Actual and Simulated Injury of *Creontiades signatus* (Heteroptera: Miridae) Feeding on Cotton Bolls[1]

J. Scott Armstrong,[2] Randy J. Coleman and Brian L. Duggan[3]

USDA, ARS, Beneficial Insect Research Unit, Weslaco, Texas 78596 USA

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Abstract The actual feeding injury of *Creontiades signatus* Distant (Heteroptera: Miridae) was compared with a simulated technique during 2005, 2006 and 2008 by injecting varying dilutions of pectinase into cotton bolls at different boll sizes (ages) and into 2 or 4 locules to determine if such a technique could be used to reduce the time and labor involved with conducting economic injury level studies in the field. The most accurate simulation occurred in 2008 by injecting 1 µL of 10% pectinase into all 4 locules of a cotton boll. This improved the relationships of injury score to seed cotton, seed, and lint weights. The youngest boll age class of ≥ 2 cm diam. (2 d of age) was not significantly more damaged than the medium age ≥ 2.5 cm (8 d of age) bolls, and both sustained significantly more injury than the large boll classification of ≥ 3 cm (12 d of age). However, small bolls were at least 3 times more likely to abscise than medium-sized bolls, and large bolls did not abscise regardless of treatment. Some damage was observed for large bolls from the injected and actual feeding compared with the controls, but the lint and seed weights were not significantly different for any of the treatments including the controls. Our study characterizes the feeding injury caused by *C. signatus* and describes a simulated technique that may be used to further economic injury studies.

Key Words *Creontiades signatus*, cotton, simulated, damage

Over the last few years, there has been confusion over the identity of a green mirid with a short history of infesting and causing economic damage to cotton, *Gossypium hirsutum* L. by feeding on the developing bolls in South Texas cotton growing regions. The first reports of it being a pest of cotton were made in Integrated Pest Management Newsletters and Extension Guides by Extension entomologists from the Upper Coastal Bend (Fromme 2001, Parker 2006) and South Texas (Norman and Sparks 2002). Economic infestations from these reports indicated concern for feeding injury to cotton bolls as opposed to developing florets (squares) because of the lateness of the infestations. The densities of this mirid pest have increased steadily over the last few years not only in cotton, but also in soybeans, *Glycine max* (L.) Merr. Although little is known on the available host plants of *Creontiades signatus* Distant, soybeans are a viable reproducing host (J.S.A., unpubl. data) grown as spring and fall-planted crops in the Lower Rio Grande of South Texas. Because 2 crops of soybeans are grown in a single growing season, they are available as a reproducing host for 6 months during the year, increasing the densities and potential for *C. signatus* to increase as a pest of cotton. More information is needed on the temporal patterns of *C. signatus* and its relationship with the cropping sequence of cotton, soybeans, other crops, and alternate host plants.

[1]Received 07 May 2009; accepted for publication 13 September 2009.
[2]Address inquiries (email: scott.armstrong@ars.usda.gov).
[3]Monsanto, St. Louis, MO 63167.

Molecular techniques and taxonomic observations were used to help differentiate closely-related species within the genus *Creontiades* and deduce that the green mirid was *C. signatus*, probably a native species (Coleman et al. 2008). Further research that reports the oviposition site preference of *C. signatus* on okra leaf and normal leaf cotton genotypes, and the isolation of the pathogens feeding injury to cotton bolls has recently been reported (Armstrong et al. 2009a, b.).

An increase in the knowledge of management and bioeconomics is needed for *C. signatus* infesting cotton considering that other bug pest complexes have significantly increased in pest status, coincidentally following initiation of boll weevil eradication programs. For example, the tarnished plant bug, *Lygus lineolaris* (Palisot de Beavois), has increased in pest status in the southern and midSouth cotton belt regions following boll weevil eradication (Layton 2000) to the point that it has developed insecticide resistance to different classes of insecticides (Snodgrass 1996, Snodgrass et al. 2009). The stink bug complex in the southeastern cotton belt regions has increased in pest status following boll weevil eradication (Greene et al. 1999, Turnipseed et al. 2004, Willrich et al. 2004) and introduction of *Bt* cotton. However, the pest status of all bug complexes has not necessarily increased or is not related to boll weevil eradication. California and Arizona have perennial problems with *Lygus hesperus* Knight and *L. elisus* Van Duzee (Heteroptera: Miridae) in alfalfa and cotton (Godfrey et al. 2008, Ellsworth and Naranjo 2008).

There has been no research to determine standard sampling methods, action thresholds, or insecticide recommendations for *C. signatus* infesting cotton in the U.S., and the current recommendations for management are listed as the same for *Lygus* spp. for lack of such information. *Creontiades signatus* may become more of a concern as a pest of South Texas cotton as insecticide use for boll weevil increases under active eradication. Therefore, our objectives were to: (1) characterize the injury caused by *C. signatus* feeding on cotton bolls of different age classes, and (2) compare actual feeding damage to a simulated damage technique to determine the usefulness for conducting economic injury studies. If a comparable feeding injury technique is developed for this mirid pest, it could expedite economic injury studies that would aid in management decisions.

Materials and Methods

Insect rearing. A colony of *C. signatus* was initiated in January 2005 by collecting adults from rocket mustard, *Sisymbrium irio* L., and pigweed, *Amaranthus* spp., from several locations in Hidalgo Co., TX. The collected adults were maintained by providing "Texas Pinkeye" pea pods, *Vigna unguiculata* L., for feeding and oviposition and supplementing with field corn ears, *Zea mays* L., in ventilated Tupperware® containers held in environmental chambers at 27°C, 65% RH, and a 14:10 h L:D photophase. About once a week, newly-collected adults from Hidalgo and Cameron counties were mixed with the laboratory colony to help maintain a vigorous genetic background.

Feeding injury to bolls. The cotton plots used in these studies for 2005, 2006 and 2008 were all within a larger block of "Stoneville 474" planted on 100-cm row centers at the USDA-ARS Farms, Weslaco, TX, on March 2, 9, and 5, respectively. In 2008, the cotton plots were maintained within a screened field-cage (15 × 30 m) because of a longer infestation period required for small, medium and large-sized cotton bolls, and to lessen the effects of malathion applications for boll weevil eradication. Within each block of cotton planted for all 3 yrs, a randomized block was used with 1 linear-m-row of cotton serving as a replicate, and alternating rows used as buffers between microplots.

A first-position cotton boll from different plants within the 1 row m was used for all treatments administered to the cotton bolls. Cotton bolls were aged by removing day-old blooms and inspecting for any preexisting external damage. If the young bolls were devoid of any injury, they were tagged, labeled and enclosed with bottomless 355-ml polystyrene cups covered with nylon hosiery (Greene et al. 1999). The hosiery sleeve was secured to the boll peduncle and at the cage top with a twist-tie.

One wk later, the first-position bolls were used for the different treatments on different plants in the 1-m row. In 2005, the 3 treatments were: (1) introduction of one adult, (2) injection of 1 μL of a 10% solution of pectinase (19 units/ml protein extract from *Aspergillus niger* Teigh. in 40% glycerol (Sigma-Aldrich, St. Louis, MO), diluted in distilled water via a 50 μL Hamilton® repeating syringe (Hamilton Co., Reno, NV) to 2 opposing locules of each boll with a 0.41-mm diam needle, and (3) untreated controls that were enclosed with no bug or injection. In 2006, the experiment included the addition of 5 and 15% pectinase dilutions injected into opposite locules of each boll, with 20 replicates for each treatment. In 2008, small (≤ 2.0 cm diam, < 4 d of age), medium (2 - 3 cm diam, < 8 d of age) and large (≥ 3 cm in diam, < 12 d of age) first-position bolls were injected into all 4 locules with the 10% pectinase, infested with *C. signatus*, or were controls, with 40 replicates for each treatment. The bugs and nylon hosiery sleeves were removed after 72 h. When the cotton reached harvestable maturity, the number of bolls that remained attached to the plant versus those that had abscised were noted. Bolls that remained attached were taken to the laboratory, and injury was rated on a 0 - 4 scale; a value of 0 being no damage, whereas a score of 4 represented a boll that sustained injury to all 4 locules of the boll according to Lei et al. (2003). Abscised bolls received a damage score of 4, and no yield parameters from these bolls were included in further statistical analysis. Bolls were individually weighed, followed by manual delinting to obtain lint and seed weights. Seeds were rated as good (viable), undeveloped (unfertilized ovules), or injured. The fixed effect variables of injury score, seeds, seed weight, lint weight, seed-cotton weight, and number of growth warts on the inside of the capsule caused by feeding were subjected to analysis of variance using the PROC MIXED model (SAS Institute 2001) with the degrees of freedom adjusted in the model statement using the Satterthwaite method (Littell et al. 1996). Treatment means from the fixed effects were tested using the LS means statement, but significance of differences among means was tested using Tukey-Kramer test ($\alpha = 0.05$).

Results

Actual and simulated feeding injury. The fixed effects of injury score ($F = 73.6$; df = 2, 84.6; $P < 0.001$), seed cotton weight ($F = 13.16$; df = 2, 77.6; $P < 0.001$), number of injured seeds ($F = 45.48$; df = 2, 77.6; $P < 0.001$), growths on the capsule wall ($F = 29.39$; df = 2, 77.6; $P < 0.001$), lint weight ($F = 18.14$; df = 2, 77.6; $P < 0.001$) and seed weight ($F = 9.47$; df = 2, 77.6; $P = 0.003$) were significantly different for 2005 where *C. signatus* were enclosed with bolls or the bolls were injected with 10% pectinase on opposing locules and compared with the controls (Table 1). The pectinase and mirid treatments both lost 10% (3) of the bolls, whereas the control lost 6.7% (2).

The 10% pectinase injection injury score was significantly lower (0.7) than the bug-infested score (1.8), whereas the control sustained minimal injury. Mean seed cotton weights were 29% lower than the *C. signatus*-infested bolls, and 23% lower than the 10% pectinase injection when compared with the control, respectively. There was an average of 9.9 injured seeds from the *C. signatus*-infested bolls compared 2.9 from

Table 1. Cotton boll injury, abscission rates, and yield parameters associated with *Creontiades signatus* feeding and pectinase injections to 8 d old cotton bolls at the time of injection/infestation, 2005 and 2006.

Year	Treatment	Injury Score	Percent Abscised Bolls	Seed cotton weight (g)	# Injured seeds	# Growths on capsule wall	Lint weight (g)	Seed weight (g)
2005	Control	0.01 ± 0.01 c	6.7	5.1 ± 0.20 a	0.0 ± 0.0 c	0.0 ± 0.0 c	2.0 ± 0.08 a	3.0 ± 0.12 a
	C. signatus	1.8 ± 0.15 a	10.0	3.6 ± 0.23 b	9.9 ± 0.34 a	6.5 ± 0.94 a	1.3 ± 0.09b	2.3 ± 0.14 b
	10% pectinase	0.7 ± 0.06 b	10.0	4.7 ± 0.19 a	2.9 ± 0.57 b	2.1 ± 0.04 b	1.9 ± 0.8 a	2.9 ± 0.12 a
2006	Control	0.01 ± 0.01 c	0.0	4.2 ± 0.28 a	0.0 ± 0.0 c	0.0 ± 0.0 c	1.6 ± 0.1 a	2.61 ± 0.18 a
	C. signatus	2.4 ± 0.28 a	25.0	2.0 ± 0.30 b	13.3 ± 1.9 a	8.5 ± 1.4 a	0.7 ± 0.0 b	1.26 ± 0.20 b
	5% pectinase	1.3 ± 0.24 b	15.0	2.5 ± 0.23 ab	7.9 ± 0.9 b	2.9 ± 0.4 b	0.9 ± 0.1 b	1.59 ± 0.13 b
	10% pectinase	2.0 ± 0.27 ab	20.0	2.2 ± 0.23 b	7.4 ± 1.0 b	2.7 ± 0.4 b	0.8 ± 0.1 b	1.37 ± 0.15 b
	15% pectinase	2.0 ± 0.31 ab	30.0	2.1 ± 0.26 b	8.9 ± 1.0 a	3.2 ± 0.4 b	0.8 ± 0.0 b	1.32 ± 0.11 b

Least-square means ± SEM; means in a column within year of study followed by the same small letter are not significantly different, whereas those followed by a different letter are significantly different, Tukey-Kramer test, ($\alpha = 0.05$).

the 10% pectinase injection, indicating that *C. signatus* are probing and actively feeding on the developing embryos inside all 4 locules of the cotton boll. The number of growths on the capsule wall was significantly higher for the infested bolls when compared with the pectinase injection; an indication that *C. signatus* are seeking and actively feeding on the developing seed embryos. Lint weights and seed weights for the control and 10% pectinase injections were not significantly different from each other, but both were significantly higher than the *C. signatus* infestation.

The results of the 2006 field experiment included the addition of 5 and 10% pectinase injections to improve simulation of the feeding damage. The fixed effects of injury score ($F = 13.44$; df = 4, 93.8; $P < 0.001$), seed cotton weight ($F = 6.60$; df = 4, 77.0; $P < 0.001$), number of injured seeds ($F = 20.74$; df = 4, 77.0; $P < 0.001$), growths on the carpal wall ($F = 22.55$; df = 4, 77.0; $P < 0.001$), lint weight ($F = 7.10$; df = 2, 77.0; $P < 0.001$) and seed weight ($F = 6.72$; df = 4, 77.0; $P < 0.001$) were all significant in the 2006 experiment. No bolls abscised from the controls, whereas 25% of the bug-infested bolls were lost compared with 15, 20 and 30% for the 5, 10, and 15% pectinase injections, respectively. The *C. signatus*-injury score was statistically similar to the 10 and 15% pectinase injections; however, the 10 and 15% injections were not significantly higher than the 5% pectinase injection (Table 1). The control for seed cotton, lint and seed weights were statistically lower than all pectinase treatments (5, 10, 15%), but statistically similar to the bug-infested bolls. The number of injured seeds for the *C. signatus*-infested bolls was numerically higher but not significantly different from the 15% pectinase injections, whereas the 10% and 5% were not significantly different from each other, but both were significantly higher than the control. The number of growth warts on the capsule wall was statistically higher for *C. signatus*, followed by a similarity in the pectinase treatments, which were statistically higher than the control.

Boll abscission was 35.0 and 38.6% for small bolls infested and injected with 10% pectinase in 2008, respectively, whereas the control lost 5% of the bolls. Medium-sized bolls were abscised 7.5% of the time for both treatments compared with none for the controls. There was no abscission for any of the large boll treatments. The treatment effects for pectinase dilution ($F = 273.38$; df = 2, 342; $P < 0.001$) and boll size ($F = 6.39$; df = 2, 342; $P < 0.002$) were significant factors in the model for injury score (Table 2). Small and medium-sized boll injury scores were significantly higher for the infested or injected treatments when compared with the controls. Feeding injury, other than *C. signatus* feeding symptoms, was present in the controls for all 3 boll sizes. Large bolls that were injected or infested were almost identical in injury score, and both were significantly higher than the control. The number of growths on the carpal wall ($F = 289.55$; df = 2, 342; $P < 0.001$) were all significant from each other, with the *C. signatus* infested having higher numbers than the simulated or control. When the interiors of the carpal walls were being inspected, it was obvious that the bugs pierce and probe the boll during the process of feeding. Seed cotton weights ($F = 19.75$; df = 330, 6.02; $P < 0.002$) were statistically similar for the pectinase and *C. signatus* treatments within small and medium bolls, and both were significantly lower than the controls. However, all yield parameters for the large bolls were statistically similar but the injury scores were higher for the bug and simulated technique.

Discussion

Improvements were made with each year of study in simulating the injury caused by *C. signatus* feeding to cotton bolls. More accurate injury simulations were made in 2006

Table 2. Cotton boll injury, abscission rates and yield parameters associated with Creontiades signatus feeding and pectinase injections to small, medium and large cotton bolls at the time of injection/infestation, 2008.

Boll size	Treatment	Injury Score	Percent Abscised Bolls	Seed cotton weight (g)	# Growths on capsule wall	# Injured seeds	Lint weight (g)	Seed weight (g)
Small	Control	0.09 ± 0.02 b	5.0	2.51 ± 0.31 a	0.0 ± 0.0 c	0.11 ± 0.0 c	0.90 ± 0.19 a	1.61 ± 0.20 a
	C. signatus	3.30 ± 0.21 a	35.0	1.91 ± 0.33 b	7.6 ± 0.84 a	9.9 ± 0.34 b	0.70 ± 0.12 b	1.21 ± 0.21 b
	10% pectinase	3.47 ± 0.08 a	38.6	1.97 ± 0.14 b	4.1 ± 0.16 b	11.3 ± 0.57 a	0.71 ± 0.14 b	1.26 ± 0.08 b
Medium	Control	0.05 ± 0.01 b	0.0	2.82 ± 0.18 a	0.0 ± 0.0 c	0.02 ± 0.0 b	1.0 ± 0.078 a	1.82 ± 0.11 a
	C. signatus	3.39 ± 0.19 a	7.5	1.95 ± 0.21 b	8.5 ± 0.94 a	13.6 ± 1.9 a	0.69 ± 0.084 b	1.26 ± 0.13 b
	10% pectinase	3.52 ± 0.10 a	7.5	1.69 ± 0.12 b	5.5 ± 0.04 b	14.9 ± 0.9 a	0.52 ± 0.039 b	1.17 ± 0.08 b
Large	Control	0.12 ± 0.11 b	0.0	4.05 ± 0.11 b	0.1 ± 0.0 c	0.0 ± 0.0 c	1.51 ± 0.06 a	2.53 ± 0.07 a
	C. signatus	1.72 ± 0.32 a	0.0	3.87 ± 0.19 b	5.5 ± 0.44 a	3.9 ± 1.0 a	1.48 ± 0.08 a	2.38 ± 0.12 a
	10% pectinase	1.63 ± 0.21 a	0.0	3.82 ± 0.14 b	3.1 ± 0.04 b	2.9 ± 1.1 b	1.47 ± 0.07 a	2.45 ± 0.08 a

Least-square means ± SEM; means within the same column for each boll sized followed by the same small letter are not significantly different, whereas those followed by a different letter are significantly different, Tukey-Kramer test, (α = 0.05).

over 2005 with regards to the percent abscising, damage score and number of injured seeds. The *C. signatus* and 10% pectinase injury means were higher and statistically similar in 2006 compared with those of 2005. This can be at least partially explained by increased drought stress in 2006. The test area should have been irrigated one more time, and the combination of plant bug feeding injury and drought stress can magnify abscission rates (Stewart and Sterling 1989, Armstrong and Camelo 2003). Additional improvements were made with the lint and seed weights, where none of the injection treatments were statistically different than the *C. signatus* infestation and all were significantly lower than the control. However, from all the improvements made from 2005 - 2006, the best estimates for simulating feeding injury came from 2008 by increasing the number of 10% pectinase injections from 2 - 4 locules for each boll. This improvement came from realizing that *C. signatus* probe the bolls multiple times with their stylets to reach the developing embryos. Increased numbers of growthy warts on the inside of the boll are indicative of probing with the stylets. Smaller bolls are more susceptible to adult feeding as evident by increases in injury to seed and fiber development. Larger bolls are less susceptible to feeding injury, but it does not always relate to loss in yield as was apparent from the 2008 study, which indicates that the damage rating may over estimate losses in lint and seed. Although we used adult *C. signatus* to infest cotton bolls, Zink and Rosenheim (2005) measured the mouthpart (stylet) lengths of nymphal and adult *L. hesperus* and discovered that even though the younger instars spend more time feeding on anther sacs, more injury and abscission were inflicted by adults, and this was correlated with the length of the stylets. Our study confirms that as the cotton bolls grow and increase in diameter, they also become less vulnerable to feeding injury from adult *C. signatus*. The injury noted from enclosing the bolls with *C. signatus* was characteristically similar to that of *C. dilutus* from Australia (Lei et al. 2003), and *L. lineolaris* (Pack and Tugwell 1976) and stink bugs (Heteroptera: Pentatomidae) (Greene et al. 1999) from the U.S. However, we recognized during the process of comparing pectinase and *C. signatus* treatments that there were marked increases in the number of injured seeds from actual feeding. *Creontiades signatus* prefers to feed on the developing seeds, and the resulting damage to fiber development was higher than when the boll was injected into 4 locules as opposed to 2. The 2008 methodology appears to approximate *C. signatus* feeding well enough that it can be used to conduct economic injury studies realizing that bolls > 12 d of age or > 3 cm diam may show feeding injury, but this may not have an impact on lint and seed yields.

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