Vibrational signals in a leafminer parasitoid system: 
the sensory ecology of a leafminer in a tritrophic context

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1 Summary

Late instar larvae and pupae of the spotted tentiform leafminer, *Phyllonorycter malella* (Ger.) (Lepidoptera: Gracillariidae), show characteristic avoidance reactions when attacked by one of their enemies, the polyphagous ectoparasitoid *Symphiesis sericeicornis* Nees (Hymenoptera: Eulophidae). Vibrations elicited by the parasitoid when searching for hosts on apple leaves are suspected to be the primary cues by which leafminers detect the presence of their opponents. This study focuses on the sensory ecology of the leafminer in a tritrophic context: the parasitoid as emitter of vibratory signals, the leafminer as receiver and the plant as transmission channel.

In the first part, parasitoid vibrations were characterised as a potential releasing stimulus of evasive behaviour in the leafminer. Vibrations produced by a foraging parasitoid were measured on a mine with a laser Doppler vibrometer. By recording concurrently the behaviour of the parasitoid on video, vibrations could be assigned to particular behaviours. Subsequently, vibrations were characterised by their dominant frequencies and intensities.

The behaviours *Landing* and *Take-off* both produced strong impact-like vibrations characterised by an initial irregular phase during which frequencies up to 25 kHz occurred followed by a slow decaying regular phase. Vibrations elicited by *Moving*, *Standing* and *Probing* showed no clear temporal pattern. During *Probing*, dominant frequencies of up to 5.6 kHz were observed frequently at intensities well above the background noise (>10 dB). During *Moving* and *Standing*, vibrations were more scarce and of lower frequencies and intensities. Due to their impact-like nature, vibrations produced by *Landing* and *Take-off* are probably not specific to the parasitoid. Vibrations produced by *Moving* and *Standing* are difficult to
detect and not reliable because of their non-specificity. Therefore, only Probing provides a reliable and detectable source of information for the host. The vibrations elicited during Probing could account for the evasive behaviour that is observed in this and other leafminers.

In the second part, the vibratory environment of the leafminer was investigated. Other potential sources of vibrations induced in apple leaves such as falling waterdrops, air movement and moving insects were studied. Waterdrops produced impact-like vibrational signals in which a short initial irregular phase is followed by an exponentially decaying regular phase. The vibration pattern is similar to that of a landing parasitoid. The irregular phase contains information on the vibration source, while the regular phase is determined mainly by leaf mechanics and the experimental set-up. Wind induced constant leaf movements which raised the background noise level up to at least 25 kHz, hence probably interfering with the detection of vibrational signals by the leafminer. Moving insects produced vibrations with a similar temporal pattern and frequency composition as those triggered by a moving parasitoid. However, intensity and probability of occurrence were higher for the ant and the beetle, which was probably due to their greater weight. It is argued that interference by rain and wind may play only a minor role in the field situation, as parasitoids forage for hosts only under dry conditions and low wind speeds.

In the third part, different vibration parameters were investigated for their capacity to elicit defence reactions in leafminer pupae. In addition, mines were studied regarding their vibration transmission properties. Defence reactions can be elicited over a broad range of frequencies and pupae are differentially sensitive to different frequencies. The question to which frequency range pupae are tuned cannot be answered from threshold curves, because different ways of displaying thresholds (as displacement, velocity or acceleration) yield different sensitivity minima. Thresholds of concealed pupae were about twice as high as thresholds of free pupae.
Mine tissue strongly attenuates vibrations. Damping is a non-linear function of frequency and thus highly dependent on the exact position of sender and receiver on the mine. Interferences of vibrations travelling several times across the leaf and the heterogeneity of mine tissue may account for this non-linearity. Mechanoreceptors of pupae, e.g. the prominent body hairs, receive vibratory stimuli via the silk threads spun all over the inner mine surface and not directly from the plant tissue. Therefore it is hypothesised that leafminers make use of vibration components in the direction of silk threads (longitudinal vibrations), which are less subject to attenuation, rather than of components perpendicular to the silk web (transverse vibrations). This would be in agreement with results from other arthropods using silk in perception of vibratory signals.

The relatively high reaction thresholds of leafminer pupae compared to other arthropods may be explained by the defence strategy of the immobile pupae.
2 Zusammenfassung

Altlarven und Puppen der Apfelblattminiermotte *Phyllonorycter malella* (Ger.) (Lepidoptera: Gracillariidae) zeigen charakteristische Abwehrreaktionen, wenn sie vom polyphagen Ectoparasitoiden *Sympiesis sericeicornis* Nees (Hymenoptera: Eulophidae) attackiert werden. Eine Möglichkeit der Erkennung stellen Vibrationen dar, die vom Parasitoiden während seiner Suche auf dem Blatt erzeugt werden und die vom Wirt benutzt werden, um die Anwesenheit eines Feindes zu erkennen. In dieser Arbeit wird die sensorische Ökologie eines Blattminierers im tritrophischen Kontext untersucht: der Parasitoid als Sender von Vibrationssignalen, der Minierer als Empfänger und die Pflanze als Signalübermittler.


hervorgerufene Interferenzen im Feld wahrscheinlich nur eine untergeordnete Rolle spielen, da der Parasitoid ausschließlich bei trockenem Wetter und geringen Windgeschwindigkeiten auf Nahrungssuche geht.

Erklärung stimmt mit Ergebnissen aus Untersuchungen an anderen Arthropoden, die ebenfalls Seidenfäden bei der Übertragung von Vibrationssignalen benutzen, überein. 
Die relativ hohen Reaktionsschwellen von Puppen könnten durch deren stationäre Situation innerhalb der Mine und deren damit verbundene Abwehrstrategie erklärt werden.
3 Introduction

Pest management with insects as natural enemies has been practised intensively in agriculture for about 100 years (Simmonds et al., 1976), but now receives increased attention. It is generally accepted as an environmentally sound alternative to chemical control (DeBach and Rosen, 1991), and is a well-established measure of pest control in organic farming and integrated pest management (IPM, Dorn, 1993a; DeBach and Rosen, 1991). Although there is a great demand for effective biocontrol agents, much of the selection of agent is arbitrary due to practical constraints (Waage, 1990). This has been considered one of the main reasons for the low success rates (Waage, 1990; DeBach and Rosen, 1991). In general, it is unknown why some agents have been successful and others not. Moreover, an agent that was effective in one situation may fail under different conditions. For example, the parasitic wasp Eretmocerus serius Sylvestri (Hymenoptera: Aphelinidae) provided complete control of the citrus blackfly in Cuba and in the Caribbean, but was found to be ineffective in Mexico (DeBach and Rosen, 1991). Hence, biocontrol has remained a largely unpredictable discipline (van Lenteren, 1980; Tauber et al., 1985). To gain accuracy in the prediction of agent effectiveness it was suggested that selection criteria should be evaluated and natural enemies scored according to the characteristics that are important for success (van Lenteren et al., 1982; van Lenteren, 1986; Waage, 1990). However, the fact that the pest insect itself may greatly influence the success of a biocontrol agent has remained largely uninvestigated (Gross, 1993). The success of a hunting parasitoid, for example, depends to a great extent on the potential of its host to escape. This also applies to the polyphagous ectoparasitoid Sympiesis sericeicornis Nees (Hymenoptera: Eulophidae) and one of its hosts, the spotted tentiform leafminer Phyllonorycter malella (Ger.) (Lepidoptera:
Leafminers of the genus *Phyllonorycter* are serious pests on apple in Italy and Japan (Dorn, 1993b) as well as in North America (Pottinger and LeRoux, 1971; Reissig *et al.*, 1982; Vincent and Mailloux, 1992; Blommers, 1994): among others, severe infestations can cause premature fruit drop, and reduced fruit size and fruit set in the following season. Previous work of our group investigated the combined influence of parasitism and a juvenile hormone analogue on apple leafminer populations (Dorn, 1993b). *Sympiesis* spp. were found among the most frequent parasitoid species of *Phyllonorycter* leafminers (Balázs, 1989; Casas and Baumgärtner, 1990; Maier, 1994; Maier and Weseloh, 1995). However, despite the economic importance of *Phyllonorycter* leafminers, studies on the mechanisms responsible for the control of leafminers by their natural enemies are almost non-existent (but see the approaches by Casas, 1988, 1989, 1990; Casas *et al.*, 1993).

The first three instars of *P. malella* are sap-feeders and live in flat surface mines on the underside of apple leaves. Later instars (L4 and L5) are tissue feeders and produce voluminous tentiform mines by spinning silk threads on the inner mine surface. *P. malella* pupates inside the mine in a cocoon spun by the last larval instar (L5). The conspicuous tentiform mines, which harbour the last two larval instars or the pupae, are visited by the ectoparasitoid *S. sericeicornis* during host searching.

In a field study, Casas (1989) reported that in 10% of the cases, *S. sericeicornis* abandons larvae of *P. malella* without parasitising them, sometimes after a long game of hide and seek during which the leafminer tries to avoid the parasitoid's ovipositor. He hypothesised that both parasitoid and leafminer detect and identify each other by vibrations triggered by their opponent. The question arose, why *S. sericeicornis* was such a successful parasitoid species despite the potential of its host to escape. To understand the success at the population level, a deeper knowledge of the mechanisms involved at the behavioural and sensory
levels is necessary. Such studies are situated within the conceptual framework of physiological structured population modelling (Metz and Dieckmann, 1986).

Vibratory communication in this leafminer-parasitoid system from the perspective of the parasitoid was studied by Meyhöfer (1996). The present thesis focuses on the sensory ecology of the leafminer in a tritrophic context: the parasitoid as emitter of vibratory signals, the plant as transmission channel and the leafminer as receiver. The research questions addressed in the following chapters are:

Does the parasitoid produce detectable vibrations during host searching on apple leaves and do these vibrations constitute suitable cues for the leafminer to detect parasitoid presence?

Are different vibrations to which the leafminer is exposed in the field distinguishable or do they otherwise influence the detectability of vibrations?

Does the leafminer react to vibrations and which vibration parameters elicit a response?

Does the plant as the transmission channel of vibrations modify the signals?
4 The technical equipment

4.1 Vibration measurements

4.1.1 General set-up

Vibrations were measured with a laser Doppler vibrometer (Dantec 41X62 Compact Laser Vibrometer, 2mW He-Ne-Laser). The output of the vibrometer is a voltage directly proportional to the instantaneous velocity (i.e. speed in the direction of the laser beam) of the surface of an object at the measurement point (Dantec, 1991).

To reduce background vibrations, the laser unit of the vibrometer, as well as the object to be measured, were mounted on an air-buffered vibration-damped table (Photon Control, Cambridge). The analog output of the vibrometer was passed through a programmable low-pass filter (Stanford Research SR650, attenuation 105 dB per octave; Figure 1) to avoid aliasing.

![Figure 1: Set-up of equipment used for vibration measurements.](image-url)
effects during digitising. The anti-aliasing low-pass filter was set to 25 kHz to attenuate higher frequencies which occurred when measuring without the built-in filters of the frequency tracker. The filtered vibration signals were sampled with a Mac Adios II/16 A/D converter and stored on a Macintosh Quadra 800 computer using the software SoundScope (GW Instruments, 1993). The monitor output of the vibrometer was followed on an oscilloscope (Hameg HM 205-3) to enable an optimal adjustment of the vibrometer during the experiments.

### 4.1.2 Measurements outside the calibrated range

The vibrations to be measured were expected to be of extremely low amplitude. The Laser Doppler vibrometer (LDV) is suitable for such measurements, since it enables contactless recording of instantaneous vibrations by means of the Doppler effect without loading the object to be measured (Dantec, 1991), and thus without changing its mechanical properties (in contrast to accelerometers and displacement receivers).

The Dantec LDV chosen for conducting the experiments detects frequencies over a wide range from DC to 26 kHz (analog output). However, there is a trade-off between highest detectable frequency and

### Table 1: Sensitivity as function of tracker range (after Dantec, 1991).

<table>
<thead>
<tr>
<th>Tracker input range (Doppler frequency)</th>
<th>Max. vibration frequency (kHz; analog out)</th>
<th>Velocity range (m/s)</th>
<th>Max. acceleration (mm/s²)</th>
</tr>
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<tr>
<td>3-33 kHz</td>
<td>2.4</td>
<td>2.1<em>10⁻⁶ - 4.2</em>10⁻³</td>
<td>0.2</td>
</tr>
<tr>
<td>10-100 kHz</td>
<td>7.4</td>
<td>6.3<em>10⁻⁶ - 13</em>10⁻³</td>
<td>1.8</td>
</tr>
<tr>
<td>33-333 kHz</td>
<td>24</td>
<td>21<em>10⁻⁶ - 42</em>10⁻³</td>
<td>20</td>
</tr>
<tr>
<td>0.1-1.0 MHz</td>
<td>26</td>
<td>63*10⁻⁶ - 0.13</td>
<td>180</td>
</tr>
</tbody>
</table>
sensitivity to vibration amplitude (Table 1). The highest detectable frequency further depends on the sampling rate during digitizing. The sampling frequency of the A/D converter on the MacAdios board together with the digitizer chosen in SoundScope is limited to an upper range of 50 kHz (GW Instruments, 1993). Hence, the highest detectable frequency had a theoretical upper limit of 25 kHz (half the sampling rate; Schrüfer, 1992). The detectable frequency range of the most sensitive tracker setting of the LDV (3-33 kHz; Table 1) was considered insufficient for my purposes. For the second most sensitive setting (10-100 kHz) a linear frequency response up to 7.4 kHz is guaranteed by the manufacturer (Dantec, 1991). This is still considerably below the theoretical limit of the equipment. The tracker range 33-333 kHz covers this frequency range, but has a three times lower amplitude sensitivity as the former range. Therefore, I carried out an experiment to determine the actual accuracy of the tracker range of 10-100 kHz outside the calibrated range in order to evaluate its suitability. Vibration amplitudes of standardised frequencies higher than the maximal vibration frequency stated by the manufacturer were measured in the tracker range of 10-100 kHz and compared to results obtained in a range suitable to accurately detect such high frequencies (33-333 kHz).

4.1.2.1 Material and methods

Set-up
Sine wave stimuli synthesised by a function generator (Stanford Research DS345) were fed into an electromagnetic vibration exciter (B&K 4810) via a power amplifier (B&K 2706). For better reflection of the laser beam and a better signal-to-noise ratio, a small piece of retroreflective tape (Scotchlite, 3M; ~ 1 mm²) was glued on the moving platform of the vibration exciter. Vibrations of the shaker were measured with the laser
Doppler vibrometer. The set-up of the vibrometer and accompanying equipment was the same as described in the previous section.

Experimental procedure
Frequencies of 1, 2, 3, 4, 6, 8, 10, 12, 15, 17 and 20 kHz were used in the experiment. Vibrations of each frequency were recorded in both tracker ranges, 10-100 kHz and 33-333 kHz. Vibration data acquisition had a sampling frequency of 50 kHz. On the monitor of the oscilloscope, the peak-to-peak velocity amplitude of vibrational signals was adjusted between 40*10^{-6} m/s and 200*10^{-6} m/s, i.e. within the normal working range of both tracker ranges, using the settings of the power amplifier.

Data analysis
Voltage was converted to velocity by multiplication with respective calibration factors (Dantec, 1991). The mean peak-to-peak velocity amplitude (± SD) was calculated from a series of consecutive sine vibration periods measured (n=14-29 for different frequencies). For a comparison of both tracker ranges, it was assumed that the tracker range of 33-333 kHz detects all frequencies correctly. Therefore the mean peak-to-peak velocity amplitude of the tracker range of 33-333 kHz was set as 100%. The mean peak-to-peak velocity amplitude of the tracker range of 10-100 kHz was expressed as percentage of the former.
Figure 2: Accuracy of detection of the velocity amplitude in the tracker range 10-100 kHz expressed relative to tracker range of 33-333 kHz as function of frequency. The thick dashed line plots the linear decrease of accuracy at frequencies higher than the upper frequency limit of the tracker range of 10-100 kHz.

4.1.2.2 Results

The frequency of sine vibrations was detected correctly at all frequencies tested. With the exception of 8 kHz, the tracker range of 10-100 kHz
always detected velocity amplitudes lower than those found in the range of 33-333 kHz (Figure 2). The variability of amplitude detection was high (8%-28% for 10-100 kHz; 9%-33% for 33-333 kHz). The standard deviations of the measured amplitude ranges were separated only at the highest frequency tested (20 kHz). Assuming that 7.4 kHz is the limit up to which vibration amplitudes were correctly detected, as stated by the manufacturer, the accuracy of amplitude detection of higher frequencies decreased approximately linearly with increasing frequency (\( y = 126.15 - 3.08 * x, r^2 = 0.81; y: \text{relative vibration amplitude (in %); } x: \text{frequency (between 8 kHz and 20 kHz)} \)). At 20 kHz, the vibration amplitude was underestimated by about 35%.

4.1.2.3 Discussion

There is a decrease in accuracy of vibration amplitude detection at frequencies higher than the upper limit guaranteed by the manufacturer. However, the great variability of vibration amplitude detection, which is found already at low frequencies, leads to an overlap of amplitude ranges up to 15 kHz. Given that the high variability of both ranges is considered acceptable for accurate measurements by the manufacturer and further that the frequency of all sine vibrations tested is detected correctly, I consider the tracker range of 10-100 kHz suitable with respect to sensitivity and frequency range for measurements of vibrations up to 10 kHz, but vibration amplitudes of higher frequencies may be underestimated, depending on their frequency. Therefore, the tracker range of 10-100 kHz can be considered appropriate for measurements of vibrations induced in apple leaves by small insects, such as foraging parasitoids.

The high variability of vibration amplitude may be due not only to the accuracy of the vibrometer, but to variability of the vibration exciter in
producing vibrations. The sampling frequency also contributes to the variation. Vibration extremes may not always be detected if sampling events and maximum/minimum amplitude do not coincide. This occurs frequently at a low sampling frequency relative to the measured vibration frequencies.

4.2 Calibration of the vibration exciter

4.2.1 Material and methods

Set-up
To study the accuracy of the vibration exciter, sine wave stimuli synthesised by a function generator (Stanford Research DS345) were fed into a electromagnetic vibration exciter (B&K 4810) via a power amplifier (B&K 2706). The settings of the power amplifier were as follows: gain control in centre position, attenuation switch at 0 dB, and power source at 1.8 A. Vibrations produced by the vibration exciter were measured with a laser Doppler vibrometer (Dantec 41X62 Compact Laser Vibrometer, 2mW He-Ne-Laser). The output of the vibrometer is a voltage directly proportional to the instantaneous velocity (i.e. speed in the direction of the laser beam) of the surface of an object at the measurement point (Dantec, 1991).

The analog output of the vibrometer was passed through a programmable low-pass filter (Stanford Research SR650, attenuation 105 dB per octave) to avoid aliasing effects during digitising. The filtered vibration signals were sampled with a Mac Adios II/16 A/D converter and stored on a Macintosh Quadra 800 computer using the software SoundScope (GW Instruments, 1993). The monitor output of the vibrometer was followed on
an oscilloscope (Hameg HM 205-3) to enable an optimal adjustment of the vibrometer during the experiments. Vibration data acquisition had a sampling frequency of 50 kHz. This allowed detection of frequencies up to 25 kHz. The anti-aliasing low-pass filter was set to 25 kHz to attenuate higher frequencies. The frequency tracker of the vibrometer was set in the range 0.1-1.0 MHz so velocities in the range $63 \times 10^{-6} - 0.13$ m/s and a maximal acceleration of 180 m/s$^2$ could be detected (Dantec, 1991). Internal filters were not installed.

For better reflection of the laser beam and a better signal-to-noise ratio, a small piece of retroreflective tape was glued on the platform of the vibration exciter (Scotchlite, 3M; $\sim 1$ mm$^2$). To reduce background vibrations, the laser unit of the vibrometer as well as the vibration exciter were mounted on an air-buffered vibration-damped table (Photon Control, Cambridge).

**Data analysis**

The average peak-to-peak velocity of recorded sine waves was calculated from ten successive periods for different voltages at each frequency. Acceleration ($Acc$) and displacement ($Dis$) were obtained from velocity ($Vel$) by differentiation and integration, respectively. Provided that the velocity is a sine wave of a particular frequency $f$ and maximal amplitude $a_{vel}$, the mathematical function of velocity amplitude over time $t$ would be:

$$Vel = f(t) = a_{vel} \cdot \sin(2\pi f \cdot t).$$

Differentiation yields the acceleration ($Acc$):

$$Acc = f'(t) = a_{vel} \cdot 2\pi f \cdot \cos(2\pi f \cdot t),$$

with the maximal acceleration amplitude ($a_{acc}$): $a_{acc} = a_{vel} \cdot 2\pi f$. 
Figure 3: Vibrometer measurements of peak-to-peak velocity of the vibration exciter at different frequencies and input voltages. Frequencies refer to pure sine stimuli. Input voltage refers to peak-to-peak voltage of pure sine waves generated by the function generator and amplified by the power amplifier. The upper graph shows the frequencies of 100, 200, 500, 800 and 1000 Hz, the lower graph the frequencies of 1000, 2000, 3000 and 4000 Hz.
Integration of the velocity yields the displacement (Dis):

\[ \text{Dis} = \int f(t) \, dt = \frac{a_{\text{vel}}}{2\pi f} (1 - \cos(2\pi f \cdot t)) \]

with the maximal displacement amplitude \( a_{\text{dis}} = \frac{a_{\text{vel}}}{2\pi f} \).

The peak-to-peak velocity, acceleration and displacement were obtained by multiplying the maximal amplitudes with a factor of 2. The peak-to-peak amplitudes were calculated for each frequency and voltage tested.

4.2.2 Results

The peak-to-peak velocity output of the shaker is related linearly to the peak-to-peak output voltage of the function generator (Figure 3):

\[ \text{velocity}_{pp} = a + b \times \text{voltage}_{pp} \]

The increment \( b \) is frequency dependent. Because at 0 V input the shaker produces no vibrations, the functions were fitted to pass through the origin (i.e. intercept \( a = 0 \)). Increments were obtained for each frequency by dividing the velocity at the highest tested voltage by the corresponding voltage. Increments were used as calibration factors for transformation of velocity into voltage for each frequency (Table 2). In all experiments, peak-to-peak velocity values of sine stimuli were obtained by multiplication of peak-to-peak voltage with the calibration factor of the particular frequency. The peak-to-peak acceleration was obtained from the peak-to-peak velocity by multiplication with \( 2\pi f \), the peak-to-peak displacement was obtained from the peak-to-peak velocity by multiplication with \( \frac{1}{2\pi f} \) (calibration factors for each frequency \( f \) are given in Table 2).
Table 2: Calibration factors for transformation of peak-to-peak voltage output of the function generator (in V) into peak-to-peak velocity output of the vibration exciter (in mm/s), peak-to-peak velocity into peak-to-peak displacement (in mm), and peak-to-peak velocity output (in mm/s) into peak-to-peak acceleration (in mm/s²) for all frequencies used in experiments.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Vpp-&gt;Vel</th>
<th>Vel-&gt;Dis</th>
<th>Vel-&gt;Acc</th>
</tr>
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<tr>
<td>100</td>
<td>2150.0</td>
<td>1.592E-03</td>
<td>628</td>
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<tr>
<td>200</td>
<td>744.0</td>
<td>7.958E-04</td>
<td>1257</td>
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<td>500</td>
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<td>800</td>
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<tr>
<td>1000</td>
<td>95.0</td>
<td>1.592E-04</td>
<td>6283</td>
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<tr>
<td>2000</td>
<td>36.0</td>
<td>7.958E-05</td>
<td>12566</td>
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<tr>
<td>3000</td>
<td>18.6</td>
<td>5.305E-05</td>
<td>18849</td>
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<tr>
<td>4000</td>
<td>12.9</td>
<td>3.979E-05</td>
<td>25132</td>
</tr>
</tbody>
</table>

4.2.3 Discussion

The experiments showed that the amplitude of the vibration exciter could not be directly inferred from the input voltage of the exciter because it is frequency dependent. Moreover, different vibration parameters (displacement, velocity, acceleration) need separate transformations all of which are also frequency dependent. Given e.g. a sine wave of 100 Hz and one of 1000 Hz, each with the same peak-to-peak velocity amplitude of 1 mm/s, the displacement of the 100 Hz wave will be 10 times higher than that of the 1000 Hz wave (100 Hz: 1.6 \cdot 10^{-3} \text{ mm}; 1000 Hz: 1.6 \cdot 10^{-4} \text{ mm}). In contrast, the acceleration of the 100 Hz sine wave will be 10 times lower than that of the 1000 Hz wave (100 Hz: 628.3 mm/s²; 1000 Hz: 6823 mm/s²).
4.3 Conclusions

The tracker range of 10-100 kHz of the laser Doppler vibrometer was considered appropriate for measurements of vibrations induced in apple leaves by small insects, such as searching parasitoids. Sine vibration performance of the vibration exciter is documented in the range 100-4000 Hz. Thus, the vibration exciter is sufficiently characterised for the interpretation of biotest results using sine vibrations as stimuli.
5 Parasitoid vibrations as potential releasing stimulus of evasive behaviour in a leafminer

5.1 Introduction

Sensory stimuli which reach endophytic insects are widely different from those that free-living insects experience. Visual and olfactory signals from the environment are strongly filtered, if not completely blocked by the plant material surrounding the endophyte. Mechanostimuli (sound and vibration), on the other hand, are transmitted through plant material very well (Michelsen et al., 1982). Vibratory communication has been studied in detail in a number of free-living arthropod groups that use plants as transmission channels: e.g. in alderflies (Megaloptera) (Rupprecht, 1975); in leafhoppers, planthoppers and cydnid bugs (Homoptera) (Michelsen et al., 1982; Butlin, 1993); in grasshoppers (Orthoptera) (Kalmring, 1985); in ants (Hymenoptera) and butterflies (Lepidoptera) (DeVries, 1990); in parasitic wasps (Hymenoptera) (Field and Keller, 1993); in lacewings (Neuroptera) (Devetak and Pabst, 1994); in spiders (Araneae) (Schmitt et al., 1994:); etc. In contrast, vibratory communication involving endophytic insects has rarely been studied, probably because of the inherent difficulty of measuring these systems.

The two last larval instars (L4 and L5) and the pupae of the apple leafminer *Phyllonorycter malella* (Ger.) (Lepidoptera: Gracillariidae) live in a tentiform mine. This special type of mine allows the inhabitant considerable freedom of movement. It was occasionally observed that leafminers react with evasive behaviour when attacked by the polyphagous ectoparasitoid *Sympiesis sericeicornis* Nees (Hymenoptera: Eulophidae). Field observations in this system showed that the parasitoid abandons the
host without parasitising it in 10% of the cases, sometimes after a long game of 'hide-and-seek' (Casas, 1989). The most prominent reaction of an attacked leafminer was denoted as 'wriggling' (Meyhöfer et al., 1994): the host violently moves with its whole body in order to escape stings from the ovipositor. This evasive behaviour is also known from other leafminers (Connor and Cargain, 1994, and E. F. Connor personal communication) and from a whole range of free living lepidopterous larvae and pupae (Gross, 1993). 'Wriggling' was observed in three different situations: after mechanical disturbance; when an insect, not necessarily harmful to the leafminer, was landing on the leaf; and during host location and attack behaviour by the parasitoid. This lead to the hypothesis that the host uses vibrations to perceive a foraging parasitoid. Such a hypothesis could explain the presence of hair sensillae on the body of the late instar host larvae and pupae (see also Pottinger and LeRoux, 1971, for a comparison with a sibling species). A visual comparison of their external morphology (Plate 1) with known types of hairs (McIver, 1985) suggests that they probably serve as mechanoreceptors.

In this chapter I characterise and quantify the vibrations that are produced during the foraging of female S. sericeicornis on apple leaves infested by the leafminer P. malella.

5.2 Material and Methods

Insects and Plants

Insects and plants were used from our continuous laboratory rearing as described in Casas and Meyhöfer (1994). The laboratory strain of P. malella was established in 1989 with moths collected in the region of Emilia Romana (Italy) and reared on apple seedlings (Malus sylvestris cf. Golden Delicious). The parasitoid strain originated from material collected
in autumn 1993 in South Tirol (Italy). Experiments were conducted in spring 1994. Eight apple leaves with a single mine were used for vibration measurements (fresh weight 93±42 mg, dry weight 40±12 mg, length of leaf lamina 56±7 mm, width of leaf lamina 29±3 mm, values are means ± SD). Only leaves with either paralysed hosts or empty mines were used to ensure that vibrations were exclusively triggered by the parasitoid. The weight of a female *S. sericeicornis* was approximately 0.7 mg, the weight of a leafminer was about 1 mg.

*Experimental set-up*

Vibrations of foraging female *S. sericeicornis* were measured with a laser Doppler vibrometer (Dantec 41X62 Compact Laser Vibrometer, 2mW He-Ne-Laser). The output of the vibrometer is a voltage directly proportional to the instantaneous velocity (i. e. speed in the direction of the laser beam) of the surface of an object at the measurement point.

![Figure 1: Preparation of leaves. Drawing by A. Björnsen.](image)

Vibrations were measured on the upper side of the leaf surface on the centre of the mine. For better reflection of the laser beam and a better signal-to-noise ratio, a small piece of retroreflective tape was glued on the mine centre (Scotchlite, 3M; ~ 1 mm²). The extra weight added was very small (< 0.1 mg) compared with the weight of the leaves. Therefore, its
influence on the mechanical behaviour of the leaf was considered to be negligible (see Michelsen et al., 1982).

Experiments were conducted using the cantilever set-up described by Meyhöfer et al. (1994) for measurements of leafminer vibrations: leaves were cut off from their plants and their petioles were placed in a water-filled vial through a hole in its lid. The petiole was fixed to the lid with plasticine at a distance of 0.5 cm to the leaf base (Figure 1).

Leaves were adjusted so that the leaf surface was perpendicular to the laser beam. To avoid leaf vibrations due to air turbulence, prepared leaves were placed in a closed glass box (17 x 11 x 10 cm). The laser beam passed through the lid of the glass box. To reduce background vibrations, the laser unit of the vibrometer as well as the glass box containing the apple leaf to be studied were mounted on an air-buffered vibration-damped table (Photon Control, Cambridge). Female S. sericeicornis, which had prior oviposition experience, were released singly into the glass box. Females were investigated more than once on the same leaf, and on different leaves.

The analog output of the vibrometer was passed through a programmable low-pass filter (Stanford Research SR650, attenuation 105 dB per octave) to avoid aliasing effects during digitising. The filtered vibration signals were sampled with a Mac Adios II/16 A/D converter and stored on a Macintosh Quadra 800 computer using the software SoundScope (GW Instruments, 1993). The monitor output of the vibrometer was followed on an oscilloscope (Hameg HM 205-3) to enable an optimal adjustment of the vibrometer during the experiments.

Alongside the vibration recordings, the behaviour of the parasitoid on the leaf was recorded with a video camera (Panasonic WV-BL600) equipped with a macro zoom lens (Computar 18-108/2.5). A mirror was placed behind the leaf to enable observations on both the upper and the lower side of the leaf. Video data were stored on a recorder (Panasonic AG-7355) at a rate of 50 half-frames per second, i.e. one video image every 20 ms. A
time code generator (iec Pro Gamma, Noldus) provided time information for each video image.

To synchronise video and vibration recordings, a light emitting diode (LED) was placed in the field of vision of the video. The computer programme that controlled the vibration recording flashed the diode once directly prior to data acquisition. With this method, the beginning of vibration recording could be assigned to a video image with an accuracy of 20 ms (Figure 2a). During a recording session, the beginning of a parasitoid behaviour could be determined from the video images with an accuracy of 20 ms (i.e. one half-frame). Therefore, during recording the vibratory events could be assigned to a behaviour of the parasitoid with a constant maximal error of 40 ms (Figure 2b).
**Behavioural categories**

Based on the definitions in Casas (1989), the behaviour of foraging female parasitoids on the leaf was divided into the following categories:

*Landing:* landing on the leaf, after approach by flight.

*Take-off:* departure from the leaf by flight.

*Moving on the leaf lamina:* change of location on the leaf lamina outside the mined area. Movements were in general much quicker compared with movements on the mine.

*Moving on the mine:* change of location on the mine. The movements on the mine were considered as searching activity. In general, the female moved slowly, often examining the mine surface intensely with the tips of her antennae seemingly to find a suitable spot for ovipositor insertion.

*Standing:* all actions either on the leaf lamina or on the mine without a change of location, i.e. resting, stiffness, cleaning behaviour.

*Probing:* started when the tip of the abdomen was put on the mine surface to position the ovipositor. It ended when the body axis of the parasitoid was parallel to the leaf surface again after the ovipositor was removed from the mine. We did not distinguish if the ovipositor had been inserted into the mine or not.

**Data acquisition**

Vibration data acquisition had a sampling frequency of 50 kHz. This allowed detection of frequencies up to 25 kHz. The anti-aliasing low-pass filter was set to 25 kHz to attenuate higher frequencies. The frequency tracker of the vibrometer was set in the range 10-100 kHz so velocities in the range $6.3 \times 10^{-6} - 13 \times 10^{-3}$ m/s and a maximal acceleration of 1.8 m/s² could be detected (Dantec, 1991). A linear frequency response of the vibrometer up to 7.4 kHz at this tracker range is reported by the manufacturer. Calibration experiments with sinusoidal vibrations of different frequencies produced by a vibration exciter (Brüel and Kjaer 4810
+ 2706) controlled by a function generator (Stanford Research DS345) were conducted (chapter 4). No deviations from linearity were observed up to 10 kHz with this setting. In the range 10 - 20 kHz, the frequency of a sinusoidal vibration was still detected correctly. However, the accuracy of the corresponding intensities decreased approximately linearly with increasing frequency. At 20 kHz the velocity amplitude was underestimated by 30%. Thus, vibrations above 10 kHz were identified correctly in the frequency domain, but underestimated in their intensity depending on their frequency.

In each experiment, vibrations were recorded for 50 s. Using the information on the video tapes, the records were cut into segments according to the behaviour of the parasitoid (Figure 3a,b). Segments of vibratory signals triggered by the same behaviour were denoted as replicates. One record may therefore contain several replicates.

The total recording time was c. 30 min of various foraging activities, and the acquired vibration data amounted to 112 MB. All records in which parasitoids contacted the leaf were included.
Vibration recording (50 sec)

Vibrations of a behavioural event (replicate)

Analysis of frequency content in successive intervals of 40 msec

Spectrum (signal-to-noise ratio)

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3 kHz</td>
<td>11 dB</td>
</tr>
<tr>
<td>2.3 kHz</td>
<td>7 dB</td>
</tr>
<tr>
<td>0 kHz</td>
<td>0 dB</td>
</tr>
</tbody>
</table>

Characteristic:
Dominant frequency
Intensity

Figure 3: Frequency analysis of vibration recordings. S: Standing, Mm: Moving on the mine, P: Probing. Details are given in the text.

Frequency analysis

Frequency spectra were calculated by Fast Fourier Transformation (FFT, on 2048 points) of the velocity. Intensities were calculated as uncalibrated sound intensity levels (SIL). As in acoustics, intensities were given on a logarithmic scale as decibel values (dB). The same algorithm as in Meyhöfer et al. (1994) was used:
SIL = 10 \cdot \log_{10} \left( \frac{2}{\text{FFTpoints} \sqrt{\text{real}^2 + \text{imag}^2}} \right), \text{ where real is the real part of}

the complex notation of the FFT, imag the imaginary part of the complex notation of the FFT and \text{FFTpoints} the number of points used for the FFT. The algorithm returns a spectrum of 1024 dB values for frequencies between 0 and 25 kHz. To gain accuracy in the location of frequency peaks, spectra were smoothed by calculating a 5-point moving average, and afterwards by compressing the number of dB values to 512 by calculating the average of two successive points. The resulting spectrum has a frequency resolution of about 49 Hz.

![Frequency spectrum](image)

**Figure 4:** Range of background noise during all experiments.

Each replicate was divided into successive intervals of 40 ms (Figure 3 c) which corresponds to the time resolution that could be assigned to an action of a foraging parasitoid. The frequency spectrum was calculated for each interval. A 40 ms time interval contained 2000 sample points (sampling frequency of 50 kHz). To obtain the 2048 points necessary for the FFT (the
number of source points to perform a FFT has to be a power of two), the first 48 points of the following 40 ms interval were appended to the current interval. The background noise level was estimated for each replicate by calculating the average + 2 SD of ten spectra of the closest time series in which the parasitoid was not on the leaf. It was calculated at least once for each experiment, as the background noise was found to vary from leaf to leaf and over time for the same leaf. The spectra were obtained from 40 ms intervals as described above. The range of the background noise during all experiments is given in Figure 4. Every frequency spectrum obtained from a foraging female was compared to a spectrum of the background noise by calculating signal-to-noise ratios (Figure 3d). These ratios were obtained by subtracting the background noise spectra (in dB) from the dB values of frequency spectra obtained from foraging parasitoids. The obtained signal-to-noise ratio vibration spectra were characterised by their dominant frequency, i.e. the location of the frequency peak with the maximum intensity (Figure 3e).

**Table 1: Range of frequency classes.**

<table>
<thead>
<tr>
<th>Frequency class</th>
<th>Frequency range (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 178</td>
</tr>
<tr>
<td>2</td>
<td>178 - 355</td>
</tr>
<tr>
<td>3</td>
<td>355 - 708</td>
</tr>
<tr>
<td>4</td>
<td>708 - 1410</td>
</tr>
<tr>
<td>5</td>
<td>1410 - 2820</td>
</tr>
<tr>
<td>6</td>
<td>2820 - 5620</td>
</tr>
<tr>
<td>7</td>
<td>5620 - 11,200</td>
</tr>
<tr>
<td>8</td>
<td>11,200 - 22,400</td>
</tr>
</tbody>
</table>

The dominant frequencies of each replicate were then sorted by their intensities. Only segments in which the signal-to-noise ratio of the dominant frequency was higher than 3, 5, 7, or 10 dB were further
analysed. These thresholds correspond to intensities at least twice, three times, five times, or ten times the intensity of the background noise. The frequency scale was divided into 8 frequency classes corresponding to octave bands from <178 Hz to 22.4 kHz (Table 1) and dominant frequencies were assigned accordingly to the different frequency classes. The analysis is based on 35 recording sessions of 8 *S. sericeicornis* foraging on 8 apple leaves.

Figure 5a (legend on next page)
Figure 5a,b: Velocity time course and spectrogram of typical vibrations triggered by a female *S. sericeicornis* during *Landing* and *Take-off* (ir: irregular phase, re: regular phase). Spectrograms were calculated from the velocity series using the software SoundScope (settings: 512 FFT-points, Hamming window 184 Hz (8ms), intensity range: black 30 dB to white 0 dB, frame advance 0.1 ms, no 6 dB pre-emphasis). The frequency bar at 2.5 kHz belongs to the background noise (see Figure 4).
5.3 Results

**Short-lasting behaviours: Landing and Take-off**

*Landing*: The impact by an alighting parasitoid resulted in a vibrational signal in which a short initial irregular phase followed by a longer regular phase could be discriminated (Figure 5a). The irregular phase was characterised by irregular changing velocity amplitudes, which caused broad frequency bands up to our measurement range of 25 kHz, and presumably above. The maximal velocity of the leaf surface during the irregular phase was $5.3 \pm 2.4$ mm/s (mean ± SD; \(n=11\)). A parasitoid caused higher velocity amplitudes when approaching the leaf from a greater distance (e.g. from the glass box; \(n=8\)) than from a point close by (e.g. the mirror or the edge of the vial; \(n=3\)). The irregular vibrations merged into regular oscillations with a basic frequency between 11 and 16 Hz. There was no sharp transition between the first irregular and the second regular phase and we set the border between both phases by eye. The duration of the irregular phase was between 20 and 100 ms. The amplitude of the basic oscillation decreased in the course of the regular phase (Figure 5a). Since the absolute duration of the basic oscillation could not be determined accurately, we calculated the half life of the amplitude for each recording. The period of the basic oscillation and the attenuation from one maximum to the next were constant for five successive maxima. Therefore, we approximated the basic oscillation by a damped harmonic oscillation of the form \(y = \sin(2\pi ft) \cdot e^{-kt}\), with \(y\) being the vibration amplitude, \(f\) the frequency of the basic oscillation, \(t\) the time, and \(k\) the damping constant. The amplitude of the basic oscillation decreased exponentially with a half life of $137 \pm 28$ ms (mean ± SD; \(n=9\)). When the impact on the leaf was weak (e.g. approach from a short distance), the regular phase was not clear enough to discern the basic frequency \((n=2)\). The basic oscillation was superimposed with dominant high frequencies directly proportional to the
absolute value of the velocity of the basic oscillation during the whole regular phase (Figure 5a).

Take-off: Vibrations produced when a female took off can be separated into two phases with the same broad characteristics as described for Landing. The initial, irregular phase during Take-off was much shorter (< 20 ms, n=12) than in Landing (Figure 5b). The maximal velocity during this phase was 2.7 ± 1.3 mm/s (mean ± SD), which is about half the velocity elicited by a landing parasitoid. There was no difference between the regular phases triggered by Landing or Take-off (basic oscillation: frequency between 11 and 16 Hz; half life of 147 ± 28 ms (mean ± SD)).

Long lasting behaviours: Moving, Standing and Probing

The duration of these behaviours was highly variable, ranging from < 1 to > 30 s (Table 2). The maximal velocity elicited by Probing was much higher than for the other long lasting behavioural states (Table 2). Velocities elicited during Probing were about half as high as velocities triggered by Take-off and a quarter as high as triggered by Landing. For Probing, 97 % of replicates contained velocities clearly higher than in the background noise (mean + 2SD = 0.2 mm/s), while for Moving and Standing a high proportion of replicates contained no velocities higher than the background noise (Table 2).
Figure 6: Velocity time course and spectrogram of typical vibratory signals triggered by a female *S. sericeicornis* during *Standing, Moving on the leaf lamina, Moving on the mine* and *Probing*. To allow a direct comparison with *Landing* and *Take-off*, the spectrogram settings were chosen the same as for Figure 5. The frequency bar at 2.5 kHz belongs to the background noise (see Figure 4).
Table 2: Duration of behavioural events and maximal velocity triggered during such an event. \( n \) = total number of replicates in a behavioural class; \( \%n_{\text{eff}} \) = percentage of replicates in which the maximal velocity exceeded the maximal velocity of the background vibrations (mean + 2SD = 0.2 mm/s).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Duration (s)</th>
<th>Max. Velocity (mm/s)</th>
<th>( n )</th>
<th>( %n_{\text{eff}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background</td>
<td>5.9 ± 6.6</td>
<td>0.10 ± 0.05</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Standing</td>
<td>8.7 ± 9.4</td>
<td>0.28 ± 0.27</td>
<td>28</td>
<td>53</td>
</tr>
<tr>
<td>Moving on the leaf lamina</td>
<td>6.7 ± 5.2</td>
<td>0.23 ± 0.27</td>
<td>48</td>
<td>25</td>
</tr>
<tr>
<td>Probing</td>
<td>7.5 ± 5.2</td>
<td>1.49 ± 1.08</td>
<td>31</td>
<td>97</td>
</tr>
</tbody>
</table>

In contrast to vibrations triggered by short lasting behaviours, the velocity amplitude of vibrational signals elicited by long lasting behaviours was irregular, without a clear temporal pattern. During a single behaviour, phases with obvious vibration signals alternated with phases indistinguishable from the background noise (Figure 6). We characterised the signals quantitatively by calculating the probability of their occurrence during two different kinds of intervals. Intervals of the first kind lasted 40 ms and corresponded to the smallest time interval for which we could assign a behavioural event with good confidence. The second kind of interval was variable in time and represented the integration of intervals of the first kind over the period of time needed for completion of a behavioural event. Thus, using intervals of the first kind we investigated the probability of occurrence of a vibration signal in time ('time scale'), and using intervals of the second kind we investigated the probability of occurrence of a vibration signal at least once during a behavioural event ('behavioural scale').
Figure 7: Probabilities that a vibrational signal whose dominant frequency exceeds a certain threshold above the background noise occurs at least once in the course of a complete behavioural event.

*Behavioural scale:* During complete behavioural events of *Probing*, vibrations with dominant frequencies of intensities at least ten times higher (>10 dB) than the background noise were triggered with almost certainty (97% of replicates, Figure 7). For the other behaviours, dominant frequencies of such high intensities were rarely found, while vibrations with dominant frequencies of low intensities (at least 3 dB above background noise) occurred quite often. The probability that vibrations of a given intensity above the background noise were elicited was consistently highest during *Probing* and lowest during *Standing*, with *Moving* (either on the leaf lamina or on the mine) being intermediate (Figure 7). In all behavioural states, the lowest threshold (>3 dB above background noise) was exceeded by dominant frequencies of all frequency classes (Figure 8). However, dominant frequencies of high intensities (>10 dB), that were
triggered during *Probing*, originated primarily from frequency classes 4 to 6 (708-5620 Hz). It should be noted that dominant frequencies triggered during *Moving on the leaf lamina* belonged to low frequency classes, while dominant frequencies triggered during *Moving on the mine* belonged to high frequency classes.
Figure 8: Probabilities that dominant frequencies of a certain frequency class and intensity occur at least once in the course of a complete behavioural event.
Figure 9: Probabilities that the dominant frequency of a 40 ms interval exceeds a certain threshold above the background noise, calculated for different behavioural classes.

Time scale: The analysis of the same signals at the 40 ms time scale showed that vibrations of high intensity occurred as rare events (Figure 9). **Probing** was the only behaviour with abundant vibrations of high intensities (> 10 dB; Figure 9 and 10). The dominant frequency of such vibrations was located in frequency classes 3 to 6 (355-5620 Hz; Figure 10). In **Moving on the leaf lamina**, the dominant frequency was most likely in the lowest frequency class.
Figure 10: Probabilities that the dominant frequency of a 40 ms interval is located in a certain frequency class, calculated for different behavioural classes.
5.4 Discussion

**Implications and origin of the high variability between replicates**

In the experiments, some replicates show no vibrations over the total duration of the behaviour, while in other replicates vibrations were found in a large number of 40 ms intervals. This variability between replicates provides one reason to analyse the vibrations on both the behavioural and the time scale. There is *a priori* a simple theoretical relation between the two different scales: let \( p \) be the probability that a vibratory event would occur in a 40 ms interval. Then \( q = 1 - p \) would be the probability that this event would not occur during a 40 ms interval. The number of 40 ms intervals \( i \) comprised in a behavioural event can be obtained from the average duration of a behavioural event. The probability \( P \) that at least one vibratory signal would occur during a complete behavioural event is then \( P = 1 - q^i \). This relationship does not fit the data, as the assumption of independence between 40 ms segments underlies the calculation of \( P \). The strong heterogeneity between replicates implies that the relationship between the two scales is therefore more complex.

Another reason for the usefulness of the two different scales is that the restriction to a single occurrence at the behavioural level for scoring replicates discards any information about multiple occurrences. On the behavioural scale a replicate with a single occurrence will be scored the same as a replicate with a large number of occurrences. In contrast, when analysed at the 40 ms time scale, replicates with multiple occurrences will result in a marked increase of the probability \( p \). It appears that the most important contributors to the high variability between replicates are the varying distance between the parasitoid and the point of measurement, the different leaf characteristics, and the heterogeneity of the plant material through which vibrations were travelling (see also Michelsen *et al.*, 1982; Barth *et al.*, 1988).
The characterisation of parasitoid vibrations on both the behavioural and the time scale also provides a useful tool for future work, in which different kinds of vibratory signals will be played to the leafminer to elicit evasive behaviour. Combination with an analysis of the behavioural coupling between leafminer and parasitoid will yield profitable information, whether the leafminer uses single vibratory events or whether it integrates vibratory events over a period of time to detect the presence of a parasitoid.

Vibrational signals produced by different behaviours

Landing and Take-off imparted relatively strong forces to the leaf. Studies on the velocity of vibration propagation in stems of different plant species (Michelsen et al., 1982) suggest that, in my experiments, induced vibrations travelled several times across the leaf during the fade away period. Because the duration of the irregular phase approximately corresponds to the duration of the actual landing or take-off of the parasitoid, I assume that vibrations during the irregular phase are directly elicited by the impact of the parasitoid on the leaf. Since the insect does not impart force onto the leaf during the regular phase, vibrations of the regular phase must be reverberations which travel several times across the leaf. The fact that there are no differences between the regular phases of Landing and Take-off, indicates that vibrations during the fade away depend to a high degree on the set-up and the leaf properties, rather than on what actually caused such vibrations. Therefore, I suppose that vibrations during the regular phase are of low informational value for the leafminer.

When a parasitoid is standing on a leaf, it is not necessarily immobile. Vibrations elicited during Standing most probably originate from actions such as cleaning, lifting and lowering the wings and dropping faeces. Moving on the leaf lamina show vibrations of higher intensities more often than Moving on the mine, despite the fact that in the latter case the impact point and the measurement point are closer to one another. In both cases,
vibrations are triggered mainly by leg movements. The quicker movements outside the mined area may induce stronger vibrations in the leaf than the slow movements on the mined area. During Probing, the strongest vibration peaks occur when the ovipositor is inserted into the mine, when it is withdrawn, or when the parasitoid is swinging around the inserted ovipositor, most probably to locate the host by touching it. However, during both ovipositor insertion and withdrawal several distinct vibration peaks occur rather than a continuous complex of vibration peaks (= "pulse complex" as defined by Meyhöfer et al., 1994). Therefore, I assume that neither ovipositor insertion into the mine nor its withdrawal is a smooth process.

Reliability and detectability of vibrational signals

The type of system in which vibrational communication is studied will to a great extent determine the characteristics of the signals involved. In mating systems, the subject of most studies on vibrational communication, both mating partners, sender and receiver, benefit from successful communication. For easy identification of the partner, signals will generally have evolved to be easily detectable and reliable. In predator-prey and parasitoid-host systems, however, producing signals usually is disadvantageous to the sender, since it may lead to detection and identification by its opponent. In most studies on such systems, however, only vibrations elicited by the prey/host and used by the predator/parasitoid for attack have been investigated (e.g. Brownell, 1977; Lang, 1980; Klärner and Barth, 1982; Bleckmann, 1985; Barth et al., 1988; Sugimoto et al., 1988). With a few exceptions (e.g. Camhi et al., 1978; Tautz and Markl, 1978; Gnatzy and Kämper, 1990), the possibility of the prey/host to detect and escape from its enemies has not been discussed. In the parasitoid-leafminer system, there is a strong behavioural coupling between the opponents, both being sender and receiver of vibrations (Casas, 1994;
The parasitoid as a sender of vibrational signals has a strong disadvantage in alerting the leafminer to its presence while the leafminer as a receiver benefits from this information which enables it to initiate evasive actions. The reverse situation is also true: the moving leafminer may give useful information to the parasitoid about its location and suitability. Thus, selection should act on both opponents for vibratory inconspicuousness.

Minimising vibrations during foraging is one way for the parasitoid to accomplish this. The detectability of vibrations is highly dependent on the intensity and frequency composition of the background noise. This is well known in humans and vertebrates, but also applies to insects (Ehret et al., 1982; Römer et al., 1989). Vibrational signals of a foraging parasitoid may be masked by environmental vibrations. Wind and rain are major sources of vibrations that determine the background noise level for plants in the field. Both raise the noise level over the whole frequency scale, but low frequencies increase more in intensity than high frequencies (Barth et al., 1988; chapter 6). Therefore, high frequencies should be easier to detect than low frequencies in a noisy environment. Because Landing, Take-off and Probing produce the highest frequencies, these are the parasitoid behaviours that are most likely to be detected by a leafminer in the field.

Producing vibrations which might originate from other sources would be another strategy for vibratory inconspicuousness (e.g. vibratory camouflage: Barth et al., 1988). Such unspecific signals have a low reliability and therefore should not elicit evasive behaviours in the leafminer. Landing, Take-off, Moving and Standing are behaviours not specific to a parasitoid. Many insects show these behaviours. I assume that vibrations elicited during these behaviours by other insects are very similar to vibrations elicited by S. sericeicornis during the same behaviour. This is supported by studies on ants moving on apple leaves (chapter 6). Furthermore, waterdrops falling on apple leaves (simulated rainfall)
produce vibrations with similar temporal and frequency pattern as *Landing* and *Take-off* (chapter 6). Therefore, I expect no behavioural response of leafminers tuned to vibrations triggered by *Landing, Take-off, Moving* and *Standing*. *Probing* is the only behaviour that is characteristic of parasitoids. Vibrations triggered by a probing parasitoid were clearly distinct from vibrations triggered by other behaviours with respect to density and frequency content. Vibrations of high intensity were measured almost every time a parasitoid probed and occurred often on a shorter time scale during *Probing*. Therefore, in addition to their high detectability, vibrations triggered during *Probing* offer reliable signals through which a leafminer could detect the presence of a parasitoid. In a recent study, Meyhöfer *et al.* (submitted) showed that the insertion of the ovipositor without touching the host had indeed a marked influence on the behaviour of leafminer larvae, whereas other behaviours of the parasitoid did not elicit a behavioural response.

5.5 Conclusions

Only *Probing* provides a reliable and detectable source of information for the host to detect a foraging parasitoid, whereas other behaviours of the parasitoid produce either unspecific vibrations or signals difficult to detect. The vibrations elicited during *Probing* could account for the evasive behaviour that is observed in this and other leafminers.
Plate 1: Scanning electron micrographs of *P. malella* larva. **A**: Ventrolateral view of thoracic part of fifth instar larva (L5). **B**: Typical long hair sensilla located on the upper side of first thoracic segment of L5. No pores or openings were found, indicating that the hair has no chemoreceptive property. The hair arises from a socket and can be articulated at the base. These are indications that the hair has mechanoreceptive properties (McIver, 1985). **C**: Socket and basal part of shaft of long hair sensilla. **D**: Thoracic leg of L5. **E**: Abdominal leg of L5. Short hairs on both leg types show the same characteristic features for mechanoreceptors as long hair sensillae. Short hairs are located on the underside of larvae and presumably are in direct contact with mine tissue. Therefore, they are likely involved in perception of vibrations from the substrate. **F**: Ventrolateral view of abdominal part of fifth instar larva, showing segments A1-A5. Note the prominent long body hairs on the upper side of the larva. Scale bars indicate 100 μm (A, F) or 10 μm (B-E), respectively. Acceleration voltage was 15 kV for all pictures.
6 On the vibratory environment of a leafminer

6.1 Introduction

The evolution of senses and their integration into behavioural patterns is likely to have been strongly influenced by the environment of the animals in question. This also applies to the vibration sense of *Phyllonorycter* leafminers and their vibratory environment. Larvae and pupae of *P. malella* (Ger.) (Lepidoptera: Gracillariidae) show characteristic avoidance reactions when attacked by one of their enemies, the parasitic wasp *S. sericeicornis* Nees (Hymenoptera: Eulophidae). One of the problems these leafminers are confronted with is the question how to distinguish between vibrations triggered by their natural enemies (Bacher *et al.*; 1996) and the various other types of vibrations to which they are exposed. These non-relevant sources include vibrations elicited by other insects, which cause no harm to the leafminer, and the vibratory background noise. Wind and rain are major sources of vibration that determine the background noise level for plants in the field. Besides of leafminer enemies, ants were among the most frequently observed insects on apple leaves.

In this chapter I characterise and quantify the vibrations that are triggered by falling waterdrops, air movements and ants or beetles moving on apple leaves. The information content and specificity of the different types of vibrations is discussed and related to the situation in the field.
6.2 Material and Methods

Experimental set-up
Vibrations were measured on the centre of the upper leaf surface of eight apple leaves (Malus sylvestris cf. Golden Delicious) with a laser Doppler vibrometer (Dantec 41X62 Compact Laser Vibrometer, 2mW He-Ne-Laser) as described in detail in Bacher et al. (1996). To avoid leaf vibrations due to air turbulence, prepared leaves were placed in an open glass box (17 x 11 x 10 cm) for measurements of waterdrop induced vibrations. The box was closed with a glass top during recordings of vibrations elicited by moving insects. The box was removed for investigations on air movement 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 waterdrops of 15 mg were produced with an adjustable Eppendorf pipette fixed at a distance of 33-35 cm above the leaves. The pipette was adjusted such that the falling waterdrops landed on the major leaf vein. One target point was chosen at half the distance from the vibration measurement point to the leaf tip (‘apical’), a second at half distance to the leaf base (‘basal’). The accuracy of the target hits was controlled with the video recordings. Lateral deviations of the impact 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. Five leaves were investigated (Table 1): three were taken from our laboratory culture and were thin and soft and not infested by leafminers (leaves 1-3). One experimental leaf had a single mine on it containing an
immobile host (leaf 5). In contrast to the other leaves, the measurement point was chosen on the centre of the mine in this leaf to allow direct comparisons with results of Bacher et al. (1996). One apple leaf of the same dimensions as the others was taken from an apple tree growing in the field (leaf 4). It was considerably heavier and stiffer as the leaves from the laboratory culture (Table 1).

### Table 1: Characteristics of leaves used in the experiments.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Infested</th>
<th>Fresh weight (mg)</th>
<th>Dry weight (mg)</th>
<th>Length of leaf lamina (cm)</th>
<th>Width of leaf lamina (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf 1</td>
<td>Laboratory</td>
<td>-</td>
<td>60</td>
<td>7.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Leaf 2</td>
<td>Laboratory</td>
<td>-</td>
<td>179</td>
<td>6.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Leaf 3</td>
<td>Laboratory</td>
<td>-</td>
<td>162</td>
<td>6.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Leaf 4</td>
<td>Field</td>
<td>-</td>
<td>532</td>
<td>7.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Leaf 5</td>
<td>Laboratory</td>
<td>+</td>
<td>233</td>
<td>6.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Leaf 6</td>
<td>Laboratory</td>
<td>-</td>
<td>308</td>
<td>8.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Leaf 7</td>
<td>Laboratory</td>
<td>-</td>
<td>244</td>
<td>6.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Leaf 8</td>
<td>Laboratory</td>
<td>-</td>
<td>110</td>
<td>7.0</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Air movements were produced by means of a hair dryer (Satrap profi 1500 diffuser, heating disabled). Leaves were horizontally adjusted at a distance of 3 cm from the vibration damped table they stood on. The hair dryer was placed at a distance of 80 cm from the leaves and had no direct contact to the table. 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 of 1.5 and 0.75 m/s, respectively, measured with an A100R switch anemometer connected to a CR10 datalogger (Campbell Scientific), at the position of the leaves. Leaves 6 and 7 were adjusted either to achieve an air flow in direction of the longitudinal axis with the leaf tip pointing towards the hair dryer or, in a different
experiment, an air flow perpendicular to the longitudinal leaf axis. Two recordings of 10 s each were made of each leaf in each adjustment. Vibrations triggered during movements of an ant collected from an apple tree in the field (probably *Larius niger*, 1.2 mg weight) and a beetle (*Leptinotarsa decemlineata*, 19.6 mg weight) were recorded on an uninfested, laboratory cultured apple leaf (leaf 8). Movements comprised all actions with change of the location on the leaf. In general, the ant was running at a high speed across the leaf while the beetle was walking rather slowly.

*Data acquisition and analysis*

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). Preliminary experiments showed that drop-outs were produced frequently when trying to measure vibrations produced by falling waterdrops and wind with the vibrometer settings in the most sensitive range. Therefore, the frequency tracker of the vibrometer was set in the less sensitive range of 0.1-1.0 MHz so that velocities from $63 \times 10^{-6}$ to 0.13 m/s and a maximal acceleration of $180 \text{ m/s}^2$ 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. Vibration recordings (in Volt) were converted to velocity. Frequency spectra were calculated from 40 ms intervals of the velocity time series by Fast Fourier Transformation and compared to spectra of the background noise by calculating signal-to-noise ratios (see Bacher *et al.*, 1996). The obtained signal-to-noise ratio vibration spectra were characterised by their dominant frequency, i.e. the location of the frequency peak with the maximum intensity. The frequency scale was divided into 8 frequency classes corresponding to octave bands from $<178 \text{ Hz}$ to 22.4 kHz (Table 2) and dominant frequencies were accordingly assigned to the different frequency classes.
Table 2: Range of frequency classes.

<table>
<thead>
<tr>
<th>Frequency class</th>
<th>Frequency range (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 178</td>
</tr>
<tr>
<td>2</td>
<td>178 - 355</td>
</tr>
<tr>
<td>3</td>
<td>355 - 708</td>
</tr>
<tr>
<td>4</td>
<td>708 - 1410</td>
</tr>
<tr>
<td>5</td>
<td>1410 - 2820</td>
</tr>
<tr>
<td>6</td>
<td>2820 - 5620</td>
</tr>
<tr>
<td>7</td>
<td>5620 - 11,200</td>
</tr>
<tr>
<td>8</td>
<td>11,200 - 22,400</td>
</tr>
</tbody>
</table>

I refrained from applying statistical tests for comparisons, because differences important for the research questions were considered to be obvious without testing. Moreover, the data compared were not collected at random, and were transformed and pooled already before comparing, thus not fulfilling the basic assumptions of conventional statistical tests.

6.3 Results

Waterdrops

The impact of a falling waterdrop on an apple leaf resulted in a vibrational signal in which a short initial irregular phase could be distinguished from a following longer regular phase (Figure 1). The irregular phase was characterised by irregularly changing velocity amplitudes, which caused broad frequency bands up to our limit of measurement at 25 kHz, and presumably above. There was no sharp transition between the irregular and the regular phase (Figure 1) and we set the border between both phases by eye. The duration of the irregular phase appeared to be independent of the impact point in cultured leaves (Table 3). However, in the field collected leaf (leaf 4) basal application of the impact resulted in a short irregular
phase, consisting of almost a single vibration peak, while the duration of the irregular phase was in the same range as in laboratory cultured leaves when the impact was applied apical of the leaf centre. The maximal velocity during the irregular phase ranged between 76.1 and 137.1 mm/s (Table 3) and was found to be independent of the impact point. Velocities of the field collected leaf were not remarkably different from those of laboratory cultured leaves, but ranged at the lower end.

The irregular vibrations merged into regular oscillations with a basic frequency between 5.7 and 11.7 Hz. The frequency of the regular phase was dependent on the impact point: in all leaves it was lower when the impact was applied apical of the leaf centre (Table 3). The amplitude of the basic oscillation decreased in the course of the regular phase (Figure 1). The period of the basic oscillation and the attenuation from one maximum to the next were constant for five successive maxima. Therefore, I approximated the basic oscillation by a damped harmonic oscillation of the form \( y = \sin(2\pi ft) \cdot e^{-kt} \), with \( y \) being the vibration amplitude, \( f \) the frequency of the basic oscillation, \( t \) the time, and \( k \) the damping constant. The amplitude of the basic oscillation decreased exponentially with a half life of 163 ± 37 ms (mean ± SD; \( n=20 \)). 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 (Table 1) was found. The basic oscillation was superimposed with dominant high frequencies directly proportional to the absolute value of the velocity of the basic oscillation during the whole regular phase (Figure 1). There was no difference of vibration characteristics between infested leaves and the uninfested leaf (Table 3).
Figure 1: Velocity time course and spectrogram of a typical vibrational signal triggered by a waterdrop falling on an apple leaf. The spectrogram was calculated from the velocity series using the software SoundScope (settings: 512 FFT-points, Hamming window 184 Hz (8 ms), intensity range: black 30 dB to white 0 dB, frame advance 0.1 ms, no 6 dB pre-emphasis). For a comparison, a typical vibration triggered by a landing parasitoid was plotted on the same scale (data from Bacher et al., 1996).
Table 3: Characteristics of vibrations induced by waterdrops falling on apple leaves. Leaves 1-3, 5: laboratory cultured, leaf 4: field collected. Leaves 1-4: uninfested, leaf 5: infested.

<table>
<thead>
<tr>
<th></th>
<th>Irregular phase</th>
<th></th>
<th>Regular phase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration (ms)</td>
<td>Maximal velocity (mm/s)</td>
<td>Frequency (Hz)</td>
<td>Half life (ms)</td>
</tr>
<tr>
<td><strong>Position of impact point</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>25</td>
<td>93.8</td>
<td>10.3</td>
<td>173</td>
</tr>
<tr>
<td>basal</td>
<td>29</td>
<td>127.1</td>
<td>10.4</td>
<td>190</td>
</tr>
<tr>
<td>apical</td>
<td>21</td>
<td>137.1</td>
<td>7.1</td>
<td>175</td>
</tr>
<tr>
<td>apical</td>
<td>26</td>
<td>113.9</td>
<td>5.7</td>
<td>263</td>
</tr>
<tr>
<td>Leaf 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>27</td>
<td>119.9</td>
<td>10.0</td>
<td>147</td>
</tr>
<tr>
<td>basal</td>
<td>25</td>
<td>76.1</td>
<td>10.5</td>
<td>88</td>
</tr>
<tr>
<td>apical</td>
<td>23</td>
<td>132.6</td>
<td>9.3</td>
<td>110</td>
</tr>
<tr>
<td>apical</td>
<td>22</td>
<td>136.6</td>
<td>9.1</td>
<td>112</td>
</tr>
<tr>
<td>Leaf 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>12</td>
<td>134.8</td>
<td>9.8</td>
<td>152</td>
</tr>
<tr>
<td>basal</td>
<td>9</td>
<td>80.1</td>
<td>9.4</td>
<td>185</td>
</tr>
<tr>
<td>apical</td>
<td>17</td>
<td>136.9</td>
<td>6.6</td>
<td>218</td>
</tr>
<tr>
<td>apical</td>
<td>16</td>
<td>137.1</td>
<td>6.5</td>
<td>157</td>
</tr>
<tr>
<td>Leaf 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>2</td>
<td>96.5</td>
<td>11.7</td>
<td>149</td>
</tr>
<tr>
<td>basal</td>
<td>1</td>
<td>98.7</td>
<td>11.4</td>
<td>157</td>
</tr>
<tr>
<td>apical</td>
<td>14</td>
<td>105.8</td>
<td>10.9</td>
<td>158</td>
</tr>
<tr>
<td>apical</td>
<td>28</td>
<td>118.9</td>
<td>10.9</td>
<td>153</td>
</tr>
<tr>
<td>Leaf 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>basal</td>
<td>14</td>
<td>129.5</td>
<td>10.8</td>
<td>172</td>
</tr>
<tr>
<td>basal</td>
<td>16</td>
<td>136.4</td>
<td>10.7</td>
<td>164</td>
</tr>
<tr>
<td>apical</td>
<td>15</td>
<td>135.9</td>
<td>9.9</td>
<td>166</td>
</tr>
<tr>
<td>apical</td>
<td>21</td>
<td>133.1</td>
<td>9.8</td>
<td>173</td>
</tr>
</tbody>
</table>

Air movements

Air movements induced irregular vibration amplitudes in apple leaves, producing broad frequency bands up to the limit of measurement at 25 kHz (Figure 2). However, intensities decreased with increasing frequency. A regular basic oscillation could be discriminated in the irregular time course.
Table 4: Characteristics of vibrations induced by air movements in apple leaves.

<table>
<thead>
<tr>
<th>Relative wind speed (m/s)</th>
<th>Leaf adjustment relative to direction of air flow</th>
<th>Max. velocity (mm/s)</th>
<th>Frequency of basic oscillation (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>parallel</td>
<td>100</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>parallel</td>
<td>120</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>perpendicular</td>
<td>120</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>perpendicular</td>
<td>130</td>
<td>10.0</td>
</tr>
<tr>
<td>Leaf 6</td>
<td>parallel</td>
<td>60</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>parallel</td>
<td>50</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>perpendicular</td>
<td>50</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>perpendicular</td>
<td>60</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>parallel</td>
<td>100</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>parallel</td>
<td>80</td>
<td>8.6</td>
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<tr>
<td></td>
<td>parallel</td>
<td>70</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>perpendicular</td>
<td>80</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>perpendicular</td>
<td>80</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>parallel</td>
<td>40</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>parallel</td>
<td>40</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>perpendicular</td>
<td>30</td>
<td>12.7</td>
</tr>
</tbody>
</table>

The frequency of the basic oscillation appeared to be dependent on the direction of air flow relative to the alignment of leaves (Table 4). Air flows perpendicular to the leaf axis produced a higher frequency than parallel air flows. There was little effect of wind speed on the frequency of the basic oscillation. In contrast, the maximal velocity induced in leaves was highly dependent of wind speed, but independent of wind direction.
Figure 2: Velocity time course and spectrogram of typical vibratory signals triggered by air movements of high (1.5 m/s, upper graph) and low speed (0.75 m/s, lower graph). Scales and spectrogram settings were chosen the same as for Figure 1.
Moving insects

The velocity amplitude of vibrational signals elicited by insects moving on an apple leaf was irregular, without a clear temporal pattern. During a single behaviour, phases with obvious vibration signals alternated with phases indistinguishable from the background noise (Figure 3). The maximal velocity elicited by the beetle (3.17±2.45 mm/s; mean±SD) was much higher than velocities elicited by the ant (0.39±0.16 mm/s; mean±SD). I characterised the frequency content of the signals quantitatively by calculating the probability of the occurrence of the dominant frequency during two different kinds of intervals. Intervals of the first kind lasted 40 ms. The second kind of interval was variable in time and represented the integration of intervals of the first kind over the period of time needed for completion of a behavioural event, i.e. moving on the leaf lamina. Thus, using intervals of the first kind I investigated the probability of occurrence of a vibration signal in time (‘time scale’), and using intervals of the second kind I investigated the probability of occurrence of a vibration signal at least once during a behavioural event (‘behavioural scale’).
Figure 3: Velocity time course and spectrogram of typical vibratory signals triggered by an ant and a beetle moving on an apple leaf. Time scale and spectrogram settings were chosen the same as for Figure 1.
Figure 4: Probabilities that dominant frequencies of a certain frequency class and intensity occur at least once in the course of a complete behavioural event when an ant or a beetle move on an apple leaf.

*Behavioural scale:* The lowest threshold (>3 dB above background noise) was exceeded by dominant frequencies of the first 4 frequency classes, i.e.
frequencies <1.41 kHz, for vibrations triggered by a moving ant (Figure 4). Low frequencies (class 1) were found in all replicates at intensities >5 dB. Higher intensities, however, occurred as rare events. Dominant frequencies elicited by a moving beetle were found over the whole spectrum of frequency classes (Figure 4). They were triggered with high probability even at intensities >10 dB.

*Time scale:* The analysis of the same signals at the 40 ms time scale showed that vibrations of high frequencies and intensities occurred as rare events (Figure 5). The dominant frequency of vibrations caused by both the ant and the beetle was most likely located in the lowest frequency class. Abundant vibrations of high intensities (> 10 dB) were triggered only by the beetle and occurred only in the first frequency class (Figure 5).
Figure 5: Probabilities that the dominant frequency of a 40 ms interval is located in a certain frequency class when an ant or a beetle move on an apple leaf.
6.4 Discussion

Information contents of vibratory signals

Application of a well defined impact on different positions of a leaf and on different leaves provides information about which parts of the triggered vibrational signal are determined by the impact source and which by the leaf structure and the set-up. Only the former parts are important for the leafminer because they contain potential information on their origin. In the following I will discuss the results with respect to their information contents.

Waterdrops imparted relatively strong forces to the leaf. The impact is delivered to the leaf during a very short time span, but induced vibrations can be measured long after the impact ceased. Because the duration of the irregular phase approximately corresponds to the duration of the actual drop hitting the leaf, I assume that vibrations during the irregular phase are directly elicited by the impact of the drop on the leaf. The vibration characteristics of the irregular phase are to a large extent independent of the impact point and leaf (but see leaf 4, duration of the irregular phase, impact point apical). It therefore seems likely that the irregular phase contains specific information about the vibration source. Since no force is imparted to the leaf during the regular phase, vibrations of the regular phase must be reverberations which travel across the leaf for several times. The fact that the characteristics of the regular phase are dependent on the impact point and differ between individual leaves indicates that vibrations during the fade away depend to a high degree on the set-up and the leaf properties, rather than on the actual source of such vibrations. Therefore I suppose that vibrations during the regular phase are of low informational value for the leafminer. However, considering the frequency of the basic oscillation, the leafminer may derive information on the location of the impact point.
Constant air movements continually induce vibrations in apple leaves. The frequency of the basic oscillation contains information on wind direction. However, it seems possible that the same effect can be elicited with different wind speeds, making this characteristic a source of information not very reliable for the leafminer. In contrast, the maximal velocity seems to be a good indicator of wind speed.

The quicker movements of the ant compared to the beetle do not outweigh the greater impact of movements of the latter on leaves due to its greater weight. It seems that the intensity of vibrations elicited by moving insects contains information on their size rather than on their speed of movement.

Implications for the sensory world of a leafminer

The problem facing a leafminer is how to distinguish between the various types of vibrations to which it is exposed. Ideally, it should only react with evasive behaviour when attacked by an enemy, e.g. a parasitoid, and therefore should discriminate the characteristics of parasitoid elicited vibrations from vibrations of other origins. Vibrations produced by waterdrops show a similar temporal pattern as vibrations produced by a parasitoid landing on an apple leaf or departing by flight (Bacher et al. 1996). Both differ considerably in their amplitude, most likely due to the different weight of waterdrops and parasitoid. However, amplitude probably is of limited use for distinguishing types of vibrations, because distance and spatial relations between sender and receiver differ greatly under natural conditions (Barth et al., 1988). It seems that impacts originating from a variety of different sources produce vibrations with the same broad characteristics. Thus impact-like vibrations constitute no reliable source to infer the nature of their origin, and are probably of low informational value for the leafminer.

Vibrations elicited by a moving ant are similar in temporal pattern and frequency content to those triggered by a parasitoid moving on an apple
leaf. Vibration intensity and probability of occurrence differ, again mainly due to weight differences. The heavier beetle induced even more intense vibrations containing additional high frequency components. Therefore it can be concluded that vibrations elicited by moving insects contain information about their size. Insects of similar size as a parasitoid but not harmful to the leafminer most probably trigger similar vibrational signals, which thus are difficult to distinguish from vibrations produced by a moving enemy. However, no vibrations produced by either the ant or the beetle were similar to vibrations triggered by a parasitoid when inserting its ovipositor into the mine (Probing: Bacher et al., 1996) suggesting that vibrations elicited by a probing parasitoid might be the decisive source of information for the leafminer to detect the presence of its enemy (Meyhöfer et al., submitted; Bacher et al., 1996 and chapter 5).

Besides effecting signal reliability, the level of the background noise will also determine the detectability of vibrational signals (Ehret et al., 1982; Römer et al., 1989). Wind and rain are major sources of vibrations that determine the background noise level for plants in the field. Both raise the noise level over the whole frequency scale, but low frequencies increase more in intensity than high frequencies (see also Barth et al., 1988). Therefore, high frequencies should be easier to detect than low frequencies in a noisy environment.

In the field, however, the level of environmental noise produced by rain and wind may be of minor importance for masking parasitoid vibrations as these laboratory data suggest. Casas (1989) found females of S. sericeicornis searching for hosts only at low wind speeds (< 0.6 m/s) during periods when it was not raining. Thus, in the field parasitoids hunt only at low background noise levels. This will be advantageous for the leafminer, since low noise levels facilitate detection and recognition of parasitoids, which are prerequisites for the leafminer to escape parasitization. However, it will also benefit parasitoids if they use
vibrational cues in host location and identification (Casas, 1989; Meyhöfer et al., submitted).

Apple leaves growing under natural conditions differ in some structural characteristics such as thickness or stiffness from those raised in growth chambers. This seems to have consequences on single aspects of transmission properties such as differences in the duration of the irregular phase depending on the impact point. However, it is unknown if these aspects of vibratory signals are of any informational value to the leafminer. Hence, before generalising the results obtained from laboratory cultured leaves for field conditions, more studies on the consequences of vibration modification through plant material on behavioural interactions of leafminer and parasitoid are required.

6.5 Conclusions

Vibrations produced by moving insects had a temporal pattern and frequency composition similar to those triggered by a moving parasitoid. Waterdrops produced impact-like vibrational signals similar to that of a landing parasitoid. Wind induced constant leaf movements which raised the background noise level up to at least 25 kHz. Interference by rain and wind may play only a minor role in the field situation, as parasitoids forage for hosts only under dry conditions and low wind speeds. None of the studied vibration sources mimicked vibrations produced by a probing parasitoid, thus supporting the hypothesis that the latter might serve the leafminer to detect parasitoid presence.
7 Substrate vibrations as elicitors of defence behaviour in leafminer pupae

7.1 Introduction

Late instar larvae and pupae of the leafminer *Phyllonorycter malella* (Ger.) (Lepidoptera: Gracillariidae) show characteristic avoidance reactions when the leafminer is attacked by one of its parasitoids, the eulophid wasp *Sympiesis sericeicornis* Nees (Hymenoptera: Eulophidae). While pupae can only express a behaviour denoted as “wriggling”, larvae show a more complex reaction pattern (Meyhöfer *et al.* submitted) the reason for which is still not understood properly. In addition to wriggling, which is considered an active defence (Gross, 1993), larvae may also escape ovipositor stings by changing their location inside the mine. Avoidance behaviour seems to increase the handling time by the parasitoid, which in about 10% of the cases leads to the parasitoid abandoning the mine without parasitizing the host (Casas, 1989). Therefore, it seems likely that avoidance behaviour has an adaptive value for the leafminer. For avoidance behaviour to be effective, the leafminer has to detect parasitoid presence at a stage that allows for an effective defence. We hypothesized that *P. malella* uses vibrations produced by female parasitoids while searching on mined apple leaves to detect its opponent (Bacher *et al.*, 1996). It was demonstrated that these vibrations provide both detectable and reliable cues for the leafminer to identify its enemy (Bacher *et al.*, 1996). However, the question which signal parameters are actually involved in eliciting avoidance behaviour remained to be answered.
The aim of this study was to investigate if *P. malella* responds to substrate vibrations and to determine which frequency components are involved in eliciting such a reaction. Pupae of *P. malella* were chosen as the study object because of their monotypic behavioural reaction. The behaviour of the pupae in response to direct as well as indirect stimulation was investigated. In addition, the effect of the mine as a transmission channel on vibration characteristics was studied.

### 7.2 Material and methods

**General set-up**

Vibrations were produced by means of an electromagnetic vibration exciter (B&K 4810) connected to a power amplifier (B&K 2706). The vibration exciter stood on an air-buffered, vibration damped table (Photon Control, Cambridge) to attenuate background vibrations. Pupae were placed directly onto the moving platform of the vibration exciter in experiments where stimuli were offered to free pupae. In experiments where stimuli were offered to pupae concealed in their leaf mines, leaves with a single mine were detached and their petioles placed in a water-filled vial through a hole in its lid. The petiole was fixed to the lid with plasticine at a distance of 0.5 cm from the leaf base. The vial was fixed on a small, vertically adjustable table such that the leaf was horizontal. The adjustable table holding the leaf was placed on the air buffered table next to the vibration exciter. All equipment besides the vibration exciter and the adjustable table emitting unwanted vibrations was removed from the air-buffered table. The top of the vibration exciter was equipped with a sharpened screw. Using correction fluid, the tip of the screw was glued to the lower epidermis of the mine at a distance of approximately 1 cm from the pupa inside the mine in the direction of the leaf margin. The behaviour of the pupae inside the
mines was monitored through the lower epidermis of the leaf by means of a video camera (Panasonic WV-BL600) equipped with a macro zoom lens (Computar 18-108/2.5). The mine was illuminated from above by a glass fibre lamp (Fiberoptic-Heim AG, L100) to facilitate observations.

To study the transmission of external vibrations inside the mine, leaves were prepared in the same way as described above. At the location of the pupa, a small window was cut into the upper side of the mine through which the pupa was removed. A tiny piece of retroreflective tape was glued to the silk threads of the pupal cocoon inside the mine (Scotchlite, 3M; ~ 1 mm\(^2\)) with correction fluid. Vibrations on the inner mine surface were measured with a Laser Doppler vibrometer (Dantec 41X62 Compact Laser Vibrometer, 2mW He-Ne-Laser, tracker range 0.1-1.0 MHz) at the location of the retroreflective tape.

**Generating the vibrational signals**

Sine wave signals were generated by a function generator (Stanford Research DS345) and fed directly into the power amplifier. Frequencies of 100, 200, 500, 800, 1000, 2000, 3000 and 4000 Hz were used in the experiments. This range comprised the most prominent frequencies elicited by parasitoids during host location on mined apple leaves (Bacher et al., 1996).

Noise signals were generated on a Macintosh Quadra 800 Computer using the software SoundScope (GW Instruments, 1993). A ± 1 V Gaussian white noise signal (duration = 2 s, sampling frequency = 50 kHz, rise and fall times = 0.1 s) was filtered with digital bandpass filters (WLFDAP, Zola Technologies, Inc.: filter length 63 points, stopband attenuation 80 dB, sampling frequency 50 kHz). Filters had the bandwidth of octaves and the border frequencies were chosen according to Bacher et al. (1996), i.e. band 1 from 178-355 Hz, band 2 from 355-708 Hz, band 3 from 708-1410 Hz, band 4 from 1410-2820 Hz, and band 5 from 2820-5620 Hz. Due to
technical limitations of the equipment other frequency bands were not tested. The band limited noise signals were fed into the power amplifier via a D/A converter on a Mac Adios II/16 board (GW Instruments). The intensities were varied by multiplying the original stimuli by different factors (step size 0.5).

**Experimental procedure**

*Free pupae, sine stimuli:* The power amplifier was set to 20 dB attenuation and the gain control was adjusted to the middle position. Pupae were dissected out of their leafmines and placed on top of the platform of the vibration exciter. Pupae had to rest for at least 3 minutes without moving the abdomen prior to each experiment. Each individual was tested with the frequencies 100, 200, 500, 800, 1000, 2000, 3000 and 4000 Hz in random sequence. The intensity of the sine stimulus of a chosen frequency was increased on the function generator from zero in steps of 0.01 Vpp (Volt peak-to-peak) until the pupa showed wriggling behaviour. The duration of each intensity step was approximately 2 s, without a rest period between two intensities. The voltage at which individuals started “wriggling” (= reaction threshold) was recorded for each frequency. If the tested animal did not show a behavioural response until 1 Vpp, the test was terminated and the result was classified as “no reaction”. The experiment was resumed by testing the next frequency. Experiments were counted only if individuals showed a behavioural response to at least three of the tested frequencies. Valid results were obtained from 15 pupae.

*Concealed pupae, sine stimuli:* The test procedure was the same as for free pupae. Unlike the previous experiment, intensities were increased until the signal was clipped by the power amplifier. Animals that did not show a behavioural response until the maximal intensity were classified as “no response”. Valid results were obtained from 9 pupae.
Concealed pupae, bandlimited noise stimuli: The power amplifier was set to 10 dB attenuation and the gain control was adjusted to the middle position. Frequency bands 1 to 5 were tested with each individual in random sequence. Intensities were increased from zero until a behavioural response was elicited. Each stimulus intensity was given three times with a pause of approximately 10 s in between. Pupae had to rest for at least 3 min before the next frequency was tested. Experiments were counted if individuals showed a behavioural response to at least three of the tested frequency bands. Valid results were obtained from 5 pupae.

Transmission of vibrations in mines: The power amplifier was set to 20 dB attenuation and the gain control was adjusted in the middle position. Sine waves of all frequencies used in the other experiments were tested at varying intensities. Three leaves were measured.

Data analysis
The behavioural thresholds (in Vpp) were converted to velocity. For sine wave data, displacement and acceleration were calculated from velocity. Peak-to-peak data of experiments in which sine stimuli were used were transformed to RMS values by division by $\sqrt{2}$. For a direct comparison, the amplitudes of the bandlimited noise stimuli were also given as RMS values by numerical calculation of the standard deviation multiplied by a factor of 2 (SoundScope, 1993). Thus, data are presented as “effective” amplitudes in both directions of the mean.

If not indicated differently, values are given as mean ± SD. For the calculation of means, all experiments in which the pupae showed no response were discarded.
7.3 Results

*Free pupae*

Pupae reacted to all frequencies in the range tested (Figure 1). However, the threshold curves differed considerably, depending on the stimulus parameter (displacement, velocity, acceleration) plotted. Displacement thresholds were highest at 100 Hz (on average 45 ± 25 µm) but decreased steadily over more than two orders of magnitude with increasing frequency to an average of 0.16 ± 0.07 µm at 4 kHz. Velocity thresholds showed a similar tendency to decrease with increasing frequency, but decreased only half an order of magnitude, from an average of 28 ± 16 mm/s at 100 Hz to an average of 5.5 ± 1.9 mm/s at 1 kHz. Between 2 kHz and 4 kHz the threshold remained nearly constant between 3.1 and 4.2 mm/s. In contrast, the acceleration threshold curve showed a positive relationship with frequency. The lowest behavioural threshold occurred at 100 Hz (17.7 ± 9.8 m/s²) and the highest at 4 kHz (104 ± 48 m/s²). Thus, the results did not reveal a frequency window to which pupae of *P. malella* are especially sensitive.

Up to 1 kHz all individuals tested showed a behavioural response to intensities below the arbitrarily chosen highest test amplitude of 1 Vpp. Above 1 kHz the percentage of animals reacting decreased with increasing frequency to only 57% of the tested individuals responding at 4 kHz. This indicates that the actual threshold values may be underestimated at high frequencies.
Figure 1: Reaction thresholds of free pupae to sine stimuli (mean ± SD). Data are displayed as RMS displacement, velocity and acceleration amplitudes. The solid line indicates the arbitrarily chosen highest intensity (1 Vpp) tested. In the lowest graph the percentages of responding animals are given at each frequency. N=14.
**Concealed pupae**

The threshold values of concealed pupae to sine stimuli were generally higher than those of free pupae (Figure 2). At frequencies up to 1 kHz the threshold values were about twice as high as thresholds of free pupae (between 1.5 times at 200 Hz and 2.4 times at 800 Hz). At higher frequencies, however, no reliable threshold values were found due to the low percentage of responding animals. The few animals responding indicate that far higher amplitudes are necessary to elicit wriggling behaviour in concealed pupae at high frequencies (more than 5-fold, compared to free pupae).

Reaction thresholds of concealed pupae to bandlimited stimuli ranged between $8.4 \pm 3.0$ mm/s at band 1 and $4.6 \pm 0.9$ mm/s at band 3 (Figure 3). These thresholds were 4.2 to 5.9 times below the thresholds for sine stimuli in the same frequency range (200 - 1000 Hz). No behavioural reaction was elicited in response to stimuli of frequency bands 4 and 5 up to the highest intensities tested.
Figure 2: Reaction thresholds of pupae concealed in their leafmines to sine stimuli (mean ± SD). The data are displayed as RMS displacement, velocity and acceleration amplitudes. The solid line indicates the highest intensity tested (until the amplifier clipped the signal). In the lowest graph the percentages of responding animals are given at each frequency. N=9.
Figure 3: Reaction thresholds of pupae concealed in their leafmines to bandlimited noise stimuli (mean ± SD). The data are displayed as RMS velocity amplitudes. The horizontal bars indicate the width of the frequency bands. The solid line indicates the highest intensity tested (until the amplifier clipped the signal). The percentages of responding animals are given at each frequency band tested. N=5.
Figure 4: Velocity amplitudes of the vibration exciter (circles) and of the inner mine surface (squares) at a distance of 1 cm from the tip of the vibration exciter depending on the input voltage and frequency (data from leaf 2).
Table 1: Ratios of peak-to-peak velocities produced by the vibration exciter to velocities measured on the inner mine surface at a distance of 1 cm from the tip of the vibration exciter (Damping factors).

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Leaf 1</th>
<th>Leaf 2</th>
<th>Leaf 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3.9</td>
<td>13.6</td>
<td>13.3</td>
</tr>
<tr>
<td>200</td>
<td>4.7</td>
<td>18.1</td>
<td>11.0</td>
</tr>
<tr>
<td>500</td>
<td>3.2</td>
<td>8.9</td>
<td>7.0</td>
</tr>
<tr>
<td>800</td>
<td>3.6</td>
<td>36.6</td>
<td>15.7</td>
</tr>
<tr>
<td>1000</td>
<td>3.6</td>
<td>128.4</td>
<td>20.0</td>
</tr>
<tr>
<td>2000</td>
<td>3.2</td>
<td>181.8</td>
<td>16.3</td>
</tr>
<tr>
<td>3000</td>
<td>10.3</td>
<td>113.4</td>
<td>12.6</td>
</tr>
<tr>
<td>4000</td>
<td>11.1</td>
<td>16.1</td>
<td>81.6</td>
</tr>
</tbody>
</table>

Transmission of vibrations in mines

Mine tissue significantly attenuated vibrations. Vibrations were damped linearly with increasing stimulus intensity (Figure 4). Different frequencies were transmitted differentially well in the mine (Table 1). Moreover, the damping factors of frequencies (i.e. the velocity amplitude of the vibration exciter divided by the velocity amplitude of the inner mine surface at the measurement point) varied considerably between leaves (e.g. up to 56-fold at 2 kHz between leaf 1 and leaf 2). The great heterogeneity in vibration damping indicates that the transmission of vibratory signals is highly dependent on the exact position of sender and receiver on the leaf.
7.4 Discussion

Threshold curves and frequency sensitivity

The results of this study demonstrate that pupae of *P. malella* react to vibratory stimuli and that they are differentially sensitive to different frequencies.

The question whether the pupae are tuned to a specific frequency range cannot be answered unequivocally on the basis of the available data. Interpreting a dip in a threshold curve of velocity, acceleration or displacement as a sensitivity maximum of the insect would be justified only if the relative role of the various stimulus parameters is known. However, since different threshold minima are obtained depending on which vibration parameter is considered it seems impossible to judge from a biotest which stimulus parameter is relevant, and hence which frequency range the animal is tuned to. This problem seems to apply to a broad range of studies on behavioural or electrophysiological response thresholds to mechanostimuli (e.g. Michelsen *et al.*, 1986; Dambach, 1972). The three parameters cannot be tested independently, because it is impossible to vary one parameter of a mechanical stimulus (e.g. acceleration) without affecting the others (in this example displacement and velocity). A further problem lies in the fact that different receptors may respond to different vibration parameters. To draw more detailed conclusions it would be necessary to locate a single receptor instead of non-specific stimulation of all receptors of an animal.

Already in one of the pioneer studies on threshold values to vibratory stimuli, Autrum (1941) discussed the difficulty to assign one of the three vibration parameters to a receptor organ. His suggestion, that the parameter assuming nearly identical values over a broad range of frequencies must be the one relevant for a given receptor organ, is still followed in recent studies (e.g. Markl and Tautz, 1975; Kalmring, 1985; Kirchner *et al.*, 1994;
Breithaupt and Tautz 1990). However, Autrum himself (1941) pointed out that his argumentation provides no proof and can, thus, be only a crude estimate of the relevant vibration parameter. Moreover, the assumption underlying his argumentation is that a receptor acts as frequency-independent. However, it is now widely accepted that mechanoreceptors do not work equally well at all frequencies (e.g. Barth and Blickhan, 1984, and references therein).

One solution to overcome these obstacles may be to (i) locate a receptor relevant for eliciting a behavioural response and then (ii) study its structure and mechanical properties. Since the transduction mechanism of a mechanical stimulus into a receptor current seems to be primarily a uniform process of stretching the dendritic membrane (French, 1988), the auxiliary cuticular structures have been suggested as the components of a receptor organ selecting the adequate stimulus parameter from a variety of potential mechanical stimuli (Barth and Blickhan, 1984; or the whole receptor organ itself: Dambach, 1972). This approach has been followed in the well studied cases of caterpillar filiform hairs (Markl and Tautz, 1975; Tautz, 1977; 1978a,b; 1979) and of spider trichobothria (Barth, 1982; Barth et al. 1993; Humphrey et al., 1993). A different approach may be the determination of the exact moment of response of receptors showing a phase-locked response to sine stimuli. In such receptors action potentials are elicited at exactly the same point in each period of a given sinusoidal vibration (French and Wong, 1976; Tautz, 1978b; Kühne, 1982). Since the displacement, velocity and acceleration maxima of a sine vibration are separated by \( \pi/2 \), it seems possible to link the response moment to one of these maxima. However, the time delay between actual reception of the stimulus and the triggered action potential has to be considered.
Natural vibratory stimuli and the sensitivity of other arthropods

During the location of the host inside the mine, the ectoparasitoid *S. sericeicornis* elicits the most prominent vibrations when inserting its ovipositor into the mine. During such probing behaviour velocities of the leaf surface up to 8.9 mm/s were measured (average 3.0 mm/s; for a direct comparison with thresholds of this study the values were multiplied by a factor of 2 to obtain amplitudes in both directions of the mean; Bacher *et al*., 1996). These vibrations are in the same intensity range as behavioural thresholds of *P. malella* pupae to bandlimited noise stimuli. Moreover, bandlimited noise stimuli are similar in frequency composition to the broadbanded natural stimuli. This suggests that vibrations elicited by a probing parasitoid could be perceived by the host. In contrast, the duration of single naturally occurring vibrational stimuli is much shorter (approximately 1-2 ms) than the bandlimited noise stimuli used in our experiments (Bacher *et al*., 1996). However, such vibration peaks occur frequently during probing behaviour by a parasitoid. Although the intensity and frequency composition of natural vibrations are similar to bandlimited noise stimuli evoking defence behaviour in leafminer pupae, the importance of the temporal pattern of vibrational stimuli in eliciting a behavioural response remains to be investigated.

There are only few studies on behavioural thresholds in arthropods regarding vibrations transmitted through solid substrate. For example, the displacement thresholds releasing prey capture behaviour in the wandering spider *Cupiennius salei* are in the same range as those for *P. malella* (Hergenröder and Barth, 1983; Table 2). On the other hand there are examples indicating that insects can be much more responsive to vibrations than *P. malella* pupae. For example, the damp-wood termite *Zootermopsis nevadensis* shows an alarm reaction to vibration intensities 60 times below the threshold of leafminer pupae (Kirchner *et al*., 1993), and the honeybee *Apis mellifera* is nearly 15 times more responsive than *P. malella* to
vibrations of the comb (Michelsen et al., 1986; Table 2). Thus, the behavioural thresholds of *P. malella* pupae are relatively high compared to other arthropods. The fact that *P. malella* pupae do not respond at lower intensities, however, does not mean that they might not perceive such vibrations. In spiders, receptor thresholds are lower by as much as 40 to 60 dB than the thresholds for the release of prey catching behaviour (Klárner and Barth, 1982; and references therein).

**Table 2**: Comparison of reaction thresholds of *P. malella* pupae with other arthropods.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Cupiennius salei</th>
<th><em>P. malella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>100 Hz Sine</td>
<td>70 μm</td>
<td>83 μm</td>
</tr>
<tr>
<td>400 Hz Sine</td>
<td>25 μm</td>
<td>ca 10 μm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Zootermopsis nevadensis</th>
<th>Apis mellifera</th>
<th><em>P. malella</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 kHz Sine</td>
<td>0.07 μm</td>
<td>0.3 μm</td>
<td>4.3 μm</td>
</tr>
</tbody>
</table>

*The plant as transmission channel*

A great part of the heterogeneity in vibration damping may be caused by interferences of vibrations travelling several times across the leaf during the stimulus period inducing standing waves where amplifications and

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1 After Hergenröder and Barth (1983)
2 After Kirchner et al. (1993)
3 After Michelsen et al. (1986)
extinctions occur at points close to each other. The great damping factors may therefore be measured at a point close to a vibration node (extinction). However, since all damping factors were greater than 3 (Table 1), the actual attenuation of vibrations travelling 1 cm through mine tissue can be assumed to be greater than factor 3. Assuming further the velocity of vibration propagation to be in the same order of magnitude as known from other plants (i.e. about 50-100 m/s; Michelsen et al., 1982), such interference phenomena will occur when stimuli durations are longer than 1 ms, because vibrations will then travel more than once across the leaf. Therefore, reverberations should occur almost each time natural stimuli are applied to apple leaves (see Bacher et al., 1996; chapter 5). Variations in leaf and cocoon structure (Plate 1 C-F) may also contribute to the heterogeneity of vibration damping.

Non-linearity in vibration propagation seems to apply to a broad range of plants used by arthropods in vibratory communication (Barth, 1989; Michelsen et al., 1982). As a consequence, the frequency content of vibrational signals travelling through plant tissue will be unpredictable to a large extent for communicating animals. Responsiveness to a broad range of frequencies will enhance the chance to perceive vibratory signals through plant tissue. Moreover, broadbandedness as such may be a parameter potentially used by leafminers for signal recognition, as indicated by the lower thresholds to noise stimuli compared to sine stimuli. The damping properties of mine tissue may explain the higher threshold values of concealed pupae compared to free pupae. However, behavioural thresholds of concealed pupae to vibrations up to 1 kHz were only twice as high as thresholds of free pupae, whereas mine tissue attenuated vibration amplitudes between 3.2 and 128 times in the same frequency range. When we consider these damping factors it seems that concealed pupae have relatively lower reaction thresholds than free pupae. This can be explained
by a better responsiveness of pupae in their normal environment or a better coupling between mine tissue and mechanoreceptors of pupae.

Inside their leafmines, bodyhairs of the pupae, which most likely serve as mechanoreceptors (Plate 1 A+B), do not directly contact plant tissue, but rather the silk threads that surround them (spun by late larval instars all over the inner mine surface; Plate 1 D+E). Therefore we assume that leafminers receive vibratory signals via the silk threads. Even though we measured vibrations on the silk layer of the inner cocoon surface, our set-up restricted us to detect only vibrations perpendicular to the surface (transverse vibrations). Measurements in spider orbwebs revealed that silk threads heavily attenuate transverse and lateral vibrations, while longitudinal vibrations (i.e. motion directed along the thread axis) propagate with very little attenuation (Masters, 1984a; Landolfa and Barth, submitted). Spiders preferentially use the latter vibration components for orientation/guidance within their webs (Klärner and Barth, 1982; Masters, 1984b). The seemingly higher sensitivity of concealed pupae compared to free pupae could be explained at least partly, if leafminers, too, would make use of the longitudinal component of vibrations along silk threads.

The defence strategy of P. malella pupae

Late instar larvae of P. malella (L4 and L5) often change their behaviour in response to the insertion of the ovipositor by S. sericeicornis into the mine during the host location process (Meyhöfer et al., submitted). Such a response is found less often in pupae (R. Meyhöfer, unpublished). The different situation of pupae compared to larvae may account for the lower responsiveness of the former. While larvae enjoy a considerable freedom of movement inside the mine and, thus, are able to actively escape the ovipositor, pupae are sessile. Their position inside the mine is fixed by a silken cocoon spun before pupation. In general, wriggling behaviour is believed to deflect the ovipositor during parasitization trials (Gross, 1993).
In the laboratory we often observed that a heavily wriggling pupa could not be penetrated by the ovipositor of a female parasitoid (S. Bacher, personal observation). A searching *S. sericeicornis* female most probably discriminates between mines containing suitable and unsuitable hosts according to the vibrations released by their inhabitant (Casas, 1989). Females soon abandon mines containing unsuitable hosts (paralyzed host, empty mine) (Casas, 1989). Assuming that vibrations triggered by the leafminer serve as stimuli for the parasitoid to continue probing it would be a reasonable strategy for the host to remain still if the parasitoid probes at a distance. However, if the ovipositor is within reach of the host, an active defence behaviour such as wriggling is likely to become the better alternative to remaining motionless and risk being stung for a host that cannot change location. If the parasitoid is near the host, the leafminer will receive stronger signals than if the parasitoid probes far away from the host, because intensity is a function of distance. This scenario might well explain why wriggling should be triggered only at relatively high vibrational thresholds.

The strategy of being motionless for as long as possible seems to be optimal only for a host that cannot change its location inside the mine. The mobile stages of *P. malella* seem to follow a different behavioural pattern of active avoidance, even if the parasitoid is still far away on the mine (Meyhöfer et al., submitted). The effect or the benefit of the avoidance behaviour of larvae during such a “cat-and-mouse-game” remains to be investigated.
7.5 Conclusions

Defence reactions can be elicited over a broad range of frequencies. Different reaction thresholds at different frequencies indicate that leafminers discriminate between frequencies. Mine tissue strongly attenuates vibrations. Damping is a non-linear function of frequency and thus highly dependent on the exact position of sender and receiver on the mine. Mechanoreceptors of pupae, e.g. the prominent body hairs, receive vibratory stimuli via the silk threads spun all over the inner mine surface and not directly from the plant tissue. It is therefore likely that leafminers make use of vibration components in the direction of silk threads (longitudinal vibrations), which are less subject to attenuation, rather than of components perpendicular to the silk web (transverse vibrations).

The relatively high reaction thresholds of leafminer pupae compared to other arthropods may be explained by the defence strategy of the immobile pupae.
Plate 1: Scanning electron micrographs of *P. malella* pupa (A, B) and mine (C-F). A: pupa; B: abdominal segments A3-A5 with prominent hair sensillae; C: inside of a late instar mine shown from below. The lower epidermis was removed to allow view of the inner surface of the roof of the tentiform mine; D: lower epidermis with silk threads causing the wrinkling of the former; E: roof of mine showing the silken net spun all over the inner surface; F: detail of the inside showing the heterogeneity of the mine tissue. fh: frass hole, le: lower epidermis, lv: leaf vein, mc: mine cavity, mlv: main leaf vein, pp: palisade parenchyma, sp: spongy parenchyma, st: silk threads. Scale bars indicate 100 μm. Acceleration voltage was 15 kV for all pictures.
8 General discussion

This study is embedded in the context of elucidating the key mechanisms leading to success or failure of a biocontrol agent. Such knowledge should help in predicting the outcome of biocontrol attempts with better accuracy. The organisms used, a parasitoid, a leafminer and apple plants, represent both an economically important agricultural system as well as a model for systems involving endophytic host insects. The purpose of this investigation was to reveal the role of vibrations emitted by the foraging parasitoid in its detection by the host and as a releaser of host evasive behaviour. To consider the active role of the host insect in the interaction with its natural enemy is a rather unusual focus in parasitoid research in general and especially in parasitoid leafminer systems.

The results of this study reveal the great significance of vibrations for P. malella in detecting the parasitoid S. sericeicornis and in eliciting avoidance behaviour: Vibrations elicited during the insertion of the ovipositor into the mine, which is a behaviour specific for leafminer parasitoids, provide both detectable and reliable cues for the leafminer by which it could recognise the presence of its enemy. In contrast, other behaviours of the parasitoid trigger non-specific vibrations which could also originate from other insects moving on apple leaves. Vibrations of abiotic origin raise the background noise level over the whole frequency scale, thus complicating vibratory communication between the opponents, but may be of minor importance in the field since parasitoids forage only under conditions of dry weather and low wind speed. In leafminer pupae, defensive reactions are elicited over a wide frequency range, and pupae seem to discriminate between different frequencies. Vibrations transmitted through mine tissue are strongly attenuated. However, the prominent body
hairs of leafminer late instar larvae and pupae, which most likely are involved in mechanoreception, receive vibrations via the silk threads spun all over the inner mine surface by late instar larvae rather than directly from the plant tissue. It is likely that silk threads transmit vibrations with less attenuation than plant tissue and therefore enable the leafminer to identify different vibration types with high accuracy.

Following the bottom-up approach of this study, the results will be discussed at different levels: They will be placed in the context of sensory physiology and behavioural ecology, possible evolutionary paths for the development of evasive behaviour in this leafminer species will be discussed, and finally the potential value of the results for explaining field observations will be outlined.

On the sensory physiology - the release mechanism of evasive behaviour

Late instar larvae and pupae of *P. malella* react with evasive behaviour to a variety of different vibratory signals. These include vibrations triggered by a foraging parasitoid (chapter 5), but also vibrations of insects harmless to the leafminer (chapter 6) and of abiotic origin (chapters 6 and 7). Moreover, leafminers seem to discriminate between different frequencies (chapter 7). Since vibrations triggered by a parasitoid do not have a repetitive time pattern, one hypothesis about the release mechanism in *P. malella* could be that a behavioural response is elicited when a leafminer receives a vibratory signal that exceeds an absolute threshold once. An alternative hypothesis is that the receiver may have to be stimulated repeatedly before an evasive reaction is evoked. In this case, the density of vibratory signals when integrated over a time period would be a criterion for assessing the presence of a parasitoid. A combined analysis of temporal pattern and frequencies that elicit evasive behaviour, taking into account the vibratory signals triggered by a foraging parasitoid, will allow a deeper understanding of vibration parameters relevant for eliciting evasive
behaviour. It should be feasible to test these alternative hypotheses on pupae in a biotest similar to that used in chapter 7. As for larvae, however, testing of vibration parameters that elicit evasive behaviour will be much more complicated because behavioural responses of larvae to attack by *S. sericeicornis* are manifold and not always compelling (Meyhofer *et al.*, submitted). A biotest would have to be conducted in a way that allows larvae to move freely in their natural environment, i.e. inside their mines. Larval behaviour would have to be monitored continuously and behavioural changes would have to be analysed statistically, e.g. as changes in transition probabilities between different behaviours (following the work of Meyhöfer *et al.*, submitted). Although difficult to conduct, such a biotest will be rewarding to complement the results already obtained on *P. malella* pupae, and considerable effort should be spent on the development of a suitable set-up.

*On the behavioural ecology - effectiveness of evasive behaviour*

It is known from field studies that *S. sericeicornis* leaves larvae of *P. malella* unparasitised in about 10% of the cases (Casas, 1989). However, the behaviours that lead to successful escape of individual leafminers are unknown. To understand the evasive behaviours of leafminers, and finally their escape strategy (e.g. as outlined in chapter 7), the mechanics of single actions have to be studied with respect to escape/defence potential of the host.

In contrast to the results of the field study, all hosts were successfully parasitised in a laboratory study on the behavioural coupling between leafminer and parasitoid (Meyhöfer *et al.*, submitted). These findings suggest that a parasitoid is more likely to abandon a suitable host in the field. This may be mediated through a lower motivational state of individual parasitoids or the greater number of alternative host patches available in the field (as predicted by foraging theory; summarised in
Godfray, 1994). It indicates that the parasitoid determines to a considerable extent the success of the hosts evasive behaviour. This has to be considered in an explanation of the escape strategy of leafminers.

Evasive behaviour of leafminers may prevent their paralysation by the parasitoid, but does not necessarily hinder the parasitoid from depositing eggs in the mine. During this study it was observed occasionally that female parasitoids were unable to penetrate heavily wriggling pupae, but nevertheless laid several eggs in the mine outside the pupal cocoon (personal observation). Whether the newly hatched parasitoid larvae are able to survive on non-paralysed hosts will depend on their success in moving actively towards the host and mounting it. This is likely to be difficult in the case of unparalysed mobile host larvae, which can easily move away, but seems feasible in the case of stationary pupae. However, the cocoon spun by the last larval instar of the leafminer may hinder parasitoid larvae from reaching their host. On the other hand, when pupae are paralysed it seems possible for the parasitoid to deposit an egg directly inside the cocoon. Investigations of such scenarios might contribute to understanding the necessity of paralysis for successful parasitisation.

**On the evolution of evasive behaviour**

A prerequisite for the evolution of any characteristic is its adaptiveness, i.e. it has to increase the fitness of individuals possessing this characteristic. Possible benefits of evasive behaviour to leafminers are (I) increased handling time of hosts by the parasitoid and (II) the fact that about 10% of individuals escape parasitisation (Casas, 1989). If parasitoids are time-limited (and not egg-limited), an increase in handling time may favour not-attacked individuals of the leafminer by increasing the probability that the latter do not encounter a parasitoid. However, the beneficiaries of such a scenario, which are the leafminer individuals that transmit their genes to the next generation, are the not-attacked individuals, who are not
necessarily those that developed evasive behaviour. On the other hand, the percentage of leafminers that survived attacks of *S. sericeicornis* is low compared to insect species in which the advantage of evasive behaviour is more obvious (see Gross, 1993). However, escaping parasitisation of *S. sericeicornis* may not necessarily be the only (not even primary) function of the observed evasive behaviour in *P. malella*. It may e.g. also serve to escape other enemies. Therefore, studies on the behavioural interactions of *Phyllonorycter* leafminers with other parasitoid and predator species should be carried out to obtain a more detailed insight in the significance of evasive behaviour.

*On the field situation*

Plant tissue strongly modifies vibrations (chapter 7). Data on transmission of vibration in leaves indicate that some characteristics of vibratory signals are differentially modified in laboratory cultured and field collected leaves (chapter 6). Apple leaves from laboratory cultured seedlings, which were used in most experiments, differ in many respects from leaves in the field (chapter 6). In general, leaves grown outside are thicker and larger than leaves cultured in the laboratory. Moreover, *Phyllonorycter* mines in the field have significantly thicker silk layers (personal observation). These factors, and others such as the fixation and position of leaves on branches, may influence the mechanical properties of leaves, and thus the transmission and modification of vibratory signals. Experiments conducted in this study represent the first approach to describe vibrations induced in apple leaves (chapters 5-7). It was reasonable to use standardised leaves in these studies for a comparison between vibrations of different origins and for the interpretation of behavioural studies in which a similar set-up was used (Meyhöfer *et al.*, submitted). To generalise the results, however, the structure and variability of leaves grown on apple trees in the field, their influence on modification of vibratory signal characteristics and the
informational value of the latter for the leafminer have to be taken into account.

This study shows that it is most likely that vibrations elicited by probing of the parasitoid trigger a defence reaction in host larvae and pupae which may lead to failure of parasitisation in a certain proportion of attacks. Quantitative assessments of this phenomenon, however, require more studies. Moreover, it needs to be investigated how far other sensory modalities are involved in eliciting evasive behaviour. To link the sensory level to the population dynamics more information on life history traits such as development, reproduction, survival, predation and dispersal have to be considered (see e.g. Sabelis and van der Meer, 1986). At the present stage of research, statements about the role of evasive behaviour of the host in determining the success rate of a biocontrol agent are difficult to make. However, further studies on the effectiveness of evasive behaviour against *S. sericeicornis* and other enemies, as outlined in the previous paragraphs, could shed light on the importance of such behaviour for *P. malella*, and for management of apple leafminer populations with natural enemies.
9 References


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