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SEISMIC BEHAVIORS OF A LEAFMINER, *ANTISPILA NYSAEFOLIELLA* (LEPIDOPTERA: HELIOZELIDAE)

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ABSTRACT

This paper presents the first descriptions of 2 distinct behaviors of the Tupelo leafminer, *Antispila nysaefoliella* Clemens (Lepidoptera: Heliozelidae). Through the use of specialized morphological structures, the leafminers use these behaviors to generate substrate-borne vibrations that can be emitted as audible sounds to humans. Scanning electron micrographs of these structures are presented. In 1 behavior, the larvae "tick" their abdomens back and forth rhythmically; and in the other, they "rattle" their abdomens in short rapid pulses. These are named for the sounds produced. Previous studies have shown that parasitoids emit substrate vibrations while walking and probing during their search for hosts from the leaf surface. The experimental results show that the larvae of *A. nysaefoliella* are sensitive to vibrational stimuli in general by "wriggling to all frequencies", but "ticked" only to some frequencies which are characteristic of parasitoid probing behavior, and "rattled" rarely.

Supplementary material (videos) online at http://www.fcla.edu/FlaEnt/fe914.htm

Key Words: anti-parasitoid, defense, host-parasitoid, plant-insect, signals, sound playback, vibrations

RESUMEN

Se describen dos comportamientos distintos observados en *Antispila nysaefoliella* Clemens (Lepidoptera: Heliozelidae), insecto minador de hoja especializado en arboles de goma negra *Nyssa sylvatica* Marsh (Cornaceae). Ambos comportamientos son realizados utilizando estructuras morfológicas especializadas que generan vibraciones en la superficie de las hojas. En el primer tipo de vibración, las larvas del tupelo utilizan movimientos "pendulares" de sus abdómenes hacia atrás y adelante de forma rítmica. En la segunda vibración, los movimientos son producidos por una serie rápida de sonidos ruidosos cortos originados en el abdomen, los que son denominados movimientos de "cascabeleo". Se presentan microfotografías obtenidas por escaneo de electrones de las estructuras especializadas involucradas en la creación de vibraciones. Los resultados demostraron que las larvas de tupelo son sensitivas a todo tipo de vibraciones, pero responden de manera diferente. Las larvas utilizan "serpenteos" como respuesta general, movimientos "pendulares" solo a determinadas frecuencias, y raramente utilizan "cascabeleos".

Translation provided by the author.

The use of substrate-borne vibrations for communication is prevalent among many insects (Roces & Holldobler 1996; Cummings et al. 1999; Cokl & Doberlet 2003; Virant-Doberlet & Cokl 2004; Cocroft 2005; Cocroft & Rodriguez 2005; Casas & Magal 2006; Castellanos & Barbosa 2006; Hoch et al. 2006; Casas et al. 2007). For insects living on plants, vibrations are especially effective for signal transmission because signalers typically have small body sizes and use short-range communication (within individual plants) (Bennet-Clark 1998; Cokl & Doberlet 2003; Cokl et al. 2004; Cocroft et al. 2006). The majority of studies on vibrational communication, thus far, have been aimed at understanding its utility in mate selection (Cokl et al. 2004; Rodriguez et al. 2004; Moraes et al. 2005; Virant-Doberlet & Zezlina 2007) or complex social interactions (Roces & Holldobler 1996; Cocroft 1999, 2005; Cummings et al. 1999; Cocroft & Rodriguez 2005). However, researchers are beginning to uncover the role of vibrations in food-finding and mediating interactions between prey and predators (or hosts and parasitoids) (Bacher et al. 1996; Meyhöfer et al. 1997; Djemai et al. 2004; Evans et al. 2005; Castellanos & Barbosa 2006). Some predatory wasps that search for concealed prey have evolved specialized traits, such as modified antennae to tap on substrates to detect prey position through vibrational sounding (Meyhöfer & Casas 1999; Broad & Quicke 2000; Djemai et al. 2004), and likewise, their prey have evolved sensory apparatus to detect the presence of their potential attackers and respond with defensive or evasive behaviors (Connor & Cargain 1994; Bacher et al. 1996; Bacher et al. 1997; Djemai et al. 2000, 2001; Castellanos & Barbosa 2006).

The adaptations of leaf-mining larvae to detect and evade attack by their parasitoids are likely to be under intense selection. Those parasitoids that hunt for concealed prey have been found to release vibrations that provide information for the host hiding beneath the leaf surface (See Video 1), which subsequently can trigger host evasive responses (Meyhöfer et al. 1994; Bacher et al. 1996; Meyhöfer & Casas 1999). In this paper, I provide the first descriptions of vibration-producing behaviors and associated morphology of a leaf-mining moth, *Antispila nysaefoliella* Clemens (Lepidoptera: Heliozelidae), which specializes on black gum, *Nyssa sylvatica* Marsh (Cornaceae).

MATERIALS AND METHODS

Natural History and Observations

In general, the shape and design of a leaf mine depends on both the feeding morphology and behavior of the species, as well as, the leaf morphology of the host plant (Needham et al. 1928; Hering 1951). The mines of *A. nysaefoliella* always begin as solitary mines (1 larva per mine) beside a leaf vein and expand radially to form blotches that become more oblong-shaped at later instars (Fig. 1). By the time of pupation, mines are 2.48 ± 0.56 cm^2 in size, and larvae are 3.18 ± 0.07 mm in length and weigh up to 0.472 ± 0.22 mg (dry weight) (mean ± 1 SD, n = 30). For 7 consecutive field seasons (2001-07), I have observed the behaviors of the larvae of *A. nysaefoliella* in my study population, which is located within a mixed deciduous forest in Clarke County, VA (39° 00.85' N, 78° 03.88' W). I have observed the same behaviors in larvae upon random encounters in locations throughout northern Virginia and Washington, D.C. where there are black gum trees.

During the peak of larval activity in 2001 (25 Aug to 3 Sep) at my study site in Clark Co., I set a mini-digital video camera on a tripod in front of leaves (with larvae) for 1-h intervals throughout the day (0900-1800 h) to record larval behaviors and other activity at the leaf (e.g., visitations by parasitoids). Because *A. nysaefoliella* feed on both upper and lower tissue layers, mines are translucent and larvae can be observed easily inside their mines, especially when sunlight illuminates the background (Fig. 1 and Videos 2-4, online). In total, I recorded 20 h of observation on 20 leaves with variable number of larvae per leaf (total = 144 larvae). All activity and behaviors were described and scored on computer monitors, based on frame-by-frame enhancements whenever necessary to score rapid movements and activity. The resolution of the video images did not allow for identification of visiting insects. However, a best guess at whether the insect might be a searching parasitic wasp could be made based on the behavioral (search) pattern of the insect. See Video 2 for an example of a visit by a potential wasp.

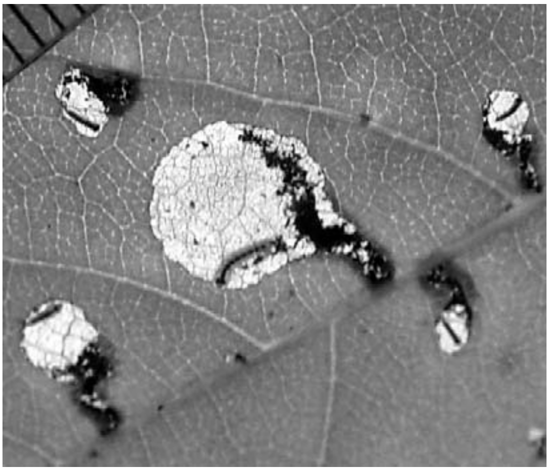

Fig. 1. Larvae of *Antispila nysaefoliella* in their mines on black gum, *Nyssa sylvatica*, pictured here from the lower leaf surface. These 6 mines are the result of approximately 1-6 d (smallest to largest) of feeding. Note: The dark sclerotized band (dorsal side) is visible because larvae feed dorsal-side down. Scale (1 mm) is in top left corner.

Recording Vibrations

In a subsequent field season in 2004, leaves with mines (and larvae) were collected from the field and brought to the lab of Prof. R. B. Cocroft at the University of Missouri, Columbia. Recordings of the vibrations caused by *A. nysaefoliella* larvae were taken under controlled conditions (enclosed room, ~23 C) by a laser-vibrometer (Polytec Compact Laser Vibrometer CLV 1000 with a CLV M030 decoder module, at a sensitivity of 25 mm s^{-1} V^{-1}; Polytec Inc., Auburn, MA). Tiny (~1-2 mm square) pieces of reflective tape were placed roughly in the center of the leaf on the leaf-midrib, and the width of the laser beam was ~0.5 mm. Signals were high-pass filtered at 80 Hz by a Krohn-Hite 3202 filter and low-pass filtered at 5000 Hz (44100 Hz sampling rate, 16 bit resolution). Data output was recorded with a Dell desktop computer with a national Instruments acquisition board (44, 100 Hz sampling rate, 16-bit resolution) and a custom program written in Labview (v. 6.0; national Instruments, Austin, TX). Files were then exported as .wav files on a Macintosh G3 computer with OS 9.2.2.

Scanning Electron Microscopy

Larvae in late-instars were fixed in glutaraldehyde and post-fixed in osmium tetroxide for morphological observations. Specimens were dehydrated in acetone of varying concentrations (i.e., 50%, 70%, 80%, 90%, 100%) prior to critical point drying. They were then glued to mounting stubs in various orientations and sputter coated with gold.

RESULTS

Wriggles, Ticks, and Rattles

Four different behaviors were identified from the field observations and video recordings. These are "wriggling without" and "wriggling with" displacement (i.e., where the body travels a measurable distance within the mine), "ticking" (rhythmic movement of abdomens back and forth like a clock pendulum), and "rattling" (rapid pulse of abdomen like the rattle of a rattlesnake). Ticks are slow and rhythmic (Video 3, online), and rattles are very rapid and occur in short bursts that often punctuate bouts of ticking (Video 4, online). All of these described behaviors are distinct from general feeding activity where, during feeding, larvae remain motionless and with their heads pointed toward the green leaf tissue at the mine periphery. When they wriggle, tick, or rattle, they typically pull away from the edge of the mine and move towards the center.

The average time that an individual larva spent wriggling, ticking, or rattling during the 60-min observation was, respectively, 3.00 ± 0.45, 1.10 ± 0.25, and 0.01 ± 0.00 min on leaves without any insect (i.e., potential parasitoid) visitations, and 2.28 ± 0.27, 1.19 ± 0.18, and 0.02 ± 0.01 min (mean ± 1 SE) on leaves with an insect visitation. Flying insects appeared in 11 of the 20 observations; and there were typically 1 ($n = 7$) or 2 insect visits ($n = 4$) per 1-h observational period for those 11 leaves that did receive a visit. The average time that an insect stayed on a leaf was 4.8 ± 0.3 min (1 SD). Multivariate analysis of variance of the time spent by each larva performing each behavior (wriggle, tick, rattle) in the presence or absence of an insect visitation showed that an individual spent a significantly greater amount of time ticking when there was an insect visitation than when there was not ($F_{1,113} = 5.182, P = 0.025$). However, there were no effects on the amount of time spent wriggling ($F_{1,113} = 0.336$, $P = 0.563$) or rattling ($F_{1,113} = 2.245, P = 0.137$). Leaf, group size, and the time that a visiting insect stayed on a leaf were entered as covariates.

The tick and rattle behaviors are named for the sounds that they emit, and are distinct in their spectral qualities (Fig. 2). *Antispila nysaefoliella* larvae appear to be able to create substrate-borne vibrations with specialized sclerotized morphological structures which line the dorsal side of the abdominal segments and occur as points on the last caudal segment and on the tip (Figs. 1, 3). All such morphology and sclerotization is lost at pupation. The mine epidermis may be acting as a tympanic membrane, and hence, may be transmitting the vibrations that are produced by the larval movements into sounds that can be clearly audible to the human ear (even when standing 2 m below the canopy). Larvae that have been removed from their mines do not generate these sounds, despite their ticking and rattling motions in response to being removed from their mines.

DISCUSSION

To the best of my knowledge, no other leaf-mining species has been reported to exhibit the ticking and rattling behaviors or the associated morphological structures that I have observed in *Antispila nysaefoliella*. However, the use of vibrations, acoustic signals, and body-waving behaviors in defensive or aggressive interactions has been reported for the larvae of other Lepidoptera (Yack et al. 2001; Fletcher et al. 2006; Brown et al. 2007). Support also comes from observations in other insect taxa, where the vibrations caused by the knocking and scraping of gasters on substrate by several species of ants (Formicidae: Dolicherinae) and the head-drumming of the damp-wood termite, *Zootermopsis nevadensis* (Isoptera: Termopsidae) appear to func-

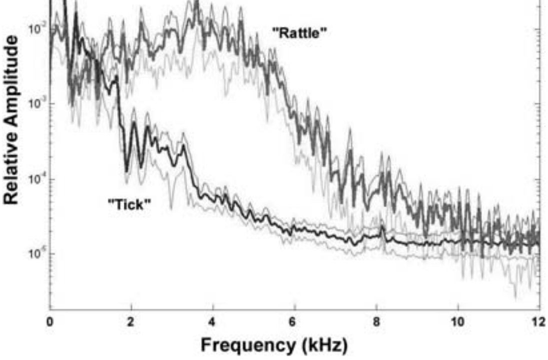

Fig. 2. The fast Fourier transforms of the frequency spectra of the tick and rattle signals.

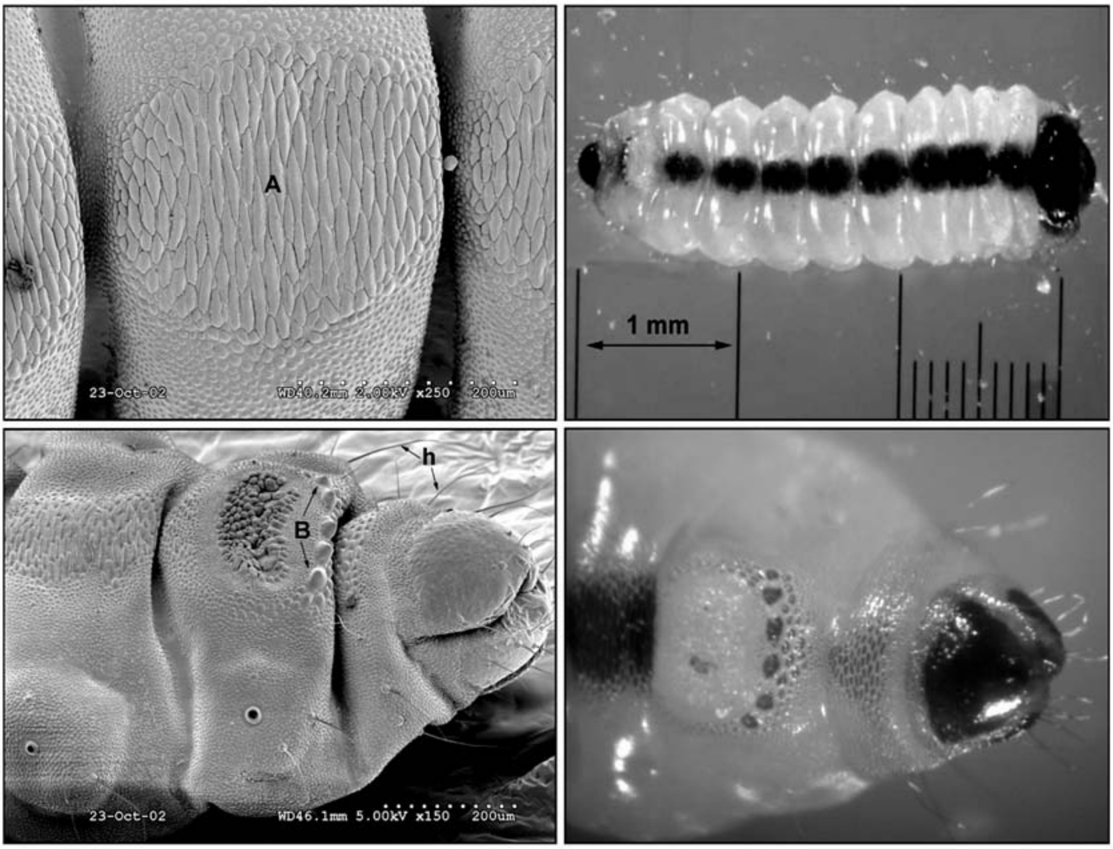

Fig. 3. Scanning electron micrographs and 100X images of specialized structures for producing substrate vibrations. Top panels: (A) Interdigitating sclerotized ridges along dorsal surface. Bottom panels: (B) - Sclerotized pointed bumps and sclerotized caudal tip. Note also the sensory hairs (h) that outline the body. Scale is provided in the lower right of each SEM, and distance between tick marks represents 200 μm.

tion as alarm calls (Kirchner et al. 1994; Rohe & Rupprecht 2001). The results presented here suggest that the ticking and rattling behaviors in *A. nysaefoliella* are important for generating vibrations, which has a well-documented adaptive role in mediating insect interactions (Meyhöfer et al. 1997; Cokl & Doberlet 2003; Djemai et al. 2004; Cocroft & Rodriguez 2005; Drosopoulos & Claridge 2006).

Several lines of evidence support the hypothesis that vibration production in *A. nysaefoliella* operates as a defense against parasitoids. First, parasitism is the most important mortality source of leaf-mining insects (Hawkins et al. 1997; Low 2008), which suggests that selection by parasitoids is intense and may drive the evolution of defense mechanisms in leafminers that match the mode(s) of attack of their enemies (i.e., vibrations). Second, because parasitoids of leafminers use vibrations to search for hosts from the leaf surface after detecting the mines from flight, generating seismic waves (substrate-borne vibrations) can be an effective tactic for disrupting or increasing the costs of the search process of parasitic wasps (Djemai et al. 2004). Last, the loss of the specialized morphology associated with vibration production at pupation suggests a function specialized for the larval stage (a time during which they are most vulnerable to parasitism).

Although this study supports the importance of vibrations in *A. nysaefoliella*, more work is clearly needed to fully understand the context(s) and function(s) of their vibration-generating behaviors. Future studies will require sophisticated equipment and more experimentation to test alternative hypotheses relating to the adaptive value of transmitting substrate vibrations and whether the behaviors are functioning as direct (individual) evasive responses, conspecific warning signals, or honest signals that may cause parasitoids to give up their search more quickly (Maynard-Smith & Harper 2004). Because most leaf-mining species are faced with similar constraints and natural enemies, there may be other leaf-mining species with similar behaviors and morphologies that have yet to be discovered or recognized. This would be an exciting area of research for future studies.

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