

VERDE PLANT BUG ASSOCIATION WITH BOLL DAMAGE INCLUDING COTTON BOLL ROT AND POTENTIAL IN-SEASON INDICATORS OF DAMAGE

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Abstract

Cotton along the Gulf Coast of south Texas has experienced loss from cotton boll rot especially during the last 10 to 15 years, and stink bugs and plant bugs (Hemiptera: Pentatomidae and Miridae) that feed on cotton bolls have been suspected in introducing the disease. A replicated grower field survey was done to capture a representation of these sucking bug species and subsequent boll injury, including cotton boll rot, in 2010 and 2011. This survey was paired with a controlled field cage experiment that isolated feeding by verde plant bug, *Creontiades signatus* Distant (Hemiptera: Miridae). Verde plant bug was the dominant boll-feeding sucking bug species (~99% of insects collected) during peak to late bloom in cotton fields within 8 km of coastal waters (average of 0.42 bugs per plant), while it was not detected in inland fields. Cotton boll rot was found on up to 25% of the open bolls inspected, the disease was concentrated in coastal fields where verde plant bug was found. Isolating verde plant bug feeding further implicated it in introducing cotton boll rot. Verde plant bug-infested plants had significantly higher incidence of insect-punctured bolls and locules (15-35% in infested plants) and disease symptom incidence (5-27% in infested plants) than uninfested plants. All bolls with symptoms of disease tested positive for bacteria. Based on field data from 14 fields, stepwise regression using 4 independent variables (verde plant bug per plant [using a beat bucket or sweep net], proportion of green bolls with signs of external feeding, proportion of green bolls with signs of internal feeding, and proportion of green bolls with signs of boll rot) identified a one independent variable model as the best indicator of at-harvest boll damage. Verde plant bug per plant using a beat bucket was the selected model. Input of other variables did not increase the informative value of the regression using beat bucket sampling for verde plant bugs as an indicator of at-harvest damage. Because verde plant bug presence is an early step in the sequence of events leading to boll damage, opening bolls to verify internal feeding may be useful to supplement the beat bucket sampling method. From a pest and disease monitoring viewpoint, an in-season insect monitoring program is justified and needed most critically for fields close to coastal waters.

Introduction

Stink bugs and plant bugs (Hemiptera: Pentatomidae and Miridae) that feed on cotton bolls have reached elevated past status in cotton, *Gossypium hirsutum* L. (Malvaceae), during the last 10 to 15 years, including along the Gulf Coast of south Texas. Insecticide sprays, which traditionally controlled these sucking bugs, have been reduced following the advent and success of transgenic Bt (*Bacillus thuringiensis*)-cotton for heliothine control and boll weevil eradication (Edge et al. 2001, Allen 2008).

Stink bugs have been shown to cause damage to cotton bolls: boll abscission, lint staining and loss, and seed loss (Greene et al. 2001). Loss is magnified when bacteria causing cotton boll rot are introduced during feeding, as shown for the southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Medrano et al. 2007). Along the Gulf Coast cotton growing region of south Texas, several species of stink bugs occur on cotton; although their abundance and species composition is variable throughout the region (Hopkins et al. 2009). In addition, a mirid, the verde plant bug, *Creontiades signatus* Distant (Hemiptera: Miridae), emerged as a threat along the Gulf Coast during this time period of increased boll damage and cotton boll rot. Like the stink bugs, verde plant bug is able to injure cotton bolls, resulting in lint and seed staining, and possibly introduction of cotton boll rot (Armstrong et al. 2010).

To complement greenhouse studies verifying that microbes are associated with verde plant bug feeding injury to cotton bolls (Armstrong et al. 2009c), we investigated whether feeding by verde plant bug is associated with cotton

boll rot in the field. A replicated grower field survey was done in 2010 and 2011 to capture a range of sucking bug species and observe subsequent boll injury, including cotton boll rot, occurring in cotton along the Gulf Coast region of Texas. The vast majority of boll-feeding sucking bugs collected were verde plant bug, which allowed a presumptive association of this insect's feeding to any cotton boll rot that was subsequently detected. To further strengthen the association of verde plant bug and transmission of boll-rotting organisms, a controlled field cage experiment was done to compare characteristics of insect feeding and cotton boll rot on plants exposed to and protected from verde plant bug adults.

Materials and Methods

Field Survey

In 2010, sucking bugs were collected from 80-200 plants in each of 15 cotton fields along the Gulf Coast region of Texas. Sucking bugs were collected using a beat bucket and sweep net during peak to late bloom (about 10 to 7 nodes above white flower [Kerby et al. 2010]) and were identified and counted in the field. Nine and six fields were designated as coastal and inland, respectively. Fields within 8 km of the nearest coastline, inland bay, or coastal waterway were designated coastal ($n = 8$); fields exceeding this 8 km demarcation were designated as inland ($n = 6$). Locations by county and GPS coordinates were Calhoun County (28.580N/96.664W, 28.551N/96.645W, and 28.550N/96.65W), Aransas County (28.098N/97.218W), Nueces County (27.781N/97.561W, 27.776N/97.562W, 27.770N/97.562W, 27.725N/97.668W, 27.708N/97.668W, 27.708N/97.668W, and 27.708N/97.644W), Kleberg County (27.437N/97.848W, 27.426N/97.875W, and 27.446N/97.910W) and Cameron County (36.199N/97.485W and 26.193N/97.488W). Randomly selected green bolls from 1.0 to 3.5 cm in diameter ($n = 120$ to 150) were inspected externally for signs of stylet probing, then opened and inspected for signs of probing on the inner carpel wall and initial signs of cotton boll rot developing in the locules (Medrano et al. 2007). The same fields were revisited near harvest to assess damage to open bolls. At each field, randomly selected bolls ($n=150$) judged as harvestable (i.e., expected to be fully open in time for mechanical harvest [Jenkins et al. 1990]) were scored for damage using a five class locule damage scale. The scale ranged from 0 (no damage), through an incremental 1 to 3 gradation as damage progressively worsened within each locule and affected additional locules, to 4 (severe damage to all locules) (Lei et al. 2003).

Drought conditions were severe during 2011: about 8.3 cm of rainfall April 1 through Aug. 30 compared with 45.7 cm in 2010 and a 35.5 cm average over 125 years (Corpus Christi station, National Weather Service 2011). Sucking bug populations were very low but verde plant bug was detected at potentially damaging levels in the same locality of two coastal fields observed the previous year (Rio Hondo in the Lower Rio Grande Valley, and the Texas AgriLife Research and Extension Center, Corpus Christi). At these fields, verde plant bug was counted during peak to late bloom, and green and open bolls were scored for feeding injury and damage as previously described. During both years, fields were planted to multiple cultivars adapted to the region. Insecticides were used in some fields at early squaring for cotton fleahopper control, but sampling never occurred within two weeks of an application.

Controlled Field Cage Experiment

In 2010 and 2011 at the Texas AgriLife Research and Extension Center (Corpus Christi, TX), we exposed caged cotton plants to verde plant bug and measured boll injury and cotton boll rot of green bolls, and compared results to those from non-infested caged plants. Adult verde plant bugs were obtained from a laboratory colony that was established and periodically replenished with field-collected bugs from several wild and cultivated host plants in the Lower Rio Grande Valley (JSA, pers. obs.). Green bean and corn were the food source, harborage, and oviposition substrate for the colony (Armstrong 2010). Adults were taken from the colony for experimental use within two months of the last field collection used to replenish the colony. The procedure was designed to maintain microbes associated with feeding in the colony while providing a ready and uniform source of bugs for the experiment. In 2010, infestation rates for the field experiment were 0 (control), 0.25, and 2 verde plant bugs per plant. The treatment cages were replicated 3 to 4 times in a randomized complete block design. Full plant cages made of organza cloth were placed over randomly selected groups of plants (12 plants per cage) when plants were at peak bloom (about 10 to 7 nodes above white flower), which is consistent with the time period of verde plant bug occurrence in commercial fields of the region. Prior to caging, no stink bug and verde plant bug activity was detected for the previous three weeks, and plant inspection also indicated no feeding activity prior to caging. Two days before infesting, all cages were sprayed with short-residual pyrethrins (0.02% by volume, United Industries, St. Louis, MO). At infestation, randomly selected adults from the colony were placed in one-ounce portion cups in the morning and released at the base of the cages by 10:00 AM at the designated infestation rates. Seven days after the

infestation, bugs were killed with a combination of pyrethrins and a longer residual pyrethroid (zeta-cypermethrin, FMC, Philadelphia, PA). Cages were left on the plants to further avoid incidental insect feeding. Two weeks later during late bloom, half the green bolls were harvested. In 2011, the experiment was repeated with the following modifications. Infestation rates were changed to 0, 2, and 4 verde plant bug per plant based on preliminary results coming from a companion economic threshold study. Also, plants per cage were reduced to four to avoid plant crowding that was experienced in 2010, and all green bolls from the cages were harvested. All other procedures related to insect infestation and spraying were the same as in 2010.

At late bloom before bolls began to open, green bolls ($n \geq 40$ per treatment) were randomly selected across replications of each treatment for detailed insect injury and microbiological analyses, according to methods described in Medrano et al. (2007). Bolls were individually surface sterilized for 10 min in a 0.5% sodium hypochlorite solution then rinsed for 2 min in sterile water three times. Macroscopic evidence of insect feeding on the outer and inner boll, and symptoms of infection of lint and seed tissue were recorded after excising carpel walls with a sterile scalpel. Diseased bolls were scored for severity using a scale of 0 – 5 for each locule: 0 (no disease symptoms), 1 (1 to 24%), 2 (25 to 49%), 3 (50 to 74%), 4 (75 to 99%), and 5 (100% rotted tissue).

Data Analyses

To evaluate species composition in the field survey, numbers and percentages for each species relative to the total number of sucking bugs collected fields were calculated separately (adjusted to a per field basis of 120 plants) for the coastal and inland fields in 2010. A χ^2 (Pearson's) goodness-of-fit test was used to test equality of the proportions of species collected in coastal and inland (Chapter 13, Freund and Walpole 1980).

For fields sampled in 2010 and the two fields in 2011, boll data were used to calculate proportion of bolls with signs of cotton boll rot for green bolls inspected during peak to late bloom and open bolls inspected near harvest, and average damage score of open bolls (0-4 scale). Verde plant bug per plant for the beat bucket and sweep net methods during peak to late bloom was also calculated for each field. These field averages were used to build five simple linear regression models to compare prediction of damage scores with these in-season indicators separately. A qualitative independent variable was also included to distinguish coastal fields from inland fields (Chapter 10, Neter et al. 1985). Damage score (y) was regressed on the following: verde plant bug per plant using a beat bucket, verde plant bug per plant using a sweep net, proportion of green bolls with signs of external feeding, proportion of green bolls with signs of internal feeding, and proportion of green bolls with signs of boll rot, each considering whether the relationship differed between coastal and inland fields. Next, two sets of four independent variables were entered into stepwise regression using a 0.15 variable selection significance level. The four independent variables were: verde plant bug per plant using either the beat bucket or the sweep net (x_{1b} and x_{1s} , respectively), proportion of green bolls with signs of external feeding (x_2), proportion of green bolls with signs of internal feeding (x_3), and proportion of green bolls with signs of boll rot (x_4). Inspection of residuals showed no pattern of deviation from linear regression assumptions; therefore neither data transformations nor curvilinear functions were considered.

For the controlled field cage experiment each year, percentages of insect-punctured bolls and locules, number of punctures per boll, percentages of diseased bolls and locules, and amount of disease per boll were calculated from uninfested plants and plants from each infestation level. A χ^2 (Pearson's) goodness-of-fit test was used to test equality of the frequencies of the measurements between treatments.

Results

Field Survey

Plant bugs and stink bugs were much more numerous in coastal fields (mean = 0.61 bugs per plant, ranging from 0.045 to 1.59 per plant using a beat bucket) than in inland fields from peak to late bloom (mean = 0.06 bugs per plant, ranging from 0 to 0.23 per plant using a beat bucket) ($\chi^2 = 75.3$, $df = 1$, $P < 0.0001$) in 2010. Verde plant bug averaged 0.42 bugs per plant in coastal fields and its abundance was quite variable among fields inspected (0-1.56 bugs per plant). Both verde plant bug nymphs and adults were collected. In comparison, it was not detected in inland fields (Table 1). In collections from the same fields earlier in the season, over 99% of the 216 (early-season squaring) and 140 (early bloom) insects collected were cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae). Cotton fleahopper feeds on squares and very small bolls, primarily causing abscission (Bell et al. 2006). Excluding cotton fleahopper and rice stink bug, *Oebalus pugnax* (F.) (Hemiptera: Pentatomidae) (which

does not feed on cotton bolls), verde plant bug represented the vast majority of sucking bugs in coastal fields that fed on bolls (~99 % of insects collected) (Table 1).

Table 1. Species composition of plant bugs and stink bugs (Hemiptera: Miridae and Pentatomidae) from peak to late bloom, estimated with beat bucket sampling, in coastal and inland cotton fields of the Gulf Coast Region of south Texas, 2010.

Species ^b	Coastal ^a		Inland ^a	
	No. insects (%)	% boll-feeding	No. insects (%)	% boll-feeding
Cotton fleahopper ^c	258 (30.2)	-	42 (95.5)	-
Rice stink bug ^c	2 (0.2)	-	2 (4.5)	-
Verde plant bug	589 (68.8)	98.9	0 (0)	0
Lygus spp.	2 (0.2)	0.3	0 (0)	0
Green stink bug spp.	5 (0.6)	0.8	0 (0)	0

^a Coastal (n=9, total plants inspected=1,400) and inland (n=6, number of plants inspected=720) fields. Data were adjusted to a per field basis of 120 plants for coastal and inland field comparison.

^b Scientific names for mirids: cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter); verde plant bug, *Creontiades signatus* Distant; and *Lygus*, not identified to species, but likely *Lygus lineolaris* (Palisot de Beauvois), based on past regional records (Esquivel and Mowery 2007). Scientific names for pentatomids: rice stink bug, *Oebalus pugnax* (F.); and stink bugs were not identified to species, but likely mixture of southern green stink bug, *Nezara viridula* (L.) and green stink bug, *Acrosternum hilare* (Say), based on past regional records (Hopkins et al. 2009).

^c Species was excluded from the boll-feeding sucking bug calculation.

In 2011, drought conditions apparently led to much lower insect activity; therefore monitoring activity was limited to two coastal fields where verde plant bug was found at potentially damaging levels, and other plant bugs and stink bugs were not detected. During both years, cotton boll rot and damage of open bolls was subsequently detected, and the damage was concentrated in coastal fields where verde plant bug was found. Cotton boll rot was found in up to 25% of the open bolls, and it was most common in coastal fields where verde plant bug was detected. Cotton boll rot detected in inland fields never exceeded 8%. Cotton boll rot was mostly seen on bolls on the upper and outer portion of the plant, which is consistent with verde plant bug feeding occurring most frequently on small to mid-sized bolls during peak to late bloom (Armstrong et al. 2009a). For both inland and coastal fields, there was a strong linear relationship of boll rot in open bolls to damage score. This trend was apparent especially in coastal fields where cotton boll rot magnified damage (i.e., the qualitative variable of field distance to the coast and inland bays and waterways was significant) (Fig. 1).

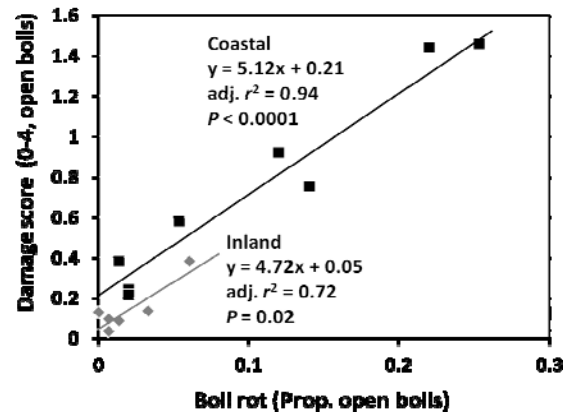


Figure 1. Regressions of presence of cotton boll rot near harvest (proportion of open bolls with signs of cotton boll rot) to damage of open bolls near harvest (average of a 5 class locule damage score, 0-4) for coastal and inland fields. Symbols indicate coastal fields within 8 km of the coastline and inland bays (squares) and fields further inland (diamonds). Fields were located from Port Lavaca to Rio Grande Valley, south Texas, 2010-2011.

Signs of external green boll feeding were very common in all fields ($>70\%$ of bolls), but the proportion of green bolls with signs of external feeding was not indicative of the damage score at harvest (adjusted $r^2 = 0.04$, $P = 0.13$). In contrast, the proportion of green bolls with signs of internal feeding was a moderately good indicator of the damage score at harvest represented well by a common regression of coastal and inland fields ($P = 0.004$) (Fig. 2). The use of insect monitoring as a possible indication of subsequent damage appears to have value as the damage score was linearly related to the number of verde plant bugs per plant during peak to late bloom using the beat bucket ($P = 0.0004$) and the sweep net ($P = 0.009$) (Fig. 3). No verde plant bugs were detected in inland fields; therefore one regression was estimated.

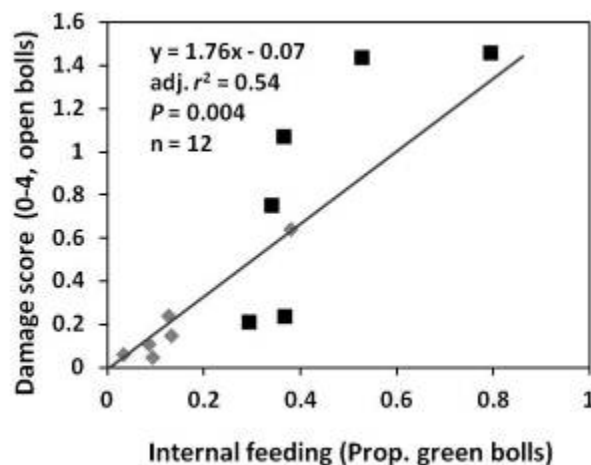


Figure 2. Regression of damage score to presence of internal feeding (proportion of green bolls with internal signs of feeding) by cracking green bolls. Symbols indicate coastal fields within 8 km of the coastline and inland bays (squares) and fields further inland (diamonds). Fields were located from Port Lavaca to Rio Grande Valley, southern Texas, 2010-2011.

The stepwise procedure identified the beat bucket procedure as the most expedient time to collect samples. A one independent variable model was identified when using the beat bucket ($y = 2.06x_{1b} + 0.27$; adjusted $r^2 = 0.74$; $F = 32.3$; $df = 1, 10$; $P = 0.0002$). Because verde plant bug presence is an early step in the sequence of events leading to

boll damage, observing signs of internal feeding (cracking green bolls) would be valuable to verify that verde plant bug is damaging the bolls.

Controlled Field Cage Experiment

In both 2010 and 2011, the plant enclosures were successful in restricting outside infestations, boll feeding and subsequent damage to the plants in the cages where verde plant bugs were introduced. The percentage of punctured bolls and locules and the number of punctures per boll were much higher at all infestation levels compared with the uninfested treatment ($\chi^2 > 14.2$; $df = 2$; $P < 0.0009$) (Table 2). The greatest χ^2 cell contribution came from the uninfested treatment in all comparisons, and differences were not as great across the verde plant bug infestation levels. The subsequent percent diseased bolls and locules, and amount of disease per boll, followed the same pattern of much greater disease detected in the two infestation levels compared with the uninfested plants ($\chi^2 > 9.1$; $df = 1$; $P < 0.02$) (Table 2).

Table 2. χ^2 goodness-of-fit tests comparing summary statistics of insect-punctured bolls and locules and diseased bolls and locules from verde plant bug-infested and uninfested plants of a field cage experiment, Corpus Christi, Texas, 2010-2011.

Year	Infestation	# of Bolls	# of Locules	% of Punctured Bolls	% of Punctured Locules	Punctures per Boll	% Diseased Bolls	% Diseased Locules	Amount of Disease per Boll
2010	0	84	341	11.90	2.93	0.12	0.00	0.00	0.00
	0.25	96	387	35.42	17.05	2.69	17.71	9.82	1.28
	2	67	271	43.28	21.96	4.49	16.42	9.23	0.85
	χ^2								41.3
	χ^2			14.2	46.4	319	14.5	32.7	
	χ^2								
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	P			0.0009	< 0.0001	< 0.0001	0.0008	< 0.0001	< 0.0001
2011	0	81	324	2.47	0.93	0.037	2.47	0.62	0.25
	2	40	166	35.00	15.06	1.30	27.50	9.64	0.60
	4	40	160	35.00	18.12	1.08	17.50	5.00	0.42
	χ^2								9.1
	χ^2			22.9	46.2	89.7	14.6	22.9	
	χ^2								
	χ^2								
	χ^2								
	P			0.0002	< 0.0001	< 0.0001	0.0007	0.0002	0.011

Infestation is the number of verde plant bug per plant.

df = 2 for all comparisons.

% punctured and diseased bolls and amount of disease based on the boll count for each infestation. % punctured and diseased locules based on the locule count for each infestation.

Examining the bolls with disease symptoms for bacteria and fungi ($n = 7$ to 17 depending upon the treatment and year), all preparations plated positive for bacteria and none for fungi, while all controls from the uninfested treatment were negative.

Discussion

Overall, verde plant bug was the dominant boll-feeding sucking bug species along the coastal cotton growing region of south Texas in 2010 and 2011, and substantiates earlier field observations of its activity and damage to cotton (Coleman 2007). In regard to the strong concentration of verde plant bug in coastal fields, coastal seepweed and annual seepweed grow in saline and alkaline soils in the vicinity of cotton fields located near coastal waters. These plants along with weedy annual hosts may provide a resource for verde plant bug populations to increase before migration to cotton. It is worth noting that verde plant bug has been detected in inland cotton fields in other surveys, but consistently at much lower levels than in coastal fields.

The relationship of in-season verde plant bug density to early signs of cotton boll rot in green bolls was poor, but as the disease progressed to near harvest the association of in-season verde plant bug density to cotton boll rot in open bolls was significant (Fig. 3). The field cage experiment further implicated verde plant bug as introducing disease agents causing cotton boll rot (Table 2) and bacteria was associated with the rot. The better relationship of verde plant bug densities to signs of disease when bolls are open is consistent with observations of Medrano et al. (2009), who found initial feeding by southern green stink bug until full expression of cotton boll rot took several weeks. For those utilizing the practice of cracking green bolls, we caution that inspection for early signs of cotton boll rot in green bolls may be a poor indicator of final disease expression and resulting boll damage. When the focus is verde plant bug, special attention should be given to fields near coastal waters. Peak bloom is clearly a critical time for monitoring, although it may be judicious to begin sampling during early bloom to detect initial infestations of verde plant bug. On-going economic threshold, boll injury, and pathogenicity work will assist in further quantifying cotton boll rot and harvest risk associated with verde plant bug feeding. Therefore from a pest and disease monitoring viewpoint, an in-season insect monitoring program is justified and serves as an indicator of subsequent boll damage, including that caused by cotton boll rot.

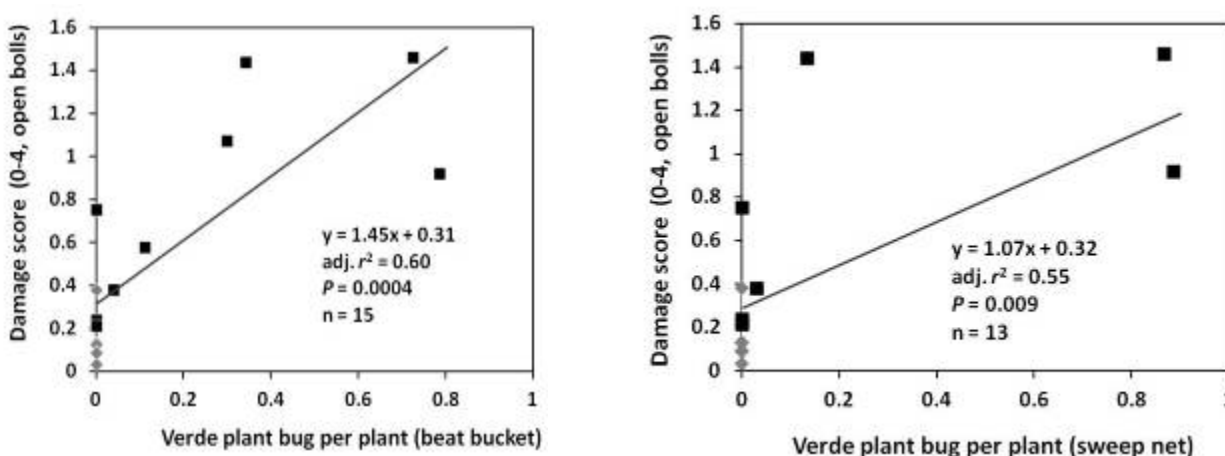


Figure 3. Regressions of damage score to number of verde plant bug detected during peak to late bloom with a beat bucket (left graph) and sweep net (right graph). Symbols indicate coastal fields within 8 km of the coastline and inland bays (squares) and fields further inland (diamonds). Fields were located from Port Lavaca to Rio Grande Valley, south Texas, 2010-2011.

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