

FEEDING DAMAGE AND SURVIVAL OF TARNISHED PLANT BUG NYMPHS IN FREGO BRACT COMPARED TO A NECTARILESS COTTON LINE

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Abstract

Field trials conducted in 2007 and 2008 in NE Arkansas were aimed at evaluating field techniques for screening cottons with host plant resistance to tarnished plant bug (*Lygus lineolaris* (Palisot de Beauvois)). Third instar nymphs were released on RBCDHGPIQH-1-97, an early fruiting, frego bract line from Texas and Arkot 9608ne, a nectariless line. Comparisons of plant bug survival and subsequent feeding injury following release at first flowers were made. White flower anther injury, COTMAN squaremap sampling, plant bug recovery, in-season mapping with square and boll dissection, and yields were included in evaluations. Results indicate significantly greater survival of nymphs on frego compared to the Arkot ne as well as levels of feeding injury. Within 5 days of manual release, anther injury was apparent in white flowers in both lines. Techniques used in the trial show promise for expanding host plant resistance screening protocols.

Introduction

Following the progression of boll weevil eradication and commercial adoption of transgenic cultivars for caterpillar control, tarnished plant bug has inherited the role of major insect pest in US Midsouth cotton. Insecticides remain the chief method for managing damaging populations of plant bugs, but research to improve host plant resistance to this important pest is on-going in Arkansas. One focus of this effort is development of field and lab techniques for expediting screening procedures for cotton lines that show promise for host plant resistance to plant bug.

Several traits have been identified to have specific positive and negative effects with response to damage by plant bugs. These include preference of cottons with extra-floral nectaries compared to nectariless types (reviewed by Jenkins and Wilson, 1996). The frego bract type (floral bracts roll inward and curl away from the flower bud or developing boll) also has been identified as a preferred type (Maredia et al, 1994).

A frego bract germplasm line, TX RBCDHGPIQH-1-97, is routinely planted in Arkansas screening trials to gauge plant bug feeding activity in small plot field research (Bourland 2004, Bourland and Jones 2008, Maredia et al 1994). For this technique, white flower anther injury is monitored daily among different cotton lines during in the first weeks of flower. In laboratory preference tests using a water foam test arena, we have found that plant bug nymphs preferentially feed on squares of this frego line compared to other cottons in choice tests (Teague et al 2007). In no-choice tests, severity of feeding injury is significantly greater with this line compared to feeding injury observed to other test lines (measured by exposing single squares to 1 nymph for 24 hrs and then counting damaged anthers (Teague unpublished)). Differences in bract morphology or square size were not significant factors in plant bug feeding preference in the arena tests (Teague et al 2007).

In this paper we summarize results from a 2 year field study designed to examine nymph survival and temporal dynamics of white flower injury and other feeding injury symptoms following manual infestations of plant bug nymphs in small plots planted with the preferred frego cotton line and a nectariless line.

Materials and Methods

The field experiment was conducted at the Cooperative University Research Farm at the Judd Hill Plantation in Poinsett County, in NE Arkansas near Trumann. Two cotton lines were tested: 1) Arkot 9608ne, (AR ne), a nectariless germplasm line developed in Arkansas (Bourland and Jones 2008), and 2) RBCDHGPIQH-1-97 (frego), an early fruiting, frego bract line. In 2007 there were four tarnished plant bug (TPB) treatments: 1) insecticides sprayed pre-flower followed by TPB manual infestation at first flowers; 2) no sprays pre-flower followed by TPB

manual infestation at first flowers; 3) plants sprayed weekly with insecticides full season; 4) no sprays or bugs. All plots were sprayed with insecticides after the 3rd week of flowering. The 2*4 split-plot factorial experiment was arranged in a randomized complete block design. The four plant bug/spray treatments were considered main plots. Three blocks were used, and each treatment combination occurred only once in each block. Plots were 8 rows wide, 50 ft long with 15 ft alleys. In 2008, there were 3 plant bug treatments: 1) no sprays pre-flower followed by TPB manual infestation at first flowers; 2) sprayed weekly with insecticides full season; 3) untreated and unsprayed until 3rd week after flowers. Manual infestations in both years were made on 11 July using 3rd instar plant bug nymphs released at 3 to 5 bugs per plant in rows 5 and 6 of each plot. Confetti-sized strips of shredded white copy paper (0.5 cm wide and 10 to 20 cm long) on which TPB nymphs were resting were laid across the terminal leaves of plants to be infested. The paper “ribbons” lined the bottom of TPB rearing containers, and the nymphs clinging to these ribbons crawled onto the plants. Bugs were released during the cool period of the morning just after dew had dried. Bugs were obtained from the USDA-ARS SIMRU plant bug colony maintained at Mississippi State University. The ARS colony was maintained on artificial diet (Cohen 2000). Eggs shipped from the Mississippi colony were allowed to hatch, and nymphs were held at the Cotton IPM Lab at ASU on ears of sweet corn until they reached appropriate stage for field release.

For the field test, a seeding rate of 3 to 4 Cruiser treated seeds/ft in rows spaced 38 inches apart was planted in the Dundee silt loam. Plots were furrow irrigated weekly as needed. Standard UA Experiment Station fertility practices were used. Insecticides and treatment timing for 2007 and 2008 are listed in Tables 1 & 2.

Table 1. Insecticide application timing, product selection and rates used for the 2007 field trial. Treatments receiving insecticide applications pre-flower were the “sprayed” and the “sprayed-bug” (spr-bug). All treatments received applications beginning 24 July, 76 days after planting.

Application Timing (treatment designation)	Application Date	Days after planting	Product (rate/acre)
insecticide pre-flower (sprayed & spr-bugs)	20-Jun	42	Trimax 1.8oz
insecticide pre-flower (sprayed & spr-bugs)	27-Jun	49	Centric 2oz
insecticide pre-flower (sprayed & spr-bugs)	05-Jul	57	Centric 2oz
BUGS Released	11-Jul	63	
post-flowers (sprayed)	16-Jul	68	Acephate 90 0.5 lb/A , Diamond 9oz
post-flowers (all treatment plots)	24-Jul	76	Baythroid 1.6 oz + Centric 2oz
post-flowers (all treatment plots)	31-Jul	83	Bidrin 3.2 oz
post-flowers (all treatment plots)	06-Aug	89	Bidrin 3.2 oz

Table 2. Insecticide application timing, product selection and rates used for the 2007 field trial. Only the “sprayed” treatments received insecticide applications pre-flower. All treatments received applications beginning 29 July, 68 days after planting.

Application Timing (treatment designation)	Application Date	Days after planting	Product (rate/acre)
pre-flower (sprayed)	18-Jun	26	Trimax 1.8oz
pre-flower (sprayed)	02-Jul	40	Diamond 9oz
pre-flower (sprayed)	08-Jul	46	Centric 2oz
BUGS Released	11-Jul	49	
post-flowers (sprayed)	16-Jul	54	Centric 2oz

post-flowers (sprayed)	22-Jul	60	Leverage 3.75 oz
post-flowers (all treatment plots)	29-Jul	68	Centric 2oz
post-flowers (all treatment plots)	06-Aug	75	Bidrin 3.2 oz
post-flowers (all treatment plots)	14-Aug	83	Bidrin 3.2 oz

Crop Monitoring -- Plants were monitored in each plot from the squaring period through physiological cutout (mean nodes above white flower=5) using the Squaremap procedure in the COTMAN™ crop monitoring system (Danforth and O’Leary 1998, Oosterhuis and Bourland 2008). Ten consecutive plants in row 5 were monitored weekly. Sampling included measurement of plant height, number of sympodia, and presence or absence of first position squares and bolls. Squaremap fruit retention data for each cotton line were analyzed separately for each sampling date using ANOVA.

Field Sampling for TPB and Injury -- Plant bug population densities were monitored using weekly drop cloth sampling. Numbers of nymphs and adults were recorded. Rows 3 and 4 were used for sampling up until first flowers. Following manual infestation, a section of release row 5 was allocated for sampling with drop cloths. One sample along 3 ft of row per plot was taken using a black drop cloth, and numbers of nymphs recorded. Post release samples were taken at a different portion of the row on each successive sample date; the aim was to obtain an estimate of survivorship of nymphs after release. Other portions of the release rows were used for plant measurements and were not sampled for bugs. Variation in average number of collected insects was analyzed using ANOVA Student-Newman-Keuls Test separately for each date.

White Flower Anther Injury -- White flower anther injury assessments were made by inspecting 10 white flowers per plot in rows 5 and 6 after initiation of flowering. Injury was categorized as either (0) no anther injury or (1) anther injury present. Sections in the release rows separate from insect sampling areas were used in plant assessments to avoid confounding observations with injury resulting from the bug sampling procedure (beating plants to dislodge insects onto drop cloths). Injury data for each cotton line were analyzed separately for each sampling date using ANOVA.

Fruit dissections -- In 2007, additional assessments were made to evaluate boll and square injury from bug feeding. Fruiting forms from 10 plants per plot were removed, mapped and dissected at 9 days after manual infestation. Whole plants from row 5 of treatment plots were cut at the base, carefully loaded into individual paper bags and transported to the field lab for evaluation. A modified “box” map procedure was used to sort fruiting forms for each group of plants by main stem node number (counting from the bottom up) and by position. Squares and bolls were sorted into labeled cups. Each fruiting form was systematically dissected and inspected for signs of plant bug feeding injury. External feeding injury symptoms also were noted for bolls. Because of time restrictions, plant samples from the spr-bug treatment were not included.

Yield -- Plots were machine harvested using a 2-row research picker. Yield totals from row 6 were used for analysis. Turnout calculations were based on hand picked 50 boll samples collected on consecutive plants and then ginned using a laboratory gin. Lint yields were analyzed using ANOVA, and means separated using LSD.

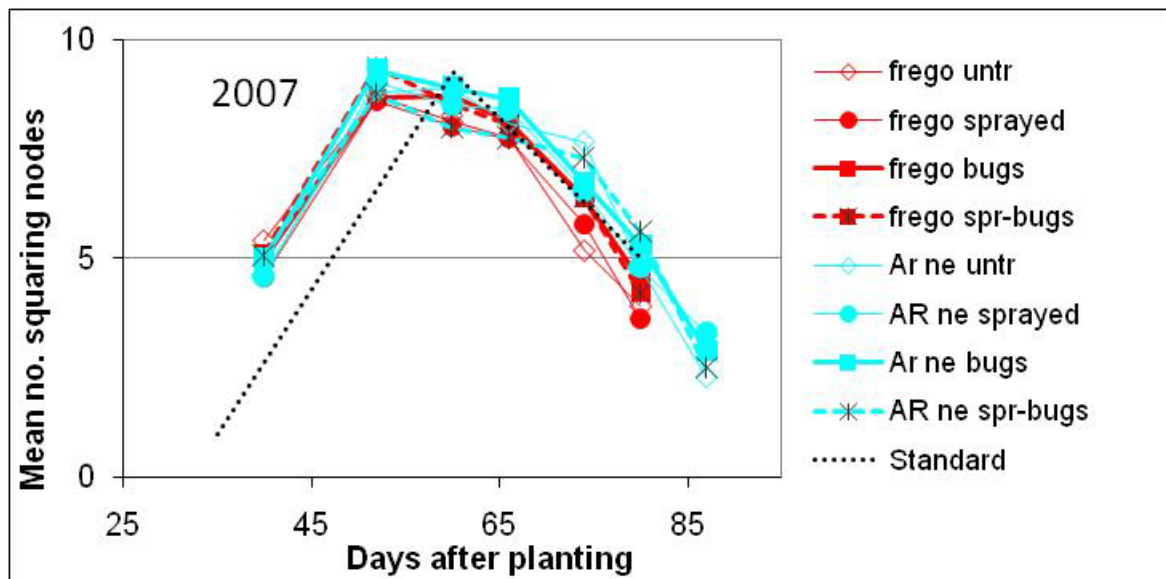


Figure 1. Growth curves for the 2007 trial with frego and AR ne with 4 plant bug/insecticide treatments, all shown in comparison with the standard curve, the COTMAN target development curve. Plant bug nymphs were released at 63 days after planting at first flowers.

Results

Crop Monitoring -- Crop growth curves depicting development of main stem squaring nodes for the two cotton lines and four spray/bug treatments show very early availability of squares in the 2007 and 2008 season relative to the standard of 35 days after planting (Figs 1, 2). Warm spring temperatures in 2007 and late planting date in 2008 resulted in rapid plant development. Growth curves and days to cutout indicate crop delay associated with plant bugs in 2008.

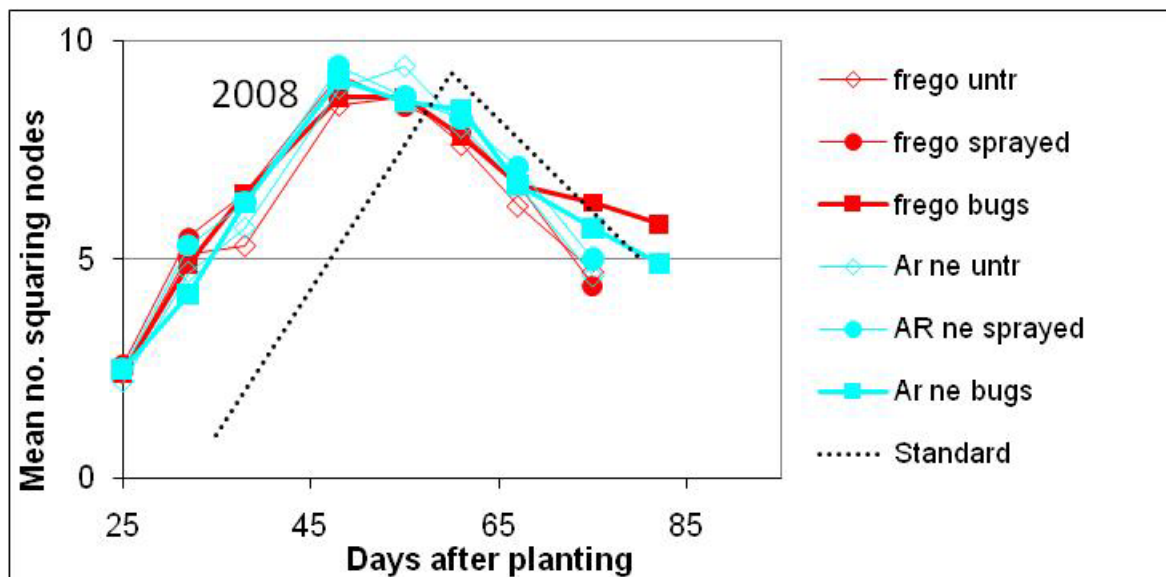


Figure 2. Growth curves for the 2007 trial with frego and AR ne with 4 plant bug/insecticide treatments, all shown in comparison with the standard curve, the COTMAN target development curve. Plant bug nymphs were released at 49 days after planting during the first week of flowers.

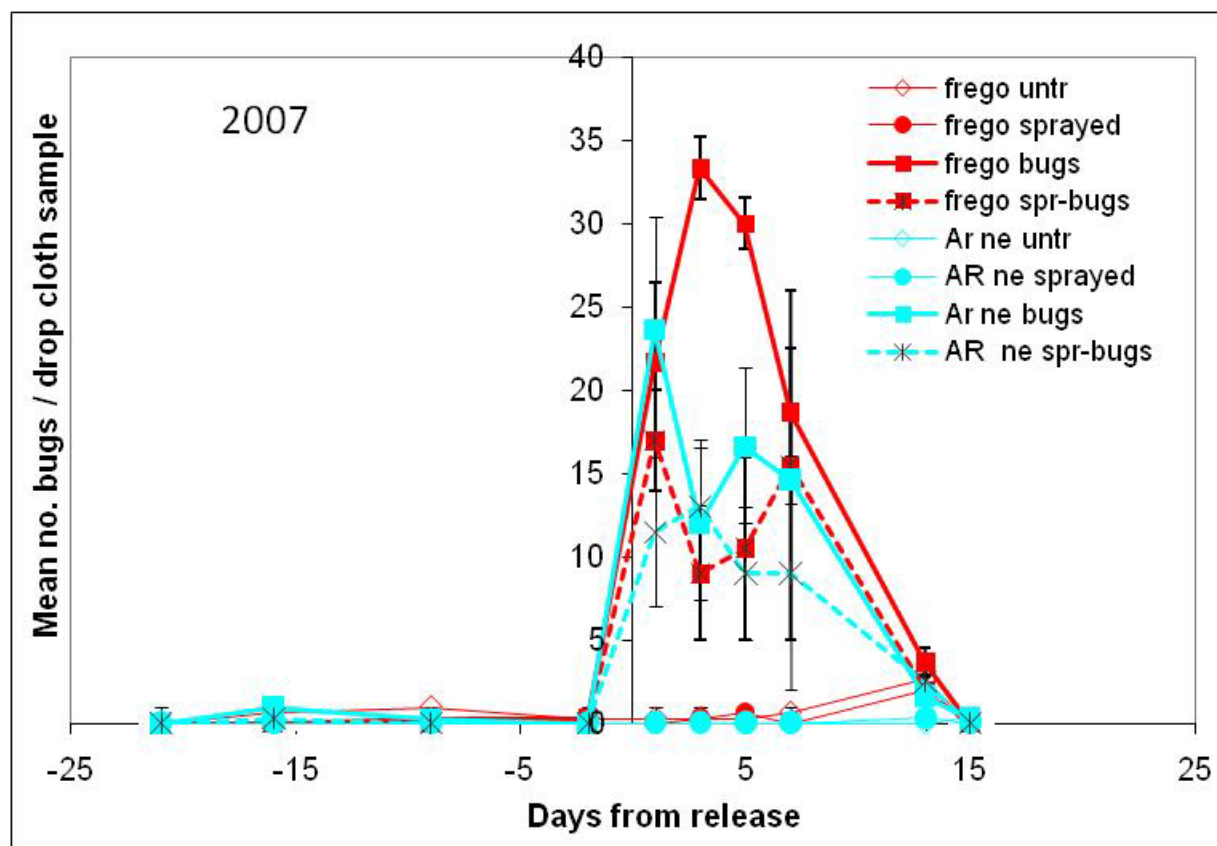


Figure 3. Results from 2007 drop cloth samples taken prior to and following release of 3rd instar plant bug nymphs in frego and AR ne cotton showing mean no. of plant bug nymphs observed per drop sample. Non infested treatments either were untreated (untr) or had received pre-flower applications of insecticides (sprayed). Treatments designated with spr-bugs were manually infested with nymphs, but also had been sprayed with insecticides pre-flower.

Field Sampling for TPB – Field population densities of plant bugs were at very low levels at the research site in both 2007 and 2008. It was assumed injury observed following the manual releases was associated with the introduced insects rather than the field population. Results from drop cloth samples are shown with release date reference point of '0' (Fig 3 and 4). For 2007, nymph numbers in drop cloth samples following the manual infestations were quite high. Over 30 bugs/3ft were observed in release plots in the frego line at 2 and 3 days after release. Numbers of nymphs recovered in AR ne were lower on these sample dates. These data indicate that nymph survival on the frego line was greater than the AR ne line. Bug numbers measured following release were lower compared to 2007; however, similar trends were observed with greater recovery of nymphs on frego compared to AR ne (Fig 4). Weather conditions in 2008 were less conducive to insect survival. Uncharacteristically high temperatures and low relative humidity in the first week of flowers likely contributed to limited successful establishment of the plant bugs nymphs (infestation rates and techniques were similar for both years).

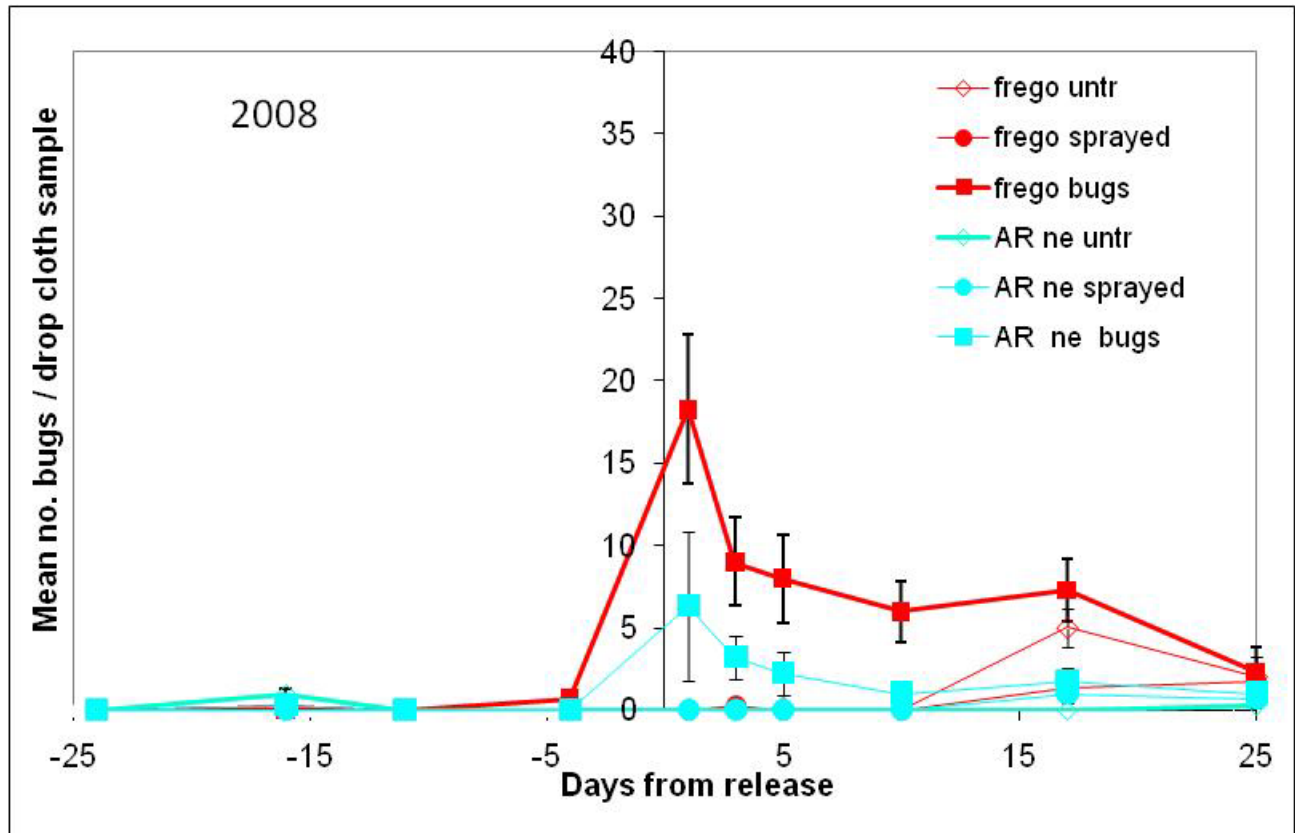


Figure 4. Results from 2008 drop cloth samples taken prior to and following release of 3rd instar plant bug nymphs in frego and AR ne cotton showing mean no. of plant bug nymphs observed per drop sample. Non infested treatments either were untreated (untr) or had received applications of insecticides (sprayed).

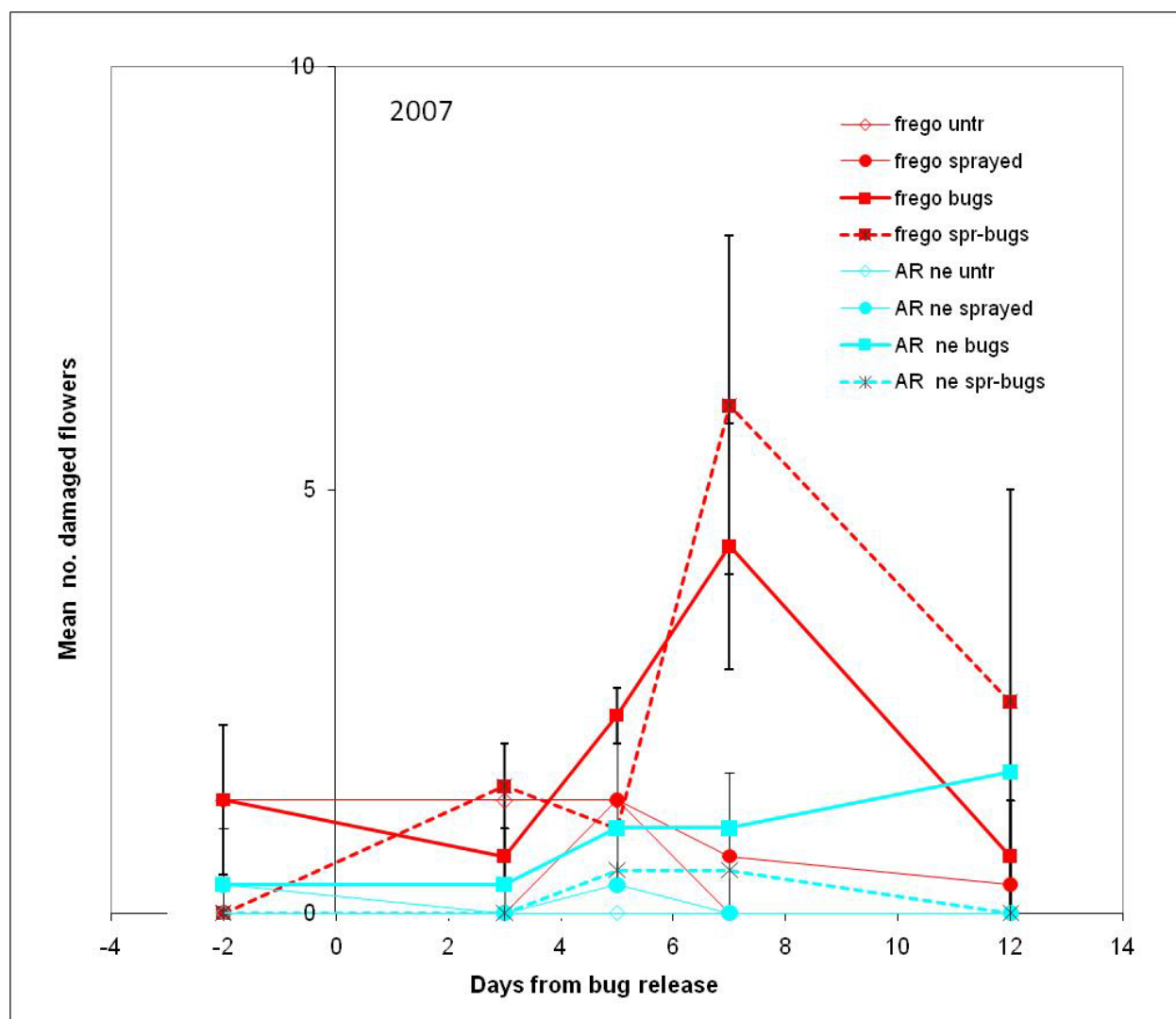


Figure 5. Mean (\pm SEM) no. of white flowers with damaged anthers prior to and following plant bug release (day 0) compared to treatments receiving insecticide or that were untreated in 2007. Injury assessments were made by counts of 10 white flowers per plot.

White Flower Anther Injury -- Numbers of flowers with anther injury reflect square feeding injury from plant bug nymphs following the manual infestations (Figs 5, 6). Damaged anthers appeared in flowers in frego bug release treatments by 5 days after bug release in both years. Few flowers with injury were noted in the untreated and unsprayed frego or in AR ne plots. In 2008 when bug survival was low, there were few flowers with anther injury noted in AR ne where bugs were released; there was only a brief rise in damaged flower counts occurring at 6 days after release. The flower sampling period was extended in 2008, and feeding injury from the single infestation of nymphs was observed to persist out to 18 days for the frego line (Fig 6). Numbers in the untreated plots increased after 2 weeks, an indication that either bugs had matured and adults were moving out of manually infested plots and/or native field population of bugs had moved into the study site.

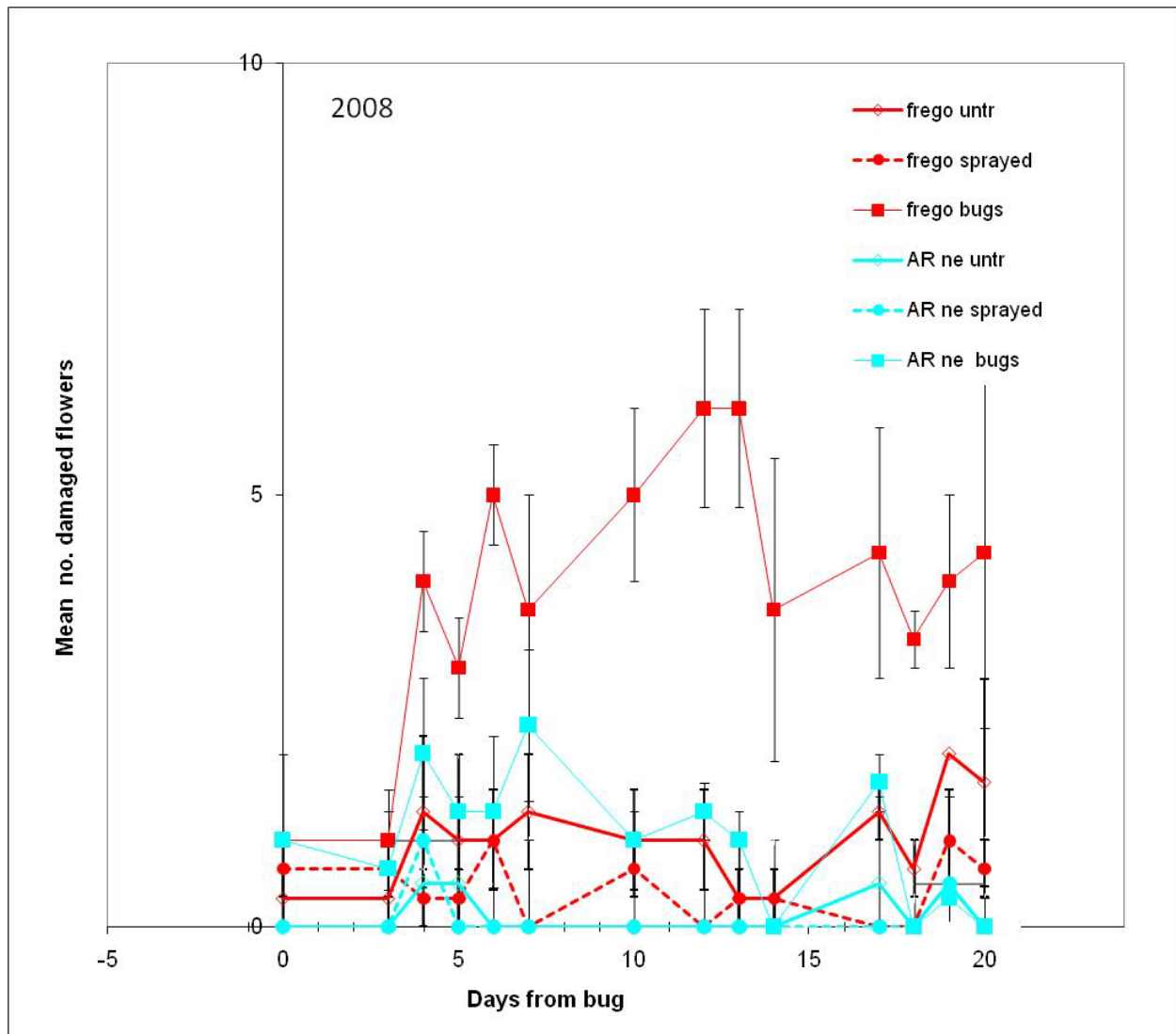


Figure 6. Mean no. (\pm SEM) of white flowers with damaged anthers prior to and following plant bug release (day 0) compared to treatments receiving insecticide or that were untreated in 2008. Injury assessments were made by counts of 10 white flowers per plot.

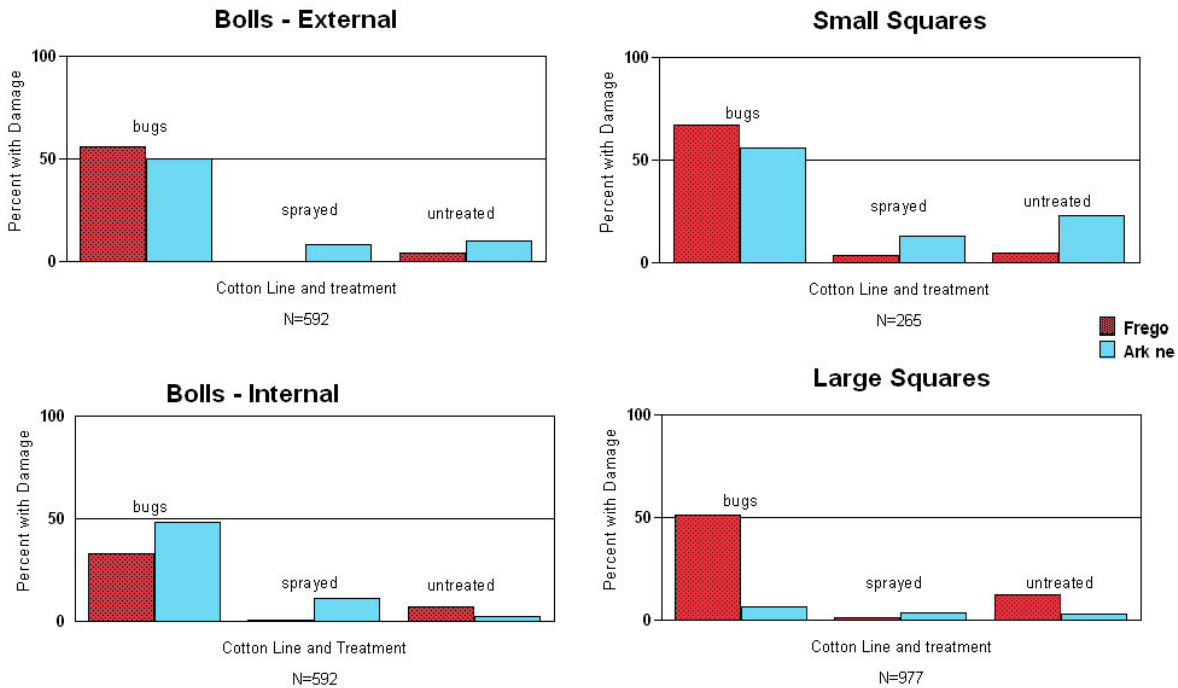


Figure 7. Results from in-season fruit injury mapping to determine location of nymph feeding damage assessed on 10 plants per plot collected 9 days after nymphs were released in 2007. Injury was significantly higher (all $p < 0.01$) where bugs were released compared to plants from sprayed or untreated plots. Where bugs were released, large squares of the frego were observed to have highest levels of injury among all treatments.

Fruit Dissections – Results from fruit and square dissections in the modified in-season “box” maps, show differences in injury among plant bug treatments; injury was greatest where bugs were released for both lines (Fig 7). External injury signs did not provide a reliable indication of internal injury to bolls (Fig 7). Feeding injury where bugs were released was similar for small squares and bolls, but was significantly higher for large squares ($>5\text{cm}$) from the frego line (Fig 7, 8). Typically, plant bug feeding on small squares results in square abscission. Larger squares typically are more tolerant because the insect feeding is localized on anthers and pollen sacks (Pack and Tugwell 1976). On small squares, the bug feeds on the totality of the floral bud, and it sheds. If the injury is not too great, and the square is retained, the anther injury is visible in the open flower. The number of damaged anthers in a white flower is a “reflection of cumulative feeding that occurred after squares reached 3mm” (Maredia et al 1994). Squares with extensive anther damage may shed as bolls (Pack and Tugwell 1976).

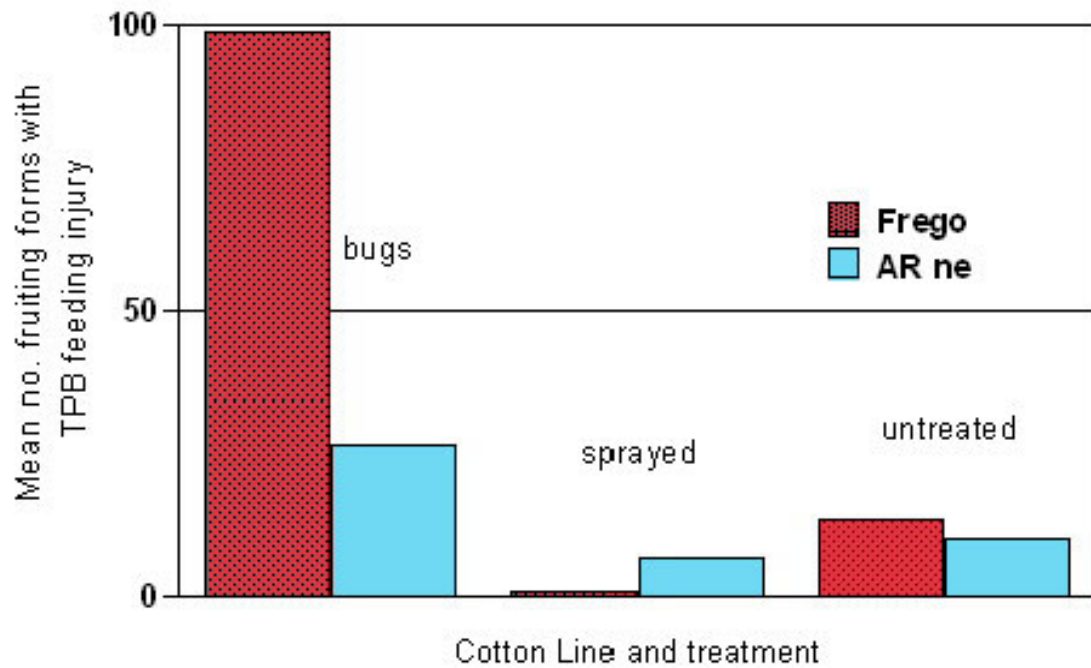


Figure 8. Mean no. of total fruiting forms per 10 plants showing signs of plant bug feeding injury following dissection of total squares, flowers and bolls on plants collected 9 days after manual infestation of nymphs compared to plants sprayed with insecticide or untreated plants. Injury was significantly higher in the frego line compared to AR ne ($P=0.001$).

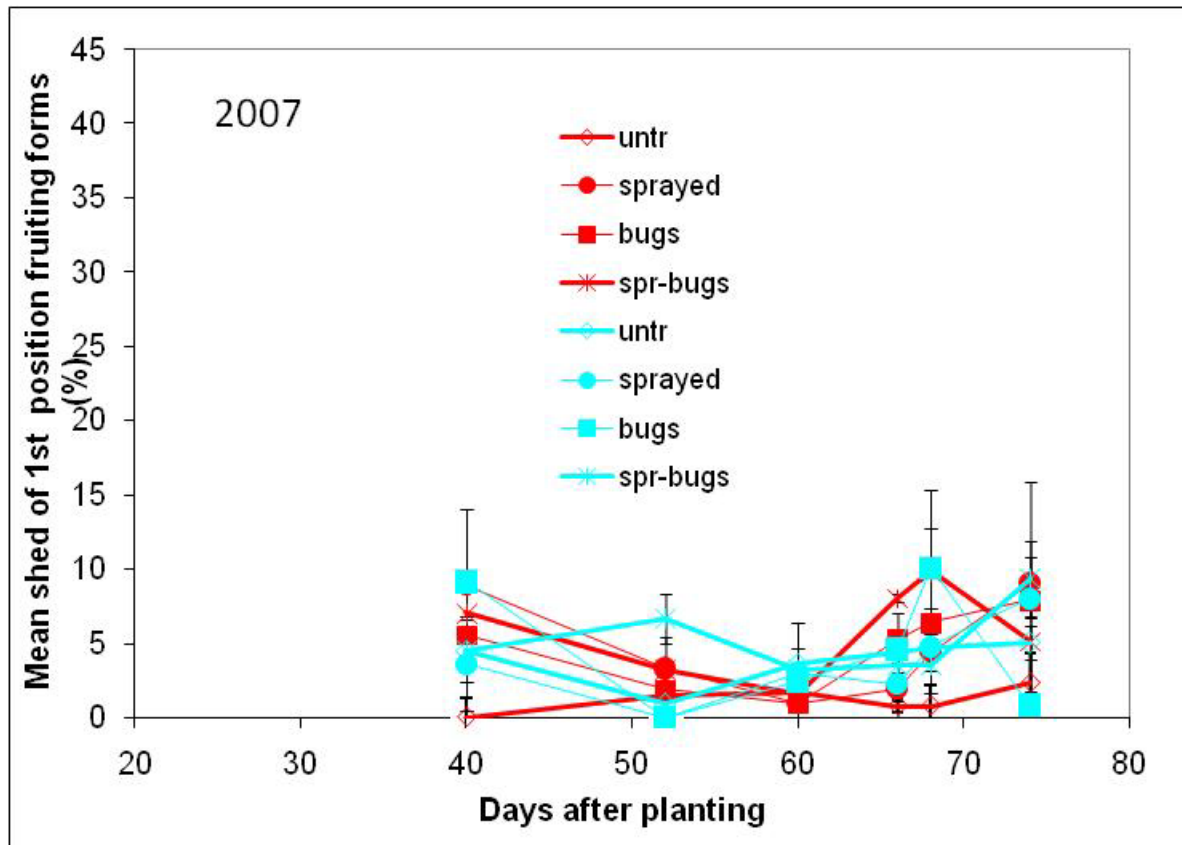


Figure 9. Mean shed (\pm SEM) of squares + bolls in first position for frego and AR ne lines in 2007 trial determined using COTMAN Squaremap sampling. No differences in square or boll shed were measured season long.

In-season plant mapping – Results from COTMAN Squaremap data collection indicate few first position square and boll sheds associated with plant bug infestations or other treatments in 2007 (Fig. 9 and 10). In the 2007 dissections, and with white flower injury counts, it is apparent that bugs were feeding on squares in both lines, but injury appears to have been insufficient to cause sheds (Fig 9). Retention was reduced in 2008 after nymphs were released (Fig 10). Shed levels were significantly higher at 61 and 67 days after planting where bugs were released in both frego and Ar ne. Differences among bug and sprayed treatments were not apparent later in the season as the crop reached physiological cutout and sheds increased among all treatments.

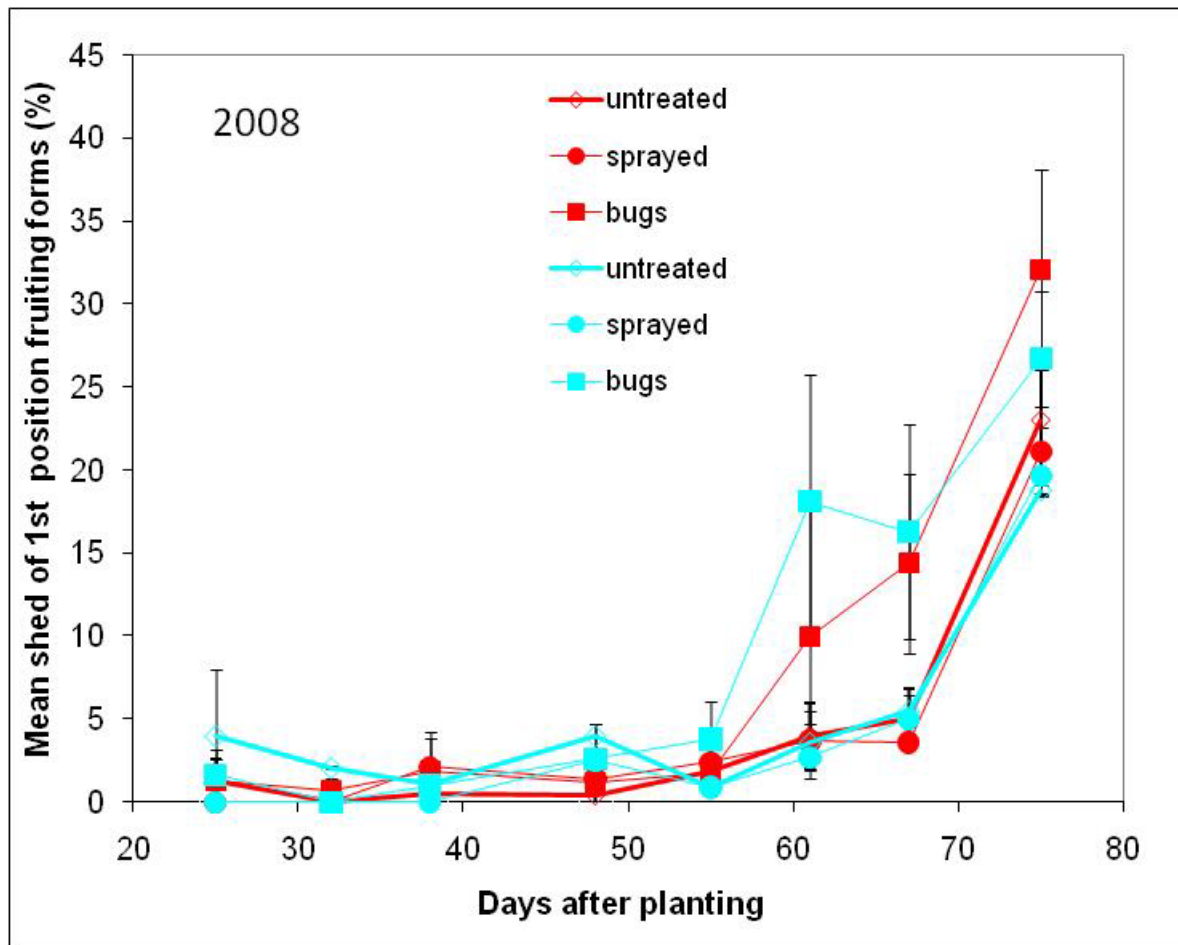


Figure 10. Mean shed (\pm SEM) of squares + bolls in first position for frego and AR ne lines in 2008 trial determined from COTMAN Squaremap samples. Treatments with manually applied nymphs had higher levels of shed in sample dates 61 and 67 days after planting ($P=0.01$).

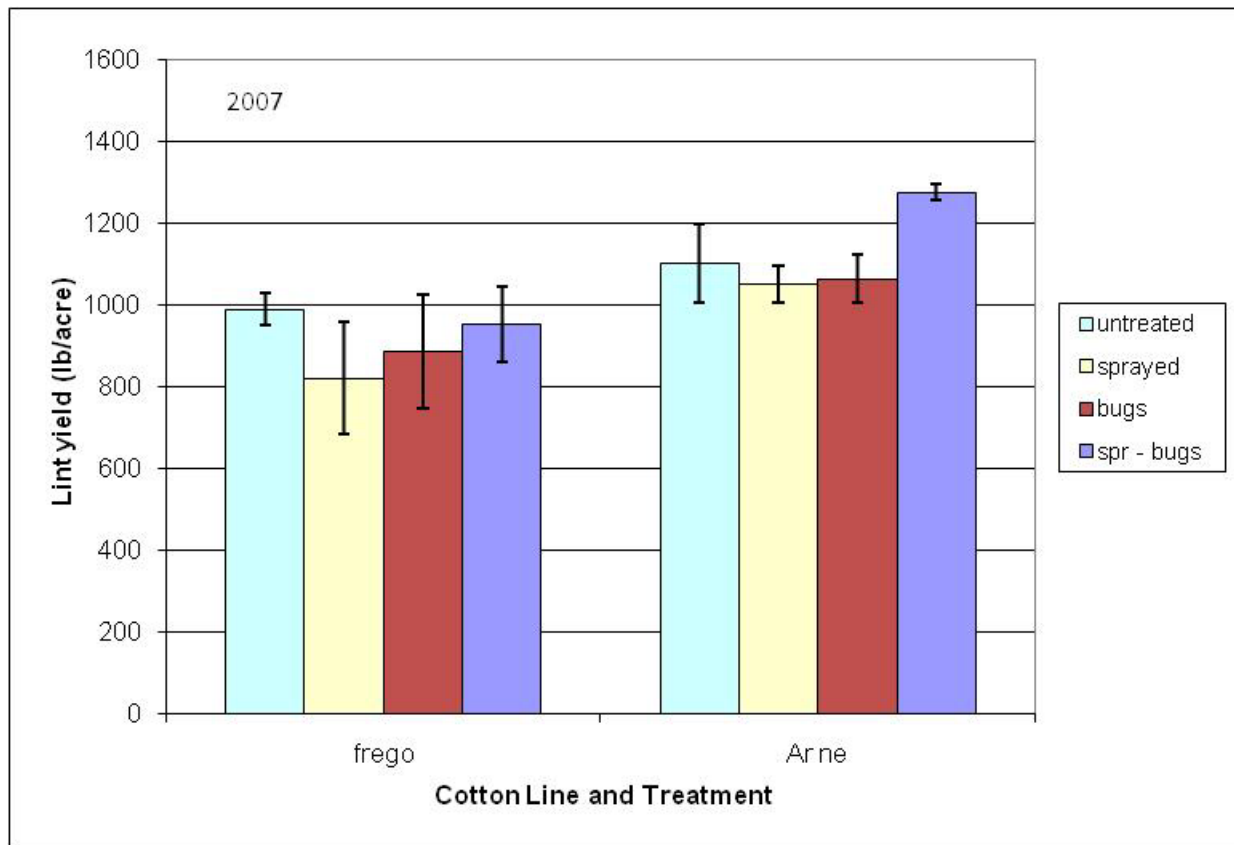


Figure 11. Mean lint yields (\pm SEM) from 2007 trial were not different among plant bug treatments; however, AR ne produced more cotton than the frego line with mean lint yields of 1109 and 908 lb/acre for AR ne and frego, respectively ($P=0.01$; $LSD=142$).

Yield – Despite successful establishment of nymphs and documented feeding injury in both lines, plant bugs did not affect lint yield in either 2007 or 2008. In 2007, yields were significantly higher in the Ar ne line than frego, but no differences between lines were observed in 2008.

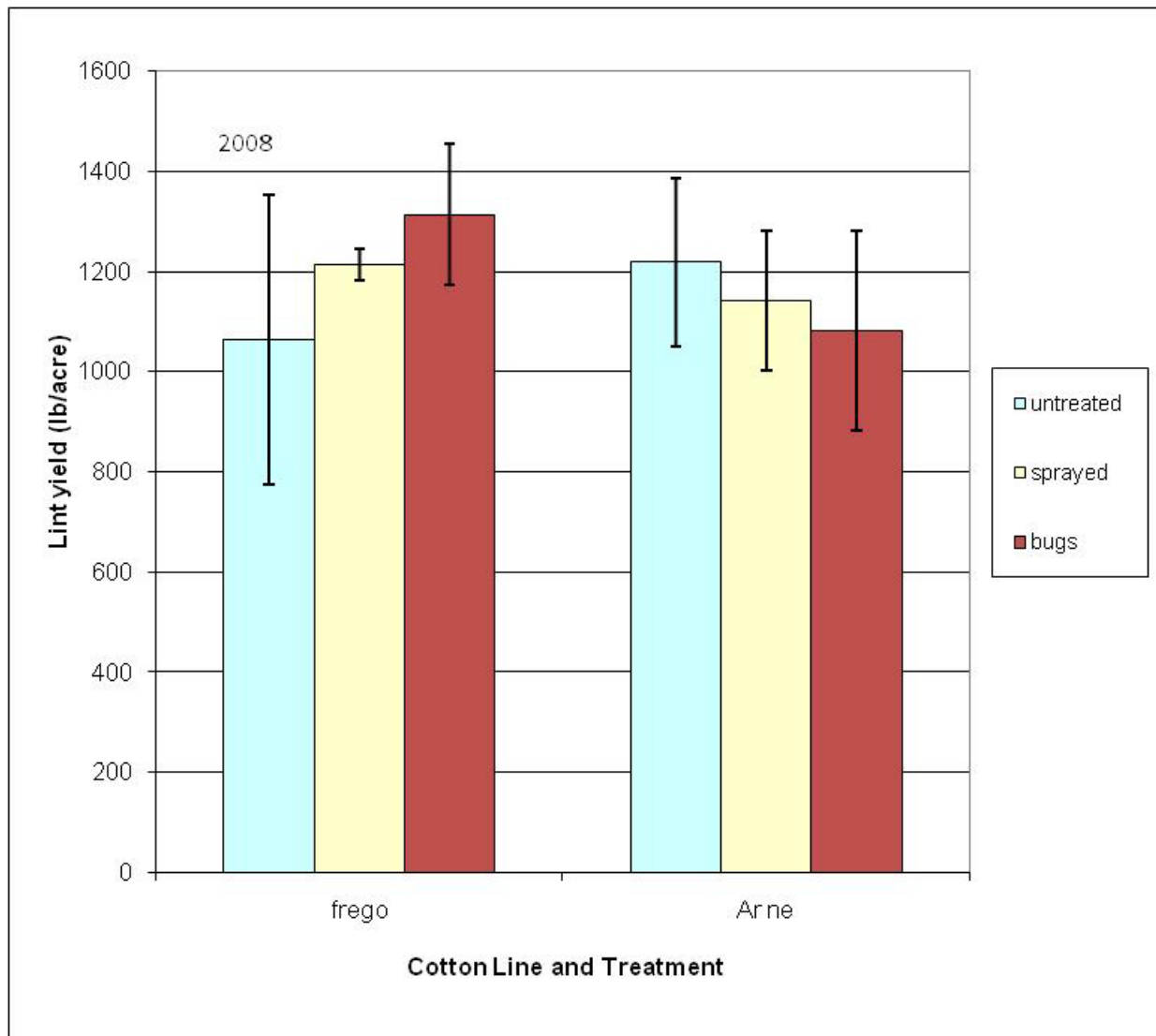


Figure 12. Mean lint yields (\pm SEM) from 2008 trial – there were no differences among plant bug treatments or cotton lines ($P>0.25$).

Conclusions

Techniques used in this trial show promise for expanding host plant resistance screening protocols. Manual infestation methods used provided information differential bug survival and subsequent feeding injury on lines with differential resistance, but the procedures are labor and time intensive. Results indicate significantly greater survival of lab reared nymphs on frego compared to the Arkot ne line as well as levels of feeding injury following infestations. Monitoring white flower anther injury was the most simple and rapid technique for assessing plant bug feeding activity. Within 5 days of manual release, anther injury was apparent in white flowers in both lines. Monitoring plant maturity using the COTMAN and NAWF sampling protocol provided information on availability of squares in late season which is an important key in deciphering nymph survival and late season movement of adult bugs. Yields were not a reliable indicator of host plant resistance characteristics of the lines tested.

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