EVALUATION AND UTILIZATION OF RESISTANCE TO TARNISHED PLANT BUG IN COTTON - SURVIVAL OF NYMPHS ON A FREGO BRACT COMPARED TO A NECTARILESS LINE IN FIELD & LABORATORY TRIALS

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Abstract

Field and laboratory trials conducted in 2010 and 2011 were aimed at evaluating techniques for screening cottons with host plant resistance to tarnished plant bug (*Lygus lineolaris* (Palisot de Beauvois)). Nymphs (either small or large) were released on terminal cuttings or in field cages of RBCDHGPIQH-1-97, an early fruiting, frego bract line that is considered susceptible to tarnished plant bug (TPB) and Arkot 9608ne, a nectariless line moderately resistant to TPB. Earlier field and lab trials have shown plant bugs prefer feeding on squares of frego compared to the Arkot line. In field caged trials as well as lab feeding trials using main stem cuttings from field collected plants, results indicated significantly greater survival of plant bug nymphs after 3 days on the frego compared to Arkot line. Very high survivorship with no differences between lines was observed in cage and main stem cuttings trials with greenhouse grown cotton. Techniques used in the trial show promise for expanding host plant resistance screening protocols. If confirmed, resistance of varieties and breeding lines could be compared simply by taking plant cuttings from existing field tests.

Introduction

Frego bract cotton is recognized as a preferred cotton type for tarnished plant bug (TPB) (Maredia et al, 1993; Jenkins and Wilson 1996). In cotton host plant resistance (HPR) research in Arkansas, a frego bract cotton line generally is included in field trials to screen new varieties and germplasm lines to gauge plant bug feeding activity (Bourland 2004). In earlier work in this Cotton Incorporated sponsored research project, we manually infested TPB nymphs in a small plot variety trial and found that numbers of nymphs in frego plots were significantly higher than in Arkot 9608ne, a nectariless line. This was interpreted as an indication of reduced survival of nymphs on the Arkot cotton – signaling an increased level of HPR. Manually infesting large numbers of TPB nymphs in field trials is costly and would not be practical in a university HPR screening program that could include hundreds of cotton strains and selections. Our aim with this project in 2010 and 2011 has been to explore use of simple and rapid field and lab bioassay techniques to evaluate plant bug survival without requiring thousands of insects every week. Because of the differences in bug survival we had observed in the earlier field work, we chose to continue to work with Arkot 9608ne and frego.

This report summarizes laboratory and field feeding studies with main stem terminal cuttings that included the upper 6 to 10 squaring nodes. Field cage testing also was included to enable us to compare the lab technique to an established, yet burdensome, field screening technique. The work included use of large nymphs (4^{th} to 5^{th} instar) as well as small, newly hatched nymphs. Working with tiny first instar nymphs poses practical challenges; however, we were interested to determine if there were differences in host plant suitability with very young insects compared to older (and hardier?) insects. It is anticipated that techniques that show promise will be used in future expanded testing with additional cotton lines.

Materials and Methods

Field Cages

The field cage experiments were conducted in Northeast Arkansas at the Judd Hill Foundation Research Farm near Trumann. Two cotton germplasm lines were tested, Arkot 9608ne (Bourland and Jones 2008) and a frego line from Texas, RBCDHGPIQH-1-97 (frego). Plots were 8 rows wide, 50 ft long with 10 ft alleys and were arranged in an RCB with 4 replications. Date of planting was 7 May 2010 and 9 May 2011. Feeding trials were held around the 3^{rd} week of squaring, prior to first flowers -- 25 June 2010 and 24 June 2011. No foliar insecticides were applied in either season. In the center rows of each plot, organdy sleeve cages, 6 inches diameter by 18 inches long, were secured to randomly selected individual plants (Figure 1). The lower end of each cage was tied around the plant main stem ca. 10 to 12 inches from the terminal. Three TPB nymphs, depending on treatment, either small (1^{st}

instar) or large nymphs (4th to 5th instar) were placed into each cage. Only large nymphs were tested in 2010. Cages were secured with twist ties. Three days after release the plants were cut below the cage, placed in paper bags, and taken to the Judd Hill field lab where TPB mortality was determined.

Figure 1. Methods used in field cage testing to evaluate survival of tarnished plant bug nymphs on a susceptible and moderately resistant cotton line.

Main stem cuttings

Plant cuttings of frego and Arkot 9608ne were collected from plants in the center portion of field plots in the Judd Hill field trial. Garden shears were used to clip main stem cuttings, 14 to 16 inches long, from the uppermost portion of cotton plants. Cuttings were placed in 5 gallon buckets with water and immediately transferred to the ASU Cotton Laboratory where they were trimmed further to remove lower leaf tissue and branches. The main stem containing the upper most 6 to 7 squaring nodes and first position squares and upper leafs were retained. The base of main

stems were re-cut on an angle and then positioned in ca. 4 X 3 inch thick cut blocks of florist's wet floral foam placed in plastic tubs partially filled with water (Figure 2).

Figure 2. Plant bug nymphs were released onto plant terminal cuttings positioned in floral water foam (Oasis). Sleeve cages were used to confine bugs. In developing the cuttings technique, we initially used non-caged plants; however, there were problems with lost and drowned bugs. The sleeve cages, supported with small wooden garden stakes, worked to confine bugs on the cuttings.

Methods varied slightly between years; improvements were adopted in 2011. In 2010, there were 3 non-caged cuttings of the same cotton line per tub; in 2011 there was one cutting per tub and cuttings were enclosed in sleeve cages. Each cutting was manually infested with 3 nymphs, either large (predominately 4^{th}-5^{th} instar) or small (predominately 1^{st} instar). Cuttings were held under grow lights at room temperature in the laboratory. After 3 days, cuttings were inspected, and numbers of bugs (dead, alive) per cutting determined. Evaluations with small nymphs were made on 11 and 15 June 2010. Evaluations with large nymphs were made on 11, 15, 21, 24, June 2010. In

2011, one test was performed on 24 June, using small and large nymphs. Cuttings and field cage tests were performed all on the same day in 2011.

Greenhouse cuttings and cage tests

Tests were performed in February 2011 using greenhouse grown frego and Arkot 9608ne. Pots (1/2 gallon with Metromix 366) were seeded 16 December in the ASU greenhouse. Plant bug test methods were similar to those employed in 2011 cuttings and field cage tests. There were three plants per pot; one plant was clipped at the base and collected for the lab cuttings trial, one plant was caged for the greenhouse cage trial, and the third plant was clipped and discarded. The remaining plant was stabilized with a wooden stake and a fabric sleeve cage placed over it. Three plant bug nymphs, either small or large, were released per cage (Figure 3). After 3 days, plants were clipped just below the cage and transferred to the laboratory for survival assessments. Cuttings from the greenhouse were handled as described for field grown plants above. Each test with greenhouse caged plants and cuttings were performed on the same day. There were 8 to10 plants per line used in each test. Tests were conducted on 9 and 12 February for small nymphs and 4 and 15 February for large nymphs.

Figure 3. Greenhouse grown frego and Arkot 9608ne plants were used in cage and cuttings trials with small and large plant bugs.

Insects used in testing

Plant bug nymphs used in 2010 trials and in greenhouse cotton tests were from a laboratory colony maintained at Arkansas State University. These insects originated from field populations collected on flowering daisy fleabane (*Erigeron* spp.) growing in fallow fields in early summer 2010 in NE Arkansas. These insects were maintained on ears of sweet corn with gel packets used as an ovipositional substrate (Cohen 2000). Plant bug nymphs used in the summer 2011 feeding trials were field collected one day prior to testing. The uppermost portions of flowering fleabane plants were cut using scissors in ca. 12 to 18 inch lengths. These were placed in 5 gal buckets and were transported back to the lab where they were positioned in moistened floral foam and held overnight in a dark, cool holding room (ca. 72^{o}F). On the morning of the test, insects were collected by shaking plants over a dark colored plastic tray. First instar nymphs were gently picked up using a camel's hair brush and placed into 20 mL glass scintillation vials. There were 3 bugs per vial. Each vial was immediately placed into 5- quart coolers with "blue ice". Large nymphs were collected using sweep nets in fleabane. Nymphs were aspirated into screened 5 gal plastic buckets lined with moistened paper towels. Back at the lab, they were held overnight at 85^{o}F and provided ears of sweet corn and water on moistened floral foam. Large bugs were handled similarly to small bugs – on the morning of the trial, three bugs were transferred to each vial and placed on blue ice. Bugs were shaken from vials into sleeve cages.

Results

Field cages

Weather conditions during the 2010 field cage study were hot and dry with daytime high temperatures of 99, 100 and 99°F during the 3 day test. Considering the extreme heat, plant bug survival was surprisingly high. Mortality levels for large nymphs in cages was slightly higher for Arkot 9608ne compared to frego plants ((AOV; P=0.14) (Figure 4). For 2011 field cage tests, daily high temperatures during testing were less extreme than 2010 with highs of 82, 96, and 96 °F. Mean mortality of both small and large plant bug nymphs was significantly higher for nymphs held in cages of Arkot 9608ne compared to frego cotton (P=0.006) (Figure 5). Mortality levels were similar among the two bug sizes, and there was not a significant bug size*cotton line interaction.

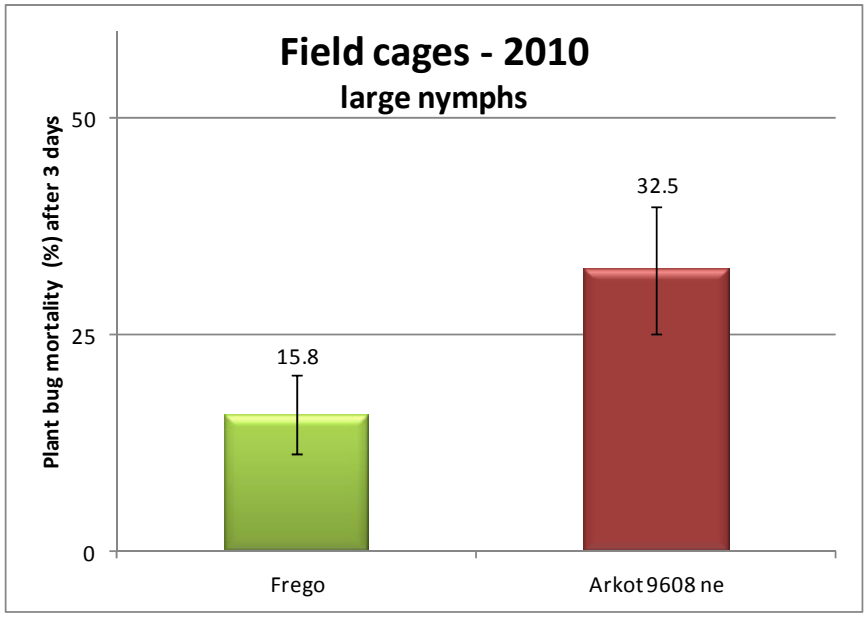

Figure 4. Mortality (mean ±SEM) of large plant bug nymphs three days after release in field sleeve cages in 2010 Judd Hill field trials. There were 20 cages per cotton line with 3 large plant bug nymphs released per cage (AOV; P=0.14) (n=20).

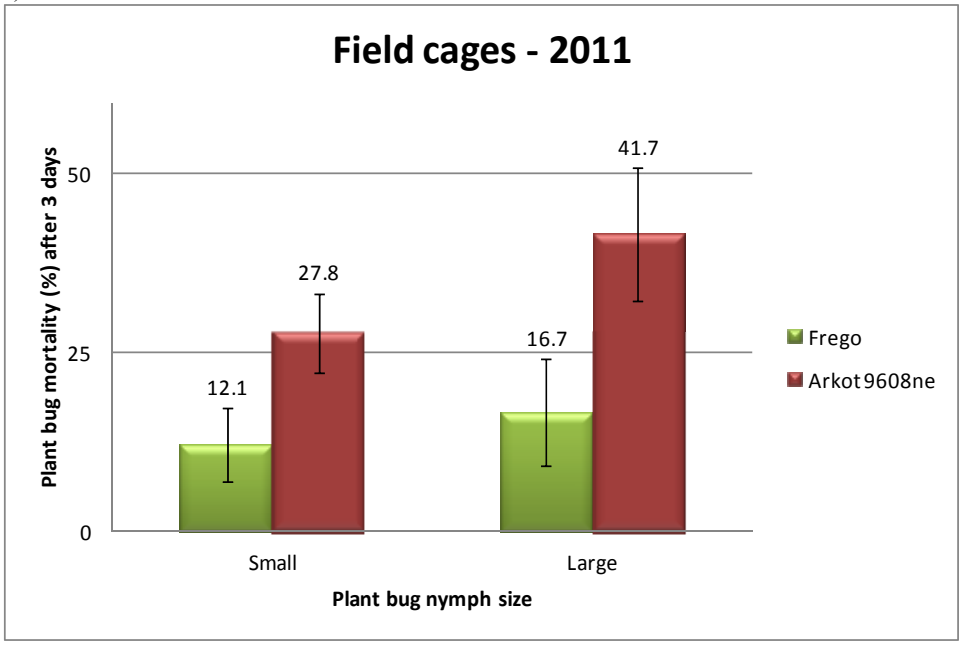

Figure 5. Mortality (mean ±SEM) of small and large plant bug nymphs three days after release in field sleeve cages (P=0.006) (n=12) in 2011 field cage trials. No significant differences in mortality were associated with bug size (P=0.18) or cotton line * bug size interactions.

Main stem cuttings

In 2010 trials with main stem cuttings, mean mortality of small plant bug nymphs was significantly lower on frego (12.5%) compared to Arkot 9608ne (41.7%) (P=0.04) (Figure 6). Similar results were observed in separate 2010 trials with large nymphs (P=0.001).

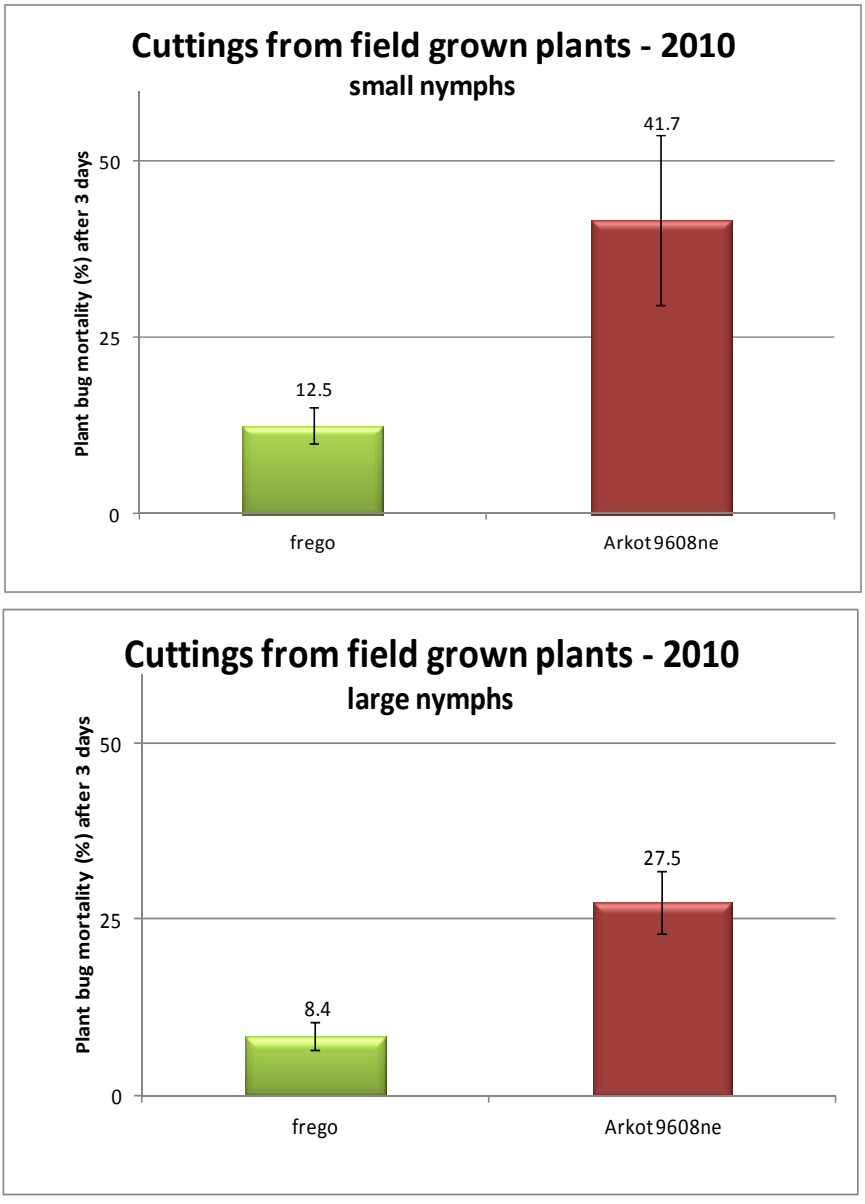

Figure 6. Mortality (mean ±SEM) of plant bug nymphs three days after release on cuttings from field grown plants of frego and Arkot 9608ne in2010; differences were observed with separate tests with small (P=0.04) (n=8) and with large nymphs (P=0.001) (n=28).

Similar results were observed in 2011 feeding trials with cuttings from field grown plants (Figure 7). Mean mortality of both small and large plant bug nymphs was significantly lower on frego compared to Arkot 9608ne (P=0.006). Mortality also was significantly higher with smaller compared to larger bugs (P=0.005). There were no significant interactions with cotton line * bug size.

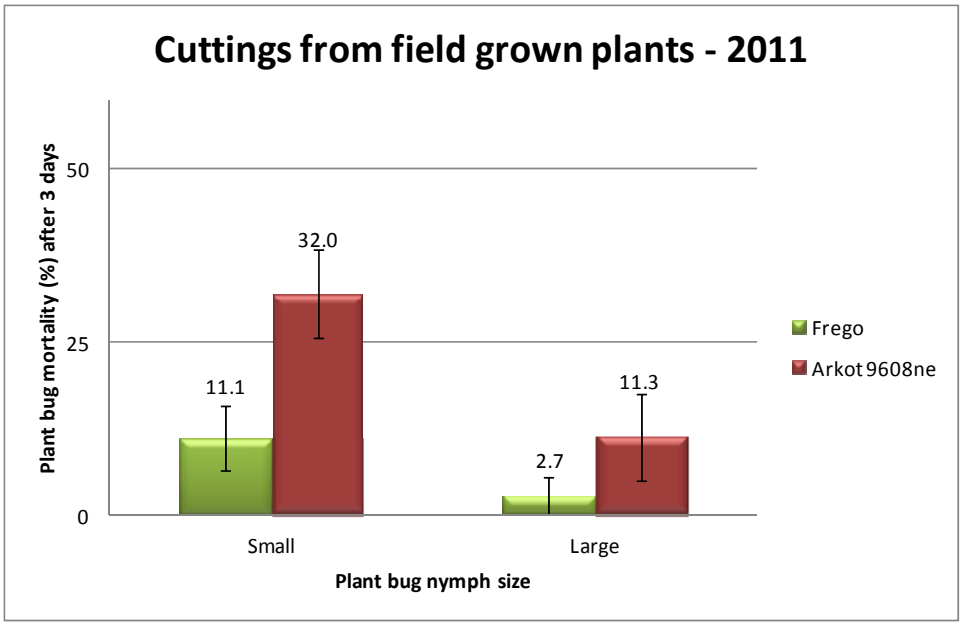

Figure 7. Mortality (mean ±SEM) of small and large plant bug nymphs three days after release on cuttings from field grown plants of frego and Arkot 9608ne in2011; significantly lower mortality was observed with frego compared to Arkot 9608ne (P=0.006) (n=12) , and mortality was significantly higher with smaller compared to larger bugs (P=0.005). There were no significant interactions with cotton line * bug size.

Greenhouse cuttings and cage tests

With greenhouse grown plants, there were no differences in large bug and small plant bug survival on the resistant Arkot9608ne compared to the susceptible frego cotton line in either the greenhouse cage or greenhouse cuttings trial (Figure 8 and 9).

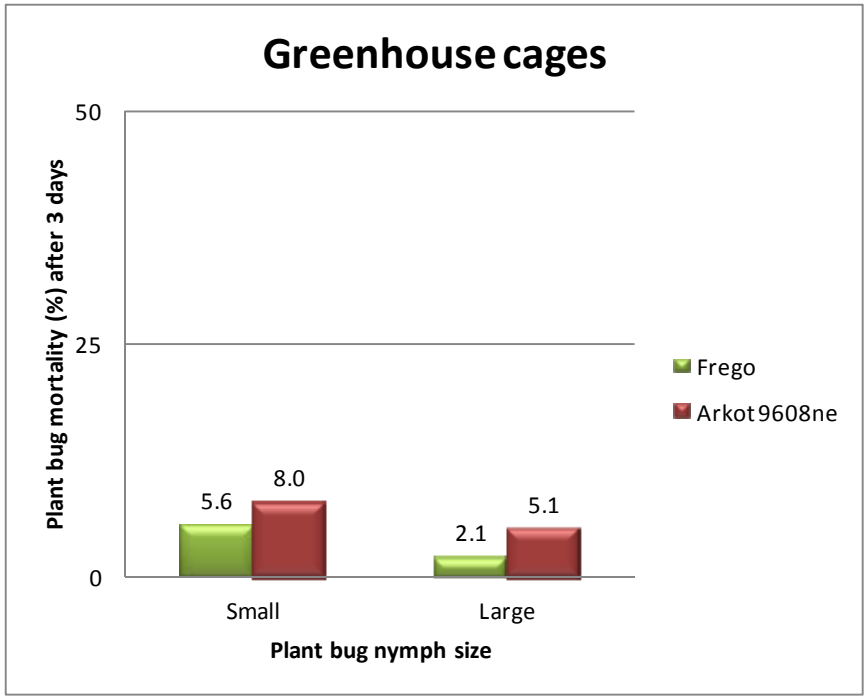

Figure 8. Plant bug mortality of plant bug nymphs three days after release on caged greenhouse plants of frego and Arkot 9608ne in2011; no significant differences in mortality were associated with either cotton line or bug size.

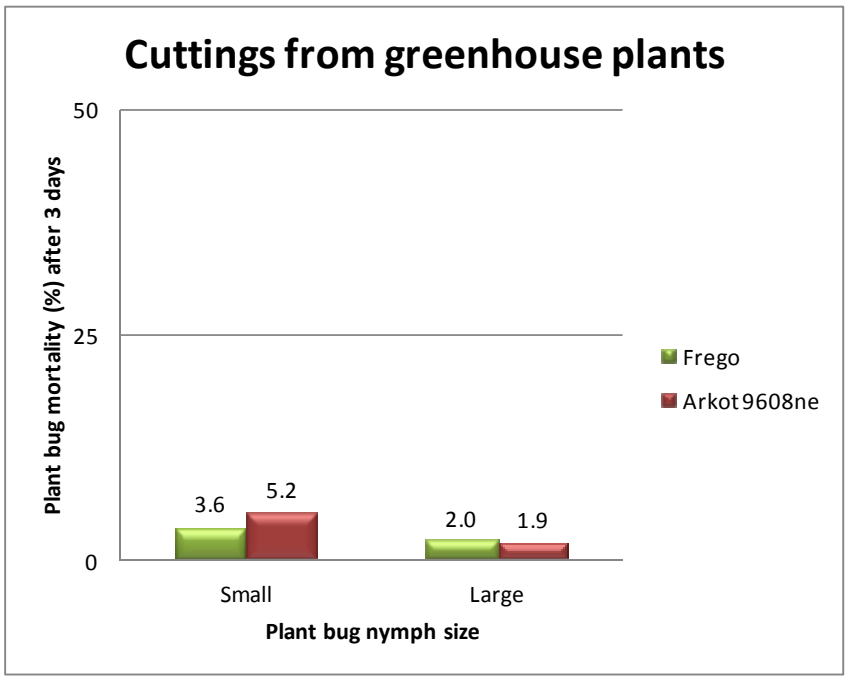

Figure 9. Plant bug mortality of plant bug nymphs three days after release on cuttings from greenhouse grown plants of frego and Arkot 9608ne in2011; no significant differences in mortality were associated with either cotton line or bug size .

Conclusions

Field and lab results from 2010 and 2011 indicate that survival of plant bugs differed when nymphs were fed a susceptible compared to a moderately resistant cotton line, and results were similar in either field cages or in the lab with field grown plant cuttings. Differences in HPR properties between the two cotton lines were not apparent in

tests with greenhouse grown plants. Young, newly hatched plant bug nymphs compared to older nymphs appeared to be more sensitive to resistant cotton lines when using cuttings. Small bugs therefore may have utility in HPR testing protocols; however, the increased handling time and higher levels of technical expertise and care required to use 1st instar nymphs compared to using older nymphs is considerable and likely would not be practical in most screening programs.

These simple feeding bioassay techniques show promise and could be helpful in evaluating plant bug resistance in new cotton germplasm. Results from cuttings tests are particularly encouraging. Employing field cage tests in HPR screening programs would be time and labor intensive. Use of main stem cuttings is less onerous and likely would be more appealing to lab workers. If the cuttings method is confirmed, resistance of varieties and breeding lines could be compared simply by taking plant cuttings from existing field tests. Also, cuttings might be made from segregating populations thereby allowing breeders to directly select for resistance to TPB. Further evaluations using additional cotton lines and with standardized handling of test insects are needed and planned.

Acknowledgements

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