SCREENING UPLAND COTTON FOR RESISTANCE TO COTTON FLEAHOPPER (HETEROPTERA: MIRIDAE)

A Thesis

by

DIWAKAR KARTHIK MEKALA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Plant Breeding

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August 2004

Major Subject: Plant Breeding

ABSTRACT

Screening Upland Cotton for Resistance to Cotton Fleahopper (Heteroptera: Miridae).

(August 2004)

Diwakar Karthik Mekala, B. S., Acharya N. G. Ranga Agricultural University

Co-Chairs of Advisory Committee: Dr. C. Wayne Smith
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Cotton (*Gossypium hirsutum* L.) crop maturity is delayed by cotton fleahopper (*Pseudatomoscelis seriatus* Reuter) (fleahopper) feeding on early-season fruit forms which increases vulnerability to late-season pests such as *Helicoverpa zea* (Boddie) and *Heliothis virescens* (Fabricius).

The objectives of this research were to evaluate methods of screening for resistance to fleahopper and to screen selected genotypes. Six fleahoppers were caged on plants in the insectary for 72 h. Numbers of live fleahoppers and percent square damage were determined 48 h following the removal of fleahoppers. Fleahopper numbers and percent square set were determined on randomly selected plants of 16 genotypes when grown under field conditions in 2002 and 2003. Across multiple sampling dates, the number of fleahoppers per plant was higher ($p=0.05$) in *G. arboreum* and Pilose (*G. hirsutum*), but no consistent differences were observed among the remaining 15 genotypes which represented several germplasm pools across the United States. Field and no-choice feeding tests suggested that Pilose, Lankart 142, Suregrow 747, and

Stoneville 474 were more resistant hairy-leaf genotypes and not different ($p=0.05$) in resistance than the smooth-leaf genotypes, Deltapine 50 and TAM 96WD-69s.

Pin-head, match-head, and one-third grown squares were removed from plants and placed on agar in petri-plates. Four fleahoppers were released per plate and allowed to feed for 48 h. Fleahopper damage, brown areas along the anthers and/or brown and shrunken pollen sacs was most evident in pin-head sized squares.

This work is dedicated to my parents and my brother for their encouragement and support.

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INTRODUCTION

Cotton is one of the major agricultural crops in the United States. Damage due to insect pests is among the many production constraints leading to decreased yields and profits. Producers depend upon insecticides for controlling certain insect pests because they have no other alternative. If the producers had cotton cultivars resistant to fleahoppers that were effective, they would not continue to spray insecticides for controlling fleahoppers. The benefits of developing cotton germplasm resistant to fleahoppers include

1. Lowering the burden on producers to control fleahoppers,
2. The producers do not have to invest time to inspect their fields or for spray operations,
3. Lowering the investment in pesticides and spray equipment, and
4. There will not be crop losses due to inaccurate timing of treatment as with insecticides, or inability to spray due to inclement weather conditions.

Current reliance on insecticide control for cotton fleahopper requires field scouting and threshold for timely insecticide treatments, time and trained personnel. Weather can also interfere with timely application. Most insecticides for fleahoppers are broad-spectrum, which adversely impact beneficial insects that help suppress outbreaks of other cotton pests. Hence, there is need for a different approach to keep these fleahoppers below the economic damage threshold.

This thesis follows the style and format of Crop Science.

Key insect pests of cotton in Texas are fleahoppers, bollworms, and tobacco budworms. The advantages associated with early maturity have made earliness a priority in most cotton breeding programs. The major advantages of earliness include reducing the losses to fiber quality and yield and lowering pest control costs for key pests such as boll weevil (*Anthonomus grandis* Boheman), tobacco budworm, and bollworm (Sprott et al., 1976; Adkisson et al., 1982). Early maturing cotton cultivars aid in minimizing production inputs such as pesticides and in turn maximize profits and energy efficiency (Sprott et al., 1976). But the benefits of earliness are lost due to early season key pests such as the fleahopper.

In recent years there has been an increase in the importance of fleahoppers as a pest, especially in Texas, relative to the boll weevil and bollworm/budworm mainly due to the success of the boll weevil eradication program and adoption of Bt cotton. The only current option for managing fleahopper is the use of insecticides. While boll weevil eradication in many cases reduces the need for early season insecticides, it will be necessary for Texas producers to continue some early season treatments to control fleahoppers, and thus they will not fully benefit from the insecticide savings and conservation of beneficial insects associated with boll weevil eradication.

Fleahopper is an early-season pest that feeds mostly on small squares which leads to delayed maturity and ultimately increases the vulnerability of cotton to late-season pests. The development of cotton germplasms resistant to the fleahopper would provide meaningful economic and environmental benefits for cotton producers.

The objectives of this project were 1) to develop reliable screening techniques to identify fleahopper resistance, and 2) to screen selected cotton genotypes for resistance in field and no-choice experiments. Genotypes were selected that had either plant morphological characters suspected of impacting fleahopper injury, such as trichome density and nectariless, or tolerance based upon field observations in breeding trials.

LITERATURE REVIEW

Upland cotton is native to Mexico and Central America. Cotton, originally a perennial plant, is grown and cultivated as an annual crop for commercial production. Even though cotton is considered as a better fiber, the cotton industry in the United States is facing stiff competition from man-made fibers. The reasons include low competitiveness in marketing due to increased production costs for land, labor, machinery, and pest control. There is need for expansion of research leading to the development of economically feasible and environmentally sound cotton production practices including pest control. Host plant resistance has been a useful control measure in a number of crop species for preventing insect injury (Harris, 1975).

Nationwide, fleahopper has been consistently ranked among the top ten most damaging insect pests of cotton. It ranked fourth, seventh, sixth, ninth, and first among top ten most damaging insect pests of cotton in 2003, 2002, 2001, 2000, and 1999, respectively (Williams, 2003). In 2003, 63,386 bales of cotton worth $18,255,123 were lost due to fleahopper infestation in Texas. The average cost of an application to control fleahoppers in Texas during 2003 was $6.46 per acre. About 37% of all fields in Texas were sprayed at least once for controlling fleahoppers in 2003. The total loss, which includes value of bales lost plus cost of spraying, was $20,561,081 in Texas during 2003.

Injury due to the fleahopper was first noted in cotton fields along the Texas coast in 1919 (Reinhard, 1926), followed by Georgia (Hunter, 1926), and South Carolina

(Eddy, 1927). Presently, it is an important pest in Texas and Oklahoma, and sometimes in mid-south states such as Arkansas, Louisiana, and Mississippi. The symptoms of damage include abortion or “blasting” of small squares, excessive vegetative growth, inhibition of fruiting branches, and shortening of internodes (Reinhard, 1926; Gaines, 1965; Hanny et al., 1977). There was an early speculation that fleahoppers transmit pathogenic viruses (Reinhard, 1926; Hunter, 1926), however later research (Painter, 1930) concluded that there is no virus transmission involved and that the whip-like plant growth might be due to extensive mechanical injury to plant tissues. Later work indicated that there is a possible secretion of growth regulators such as indole acetic acid (IAA) and ethylene precursor (ACC) (Burden et al., 1989) into the plant, and enzymatic digestion of plant tissues or loss of plant tissues by the fleahopper feeding (Flemion et al., 1954; Tingey and Pillemer, 1977). Martin et al. (1988) demonstrated that pectinases in the fleahopper saliva destroy plant cells and also elicit stress ethylene production in squares which results in square abscission.

The adult fleahopper is a small, yellowish green insect measuring about 3.4 mm in length, with a lifespan of approximately 20 d (Reinhard, 1926). It is flat with an elongated, oval outline, and prominent antennae. Nymphs resemble adults except that they are wingless. They pass through five instars and require 15-17 d to reach maturity. The female fleahoppers insert their eggs into the stems of variety of host plants. Fleahoppers overwinter in the egg stage (Reinhard, 1926) in hosts such as croton (*Croton capitatus* L.). Nymphs and adults feed on horsemint (*Monarda punctata* L.), a primary host during spring and early summer and many other wild hosts. Wooly croton

(*Croton capitatus* L.) is a common host later in the summer in Texas (Holtzer and Sterling, 1980). Nymphs start emerging in March, which usually coincides with the growth of many species of wild host plants (Hunter, 1926; Knight, 1926; Reinhard, 1926). Beerwinkle and Marshall (1999) indicated that fleahoppers are attracted to volatiles from flowering wild host plants such as false ragweed (*Parthenium hysterophorous* L.), croton, and horsemint in preference to volatiles from squaring cotton. Maturity of wild host plants in the early summer causes adult fleahoppers to migrate to cotton. Both biotic and abiotic factors such as cotton plant phenology, rainfall, and temperature affect fleahopper migration and the severity of their infestation (Reinhard, 1926; Gaines, 1933; Gaylor, 1975).

Fleahopper is controlled presently by chemical means, but the use of insecticides has disadvantages, such as destruction of natural enemies which increases the risk of secondary outbreaks of late-season pests, environmental pollution, and the build up of insecticidal resistance (Anonymous, 1969). The ideal method to solve this problem is to develop fleahopper resistant cultivars. Earlier research indicated that hairy genotypes tolerate feeding while smooth genotypes are more sensitive to feeding by fleahoppers and suffered higher yield reductions (Robinson, 1971; Walker et al., 1974; Meredith and Schuster, 1979). Cotton jassids (*Amrasca devestans* Distant, *Jacobiasca lybica*, *J. fascialis*, and *Empoasca terrae reginae*) are similar to fleahoppers as they belong to the same order Hemiptera and have piercing and sucking mouth parts. Jassids feed on the under surface or abaxial surface of the leaves. Afzal and Abbas (1944) and Parnell et al. (1949) indicated that host plant resistance to jassids is due to the leaf hairiness in cotton.

Along with trichome density, trichome length and their angles of insertion also contribute toward jassid resistance (Parnell et al., 1945). However, it has also been reported that not all hairy cotton are resistant to jassids (Parnell, 1925; Husain, 1938; Husain and Lal, 1940; Afzal and Ghani, 1946; Tidke and Sane, 1962). A positive correlation was observed between silica and tannin contents of cotton leaves with jassid resistance (Chakravorty and Sahn, 1972). Pilosity, or hairiness, in cotton has not been used in developing commercial cultivars due to a variety of reasons such as the apparent pleiotropic effects of the Pilose allele with short and coarse fiber, which makes them unsuitable for use in the textile industry (Simpson, 1947). Pubescence also increases plant debris in harvested seed cotton. Although some Pilose genotypes have shown resistance to fleahopper, they have been observed to be highly attractive for oviposition by *Heliothis* spp., when compared with moderately smooth to glabrous cottons (Robinson et al., 1980).

The nectariless trait, conditioned by recessive genes ne_1 and ne_2, removes the extrafloral nectarines present on the leaves and involucral bracts of cotton (Meyer and Meyer, 1961). This trait was found to reduce tarnished plant bug (*Lygus lineoralis* Palisot de Beauvois) and fleahopper numbers by 23-64% when compared to nectaried isolines (Schuster and Maxwell, 1974; Meredith, 1976). Cotton nectar is considered to be an important source of nutrients for insects (Butler et al., 1972). Early research by Lukefahr et al., (1970) found that the nectariless trait was not effective in reducing fleahopper numbers. However, later research suggested that nectariless had an adverse effect on nymphal emergence and survival of plant bug, *Lygus hesperus* Knight

(Benedict et al., 1981). However, the nectariless trait is associated with a 17-42% reduction in the densities of beneficial insects (Schuster et al., 1976; Adjei-Maafo and Wilson, 1983). In spite of this disadvantage, nectariless was found to be a dependable plant bug resistance trait (Lidell, 1985).

Work on host plant resistance to fleahopper has been ongoing but erratic since the 1960s, and little has been reported on developing screening techniques for identifying resistance. Several scientists (Lukefahr et al., 1968, 1970; Tingey and Pillemer, 1977; Mussett et al., 1979; Niles, 1980; Sterling et al., 1989) have reported lower fleahopper densities on glabrous than on hirsute strains of cotton regardless of the presence of nectaries or pigment glands. Walker et al. (1974) and Lukefahr et al. (1976) observed that sensitivity to injury can be greater on glabrous than on hairy cottons.

Fleahopper densities, such as those of tarnished plant bugs, are often reduced in nectariless cotton (Schuster and Maxwell, 1974; Schuster et al., 1976; Agnew et al., 1982). Certain cotton cultivars with high trichome densities were found to tolerate high fleahopper densities (Walker et al., 1974; Ring et al., 1993). Like many cotton insects, fleahoppers also have less preference for strains having high gossypol (Lukefahr and Houghtaling, 1975). Most researchers have taken fleahopper densities into consideration when identifying resistance, with little importance given to estimation of square loss or injury, as a basis of identifying resistance. Most experiments conducted by earlier researchers involved identification of anti-xenosis (non-preference) and tolerance mechanisms of resistance, with little emphasis given to the identification of antibiosis.

Screening for resistance to fleahoppers is a new challenge as techniques have not been developed which will identify resistance. Furthermore, this insect currently cannot be reared in the laboratory in large numbers to provide insects for screening.

MATERIALS AND METHODS

Genetic Material

The genotypes used for this study included eleven commercial cultivars, four breeding lines developed by the Texas Agricultural Experiment Station at College Station, four near isogenic lines of DES 119 (combinations of smooth leaves, hairy leaves, nectaried, and nectariless), Pilose, and *Gossypium arboreum*, Asiatic cotton (Table 1). These genotypes represent adapted germplasm pools from the eastern U.S. (Pee Dee genotypes), the mid-south (Deltapine, Stoneville, and Suregrow), the Texas Agricultural Experiment Station (TAM and Tamcot), the Texas Blacklands (Lankart 142), the Texas high plains (Paymaster and All-Tex), New Mexico Acala (Acala 1517-99), and California Acala (Maxxa), plus the non-adapted Pilose and the *G.arboreum* lines.

Trichome Density Measurement

Trichome density was determined for all genotypes except Pilose in order to determine the association of trichome density and fleahopper numbers and percent square damage among cotton genotypes. Trichome counts were made on bracts, leaves, and stems. Ten uniformly sized (12-15 mm diameter) squares were collected per genotype from field grown plants at peak blooming stage. A 4 mm diameter cork borer was used to punch a fixed area of the bract tissue. The numbers of trichomes per disc were counted with the aid of a dissecting microscope.

Table 1. Cotton genotypes used to evaluate host plant resistance to fleahopper in 2002 and 2003.

Genotype	Source	Year released	Trait(s) of interest	Primary area of adaptation
1. Suregrow 747 (SG 747)	Delta and Pine Land Company	1998	Hairy	Mid-South
2. Stoneville 474 (STO 474)	Stoneville Pedigreed Seed Company	1996	Hairy	Mid-South
3. Stoneville 213 (STO 213)	Stoneville Pedigreed Seed Company	1962	Hairy	Mid-South
4. Lankart 142 (LANKART)	Delta and Pineland Company	1987	Hairy	Texas Blacklands
5. Paymaster Ute (PMUTE)	Paymaster Seed Company	1996	Hairy	Texas High Plains
6. AllTex Atlas (ATLAS)	AllTex Seed Inc.	1990	Hairy	Texas High Plains
7. Acala 1517-99 (1517-99)	New Mexico Agricultural Experiment Station	2000	Hairy	New Mexico
8. Acala Maxxa (MAXXA)	California Planting Cotton Seed Distributors	1990	Hairy	San Joaquin Valley California
9. Deltapine 50 (DP 50)	Delta and Pine Land Company	1984	Smooth-leaf Hairy-stem	Mid-South
10. TAM 96WD-22h (WD-22h)	Texas Agricultural Experiment Station	NR *	Hairy	Central and South Texas

Table 1. Continued.

Genotype	Source	Year released	Trait(s) of interest	Primary area of adaptation
11. PD 6186 (PD6186)	USDA, Florence, SC	1985	Hairy	South Carolina
12. DES 119 H	Mississippi Agricultural and Forestry Experiment Station	1998	Hairy and nectaried	Mid-South
13. DES 119 H, ne	Mississippi Agricultural and Forestry Experiment Station	1998	Hairy and nectariless	Mid-South
14. Tamcot CAB-CS (CAB-CS)	Texas Agricultural Experiment Station	1986	Smooth-leaf and stem	Texas
15. TAM 96WD-69s (WD-69s)	Texas Agricultural Experiment Station	NR *	Smooth-leaf and stem	Central and South Texas
16. TAM 96WD-22s (WD-22s)	Texas Agricultural Experiment Station	NR *	Semi-smooth leaf	Central and South Texas
17. Pee Dee 22 (PD 22)	USDA, Florence, SC	NR *	Smooth-leaf and stem	South Carolina
18. DES 119 S	Mississippi Agricultural and Forestry Experiment Station	1998	Smooth-leaf and stem, nectaried	Mid-South

Table 1. Continued.

Genotype	Source	Year released	Trait(s) of interest	Primary area of adaptation
19. DES 119 S, ne	Mississippi Agricultural and Forestry Experiment Station.	1998	Smooth-leaf and stem, nectariless	Mid-South
20. Pilose	Unknown origin	NR *	Extremely hairy	Unadapted

* NR – Not Released.

Ten topmost fully expanded leaves were collected per genotype from field grown plants at peak blooming stage. A 10 mm cork borer was used to punch a fixed area of leaf (from one side of the mid-rib) for counting the numbers of trichomes under a dissecting microscope. The lower or abaxial surface of the leaf was used for counting the numbers of trichomes. Ten stems were collected per genotype from field grown plants at peak blooming stage. A 10 mm cork borer was used to make an impression of a fixed size on the stem on the topmost nodes with fully expanded leaves and the numbers of trichomes in this area was counted under the dissecting microscope.

Statistical Analysis

Trichome density was obtained per mm^{-2} area of the cork borer which was later converted into cm^{-2}. Trichome density counts on bracts, leaves, and stems were analyzed by analysis of variance (PROC GLM) using SAS®. Means among the genotypes were separated by Waller Duncan's LSD.

Field Trials: Large Field Trial, With and Without Insecticide

Field experiments were conducted at the Texas A&M Research and Extension Center, Dallas, Texas in 2002 and 2003. Ten commercial cultivars (Suregrow 747, Stoneville 474, Stoneville 213, Lankart 142, Paymaster UTE, All-Tex Atlas, Acala 1517-99, Acala Maxxa, Deltapine 50, and Tamcot CAB-CS), six upland cotton strains (TAM 96WD-22h, PD 6186, PD 22, TAM 96WD-69s, TAM 96WD-22s, and Pilose), and *Gossypium arboreum*, an Asiatic cotton, were planted in a split block arrangement

of a randomized complete block design with four replications. Pilose was included in 2003 only. The main blocks were insecticide treated and non-treated to control fleahopper populations. The insecticide treated block acted as a check wherein fleahoppers were not allowed to feed on the plants. Blocks were split to genotypes planted in single rows, 6 m x 76 cm. Thinning was practiced to maintain 40 cm between plants.

Fleahoppers were allowed to feed in the non-treated block, while in the other block, insecticide treatment was applied weekly starting at first square to prevent or minimize fleahopper feeding. To ensure sufficient population of fleahoppers, the wild hosts *Monarda* spp. and *Croton* spp. were planted on the sides of the field. These wild host plants were mowed periodically using a home lawn mower to move fleahoppers into the study plots. Thrips (*Thrips* spp.) were controlled during the early vegetative growth stages with insecticidal soap, a contact insecticide, applied in both treated and non-treated blocks. Application of insecticidal soap to control thrips was discontinued in both blocks when the plants reached the sixth true leaf stage in order to avoid killing fleahoppers in the non-treated block. Orthene®, a systemic insecticide, was applied weekly in the treated block to control fleahoppers. Early season infestation of bollworms and budworms were controlled by Safer® caterpillar killer, a microbial Bt insecticide in 2002 and Entrust® (Spinosad) in 2003. Both insecticides are selective to lepidopterous larvae and are non-toxic to fleahoppers. Planting was performed on 20 May and 28 April in 2002 and 2003, respectively. Moderate populations of fleahoppers were observed in

2002 as the planting was delayed. Planting was timely in 2003 and squaring coincided with the major fleahopper infestation.

Fleahopper Counts

Fleahopper (adult and nymph) numbers and the number of main stem nodes were counted on five plants selected randomly in each single row sub plot on three dates (20 June, 4 July, and 11 July) in 2002 and five dates (2 June, 7 June, 17 June, 23 June, and 30 June) in 2003. Periodic observation of *Lygus* bugs and early season bollworms and budworms were made to ensure that the damage to cotton squares was attributable to fleahoppers.

Square Counts

The numbers of healthy, damaged, and missing squares were counted on the same five plants selected randomly for fleahopper counts, in each single row sub plot on two dates (4 and 15 July) in 2002, separated by 12 d gap between the two counts. All squaring positions were counted on 4 July while only the first and second positions of each fruiting limb were counted on 15 July. Squares were counted on two dates (23 June and 7 July) in 2003 with 14 d gap between the counts. All the squaring positions were considered in 2003. Plant heights were measured simultaneously with square counts. Square counts were taken during the peak periods of fleahopper population and during the first three weeks of squaring when plants are most susceptible to fleahopper damage (Parker et al., 2004).

Small Field Trial: Detailed Evaluation of Fleahopper Damage

This study, conducted in 2002, included four near isogenic lines of DES 119 (combinations of smooth leaves, hairy leaves, nectaried, and nectariless) planted in a randomized complete block design with four replications. Plot size was 6 m x 152 cm. All cultural practices, including the insect control, were as described above for the large field trial.

Fleahopper Counts

Fleahopper (adult and nymph) numbers and the number of main stem nodes were counted on five plants selected randomly in each single row plot on four dates (21 June, 29 June, 7 July, and 18 July). Periodic observation of *Lygus* bugs were made to ensure that the damage to cotton squares was attributable to fleahoppers only.

Square Counts

Square counts were made on 7 and 18 July with the same methodology as described for the large field trial of 2002.

Statistical Analysis

Preliminary analysis of the fleahopper count data in large field and small field trials indicated that the fleahopper count data were not normally distributed (Shapiro-Wilk test for normality, 1965) and had no equal error variances (Levene's test for equality of variances, 1960) but followed a Poisson distribution, with variances and

means associated. This violation of one of the assumptions of analysis of variance was avoided by transforming the data as $y_t = \sqrt{(y + 0.5)}$ (Little and Hills, 1978; Gomez and Gomez, 1976). The transformed data was used for all the analysis with means reported in original units. Percent square set was calculated in both large and small field trials, based on the observations from healthy, damaged, and missing squares per plant as follows;

$$\text{Percent square set} = \frac{\text{number of healthy squares}}{\text{number of healthy + damaged + missing squares}} \times 100$$

Percent square set data from the large field trial was normally distributed (Shapiro-Wilk test for normality, 1965) and had equal error variances (Levene's test for equal variances, 1960) among all the genotypes while it was not normally distributed and had no equal error variances in the small field trial. The percent square set data from the small field trial was transformed using the formula $y_t = \log y$ (Little and Hills, 1978; Gomez and Gomez, 1976). The transformed data was used for all the analysis with means reported in original units. The average number of fleahoppers and percent square set per plant were analyzed by using the analysis of variance (PROC GLM) by SAS®. Means of fleahoppers and percent square set among the genotypes were separated using Waller Duncan's LSD. Interaction means of genotype x insecticide treatment were separated by Bayes LSD using the procedures as outlined by Smith (1978).

Field Ovipositional Preference Trial

This study was conducted in 2003 and included the same genotypes as the large field trial and planted in a randomized complete block design with four replications. Plot size was 6 m x 76 cm. All cultural practices including insect control were as described above for the large field trial. On 26 June, two plants at 14^{th} node stage, per plot were excised near ground level and placed in separate polythene bags with dry paper towels. Plants were then taken to the lab where fleahopper nymphs were shaken onto a white paper and counted. Plants were placed back in the same bag and stored in an incubator at 30^0C. Plants were removed and fleahopper nymphs counted by the same method at 3 d intervals for 15 d. Paper towels were changed at each count in order to avoid fungus development. The entire process was repeated on 17 July using only the top 3 main stem nodes and associated leaves and sympodia instead of the whole plant when the plants were at 20^{th} node stage.

Statistical Analysis

Preliminary analysis of the data indicated that the number of nymphs emerging were not normally distributed but followed a Poisson distribution, where the variances and means were associated. Hence, the data was transformed by using the formula $y_t = \sqrt{(y + 0.5)}$ (Little and Hills, 1978; Gomez and Gomez, 1976) to avoid the violation of one of the assumptions of analysis of variance. The transformed data was used for all the analysis with means reported in original units. Numbers of fleahopper nymphs emerging

were analyzed by using analysis of variance (PROC GLM) by SAS®. Genotype means were separated by Waller Duncan's LSD.

No-Choice Feeding Tests

No-choice feeding tests were conducted in the greenhouse and an insectary at the Texas A&M Research and Extension Center, Dallas, Texas in 2002 and 2003. Four replications of each of twenty-one genotypes, including ten commercial cultivars (Suregrow 747, Stoneville 474, Stoneville 213, Lankart 142, Paymaster UTE, All-Tex Atlas, Acala 1517-99, Acala Maxxa, Deltapine 50, and Tamcot CAB-CS), six upland cotton strains (TAM 96WD-22h, PD 6186, PD 22, TAM 96WD-69s, TAM 96WD-22s and Pilose), *G.arboreum*, an Asiatic cotton, and near isogenic lines of DES 119 (combinations of smooth leaves, hairy leaves, nectaried, and nectariless) were planted in 7.5 liter pots and grown in the greenhouse. Pilose was included in 2003 only and all the genotypes were replicated 5 times. These plants were transferred to an insectary where better environmental control was possible, when all of the plants reached the tenth node stage of growth.

All plants were mapped (Mauney and Henneberry, 1979) for position and size of the squares before conducting the experiment. Squares that were 3 mm or less in diameter were considered susceptible to fleahopper damage. Each plant was confined in a cage made of nylon organdy and clear plastic. The cage used for confining the insects on each plant was made of nylon organdy having cylindrical dimensions 38 cm in length and 12 cm in diameter attached to the top clear plastic having cylindrical dimensions of

10 cm in length and 12 cm in diameter. The top of the cylindrical cage was closed by a circular lid made of nylon organdy. A wooden stake was glued to the cylindrical clear plastic part of the cage so that the stake with the cage attached can be clamped to a wooden pole present in the potted plants. For the experiments, the cages were placed on individual plants in such a way that the bottom of the cage was open for introducing the fleahoppers.

A number of preliminary trials were performed to identify a suitable number of fleahoppers and to determine optimum duration of fleahopper confinement to insure damage. It was determined that six fleahoppers should be confined on each plant for 72 h. Fleahoppers were collected from wild hosts, *Monarda* spp. and *Croton* spp., growing on the sides of the fields by using a KIS Sampler (keep-it-simple sampler) (Beerwinkle et al., 1997) and placed in a transparent plastic container. Six fleahoppers were aspirated into a plastic vial. A fresh green bean (*Phaseolus vulgaris* L.) was placed in each vial to serve as a food supply. Sufficient number of such vials, each having six fleahoppers, were prepared and kept overnight to insure equal feeding capacity. The following morning, a single vial was put inside each cage and the cages were tied at the bottom around the stem with a twist tie. All the fleahoppers were released inside the cages by opening the caps of the vials. Pots were placed in a randomized complete block arrangement. In the second trial of 2003, cotton plugs were used instead of plastic caps on the vials in order to insure good aeration in the vial and avoid fleahopper mortality due to condensation. A temperature of 30^0C and a light period of 14 h light were maintained in the insectary during the experiment.

After 72 h, all fleahoppers were removed by aspiration. The numbers of live fleahoppers per plant were determined and the cages were removed. All plants were re-mapped for size and positions having healthy, damaged, and missing squares after 48 h.

Statistical Analysis

Preliminary analysis of the data indicated that the percent square damage data were not normally distributed but followed a Poisson distribution, where the variances and means were associated. This violation of one of the assumptions of analysis of variance was avoided by the transforming the data using the formula $y_t = \sqrt{(y + 1)}$ to each value in 2002 and $y_t = \sqrt{(y + 0.5)}$ to each value in 2003 (Little and Hills, 1978; Gomez and Gomez, 1976). The transformed data was used for analysis while untransformed means are reported. Number of live fleahoppers and percent square damage per plant were analyzed by using analysis of variance (PROC GLM) by SAS®. Genotype means were separated by Waller Duncan's LSD.

Lab Tests

Studies were conducted in 2003 to determine specifically which size square is preferred by fleahoppers and to determine whether it is feasible to use excised squares for screening cotton for resistance to fleahoppers. Four each of pin-head (≤ 3 mm), match-head (>3 to ≤ 6 mm), and one-third grown squares (> 6 mm) were removed from plants grown in the greenhouse and placed randomly in a circle on a solidified 2% Bacto™ Agar medium in a petri-plate. In order to avoid the fleahoppers becoming

immobilized by the agar medium, parafilm was placed on top of the agar in each plate. A hole was pierced in the parafilm and the peduncle of each square was inserted into the agar. This system kept the squares fresh for at least 72 h. Four adult fleahoppers were released per plate, with duplicate plates prepared with squares but without fleahoppers as a control. All the plates were kept in the incubator at 30^0C with 14 h of light and 10 h of dark. Fleahoppers were retained in the plates for 48 h. After an additional 24 h, each square was dissected using a razor blade and the internal tissue was examined under a microscope for fleahopper feeding. Squares having brown discoloration and shrunken anthers were considered damaged as suggested by Williams and Tugwell (2000). Each trial was replicated four times and the entire trial was repeated three times.

Statistical Analysis

Percent square damage data in the preliminary analysis indicated that the data were not normally distributed but followed a Poisson distribution, where the variances and means were associated. This violation of one of the assumptions of analysis of variance was avoided by the transforming the data by using the formula $y_t = \sqrt{(y + 0.5)}$ to each value in trial I and $y_t = \sqrt{(y + 1)}$ to each value in trial II and III (Little and Hills, 1978; Gomez and Gomez, 1976). The transformed data was used for all the analysis, while untransformed means are reported herein. Percent square damage data per petri-plate were analyzed by using analysis of variance (PROC GLM) by SAS®. Percent square damage within each square size was separated by Waller Duncan's LSD.

RESULTS AND DISCUSSION

Trichome Density Measurement

Numbers of trichomes were counted on bracts, leaves, and stems (except for stems of STO 213) in all the genotypes except Pilose, which was excluded because of its extreme high density of trichomes. Genotypes varied (p=0.01) in trichome density of bracts, leaves, and stems (Table 2).

G.arboreum had higher (p=0.05) number of trichomes on bracts than any other genotype (Table 3). SG 747 had more (p=0.05) bract trichomes than all other genotypes except STO 213 and STO 474. *G.arboreum*, SG 747, STO 213, STO 474, 1517-99, 119 H, ne, MAXXA, PD 6186, LANKART, 119 H, PMUTE, ATLAS, and WD-22h had significantly more bract trichomes than PD 22, DP 50, WD-69s, CAB-CS, 119 S, WD-22s, and 119 S, ne.

The numbers of trichomes cm^{-2} of leaf area were greater (p=0.05) in *G.arboreum* than all other genotypes, with all genotypes designated as hairy leaf types in Table 3 having more (p=0.05) leaf trichomes than the genotypes identified as having smooth leaves, except for WD-22s which was not different than PD 6186, LANKART, or WD-22h. As leaves of PMUTE averaged fewer (p=0.05) leaf trichomes than the hairy leaf types even though it had a higher number of bract trichomes, it would be considered a smooth leaf genotype.

Stem pubescence did not separate these genotypes into the smooth and hairy categories as did bract and leaf trichome numbers (Table 3). As a group and excluding

G.arboreum, the smooth leaf genotypes ranged from 1 to 9 stem trichomes cm^{-2}, including PMUTE, while the hairy leaf genotypes ranged from 2 to 15. Previous research has indicated that hairy genotypes either tolerate or resist fleahopper feeding while smooth genotypes suffer more damage from this pest (Robinson, 1971; Walker et al., 1974; Meredith and Schuster, 1979). However, extreme hairiness in cotton, such as in Pilose, has not been used in developing commercial cultivars due to a variety of reasons, including pleiotropic effects of the Pilose allele with short and coarse fibers (Simpson, 1947), increased plant debris in harvested seed cotton, attractiveness for oviposition by *Heliothis* spp., when compared with moderately smooth to glabrous cottons (Robinson et al., 1980).

These data on trichome density verify that the genotypes chosen for this study include meaningful variation in trichome numbers that would allow the determination of the impact of trichome density on fleahopper resistance in a number of genetic backgrounds.

Table 2. Mean squares for number of trichomes on bracts, leaves, and stems cm^{-2} on 19 genotypes of upland cotton and 1 Asiatic cotton genotype in the trichome study during 2002 at Dallas, TX.

Source of variation	df	Bracts	Leaves	Stems
Geno	19 †	636794 **	18046**	5773 **
Error	180 ‡	16913	558	45

** Significant at 0.01 probability level.

† Degrees of freedom for trichome numbers on stems is 18, because STO 213 is missing.

‡ Error degrees of freedom for trichome numbers on stems is 171, because STO 213 is missing.

Table 3. Mean number of trichomes cm^{-2} on bracts, leaves, and stems in 19 genotypes of upland cotton and 1 Asiatic cotton genotype in the trichome study during 2002 at Dallas, TX.

Genotypes	Leaf pubescence	Bracts	Leaves	Stems
G.arb	Hairy	934 a †	172 a	110 a
SG 747	Hairy	665 b	37 fg	7 cdefg
STO 213	Hairy	599 bc	58 de	-
STO 474	Hairy	598 bc	71 cd	15 b
1517-99	Hairy	550 cd	86 bc	11 bc
119 H, ne	Hairy	527 cd	78 bc	10 bcd
MAXXA	Hairy	511 cd	45 ef	8 cdef
PD 6186	Hairy	464 de	33 fgh	3 fgh
LANKART	Hairy	404 e	20 ghi	2 gh
119 H	Hairy	404 e	71 cd	11 bc
PMUTE	Smooth	371 e	12 i	5 defgh
ATLAS	Hairy	365 e	91 b	11 bc
WD-22h	Hairy	242 f	33 fgh	8 cdef
PD 22	Smooth	139 g	5 i	1 h
DP 50	Smooth	86 g	11 i	6 cdefg
WD-69s ‡	Smooth	76 g	11 i	1 h
CAB-CS ‡	Smooth	72 g	4 i	1 h
119 S	Smooth	66 g	2 i	9 cde
WD-22s	Smooth	60 g	18 hi	8 cdef
119 S, ne	Smooth	53 g	3 i	4 efgh
Mean		359	43	10
C.V. (%)		36	54	56

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
‡ Smooth-leaf and stem.

Field Trials: Large Field Trial, With and Without Insecticide

Fleahopper counts

Fleahopper (nymph and adult) counts were made in 2002 and 2003 to determine if resistance, i.e., non-preference, could be detected among the 17 genotypes representing 7 germplasm pools and varying morphology, i.e., smooth and hairy phenology, and earliness. Fleahopper numbers were well above the economic threshold level of 10 to 15 fleahoppers per 100 terminals recommended for the Texas Blacklands region (Parker et al., 2004) in the non-treated block both in 2002 and 2003. Since planting date varied across years and sampling was performed at different plant growth stages each year, analysis of variance were performed within each year. All the fleahopper count data were transformed using appropriate factors, in such a way that the data were close to normal distribution.

In 2002 and 2003, total number of fleahoppers differed ($p=0.01$) within sampling dates, insecticide treatments, and genotypes (Tables 4 and 5). The treatment x sampling date interaction was significant each year, as were genotype x treatment in 2002 and all genotype interactions in 2003; hence the genotypes were separated within each treatment. The genotype x date interaction and genotype x date x treatment interaction was not significant in 2002; hence the genotypes were not separated by date. However, in 2003 as all genotype interactions were significant hence genotypes were separated within treatments and within each sampling date.

The mean numbers of fleahoppers per plant were multiplied by 100 for ease of comparison with the economic thresholds (Tables 6 and 7). In 2002, perhaps due to

Table 4. Mean squares for total number of fleahoppers between sample dates on 15 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled in the large field trial during 2002 at Dallas, TX (number of fleahoppers data transformed).

Source of variation	df	Mean squares
Date	2	1.005 **
Error A	6	0.121
Treat	1	10.504 **
Treat*Date	2	0.850 **
Error B	9	0.129
Geno	15	0.103 **
Geno*Date	30	0.037
Geno*Treat	15	0.074 *
Geno*Date*Treat	30	0.042
Error C	258	0.041

*, ** Significant at 0.05 and 0.01 levels of probability, respectively.

Table 5. Mean squares for total number of fleahoppers between sample dates on 16 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled in the large field trial during 2003 at Dallas, TX (number of fleahoppers data transformed).

Source of variation	df	Mean squares
Date	4	1.185 **
Error A	12	0.265
Treat	1	27.079 **
Treat*Date	4	2.581 **
Error B	15	0.295
Geno	16	0.816 **
Geno*Date	64	0.168 **
Geno*Treat	16	0.615 **
Geno*Date*Treat	64	0.150 *
Error C	480	0.106

*, ** Significant at 0.05 and 0.01 levels of probability, respectively.

Table 6. Mean number of fleahoppers per 100 plants on 15 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled in the large field trial in 2002 at Dallas, TX.

Genotypes	Leaf pubescence	T	NT
1517-99	Hairy	5	43 abc †
G.arb	Hairy	3	63 a
STO 213	Hairy	2	50 ab
CAB-CS ‡	Smooth	2	40 abc
WD-22s	Smooth	2	33 bc
PD 6186	Hairy	2	33 bc
ATLAS	Hairy	0	40 abc
LANKART	Hairy	0	25 bc
PD 22	Smooth	0	25 bc
PMUTE	Smooth	0	18 c
SG 747	Hairy	0	23 c
DP 50	Smooth	0	24 bc
STO 474	Hairy	0	33 bc
WD-22h	Hairy	0	33 bc
MAXXA	Hairy	0	35 bc
WD-69s ‡	Smooth	0	20 c
Mean		< 1	34
CV (%)		1150	174

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
T = Treated and NT = Non-treated blocks.
‡ Smooth-leaf and stem.

Table 7. Mean number of fleahoppers per 100 plants on 16 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled in large field trial in 2003 at Dallas, TX.

Genotypes	Leaf pubescence	2 Jun		7 Jun		17 Jun		23 Jun		30 Jun	
		T	NT	T	NT	T	NT	T	NT	T	NT
G.arb	Hairy	60	25	0	60 b †	30	40 cdef †	0	25	0	60 cdefg †
Pilose	Smooth	35	75	0	110 a	30	170 a	0	85	0	310 a
DP 50	Smooth	30	15	0	15 cde	10	40 cdef	10	10	0	30 fg
ATLAS	Hairy	25	20	0	25 cde	5	75 bc	0	145	0	110 bc
PD 22	Smooth	25	5	0	20 cde	15	50 bcdef	0	15	0	45 defg
1517-99	Hairy	15	10	0	35 c	10	80 b	0	115	0	75 bcdef
STO 213	Hairy	15	0	0	25 cde	5	20 ef	0	90	0	80 bcdef
STO 474	Smooth	15	25	0	15 cde	15	65 bcd	0	70	0	65 cdefg
MAXXA	Hairy	10	25	0	25 cde	10	30 def	0	15	0	125 b
LANKART	Hairy	10	5	0	10 de	0	20 ef	0	40	0	20 g
CAB-CS ‡	Smooth	10	65	0	30 cd	0	15 f	0	55	0	50 defg
SG 747	Hairy	10	15	0	10 de	0	55 bcde	0	60	0	40 efg
WD-22h	Hairy	10	10	0	15 cde	0	25 ef	15	170	0	95 bcd
WD-22s	Smooth	10	15	0	15 cde	10	20 ef	5	30	0	30 fg
PD 6186	Hairy	5	15	0	15 cde	10	25 ef	0	85	0	85 bcde
PMUTE	Smooth	0	15	0	5 e	10	20 ef	5	80	0	65 cdefg
WD-69s ‡	Hairy	0	5	0	5 e	10	15 f	0	30	0	15 g
Mean		17	20	0	26	10	45	2	66	0	76
CV (%)		267	335	0	158	308	146	717	125	0	117

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
‡ Smooth-leaf and stem.

delayed planting, large fleahopper densities were not observed; hence sampling was performed only three times. When planted at an appropriate time in 2003, relatively higher fleahopper densities were observed and sampling was performed on five dates.

In 2002, no significant differences were observed in the treated block among all the genotypes for mean number of fleahoppers per 100 plants, however in the non-treated block *G.arboreum* and STO 213 had higher (p=0.05) mean number of fleahoppers than SG 747, WD-69s, and PMUTE (Table 6). The plants averaged 6^{th}, 9^{th}, and 14^{th} node stages on the three sample dates 20 June, 4 July, and 11 July in 2002.

On 2 June 2003, as the plants were in an early growth stage (6^{th} node stage), no significant differences in mean number of fleahoppers were observed among the genotypes, in either the treated or non-treated (Table 7). When the plants averaged the 7^{th}, 11^{th}, and 15^{th} node stages on 7 June, 17 June, and 30 June, respectively, differences (p=0.05) in the fleahopper numbers were observed among the genotypes grown under non-insecticide treated conditions. On sample dates 7 and 30 June, fleahopper counts were made just after insecticide treatment, hence no fleahoppers were observed in the treated block. No differences were observed among the genotypes in mean number of fleahoppers in the treated block on sample dates 2 Jun, 17 Jun, and 23 Jun. In the non-treated block on 7 June, Pilose harbored higher (p=0.05) numbers of fleahoppers than other genotypes, followed by *G.arboreum*. WD-69s and PMUTE had the fewest fleahoppers on 7 June 2003, but not significantly fewer than 11 other genotypes. On 17 June, in the non-treated block, Pilose again had higher (p=0.05) numbers of fleahoppers than other genotypes. Pilose, 1517-99, ATLAS, STO 474, and SG 747 had higher

(p=0.05) numbers of fleahoppers than CAB-CS and WD-69s. Pilose, in the non-treated block on 30 June had higher (p=0.05) numbers of fleahoppers than all other genotypes, with MAXXA having higher numbers of fleahoppers than STO 474, PMUTE, *G.arboreum*, CAB-CS, PD 22, SG 747, DP 50, WD-22s, LANKART, and WD-69s. Again WD-69s had the fewest fleahoppers but not significantly fewer than STO 474, PMUTE, *G.arboreum*, CAB-CS, PD 22, SG 747, DP 50, WD-22s, and LANKART. On most of the sample dates in 2002 and 2003, in the insecticide treated block, high coefficient of variation was observed for the total number of fleahoppers which was because of high number of zero values in all the genotypes.

As individual sampling dates in 2002 and 2003 did not clearly separate genotypes, fleahopper counts were averaged across sampling dates. This is not statistically correct but appears to be scientifically sound since the fleahopper is a mobile pest and single sample dates would reflect only an extremely narrow view of a season long phenomenon. The average numbers of fleahoppers across all the genotypes were not significantly different between 2002 and 2003 (Table 8). Insecticide treatment and cotton genotype affected (p=0.01) fleahopper numbers as expected but these genotypes did not respond (p=0.01) the same to insecticide treatment.

To address the issue of preference as a food source by the fleahoppers among the cotton genotypes, means were separated within insecticide treatments and the interaction means were separated using $\sqrt{(4\ s^2/4)}$ as the standard error for the difference between interaction means rather than $\sqrt{(2\ s^2/4)}$, which is the appropriate standard error for calculating least significant differences (LSD) among genotypic means. Separation of

genotypic means within treatments will identify cotton genotypes that suffer more or less fleahopper feeding damage within a given insecticide regime. However, separation of the interaction means would identify cotton genotypes that are more or less preferred by the fleahoppers as a food source. The rationale here is that a genotype averaging low numbers of fleahopper when not treated with insecticide could be considered non-preferred compared with a genotype with a high average numbers of fleahoppers, e.g., 40 versus 80. However, if these same cotton genotypes averaged 30 and 70 fleahoppers, respectively, in the treated block, thereby each having a NT-T average of 10. Separation of the interaction means, i.e., 10 in this example, would suggest no difference in susceptibility/resistance. When these cotton genotypes were not treated with insecticide to control fleahoppers, WD-69s harbored fewer ($p=0.05$) fleahoppers than 10 of the remaining 16 genotypes (Table 9). SG 747, WD-22s, PMUTE, PD 22, DP 50, and LANKART were not different ($p=0.05$) than WD-69s. This suggests that these 7 genotypes are less preferred by the fleahoppers. These data are especially encouraging since 4 of the 7 have relatively smooth leaves and conventional wisdom is that smooth leaf genotypes are more susceptible to fleahoppers than more hairy leaf genotypes (Smith personal communication, 2004). Separation of the interaction means indicated that all upland genotypes responded the same to insecticide treatment.

Table 8. Mean squares for average number of fleahoppers on 16 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled in the large field trial during 2002 and 2003 at Dallas, TX (number of fleahoppers data transformed).

Source of variation	df	Mean squares
Yr	1	0.151
Error A	6	0.032
Treat	1	9.092 **
Treat*Yr	1	0.001
Error B	6	0.035
Geno	16	0.172 **
Geno*Yr	15 †	0.020
Geno*Treat	16	0.117 **
Geno*Yr*Treat	15 †	0.026
Error C	182	0.017

** Significant at 0.01 level of probability.

† Degrees of freedom are 1 less for Yr x Geno and Yr x Treat x Geno as Pilose was included in 2003 only.

Table 9. Mean separation for average number of fleahoppers per 100 plants in 16 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled in the large field trial during 2002 and 2003 at Dallas, TX.

Genotypes	Leaf pubescence	NT	T	NT-T
Pilose	Hairy	150 a †	13 a †	137 a †
ATLAS	Hairy	58 b	3 b	55 b
1517-99	Hairy	53 bc	5 b	48 b
G.arb	Hairy	53 bc	10 a	43 b
WD-22h	Hairy	50 bc	3 b	47 b
STO 213	Hairy	47 bc	3 b	44 b
CAB-CS ‡	Smooth	42 cd	2 b	40 b
STO 474	Hairy	41 cde	3 b	38 b
PD 6186	Hairy	40 cdef	2 b	38 b
MAXXA	Hairy	40 cdef	2 b	38 b
SG 747	Hairy	30 defg	1 b	29 b
PMUTE	Smooth	28 efg	2 b	26 b
WD-22s	Smooth	28 efg	3 b	25 b
PD 22	Smooth	26 fg	4 b	22 b
DP 50	Smooth	23 g	5 b	18 b
LANKART	Hairy	22 g	1 b	21 b
WD-69s ‡	Smooth	17 g	1 b	16 b
Mean		41	3	40
CV (%)		81	251	

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
‡ Smooth-leaf and stem.

Percent Square Set

Percent square set data were not combined for the two years because plants were at different growth stages in each year when counts were made. In 2002, square counts were made when the plants averaged 9th and 15th node stages on 4 and 15 July, while in 2003, square counts were made when the plants averaged 13th and 17th node stages on 23 June and 7 July, respectively. Percent square set within each year was not combined across sample dates for the same reason.

In 2002, insecticide treatments and genotypes differed (p=0.01), and genotypes did not respond the same within the treatments, hence genotypes were separated within treatments (Table 10). Plants within genotypes did not vary in percent square set. No significant differences among the genotypes were observed in the treated blocks on either sample date in 2002, however genotypes were different (p=0.01) in the non-treated block (Tables 11 and 12). On 4 July, LANKART had higher (p=0.05) percent square set than all other genotypes except SG 747, WD-22h, STO 474, and DP 50 when not treated with insecticide to control fleahoppers. LANKART, SG 747, WD-22h, STO 474, DP 50, and WD-69s had significantly higher percent square set than PMUTE, PD 22, MAXXA, and *G.arboreum*. Significant differences in t-statistic were observed for percent square set between treated and non-treated blocks in all the genotypes except LANKART. This means that LANKART reacted the same to the insecticide treated and non-treated for percent square set. On 15 July, SG 747 had higher (p=0.05) percent square set than all other genotypes except STO 474, PMUTE, LANKART, WD-22h, and WD-69s when not treated with insecticide to control fleahoppers (Table 12). DP 50 had

Table 10. Mean squares for percent square set on 15 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled during 2002 in large field trial at Dallas, TX.

		Mean squares	
Source of variation	df	4 Jul	15 Jul
Treat	1	161759.528 **	83338.148 **
Error A	3	2414.843	190.547
Geno	15	1802.291 **	3449.280 **
Geno*Treat	15	1591.498 **	3805.788 **
Error B	86	363.241	215.086
Plants	4	370.165	428.409
Plants*Treat	4	334.687	225.040
Plants*Geno	60	342.116	186.682
Plants*Treat*Geno	60	374.609	185.589
Error C	352 ‡	247.826	142.367

** Significant at 0.01 level of probability.

‡ Error C degrees of freedom is 363 on 15 Jul, difference due to missing values.

Table 11. Mean percent square set per 20 plants on 15 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled on 4 Jul, 2002 in large field trial at Dallas, TX.

Genotypes	Leaf Pubescence	T	NT	T-NT	Value of t-statistic
MAXXA	Hairy	100	50 f †	50	8.01 **
STO 213	Hairy	100	64 cdef	36	5.02 **
WD-22s	Smooth	100	64 cdef	36	6.60 **
ATLAS	Hairy	100	62 def	38	8.15 **
WD-69s ‡	Smooth	99	68 bcde	31	5.30 **
SG 747	Hairy	99	82 ab	17	4.46 **
WD-22h	Hairy	99	77 abc	22	3.05 **
PD 6186	Hairy	99	64 cdef	35	5.30 **
STO 474	Hairy	99	76 abcd	23	3.29 **
PMUTE	Smooth	99	54 f	45	8.31 **
DP 50	Smooth	99	74 abcd	25	5.95 **
LANKART	Hairy	99	86 a	13	1.72
PD 22	Smooth	98	53 f	45	10.28 **
CAB-CS ‡	Smooth	98	62 def	36	6.25 **
1517-99	Hairy	98	57 ef	41	7.16 **
G.arb	Hairy	97	27 g	70	9.03 **
Mean		99	63		
CV (%)		4	36		

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
** Significant at 0.01 level of probability.
T=Treated and NT = Non-treated blocks.
‡ Smooth-leaf and stem.

Table 12. Mean percent square set per 20 plants on 15 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled on 15 Jul, 2002 in large field trial at Dallas, TX.

Genotypes	Leaf pubescence	T	NT	T-NT	Value of t-statistic
PD 22	Smooth	99	67 d †	32	7.56 **
PMUTE	Smooth	97	83 ab	14	4.41 **
G.arb	Hairy	97	4 e	93	23.31 **
CAB-CS ‡	Smooth	97	72 cd	25	6.32 **
WD-69s ‡	Smooth	95	79 abc	16	4.86 **
SG 747	Hairy	95	86 a	9	2.31 *
DP 50	Smooth	95	76 bc	19	4.09 **
1517-99	Hairy	95	72 cd	23	6.14 **
STO 474	Hairy	95	83 ab	12	3.09 **
WD-22s	Smooth	94	67 d	27	5.50 **
ATLAS	Hairy	93	68 d	25	5.73 **
WD-22h	Hairy	93	79 abc	14	1.84
PD 6186	Hairy	92	68 d	24	6.43**
LANKART	Hairy	91	83 ab	8	1.82
STO 213	Hairy	91	72 cd	19	6.04 **
MAXXA	Hairy	86	65 d	21	4.06 **
Mean		94	70		
CV (%)		10	20		

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
*, ** Significant at 0.05 and 0.01 levels of probability, respectively.
T = Treated and NT = Non-treated.
‡ Smooth-leaf and stem.

significantly higher percent square set than all the remaining genotypes except 1517-99, STO 213, and CAB-CS. However, no significant differences in percent square set were observed among WD-22h, WD-69s, DP 50, 1517-99, STO 213, CAB-CS. Significant differences in t-statistic were observed for percent square set between treated and non-treated, in all the genotypes except LANKART and WD-22h. This means that LANKART and WD-22h reacted the same to the insecticide treated and non-treated for percent square set.

On both sample dates in 2002, significantly higher percent square set was found in some of the hairy genotypes such as LANKART, SG 747, STO 474, and WD-22h and smooth genotypes such as DP 50 and WD-69s when compared to highly susceptible *G.arboreum*, when not treated with insecticide to control fleahoppers. Most researchers (Walker et al., 1974; Lukefahr et al., 1976) observed that plant damage due to fleahopper feeding was greater on glabrous than on hairy cottons. But, from this field trial, even some smooth genotypes were observed to have lesser square damage than certain hairy genotypes due to fleahopper feeding.

In 2003, on both 23 June and 7 July, insecticide treatments and genotypes differed ($p=0.01$), and genotypes did not respond the same within treatments (Table 13), hence genotypes were separated within the treatments. There were significant differences among the genotypes in percent square set on both sample dates in the treated and non-treated blocks except in the treated block on 23 June. On 23 June, Pilose had a higher ($p=0.05$) percent square set than other genotypes except SG 747 and DP 50 when not treated with insecticide to control fleahopppers (Table 14). Pilose, SG 747, DP

50, PD 6186, STO 213, LANKART, STO 474 had significantly higher percent square set than all other genotypes except WD-22h, WD-22s, WD-69s, ATLAS, and 1517-99. Differences (p=0.01) in t-statistic were observed for percent square set between treated and non-treated in all the genotypes except DP 50. This means that DP 50 reacted the same to the insecticide treated and non-treated for percent square set. On 7 July, Pilose had higher (p=0.05) percent square set than all other genotypes when not treated for fleahoppers (Table 15). Percent square set was significantly higher in Pilose, STO 213, SG 747, DP 50, STO 474, WD-69s, 1517-99, WD-22h, and LANKART than PMUTE, PD 6186, CAB-CS, and PD 22. Significant differences in t-statistic were observed for percent square set between treated and non-treated in all the genotypes, which means that all the genotypes did not react the same to the insecticide treated and non-treated. Again on both sample dates in 2003, percent square set was significantly higher in hairy genotypes such as Pilose, SG 747, STO 474, LANKART, and WD-22h and smooth genotypes such DP 50 and WD-69s when compared to the highly susceptible *G.arboreum*.

The t-tests were used to find differences in the percent square set between treated and non-treated within each genotype but, this test is not sufficient to separate interaction means which could elucidate differences in susceptibility to fleahopper feeding. Again, as discussed in the fleahopper count results in addressing the issue of preference as a food source by the fleahoppers, genotypes means for percent square set were separated within insecticide treatments as reported above and the interaction means

Table 13. Mean squares for percent square set on 16 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled in large field trial during 2003 at Dallas, TX.

		Mean squares	
Source of variation	df	23 Jun	7 Jul
Treat	1	81855.644 **	98872.947 **
Error A	3	1194.999	591.751
Geno	16	4215.858 **	8444.583 **
Geno*Treat	16	4615.839 **	1413.184 **
Error B	96	206.800	147.898
Plants	4	44.340	20.968
Plants*Treat	4	53.715	37.561
Plants*Geno	64	96.793	103.790
Plants*Treat*Geno	64	128.764	75.766
Error C	408	102.269	102.874

** Significant at 0.01 level of probability.

Table 14. Mean percent square set per 20 plants on 16 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled in large field trial on 23 Jun, 2003 at Dallas, TX.

Genotypes	Leaf pubescence	T	NT	T-NT	Value of t-statistic
G.arb	Hairy	100	0 g †	100	279.00 **
SG 747	Hairy	100	88 ab	12	3.94 **
STO 213	Hairy	98	84 bcd	14	4.70 **
STO 474	Hairy	98	81 bcd	17	5.79 **
Pilose	Hairy	98	92 a	6	2.58 **
LANKART	Hairy	97	82 bcd	15	3.42 **
WD-22s	Smooth	97	78 cde	19	4.20 **
PD 22	Smooth	95	73 e	22	4.44 **
PD 6186	Hairy	95	84 bcd	11	3.19 **
MAXXA	Hairy	95	73 e	22	5.98 **
WD-69s ‡	Smooth	95	78 cde	17	4.29 **
1517-99	Hairy	95	78 cde	17	7.24 **
ATLAS	Hairy	95	78 cde	17	4.56 **
PMUTE	Smooth	94	63 f	31	6.75 **
WD-22h	Hairy	92	79 cde	13	3.40 **
CAB-CS ‡	Smooth	92	56 f	36	7.70 **
DP 50	Smooth	90	85 abc	5	1.38
Mean		96	74		
CV (%)		5	18		

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
** Significant at 0.01 level of probability.
T = Treated and NT = Non-treated blocks.
‡ Smooth-leaf and stem.

Table 15. Mean percent square set per 20 plants on 16 genotypes of upland cotton and 1 Asiatic cotton genotype when fleahoppers were controlled and not-controlled in large field trial on 7 Jul, 2003 at Dallas, TX.

Genotypes	Leaf pubescence	T	NT	T-NT	Value of t-statistic
WD-69s ‡	Smooth	98 a	78 bcd	20	7.56 **
Pilose	Hairy	98 a	89 a	9	5.24 **
SG 747	Hairy	98 a	81 bc	17	8.43 **
STO 213	Hairy	97 ab	81 bc	16	5.91 **
WD-22h	Hairy	97 ab	78 bcd	19	7.28 **
PMUTE	Smooth	97 ab	60 e	37	9.53 **
STO 474	Hairy	97 ab	80 bc	17	6.16 **
WD-22s	Smooth	96 abcd	74 bcd	22	7.18 **
LANKART	Hairy	96 abcd	74 bcd	22	5.88 **
1517-99	Hairy	95 abcde	78 bcd	17	6.96 **
DP 50	Smooth	95 abcde	80 bc	15	4.49 **
ATLAS	Hairy	94 bcde	74 bcd	20	5.41 **
MAXXA	Hairy	93 cde	72 d	21	7.41 **
PD 6186	Hairy	93 cde	65 e	28	8.49 **
CAB-CS ‡	Smooth	93 de	51 f	42	11.11 **
PD 22	Smooth	92 e	62 e	30	5.54 **
G.arb	Hairy	58 f	0 g	58	12.82 **
Mean		93	69		
CV (%)		7	18		

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
** Significant at 0.01 level of probability.
T = Treated and NT = Non-Treated blocks.
‡ Smooth-leaf and stem.

were separated using $\sqrt{(4\ s^2/4)}$ as the standard error for the difference between interaction means rather than $\sqrt{(2\ s^2/4)}$ for the same reason as noted earlier.

In 2002, on 4 July, the mean differences in percent square set between treated and non-treated indicated that LANKART, SG 747, WD-22h, DP 50, and STO 474 reacted differently (p=0.05) in their ability to set more squares than *G.arboreum*, while on 15 July, all the genotypes reacted differently (p=0.05) in their ability to set more squares than *G.arboreum*, but not different from each other when not treated with insecticide to control fleahoppers (Table 16).

On 23 June 2003, Pilose, DP 50, PD 6186, SG 747, WD-22h, STO 213, and LANKART reacted differently (p=0.05) in their ability to set more squares than CAB-CS and *G.arboreum*, while on 7 July, *G.arboreum* expressed greater (p=0.05) sensitivity to fleahopper feeding than all other genotypes. All other genotypes were not different in their reaction to insecticide treatment than Pilose which expressed the greatest numerical resistance to fleahopper feeding.

Even though in 2002 and 2003, statistically no clear differences were observed among the adapted cotton genotypes in the mean differences in percent square set between treated and non-treated, numerically lower square damage was observed in certain hairy genotypes such as Pilose, LANKART, SG 747, WD-22h, and STO 474 and smooth genotypes such as DP 50 and WD-69s.

In both 2002 and 2003, surprisingly WD-69s, a smooth-leaf and stem genotype was observed as having fewer fleahoppers than most of the hairy and smooth genotypes

and on most sample dates it had relatively higher percent square set than all other smooth genotypes except DP 50, which also has hairy stems.

Small Field Trial: Detailed Evaluation of Fleahopper Damage

In order to determine differences in fleahopper preference and percent square set between nectaried and nectariless isolines of cotton, fleahopper (nymph and adult) and square counts were made on four and two sample dates, respectively, in 2002. There were no significant differences among sample dates nor date x genotype interaction, however there were differences ($p=0.05$) among the genotypes for fleahopper density across dates (Table 17). 119 H, which is a hairy and nectaried isoline of the upland cotton cultivar DES 119, had higher ($p=0.05$) mean number of fleahoppers per 100 terminals than 119 S, ne, a smooth and nectariless isoline (Table 18). However, no differences were observed among 119 H, 119 H, ne, and 119 S for mean number of fleahoppers per 100 terminals. Similarly, no differences were observed between 119 S and 119 S, ne. These data are similar to those reported above for hairy versus smooth genotypes.

Table 16. Mean differences in percent square set among cotton genotypes between treated and non-treated with insecticide for control of fleahoppers in 2002 and 2003.

				T-NT	
Genotypes	Leaf pubescence	4 Jul, 2002	15 July, 2002	23 Jun, 2003	7 July, 2003
G.arb	Hairy	69 a	93 a	99 a	58 a
MAXXA	Hairy	50 ab	21 b	22 bc	21 bcd
PD 22	Smooth	46 ab	32 b	22 bc	30 bcd
PMUTE	Smooth	45 ab	14 b	22 bc	36 abc
1517-99	Hairy	40 ab	23 b	17 cd	17 cd
ATLAS	Hairy	38 ab	25 b	17 cd	20 bcd
CAB-CS ‡	Smooth	36 ab	24 b	35 b	42 ab
STO 213	Hairy	36 ab	19 b	15 cde	16 cd
WD-22s	Smooth	36 ab	27 b	19 cd	23 bcd
PD 6186	Hairy	35 ab	29 b	11 cde	28 bcd
WD-69s ‡	Smooth	30 ab	16 b	17 cd	21 bcd
STO 474	Hairy	25 b	11 b	17 cd	17 cd
DP 50	Smooth	25 b	19 b	5 de	15 cd
WD-22h	Hairy	22 b	12 b	14 cde	19 cd
SG 747	Hairy	17 b	10 b	11 cde	18 cd
LANKART	Hairy	13 b	8 b	15 cde	21 bcd
§ Pilose	Hairy	-	-	1 e	9 d

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Bayes LSD.
§ Pilose included only in 2003.
T = Treated and NT = Non-treated blocks.
‡ Smooth-leaf and stem.

Schuster and Maxwell (1974), Schuster et al. (1976), and Agnew et al. (1982) stated that fleahopper densities, like tarnished bugs are often reduced in nectariless cotton. However, no differences in the fleahopper densities were observed between nectaried and nectariless genotypes within these isolines of hairy and smooth. But, hairy and nectaried isoline had significantly higher fleahopper numbers than the smooth and nectariless isoline.

Square counts and percent square set were determined in all 4 genotypes twice, with an 11 d interval between counts. These counts were made in mid and late-season when fleahopper numbers were expected to be high. Percent square set data was not combined for the two sample dates as the plants were at different growth stages on 7 and 18 July. No significant differences were found among genotypes for percent square set on either date (Table 19). However, on 7 July, there was a significant interaction between genotypes and plants suggesting some plant to plant variability in one or more of the isolines. Percent square set data are shown in Table 20.

Table 17. Mean squares for number of fleahoppers between the sample dates on 4 isolines of DES 119 (combinations of smooth leaves, hairy leaves, nectaried, and nectariless) in the small field trial during 2002 at Dallas, TX (number of fleahoppers data transformed).

Source of variation	df	Mean squares
Date	3	0.050
Error A	9	0.039
Geno	3	0.108 *
Geno*Date	9	0.017
Error B	36	0.035
Plants	4	0.044
Plants*Geno	12	0.025
Plants*Date	12	0.030
Plants*Geno*Date	36	0.025
Error C	192	0.029

* Significant at 0.05 probability level.

Table 18. Mean number of fleahoppers per 100 terminals on 4 isolines of DES 119 (combinations of smooth leaves, hairy, nectaried, and nectariless) across four sample dates in the small field trial during 2002 at Dallas, TX.

Genotypes	Leaf pubescence	Fleahopper numbers
119 H	Hairy, nectaried	20 a †
119 H, ne	Hairy, nectariless	18 a
119 S	Smooth, nectaried	10 ab
119 S, ne	Smooth, nectariless	4 b
Mean		13
CV (%)		270

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.

Table 19. Mean squares for percent square set on 7 and 18 July in 4 isolines of DES 119 (combinations of smooth leaves, hairy leaves, nectaried and nectariless) in the small field trial during 2002 at Dallas, TX (percent square set data transformed).

		Mean squares	
Source of variation	df	7 Jul	18 Jul
Geno	3	0.076	0.097
Error A	9	0.219	0.027
Plants	4	0.123	0.053
Plants*Geno	12	0.254 **	0.033
Error C	48	0.099	0.036

** Significant at 0.01 level of probability.

Table 20. Mean percent square set on 7 and 18 July in 4 isolines of DES 119 (combinations of smooth leaves, hairy leaves, nectaried, and nectariless) in the small field trial during 2002 at Dallas, TX.

		Percent square set	
Genotypes	Leaf pubescence	7 Jul	18 Jul
119 S	Smooth, nectaried	74	77
119 H	Hairy, nectaried	71	79
119 S, ne	Smooth, nectariless	66	73
119 H, ne	Hairy, nectariless	66	85
Mean		69	79
CV (%)		24	17

Field Ovipositional Preference Test

Ovipositional preference is another method used for identifying resistance. As it is time-consuming to count the number of eggs laid by fleahoppers, requiring microscopy, an easier way of counting the number of nymphs emerging per plant was necessary for identifying ovipositional preference. Two trials were conducted during 2003. In the first trial, 2 plants per plot were excised near the ground level and kept in separate polythene bags with dry paper towels. Plants were then taken to the lab where fleahopper nymphs were shaken onto a white paper and counted. Plants were placed back in the same bag and stored in an incubator at 30^0C. Plants were removed and fleahopper nymphs counted by the same method at 3 d intervals for 15 d. The entire process was repeated using only the top 3 main stem nodes and associated leaves and sympodia instead of the whole plant in the second trial.

There were no significant differences between the trials and trial x genotype interaction, however genotypes were different ($p=0.01$) as expected (Table 21). WD-69s had lower ($p=0.05$) numbers of nymphs than Pilose and WD-22h (Table 22). All other genotypes did not differ in density of nymphs.

Even though the number of nymphs emerging could not be separated statistically between hairy and smooth genotypes, numerically higher number of nymphs emerged from hairy genotypes than smooth genotypes except for *G.arboreum* and SG 747. There might be an evolutionary significance associated with this type of behavior by the female fleahoppers in preferring to lay more eggs on hairy genotypes than smooth genotypes.

Higher number of trichomes might be useful for the emerging fleahopper nymphs for shelter from wind and rain.

No-Choice Feeding Tests

In 2002, one trial of a no-choice feeding test was performed while two trials were conducted in 2003. In 2003, the methodology of handling and releasing the fleahoppers into the cages was different between the two trials. In the second trial of 2003, plastic plugs were replaced by cotton plugs in the vials to avoid condensation problems. Fleahoppers appeared to be more active when cotton plugs were used instead of plastic plugs. Apparently cotton plugs enabled air movement from the vial to the outside atmosphere and hence the condensation problems encountered with the plastic plugs were avoided.

Table 21. Mean squares for number of nymphs emerging per 8 plants between two trials on 16 genotypes of upland cotton and 1 Asiatic cotton genotype in field ovipositional preference test during 2003 at Dallas, TX (number of nymphs emerging data were transformed).

Source of variation	df	Mean squares
Trial	1	0.825
Error A	3	0.162
Geno	16	2.521 **
Geno*Trial	16	0.592
Error B	96	0.532

** Significant at 0.01 probability level.

Table 22. Number of nymphs emerging per 8 plants on 16 genotypes of upland cotton and 1 Asiatic cotton genotype in the field ovipositional preference test during 2003 at Dallas, TX.

Genotypes	Leaf pubescence	Number of nymphs emerged
Pilose	Hairy	58 a †
WD-22h	Hairy	21 b
1517-99	Hairy	17 bc
ATLAS	Hairy	16 bc
LANKART	Hairy	16 bc
STO 474	Hairy	15 bc
PD 6186	Hairy	15 bc
MAXXA	Hairy	15 bc
STO 213	Hairy	14 bc
DP 50	Smooth	12 bc
CAB-CS ‡	Smooth	12 bc
PMUTE	Smooth	9 bc
G.arb	Hairy	9 bc
WD-22s	Smooth	8 bc
PD 22	Smooth	8 bc
SG 747	Hairy	8 bc
WD-69s ‡	Smooth	7 c
Mean		15
CV (%)		40

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
‡ Smooth-leaf and stem.

No significant differences in percent square damage or number of live fleahoppers retrieved from the caged plants were observed neither across trials nor for the trial x genotype interaction (Tables 23 and 24). However, genotypes differed ($p=0.01$) for percent square damage (Table 23). WD-69s had lower ($p=0.05$) percent square damage than all other genotypes except DP 50, WD-22s, SG 747, STO 474, PD 6186, WD-22h, 119 H, ne, 1517-99, Pilose, and 119 H from the no-choice fleahopper feeding (Table 25).

In the field trails, which were preference tests, hairy genotypes such as Pilose, SG 747, STO 474, STO 213, LANKART, and WD-22h and smooth genotypes such as DP 50 and WD-69s had higher percent square set. However, in the no-choice tests conducted in the insectary, hairy genotypes such as SG 747, STO 474, WD-22h, and Pilose and smooth genotypes such as WD-69s and DP 50 had lower percent square damage. This suggests that SG 747, STO 474, WD-22h, Pilose, DP 50, and WD-69s may carry a level of resistance to fleahopper feeding relative to the other genotypes in this study.

Table 23. Mean squares among 3 trials for percent square damage on 20 genotypes of upland cotton and 1 Asiatic cotton genotype (Pilose included in 2003 only) in no-choice feeding tests during 2002 and 2003 at Dallas, TX (percent square damage data transformed).

Source of variation	df	Mean squares
Trials	2	48.717
Error A	11 †	32.077
Geno	20	18.633 **
Geno*Trials	39	6.093
Error B	209	5.734

** Significant at 0.01 probability level.
† Error A degrees of freedom are 11, (Rep) + (Trial x Rep) and the number of replications were 4 and 5 in 2002 and 2003, respectively.

Table 24. Mean squares among 3 trials for live fleahoppers obtained on 20 genotypes of upland cotton and 1 Asiatic cotton genotype (Pilose included in 2003 only) in no-choice feeding tests during 2002 and 2003 at Dallas, TX.

Source of variation	df	Mean squares
Trials	2	45.646
Error A	11 †	13.440
Geno	20	1.606
Geno*Trials	39	2.340
Error B	209	1.849

** Significant at 0.01 probability level.
† Error A degrees of freedom are 11, (Rep) + (Trial x Rep) and the number of replications were 4 and 5 in 2002 and 2003, respectively.

Table 25. Mean percent square damage and number of live fleahoppers obtained on 20 genotypes of upland cotton and 1 Asiatic cotton genotypes in the no-choice feeding tests during 2002 and 2003 at Dallas, TX.

Genotypes	Leaf pubescence	Percent square damage	Live fleahoppers
G.arb	Hairy	45 a	1.4
CAB-CS ‡	Smooth	38 ab	1.4
PD 22	Smooth	32 bc	1.8
PMUTE	Smooth	25 cd	1.2
ATLAS	Hairy	22 cde	1.7
MAXXA	Hairy	20 cde	1.1
119 S	Smooth	19 cdef	1.5
LANKART	Hairy	19 cdef	1.2
119 S, ne	Smooth	17 defg	1.5
STO 213	Hairy	16 defg	1.4
119 H	Hairy	14 defgh	1.4
Pilose	Hairy	14 defgh	1.5
1517-99	Hairy	13 efgh	2.6
119 H, ne	Hairy	12 efgh	1.1
WD-22h	Hairy	10 efgh	1.8
PD 6186	Hairy	9 efgh	1.4
STO 474	Hairy	7 fgh	1.1
SG 747	Hairy	6 gh	0.9
WD-22s	Smooth	6 gh	1.5
DP 50	Smooth	5 gh	1.1
WD-69s ‡	Smooth	2 h	1.6
Mean		17	1.4
CV (%)		110	95

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.
‡ Smooth-leaf and hairy stem.

Lab Tests

Preliminary trials (data not reported) were conducted to determine the length of time excised squares will be remain green and relatively turgid when placed on 2% agar in covered petri-plates and held at 30^0C in an incubator. Squares remained green and turgid for at least 3 d. In 2003, three trials were conducted to determine specifically which size square is preferred by fleahoppers.

In trials I and II, all three size squares, pin-head, match-head, and one-third grown, were used. Treatments (with and without fleahoppers) ($p=0.05$), square size, and square size x treatment interaction differed ($p=0.01$) for percent square damage in trials I and II (Table 26). Hence, square sizes were separated within each treatment. Percent square damage was higher ($p=0.05$) in pin-head size squares when compared with match-head and one-third grown squares in the petri-plates where fleahoppers were allowed to feed for 2 d (Table 27). No differences were observed among the square sizes in the petri-plates where there were no fleahoppers. Means in Table 27 suggest that the square size x treatment interaction was magnitude of response interaction and of no importance in this study.

In trial III, only pin-head and match-head size squares were used as no fleahopper damage was observed in the one-third grown squares in trials I and II. Treatments (with and without fleahoppers), square size, and treatment x square size interaction differed significantly for percent square damage in trial III (Table 28), hence square sizes were separated within each treatment. Percent square damage again was higher in pin-head size squares when compared to match-head size squares in petri-

plates where fleahoppers were released (Table 29). No differences were observed in percent square damage within square sizes in petri-plates where there were no fleahoppers.

Percent square damage was found to be significantly higher in pin-head sized squares when compared with match-head or one-third squares in all the trials (Tables 27 and 29). Plant bugs such as *Lygus lineolaris* prefers to feed on male reproductive tissue (i.e. staminal columns and developing anthers) in small square buds, which might be due to the considerable proportional area occupied by this tissue (Williams and Tugwell, 2000). In the larger square buds the relative proportion of the male reproductive tissue decreases as the female reproductive tissue grows. Along with the size of the square, differential levels of phytochemicals and nutrient contents have an influence on plant bug feeding. Studies with fleahoppers such as the one conducted by Chan et al. (1978) on the influence of four classes of cotton phytochemical constituents, *viz*., condensed tannins, flavonoids, terpene aldehydes, and cyclopropenoid fatty acids on larvae of *Heliothis virescens* might lead to a clearer understanding of host resistance toward fleahoppers. Also, there is a need for an in-depth analysis of the phytochemical contents in different sizes of square buds.

Table 26. Mean squares for percent square damage with and without fleahoppers in trials I and II in the lab tests, where 4 fleahoppers were released per petri-plate and allowed to feed for 2 d during 2003 at Dallas, TX (percent square damage data transformed).

Source of variation	df	Mean squares
Treat	1	43.896 *
Error A	8	5.401
Trial	1	8.482
Trial*Treat	1	1.386
Error B	8	3.428
Sqsz	2	107.919 **
Sqsz*Treat	2	52.360 **
Sqsz*Trial	2	0.908
Sqsz*Treat*Trial	2	7.128
Error C	32	3.087

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

Table 27. Mean percent square damage with and without fleahoppers in trials I and II in the lab tests, where 4 fleahoppers were released per petri-plate and allowed to feed for 2 d during 2003 at Dallas, TX.

	Percent square damage	
Square size	With fleahoppers	Without fleahoppers
Pin-head (≤ 3 mm)	73 a †	15
Match-head (>3 to ≤6 mm)	15 b	10
One-third grown (> 6 mm)	0 c	5
Mean	29	10
CV%	48	129

† Means within a column followed by the same letter are not different at K = 100 (approximates p = 0.05) according to Waller-Duncan LSD.

Table 28. Mean squares for percent square damage with and without fleahoppers in trial III in the lab tests, where 4 fleahoppers were released per petri-plate and allowed to feed for 2 d during 2003 at Dallas, TX (percent square damage data transformed).

Source of variation	df	Mean squares
Treat	1	74.768 **
Error A	4	2.601
Sqsz	1	29.646 **
Sqsz*Treat	1	9.180 *
Error B	8	0.972

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

Table 29. Mean percent square damage with and without fleahoppers in trial III in the lab tests, where 4 fleahoppers were released per petri-plate and allowed to feed for 2 d during 2003 at Dallas, TX.

	Percent square damage	
Square size	With fleahoppers	Without fleahoppers
Pin-head (≤3 mm)	76 a †	16
Match-head (>3 to ≤6 mm)	24 b	8
Mean	50	12
CV%	27	118

† Means within a column followed by the same letter are not different at $\alpha = 0.05$ according to t-Test LSD.

Correlation Studies

Correlation studies were conducted to determine the associations between fleahopper density, percent square set, and trichome density. *G.arboreum* and Pilose were excluded in these studies because these genotypes are not adapted to the U.S. in general and specifically the north. As expected, fleahopper density was positively correlated with trichome density on bracts, leaves, and stems (P=0.01) under field culture (Table 30). Similarly, a positive correlation was observed between percent square set and trichome density on bracts (P=0.01), leaves (P=0.02), and stems (P=0.01). However, the correlation coefficient values were low for fleahopper densities and percent square set with trichome densities on bracts, leaves, and stems suggesting an extremely weak association. These associations suggest that smoother leaf genotypes can be developed that will not be more susceptible to feeding and feeding damage than hairy-leaf types. Fleahopper density and percent square set were negatively correlated (Table 31), however, the correlation coefficient was extremely low, again suggesting little association. This is disturbing since it implies that low to moderate levels of decrease in preference or attractiveness may not result in reduced damage.

Nymphal emergence was positively correlated with the trichomes numbers on bracts (P=0.59) and leaves (P=0.55), and negatively correlated with trichome numbers on stems (P=0.58) (Table 32). However, the correlation coefficients were extremely low, suggesting little association.

Table 30. Pearson's correlation coefficients and the probability of larger r value, among trichome density measurements for 15 genotypes and total fleahopper numbers and percent square set in large field trial during 2002 and 2003 at Dallas, TX (*G.arboreum* excluded).

	Trichomes on bracts	Trichomes on leaves	Trichomes on stems
Fleahopper	0.21 †	0.17	0.11
density	0.01 ‡	0.01	0.01
Percent	0.11	0.07	0.11
square set	0.01	0.02	0.01

† Pearson correlation coefficients.
‡ Probability of a larger r value.

Table 31. Pearson's correlation coefficients and the probability of larger r value, between total fleahoppers numbers and percent square set for 15 genotypes in 2002 and 2003 at Dallas, TX (*G.arboreum* excluded).

	Fleahopper density	Percent square set
Fleahopper	1.00 †	-0.07
Density	0.00 ‡	0.10

† Pearson correlation coefficients.
‡ Probability of a larger r value.

Table 32. Pearson's correlation coefficients and the probability of larger r value, among trichome density measurements for 15 genotypes and fleahopper nymph emergence in 2003 at Dallas, TX (*G.arboreum* excluded).

	Trichomes on bracts	Trichomes on leaves	Trichomes on stems
Nymphal	0.10 †	0.11	-0.11
emergence	0.59 ‡	0.55	0.58

† Pearson correlation coefficients.
‡ Probability of a larger r value.

CONCLUSIONS

Screening for resistance to fleahopper is an important task toward developing cotton germplasm with resistance. The major aim of these experiments was to develop a reliable method to screen genotypes for resistance to fleahopper.

The research reported at this juncture directs the following conclusions:

1. Insect counts from the field trials were useful in determining the fleahopper preference. Comparison of average fleahopper numbers between the years was found to be useful in differentiating fleahopper preference among cotton genotypes. Numerically, higher numbers of fleahoppers were observed on hairy genotypes than on smooth genotypes.

2. Hairy genotypes such as Pilose, LANKART, SG 747, WD-22h, and STO 474 and smooth genotypes such as DP 50 and WD-69s had numerically higher percent square set than other genotypes in the field trial. Hence these genotypes were relatively resistant based on the percent square set.

3. Trichome and fleahopper density were weakly and positively correlated, indicating that trichomes have some influence on fleahopper density. Surprisingly, even though Pilose had greater density of fleahoppers than any other genotype, it retained a higher percent of squares suggesting it is tolerant to fleahopper damage. However, *G.arboreum* a hairy genotype was found to be highly susceptible to fleahopper damage, hence resistance to fleahoppers may or may not be associated to trichome density but there might be some other trait which is enabling certain cotton genotypes to have lesser

square damage. Or it may be trichome length and not the trichome density that is responsible for inducing resistance in cotton toward fleahoppers. From the physical comparison of trichome lengths, *G.arboreum* had short and flat trichomes while Pilose had long and erect trichomes, which might act as a physical barrier for the fleahoppers to feed.

4. Smooth and nectariless isoline of DES 119 was less preferred by fleahoppers when compared with nectaried and nectariless isolines of DES 119 H. However, no differences in square damage by fleahoppers were observed among these genotypes.

5. Non-preference for oviposition was used as a mechanism of resistance for differentiating resistant genotypes from susceptible. Numerically, higher numbers of fleahoppper nymphs emerged from hairy-leaf genotypes than from smooth-leaf genotypes.

6. Percent square damage was lower in WD-69s, SG 747, 1517-99, DP 50, LANKART, and Pilose when compared to PD 22, CAB-CS, MAXXA, and *G.arboreum* in the no-choice feeding tests.

7. Pin-head size squares are preferred by fleahoppers for feeding compared with match-head and one-third grown squares.

FUTURE CONSIDERATIONS

Field trials should be continued for identifying cotton genotypes resistant to fleahopper. Fleahopper counts should be made starting from the 6th node stage of crop growth until the full bloom stage. Visual counts for fleahoppers can be made when the plants are at first squaring, however visual counts would be stressful and time-consuming when the plants start putting more nodes. Hence, beat bucket sampling technique (Knutson and Wilson, 1999; Muegge et al., 2003) can be very useful for counting fleahoppers on individual plants as it is easy, reliable, and less time-consuming. However, there is possibility that the plants used for beat bucket sampling might have additional factors that cause the square loss other than insects, such as human damage. Hence, it is advisable to have separate fields for fleahopper counts and square counts, provided that there is no limitation for land and seed. By having separate field for square counts, destructive sampling can be performed wherein the sample plants can be collected from field and mapping for healthy, damaged, and missing squares can be made in the lab instead of performing it in the field.

Even though the data from field tests are much more reliable, these tests are time-bound and require a lot of effort and space. Hence, an easier technique to screen cotton genotypes can be used such as the one performed in the lab using cotton squares on agar medium in the petri-plates with a known number of fleahoppers per plate. And also these tests can be conducted year round provided there is availability of fleahopppers. Efficient rearing techniques have been identified for fleahoppers (Lopez and Parker

personal communication, 2004) which will provide these insects in sufficient numbers for conducting different tests year round. Field and no-choice test results suggested that trichomes may not be a trait contributing towards host plant resistance from fleahopper feeding. Hence, tests can be performed using cotton squares with and without bracts as a food source for fleahoppers.

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