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ARTHROPOD MANAGEMENT

Cotton Fleahopper (Heteroptera: Miridae) Responses to Volatiles from Selected Host Plants

Kenneth R. Beerwinkle* and Henry F. Marshall

INTERPRETIVE SUMMARY

Several field studies have indicated that cotton fleahoppers prefer some wild host plants instead of cotton. We conducted laboratory studies to determine if odors from selected flowering weed host plants were more attractive to fleahoppers than were odors from squaring cotton plants. If so, synthetic attractants that mimic the odors from the preferred wild plants might be developed and used to cause fleahoppers to concentrate in restricted areas of cotton or other crops where they could be controlled with applications of insecticides; thus, alleviating the need for broadcast spraying of whole cotton fields.

Alternatively, synthetic attractants might be combined with a killing agent to formulate attract-and-kill baits that would be selective for fleahoppers. We tested three different flowering weed host plants and found that fleahoppers were attracted by odors from each in preference to odors from squaring cotton plants. Further, we were able to collect the chemicals in the weed odors and then revolatilize them. Odors from the revolatilized chemicals continued to be attractive to fleahoppers. These results indicate there is a good possibility the attractant chemicals in the odors emitted from the weed hosts tested can be isolated and identified, thereby enabling the formulation of synthetic mimics of these attractive odors.

ABSTRACT

Several field studies have indicated that cotton fleahoppers *(Pseudatomoscelis seriatus* Reuter) prefer some wild host plants instead of cotton (*Gossypium hirsutum* L.). The relative attractiveness of volatiles from selected host plants to adult cotton fleahoppers was determined in a series of two-choice olfactometer bioassays. We found that fleahoppers were attracted by volatiles from each of three flowering wild hosts - false ragweed (*Parthenium hysterophorus* L.), croton (*Croton capitatus* Michx.), and horsemint (*Monarda punctata* L.) - in preference to volatiles from squaring cotton. The insects preferred false ragweed volatiles to those of croton and horsemint, which were comparable in attractiveness. Revolatilized chemical compounds, collected from the head-space volatiles of each of the three wild host plants tested, retained their attractiveness. These results indicate reasonably good potential for successful isolation and identification of the preferred attractants, and the subsequent development of synthetic mimic attractants that may be useful in the development of new attractant-based biorational management techniques for cotton fleahoppers.

The cotton fleahopper is an important early-season pest of cotton in Texas, Oklahoma, Louisiana, and Mississippi that caused estimated losses of more than $36 million in 1997 (Williams, 1998). Adults and nymphs damage young cotton plants by sucking the sap from young squares and terminal growth, causing excessive fruit shedding and abnormal whip-like growth of the plant (Reinhard, 1926).

Fleahoppers feed and reproduce on a large number of wild plants (Reinhard, 1926, 1927; Schuster et al., 1969), with the most important species belonging to the genera *Oenothera*, *Monarda*, *Solanum*, and *Croton* (Hixson, 1941). They overwinter as diapausing eggs that are inserted during autumn under the bark on woody stems of senescent plants, primarily *Croton* spp. in Central Texas.

Egg diapause is broken in the spring with the onset of warmer temperatures and increased rainfall. Newly hatched nymphs feed on an assortment of spring weed species, including *Oenothera* and

Kenneth R. Beerwinkle, Agricultural Engineer, Areawide Pest Management Research Unit, USDA, ARS, SPARC, 2771 F&B Road, College Station, TX 77840; Henry F. Marshall, Chemist, Areawide Pest Management Research Unit, USDA, ARS, SPARC, 2771 F&B Road, College Station, TX 77840.
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Monarda, and mature to the adult stage in 9 to 20 d, depending on temperatures (Little and Martin, 1942). The fleahoppers complete one or more generations on the weed hosts.

As the season progresses, the wild host plants mature and become increasingly less attractive to adult fleahoppers, which then migrate to cotton (Almand et al., 1976). This migration usually occurs as cotton is beginning to develop young flower buds (pinhead squares). The cotton plant is most attractive and susceptible to fleahopper attack during the first few weeks of squaring.

In cotton production areas where the boll weevil is an important pest, fleahoppers and emerging overwintered weevils move into squaring cotton at about the same time. Conventional management strategies usually involve two or more early-season insecticide applications to control both pests. The insecticides applied to control fleahoppers and boll weevils also frequently kill many arthropods that are important natural enemies of *Helicoverpa* and *Heliothis* spp. The suppression of populations of beneficial arthropods by early-season insecticide applications can lead to damaging outbreaks of *Helicoverpa* and *Heliothis* spp. that otherwise would have been held below economic thresholds (Anonymous, 1973; Gaines, 1942; Ewing and Ivy 1943; Ridgway et al., 1967).

Several reports (Reinhard 1926; Little and Martin, 1942; Holtzer and Sterling, 1980) have indicated that fleahoppers prefer flowering weed hosts such as horsemint and croton over cotton. Our objective was to evaluate the relative attractiveness of volatiles from selected wild host plants and fruiting cotton to adult fleahoppers using two-choice olfactometers.

MATERIALS AND METHODS

Fleahoppers

Both laboratory-reared and field-collected adult fleahoppers were used in the bioassays. Laboratory fleahoppers were reared using methods similar to those described by Breene et al. (1989). Senescent stems of croton containing overwintering fleahopper eggs were collected in Brazos County near College Station, TX, during late February 1996 to 1998 and held in burlap bags in storage at about 10 °C. Croton stems were removed from storage as needed, cut to uniform lengths of about 13 cm, and loosely packed into open ended cans (1.1 kg coffee cans with both ends screened). The cans containing the croton stems were then immersed in tap water for 15 min, allowed to drain for about 30 min, and placed in an environmental chamber maintained at about 27 °C with a relative humidity of 65 to 85% and a 12:12 [light: dark] photoperiod. The water immersion process was repeated every 2 d to simulate spring rains and initiate fleahopper eclosion.

Fleahopper nymphs began to emerge after 5 to 14 d of this alternating wet-dry cycle, and were collected daily by vigorously shaking the cans over a collection funnel that emptied the nymphs and loosened plant debris into a 0.5-L wide-mouth polyethylene bottle (Consolidated Plastics Co., Twinsburg, OH). After placing a 4-cm-long section of fresh green bean (*Phaseolus* spp.) and a 0.7-cm thick slice of potato (*Solanum tuberosum* L.) on a screen platform in the bottom of the bottle to furnish moisture and food for the nymphs, the bottle was closed with a nylon organza-covered cap.

Green bean and potato slices were replaced four to six times per week. When nymphs were third to fourth instars, they and their accompanying croton debris were transferred to a 0.5-L container (8.3-cm diameter by 8.5-cm depth) that was light-tight except for a 1.3-cm diameter orifice on the side of the container near the bottom.

The orifice was connected to a 2.0-cm-long section of clear Tygon plastic tubing that formed a closed passageway to a clear plastic container (9.5-cm diam. by 6.5-cm depth) that had ventilation ports covered with fine nylon mesh and contained sections of green bean and potato. The connected containers were held in an environmental chamber for about 24 h so that most of the nymphs, attracted by light (Breene and Sterling, 1988) and food, left the debris in the darkened container and moved into the lighted container. The debris-free nymphs were then returned to their original containers until they matured to adults in 18 to 21 d.

When dense populations of wild fleahoppers were present on flowering croton plants around College Station, TX (usually between late July and mid-October each year), we used field-collected cotton fleahoppers in olfactometer experiments. Feral fleahoppers were collected by sweeping croton plants

with a KISS (Keep-It-Simple Sampler) engine-driven pneumatic sampler (Beerwinkle et al., 1997). The collections were returned to the laboratory for processing.

Fleahoppers were separated using the same procedure described above for separating the laboratory-reared nymphs from croton debris. In addition, some hand separation was required to remove unwanted spiders and nontarget insects.

Olfactometer experiments were usually conducted <48 h after capture of the feral fleahoppers, which were primarily late instars and adults. They were maintained during this time in an environmental chamber supplied with green bean and sliced potato for food as described for laboratory rearing.

Olfactometers

Two-choice olfactometers (Fig.1) were constructed of clear acrylic. The arena enclosure (A, Fig.1) was constructed with 3.2-mm-thick sheets in a triangular shape with a base width of 20.3 cm, a truncated apex height of 34.3 cm, and a wall height of 3.8 cm. Two combination volatile-source/fleahopper-capture chambers (B1 and B2, Fig.1) were each fabricated with a pair of hinged clear plastic boxes (5.1 by 5.1 by 5.1-cm, Ward's Natural Sciences, Rochester, NY) stacked on top of each other. The bottom of the upper (volatile source) chamber was glued to the top of the lower (capture) chamber.

Orifices, 3.0 cm in diameter and centered in the bottom of the upper chamber and the top of the lower chamber, respectively, were separated with fine-mesh nylon screen (14.6 by 14.6 threads per cm, McMaster-Carr Supply Co., Chicago, IL). Two 2.2-cm diameter orifices in the base wall of the triangular arena enclosure, each centered about 3.3 cm from their respective base corners, opened into orifices in the respective capture chambers. Each of the two orifices in the base wall of the arena was fitted with a 2.0-cm long, horizontally oriented mesh nylon cone with a 3.0-mm diameter opening at the apex. Each cone was attached with hot glue so it extended into the capture chamber to restrict escape of attracted fleahoppers.

Metered prepurified air from the air supply system described by Beerwinkle et al. (1996) flowed with positive pressure through individual tubes

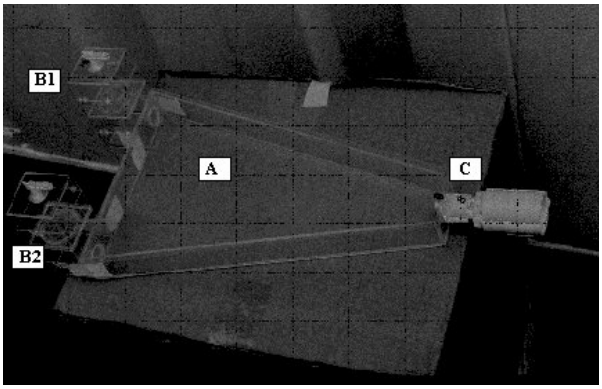

Fig. 1. Two-choice cotton fleahopper olfactometer: A, arena enclosure; B1 and B2, volatile-source/fleahopper-capture chambers; C, exhaust fitting.

connected to inlet fittings at the tops of each of the two volatile source chambers (B1 and B2, Fig.1) at a flow rate of about 1.2 L min^{-1}.

The air passed through each of the pairs of volatile-source chambers and the fleahopper-capture chambers into the arena, and then was exhausted from the arena through a fine mesh, nylon screen covering the passageway through the PVC fitting (C., Fig.1) into a section of flexible polyvinyl tubing that connected the olfactometer to an exhaust manifold serving three additional olfactometers. The manifold was connected to an exhaust duct.

A squirrel-cage blower (Model 4C442, W.W. Grainger, Houston, TX), mounted in series with the exhaust duct, pulled air from the olfactometers and delivered it to the outside of the laboratory. The capacity of the fan was regulated such that the internal pressure in each of the olfactometer arenas was maintained at a slight negative pressure (≈0.4-mm water column) relative to the ambient external chamber pressure in the laboratory. Thus, each olfactometer unit was effectively a closed system and no test volatiles escaped into the surrounding room atmosphere.

Arena enclosure internal pressures were monitored with a wall-mounted manometer (Mark II Series Model MM-80, Dwyer Instruments, Michigan City, IN). The room was maintained at about 27°C with a relative humidity of 65 to 85%.

Bioassay Experiments

Typical assays were conducted by comparing fleahopper responses to pairs of volatile sources. After the volatile sources were in place, about 40 to

50 adult fleahoppers were lightly anesthetized with CO_2 and placed in the enclosed arena where they were exposed to air streams carrying the volatiles. After 4 to 5 h of exposure to the volatile choices, the olfactometers were inspected to determine the numbers of fleahoppers that had responded to the respective volatile sources, the number of dead fleahoppers in the arena, and the number of fleahoppers in the arena that were alive but had not responded to the volatiles.

Four sets of bioassay experiments were conducted. In the first set of experiments, responses of fleahoppers to each of seven different sources of plant volatiles were compared with their responses to blank volatile sources (air only). The seven plant volatile sources included bouquets of young cotton flower buds (squares) and bouquets of plant parts from each of six different selected wild hosts, including blooms and other parts of croton and horsemint, and blooms of cutleaf evening primrose (*Oenothera laciniata* Hill), false ragweed (*Parthenium hysterophorus* L.), firewheel (*Gaillardia pulchella* Gray), and the common sunflower (*Helianthus annuus* L.).

These six wild host plants were selected for this series of experiments for the following reasons. Horsemint, croton, and cutleaf evening primrose previously were identified by several investigators (Reinhardt, 1926; Hixson, 1941; Almand et al., 1976; Holtzer and Sterling, 1980) as three of the more important wild hosts of cotton fleahoppers. False ragweed was selected because preliminary tests indicated that it was attractive to fleahoppers. Firewheel was selected for testing because a collaborator observed heavy infestations of fleahoppers on flowering firewheel plants in Bell County, TX, during mid-June 1996. Finally, common sunflower was tested because it was readily available and it has been identified as a fleahopper host in the Rio Grande Valley of Texas (Schuster et al., 1969). Typical bouquets of plant parts filled the volatile source chambers about one-half full (≈65 cm^3) when loosely packed.

In the second set of experiments, responses of fleahoppers to volatiles from bouquets of plant parts from each of croton, horsemint, and false ragweed were compared with responses to volatiles from fruiting terminals of cotton plants in paired-choice assays. In similar paired-choice assays, the third set of experiments compared fleahopper responses with all possible paired choices between volatiles from croton, horsemint, and false ragweed.

The fourth set of experiments examined the responses of fleahoppers to revolatilized odors from compounds originally adsorbed from head-space volatiles emanating from bouquets of croton, horsemint, or false ragweed. The head-space volatile compounds were collected from bouquets of blooms by adsorption on Super Q resin (80/100 mesh, Allteck Associates, Deerfield, IL) filled glass columns (0.25 by 10.5 cm). The columns were filled with 2.5 cm of resin that was held in place with glass wool plugs. Bouquets of blooms were loosely packed in acrylic cylinders (8 cm i.d. by 20 cm height) that were airtight except for an inlet port at the bottom and an outlet port at the top. Air was pulled by vacuum at a flow rate of about 200 mL min^{-1} through a resin-filled trap column before passing through the plant bouquet and a second resin-filled collection column.

Collection periods were generally about 3 h, and the adsorbed volatile components were eluted with methylene chloride to give an eluate volume of 1.0 mL. Individual lures for olfactometer bioassays were prepared by pipetting 200 µL of eluate on cotton dental rolls. In the olfactometer experiments, fleahopper responses to the revolatilized head space volatile compounds were compared with their responses to dental roll blanks containing 200 µL methylene chloride.

Only a portion of the fleahoppers exposed to pairs of volatile sources in individual assays in the four sets of experiments responded by entering a capture chamber of choice in the olfactometer. Some died in the arena of apparently natural causes (usually <15%), while others either were not attracted by the test volatiles or otherwise failed to respond for various reasons. The number of fleahoppers exposed to volatiles in each test was adjusted for the observed natural mortality before calculating the percentages responding to each of the two choices. A ratio of the percent responses to one volatile source relative to the other was used as an indicator for assessing the degree of preference for the preferred volatile in each series of two-choice experiments and for comparing the relative attractiveness of different volatiles across experiments.

Response data in percentages from the various bioassay experiments were transformed using the

inverse sine transformation prior to statistical analyses. Differences in mean response levels for field-collected and laboratory-reared fleahoppers to the various attractants were evaluated using ANOVA, and differences in mean response levels to paired volatile sources were compared with paired-sample t-tests.

RESULTS AND DISCUSSION

Fleahopper responses were significantly positive ($P < 0.01$, paired t-test) to each of the plant volatiles tested against air blanks in the first set of experiments, and response ratios ranged from a low of 1.59 for laboratory-reared insects exposed to cutleaf evening primrose volatiles to a high of 4.00 for feral insects exposed to false ragweed volatiles (Table 1). In each experiment in which both field and laboratory fleahoppers were exposed to the same pairs of volatiles (croton, horsemint, and false ragweed blooms, respectively, versus air blanks), the mean percentage of total response for field-collected fleahoppers was significantly higher than for laboratory-reared fleahoppers ($P < 0.05$, Table 1) suggesting that the feral fleahoppers were more vigorous and responsive than those reared in the laboratory. The percentages of feral and laboratory fleahoppers that responded to air blanks were similar, but comparatively greater percentages of feral fleahoppers responded positively to the respective plant volatile sources, resulting in correspondingly higher levels of total response and larger response ratios. Thus, the response behaviors of the feral and laboratory fleahoppers were qualitatively similar but quantitatively different. These results indicate that fleahoppers from both sources may be used to bioassay attractant volatiles, but that results obtained for fleahoppers from the two different sources are not directly comparable.

The rank of plant/blank response ratios (Table 1) for laboratory-reared fleahoppers indicated that, among the volatiles tested, those from false ragweed, horsemint, croton, and firewheel were more likely to attract fleahoppers than were volatiles from squaring cotton plants. Results of the second set of experiments in which fleahoppers were exposed to paired choices between volatiles from bouquets of false ragweed, horsemint, and croton blooms, respectively, and squaring cotton confirmed that fleahoppers were attracted by volatiles from each of the three wild host plants in preference to volatiles from fruiting cotton plants. The mean percent

Table 1. Adult cotton fleahopper mean responses (±SE) to volatiles from bouquets of selected plant parts and air blanks in two-choice olfactometers.

Plant material	Fleahopper type†	No. tests	Mean percent total response‡	Mean percent response §		Ratio Plant/Blank
				Plant	Blank	
F. Ragweed blooms	Field	24	80 ± 3*¶	64 ± 3	16 ± 2***	4.00
	Lab	8	67 ± 4	49 ± 4	18 ± 2***	2.72
Horsemint blooms	Field	31	78 ± 2***	60 ± 3	18 ± 2***	3.33
	Lab	95	59 ± 2	41 ± 1	18 ± 1***	2.28
Croton blooms	Field	62	81 ± 2***	62 ± 2	19 ± 1***	3.26
	Lab	51	53 ± 2	35 ± 2	18 ± 1***	1.94
Croton (prebloom)#	Lab	18	68 ± 2	50 ± 2	18 ± 2***	2.78
Firewheel blooms	Lab	39	56 ± 2	39 ± 2	17 ± 1***	2.29
Cotton squares††	Lab	29	46 ± 3	30 ± 2	16 ± 2***	1.88
Sunflower blooms	Lab	12	47 ±4	30 ± 3	17 ± 1**	1.76
Horsemint (prebloom)#	Lab	8	65 ± 4	41 ± 3	24 ± 3**	1.71
Cutleaf E. Primrose	Lab	14	70 ± 3	43 ± 2	27 ± 2***	1.59

* Significant at $P = 0.05$.

** Significant at $P = 0.01$.

*** Significant at $P = 0.001$.

† "Lab" indicates test fleahoppers were laboratory-reared, and "Field" indicates they were field-collected.

‡ Mean total percentage of exposed fleahoppers per test that responded to the two volatile sources.

§ Mean percentages of fleahoppers that responded to each of the two choices. Statistical differences in the means were evaluated with the paired-sample t-test.

¶ Statistical differences in responses of laboratory-reared and field-collected fleahoppers to volatiles of the same plant parts in the respective sets of tests were evaluated with ANOVA.

Prebloom croton and horsemint bouquets were comprised of leaves and stems from the top parts of the young plants.

†† Cotton squares ranged from pinhead to one-third-grown in development and bouquets included adjacent stems and leaves.

Table 2. Adult cotton fleahopper mean responses (±SE) to volatiles from wild plant bouquets of croton, horsemint, and false ragweed, respectively, versus bouquets of cotton squares and branches in two-choice olfactometers.

Wild plant	Fleahopper type	No. tests	Mean percent total response †	Mean percent response ‡		Ratio Plant/C. squares
				Plant	Cotton squares	
Croton	15 Lab § 10 Field	25 ¶	62 ± 3	37 ± 2	25 ± 2***	1.48
Horsemint	Lab	16 #	73 ± 3	44 ± 3	29 ± 3**	1.52
False Ragweed blooms	Field	17	72 ± 5	51 ± 4	21 ± 2***	2.43

** Significant at $P = 0.01$.
*** Significant at $P = 0.001$.
† Mean total percentage of exposed fleahoppers per test that responded to the two volatile choices.
‡ Mean percentages of fleahoppers that responded to each of the two choices. Statistical differences in the means were evaluated with the paired-sample t-test.
§ No significant difference in responses of laboratory-reared and field-collected fleahoppers (ANOVA, α=0.05) so results were combined for this analysis.
¶ Croton plant bouquets included 8 bouquets of upper leaves and stems of young prebloom plants and 17 bouquets of blooms with no significant difference in fleahopper responses (ANOVA, $\alpha = 0.05$).
# Horsemint plant bouquets included 4 bouquets of upper leaves and stems of young prebloom plants and 12 bouquets of blooms with no significant difference in fleahopper responses (ANOVA, $\alpha = 0.05$).

Table 3. Adult cotton fleahopper mean responses (±SE) to respective pairs of volatiles from bouquets of croton, horsemint, and false ragweed blooms.

Plant 1 vs. Plant 2	No. tests	Mean percent total response †	Mean percent response ‡		Ratio Plant 1/Plant 2
			Plant 1	Plant 2	
Croton vs. Horsemint	16	73 ± 3	40 ± 3	33 ± 4	1.21
False Ragweed vs. Croton	17	86 ± 2	51 ± 3	35 ± 3*	1.46
False Ragweed vs. Horsemint	16	70 ± 2	43 ± 3	27 ± 2**	1.59

* Significant at $P = 0.05$.
** Significant at $P = 0.01$.
† Mean total percentage of exposed fleahoppers per test that responded to the two volatile choices.
‡ Mean percentages of fleahoppers that responded to each of the two choices. Statistical differences in the means were evaluated with the paired-sample t-test.

responses of fleahoppers were significantly higher ($P < 0.01$, paired t-test) to volatiles from each of the wild host plants compared with their responses to volatiles from fruiting cotton plant parts (Table 2).

The range and rank of the wild host plant/fruiting cotton plant response ratios (Table 2) indicated that the volatiles from croton and horsemint plants were comparable in their superior attractiveness to volatiles from fruiting cotton; and the attractiveness of volatiles from false ragweed was superior to the attractiveness of volatiles from croton and horsemint.

When fleahoppers were exposed to all possible combinations of paired choices between volatiles from croton, horsemint, and false ragweed in the third set of experiments (Table 3), a similar trend was observed. The mean percent response of fleahoppers to croton volatiles was numerically higher than the response to horsemint volatiles, but the response percentages were not statistically different. However, fleahopper responses to false ragweed volatiles were significantly higher than their responses to either croton or horsemint volatiles ($P < 0.05$, paired t-test).

Results from the fourth set of experiments (Table 4) - in which fleahoppers were exposed to choices between revolatilized head space volatile compounds collected from croton, horsemint, and false ragweed, respectively, and air blanks - were significantly positive for each of the three volatile sources ($P < 0.001$, paired t-test). These results suggest there is good potential for isolating and identifying the active attractant chemical compounds in the volatiles of each of the three weed species tested.

Successful identification of the active chemicals would enable formulations of synthetic attractants that might be useful for developing new attractant-based biorational management techniques for fleahoppers. Synthetic attractants might be used to cause feral fleahoppers to concentrate in defined areas of cotton or some suitable factitious crop where they could be controlled with applications of insecticides only in the defined areas; thus,

Table 4. Adult field-collected cotton fleahopper mean responses (±SE) to revolatilized chemicals collected from head space volatiles (HSV) from croton, horsemint, and false ragweed, respectively, versus methylene chloride blanks.

Plant	No. tests	Mean percent total response †	Mean percent response ‡		Ratio HSV/Blank
			HSV	Blank	
Croton	27	72 ± 2	48 ± 2	24 ± 2***	2.00
Horsemint	49	51 ± 3	34 ± 2	17 ± 1***	2.00
False Ragweed	20	81 ± 3	51 ± 3	29 ± 3***	1.76

*** Significant at $P = 0.001$.

† Mean total percentage of exposed fleahoppers per test that responded to the two volatile choices.

‡ Mean percentages of fleahoppers that responded to each of the two choices. Statistical differences in the means were evaluated with the paired-sample t-test.

alleviating the need for broadcast spraying of whole cotton fields. Alternatively, synthetic attractants might be combined with biologically active materials to formulate attract-and-kill baits that are selective for fleahoppers. Successful development of these techniques holds potential for improving management of fleahopper pests through a reduction in use of synthetic pesticides and, coincidentally, a decrease in the detrimental effects on early-season beneficial insect faunas in cotton. Such techniques could contribute greatly to improved integrated pest management (IPM) strategies for cotton production, especially in areas where boll weevils have been eradicated.

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