BIOLOGICAL CONTROL **11,** 147–153 (1998)
ARTICLE NO. BC970593

Leaf Vibrations and Air Movements in a Leafminer–Parasitoid System

Jérôme Casas,* Sven Bacher,†[,1] Jürgen Tautz,‡ Rainer Meyhöfer,†[,2] and Dominique Pierre*

**Institut de la Recherche sur la Biologie des Insectes, IRBI-CNRS UPRESA 6035, Université de Tours, Parc Grammont, F-37200 Tours, France; †Plant Sciences, Applied Entomology, Swiss Federal Institute of Technology (ETH), Clausiusstrasse 25, CH-8092 Zurich, Switzerland; and ‡Biozentrum, Lehrstuhl für Verhaltensphysiologie und Soziobiologie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany*
E-mail: casas@univ-tours.fr

Received October 1, 1996; accepted October 1, 1997

This paper analyzes the vibratory environment of the leafminer *Phyllonorycter malella* (Ger.) (Lep. Gracillariidae) and its parasitoid *Sympiesis sericeicornis* Nees (Hym. Eulophidae). Previous studies established that both the host and the parasitoid not only produce but also use leaf vibrations: the former in order to escape ovipositor stings, the latter as a physical cue in the foraging process. First we characterize vibration patterns produced by simulated wind and rain and discuss the influence of these background vibrations on the host–parasitoid interaction. Second, we present a method of producing leaf vibration patterns of high repeatability. This technique allows us to characterize the influence of the leaf as modifier of vibratory signals. We discuss how *S. sericeicornis* could use such spatially variable vibratory information for between-mine foraging. Finally, a combined laser Doppler vibrometry–laser Doppler anemometry study revealed that leaf vibrations induce air movements in their immediate surroundings with characteristic temporal and spatial structures. We discuss if and how host-related information contained in the air particle displacement can be used by parasitoids. © 1998 Academic Press

KEY WORDS: ***Phyllonorycter malella; Sympiesis sericeicornis;*** **leafminer; apple; host–parasitoid interaction; vibrations; air particle movement; host searching; foraging behavior; plant biomechanics; laser Doppler anemometry.**

INTRODUCTION

Parasitoids attacking endophytic hosts rely on a whole array of chemical and physical stimuli to locate their host. Vibrations play a major role in the behavioral interaction between *Sympiesis sericeicornis* Nees (Hym. Eulophidae) and the apple tentiform leafminer *Phyllonorycter malella* (Geer.) (Lep. Gracillariidae). During a foraging bout on a mine, *S. sericeicornis* alters its behavior according to vibrations produced by the moving and wriggling host (Meyhöfer *et al.*, 1994, 1997b). The host reacts evasively to insertions of the ovipositor in the mine, even when the insertion is far from the host (Meyhöfer *et al.*, 1997b). The results of the latter study, based on an analysis of combined ethograms, have been recently supported by experimental biotests using synthetic stimuli (Bacher *et al.*, in press). Inserting the ovipositor is the only parasitoid behavior that triggers vibrations specific to a foraging parasitoid (Bacher *et al.*, 1996). This succession of changes in locations and behaviors sometimes enables the host to escape parasitism. Escape rates range from 10% of the attacks in this species (Casas, 1989) to up 80% in other leafminer–eulophid systems (Connor and Cargain, 1994; Connor, pers. commun.).

The background noise in the field may mask the vibrations produced by insects. Wind and rain are a major source of such disturbance. The first aim of this paper is to study the vibration patterns produced by these two abiotic factors and discuss their influence on the host–parasitoid interaction. The reader is referred to Ewing (1989) and Bailey (1991) for a general survey of the use of vibrations in insects.

The variability in intensity, frequency composition, and temporal pattern of vibrations for the same behavior is high, both for the host and for the parasitoid (Meyhöfer *et al.*, 1994; Bacher *et al.*, 1996). This natural and unavoidable variability has consequences for both the insects and us. Insects may have to concentrate on a few cues in a signal and discard all the remaining information that varies too much to be reliable. For us, a major difficulty lays in distinguishing, in a series of signals, the variability caused by the applications of varying forces to a leaf from the variability caused by the use of different leaves or different measuring

[1] Present address: Zoological Institute, Synecology, University of Berne, Baltzerstrasse 3, CH-3012 Bern, Switzerland.

[2] Present address: Institute of Plant Diseases and Plant Protection, University of Hannover, Herrenhaeuser Strasse 2, D-30419 Hannover, Germany.

1049-9644/98 $25.00

points. Thus, the second aim of this paper is to develop a method which ensures a high repeatability in the vibration patterns. We analyze the vibration patterns of a leaf using this new method.

Leaf vibrations induce air particle movements above and below the leaf lamina. Thus, a parasitoid on a leaf may perceive air movements and may extract information about a host's location and behaviors from the directions and velocities of particles. We question the temporal and spatial structure of air particle movements triggered by leaf vibrations and discuss their potential use by parasitoids. We present preliminary results of a combined laser Doppler vibrometry–laser Doppler anemometry study of air particle movements around a vibrating leaf. In the spirit of the symposium, the paper is written in a prospective fashion and we rely mainly on examples for illustrating our points. A thorough analysis of some of the experiments will be published elsewhere.

METHODS

Rain and Wind

Vibrations were measured on the center of the upper leaf surface of apple leaves (*Malus sylvestris* cf. Golden Delicious) with a laser Doppler vibrometer (Dantec 41X62 Compact Laser Vibrometer, 2 mW He-Ne-Laser) as described in detail in Bacher *et al.* (1996). To avoid leaf vibrations due to air turbulence, measurements of water drop-induced vibrations were carried out on three uninfested leaves which were placed in an open glass box (17 × 11 × 10 cm). The leaves taken from our laboratory culture were thin and soft and had similar characteristics (weight, 179 and 162 mg for leaves 2 and 3; length of leaf lamina; 7.1, 7, and 6.6 cm for leaves 1 to 3; width of leaf lamina, 3.4, 3, and 3.6 cm, respectively). The cantilever setup is described in Meyhöfer *et al.* (1994) and Bacher *et al.* (1996): leaves without mines were cut off from their plants and their petioles were placed in water-filled vials through a hole in its lid. The petiole was fixed to the lid with plasticin. The box was removed for investigations on wind-induced vibrations. Vibration data were stored on a Macintosh Quadra 800 computer and analyzed with the software SoundScope (GW Instruments, 1993). Alongside the vibration recordings, leaves were videotaped (recorder: Panasonic AG-7355; camera: Panasonic WV-BL600; lens: Computar 18-108/2.5).

Standardized water drops of 15 mg were produced with an adjustable Eppendorf pipet fixed at a distance of 33–35 cm above the leaves. The pipet was adjusted such that the falling water drops landed on the major leaf vein. One target point was placed halfway between the measurement point and the leaf tip (apical), a second one halfway between the measurement point and the basis of the leaf (basal). The accuracy of the target hits was controlled with the video recordings. Lateral deviations from the target point caused lateral movements of the leaf during which the vibrometer temporarily lost track of the signal (drop-outs). Recordings were considered valid only when drop-outs shorter than 4 ms occurred. Four valid recordings were made of each leaf at each measurement point.

Air movements were produced by means of a hair dryer (Satrap profi 1500 diffuser, heating disabled). Two uninfested leaves from our rearing were horizontally fixed at a distance of 3 cm from the vibration damped table on which they stood. The hair dryer was placed at a distance of 80 cm from the leaves and was not in direct contact with the table. Leaves were adjusted to receive the air flow along their longitudinal axis with the leaf tip pointing toward the hair dryer and, in a different experiment, to receive the air flow perpendicular to the leaf axis. Two recordings of 10 s each were made of each leaf in each adjustment. In a preliminary experiment in which the air flow was directed away from the leaf it was assured that vibrations emitted by the running dryer were not transmitted to the leaves on the table. The switch positions "high" and "low" produced wind speeds reaching the leaf at 1.5 and 0.75 m/s, respectively. Wind speed was measured with an A100R switch anemometer connected to a CR10 datalogger (Campbell Scientific). With a few minor adaptations to the kind of signals tested in this study, data acquisition and analysis were done in the way described by Bacher *et al.* (1996). The frequency tracker of the vibrometer was set in the less sensitive range of 0.1–1.0 MHz so that velocities from 63×10^{-6} to 0.13 m/s and a maximal acceleration of 180 m/s^2 could be detected (Dantec, 1991). A linear frequency response of the vibrometer up to 26 kHz at this tracker range is reported by the manufacturer.

Falling Balls

Vibrations of leaves impacted by small metal balls were measured with a laser Doppler vibrometer (same as above). A small metal ball (2.1 mg) was dropped from a height varying from 4 to 7.6 cm on a cantilever leaf held in horizontal position (setup as above). The metal sphere was held by an electromagnet and dropped onto the leaf when the current was turned off. The impact point was the tip of the leaf, as the occurrence of drop-outs and multiple rebounds was minimal there. Measuring points were (i) the central vein in the middle of the leaf and (ii) the central vein at the basis of the leaf. Three replicates were obtained for each combination of leaf, impact, and measuring points, giving a total of 42 measurements on 7 leaves. The recording proceeded as above.

Air Particle Displacement

The same cantilever setup as above was used for the measurements of air particle movements. The setup, as well as the falling ball device were placed in a glass box. In this experiment, the leaves and the falling ball device were placed inside a glass box which was filled with gas DEMS (particle size max. 5 µm). Experiments started as soon as the air within the box was at rest, usually within 5 min. Vibrations of leaves were measured with a laser Doppler vibrometer (LDV) (Polytec OFV 210VC, sensor head OFV 300). A laser Doppler anemometer from Dantec (LDA) recorded the air displacement in the vertical direction. The balls fell vertically on the leaf from a height of 2.5 cm. The impact point was at halfway between the central vein and the leaf border, in the middle of the leaf. This impact position was considered to reflect more accurately the source of vibrations in natural situations, but had the disadvantage of producing frequent drop-outs and multiple rebounds. Drop-outs were filtered out after recording and experiments with multiple rebounds were discarded. The LDV measuring points were placed directly below the impact point and symmetrically opposite (taking the central vein as the symmetry axis). The LDA measuring points were placed at different distances above or below the LDV measuring points. The data were recorded with a DAT recorder (Bio-Logic DTR 1800, highest sampling frequency 20 kHz) and subsequently transferred to a microcomputer (Labview 3.0.1., National Instruments).

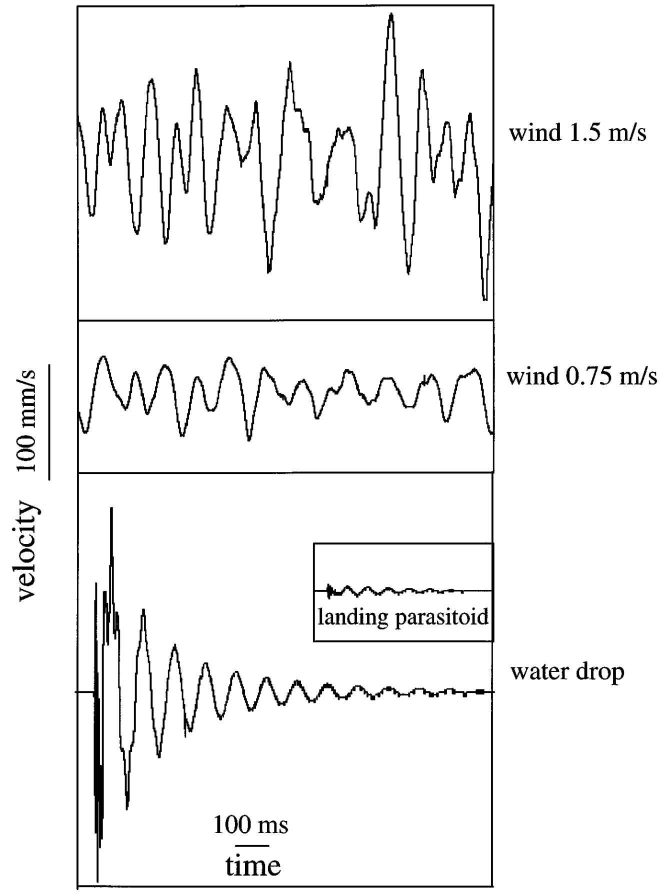

FIG. 1. Leaf vibrations caused by wind of high and low speed, a water drop, and a landing parasitoid.

RESULTS

Rain and Wind

The impact of a falling water drop on a leaf resulted in a vibratory signal which was similar to the vibrations triggered by landing parasitoids (Fig. 1) and falling balls (see below). Although the signal produced by a water drop was much stronger than the one produced by a landing parasitoid, an irregular and a regular phase were observed in both cases. In the irregular phase caused by a water drop, the frequencies were high, spanning the whole frequency range up to 25 kHz, and the duration ranged from 9 to 29 ms (12 measurements). The maximal velocity during the irregular phase ranged between 76.1 and 137.1 mm/s (Table 1) and was found to be independent of the impact point. The irregular phase merged into regular oscillations with a basic frequency between 5.7 and 10.5 Hz (12 measurements). The frequency of the regular phase was dependent on the impact point: in all leaves it was lower when the impact was applied apically (Table 1). The amplitude of the basic oscillation decreased exponentially with a half life of 163 ± 37 ms (mean ± SD; $n = 12$). The half-life was independent of the impact point, but appeared to differ slightly between individual leaves, although no obvious correlation with single leaf characteristics (weight, dimensions) was found.

Air movements induced irregular vibration amplitudes in apple leaves, producing broad frequency bands up to the limit of measurement at 25 kHz (Fig. 1). However, intensities decreased with increasing frequency. The basic oscillations were independent of the wind speed and ranged between 6.8 and 9.9 Hz (two leaves, nine measurements) for a parallel flow and 6.7 and 13.6 Hz for a perpendicular flow (two leaves, seven measurements). The maximal velocity, however, was independent on flow direction but dependent on the relative wind speed. It ranged from 30–60 mm/s for low wind speed to 70–130 mm/s for high wind.

Falling Balls

Vibrations produced by impacts of falling balls were highly repeatable (three replicates on one leaf are

TABLE 1

Characteristics of Vibrations Induced by Water Drops Falling on Apple Leaves

	Position of impact point	Characteristics of irregular phase: Duration (ms)	Characteristics of irregular phase: Maximal velocity (mm/s)	Characteristics of regular phase: Basic frequency (Hz)	Characteristics of regular phase: Half-life (ms)
Leaf 1	Basal	25	93.8	10.3	173
	Basal	29	127.1	10.4	190
	Apical	21	137.1	7.1	175
	Apical	26	113.9	5.7	263
Leaf 2	Basal	27	119.9	10.0	147
	Basal	25	76.1	10.5	88
	Apical	23	132.6	9.3	110
	Apical	22	136.6	9.1	112
Leaf 3	Basal	12	134.8	9.8	152
	Basal	9	80.1	9.4	185
	Apical	17	136.9	6.6	218
	Apical	16	137.1	6.5	157

shown in Fig. 2). A time–frequency analysis using spectrograms (not shown) showed that this is also true for high frequencies (up to 25 kHz). The same repeatability was found in all 42 impacts we measured, over a whole range of conditions of impact and measurement locations, weight and geometry of the leaf, and height of the falling ball. The vibrations consisted of an irregular phase, followed by a regular phase, as above. The pattern of vibrations changed markedly from the middle of the leaf to the basis of the leaf (Fig. 3). At the basis of the leaf, velocity dropped abruptly at the onset of the regular phase and was at least an order of magnitude lower than that seen in the middle of the leaf. In the middle of the leaf, frequencies higher than 5 kHz were still measured 600 ms after the impact (time–frequency analyses using spectrograms). At the basis of the leaf, on the other hand, they were restricted to the first half of the irregular phase and lasted less than 50 ms. The attenuation in intensity of vibrations from the tip to the basis of the leaf led to a reduction of approximately 50% in the time required for the basic oscillations to be lost in the background noise. These general patterns were observed in all measurements.

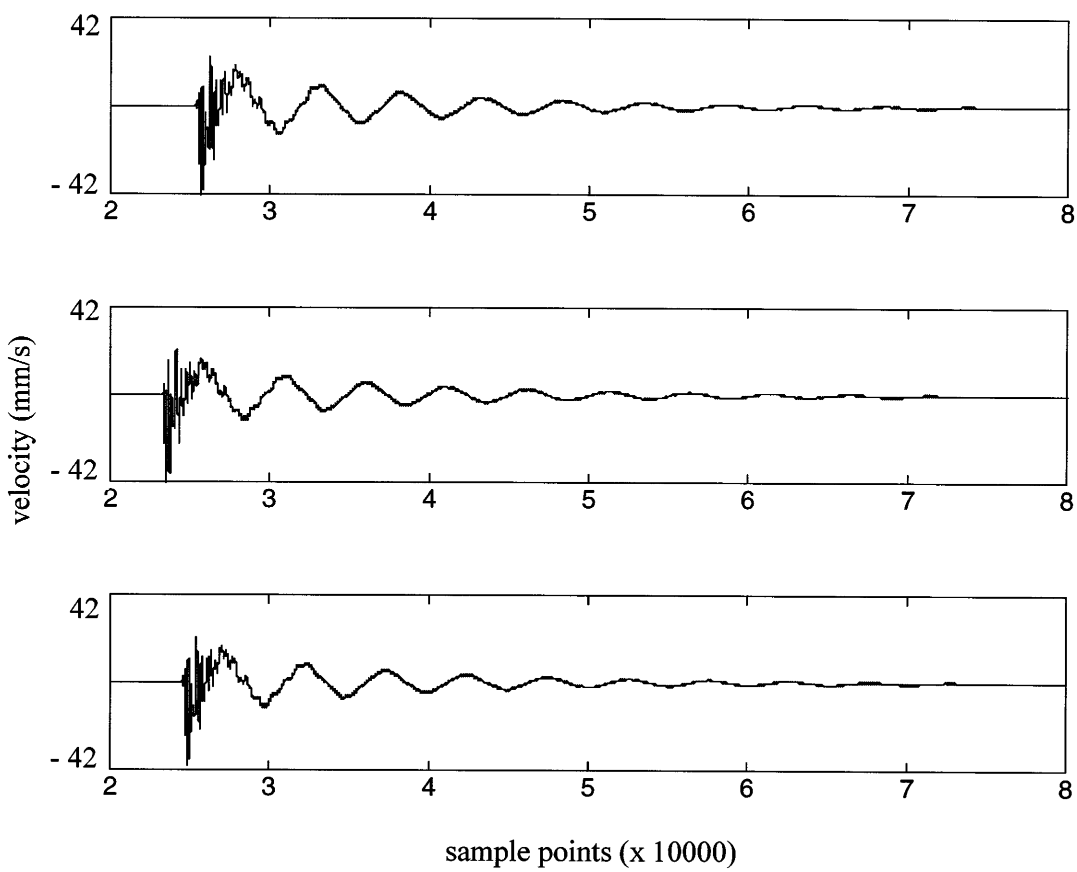

FIG. 2. Three replicates of vibrations produced by a metal ball falling on the tip of a leaf. The time origin varies from one replicate to the next.

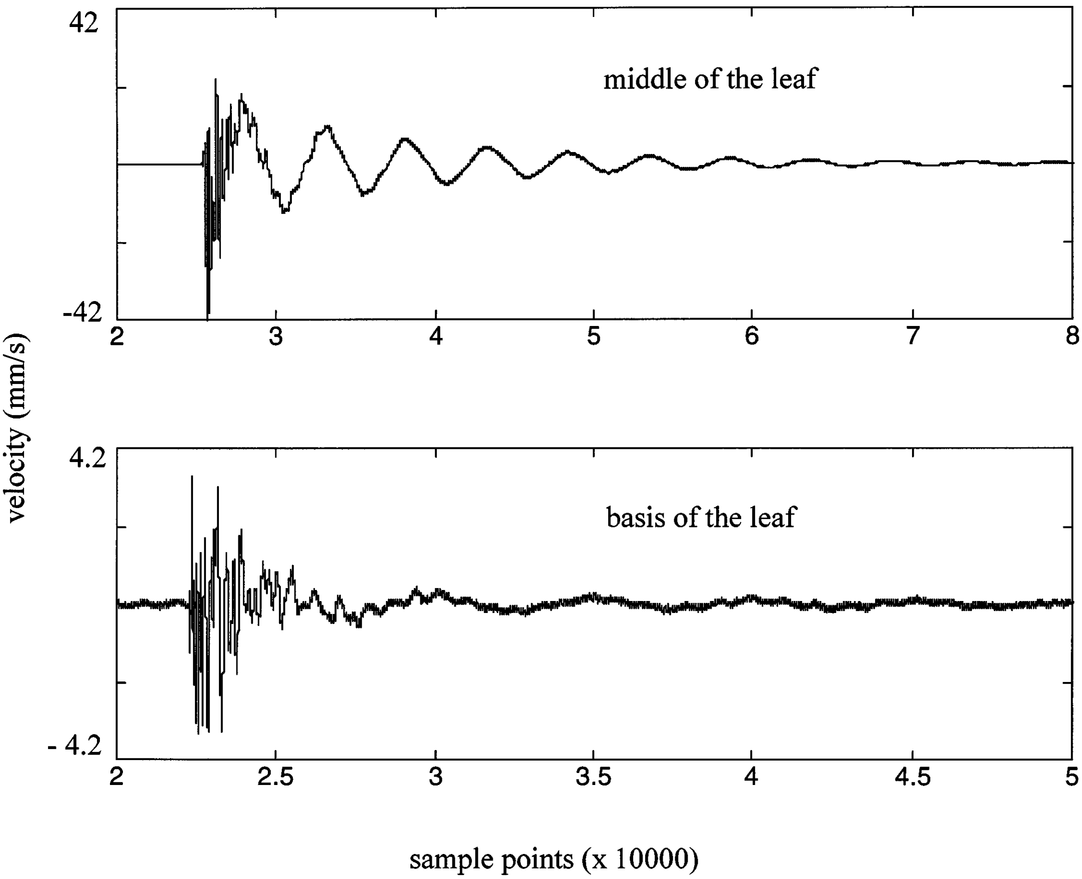

FIG. 3. Vibrations of the middle and the basis of the same leaf induced by a metal ball falling on its tip. Note the 10-fold difference in amplitude and the different time scales. The time origin differs between the two graphs.

Air Particle Displacement

Leaf vibrations produced by falling balls were well transmitted through the air. Visual comparison of LDV and LDA time series reveals the same broad temporal structure of an irregular phase, followed by a regular phase (Fig. 4). The basic oscillations of the leaf vibrations were clearly distinguishable in the air. The intensity of all frequencies was strongly attenuated in the air, compared to their equivalent in the leaves, particularly at high frequencies. This was observed in the lack of very high velocity peaks during the irregular phase and was confirmed by a time–frequency analysis (not shown).

DISCUSSION

The Abiotic Vibratory Environment

The level of background noise has a strong influence on the detectability of vibratory signals in general (Ehret *et al.*, 1982; Römer *et al.*, 1989). Rain and wind are the major abiotic sources of vibrations increasing the background noise level in plants in the field. Although they both raise the noise level over the whole frequency scale, their effect is specially marked at low frequencies. This is particularly obvious for the vibrations induced by the wind and is in accordance with the only other study of this kind of which we are aware (Barth *et al.*, 1988).

The results are significant for understanding the use of vibrations by the host, as *S. sericeicornis* and other leafminer parasitoids do not forage under rainy or windy (>0.6 m/s) conditions (Casas, 1989, and pers. observation). We sometimes observed a wriggling behavior characteristic of escape movements (Meyhöfer *et al.*, 1994; Bacher *et al.*, in press) when leaves were manually tapped or when flies landed on their surface (personal observations). These disturbances are similar to those produced by water drops, falling balls, or landing parasitoids. Insertions of the ovipositor in a mine also trigger vibrations which have two characteristics in common with vibrations pro-

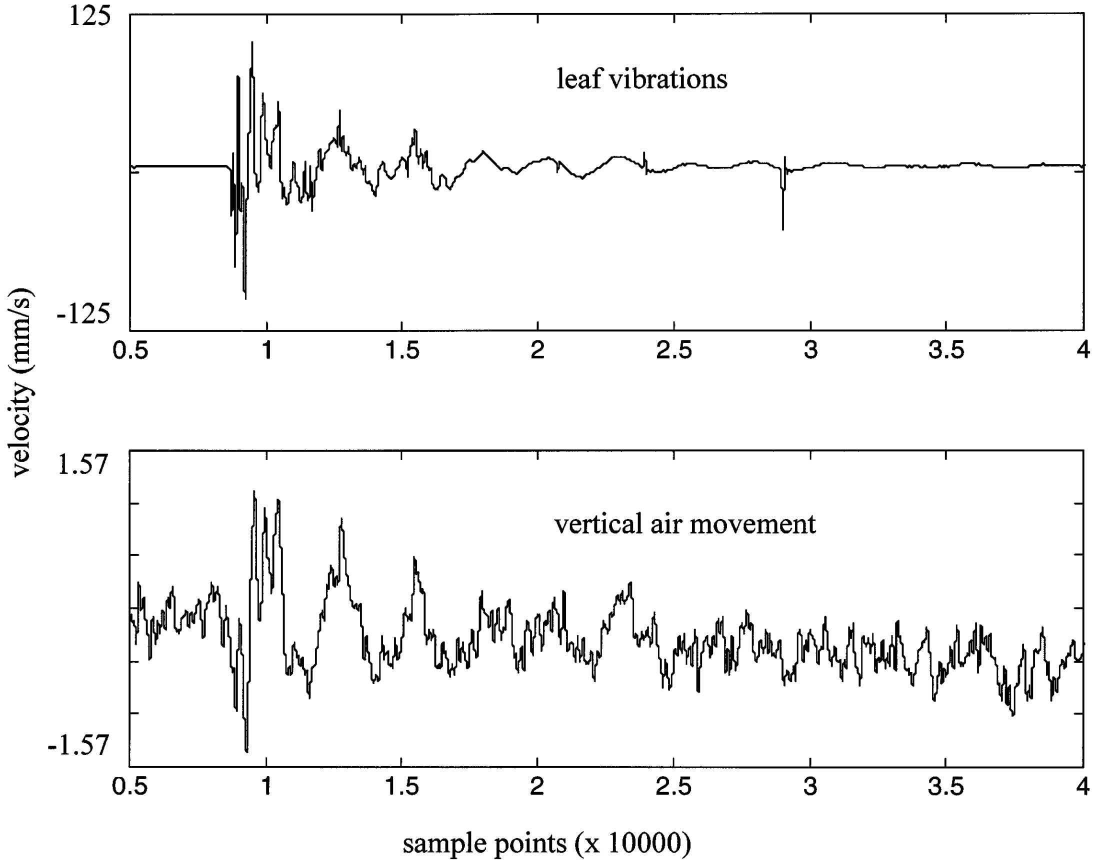

FIG. 4. Vibrations of the leaf lamina and simultaneous vertical air displacement 1 cm above the leaf surface. Note the two order of magnitude difference in amplitude. For the purpose of illustration, drop-outs were filtered out using a Butterworth lowpass filter of order 9 with a cutoff frequency of 100 Hz.

duced by impact-like events as the ones just described: high frequencies of high intensity over a short period of time. It is possible that these characteristics alone are sufficient for the leafminer to react defensively. Why does a leafminer sometimes react so indiscriminately? Ideally, it should react defensively only to those vibrations produced by insects representing a threat. The advantages of such a behavior can be understood if the costs incurred by reacting to a false alarm are much lower than those incurred by not responding to a foraging parasitoid. In the field, a leafminer could potentially use the repetitive nature of vibrations produced by rain drops and the high level of background noise due to wind as signals that it is safe from parasitoid attacks. An interesting experiment to carry out along these lines would be to test the behavioral reaction to impacts under high background noise.

As for the parasitoid, rain and wind do set the microclimatic conditions under which they can forage. What remains to be assessed now is whether the window of foraging is limited by rain and wind per se or if the background noise level gets high enough to mask vibratory signals triggered by the host.

Spatially Varying Information on a Leaf

The excellent replicability of the falling ball method enabled us to establish beyond doubt the existence of a large decrease in intensity of vibrations from the tip to the basis of the leaf, as first suggested in Meyhöfer *et al.* (1994). This spatial pattern is characteristic for a fixed cantilever, where, by definition, there is no movement at the fixed end. A leaf attached to a stem is also a cantilever, but the attachment point is not fixed. This causes slightly different patterns of vibrations. Indeed, an analysis of vibrations of leaves of small seedlings showed that vibration patterns were different in the regular phase, with a larger decrease of intensity, for the high frequencies in particular (R.M. pers. observation). Regardless of the flexibility at the attachment point, the basis of a leaf has less freedom of movement than the tip, leading to a spatially varying pattern of vibrations. Spatially varying information is a necessary condition for taxis, and *S. sericeicornis* may use it for between-mines foraging, as field observations suggest. After landing on a leaf harboring several mines of varying quality (empty, dead hosts an healthy hosts), *S. sericeicornis* goes straight to those mines inhabited by suitable hosts, the only ones producing vibrations (Casas, 1989). Unsuitable hosts would be encountered more frequently if the information was restricted to the presence or absence of vibrations on the leaf.

Air Particle Displacement Contains Information about Leaf Vibrations

A systematic survey of sensilliae on the body of *S. sericeicornis* did not reveal the existence of any hairs which could be used for receiving air particle displacement (Meyhöfer *et al.*, 1997a). However, the Johnston's organ, which is known to measure air movements (Kirchner, 1994), could function as such a receptor, or the leg itself may react to air vibrations directly, not via substrate vibrations (Shaw, 1994). Leaf vibrations are well transmitted through the air. Air particle displacement triggered by leaf vibrations has a temporal pattern following closely the substrate vibrations and extends well beyond the boundary layer of the leaf. Hence, air movements represent an additional channel of information for *S. sericeicornis* and maybe for a whole range of parasitoids foraging on endophytic hosts. Furthermore, air movement contains both chemical and physical information, and their relative importance is still to be determined. These exciting prospects will hopefully foster interest in the physical ecology of host location in parasitic wasps.

ACKNOWLEDGMENTS

We gratefully acknowledge the helpful comments provided by Ana Rivero. We also thank the reviewers who made several suggestions for further experiments.

REFERENCES

Bacher, S., Casas, J., and Dorn, S. 1996. Parasitoid vibrations as potential releasing stimulus of evasive behaviour in a leafminer. *Physiol. Entom.* **21,** 33–43.

Bacher, S., Casas, J., and Dorn, S. Substrate vibrations elicit defensive behaviour in a leafminer pupae. *J. Insect Physiol.*, in press.

Bailey, W. J. 1991. "Acoustic Behaviour of Insects: An Evolutionary Perspective." Chapman and Hall, New York.

Barth, F. G., Bleckmann, H., Bohnenberger, J., and Seyfarth, E. A. 1988. Spiders of the genus *Cupiennius* Simon 1891 (Araneae, Ctenidae) II. On the vibratory environment of a wandering spider. *Oecologia* **77,** 194–201.

Casas, J. 1989. Foraging behaviour of a leafminer parasitoid in the field. *Ecol. Entom.* **14,** 257–265.

Connor, E. F., and Cargain, M. J. 1994. Density-related foraging behaviour in *Clostocerus tricinctus*, a parasitoid of the leaf-mining moth, *Cameraria hamadryadella*. *Ecol. Entom.* **19,** 327–334.

Dantec 1991. User's manual—41X62 Compact Laser Vibrometer. Dantec Custom Designed Systems Department, Measurement Technology Division, Odense, Denmark.

Ehret, G., Moffat, A. J. M., and Tautz, J. 1982. Behavioural determination of frequency resolution in the ear of the cricket, *Teleogryllus oceanicus*. *J. Comp. Physiol. A* **148,** 237–244.

Ewing, A. W. 1989. "Arthropod Bioacoustics: Neuroethology and Behaviour." Edinburgh University Press.

Kirchner, W. H. 1994. Hearing in honeybees: The mechanical response of the bee's antenna to near field sound. *J. Comp. Physiol. A* **175,** 261–265.

Meyhöfer, R., Casas, J., and Dorn, S. 1994. Host location by a parasitoid using leafminer vibrations: Characterizing the vibrational signals produced by the leafmining host. *Physiol. Entom.* **19,** 349–359.

Meyhöfer, R., Casas, J., and Dorn, S. 1997a. Mechano- and chemoreceptors and their possible role in the host location behaviour of *Sympiesis sericeicornis* (Hymenoptera: Eulophidae). *Ann. Entomol. Soc. Am.* **90,** 208–219.

Meyhöfer, R., Casas, J., and Dorn, S. 1997b. Vibration mediated interactions in a host-parasitoid system. *Proc. R. Soc. London B* **264,** 261–266.

Römer, H., Bailey, W., and Dadour, I. 1989. Insect hearing in the field. III. Masking by noise. *J. Comp. Physiol. A* **164,** 609–620.

Shaw, S. R. 1994. Detection of airborne sound by a cockroach "vibration detector": A possible missing link in insect auditory evolution. *J. Exp. Biol.* **193,** 13–47.