

**EFFECT OF CARBON NANOTUBES ON FEEDING BEHAVIOR OF *LYGUS HESPERUS*
(HEMIPTERA: MIRIDAE) IN COTTON: AN ELECTROPENETROGRAPHIC EVALUATION**

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Abstract

A laboratory study was conducted at Texas A&M AgriLife Research Center, Lubbock, TX to examine the impact of carbon nanotubes (CNT) on *Lygus* feeding behavior using electropenetrography. A four-channel AC-DC monitor was used to examine the feeding behavior on 7-day old bolls from CNT-treated versus untreated control plants. Behaviors were recorded at $R_i 10^7 \Omega$ for five hours. We identified three non-probing (standing still, walking, and antennation) and three probing (cell rupture, transition and ingestion) waveforms. Preliminary analysis indicated that CNT reduces frequency of ingestion events.

Introduction

The western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), is an economic pest of cotton in western United States. Several chemical pesticides have been used to manage this pest. Carbon nanotubes (CNTs) are man-made materials that range in size from 1-100 nm. Due to their novel physical and electrical properties, CNTs have widely been used in various applications such as electronics, combustion, agriculture, medicine and pharmaceuticals. CNTs have been reported to have an effect on plant growth and reproduction; however, their effects vary depending on plant species and type of CNT. Arndt et al. (2014) reported that growth and reproduction in F_1 and F_2 generations of *Daphnia magna* were affected by CNT. Also, CNT accumulation in gut lining of the black-lyre leafroller moth and the brown-headed leafroller larvae has been reported. Locomotion and mortality were negatively affected by CNT in *Drosophila* while low rate of CNT accumulation was observed in *Eisenia fetida*, earthworms. The objective of the present research was to examine the impact of CNT on *Lygus* feeding behavior using electropenetrography.

Materials and Methods

This study was conducted in the Cotton Entomology Laboratory at Texas A&M AgriLife Research Center, Lubbock, Texas, to determine the impact of CNT on feeding behavior of *Lygus* bugs using electropenetrography (EPG). Soil spiked with 1 mg/kg of CNT was applied in plastic pots in greenhouse and cotton seeds were planted in CNT-augmented versus untreated control pots. Plants were reared to fruiting in the greenhouse and the pots were transferred to a Faraday cage for the experiment. Seven-day old uniform size bolls were used in the experiment to standardize the host quality. A colony of *Lygus* bugs was maintained in the laboratory and adult bugs were used in the trial. Treatments included CNT treated cotton plants and untreated control plants. *Lygus* bugs were starved for 1 hour before start of the experiment. Gold wire (0.0015 inch; $\sim 38 \mu\text{m}$) diameter was used to tether *Lygus* using water-based silver glue. Electropenetrography consisted of a four-channel AC-DC monitor, head stage amplifier and data acquisition device (Fig. 1). Wired *Lygus* bugs were placed on selected bolls *in situ* and waveforms were recorded for five hours. A single *Lygus* bug was attached per channel. A new bug was used on a new (previously uninfested) boll for each recording. Different feeding behaviors were identified using protocol established by Cervantes et al. (2016). Waveforms produced as a result of *Lygus* feeding were compared between CNT treated and untreated control plants.

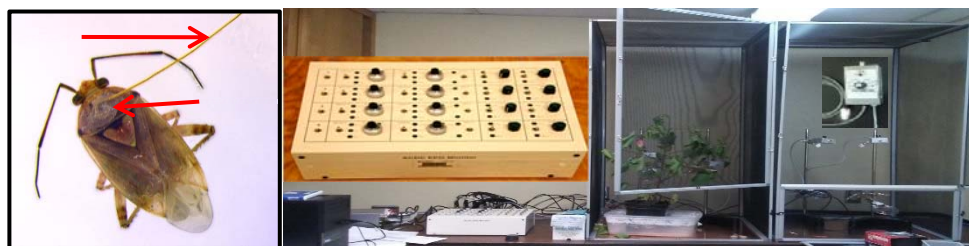


Figure 1. *Lygus hesperus* adult attached with gold wire (left), AC-DC monitor (middle), and the experimental setup inside a Faraday cage (right).

Results and Discussion

Several distinctive waveforms exhibited by *Lygus* bugs have been recorded using AC signal from control and CNT treated cotton bolls. Nonprobing waveform included standing still (S), walking (W) and antennation (A) while probing waveforms included cell rupture (CR), transition (T) and ingestion (Figs. 2-3). Standing behavior showed a straight line at the baseline level and walking behavior indicated an irregular pattern of low-amplitude peaks close to the baseline level. Cell rupture initiated with a high-amplitude peak and gradually became irregular (Fig. 4). Transition began after CR and is represented by patterned waveforms occurring in repetitive episodes. Frequency of ingestion events in control plants was higher than CNT treated plants. Repeated CR without ingestion was recorded from bolls treated with CNT. Preliminary analysis indicated that CNT reduced frequency of ingestion events, but the test probes were numerous. At present, we do not know if CNT significantly affected the feeding behavior. More recordings will be evaluated to determine if CNT impacted feeding behavior of *Lygus* bugs.

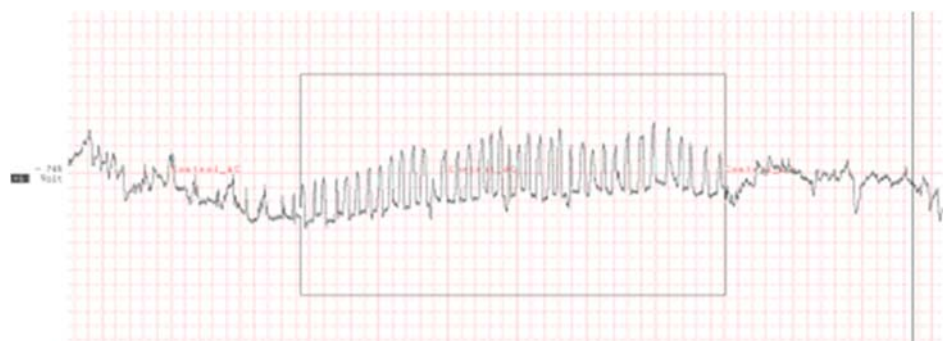


Figure 2. Ingestion waveforms recorded at $R_i 10^7 \Omega$ using AC applied signal on control plants.

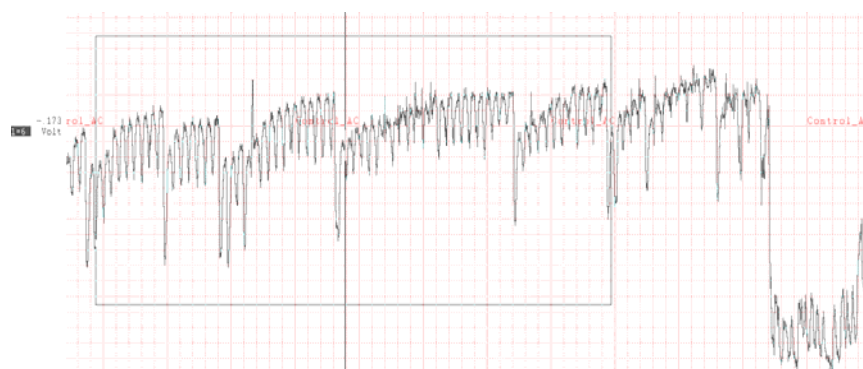


Figure 3. Fine-textured and repeated patterns of transition waveforms on control plants.

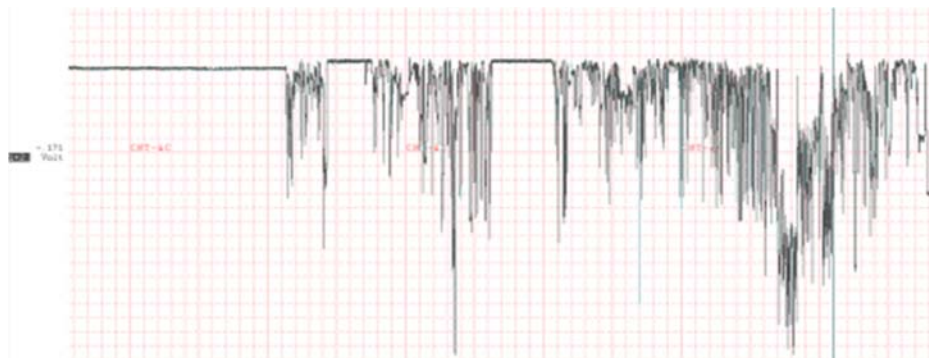


Figure 4. Repeated CR without ingestion on CNT treated cotton plants.

References

- Arndt, D.A., J. Chen, M. Moua and R. D. Klaper. 2014. Multigeneration impacts on *Daphnia magna* of carbon nanomaterials with differing core structures and functionalizations. *Environmental toxicology and chemistry*. 33: 541-547.
- Cervantes, F.A., E. A. Backus, L. Godfrey, W. Akbar, and T. L. Clark. 2016. Characterization of an EPG waveform library for adult *Lygus lineolaris* and *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton squares. *Annals of the Entomological Society of America*. 109: 684-697.