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Author: Low, Candace

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AN EXPERIMENTAL TEST OF THE SEISMIC BEHAVIORS OF ANTISPILA NYSAEFOLIELLA (LEPIDOPTERA: HELIOZELIDAE) TO VIBRATIONAL STIMULI

Candace Low
Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106 USA

Current address: Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853; E-mail: cl754@cornell.edu

Abstract

This study tested the sensitivity of the tupelo leafminer, *Antispila nysaefoliella, t*o vibrational cues and whether their unique behaviors, first reported by Low (2008), may be elicited by the frequencies matching those of parasitic wasps. In the tick behavior, a larva ticks its abdomen back and forth rhythmically, and in the rattle behavior, the larva rattles its abdomen in short rapid pulses. To test the specificity of these behaviors to vibrational cues, computer-generated signals of pure tone frequencies ranging from 1-6 kHz were played to the entire leaf of solitary larvae through airborne signal transmission. The experiment demonstrated that the larvae of *A. nysaefoliella* responded to all frequencies with wriggling movements, but ticked only in response to the frequencies that matched those generated by parasitoid probing activity. Rattling behavior was rarely elicited.

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

Resumen

Este estudio probó la sensibilidad del minador del tupelo, *Antispila nysaefoliella,* a señales en modo de vibraciones, y si este comportamiento único, primero reportado por Low (2008), puede ser provocado por las frecuencias similares a las producidas por avispas parasíticas. En el comportamiento llamado tick la larva mueve su abdomen rítmicamente hacia adelante y atrás produciendo un 'tick'. En el comportamiento llamado cascabel, la larva hace vibrar su abdomen en pulsos rápidos y cortos. Para probar la especificidad de estos comportamientos se utilizaron señales de tono puro en frecuencias entre 1-6 kHz generadas por un computador y transmitidas en forma de aire, las cuales fueron aplicadas en hojas con una larva solitaria. El experimento demostró que las larvas de *A. nysaefoliella* respondieron a todas las frecuencias encogiéndose. El comportamiento tick fue provocado solo por frecuencias similares a las producidas por parasitoides. El comportamiento de cascabel fue raramente provocado.

Vibrational cueing may be critical in mediating the interactions between leaf-mining insects and their parasitoids (Meyhöfer et al. 1997). Some leaf-mining species detect the vibrations that are released by parasitoids searching at the leaf surface, then wriggle within their mines to evade attack (Meyhöfer et al. 1994; Bacher et al. 1996; Meyhöfer & Casas 1999). Vibrations produced by wriggling can reduce the time that a parasitoid spends at a leaf before terminating its search; and thus, reduces parasitism risk. Low (2008) reported that in the tupelo leafminer, *Antispila nysaefoliella* (Lepidoptera: Heliozelidae), vibrations are not produced incidentally, but directly through the actions of unique behaviors in association with specialized morphology (sclerotized ridges and bumps).

The reported "seismic" behaviors of *A. nysaefoliella* may be an evolutionary elaboration of the wriggling observed in other species for producing defensive vibrations. Based on known interactions between hosts and parasitoids, substrate vibrations that are emitted by the host can interfere with parasitoid search cues or signal unprofitability (high search and handling costs) to parasitoids (Djemai et al. 2004). Conversely, the parasitoids that hunt for concealed prey also release vibrations that provide information for the leafminer that is hiding inside its mine (Meyhöfer et al. 1994; Bacher et al. 1996; Meyhöfer & Casas 1999). Therefore, if the ticking and rattling behaviors of *A. nysaefoliella* have evolved for a defensive function and in response to parasitic wasps, then these should be tuned to the vibrations that characterize potential danger, such as the activity of a parasitoid searching at the leaf surface.

To test if *A. nysaefoliella* larvae are indeed tuned to the vibrations of their parasitoids, I conducted a

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playback experiment using digitally produced pure-tone signals of 1-6 kHz, which spanned the range of dominant frequencies reported for the probing activity of *Sympiesis sericeicornis* Nees (Hymenoptera: Eulophidae) on leaves for the spotted tentiform leafminer, *Phyllonorycter malella* (Gerasimov) (Lepidoptera: Gracillariidae) (Bacher et al. 1996). The parasitoids that attack *A. nysaefoliella* are likely to produce similar cues to *Sympiesis sericeicornis* because they are congener species that are also similar in body size and behavior (e.g., *Pnigalio* sp. and *Closterocerus* sp.). Bacher et al. (1996) concluded that the dominant frequencies from probing (insertion of ovipositor into mine) were the most consistent at 2-3kHz, and thus would provide the most reliable information for a leafminer to detect an attacking parasitoid. In this study I ask: do these specific frequencies elicit the ticking or rattling behaviors in *A. nysaefoliella?*

Materials and Methods

Study System

The tupelo leafminer, *A. nysaefoliella*, is a specialist of blackgum, *Nyssa sylvatica* Marsh (Cornales: Nyssaceae), and the population used for this study was located within a mixed deciduous forest in Clarke County, Virginia, USA. In a previous study by Low (2008), 2 seismic behaviors were described and named "tick" and "rattle" for the audible sounds that they produce. The tick behavior is characterized by a rhythmic movement of the abdomen back and forth like a pendulum, and the rattle is a rapid pulse of the caudal segment. The rattle behavior is similar to the rattle of a rattlesnake (*Crotalus* sp.; Squamata: Viperidae) in both movement and auditory cues. Ticks are rhythmic and can last for several minutes, whereas rattles are quick rapid bursts that last only fractions of a second. Videos of the behavior can be viewed at the Infolink for supplementary files for Low (2008), http://www.fcla.edu/FlaEnt/fe91p604i.htm.

Playback Experiment

Each playback trial sequence was composed of 18 stimuli (6 frequencies at 3 amplitudes) generated using MatLab (v. 6.5 Mathworks), which were played through a stereo speaker (Pignose Model 7-100R) from a laptop computer in random order to individual larvae (N = 89). The speaker was set 20 cm away from the front of a leaf that was held in place and upright by inserting the petiole into a floral "oasis" cube, which was partially submerged in a dish of water. Each trial began when the larva stayed motionless for at least 10 min. Each stimulus recording was preceded by a 30-s silent period, and then played for 30 s. I recorded all larval activity during the experimental trials using a digital video camera (set on a tripod) and scored all behavioral responses using the video recordings after the experiment was completed. The entire experiment occurred over 6 d inside a laboratory room (~25 °C), and leaves were collected fresh from the field each d during 15-21 Aug 2002. Each experimental leaf contained only a single larva (and mine). Mines were approximately 0.8 ± 0.4 cm in diam (mean ± 1 SD).

The proportion of individuals that did not change their behavior when the signal was

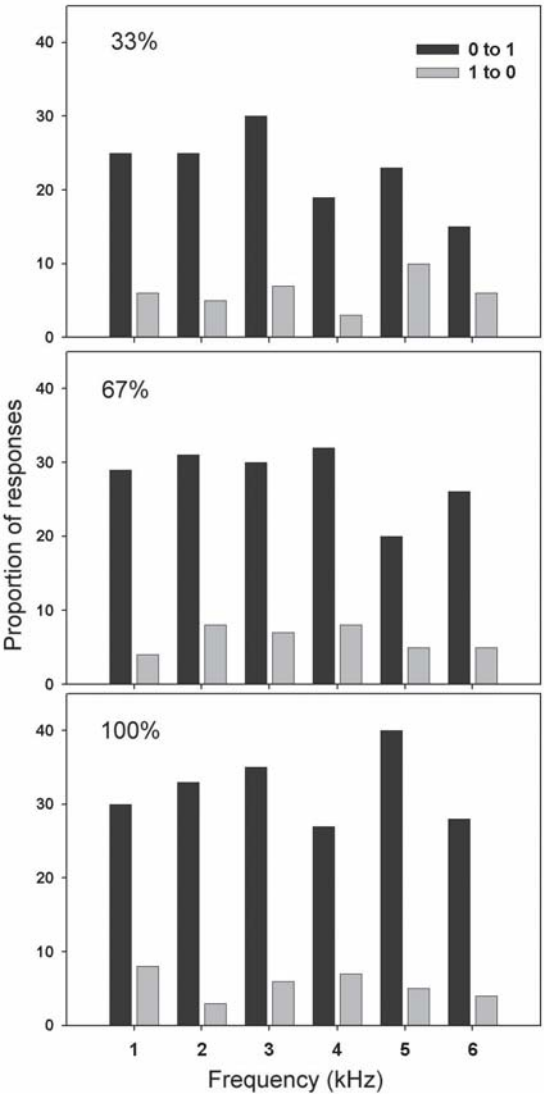

Fig. 1. The responses of larvae to playback stimuli. Behavioral transitions are defined as going from stillness during the control period to movement during the signal stimulus ("0 to 1"), and the opposite if the larva was moving during the signal, but then stopped its behavior.

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Table 1. Behavioral transitions during the sound playback experiment of 6 pure-tone frequencies at 3 relative amplitudes.

Amplitude	Frequency	*N*	No change in behavior	Change in behavior	*P*
33%	1 kHz	88	50 (0.57)	38 (0.43)	0.186
	2 kHz	88	57 (0.65)	31 (0.35)	0.338
	3 kHz	89	56 (0.63)	33 (0.37)	0.476
	4 kHz	**88**	**36 (0.41)**	**52 (0.59)**	**0.000***
	5 kHz	**89**	**30 (0.34)**	**59 (0.66)**	**0.000***
	6 kHz	89	50 (0.56)	39 (0.44)	0.153
67%	1 kHz	89	48 (0.54)	41 (0.46)	0.073
	2 kHz	**89**	**37 (0.42)**	**52 (0.58)**	**0.000***
	3 kHz	89	52 (0.58)	37 (0.42)	0.277
	4 kHz	89	55 (0.62)	34 (0.38)	0.524
	5 kHz	**89**	**22 (0.25)**	**67 (0.75)**	**0.000***
	6 kHz	87	47 (0.54)	40 (0.46)	0.079
100%	**1 kHz**	**89**	**45 (0.51)**	**44 (0.49)**	**0.018***
	2 kHz	89	56 (0.63)	33 (0.37)	0.476
	3 kHz	87	62 (0.71)	25 (0.29)	0.046
	4 kHz	89	57 (0.64)	32 (0.36)	0.390
	5 kHz	**81**	**31 (0.38)**	**50 (0.62)**	**0.000***
	6 kHz	**81**	**60 (0.74)**	**21 (0.26)**	**0.015***

Asterisks indicate statistical significance at a *p*-value of 0.0194, Which was calculated using the false discovery rate for multiple comparisons (Benjamini & Hochberg 1995).

played (either started or stopped moving) was tested against the null proportion of 0.62 in a 2-tailed binomial test. This null value was calculated from the pooled number of "no change" responses (972 out of 1579), which is also equivalent to the average proportion across tests. Statistical significance was set at a *P*-value of 0.0194 by calculation of the false discovery rate for multiple comparisons (Benjamini & Hochberg 1995).

Results

In the experimental trials, all individuals wriggled at least once, 45% ticked, 17% ticked and rattled, and 6% rattled. The transitions in behavior from the control state were predominately from stillness to movement (by wriggling, ticking, or rattling) (Fig. 1). In general, larvae responded to all frequencies at various amplitudes by wriggling, suggesting that they sensed the vibrations (Table 1). However, they responded by ticking most often to frequencies at 2-3 kHz (Fig. 2), which corresponds with the dominant signal frequencies of probing behavior by the parasitoid *Sympiesis sericeicornis* (Hymenoptera: Eulophidae, see Djemai et al. 2001). The number of observations of ticking (y) against frequency (x) can be predicted by the model: $y = 8.27x^2 - 4.19x + 1.72$ ($R^2 = 0.34$, df = 15, $F = 3.89$, $P = 0.044$; Fig. 2, bottom panel). In general, observations of the rattle behavior were too infrequent to draw any conclusions about its context or function.

Discussion

The experimental results suggest that *A. nysaefoliella* can sense vibrations in general, but respond with specialized ticking behavior to the vibrational cues that fall within the frequency range of parasitoids. Larvae responded with slight movements (wriggling) to all frequencies tested (1-6 kHz), which suggests that they sensed all signals but ticked most often in response to the dominant frequencies of parasitoid probing behavior (2-3 kHz) (Djemai et al. 2001). Because many of the parasitoids of blotch miners (e.g., *A. nysaefoliella* and *P. malella)* tend to be species generalists (but blotch specialists), this frequency range is most likely to be biologically relevant for interpreting the results of this experiment (Krombein et al. 1979; Gates et al. 2002).

The use of pure-tone signals may be adequate for isolating the frequency component of natural signals for playback, because airborne pure-tone (insect) sounds tend to propagate more accurately, and the characteristics of insect sounds and vibrations tend to be low frequency, pure-tone signals (Bennet-Clark 1998; Cocroft & Rodriguez 2005). Moreover, the thin mine epidermis is the only barrier between the larva and the external environment, and therefore, the signal received by the leafminer (via sensory hairs, Low 2008) would be minimally filtered or distorted. In contrast, signal attenuation and distortion is more likely across a leaf surface because of the differences in density (Cocroft et al. 2006).

The seismic behaviors observed for *A. nysaefoliella* provides the first hint at active signaling

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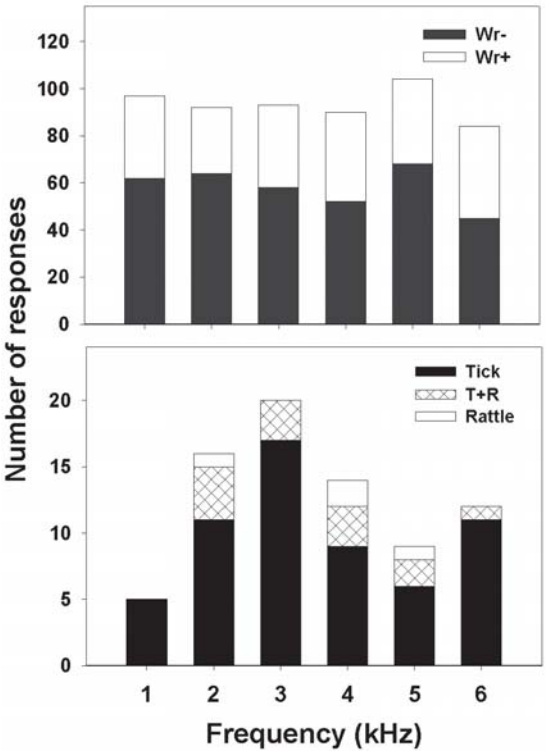

Fig. 2. Results of the playback experiment where each individual (N = 89) was tested for responses to 18 different stimuli (6 frequencies, 3 amplitudes). Each bar represents the total number of vibration-generating responses: wriggles, ticks, and rattles. In the top panel, each bar represents the total number of individuals that wriggled, divided into wriggling without (Wr) and wriggling with (Wr+) displacement. In the bottom panel, each bar represents the total number of individuals that responded by ticking only (T), rattling only (R), and both ticking and rattling (T + R). These data represent the positive responses (change in behavior) from Table 1 pooled across all amplitudes. These data represent only the "0 to 1" behavioral transitions from Fig. 1.

by a leafminer and extends the findings of many other studies that point to importance of vibrational communication in animal systems - that go beyond mate recogntion and conspecific communication (Hill 2009 and references therein). In signaling behavior, there is an intent to send information to the receiver, which then alters the receiver's behavior and increases the signaler's fitness (Bradbury & Vehrencamp 1998; Maynard-Smith & Harper 2004). Previous studies on leafminer cues (not signals) have found that parasitoids do indeed change their behavior because of the vibrational cues emitted by leafmining larvae (Djemai et al. 2004). As a consequence, the reduction in risk associated with vibration production may set the stage for intentional signals that communicate information to the parasitoid about the host's condition, such as difficulty of capture.

This study was only the first step towards understanding the adaptive significance of vibration production in *A. nysaefoliella.* Some alternative hypotheses for the function of the seismic behaviors are: individual defense against parasitoids, social (territorial or agonistic) communication, or warning conspecifics about the presence of parasitoids (via kin selection). Future work should test multiple hypotheses and determine the importance of vibrational signaling, in general, for mediating host-parasitoid interactions.

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