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Parasitoid foraging decisions mediated by artificial vibrations

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In any host–parasitoid interaction, the detection of the host in its microhabitat is a crucial step in the interaction that has a major influence on the parasitoid's fitness. We used bioassays to investigate whether the parasitoid *Sympiesis sericeicornis* (Hymenoptera: Eulophidae) uses the vibrations of its host *Phyllonorycter* sp. gr. *blancardella* (Lepidoptera: Gracillariidae) to detect it in the leaf mine. From the large variety of signals produced by the host, we identified a unitary signal that could be reproduced artificially. We then sent this signal into emptied mines to analyse the reaction of the parasitoid. The wasps reacted by increasing both the time spent foraging on the mine and the number of insertions of the ovipositor to detect the 'virtual' host. This study is the first to show that parasitoids detect host vibrations.

Adult female parasitoids spend a large proportion of their lifetime searching for hosts. During this foraging period, they use a variety of cues to make decisions about where and for how long to search at different locations (Van Alphen & Vet 1986; Godfray 1994; Vet et al. 1995; Quicke 1997; Vinson 1998). Olfactory and visual cues have been particularly well studied (reviewed in Schmidt 1991; Vinson 1991). Vibrations, even though much less well known, have been reported to be important in determining the foraging success of at least 13 species of parasitoids (Meyhöfer & Casas 1999).

The majority of parasitoids studied to date belong to the Braconidae, Eulophidae and Pteromalidae families, which normally attack endophytic Dipteran or Lepidopteran hosts. These hosts live hidden in the substrate, mining leaves or fruits, inside seed, underneath the bark of trees or in decaying fruit substrates. Because these hosts are concealed, visual and chemical stimuli might be less important for foraging parasitoids and vibrations might therefore be the main stimuli used (reviewed in Meyhöfer & Casas 1999). Reported descriptions of the behaviour of parasitoids as they approach their hosts are generally insufficient to give a clear indication of the role of vibrations. The exact mechanisms by which parasitoids use host-generated vibrations can be determined only through detailed manipulative studies like those for other cues such as semiochemicals (Vet & Dicke 1992). Sugimoto

et al. (1988) showed that playing back recorded signals produced by leafminers triggers predicted behavioural reactions by parasitoids. However, the technique used presents several major difficulties (Casas 1994). Briefly, the coupling of the recording devices, playback devices and the substrate, as well as the added mass, complicates the analysis of the signal and the associated behaviour. The problems of using inappropriate signals in the study of vibratory communication have been reviewed by Schmitt et al. (1993). To construct an appropriate bioassay, one needs qualitative and quantitative information on the host's generated signal and on the parasitoid's behavioural response.

At present, a set of data on vibrational cues is available for only one host–parasitoid system, the spotted tentiform leafminer *Phyllonorycter* spp. group *blancardella* (Lepidoptera: Gracillariidae) and its parasitoid *Sympiesis sericeicornis* Nees (Hymenoptera: Eulophidae) (Meyhöfer et al. 1994, 1997; Bacher et al. 1996, 1997; Djemai et al. 2000, 2001; Magal et al. 2000). Late-instar larvae and pupae of the spotted tentiform leafminer show characteristic defensive reactions when they are attacked by this parasitoid. Based on observations in the field, Casas (1989) hypothesized that the key stimuli involved in the parasitoid's searching behaviour are vibrations that the larvae produce while moving, feeding or escaping, or that the pupae produce by violently moving the abdomen to avoid the sting of the ovipositor. This hypothesis has been supported by the numerous results obtained so far. Previously (Djemai et al. 2001), we showed that the host reacts to synthetic parasitoid vibrations by adopting evasive behaviour as observed in the natural interaction. Furthermore, the frequency range triggering host reactions

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matches the frequency components of vibrations produced by parasitic wasps. In the present study, we carried out a bioassay to determine parasitoid reactions to host vibrations.

METHODS

Study Species

The biology of the leafminer species we studied, which is related to *Phyllonorycter blancardella* Fab. (Lepidoptera: Gracillariidae), has been described by Pottinger & LeRoux (1971). Larval development can be divided into five instars, the first three of which are sap-feeders, and the last two are tissue-feeders. The sap-feeders have a dorsoventrally compressed body and produce a flat surface mine on the lower side of the leaf. The structure of the mine changes with the development into a tissue-feeder, which spins silky threads. Its mine is shaped like a tent. Larvae and pupae are attacked by several parasitoid species (Casas & Baumgärtner 1990).

Sympiesis sericeicornis (Hymenoptera: Eulophidae) is one of the most important parasitoids attacking *Phyllonorycter* sp. gr. *blancardella*. It attacks fourth and fifth larval instars and pupae. The parasitoid immobilizes its host by injecting venom with its ovipositor before laying its eggs.

Characterizing Host Vibrations

We used leaves of apple seedlings containing mines for the experiments. We obtained the seedlings from INRA, Angers, France. We placed the petiole through a hole in the lid of a glass tube with water and used plasticine to prevent it from moving. An unwrapped section of the petiole was left between the plasticine and the leaf lamina. Temporal patterns and frequency composition of the vibratory signals during the first 50 ms are the same with this set-up as for leaves fixed to the stem (R. Meyhöfer, J. Casas, F. Wäckers & S. Dorn, unpublished data). The lower epidermis of the mined area was replaced by a clear piece of plastic attached to the leaf with scotch-tape. This replacement does not alter the vibratory signal, as temporal pattern and frequency composition of signals after impact are conserved with this set-up (Meyhöfer et al. 1994). This enabled us to examine the movements of the larva and pupa inside the mine. A mirror placed under the mined leaf allowed us to record both sides of the leaf on the same video frame. All experiments were carried out on a vibration-free table to reduce background noise.

Vibrations produced by the fourth and fifth instars and pupae were recorded with an Ometron laser vibrometer VS 100 (maximal sensitivity of 1 μm/s and maximum frequency of 50 kHz). Mined leaves were adjusted so that the surface was perpendicular to the laser beam, which was centred on the upper side of the mine. The vibration signals were sampled with a Siglab recorder (Digital Signal Process Technology, Fremont, CA, U.S.A.). We observed larval behaviour continuously through a binocular microscope to ensure that the measurements started only when the host was moving.

Pupae and larvae produced vibrational signals by moving the abdomen (pupae) or by changing location (larvae). A sharp increase in velocity indicated the beginning of a vibrational signal and a fall to the level of the background noise indicated its end. We classified recorded signals into unitary and complex ones. A unitary signal consists of a peak followed by damped oscillations. Complex signals are made of a superposition of at least two signals identified by their maximal amplitude.

To obtain a smooth approximation based on a few parameters that would allow us to compare signals, we fitted a damping oscillation to the observed signals. For each unitary signal, we fitted the damping oscillation given by the equation $Y(t) = y_{max} \times \exp(-t/\tau) \times \text{sine}(2\pi\omega/t)$, where t is time, y_{max} is the maximum amplitude, ω is the oscillation period and τ is the time constant ($\tau = T/\ln(y_{max})$, where T = signal duration; Fig. 1; Hansen & Snyder 1997). We first log transformed the absolute value of the signal, then calculated the envelope of the signal by visually placing a line through the maxima and the minima of the signal. Back calculations gave the parameters y_{max}, τ and ω.

Bioassays with Parasitoids

We sampled mines in the field and maintained them until insects emerged. Parasitoid females and males were put together to mate in a box ($30 \times 30 \times 30$ cm). After mating, 30 females were kept singly in petri dishes with water and honey. For the experiment, they were placed with mined host plants. Only mines with fourth and fifth larval instars and pupae were used. Hosts were removed from their mines within 1 min of the experiments with as little damage as possible to the mine (we made a small hole in the upper side of the leaf, to extract the host with a needle). As stimuli, we used synthetic signals that
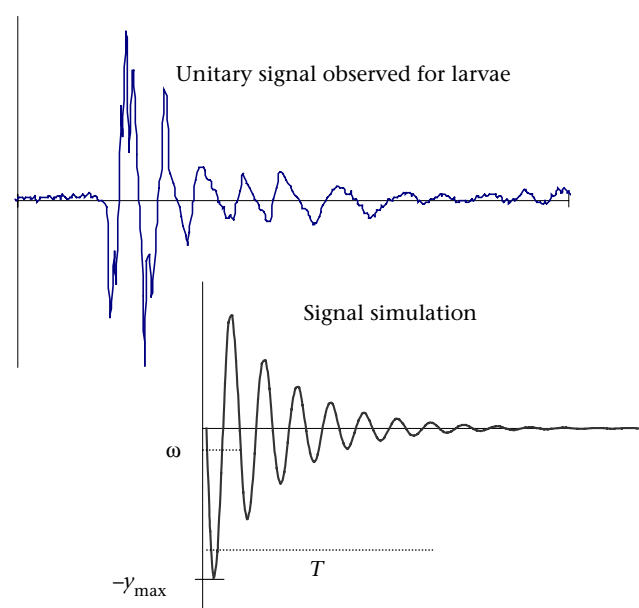
Figure 1. Unitary signal approximated by a damping oscillation. y_{max}: maximum amplitude; T: duration of the signal; ω: oscillation period.

simulated the host. We modelled the host-generated signal based on the observed maximal amplitude, the maximal frequency and the duration of unitary signals. Synthetic signals, generated with Siglab (Digital Signal Process Technology), were sinusoidal signals of a single period of 0.3×10^{-3} s with velocity of 2 mm/s. The signal was sent to the mine each second for 5 min by means of an electromagnetic vibration exciter connected to a power amplifier (V1O1-LDS PA25E Link Dynamics, Royston, Herts, U.K.). Stimulating the leaves with a single period sine wave produced a damped oscillation similar to a unitary signal in the leaves. On top of the vibration exciter was a needle with a head 0.5 mm in diameter, which permitted us to send localized vibrations on the undersides of mines which were kept intact. A laser vibrometer recorded the response of the vibrometer exciter output through the leaf on top of the mine to ensure that identical time and frequency parameters of the signal were sent and received.

We recorded the behaviour of *S. sericeicornis*, searching on single-mined apple leaves, with a video camera (Sony CDD-RGB) equipped with a macro-zoom lens (18−108/2.5), in a glass box ($30 \times 30 \times 30$ cm), starting when the parasitoid began to walk on the mine. The end of the parasitoid's foraging behaviour was defined as its departure from the leaf. We recorded each female for two periods of 5 min, with and without vibrations. Each treatment was separated by 10 min of isolation of the parasitoid outside the glass box, without mines. The order of treatments to each female was randomized. For further analysis, we used the time spent foraging on mines, which included all displacements on the mine from one location to another, and the number of ovipositor insertions in the mine.

Statistical tests were two tailed.

RESULTS

Identification of Host's Vibrations

Larvae ($N = 15$) and pupae ($N = 21$) produced unitary signals with the same average amplitude ($\overline{X} \pm \mathrm{SD} = 2.08 \pm 1.46$ mm/s; $t_{34} = 1.209$, $P = 0.235$) and with the same
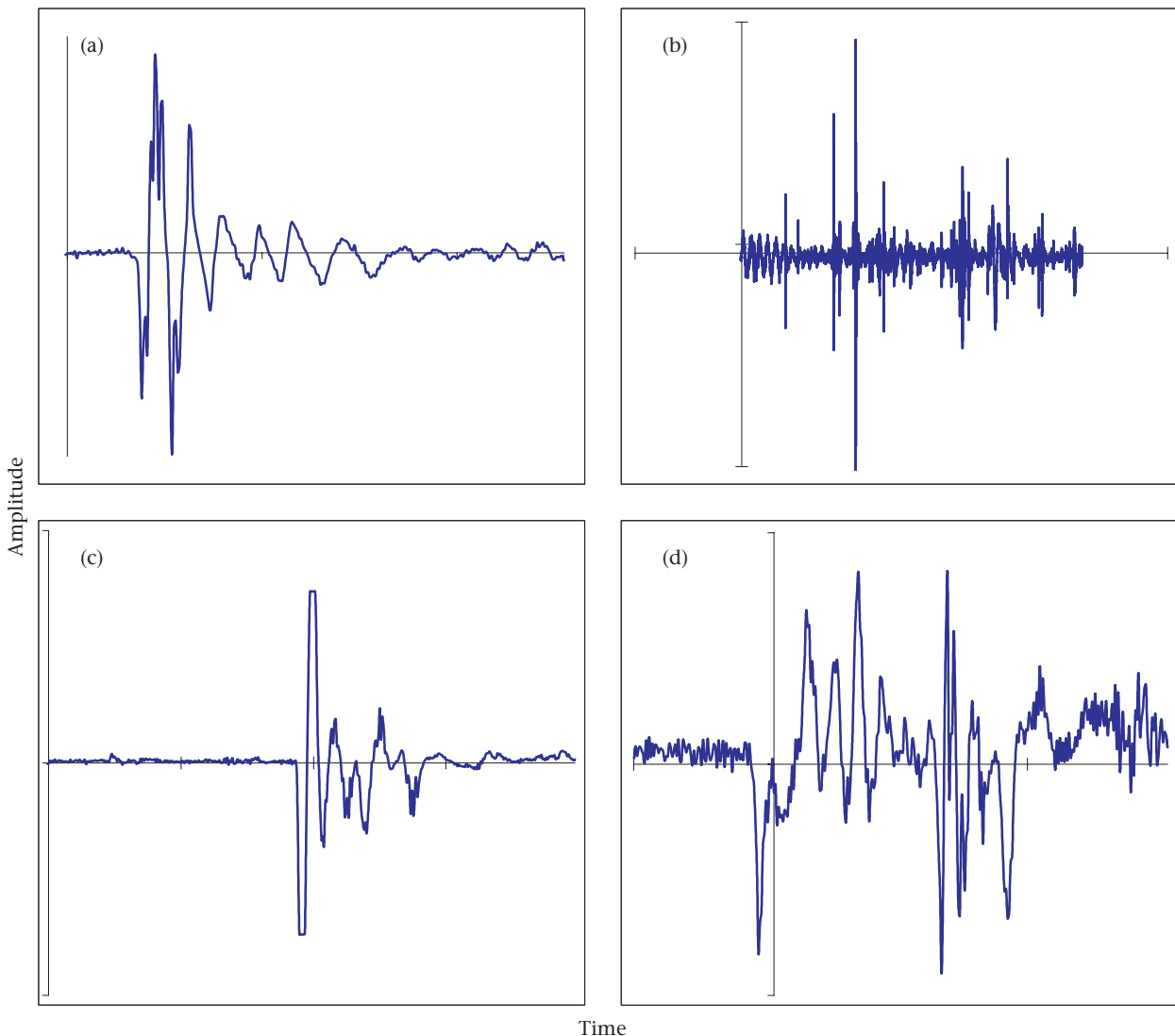
Figure 2. (a) Unitary and (b) complex signals produced by larvae. (c) Unitary and (d) complex signals produced by pupae.

average oscillation period ($\overline{X} \pm SD = 0.3 \times 10^{-3} \pm 0.24 \times 10^{-3}$ s; $t_{34} = 1.949$, $P = 0.06$; Fig. 2). Larvae produced complex signals with a mean $\pm$ SD of 5.40 ± 5.01 peaks/50 ms ($N = 30$), whereas pupae produced complex signals with a mean $\pm$ SD of 2.17 ± 1.57 peaks/50 ms ($N = 30$; Fig. 2).

Bioassays

Of 30 females, 20 left the mine within 5 min in the absence of vibrations. The other 10 females searched quickly on the leaf, outside the mine (Fig. 3). Foraging time was longer in the presence of vibrations: 93% of females stayed longer than the 5 min allowed (Wilcoxon paired-sample test: $Z = -4.198$, $N = 30$, $P < 0.001$). The parasitoids' behaviour appeared to be identical to that observed in natural interactions, from the movement of their antennae to probing. The number of ovipositor insertions was higher in the presence of vibrations (with vibrations: $\overline{X} \pm SD = 4.90 \pm 5.73$; without vibrations: 1.83 ± 3.41; Wilcoxon paired-sample test: $Z = -2.363$, $N = 30$, $P = 0.018$).

DISCUSSION

The use of substrate vibrations in communication and predator–prey interactions is widespread in arthropods. The time required to extract information from a signal depends on its complexity and on the type of information it encodes (Smith & Harper 1995; Bradbury & Vehrencamp 1998). The time constraint on the recipient will determine to what signal characteristics it will pay attention. The sensory systems of the parasitoid and the host in this system are adapted to perception of vibrations (Djemai et al. 2001), which is one way to transmit information particularly quickly, an important criterion in this type of interaction. These considerations imply that short transients (i.e. a signal with finite length) could be the feature to which both participants in the interaction are reacting.
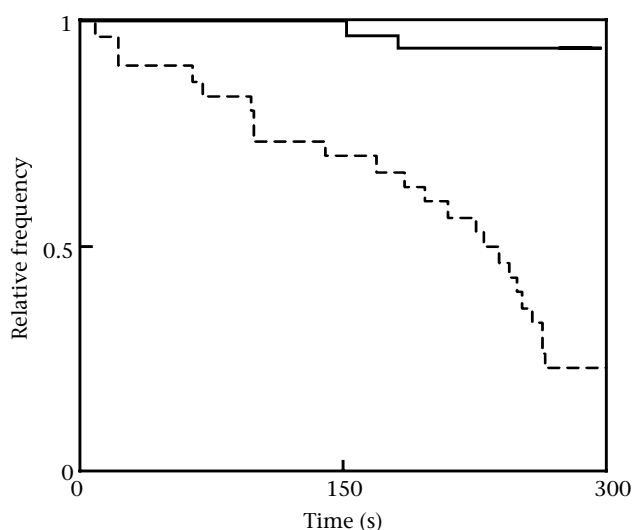
In our study, we showed that parasitoids reacted to a synthetic unitary signal, which is the simplest transient structure for signals to mimic the presence of a virtual host. We did not test other types of vibratory signals, such as white noise or square waves, as they do not belong to the repertoire of signals produced by the host. However, such a study would give valuable information on the perception abilities of this parasitoid species. In contrast, experiments with a complex synthetic signal would permit us to understand how vibratory signals can be informative about direction and distance to the source. Complex signals in their full extent may even trigger stronger behavioural reactions than unitary signals. Wasps may react to different types of vibrations with increased foraging effort, and there are certainly other sources of vibrations in their environment, as Casas et al. (1998) demonstrated. Complex signals can be interpreted as series of 'wave skeletons', the unitary signal, which can be modulated in occurrence, time and frequency. Hence, parasitoids may detect this skeleton wave in all signals and may associate it with the host. At this stage, investigations are needed that use acoustic simulations with a complex signal in a host–parasitoid system, as done for the acoustic communication of the treehopper *Umbonia crassicornis* (Cocroft et al. 2000; Miles et al. 2001).

The parasitoid *S. sericeicornis* is a generalist parasitoid attacking a large number of hosts, up to 72 species (Boucek & Askew 1968). Two-thirds of these hosts live hidden in their microhabitat usually concealed in a mine or similar microhabitat as in the case of leaftiers (insects that attach parts of a leaf or several leaves together). These hosts may all produce vibratory signals characteristic of moving hosts inside plant tissues. Broad & Quicke (2000) predicted that the evolution of vibrational sounding would be associated with parasitism of concealed hosts, and in particular of immobile hosts such as pupae. An extension of their prediction to vibration perception and use in host–parasitoid systems with mobile concealed hosts may explain the polyphagy of *S. sericeicornis* and many other allied species, which may be microhabitat specialists but host species generalists.

Figure 3. Relative frequency of female parasitoids staying on empty mines with (—) and without (- - -) vibrations for 5 min.

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