

ISOLATING AND IDENTIFYING THE MICROBES ASSOCIATED WITH GREEN MIRID FEEDING INJURY TO COTTON BOLLS

J. Scott Armstrong

USDA-ARS, Beneficial Insect Research Unit
Weslaco, TX

Enrique Gino Medrano

USDA-ARS Cotton Pathology Research Unit
College Station, TX

Jesus F. Esquivel

USDA-ARS, Areawide Pest Management Research Unit
College Station, TX

Abstract

Mirids, or plant bugs, feed on cotton bolls causing yield loss in the form of decreased lint quality and quantity. Recent research has shown that similar hemipteran pests feeding on cotton bolls can introduce boll-rotting microorganisms affecting lint quality and yield. Because the green mirid, *Creontiades signatus* Distant, is a relatively new pest of cotton in South Texas, the objective of this study was to determine the presence of microorganisms (bacteria and fungi) from the guts of field-collected *C. signatus*, and to isolate these microorganisms from tissue collected from cotton bolls that had been exposed to field-collected *C. signatus* for one week. The guts of field collected *C. signatus* were positive for up to up to 10^8 colony forming units (cfu's) of bacteria per insect, and 10^2 cfu's of filamentous fungi per insect. Eighty eight percent of cotton bolls that *C. signatus* were allowed to feed upon had up to 10^4 cfu's of bacteria and 10^2 cfu's of fungi per g of tissue indicating that the insects are capable of transmitting these organisms into cotton bolls through the process of feeding. This is the first study to examine the microbial fauna and respective microorganism concentrations in the green plant bug feeding on cotton bolls. The data presented clearly demonstrated that microorganisms are transmitted during *C. signatus* feeding/probing. The evidence that *C. signatus* feeding did not always result in disease suggests that harboring and vectoring of opportunistic cotton pathogen(s) could account for differences associated with boll damage.

Introduction

In recent years, a relatively new cotton pest identified as the green plant bug, *Creontiades signatus* Distant, has been observed causing economic damage by feeding on cotton bolls in South Texas (Armstrong and Coleman 2007). The South Texas cotton production region encompasses the Upper Coastal Bend and the Lower Rio Grande Valley, which usually represents about 600,000 ha. Damage by *C. signatus* is similar to that previously reported for stink bugs vectoring plant pathogens (Mirose et al. 2006, Medrano et al. 2007). No information is available on the phytopathogenic micro-organisms transmitted to cotton bolls by the green plant bug. Our objectives were to determine presence of microbes in the insect gut and whether these microbes were transferred into cotton tissue.

Materials and Methods

Green plant bugs, *Creontiades signatus* Distant, were collected from red root pigweed, surrounding a cotton field at the Texas AgriLife research farm located at Weslaco, TX. Green plant bugs were maintained on the same weed host tissue inside a plexi-glass cage for 24 h before being used in this study. Cotton was planted in 6.6 L pots containing a 50/50 ratio of field-collected soil and potting soil (Sunshine mix, Bellevue, WA). Cotton plants were maintained in the greenhouse by watering approximately every 10 – 14 d and fertilizing with a 5% solution of Peters fertilizer (20-20-20, N-P-K, Scott's Sierra Horticultural Products Marysville, OH) until there were sufficient cotton bolls that were 8 d of age (i.e., 2.0 – 2.5 cm in diameter).

Thirty-nine randomly selected 8-d-old cotton bolls were infested with an adult green plant bug by enclosing them in a 8 oz. bottomless styrofoam cup covered with nylon hosiery (Armstrong et al. 2007). The bugs, and an equal number of controls with no bug enclosed, were left on the plants for 7 d. At the end of 7 d, each enclosure was carefully examined to determine if the insect was still alive. Immediately after removal from the plants on June 16,

2008, twenty-four live green plant bugs, were overnight shipped to the pathology laboratory of Dr. Gino Medrano, USDA-ARS College Station, TX, and fifteen insects remained for the laboratory analysis at 14-d after exposure to bolls.

In the laboratory, the insects were surface sterilized individually in a 14 mL round bottom Falcon tube (Becton Dickinson, Franklin Lakes, NJ, USA) that contained 70% ethanol (10 mL) and were gently inverted for 8 min. The green plant bugs were rinsed twice for 1.5 min with 25 mL sterile water followed by separately placing them into a 1.1 mL microtube (SPEX SamplePrep, Metuchen, NJ, USA) that contained 0.5 mL PO_4 buffer (0.1 mol/l, pH 7.1) and a sterile 4 mm stainless steel ball (SPEX SamplePrep, Metuchen, NJ, USA). An identical steel ball was added to the tube, and then the capped tubes were placed in a 96 tube-rack to pulverize the insects. The insects were ground for 5 min at 1500 strokes/min using a 2000 Geno-Grinder (SPEX SamplePrep, Metuchen, NJ, USA) and the wash water was subjected to a 10-fold dilution (PO_4 buffer, pH 7.1). To test for the presence of microorganisms in the wash water, 100 μl aliquots were directly plated on the bacterial medium Trypticase Soy Agar (TSA) and the fungal medium Potato Dextrose Agar (PDA) amended with 100 $\mu\text{g/ml}$ chloramphenicol (Cm) and 50 $\mu\text{g/ml}$ tetracycline (Tc) (antibiotics deter bacterial growth), and then incubated for two weeks at 28°C. The insects were ground for 5 min at 1500 strokes/min using a 2000 Geno-Grinder (SPEX SamplePrep, Metuchen, NJ, USA), and then 10-fold dilution (PO_4 buffer, pH 7.1) plated on both TSA and PDA amended with Cm and Tc. Following 1 and 2 weeks of incubation at 28°C, microbial colonies were counted and expressed as colony forming units (cfu's) per insect.

Boll tissue from infested ($n=16$) and controls ($n=6$) were processed and analyzed for microbes 20 days (June 27, 2008) after the plant bugs were removed. Bolls were washed separately in 0-5% sodium hypochlorite solution for 10 min and then rinsed with sterile water for 5 min. The carpel walls of each boll were excised with a sterile scalpel, aseptically removed and visually inspected for insect feeding sites. A 0.5 g sample of the developing fiber tissue, excluding the carpel wall, was pulverized in a sterile mortar for 5 min, followed by adding 500 ml of KPO_4 , followed by 5 more min of triturating. A 100 ml aliquot was removed from the mortar into 450 μl of potassium phosphate (KPO_4) buffer. This dilution was repeated 2 more times after thoroughly shaking. The 3 dilutions were spread onto TSA and PDA amended with antibiotics, and incubated at room temperature for 2 weeks before being analyzed for cfu's of bacteria and fungi. To further characterize the visual effects of green plant bug feeding, nine infested and nine control bolls were allowed to mature in the greenhouse where they were rated for damage based on the number of locks injured during feeding (Armstrong and Coleman 2007). The lint and seed were hand separated and weighed from each infested and control bolls. These data for the control and infested bolls were compared using the PROC T-TEST procedure (SAS 2001).

Results and Discussion

To demonstrate green plant bug feeding injury to cotton bolls, a nymph, adult, and external and internal boll damage from this study are shown in Figure 1. From the 39 bolls that were infested with green plant bugs for one week, 4 of them (10.5%) aborted. Twenty nine of the 38 (76.3%) green plant bugs were still alive after one week. One bug escaped from the bag which reduced our replications to 38. The mean number of external and internal lesions was 47.3 ± 9.0 and 3.4 ± 5.94 , respectfully, on the infested bolls compared to none for the controls. The damage scores for bolls that were left to maturity were significantly higher for the infested bolls when compared to the controls (Figure 2). Cotton seed weights and lint weights were significantly higher for the controls when compared to the infested bolls (Figure 2). These descriptive and comparative statistics substantiate that the green plant bug can have the potential to cause injury and reduce the quality of cotton from both feeding injury and the introduction of boll-rotting organisms by the process of feeding.

Both bacteria and fungi were isolated from the gut of the green plant bugs, however bacteria was detected at 100% frequency (concentration ranges 10^3 - 10^8 cfu's) compared to 60% frequency (concentration ranges $<10^1$ - 10^2 cfu's) for the filamentous fungi (Table 1). The higher cfu's found in the gut could mean that the green plant bug has the potential to transmit bacteria directly to the boll during the process of feeding, however until the bacteria are more closely tracked, this can only be theorized as a possibility. Microorganisms were not detected from healthy control boll tissues that were not infested by green plant bugs (Table 1). Bacteria were detected from all bolls with evidence of insect feeding irrespective of disease development (Table 1). Fungi were isolated from 35% percent of the diseased bolls and not detected in asymptomatic bolls with evidence of insect feeding (Table 1). The data

demonstrate that phytopathological microorganisms are transmitted during *C. signatus* feeding/probing. The fact that evidence of *C. signatus* feeding did not always result in disease suggests that harboring and vectoring of opportunistic cotton pathogen(s) could account for differences associated with boll damage.

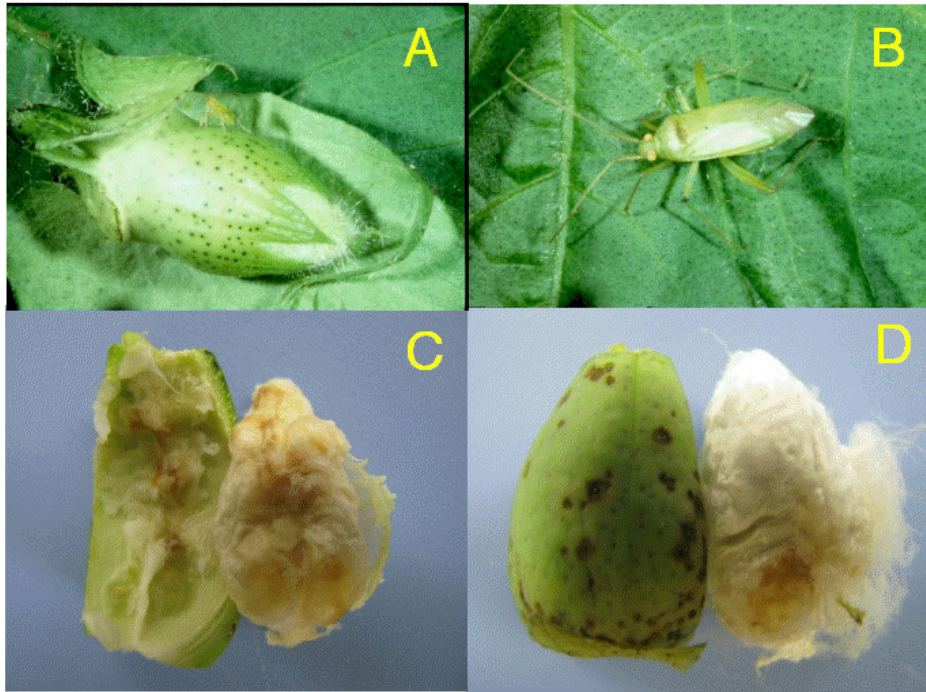


Figure 1. Green plant bug, *Creontiades signatus* Distant, nymph on a cotton square (A); Adult green plant bug on a cotton leaf (B); excised cotton boll showing internal worms, discoloured lint, and damaged seeds (C); and excised boll showing external lesions and internal damage on Stoneville '4357BGIIRF' bolls infested with adult green plant bugs (D).

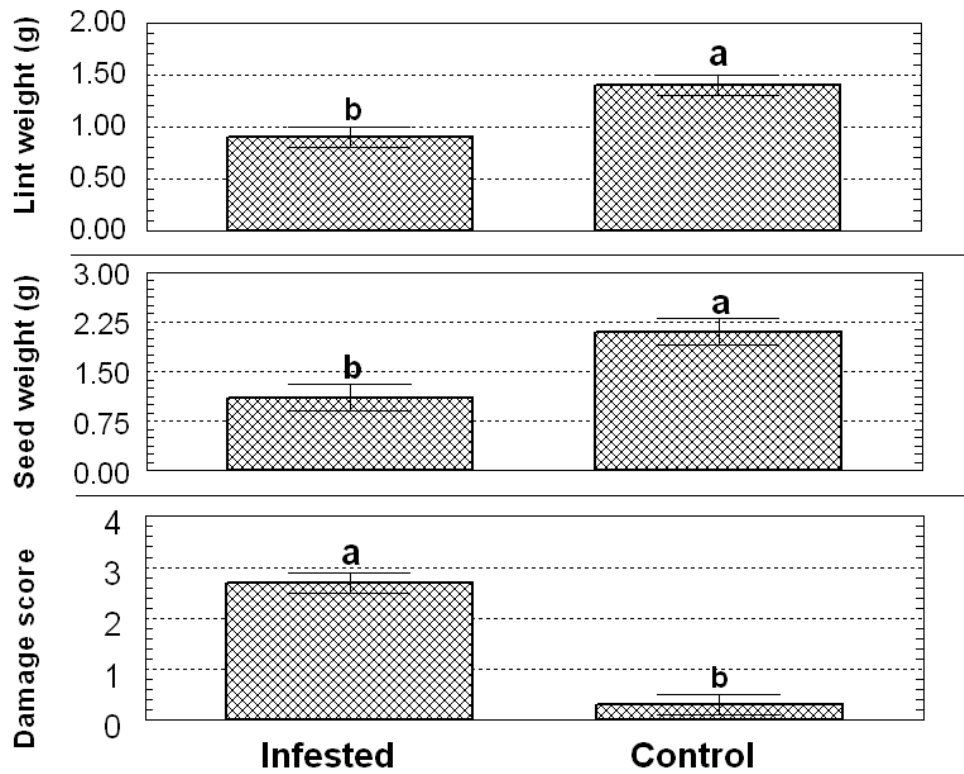


Figure 2. Damage rating (1 – 4) and yield characteristics for control cotton bolls and cotton bolls ($n=9$) infested with field-collected green plant bugs, *Creontiades signatus* Distant (Heteroptera: Miridae).

Table 1: Concentration ranges of microorganisms recovered from insects (colony forming units [cfu] / insect), lint tissue (cfu / g tissue) from bolls caged without an insect (control), or lint tissue from bolls infested with a *Creontiades signatus* Distant, and with evidence of feeding.

Sample	Bacteria (cfu)			Fungi (cfu)		
	Low	High	Isolation Frequency	Low	High	Isolation Frequency
Insects (n = 15)	10 ³	10 ⁸	100%	<10 ¹	10 ²	60%
Control Bolls (n = 6)						
No disease	<10 ¹	<10 ¹	0%	<10 ¹	<10 ¹	0%
(n = 6)						
Disease	<10 ¹	<10 ¹	0%	<10 ¹	<10 ¹	0%
(n = 0)						
Infested Bolls (n = 16)						
No disease	10 ¹	10 ⁴	100%	<10 ¹	<10 ¹	0%
(n = 2)						
Disease	10 ²	10 ⁸	100%	<10 ¹	10 ²	35%
(n = 14)						

References

- Armstrong, J. S., Coleman, R. J. and B. L. Duggan. Characterizing the damage and oviposition of a *Creontiades* plant bug to South Texas cotton. In Proc. Beltwide Cotton Conf pp. 1320 2007. (Proceedings)
- Hirose, E. P., Panizzi, A. R., De Souza, J. T., Cattelan, A. J., and Aldrich, J. R. 2006. Bacteria in the gut of southern green stink bug (Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 99: 91-95.
- Medrano, E.G., Esquivel, J.F., and Bell, A.A. 2007. Transmission of cotton seed and boll rotting bacteria by the southern green stink bug (*Nezara viridula* L.). J. Appl. Microbiol. 103: 436-444.