

# Role of Emerald Ash Borer (Coleoptera: Buprestidae) Larval Vibrations in Host-Quality Assessment by *Tetrastichus planipennis* (Hymenoptera: Eulophidae)

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**ABSTRACT** The biological control agent *Tetrastichus planipennis* Yang (Hymenoptera: Eulophidae) is a gregarious larval endoparasitoid of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an invasive cambium-feeding species responsible for recent, widespread mortality of ash (*Fraxinus* spp.) in North America. *T. planipennis* is known to prefer late-instar emerald ash borer, but the cues used to assess host size by this species and most other parasitoids of concealed hosts remain unknown. We sought to test whether vibrations produced by feeding emerald ash borer vary with larval size and whether there are any correlations between these cues and *T. planipennis* progeny number (i.e., brood size) and sex ratio. The amplitudes and rates of 3–30-ms vibrational impulses produced by emerald ash borer larvae of various sizes were measured in the laboratory before presenting the larvae to *T. planipennis*. Impulse-rate did not vary with emerald ash borer size, but vibration amplitude was significantly higher for large larvae than for small larvae. *T. planipennis* produced a significantly higher proportion of female offspring from large hosts than small hosts and was shown in previous work to produce more offspring overall from large hosts. There were no significant correlations, however, between the *T. planipennis* progeny data and the emerald ash borer sound data. Because vibration amplitude varied significantly with host size, however, we are unable to entirely reject the hypothesis that *T. planipennis* and possibly other parasitoids of concealed hosts use vibrational cues to assess host quality, particularly given the low explanatory potential of other external cues. Internal chemical cues also may be important.

**KEY WORDS** *Agrilus planipennis*, auditory cues, cryptic, vibrokinetics, vibrotaxis

The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), a cambium-feeding buprestid beetle native to Asia, was first detected near Detroit, MI, and Windsor, ON, Canada, in 2002 (Haack et al. 2002). Since then, the species has killed millions of ash (*Fraxinus* spp.) trees in northeastern North America and is expected to kill many millions more over the next decade (Kovacs et al. 2010). Consequently, controlling emerald ash borer is currently one of the most pressing challenges facing forest entomologists on the continent. A classical biological control program involving three hymenopteran parasitoid species associated with emerald ash borer in Asia is currently under way in the northeastern United States (USDA–APHIS 2007). The three species being

reared and released are *Oobius agrili* Zhang & Huang (Hymenoptera: Encyrtidae), an egg parasitoid (Zhang et al. 2005); *Tetrastichus planipennis* Yang (Hymenoptera: Eulophidae), a gregarious larval endoparasitoid (Yang et al. 2006); and *Spathius agrili* Yang (Hymenoptera: Braconidae), a gregarious larval ectoparasitoid (Yang et al. 2005).

Emerald ash borers spend most of their lives as larvae concealed beneath the bark, passing through four instars within the phloem layer. *T. planipennis* and *S. agrili* have been shown to allocate more eggs (both species) and a higher proportion of females (*S. agrili*) to larger (i.e., higher quality) emerald ash borer larvae and may parasitize late instars at a higher rate (*T. planipennis*) than early instars (Liu et al. 2007, Wang et al. 2008, Ulyshen et al. 2010b). These findings are not surprising given the selective advantage of host quality discrimination in parasitoids (Charnov et al. 1981, Charnov and Skinner 1984, Waage 1986).

Although parasitoids of exposed hosts can directly discern host size (Dijkstra 1986), *T. planipennis*, *S. agrili*, and other parasitoids of concealed hosts cannot. How parasitoids of concealed hosts assess host quality remains almost entirely unknown even though they

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have long been known to do so (e.g., Purrington and Uleman 1972). External cues that vary with host quality may be important in some cases. For example, Steidle and Fischer (2000) showed the amount of feces associated with wheat, *Triticum aestivum* L., grains infested by granary weevils, *Sitophilus granarius* (L.), increased with weevil size and strongly influenced the amount of interest exhibited by a parasitoid of the species. Internal chemical cues also may be important. For example, the hemolymph of greater wax moth, *Galleria mellonella* (L.), pupae has been shown to contain chemicals that induce oviposition (Hegdekar and Arthur 1973), but whether the concentrations of such chemicals vary with host size is not known.

Because *T. planipennisi* (Ulyshen et al. 2010a) and *S. agrili* (Wang et al. 2010) do not parasitize inactive emerald ash borer larvae, it seems likely that both species, like other parasitoids of concealed insects (Meyhöfer and Casas 1999, and references therein), rely on vibrations produced during movements or feeding to locate hosts. If such vibrations are important in locating hosts, they also may be used to assess host quality. There is some evidence that vibrational cues vary with host size in wood-boring beetles. For example, in a comparison of sounds produced by different *Anoplophora glabripennis* (Motschulsky) larval stages, Mankin et al. (2008) found first and second instars produce less vibrational energy on average than later instars. To our knowledge, however, no previous efforts have been made to determine whether such differences are used by parasitoids to distinguish host size.

Here, we present the results from a laboratory study designed to test this idea by exposing *T. planipennisi* to emerald ash borer larvae of a wide range of sizes from which accelerometer recordings had been made of their movements and feeding activity just before exposure. We hypothesized that 1) *T. planipennisi* progeny number (i.e., brood size) or the proportion of female progeny would increase with increasing initial host weight, 2) the amplitude or rate of vibrational impulses produced by feeding emerald ash borer larvae would vary with larval weight, and 3) there would be significant correlations between *T. planipennisi* progeny data and emerald ash borer vibration data.

### Materials and Methods

**Parasitoids.** A laboratory colony of *T. planipennisi*, originally collected in 2008 from Liaoning province of China, was used in this study. Only naïve wasps that had not been presented with hosts were used in the experiment. They were 3–6 wk old at the time of use and were presumed to have mated as mating activities were observed almost immediately after female emergence and both sexes were held together before being exposed to hosts.

**Emerald Ash Borer Larval Bioassays.** In total, 102 field-collected emerald ash borer larvae at various stages of development (i.e., second–fourth instars) were individually weighed, measured (i.e., head

width), and inserted into 10-cm-long large-diameter ash sticks ( $3.8 \pm 0.05$  cm; range, 2.6–5.1 [note that this is within the range of diameters used by *T. planipennisi* in the field]) and held in an incubator (25°C,  $\approx 75\%$  humidity, and a photoperiod of 16:8 [L:D] h) for 24–36 h before recordings were made. This length of time was chosen based on the previous observation that almost all emerald ash borer larvae feed actively within the first day after insertion into sticks (M.D.U., personal observations). To reduce fungal growth, the sticks were first gently scrubbed under running tap water, sealed at both ends with paraffin, held in a 0.05% bleach bath for  $\approx 5$  min, and rinsed with running tap water for 15 min. One larva was inserted into a narrow groove chiseled beneath a small flap of bark peeled from one end of each stick. The bark flaps were then held closed over the inserted larvae with thin strips of Parafilm. The heads of the inserted larvae faced away from the nearest ends of the sticks (always pointing downward) to encourage feeding in the direction with the greatest available host resource.

Immediately before recording, a drill was used to insert a 1.59-mm-diameter titanium bit into the end of each stick near the bark flap. A steel flange was attached to the bit with a screw, and an accelerometer was connected to the flange by a magnetic attachment (Mankin et al. 2008). The stick was placed on a piece of foam insulation on a table in a quiet office with air conditioning turned off to reduce external vibrations. Signals of larval movement and feeding vibrations detected by the accelerometer were passed through a charge amplifier and monitored with headphones connected to a dual-channel, digital audio recorder sampling at 44.1 kHz (24 bits). For each larva tested, an interval of  $\approx 180$  s, relatively free of interfering background noise, was stored on a computer with digital signal processing software for further analysis.

**Digital Signal Processing and Classification.** To facilitate identification of individual sound impulses produced during larval activity (Mankin et al. 2008), the stored signals were band-pass filtered between 0.2 and 5 kHz by using Raven 1.3 software (Charif et al. 2008). The impulses were identified and counted using customized Digitize, Analyze and Visualize Insect Sounds (DAVIS) software (Mankin et al. 2000). The times of impulses and their amplitudes (vibration levels measured in dB between 0.2 and four kHz, see Mankin and Benshemesh 2006) were saved in a spreadsheet. Mean rates of impulses and mean vibration amplitudes of impulses were calculated for each recording.

**Parasitoid Exposures.** Each stick from which vibrations were detected ( $n = 94$ ) was placed in a 710-ml clear plastic drinking cup (GFS.com), and five female *T. planipennisi* were added. The sticks were held upright using tacks punched through the bottoms of the cups. The cup openings were covered by fine screen held in place by lids in which 5.8-cm-diameter circular openings had been made. The cups were held in an incubator (25°C,  $\approx 75\%$  humidity, and a photoperiod of 16:8 [L:D] h) with drops of honey (i.e., food for the wasps) added to the tops of the screens (Ulyshen et al.

2010a). After 2 d, all *T. planipennis* were removed from the cups. If some were in the act of parasitizing at that time, they were given additional time to finish before being removed, never taking >2 h. Based on previous experience, 2 d is sufficient time to get high parasitism rates at a 5:1 parasitoid:host ratio (M.D.U., personal observations). We avoided longer exposure periods to be more confident about any observed relationship between initial host weight and wasp progeny number. Because a group of five females instead of a single individual was added to each cup, it is possible that more than one female contributed to each parasitized host, but we consider this unlikely considering the short exposure period. Although a 1:1 parasitoid:host ratio would have been preferable, previous experience has shown that parasitism by a single *T. planipennis* female is very unreliable, especially within a period of only two days (M.D.U., personal observations). Consequently, using small groups of females and a 2-d exposure period was a necessary compromise given the objectives of the study and the realities of working with *T. planipennis*. Two days after removing the wasps, the sticks were peeled to recover the larvae. Each emerald ash borer larva was placed in an individual Falcon petri dish (50 by 9 mm with tight-fit lid) lined with moistened filter paper and observed for parasitism. Forty-six (i.e., 49%) of the larvae were parasitized, with developing wasp larvae clearly visible beneath the cuticle. After the *T. planipennis* progeny exited the host, they were counted. Three broods failed to exit the host and were too decomposed upon dissection to count. The sex ratio was calculated for 33 broods (i.e., accurate counts could not be made for all 43 broods due to larval mortality caused primarily by fungal) once the developing progeny reached the pupal stage.

**Data Analysis.** *T. planipennis* sex ratio, emerald ash borer head width and weight, stick diameter, and sound impulse rate were  $\log(x)$ ,  $(x^{2.45} - 1)/2.45$ , square root( $x$ ),  $\log(x + 1)$ , and  $\log(x)$  transformed for normality, respectively (Sokal and Rohlf 1995). The transformation for head width was determined using the Box-Cox power transformation method (Sokal and Rohlf 1995). These transformed data were used in all subsequent analyses. All statistical analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC). To determine how emerald ash borer vibration amplitude and impulse rate varied with head width, weight and stick diameter, separate multiple linear regression analyses were performed on a data set consisting of the 94 emerald ash borer larvae that produced detectable vibrations. When significant correlations were not detected, analyses of variance were conducted after evenly assigning the larvae to three size classes (i.e., instars could not be determined with certainty) based on weight ("small" [ $<0.0236$  g;  $n = 31$ ], "medium" [ $0.0236-0.051$  g;  $n = 31$ ], and "large" [ $>0.051$  g;  $n = 32$ ]) to test the null hypothesis that there were no significant differences in vibration impulse amplitude or rate among the three size classes. If the null hypotheses were rejected, means were further separated using the Tukey-Kramer method. Similarly, to deter-

mine how *T. planipennis* progeny number and sex ratio varied with emerald ash borer head width, weight, stick diameter, and vibration impulse amplitude and rate, the same analyses as described above were performed on a data set consisting of the 43 emerald ash borer larvae from which *T. planipennis* progeny data were collected. The larvae were once again evenly assigned to size classes ("small" [ $<0.031$  g;  $n = 14$ ], "medium" [ $0.031-0.0534$  g;  $n = 14$ ] and "large" [ $>0.0534$  g;  $n = 15$ ]) for analyses of variance.

## Results

**Vibration Characteristics.** Larvae of all tested sizes produced 3–30-ms impulses of various amplitudes and rates, similar to those in previous studies of insect larval vibrations in wood (e.g., Mankin et al. 2008). Examples of an 80-s period of signals from a 9.5-mg larva and a 93-mg larva are seen in Fig. 1. Several closely spaced impulse bursts in the signal trace from the 93-mg larva are indicated by arrows, where bursts are defined as series of impulses separated by  $<0.25$  s, Mankin et al. (2008). A 60-ms expansion of the first marked burst is seen in the inset of Fig. 1. The range of amplitudes and the intervals between the three impulses in the inset is typical of the range observed for other larvae as well. Both of these larvae served as successful hosts for *T. planipennis*, with 29 progeny emerging from the 9.5-mg larva and 32 emerging from the 93-mg larva. The frequency spectra of the vibration impulses varied according to the density, elasticity, and hardness of the ash sticks, as well as their diameters, as noted in Mankin et al. (2008).

**Vibration Impulse Amplitude and Rate in Relation to Emerald Ash Borer Size and Stick Diameter.** The three parameters of the regression model (i.e., head width, larval weight and stick diameter) accounted for only 19.3 and 8.7% of the variance in vibration impulse rate ( $F = 7.2$ ;  $df = 3, 90$ ;  $P < 0.01$ ) and amplitude ( $F = 2.9$ ;  $df = 3, 90$ ;  $P = 0.04$ ), respectively. There were no significant correlations between either vibration variable and head width or larval weight (data not shown), but vibration impulse rate was significantly correlated with stick diameter ( $t = -4.13$ ,  $P < 0.01$ ). According to analysis of variance (ANOVA), there were no significant differences in vibration impulse rate among larval weight classes ( $F = 1.1$ ;  $df = 2, 91$ ;  $P = 0.4$ ), but there were differences in vibration amplitude among larval weight classes ( $F = 4.4$ ;  $df = 2, 91$ ;  $P = 0.02$ ), being significantly higher from large hosts than small hosts (Fig. 2).

**Parasitoid Progeny Number and Sex Ratio in Relation to Emerald Ash Borer Size, Vibrations, and Stick Diameter.** The five parameters of the regression model (i.e., head width, larval weight, stick diameter, vibration amplitude, and vibration impulse rate) accounted for 26.8 and 27.0% of the variance in progeny number ( $F = 2.71$ ;  $df = 5, 37$ ;  $P = 0.03$ ) and sex ratio ( $F = 2.00$ ;  $df = 5, 27$ ;  $P = 0.11$ ), respectively. There was a significant positive correlation between progeny number and stick diameter ( $t = 2.59$ ,  $P = 0.01$ ), and a strong, although not significant, positive correlation

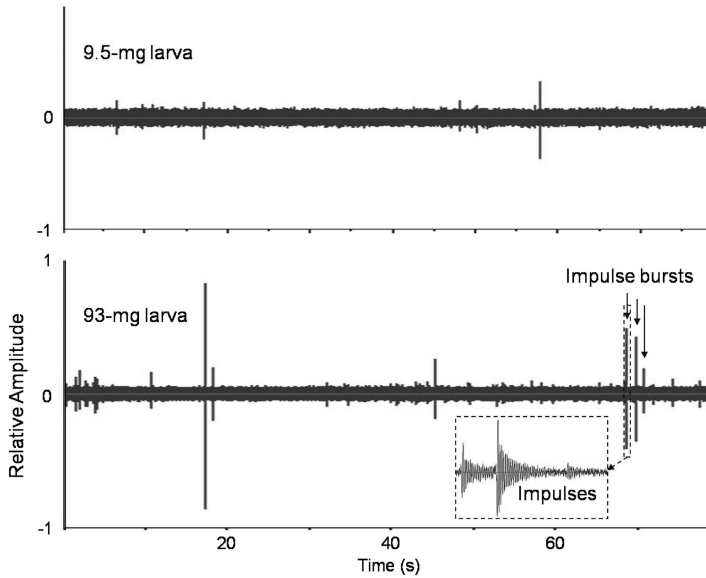


Fig. 1. Signal traces of 80-s intervals recorded from a 9.5- and a 93-mg emerald ash borer larva, with three impulse bursts marked by arrows, and a 60-ms inset showing three individual impulses in the first marked burst.

between female:male sex ratio and host weight ( $t = 1.82, P = 0.08$ ). According to ANOVA, there were no significant differences in progeny number among larval weight classes ( $F = 1.65; df = 2, 40; P = 0.2$ ), even though large hosts produced almost 20 more progeny on average than small hosts (Fig. 3). There were differences in sex ratio among larval weight classes ( $F = 3.24; df = 2, 30; P = 0.05$ ), with a greater proportion of females emerging from large hosts than small hosts (Fig. 3).

Discussion

The results from this and previous studies indicate that *T. planipennisi*, like many other parasitoid species (Godfray 1994), produces more offspring (Liu et al. 2007, Ulyshen et al. 2010b) and a higher proportion of female offspring from large hosts than small hosts, thereby supporting our first hypothesis that *T. planipennisi* progeny number or the proportion of female progeny would increase with increasing initial host

weight. Our second hypothesis that the amplitude or rate of vibrational impulses produced by feeding emerald ash borer larvae would vary with larval weight also was supported as the amplitude of vibrations produced by feeding emerald ash borer varied significantly between large and small larvae. Our third hypothesis, however, was not supported as there were no significant correlations between the *T. planipennisi* progeny data and the emerald ash borer vibration data. Consequently, the mechanism by which *T. planipennisi* assesses host quality remains unclear.

We are hesitant, however, to entirely reject the notion that *T. planipennisi* and other parasitoids of

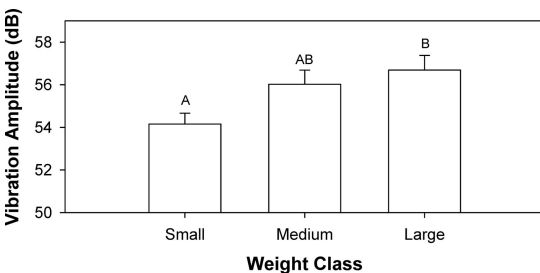


Fig. 2. Vibration amplitudes (mean  $\pm$  SE) produced by feeding emerald ash borer larvae assigned to three weight classes. Bars with different letters are significantly different based on the Tukey-Kramer mean separation test.

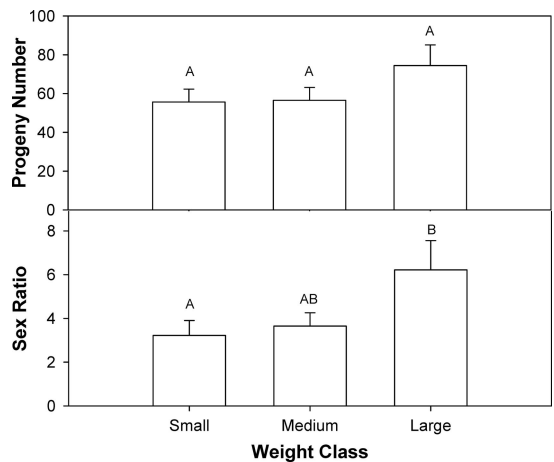


Fig. 3. Mean  $\pm$  SE *T. planipennisi* progeny number (top) and female:male sex ratio (bottom) from emerald ash borer larvae belonging to three weight classes. Bars with different letters are significantly different based on the Tukey-Kramer mean separation test.



concealed hosts use sound cues to assess host quality given that we found sound vibration amplitude to vary with host size. Several aspects of our study may have limited our ability to detect this relationship, if it exists. For example, because accelerometer recordings were made soon after drilling metal drill bits into the sticks, the emerald ash borer larvae may not have fed normally for a period after this handling, particularly considering that disturbances are known to elicit behavioral changes in other concealed insects (e.g., Djemai et al. 2001). Furthermore, given the high variability in vibration amplitude observed over the course of the recordings (data not shown), the vibration amplitude at the time of host acceptance by *T. planipennis* may have differed considerably from the mean vibration amplitudes recorded. Furthermore, because we used a 5:1 parasitoid:host ratio in our exposures, eggs may have been deposited by more than one female in some cases, possibly obscuring any relationship between host weight and clutch size. Finally, our sample size may have been too small considering the imprecision of host assessment and egg delivery by parasitoids (Godfray 1994).

Because *T. planipennis* will only parasitize actively feeding larvae (Ulyshen et al. 2010a), we suspect that movement and feeding vibrations are the main cues used in host location, if not also for distinguishing between large and small hosts. Vibration is just one of many external cues parasitoids use to locate concealed hosts that also may be used to assess host quality, however. Others include olfactory semiochemicals (e.g., hosts, host frass, microorganisms within host galleries), visual signals from plant tissue damaged by host feeding, and infrared radiation (Wang and Yang 2008). In contrast to the explanatory potential of host vibration, however, it seems unlikely that any of these other cues would be very useful to *T. planipennis* in distinguishing between active and inactive hosts or in assessing host quality. Assuming they can be detected by *T. planipennis* through intact bark, for example, the chemical signals associated with emerald ash borer frass would not be expected to give a reliable indication of host size because emerald ash borer larvae are often surrounded by the frass-filled galleries of other larvae feeding nearby. Although frass quantity was shown to be important to parasitoids of granary weevils in assessing host size (Steidle and Fischer 2000), in that system the weevils were confined to individual grains of wheat and the frass was detectable outside the grains due to small holes created by the developing larvae.

Some parasitoid species are known to locate hosts through vibrational sounding, whereby wasps tap the substrate with modified antennal segments and then detect echoes with enlarged subgenual organs in their tibia (Broad and Quicke 2000, Vilhelmsen et al. 2001). The antennae and tibia of *T. planipennis* are not obviously modified for this purpose (Yang et al. 2006), however, and evidence that vibrational sounding can be used to assess host size is lacking.

Several results from this study have important implications for mass rearing *T. planipennis* for release

against emerald ash borer. We provide, for example, the first evidence that the proportion of female progeny produced by *T. planipennis* increases with host size. This finding and the previous observation that significantly more *T. planipennis* progeny are produced from large hosts than small hosts (Ulyshen et al. 2010b) underscore the importance of using late instar emerald ash borer in rearing operations. In addition, consistent with previous work (Ulyshen et al. 2010a), our results show that the number of progeny produced per larva increases with stick diameter.

In conclusion, this study represents the first documented effort to determine whether parasitoids of concealed hosts use variations in larval feeding sounds to assess host quality. Because vibration amplitude varied significantly with host size, we are hesitant to reject the notion that *T. planipennis* and other parasitoids of concealed hosts use sound cues to assess host quality, particularly given the low explanatory potential of other external cues. More research will be needed before definitive conclusions can be reached. If external cues prove to be unimportant to *T. planipennis* and other parasitoid species in assessing host size, internal cues may be used instead (Hegdekar and Arthur 1973), assuming they vary with host size.

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