$) different between the adaxial and abaxial surfaces and among the different strains. In seven strains (Laxmi, BJA-592, PD-8619, 'Stoneville 731N', AET-5, CMS × PIMA RES, and Stoneville 1019) the epidermal attachments were impossible to count because they were hidden by numerous stellate projections. We arbitrarily classified these 1500+. No covering trichomes were found in the count areas in two strains (T-1055 and HG 10) on either surface excluding veins, and in 12 strains on adaxial surfaces; however, trichomes were observed along the major veins in each of these strains. On the adaxial surface the covering hairs ranged from 1842 per cm² in

Laxmi to 9 per cm² in TX-LY-18-72 and La-T27-1740YP, excluding the strains having no trichomes. The frequency and density of pubescence per leaf was greater on the abaxial surface, ranging from 1435 per cm² in SATU-65 to 18 per cm² in 1073-IC except those strains having either no trichomes or too many to count.

Capitate Trichomes

There is evidence that the number of pigment glands and covering trichomes in cotton can be manipulated genetically (Lee, 1968; Kohel, 1973). Capitate trichomes also can be genetically controlled in *Solanum* (Gibson, 1974) and *Medicago* (Kreitner and Sorensen, 1979). Kosmidou-Dimitropoulou et al. (1980) found that more capitate trichomes were present on the abaxial surface than on the adaxial in eight cotton strains. They also state that the ratio of capitate trichomes to epidermal (covering) trichomes varied between the abaxial and adaxial surfaces. For the 29 strains studied in this test, no significant differences were found in the number of capitate trichomes between the abaxial and adaxial surfaces. However, in nine lines, the total number of capitate trichomes was greater on the abaxial than adaxial surface; and, in the other 20 strains, more capitate trichomes were present on the adaxial than on the abaxial surface. The sum of capitate trichomes per leaf on both surfaces ranged from 12 952 per cm² in 'Stoneville 857' to 5425 per cm² in D.H.126 or more than a two-fold difference (Table 1). These data indicate that capitate trichomes in cotton probably can be manipulated by breeding techniques. We found no correlation between the number of glands and the numbers of covering trichomes, nor was any expected. A small but significant negative correlation (-0.37 , significant at the 0.01 level) was obtained between pigment glands and capitate trichomes. The number of capitate trichomes on the adaxillary and abaxillary surface was highly correlated (0.88, significant at the 0.01 level). There was no correlation between covering trichomes on the adaxial and abaxial surfaces or covering trichomes and capitate trichomes.

In 17 strains (D.H. 36, D.H. 40, D.H. 66, D.H. 121, D.H. 126, T-1055, Stoneville 7AGN, Stoneville 213, Stoneville 731N, 'DES-24,' 'Deltapine 61,' SATU-65, BJA-592, Laxmi, PD-8619, MOHG, and TX-LY-18-72) common to this test and used by White (1981) to study growth rates of *H. virescens* larvae, the number of pigment glands per cm² of leaf tissue was negatively correlated (-0.99 , significant at the 0.01 level) with larval weight after 5 days of feeding in field cages. Total capitate trichomes per leaf and covering trichome numbers on either surface were not correlated with *H. virescens* growth rates reported by White (1981).

A glabrous cotton strain with a high frequency of pigment glands should decrease *Heliothis* oviposition and larval growth rates. Frequencies of capitate trichomes do not present an effective barrier to early instar larval growth. Additional data will be necessary to determine the mechanism(s) responsible for the reduced early instar larval growth associated with pigment gland frequencies.

REFERENCES

- Beckman, C.H., W.C. Nueller, and W.E. McHardy. 1972. The localization of stored phenols in plant hairs. *Physiol. Plant Pathol.* 2:69-74.
- Bell, A.A., and R.D. Stipanovic. 1977. The chemical composition, biological activity, and genetics of pigment glands in cotton. p. 244-258. In J.M. Brown (ed.) *Proc. Beltwide Cotton Prod. Res. Conf.* Atlanta, Ga. 10-12 Jan. 1977. National Cotton Council, Memphis, Tenn.
- Cutter, E.G. 1978. *Plant anatomy. Part I. Cells and tissues.* Addison-Wesley Publishing Co., Reading, Mass.
- Gibson, R.W. 1974. Aphid-trapping glandular hairs on hybrids of *Solanum tuberosum* and *S. berthaultii*. *Potato Res.* 17:152-154.
- Guettard, J.E. 1945. Sur les crops glanduleux des plantes, leurs filets on poiles et les matieres qui en sortent. *Mem. Acad. Foy. Sci. Paris*:261-308.
- Hunter, R.C., T.F. Leigh, C. Lincoln, B.A. Waddle, and L.A. Bariola. 1965. Evaluation of selected cross-selection of cotton for resistance to boll weevil. *Arkansas Agric. Exp. Stn. Bull.* 700.
- Kohel, R.J. 1973. Genetic nomenclature in cotton. *J. Hered.* 65:291-295.
- Kosmidou-Dimitropoulou, K., J.D. Berlin, and P.R. Morey. 1980. Capitate hairs on cotton leaves and bracts. *Crop Sci.* 20:534-537.
- Kreitner, G.L., and E.L. Sorensen. 1979. Glandular trichomes on *Medicago* species. *Crop. Sci.* 19:380-384.
- Levin, D.A. 1973. The role of trichomes in plant defense. *Quart. Rev. Biol.* 48:3-15.
- Lee, J.A. 1968. Genetical studies concerning the distribution of trichomes on the leaves of *Gossypium hirsutum* L. *Genetics* 60:567-575.
- . 1973. The inheritance of gossypol level in *Gossypium*. II. Inheritance of seed gossypol in two strains of cultivated *Gossypium barbadense* L. *Genetics* 75:259-264.
- . 1977. Inheritance of gossypol level in *Gossypium*. III. Genetic potentials of two strains of *Gossypium hirsutum* L. differing widely in seed gossypol level. *Crop. Sci.* 17:827-830.
- Lukefahr, M.J. 1977. Varietal resistance to cotton insects. p. 236-237. In J.M. Brown (ed.) *Proc. Beltwide Cotton Prod. Res. Conf.* Atlanta, Ga. 10-12 Jan. 1977. National Cotton Council, Memphis, Tenn.
- , Houghtaling, J.E., and D.G. Cruhum. 1975. Suppression of *Heliothis* spp. with cottons containing combinations of resistant character. *J. Econ. Entomol.* 68:743-746.
- , ----, and H.M. Graham. 1971. Suppression of *Heliothis* populations with glabrous cotton strains. *J. Econ. Entomol.* 64:486-488.
- McMichael, S.C. 1954. Glandless boll in upland cotton and its use in the study of natural crossing. *Agron. J.* 46:527-528.
- . 1960. Combined effects of glandless genes *gl₂* and *gl₃* on pigment glands in the cotton plants. *Agron. J.* 52:385-386.
- Maxwell, F.G., J.N. Jenkins, and W.L. Parrott. 1972. Resistance of plants to insects. *Adv. Agron.* 24:187-265.
- , H.N. Lavefer, and J.N. Jenkins. 1965. Blister beetles on glandless cotton. *J. Econ. Entomol.* 58:792-793.
- Parrell, F.R., H.E. King, and D.F. Ruston. 1949. Jassiel resistance and hairiness of the cotton plant. *Bull. Entomol. Res.* 39:539-575.
- Reed, W. 1974. Selection of cotton varieties for resistance to insect pests in Uganda. *Cotton Grow. Rev.* 51:106-123.
- Reeves, R.G., and J.O. Beasley. 1935. The development of the cotton embryo. *J. Agric. Res.* 51:935-944.
- Stanford, E.E., and A. Viehoever. 1918. Chemistry and histology of the glands of the cotton plant, with notes on the occurrence of similar glands in related plants. *J. Agric. Res.* 13:419-435.
- Smith, A.L. 1964. Leaf trichomes of upland cotton varieties. *Crop Sci.* 4:348-349.
- Stephens, S.G., and H.S. Lee. 1961. Further studies on the feeding and oviposition preferences of the boll weevil (*Anthonomus grandis*). *J. Econ. Entomol.* 54:1085-1090.
- Tingey, W.M., and J.E. Laubengayer. 1981. Defense against the green peach aphid and potato leafhopper by glandular trichomes of *Solanum berthaultii*. *J. Econ. Entomol.* 74:721-725.
- Wall, W. 1970. An ultrastructural survey of glandular tissue in *Gossypium*. Ph.D. Dissertation, North Carolina State University (Diss. Abstr. 71:15944).
- Webber, I.E. 1938. Anatomy of the leaf and stem of *Gossypium*. *J. Agric. Res.* 57:269-286.
- White, W.H. 1981. Evaluation of chemical and genetic analyses as predictors of resistance of cotton to the tobacco budworm. Ph.D. Dissertation. Mississippi State University.