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Efficiency of vibrational sounding in parasitoid host location depends on substrate density

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Abstract Parasitoids of concealed hosts have to drill through a substrate with their ovipositor for successful parasitization. Hymenopteran species in this drill-and-sting guild locate immobile pupal hosts by vibrational sounding, i.e., echolocation on solid substrate. Although this host location strategy is assumed to be common among the Orussidae and Ichneumonidae there is no information yet whether it is adapted to characteristics of the host microhabitat. This study examined the effect of substrate density on responsiveness and host location efficiency in two pupal parasitoids, *Pimpla turionellae* and *Xanthopimpla stemmator* (Hymenoptera: Ichneumonidae), with different host-niche specialization and corresponding ovipositor morphology. Location and frequency of ovipositor insertions were scored on cylindrical plant stem models of various densities. Substrate density had a significant negative effect on responsiveness, number of ovipositor insertions, and host location precision in both species. The more niche-specific species *X. stemmator* showed a higher host location precision and insertion activity. We could show that vibrational sounding is obviously adapted to the host microhabitat of the parasitoid species using this host location strategy. We suggest the attenuation of pulses during vibrational sounding as the energetically costly limiting factor for this adaptation.

Keywords Echolocation · Host location · Pupal parasitoid · Substrate density · Vibration

Introduction

Parasitoid wasps in the drill-and-sting guild pierce through substrate with their ovipositor to reach their larval or pupal host within its feeding tunnel or pupation chamber (Smith et al. 1993). Some species of the hymenopteran family Ichneumonidae are well known for their exceptionally long ovipositors and their ability to parasitize hosts deeply concealed in a substrate. For example, *Megarhyssa atrata* F. and *Rhyssa persuasoria* L. attack larvae of wood-boring wasps (Siricidae) by drilling through more than 10 cm of wood (van Achterberg 1986; Le Lannic and Nénon 1999). Other ichneumonids parasitize aculeate hymenopterans through the mud or plant stem walls of their nests (Krombein 1967; Danks 1970; Eberhard 1974). Species of the tribe Pimplini are usually endoparasitoids of lepidopteran pupae (Wahl and Gauld 1998). They attack exposed hosts but also pupae concealed within leafrolls, buds, tree bark or stems of gramineous plants (Bogenschütz 1978; Gauld 1984; Fitton et al. 1988; Hailemichael et al. 1994). Successful parasitism of concealed pupae depends on the efficiency of the host location strategy employed, the morphology of the parasitoid and the physical characteristics of the substrate covering the host.

Vibrational sounding is a host location strategy that has evolved as a means of detecting immobile host stages in concealment in the parasitoid hymenopteran families Orussidae and Ichneumonidae (Broad and Quicke 2000). The wasps thereby transmit vibrations onto solid substrate via their antennae and detect the position of a potential host by analyzing the reflected signals (Wäckers et al. 1998). It is assumed that vibrational sounding is influenced by the physical properties of the substrate that supports the produced waves. Attenuation of vibration increases with distance and the density of the substrate (Dusenbery 1992) and might be impaired in wet wood (Vilhelmsen et al. 2001). The size of the parasitoid can also affect the mechanism of this sensory orientation; for example, larger females perform better

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because they are able to produce vibrations of higher intensity (Otten et al. 2001).

Ovipositor morphology is correlated with the hardness of the substrate that must be penetrated (Quicke et al. 1994, 1999; Gerling et al. 1998). The depth down to which hosts can be reached is limited by ovipositor length (Heatwole and Davis 1965; Hanks et al. 2001; Sivinski et al. 2001). Short and robust ovipositors can withstand a greater piercing force while long and thin ovipositors may start to buckle (Quicke et al. 1994, 1999). In *P. turionellae* (Ichneumonidae), females take a longer time to drill into pupae with hard cuticle and desist from their efforts if the cuticle gets too hard (Jackson 1937). The total number of ovipositor insertions by this species decreased remarkably as substrate density (paper weight) increased from 17 g m⁻² to 48–80 g m⁻² (Wäckers et al. 1998). While some studies are available on the morphological adaptation of parasitoid ovipositors and oviposition behavior, there is no information yet if and how vibrational sounding is adapted to the varying characteristics of the host microhabitat, such as the mechanical resistance of the substrate. The ability to detect and reach hosts in different solid substrates is determined by the level of specialization that is reflected in behavioral and morphological adaptation. We propose that, within the studied parasitoid guild, these factors have a substantial impact on the parasitism efficiency of hosts in natural, agricultural and forest habitats.

In the present study, we investigated the effect of substrate density and thus mechanical resistance on host location efficiency and responsiveness of two pimpline pupal parasitoids. *Pimpla turionellae* (L.) is a solitary endoparasitoid of a wide variety of lepidopteran species mainly living in woodland, hedgerows and orchards (Meyer 1925; Jackson 1937; Führer 1975; Mani et al. 1986). It attacks exposed as well as hidden pupae in diverse microhabitats including cocoons, leaf rolls, tree bark or buds and shoots of pine (Bogenschütz 1978; Fitton et al. 1988). *Xanthopimpla stemmator* (Thunberg) (Ichneumonidae) is specialized on stemborers. It parasitizes mainly lepidopteran pupae concealed in stems of gramineous plants such as maize, sugarcane or sorghum (Hailemichael et al. 1994; Moore and Kfir 1996). In order to characterize host location behavior over a wide range of substrate densities and to determine the upper limit beyond which the insects cease to show oviposition behavior, we used a paper model of a plant stem that evokes searching and oviposition behavior in both species (e.g., Hailemichael et al. 1994; Fischer et al. 2001; Otten et al. 2001). By the use of increasing paper densities we intended to simulate the attenuation of vibrations in natural plant substrates (Dusenbery 1992). We expected that not only responsiveness and number of insertion attempts would decrease with increasing substrate density but that vibrational sounding and therefore host location precision would be impaired accordingly. If vibrational sounding is adapted to the host microhabitat, then host location efficiency, i.e.,

insertion activity and precision, should be different in parasitoid species with various degrees of host-niche specialization. We tested this hypothesis and discussed the results on the basis of ovipositor morphology and body weight of parasitoids, as well as in the context of the different microhabitats of their host insects.

Materials and methods

Parasitoid rearing

The laboratory strain of *P. turionellae* originated from insects obtained from Hermann Bogenschütz at the Forest Research Institute of Baden-Wuerttemberg, Germany in 1994. *X. stemmator* was obtained from Marianne Alleyne at the University of Illinois at Urbana-Champaign, USA, in 2000. Subsequently, both parasitoid species were reared on pupae of the wax moth *Galleria mellonella* L. Adults were kept in Plexiglas containers (25×25×25 cm). *P. turionellae* was reared at 15°C, 70% relative humidity and a photophase of 16L:8D, while *X. stemmator* was reared at 23°C, 70% relative humidity during the day and at 15°C, 80% relative humidity during the night, with a photophase of 16L:8D. Wasps were fed with honey and water and were allowed to mate. Starting at an age of 5–7 days in *P. turionellae* and about 1 month in *X. stemmator*, parasitoids were provided with host pupae for oviposition and host-feeding. *X. stemmator* accepted only pupae hidden within paper rolls, whereas *P. turionellae* parasitized both covered and uncovered pupae. After having been exposed to the wasps for 3–5 h, parasitized pupae were stored at 24°C, 60% relative humidity and a photophase of 16L:8D until emergence of adults, typically after 3 weeks. Female *P. turionellae* had a lifespan of 1–2 months and female *X. stemmator* lived up to 4 months.

Experimental set-up

Ichneumonid parasitoids innately attempt to insert their ovipositors into various hollow rounded substrates. In the field, their hosts are concealed inside of leaf rolls or plant stems. Paper cylinders containing a solid section imitating a host pupa are therefore ideal experimental models. They have been used in a number of studies on host location in ichneumonid species (e.g., Henaut and Guerdoux 1982; Wäckers et al. 1998; Fischer et al. 2001; Otten et al. 2001). Since these models exclude visual and chemical host-related cues, any directed orientation of the parasitoids can be attributed to vibrational sounding (Otten et al. 2001).

Hollow cylinders (125×8 mm) were formed of white paper of seven different categories between 45 and 590 g m⁻² (referred to as 45-g and 590-g paper, respectively) (Table 1). Paper thickness was

Table 1 Density, thickness and force (mean±SD) required for penetration of the test paper categories as well as mechanically hollowed maize stems with minutens pins

Paper	Density (g m ⁻²)	Thickness (mm)	Penetration force (N)
ELCO Atlantic clipper air mail paper	45	0.06	0.3±0.02
Artoz Poseidon rag paper	110	0.18	0.7±0.03
Bristol cardboard	180	0.19	2.2±0.1
Bristol cardboard	280	0.27	3.8±0.2
Bristol cardboard	340	0.33	5.0±0.4
Bristol cardboard	440	0.39	7.2±0.4
Bristol cardboard	590	0.54	-
Maize stem (hollow)	~150	~2	0.30±0.08

Penetration force is significantly correlated with paper density (Pearson's correlation; $r=0.99$, $z=20.1$, $P<0.001$)

measured with an outside micrometer (Mitutoyo, Japan). Paper weight and the force needed for penetration of the test papers at right angles with minutens pins (0.14 mm diameter) were measured with a Mettler Toledo MT 5 balance (accuracy: ± 0.01 mg). Minutens pins were selected according to the shape and size of the ovipositor tip of the two parasitoid species. The measured penetration force was strongly correlated with paper density (Pearson's correlation; $r=0.99$, $z=20.1$, $P<0.0001$). Additionally, the density, thickness, and penetration force was evaluated for mechanically hollowed maize stems with the same methods as described for the measurement of different paper categories (Table 1).

The cylinders were offered with either a host mimic or a living host pupa. Cylinders of densities ranging from 45 up to 280 g m⁻² contained a cigarette filter (15×8 mm; Gizeh, Germany) as a solid section imitating a host pupa within a leaf roll or a plant stem (Otten et al. 2001). The filter was shifted out of the cylinder center to rule out effects of a possible preference of the wasps for this position. Both ends of the cylinder were left open. The maximal substrate density allowing for ovipositor insertion was determined with cylinders of high density ranging from 340 to 590 g m⁻². To attain a high response of the parasitoids, living *G. mellonella* pupa were hidden instead of host mimics. The ends of the cylinders with living hosts were plugged with pieces of cotton to prevent the searching parasitoids from entering.

For the trials, female parasitoids were placed individually into Plexiglas containers (18.5×8.5×7.5 cm) and exposed to a single cylinder of one of the seven densities. Paper cylinders were offered horizontally for *P. turionellae* and vertically for *X. stemmator* because of the known spatial preference of this species (Hailemichael et al. 1994). Females had access to the models with a host mimic for a period of 24 h and to the models with the hosts for 1 h. To quantify the behavioral response, the paper cylinder was subdivided into 34 sections (width: 3.7 mm) with a solid zone from sections 20–23 (host mimic with a constant length of 15 mm) or sections 19–23 (length of living host typically from >15–18 mm). At the end of an experiment the frequency and location of ovipositor insertions were scored and the parasitoid individuals were weighed (Mettler Toledo MT 5 balance; accuracy: ± 0.01 mg). The sample size for each parasitoid species in each density category consisted of 40 female wasps in trials with host mimics, and of 20 female wasps in trials with living hosts. Each parasitoid individual was used for one trial session only. Experiments with different substrate densities were randomly distributed over the observation period.

Experiments with *P. turionellae* females were carried out in a climate chamber at 20°C and 60% relative humidity with 2- to 3-week-old individuals of 37 ± 6 mg mean body weight (\pm SD). Experiments with 6- to 8-week-old *X. stemmator* females were conducted at 23°C and 70% relative humidity with individuals of 35 ± 5 mg mean body weight. Females of both species were provided with food ad libitum and had previous oviposition experience with pupae wrapped in cigarette paper (13 g m⁻²).

Ovipositor morphology

Body length (head to end of abdomen), length of ovipositor without sheaths, and diameter of the ovipositor at half length were measured for both parasitoid species under a binocular microscope using a calibrated ocular grid. For this, random samples of insects from the laboratory rearings conserved in 70% alcohol were examined.

Data analysis

Data analysis for the trials with the living host could be carried out only for *X. stemmator*, and only on 340-g and 440-g paper as the species failed to respond to 590-g paper. *P. turionellae* did not respond at all under the conditions of this trial.

The general responsiveness of the wasps to host mimics in cylinders of different paper densities, i.e., the number of individuals inserting their ovipositor *versus* the inactive ones, was analyzed for

significant differences and compared between the species using X²-tests. For the trials with living hosts, a X²-test was used to examine for significant differences in the general responsiveness of *X. stemmator* to the 340-g and 440-g paper. The effect of female body weight on the responsiveness to models with host mimic or living host was tested for each paper density using unpaired *t*-tests.

Subsequently, the effect of paper density (factor 1) and species (factor 2) on the number of ovipositor insertions into the model with the host mimic was tested by a two-way analysis of variance (ANOVA). One-way ANOVA was used to test the influence of the paper density on the number of ovipositor insertions of a single species. Ovipositor insertions on different paper densities were compared pairwise using a Student-Newman-Keuls post-hoc test. A Kruskal-Wallis test was carried out to include individuals which showed no response (no insertion) into analysis of the effect of paper density on the number of ovipositor insertions. The numbers of ovipositor insertions by *X. stemmator* into 340-g and 440-g paper cylinders with living hosts were compared for significant differences using a Mann-Whitney *U*-test, including individuals which did not respond (no insertion).

Finally, the precision of location of the host mimic or of the living host, specified by the distribution of ovipositor insertions on the paper rolls, was compared between the paper densities and, for the model with the host mimic between the two species, by an equality of variances *F*-test.

In order to describe and compare ovipositor morphology, data from 30 females per species was collected. A ratio of ovipositor length to body length was computed. Body lengths and index values were compared between the species using unpaired *t*-tests. The relationships between one and another in the variables body length, ovipositor length, and ovipositor diameter were analyzed for both species by linear regression analysis. Differences in the relationship of the three parameters to each other were tested by comparing the regression slopes in the two species using *z*- or *t*-statistics according to equality or non-equality of variances (Sachs 1992).

Results

General responsiveness

Substrate density of the cylinder model containing a host mimic significantly influenced responsiveness in both parasitoid species. Responsiveness, i.e., the proportion of females that responded to the model with ovipositor insertion versus those that did not respond, was different in all pairwise comparisons made between different substrate densities except for the two lowest densities (45-g and 110-g paper) (Table 2). Further, the two ichneumonid species differed in their responsiveness to a given substrate density except for the lowest density tested (Fig. 1; X²-tests; 45 g: X²=2.81, *df*=1, *P*=0.09; 110 g: X²=9.04, *df*=1, *P*=0.003; 180 g: X²=16.36, *df*=1, *P*<0.0001; 280 g: X²=6.14, *df*=1, *P*=0.01). While ovipositor insertions were observed in 73% of *P. turionellae* and 88% of *X. stemmator* females on the cylinders of the lowest density (45-g paper), only 2.5% of *P. turionellae* and 20% of *X. stemmator* females inserted their ovipositor into cylinders of the highest density (280-g paper).

Females of the species *X. stemmator* responded to high-density cylinder models containing living host pupae. Twelve *X. stemmator* females perforated the 340-g paper and five individuals pierced the 440-g paper. This difference in general responsiveness was significant (X²-test:

Table 2 Comparison of responsiveness (females inserting their ovipositor at least once into the model versus non-responding individuals) in *Pimpla turionellae* and *Xanthopimpla stemmator* females to the four different categories of substrate density (45-g, 110-g, 180-g, 280-g paper) used in the model with host mimic (X^2 -test; $df=1$ for all comparisons, $n=40$ per category of substrate density and per species)

Substrate density (g m ⁻²)		45	110	180
<i>Pimpla turionellae</i>				
110	X ²	0.52	-	-
	P	0.47		
180	X ²	20.05	14.68	-
	P	<0.001	<0.001	
280	X ²	41.81	34.94	7.31
	P	<0.001	<0.001	<0.01
<i>Xanthopimpla stemmator</i>				
110	X ²	0.56	-	-
	P	0.46		
180	X ²	4.59	7.81	-
	P	<0.05	<0.01	
280	X ²	36.66	42.72	18.34
	P	<0.001	<0.001	<0.001

$X^2=5.01$, $df=1$, $P=0.03$). No individual perforated the 590-g paper during the observation period of the experiments, but we observed a female repeatedly drilling into this high-density paper when given 3 h time. In contrast, the species *P. turionellae* did not respond to cylinder models of high substrate density (340-g to 590-g paper).

Influence of body weight on responsiveness

The mean body weight of responding and non-responding female *P. turionellae* was compared for all substrate densities except for the 280-g paper where only one

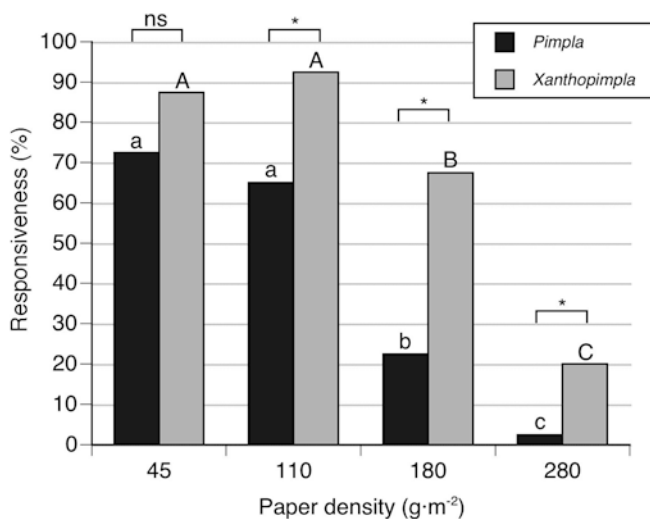


Fig. 1 Responsiveness of female *Pimpla turionellae* and *Xanthopimpla stemmator* ($n=160$ per species) to filter models of four different paper densities. Asterisk indicates significant difference ($P<0.05$) between species (X^2 -tests). Significant differences ($P<0.05$) within each species are indicated by different letters (uppercase for *X. stemmator*, lowercase for *P. turionellae*) (X^2 -tests)

female was responding. No significant differences could be detected (unpaired t -test; 45 g: $t=0.5$, $df=38$, $P=0.62$; 110 g: $t=0.8$, $df=38$, $P=0.41$; 180 g: $t=-0.3$, $df=38$, $P=0.8$). Likewise, no significant differences in mean body weight of responding and non-responding female *X. stemmator* could be detected in tests for models with host mimic (unpaired t -test; 45 g: $t=-1.7$, $df=38$, $P=0.1$; 110 g: $t=-0.6$, $df=38$, $P=0.55$; 180 g: $t=-0.4$, $df=38$, $P=0.67$; 280 g: $t=1.9$, $df=38$, $P=0.06$) and models with living host (unpaired t -test; 340 g: $t=-0.13$, $df=18$, $P=0.9$; 440 g: $t=-1.5$, $df=18$, $P=0.2$).

Number of ovipositor insertions

The analysis of pooled data showed that the mean number of ovipositor insertions on the model with host mimic was significantly influenced by substrate density (factor 1) but not by species (factor 2) (two-way ANOVA; factor 1: $F=10.3$, $df=2,157$, $P<0.0001$; factor 2: $F=1.18$, $df=1,157$, $P=0.28$). When each species was analyzed separately, the significant influence of substrate density on the number of ovipositor insertions was confirmed for both *P. turionellae* (one-way ANOVA; $F=6.3$, $df=2,61$, $P=0.003$) and *X. stemmator* (one-way ANOVA; $F=5.3$, $df=3,103$, $P=0.002$). The non-parametric approach including not responding individuals also showed the significant influence of substrate density on the number of insertions (Kruskal-Wallis test; *P. turionellae*: H corr. for ties = 61.8, $df=3$, $P<0.0001$; *X. stemmator*: H corr. for ties = 68.0, $df=3$, $P<0.0001$). Pairwise comparisons of the mean number of insertions per female *P. turionellae* revealed significant differences between the low-density substrates (45-g and 110-g paper) and the intermediate-density substrate (180-g paper; data not sufficient for the 280-g paper). For *X. stemmator* significant differences could be detected between the substrate of lowest density (45-g paper) and substrates of intermediate density (180-g and 280-g paper) as well as between 110-g and 280-g paper (Student-Newman-Keuls ANOVA post-hoc test, significance level 5%) (Fig. 2). The mean number of ovipositor insertions of *X. stemmator* differed significantly between the two high-density substrates containing living pupae (340-g and 440-g paper; analysis included zero values; Mann-Whitney U -test; $U=127.0$, number of ties = 5, $P=0.05$).

Precision of host location

The two parasitoid species differed in their precision of host location on distinct substrate densities (Table 3). Variance in the location of ovipositor insertions was significantly smaller in *X. stemmator* than *P. turionellae* on all substrate densities (280-g paper was not tested due to an insufficient amount of data) (Equality of variances F -test; 45 g: $F=2.1$, $df=29,36$, $P=0.05$; 110 g: $F=3.6$, $df=25,37$, $P<0.001$; 180 g: $F=6.1$, $df=8,26$, $P=0.006$).

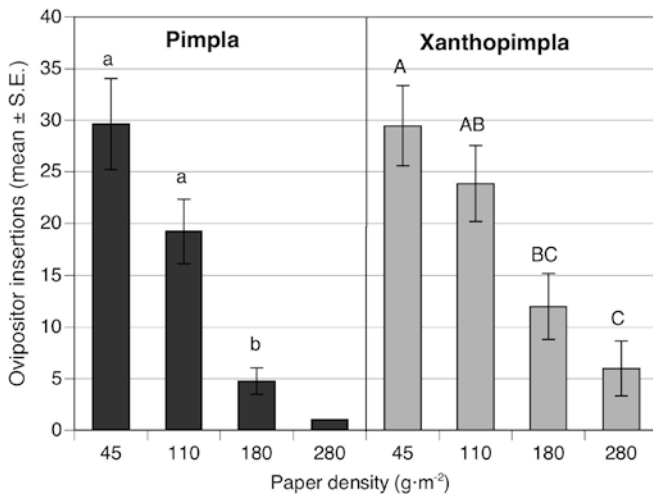


Fig. 2 Total number of ovipositor insertions (mean \pm SE) of female *Pimpla turionellae* ($n=65$) and *Xanthopimpla stemmator* ($n=107$) on filter models of four different paper densities. Significant differences ($P < 0.05$) within each species are indicated by different letters (uppercase for *X. stemmator*, lowercase for *P. turionellae*) (Student-Newman-Keuls ANOVA post-hoc test)

Precision of host location depended on substrate density (Fig. 3). Both species located the host mimic best within the 110-g paper (Table 3). In *X. stemmator*, the distribution of ovipositor insertions was significantly different between 45-g and 110-g paper (F -test; $F=2.3$, $df=36,37$, $P=0.01$), between 110-g and 180-g paper (F -test; $F=0.2$, $df=37,26$, $P < 0.0001$) and between 110-g and 280-g paper (F -test; $F=0.2$, $df=37,7$, $P=0.007$). The precision of host location decreased from low substrate densities (45-g and 110-g paper) to intermediate substrate densities (180-g and 280-g paper) in both species, and in the most obvious way in *P. turionellae*. The distribution of ovipositor insertions in this species was significantly different between 45-g and 180-g paper (F -test; $F=0.17$, $df=29,8$, $P=0.007$) and between 110-g and 180-g paper (F -test; $F=0.13$, $df=25,8$, $P=0.003$).

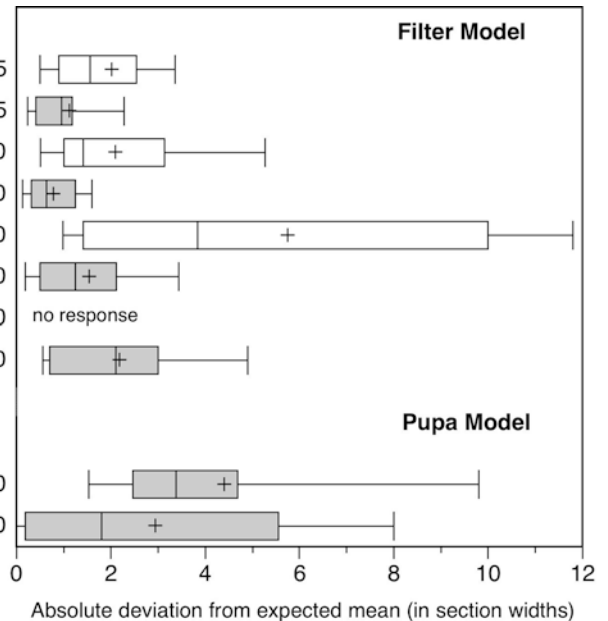


Fig. 3 Deviations of individual mean insertion locations (absolute values) of *Pimpla turionellae* and *Xanthopimpla stemmator* from the expected mean location in treatments with different paper densities (for the number of responding females see Table 3). The expected mean location corresponds to the center of the area covered by a cue (filter or pupa, respectively). Box shows 25th–75th percentile with median (vertical line) and mean (+); error bars show 10th and 90th percentiles. Units on the x-axis are equivalent to section widths on the paper cylinder

Variance in the location of ovipositor insertions of *X. stemmator* on models with living hosts was not significantly different between 340-g and 440-g paper (F -test; $F=2.9$, $df=11,4$, $P=0.23$).

Ovipositor morphology

The body length of *P. turionellae* was 11.9 ± 1.13 mm (mean \pm SD), the ovipositor length was 5.6 ± 0.46 mm,

Table 3 Ovipositor insertions of *P. turionellae* (P) and *X. stemmator* (X) in the treatments with different substrate densities

Species	Paper (g m ⁻²)	n	Ins \pm SD	Exp mean	Act mean \pm SD	Var	Dev \pm SD
X	45	40/37	28.62 \pm 22.79	21.5	20.71 \pm 1.51	2.28	-0.79 \pm 1.51
P	45	40/30	28.73 \pm 23.90	21.5	19.75 \pm 2.17	4.69	-1.75 \pm 2.17
X	110	40/38	23.42 \pm 22.17	21.5	21.62 \pm 1.00	0.99	+0.09 \pm 0.99
P	110	40/26	19.23 \pm 15.75	21.5	19.48 \pm 1.89	3.57	-2.02 \pm 1.89
X	180	40/27	12.00 \pm 16.57	21.5	22.02 \pm 2.13	4.54	+0.52 \pm 2.13
P	180	40/09	4.78 \pm 3.90	21.5	16.33 \pm 5.26	27.66	-5.17 \pm 5.26
X	280	40/08	6.00 \pm 7.62	21.5	22.78 \pm 2.56	6.57	+1.28 \pm 2.56
P	280	40/01	Insufficient data				
X	340	20/12	4.42 \pm 3.75	21	21.45 \pm 5.78	33.41	+0.46 \pm 5.78
P	340	20/-	No response				
X	440	20/05	3.20 \pm 1.64	21	23.96 \pm 3.39	11.51	+2.96 \pm 3.39
P	440	20/-	No response				
X	590	20/-	No response				
P	590	20/-	No response				

Mean number of insertions per female within 24 h for 45- to 280-g paper cylinders hiding a host mimic, and within 1 h for 340- to 590-g paper cylinders hiding a living host pupa (Ins)

Exp mean expected mean location, Act mean actual mean location, Var variance, Dev mean deviation from expected mean, SD standard deviation, n total sample size/number for responding females

and the ovipositor diameter was 0.20 ± 0.02 mm. *X. stemmator* had a body length of 12.2 ± 1.03 mm, an ovipositor length of 5.5 ± 0.55 mm, and an ovipositor diameter of 0.17 ± 0.01 mm.

The ratio of ovipositor length to body length differed significantly between the two species (unpaired *t*-test; $t = 2.59$, $df = 61$, $P = 0.01$), as *P. turionellae* had a longer ovipositor relative to the body length. Body length did not differ significantly between species (unpaired *t*-test; $t = -1.0$, $df = 61$, $P = 0.3$). Body length (bl), ovipositor length (ol), and ovipositor diameter (od) were strongly related in both species (linear regression; *X. stemmator*: bl versus ol: $y = 0.81 + 0.39x$, $r^2 = 0.52$, $F = 32.6$, $df = 1,30$, $P < 0.0001$; bl versus od: $y = 0.07 + 0.01x$, $r^2 = 0.53$, $F = 30.0$, $df = 1,27$, $P < 0.0001$; ol versus od: $y = 0.08 + 0.02x$, $r^2 = 0.63$, $F = 46.6$, $df = 1,27$, $P < 0.0001$; *P. turionellae*: bl versus ol: $y = 1.23 + 0.37x$, $r^2 = 0.80$, $F = 112.4$, $df = 1,29$, $P < 0.0001$; bl versus od: $y = 0.05 + 0.01x$, $r^2 = 0.69$, $F = 63.0$, $df = 1,28$, $P < 0.0001$; ol versus od: $y = 0.03 + 0.03x$, $r^2 = 0.70$, $F = 64.0$, $df = 1,28$, $P < 0.0001$).

When regression coefficients were compared between species significant differences could be detected for the relationship of body length and ovipositor length (slope comparison by *z*-statistics; $z = 2.62$, $P = 0.01$), as well as for the relationship of ovipositor diameter and body length (slope comparison by *t*-statistics; $t = 2.96$, $P = 0.01$). No significant difference in regression coefficients was found for the relationship of body length and ovipositor diameter (slope comparison by *t*-statistics; $t = 0.34$, $P = 0.74$).

Discussion

The host-location efficiency of *P. turionellae* and *X. stemmator* was strongly influenced by the density of the substrate covering the host mimic or living host. Increasing substrate density, i.e., increasing density of paper cylinder models containing the hidden host mimic, resulted in a decrease in the proportion of parasitoid females responding with ovipositor insertions as well as in the total number of ovipositor insertions. In general, the precision of insertion on the host mimic decreased with increasing mechanical resistance. The impact of substrate density on parasitoid performance became most obvious when low-density paper (45-g and 110-g) was compared to intermediate-density paper (180-g and 280-g). A previous investigation could not detect any influence of substrate density on distribution of ovipositor insertions in *P. turionellae* (Wäckers et al. 1998); however, it used only low-density paper (up to a maximum of 80-g) that obviously did not impair vibrational sounding. Body weight of parasitoids did not affect the results of our experiments. The females tested in our study had a relatively uniform body weight mainly within the range of the "large individuals" reported by Otten et al. (2001) for *P. turionellae*. We therefore consider that changes in parasitoid activity and performance

were solely due to properties of the substrate used in the experiments.

In *P. turionellae*, the decrease in responsiveness, number of ovipositor insertions and precision of host location from low-density substrate (45-g and 110-g paper) to intermediate-density substrate (180-g paper) was particularly obvious, with 65–73% responsiveness to low-density paper and a maximum of 23% to intermediate-density paper. With the exception of one individual, females of this species did not respond to models made of 280-g paper. Whereas the spatial distribution of ovipositor insertions on low-density substrate concentrated on the host mimic, the insertions on 180-g paper were distributed over the whole cylinder excluding the end sections. This suggests that the limit of vibrational sounding in this species lies between a substrate density represented by 110- to 180-g paper. The response of *P. turionellae* was not enhanced when host mimics were replaced by living hosts. Not a single individual perforated the models with living hosts made of 340-g paper and above. The wasps showed searching behavior on the cylinders but no probing or drilling attempts. The finding that *P. turionellae* did not try to drill through substrate of such a high density could on the one hand be due to an inability to locate the hidden host or host mimic because of stronger attenuation of pulses created during vibrational sounding. On the other hand, the penetration of such a dense substrate could be hindered by morphological constraints of the ovipositor and limitations in the applicable piercing force that is supposed to be energetically costly for the parasitoid. Our finding of the strong correlation between piercing force and paper density is consistent with that of Hagley et al. (1993) who demonstrated that the ichneumonid parasitoid *Phygadeuon wiesmanni* required higher piercing force with increasing thickness and hardness of the wall of puparia of the apple maggot. If *P. turionellae* females had been able to locate the host or its mimic by vibrational sounding, they should at least have shown probing attempts. However, this was not the case. The observed behavior is comparable to the lacking response of the females to empty cylinders without a hidden host mimic (Fischer et al. 2001).

In the field, it is likely that *P. turionellae* reaches its concealed hosts within buds or shoots of trees or beneath bark via pre-existing cracks and fissures without the need to drill on its own. The serrations of the species' ovipositor tip are much less prominent than the strong teeth found, e.g., in rhyssine ichneumonids that are able to drill through several centimeters of wood (Quicke et al. 1999). *P. turionellae* has a longer and thinner ovipositor than *X. stemmator* and can therefore reach hosts deeper within substrate. On the other hand, *P. turionellae* is restricted to less dense substrate as thin ovipositors will start to buckle sooner when penetration force is applied (Quicke et al. 1999). The findings regarding vibrational sounding efficiency and ovipositor morphology consistently indicate that *P. turionellae* is either restricted to the parasitism of hosts covered only by a thin substrate layer

like leaves or cocoons, or to hosts that are accessible by circumventing the concealing layer.

X. stemmator responded significantly better to models of all substrate densities and inserted its ovipositor generally more often than *P. turionellae* did, except for the substrate with the lowest density to which both species responded equally well. The penetration of relatively thick-stemmed grass-like maize requires a piercing force comparable to the force needed for the penetration of the lowest-density paper (45-g) which is easily accomplished by *X. stemmator* (S. Fischer et al., personal observation). Females of this species drilled through all categories of densities in the trials with hosts or their mimics, even through the substrate of the highest density (590-g), provided the observation period was prolonged. While the capability of *X. stemmator* to reach hosts in maize and sugarcane has previously been considered doubtful (Moore and Kfir 1996), our findings suggest that this substrate density does not represent the limit for ovipositor insertion in *X. stemmator* so that even hard stem walls like those of bamboo might be penetrated. Compared to *P. turionellae*, the ovipositor of *X. stemmator* is shorter relative to the body length and presumably more robust. The ovipositor tip of *X. stemmator* is also serrated in a distinctive way. Considering that ovipositor wall thickness is correlated with the hardness of the substrate it has to penetrate (Quicke et al. 1994) the ovipositor of *X. stemmator* seems to be adapted to overcome a dense substrate to reach concealed hosts.

Although the responsiveness of the insects generally decreased with increasing substrate density, more females responded to 340-g paper in models with living host than to 280-g paper in models with host mimic. We propose that the model with the living host pupa elicited a higher responsiveness in *X. stemmator* due to additional cues complementing vibrational sounding, or due to different reflectance of pulses. Pulses reflected during vibrational sounding might have been different between the two models due to the physical characteristics of the distinctive solid sections within the cylinders. Further, additional host location cues might have included vibrations caused by wriggling host pupae as demonstrated in leafminers (Bacher et al. 1997; Meyhöfer et al. 1997), air particle displacement triggered by host vibration (Casas et al. 1998), olfactory cues as shown in *P. turionellae* (Sandlan 1980), and/or thermal radiation from the host's body as described for host finding in the braconid parasitoid *Coeloides brunneri* (Richerson and Borden 1972). Since the model with the living host differed both physically and chemically from the model with the host mimic, a comparison between the behavioral responses to the two models is avoided. The model with the living host was exclusively used to investigate the capacity of *X. stemmator* to insert its ovipositor under conditions of high mechanical resistance of the substrates.

X. stemmator located the host mimic inside the cylinders more accurately than *P. turionellae*, the vari-

ance in insertion locations on the solid section being remarkably low between different substrate categories. Even on 280-g paper the insertion locations were less widely distributed than the insertions of *P. turionellae* on 180-g paper. This indicates that *X. stemmator* may use vibrational sounding more effectively at higher substrate densities. Since the body size of individuals used in the experiments was similar between the two species, *X. stemmator* females might be able to produce vibrations of higher intensity and therefore locate solidity differences more easily. *X. stemmator*, being specialized on stemborers, occupies a relatively small ecological niche to which it appears to be highly adapted.

In each parasitoid species, the efficiency of vibrational sounding depends on the hardness of the substrate the female has to deal with. The substrate density has a negative effect on the females' responsiveness, the number of ovipositor insertions, and host location precision. Comparison between the two species shows that the vibrational sounding of the parasitoids is obviously adapted to the mechanical resistance of the host microhabitat. Host location efficiency and insertion activity reflects the degree of behavioral and morphological adaptation to the parasitism of hosts concealed within substrate of varying hardness. Besides ovipositor morphology and the piercing force needed for substrate penetration during host location, the attenuation of pulses during vibrational sounding can be an energetically costly limiting factor for this adaptation.

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