

## ARTHROPOD MANAGEMENT & APPLIED ECOLOGY

### *Halyomorpha halys* (Hemiptera: Pentatomidae) Feeding Injury on Cotton Bolls

Katherine Kamminga\*, D. Ames Herbert, Michael D. Toews, Sean Malone, and Thomas Kuhar

#### ABSTRACT

**The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), is rapidly spreading from the mid-Atlantic region southward into cotton (*Gossypium hirsutum* L.) producing areas of the U.S. Stink bugs are one of the most economically damaging insect pests of cotton and feeding by stink bugs on cotton bolls can result in a reduction of lint yield and quality at harvest. Field cage studies were conducted in Virginia to determine if boll size influenced *H. halys* adult and nymphal feeding behavior. In 2011 and 2012, no-choice feeding experiments were conducted with three boll sizes (1.8, 2.8, and 3.2 cm diameter). The 2011 no-choice experiment also included the adult life stage of the native green stink bug, *Chinavia hilaris* (Say). In addition to the experiments with adults, two feeding choice experiments were conducted during 2012 with *H. halys* nymphs. The insects were placed into cages enclosing entire cotton plants with different boll sizes. Results of the no-choice experiment from 2011 indicated that feeding injury caused by *C. hilaris* was not significantly different among the different boll sizes offered, whereas injury by *H. halys* increased as boll size increased. Results from the choice experiment also revealed increasing feeding damage by *H. halys* as boll size increased. These data indicate that the addition of *H. halys* to the current stink bug complex in cotton might warrant a reevaluation of the current field scouting methods and management recommendations that show that large cotton bolls (> 3.2 cm diameter) are safe from stink bug feeding injury.**

Since the introduction of transgenic *Bacillus thuringiensis* spp. *kurstaki* Berliner cotton (Greene et al., 1999; Turnipseed et al., 1995) and the concomitant reduction in insecticide applications for lepidopteran pests, stink bugs have become one of the primary pests of cotton (*Gossypium hirsutum* L.) production. The most common stink bug pest species of cotton in the southeast U.S. are the southern green stink bug, *Nezara viridula* (L.), green stink bug, *Chinavia hilaris* [formerly *Acrosternum hilare*] (Say), and brown stink bug, *Euschistus servus* (Say). Feeding by these phytophagous stink bugs can cause wart-like growths on inner carpel walls, stained lint, and shriveled seeds resulting in economic loss to growers (Bundy et al., 2000; Emfinger et al., 2004; Wene and Sheets, 1964). Regardless of species, these stink bugs are treated as a single complex in terms of scouting methods and injury potential to cotton.

In the late 1990s, the brown marmorated stink bug, *Halyomorpha halys* (Stål), (Hemiptera: Pentatomidae), was introduced from China into Allentown, PA. This stink bug species has quickly spread across the continental U.S. and into Canada. As a highly polyphagous insect, with a plant host list spanning greater than 100 species, it is an economically important pest of many vegetables, field crops, tree fruit (Hoebeck and Carter, 2003; Hoffman, 1931; Leskey et al., 2012), and possibly tree nuts (Hedstrom et al., 2013). *H. halys* will feed on okra (Kuhar et al., 2012) and other members of the Malvaceae, including cotton.

*H. halys* was first reported in Virginia in 2004 (Day et al., 2011) and subsequently observed in a Virginia cotton field in 2012 (D.A. Herbert, unpublished data). Currently, the injury potential of *H. halys* to upland cotton in the U.S. is unknown. Previous research on this stink bug has focused primarily on feeding injury to fruit and vegetable crops such as apples, peaches, sweet corn, and tomato. The adults spend spring and early summer on wild hosts before moving to feed on agricultural commodities in the late summer to early fall (K.L. Kamminga, unpublished data). Nymphs move from wild hosts in the fall and complete their development on nearby agricultural commodities. In the mid-Atlantic region,

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K. Kamminga\*, Division of Environmental Studies and Sustainable Systems, Kentucky State University, 110 Atwood Research Building, 400 East Main St., Frankfort, KY 40601; D.A. Herbert and S. Malone, Department of Entomology, Virginia Tech, Tidewater Agricultural Research and Extension Center, 6321 Holland Rd, Suffolk, VA 23417; M. D. Toews, Department of Entomology, UGA Tifton Campus, 2360 Rainwater Rd., Tifton, GA 31793; and T. Kuhar, Department of Entomology, Virginia Tech, 216A Price Hall, Blacksburg, VA 24061

\*Corresponding author: [Kamminga@vt.edu](mailto:Kamminga@vt.edu)

growers have reported up to 100% of sweet corn ears injured by *H. halys* and up to 50% yield loss in soybeans (Leskey et al., 2012). Additionally, *H. halys* populations have caused a four-fold increase in insecticide applications in orchard crops (Leskey et al., 2012). Disruption of integrated pest management programs in other production systems has triggered concern among cotton growers and consultants.

Cotton pest management programs have changed dramatically in recent years. Previously, the economic threshold for stink bugs in cotton was based on the number of stink bugs per row foot. However, most current thresholds for stink bug scouting in cotton recommend internal assessment of medium-sized (approximately 2.4-cm diameter) bolls collected from a field for assessment of boll injury (Greene et al., 2001a). These bolls have been determined to be the size most at risk to stink bug feeding (Greene and Herzog, 1999; Greene et al., 2001a, 2009). Extension recommendations suggest application of insecticide when internal boll injury exceeds 20% of the sample population (Bacheler et al., 2007; Greene et al., 2001b, 2009). However, southeastern states recently adopted a dynamic economic threshold based on the week of bloom (Bacheler et al., 2009, 2010). The dynamic threshold suggests lower thresholds for internal injury during the third through fifth week of bloom, when the majority of the harvestable bolls are vulnerable to stink bug feeding injury. Treatment thresholds increase at the initiation of bloom and after the fifth week of bloom when proportionately less harvestable lint is susceptible to stink bug feeding. Cotton bolls are considered safe from stink bug injury once the majority have reached a diameter of 3.2 cm (Bacheler and Mott, 2005; Greene et al., 2001a), at which time those bolls are no longer scouted. Introduction of a new stink bug pest that causes feeding injury to large bolls would require changes in both boll injury thresholds and boll sampling methods.

The objective of this research was to assess the pest potential of *H. halys* on cotton and determine feeding injury caused by this stink bug species to three distinct boll sizes. Results will be critical to insect pest management programs if the *H. halys* range in North America continues to advance southward into traditional cotton growing regions.

## MATERIALS AND METHODS

**2011 No-Choice Experiment.** All experiments were conducted in cotton fields located at the Tidewater Agricultural Research and Extension Center (TAREC)

at Suffolk, VA (N 36°39.48.74; W76°44.04.13). Cotton fields were planted on 29 April with the commercial variety 'PHY 315 RF' (Dow AgroSciences, Indianapolis, IN) using extension recommended production practices in Virginia (Herbert et al., 2012). The only insecticides applied were for early season thrips management, consisting of an at-planting application of aldicarb (Temik 15G, Bayer CropScience, Research Triangle Park, NC) at 0.84 kg ai/ha and a foliar application of acephate (Orthene 97, AMVAC Chemical Corporation, Los Angeles, CA) at 0.41 kg ai/ha on 28 May.

Stink bugs used in the experiment were field collected 24 to 72 h prior to being released in the cages. *Chinavia hilaris* adults were collected from a soybean field at Virginia Beach, VA, and *H. halys* adults were collected from a community garden in Roanoke, VA. Live stink bugs were provisioned with fresh green bean pods, *Phaseolus vulgaris* L., and held in the laboratory at 25°C and 60% RH (16:8 [L:D]) immediately prior to commencing experiments.

On 2 August, stink bugs were caged individually on cotton bolls with three fixed diameters based on current scouting recommendations for stink bug injury in the southeast (Bacheler et al., 2010). The scouting guide suggests sampling bolls with diameters between 2.2 and 2.8 cm; therefore, the smallest boll sampled was 0.4 cm below 2.2 cm ( $1.8 \pm 0.1$  cm), the middle size was  $2.8 \pm 0.1$  cm, and the largest was 0.4 cm greater than 2.8 cm ( $3.2 \pm 0.1$  cm). All cotton bolls were located on either the first or second sympodial branch positions. Before caging the insects, bolls were inspected for external lesions that might have occurred prior to the experiment and those expressing lesions were excluded from the study. These bolls were excluded because prior research has indicated that there is a weak correlation between external lesions and internal warts (Blinka et al., 2010). Treatments defined at the species level included infesting the cage with a single *C. hilaris* adult, a single *H. halys* adult, or no stink bug (uninfested). Fourteen bolls of each fixed diameter were selected for each treatment. Stink bugs were caged on bolls using white nylon mesh paint strainer bags (20 × 12 cm) with drawstring closures (Mutual Dropcloth, Monroe, NC). All bolls were labeled with colored plastic "snap-on-tags" (A. M. Leonard, Inc., Piqua, OH) by treatment and boll size. Tags were positioned basal to the bolls and outside the bags.

After 48 h, all cages and stink bugs were removed, but bolls were left in place for an additional 24 h to allow time for boll feeding injury symptoms

to express (Bundy et al., 2000). During that time, bolls were protected from further insect injury with a treatment of bifenthrin (Brigade 2EC, FMC Corporation, Philadelphia, PA) applied at 0.07 kg ai/ha using a Spider Spray Trac. After the 24-h reentry interval, all cotton bolls were removed and placed in the mesh bag with the corresponding tag. Bolls were assessed for internal feeding injury by stink bugs. Stink bug feeding injury was recorded as the number of internal warts and clusters occurring on the internal carpal walls (Fig. 1) (Siebert et al., 2005; Weene and Sheets, 1964). Large clusters of internal warts were designated a value of 10 internal warts because this was the approximately value of countable warts in the clusters. This method is more conservative than Greene et al. (1999), who assigned a value (30 growths) to heavily stink bug injured bolls that fell from the plant. External injury was not assessed because of contrary accounts in the literature correlating external lesions to internal feeding injury by stink bugs (Blinka et al., 2010; Bundy et al., 2000; Willrich et al., 2004a).



**Figure 1.** Internal warts and cluster on the internal carpal wall caused by *H. halys* feeding.

**2012 No-Choice Experiment.** Methods for the no-choice tests in 2012 were similar to the 2011 experiment, except *C. hiliaris* was not evaluated because there were no populations present in local crop fields; also, *H. halys* nymphs (3<sup>rd</sup>–4<sup>th</sup> instar) were used rather than adults. The only insecticide application was acephate at 0.56 kg ai/ha, which was applied on 25 May (2-leaf stage) for thrips management. Twenty bolls of each fixed boll size per treatment were tested. At the conclusion of the trial, stink bugs were killed by hand while in the bags and the bags were left on the boll (24 h) to allow for expression of feeding injury symptoms. This experi-

ment was conducted twice in 2012 with two different commercial varieties of cotton; the first experiment was initiated on 14 August with ‘PHY 499 WRF’ (Dow AgroSciences, Indianapolis, IN) cotton and the second was initiated on 4 September with ‘DP 1034 B2RF’ (Monsanto Company, St. Louis, MO) cotton.

**2012 Choice Experiments.** On 14 August and 4 September, cotton plants were randomly chosen from a field located at TAREC. All cotton bolls were measured using a vernier caliper to the nearest tenth of a centimeter and examined for stink bug feeding lesions, following the methods of Toews et al. (2009). Although external lesions can be used to rapidly assess the potential for stink bug feeding in the field, it is not a highly accurate method compared with internal evaluation. Any bolls with obvious external lesions were removed from the plant prior to initiating the experiment.

Twenty plants with at least two small ( $1.8 \pm 0.1$  cm), medium ( $2.8 \pm 0.1$  cm), and large ( $3.2 \pm 0.1$  cm) bolls were individually caged with a mesh polyester bag ( $63.5 \times 91.4$  cm) with drawstring cord lock (Honey-Can-Do International, LLC, Berkeley, IL) covering the entire plant. Stink bugs were collected in Glenvar, VA, from sweet corn 24 to 72 h prior to being placed in the cages. Ten *H. halys* nymphs (3<sup>rd</sup>–4<sup>th</sup> instar) were released at the bottom of the plant through the drawstring opening into 10 randomly chosen cages. All stink bugs appeared to disperse randomly upward into the cages. The 10 remaining cages were not infested with stink bugs.

At 72 h, plants were cut at ground level and returned to the laboratory. Cages were removed and the numbers of dead and live stink bugs recorded. All bolls were removed from the plant and the diameter was recorded. Bolls were dissected to determine internal injury using the same protocol as the 2011 no-choice experiment.

## STATISTICAL ANALYSIS

Statistical comparisons were conducted using SAS 9.3 (SAS Institute Inc. 2012, Cary, NC) for analyses of variance or TableCurve 2D (V. 5.01, Systat Software Inc. 2002 San Jose, CA) for regression model fitting. When assessing regression models, alternative models were investigated to determine the best model fit, but the simple linear model was eventually deemed to be the most reasonable model. Data plots were created using SigmaPlot 12.3 (Systat Software Inc. 2012, San Jose, CA).

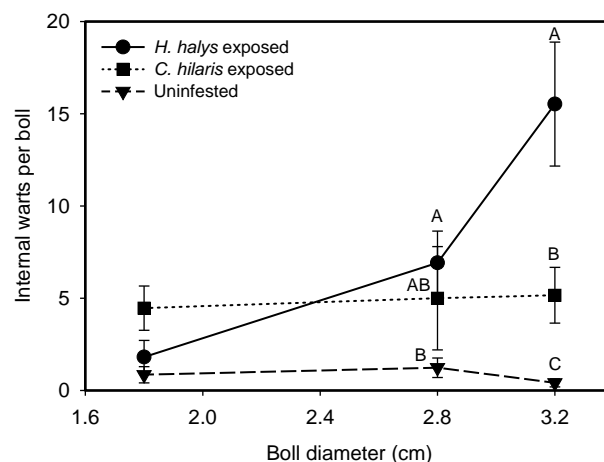
**No-Choice Experiments.** Cages found containing dead stink bugs at the end of the experiments were removed from the data set prior to analysis. By year, treatments were arranged as a two-way factorial arranged in a completely randomized design and responses were analyzed using PROC GLIMMIX. The primary response variable was number of internal warts observed on the inner carpal wall. When tests indicated a significant two-way interaction, the slice option was used to directly compare among species while holding boll size constant; differences among levels of species but within the same boll size were detected using the PDIF option. Differences in the number of internal warts across the range of boll sizes were characterized by level of species (stink bug species or uninfested cages) using trend analyses (linear and quadratic effects) because boll size was a continuous variable. Trend analyses were programmed using contrast statements.

**Choice Experiments.** Data from the two dates were combined to increase replications, plotted by treatment, and then fitted with individual linear regression equations using TableCurve 2D. Prediction equation parameters or coefficients were based on pattern of residuals and lack of fit tests (Draper and Smith, 1981). After the proper model was selected, the  $y$ -intercept and slope of each linear regression were tested using PROC REG to see if they were significantly different from zero using  $t$ -tests.

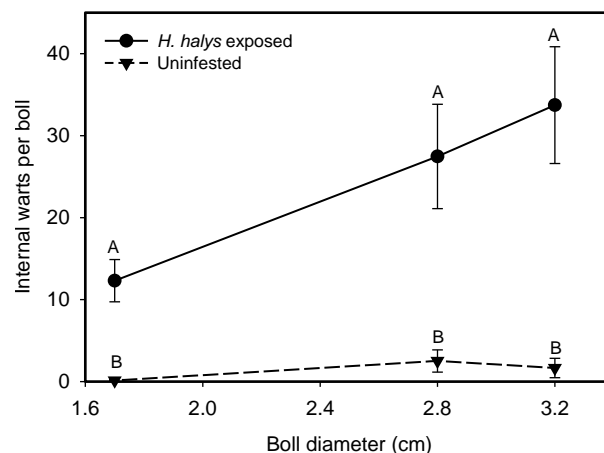
## RESULTS

**No-Choice Experiments.** There were differences among boll size and level of species treatments each year. In 2011, significant differences were detected for the level of species by boll size interaction ( $F = 5.80$ ;  $df = 4, 100$ ;  $P < 0.01$ ) and for the level of species main effect ( $F = 14.78$ ;  $df = 2, 100$ ;  $P < 0.01$ ). No differences were detected among species when controlling for the smallest boll size, but differences among species at each of the two larger boll sizes were evident (Fig. 2). Trend analyses on the 2011 data showed a significant linear response for *H. halys* ( $F = 16.45$ ;  $df = 1, 34$ ;  $P < 0.01$ ), but not for *C. hilaris* ( $F = 0.09$ ;  $df = 1, 30$ ;  $P = 0.76$ ) or uninfested bolls ( $F = 0.52$ ;  $df = 1, 36$ ;  $P = 0.48$ ). In 2012, significant differences were also detected for the level of species (*H. halys* infested vs. uninfested) by boll size interaction ( $F = 3.55$ ;  $df = 2, 217$ ;  $P = 0.03$ ) and for the level of species main effect ( $F = 55.85$ ;  $df = 1, 217$ ;  $P < 0.01$ ). Contrasts by individual boll size

showed that there were differences in the number of internal warts between the *H. halys* treated bolls and the uninfested bolls at each of the three experimental boll sizes (Fig. 3). Trend analyses on the 2012 data showed a significant linear response for *H. halys* ( $F = 7.43$ ;  $df = 1, 104$ ;  $P < 0.01$ ) but not for uninfested bolls ( $F = 0.97$ ;  $df = 1, 114$ ;  $P = 0.33$ ). Regardless of year, tests for a *H. halys* quadratic response trend were not significant ( $P = 0.45$  to  $0.54$ ).



**Figure 2.** Mean  $\pm$  SE number of internal warts by boll diameter observed on individual bolls caged with an individual *H. halys* adult (circles), individual *C. hiliaris* adult (squares), or uninfested bolls (triangles). Different letters within boll diameters signify differences between treatments ( $P < 0.05$ ).



**Figure 3.** Mean  $\pm$  SE number of internal warts by boll diameter observed on individual bolls caged with an individual *H. halys* nymph (circles) or uninfested bolls (triangles). Different letters within similar boll diameters signify differences between treatments ( $P < 0.05$ ).

**Choice Experiments.** Analyses of data suggest that bolls exposed to *H. halys* nymphs exhibited increasing numbers of internal boll warts with increasing boll diameter throughout the range of possible boll sizes from 0.5 to 3.7-cm diameter bolls (Fig. 4).

The slope of the line for linear regression of bolls exposed to *H. halys* was significant ( $F = 89.21$ ;  $df = 1, 274$ ;  $P < 0.01$ ) and had a positive slope [ $y = 6.99 (\pm 0.74)x - 6.54 (1.82)$ ;  $r^2 = 0.25$ ]. In addition, the y-intercept was significantly different than zero ( $t = 3.59$ ;  $df = 1, 274$ ;  $P < 0.01$ ). Conversely, the slope of the linear regression of all data points obtained from uninfested bolls was not significant ( $F = 2.09$ ;  $df = 1, 259$ ;  $P = 0.15$ ). Likewise, the y-intercept ( $0.01 \pm 0.29$ ) ( $t = 0.02$ ;  $df = 1, 259$ ;  $P = 0.98$ ) was not significantly different from zero.

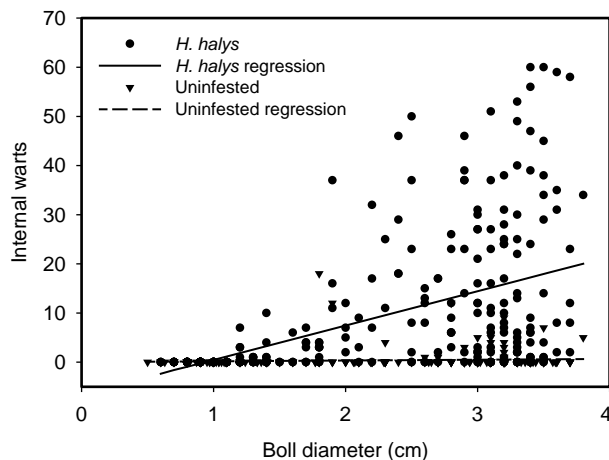


Figure 4. Scatter plot and fitted linear regression of boll diameter and number of internal warts on bolls treated with *H. halys* nymphs (circles) or uninfested (triangles).

## DISCUSSION

Current stink bug thresholds in cotton were developed for the predominant species (i.e., *C. hilaris*, *E. servus*, *N. viridula*). For example, the “Decision aid for stink bug thresholds in Southeast cotton,” is recommended for use in extension programs from Georgia to Virginia (Bachelier et al., 2010). The current recommendation is to inspect 2.2 to 2.8-cm diameter bolls for external lesions and internal injury, and the internal boll injury threshold varies according to week of bloom. This dynamic threshold considers the larger bolls (> 3.2-cm diameter) to be resistant to stink bug feeding injury (Bachelier and Mott, 2005). Here, data from choice and no-choice tests indicated that *H. halys* caused more feeding injury to larger bolls (3.2 cm) than the smaller (1.8 or 2.8 cm) bolls. Further, data from the choice tests demonstrate that *H. halys* nymphs will feed on bolls larger than 3.2 cm. As the boll diameter increased up to the largest boll included in the choice test (3.7 cm), the number of internal warts also increased.

This observation suggests that large cotton bolls are also highly susceptible to feeding injury. If *H. halys* becomes an established pest of cotton, thresholds will need to be revised to include inspecting large bolls for feeding injury.

Stink bug feeding choice tests for different boll sizes have been investigated previously for some of the primary stink bug pests in the southern U.S. Feeding choice research by Huang and Toews (2012) determined that *N. viridula* spent more time on the medium (2.1-2.5 cm) and larger (2.6-3.0 cm) sized bolls, whereas *E. servus* spent more time on bolls that were 2.1 to 2.5 and 1.1 to 1.5-cm diameter. In contrast, Hopkins et al. (2010) recorded economic injury occurring by *Euschistus* sp. feeding on large bolls (3.2 cm). We are not aware of any previously published boll choice tests for the native stink bug *C. hilaris*. These stink bugs usually appear in cotton in late summer and remain until harvest (Herbert and Toews, 2012). Our data, although limited to one experiment, show that *C. hilaris* caused similar numbers of internal warts across the three boll sizes. These data do not invalidate current thresholds for *C. hilaris* because this species was just as likely to pierce the bolls in the appropriate sampling range.

Our research showed that there was no significant difference in the number of internal warts between species for both the small and the medium sized bolls. However, there was a significant difference in boll size feeding injury by stink bug species for larger bolls. There are a few possibilities to consider as to why more feeding injury was observed by *H. halys* on the larger bolls. During the choice test, nymphs were released from the bottom of the plant. This method could have resulted in more punctures to larger bolls because lower and first position bolls are the first ones the nymphs encounter as they move up the plant and onto the branches. However, stink bugs are negatively geotactic and generally walk up to the highest point on a plant. This upward-moving behavior was also observed in the field during the tests. Moreover, the large number of internal warts on large bolls also was observed during both years of the no-choice tests. Another possibility is that it might be more difficult for stink bugs to locate seeds on larger bolls, requiring more piercing. In addition, if the seeds of older bolls are less nutritious than younger bolls, this may also cause more piercing. Future research is needed to explore these questions.

Willrich et al. (2004b) reported that native stink bug feeding on late-season cotton can result in hard-

locked and rotting bolls along with transmission of boll-rotting pathogens. Researchers in the U.S. have shown that various fruit and vegetables fed upon by *H. halys* might contain a wide variety of bacteria, fungi, and yeasts that can potentially lead to fruit rot. One yeast species, *Eremothecium coryli* (Peglion) Kurtzman, has been found commonly in almost all fruit and vegetables examined (personal communication, G. Brust and K. Rane, University of Maryland). Other yeasts also have been recovered as well as specific bacteria also found in other stink bug species. *Halyomorpha halys* caused significantly higher number of internal warts on the large bolls than *C. hilaris*. Because *H. halys* pierces cotton bolls more than the existing stink bugs, it is likely that there is greater probability of it transmitting a boll-rotting pathogen.

A final consideration relates to the best methods for sampling *H. halys* in cotton fields as the insect expands into cotton producing regions. Sweep net or shake cloth counts are standard sampling procedures used by scouts to detect stink bugs in cotton. However, these techniques have yet to be evaluated for *H. halys*. *H. halys* has a strong “startle response” that is, adults and nymphs tend to drop from the plant when there is the slightest disturbance. This habit is requiring researchers working with *H. halys* in soybean to begin exploring different field sampling methods like timed/area visual counts; a similar approach might be needed for cotton.

In summary, it appears that if *H. halys* becomes established in areas that grow cotton, it could become a major pest and would require reevaluation of boll thresholds and sampling methods. The dynamic threshold currently used in the Southeast would likely no longer be completely relevant. Most notably, because *H. halys* seems to cause significant feeding injury to larger cotton bolls, these would have to be included in any future sampling thresholds. This would result in an extended scouting period and possibly treating fields with insecticides to control stink bug later into the season.

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