WESTERN CONIFER SEED BUG, *Leptoglossus occidentalis* Heidemann: CHARACTERIZATION AND ATTRACTIVENESS OF MALE-PRODUCED SONIC AND VIBRATIONAL AGGREGATION SIGNALS

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Abstract: We tested the hypothesis that the western conifer seed bug (WCSB), *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae), use sonic and vibrational signals in addition to pheromonal signals for communication. To record sonic or vibrational signals from individual or groups of bugs of either or both sexes, we used a digital recording system, comprising computers equipped with data acquisition hardware and software, microphones sensitive to sonic and/or ultrasonic frequencies, membrane-type and piezoelectric speakers capable of emitting sonic and ultrasonic sound, and piezoelectric devices capable of emitting low-level, low-frequency vibrations to substrates. Male WCSBs produced wide band sonic and vibrational signals 20 decibels (dB sound pressure level; 0 dB = 20 μP) above ambient with two dominate frequencies of 115 +/-10 and 175 +/-15 and a distinct temporal pattern. Repeated distinct pulse trains were not observed. There was no evidence that females or nymphs produce signals, and no evidence for ultrasonic signal production by either sex. In arena bioassay experiments, male and female WCSBs preferred played-back sonic signals from males over silent control stimuli, whereas nymphs showed no preference for either stimulus, unless adults were present. Adult females preferred male-derived substrate-bound vibrational signals in a two-choice bioassay experiment, whereas adult males and nymphs did not. Use of pheromonal and sonic signals by WCSB would be adaptive, because the capacity for sonic and substrate vibrational communication persists, even if sensory adaptation or habituation occurred in response to semiochemical signals. This hypothesis is consistent with the fact that other inhabitants of complex stratified microhabitats, such as the green stink bug, *Acrosternum hilare,* have also evolved analogous multimodal communication systems.

Keywords - *Leptoglossus occidentalis*, Hemiptera, Coreidae, sonic communication, mating systems, multimodal communication systems.

1 Introduction

The polyphagous western conifer-seed bug (WCSB), *Leptoglossus occidentalis* Heidemann, (Hemiptera: Coriedae) is a serious pest of conifer seed orchards throughout western North America (Bates et al. 2001), and has recently expanded its range to include most of the northern half of the United States. Nymphs and adults damage seeds by inserting their proboscis into cones and digesting the seed contents.

A common form of communication among insects uses some form of acoustic signal propagating through a medium including airborne sound and substrate vibrations (Cocroft and Rodriguez, 2005; Greenfield 2002; Yack, 2004). Individual species of the widely studied Heteroptera employ one or more forms of the five different forms of sounds (stridulation, clicking, air expulsion, percussion, vibration) used by insects. Common forms of acoustic communication found in Heteroptera are substrate vibration and far field airborne sound (Cocroft and Rodriguez, 2005).

WCSB is often observed rapidly rocking (percussion or vibration) while on a surface like conifer cones and loudly buzzing in flight (stridulation or clicking). Evidence for aggregation signals has been observed (Blatt 1994). However, the mechanisms of communication have not yet been identified.

We tested the hypothesis that WCSBs include acoustic signals in their communication system in addition to pheromones, and report characteristics and attractiveness of male-produced signals.

2 Methods and materials

2.1 Experimental insects

Nymphs and adults of WSCBs were collected in the Kalamalka Seed Orchard, Vernon, BC, during the 2004 field-season and kept in mesh cages (30 x 30 x 50 cm) at 20-25ºC, 40-70% r. h., and a 16L: 8D photoperiod. Water, pine seeds, and Douglas-fir seedlings were provided *ad libitum*. During the 2005 field-season, nymphs and adult WCSBs were collected in the Sechelt Seed Orchard, Sechelt, BC, operated by Canfor Corporation. Insects were housed in the same mesh cages on Douglas Fir *Pseudotsuga menziesii var. menziesii* and Mugo Pine *Pinus mugo* seedlings within the cages and were kept outside. Water, pine seeds, and Douglas-fir seedlings were provided *ad libitum.* The sex of individuals was determined by the width of the posterior abdomen and the presence/absence of a marked angularity (male) or ovipositor (female).

2.2 Acquisition of sonic signals

Substrate-borne signals were recorded from WCSBs by placing two males and one female on a loudspeaker (Fig 1 A; 20-cm diam; 30 – 6000 Hz frequency response, impendence 8 ohms), or piezoelectric device (not shown) (40-mm brass-annealed; 0.06kHz resonant frequency), for 120 min. Spectral and temporal characteristics of songs were determined from recordings made from WCSBs singing on the membrane of a low- to midrange speaker, or piezoelectric devices laid flat on a vibration damping table consisting of a 50 x 50 x 1 cm steel plate resting on rubber stoppers within a soundproof container and room (Fig. 1, A). During recordings, insects were confined within a glass dome (20 cm diam) placed over the speaker without contacting its paper cone, or within Petri dishes housing the piezoelectric device. Recordings took place from 2-5 h after the beginning of the photophase. Pre-trial recordings helped differentiate between sound directly associated with the behaviour of displaying bugs and sonic by-products, such as wings hitting the glass. Potential airborne signals in the sonic range (0–24 kHz) were recorded, using an AKG CK 61-ULS condenser microphone (sensitivity: 20.0 mV/Pa; frequency response characteristics: 20 Hz – 20 kHz: flat within +/- 1 dB, reference: 0 dB = 20 μPa; AKG Acoustics, U.S., Nashville, TN 37217, USA) and sampling rates of 43.5, 50 and 100 kHz.

Software with capabilities to monitor, trigger, record, analyse, playback, and edit sonic signals was developed with Labview - Graphical Programming for Instrumentation version 7.1 (National Instruments Corporation, 11500 N Mopac Expressway, Austin, TX 78759-3504). This software was used in combination with National Instruments DAQCard-6062E and PCI-MIO-16XE-10 data acquisition cards in Pentium IV laptop and desktop computers, respectively, to record digitally at sampling frequencies noted above. The signal-to-noise ratio was improved by pre-amplifying (National Instruments SC-2040 amplifier) signals from loudspeakers before recording them on computer disk. Signals were recorded continuously, or for 1-sec intervals when the monitoring software (a virtual oscilloscope) detected sound exceeding the baseline threshold of 0.1 mV. The triggering software for audio recording was designed to: 1) stream audio data through a circular buffer at a user-defined sampling (scan) rate; 2) take user-defined numbers of pre- and post-trigger scans to acquire and place the signal into a file; 3) append subsequent audio data to that file; and 4) continue data acquisition until a user-defined time limit between trigger events was exceeded, or, the procedure was manually stopped.

Recordings were analysed in 0.1-sec time periods for time waveform, frequency spectrum, and spectrogram using the Joint Time-Frequency Analyser [sliding window fast Fourier transform (STFT), Blackman window (National Instruments)].

2.3 Experimental protocol and bioassay experiments

2.3.1 Laboratory experiments with airborne and vibrational sound

Experiments 1-8 employed a large Plexiglas™ arena (125 x 60 cm high) (Mistal et al., 2000), and tested tactic responses of walking WCSBs to played-back sonic test stimuli or silent controls. All experiments were conducted during the scotophase at 22-27°C, 40-70% r. h. Recordings were played back through the same speakers identical used for recording Exps. 1-4), or through custom-built piezoelectric speakers [44 mm Piezoelectric Ceramic Buzzer Element, 0.06kHz resonant frequency (Digi-key Corp. Thief River Falls, MN 56701)] modified for either airborne (Exps. 5-8) or substrate transmission of sound. Piezoelectric speakers were powered by a Creek OBH-21SE Headphone Amplifier, using programs developed for the DAQ boards with LabVIEW-Graphical Programming for Instrumentation (National Instruments). Airborne and vibratory stimuli were emitted at peak intensities similar to the levels measured during signal acquisition. The midrange speakers were placed 80 cm apart from each other inverted on the arena floor to impart vibrations to the floor, with stimuli randomly assigned to one of two speakers. Piezoelectric speakers were placed similarly on the arena floor without imparting vibration to the floor of the arena. For each replicate, one WCSB was released from a Petri dish in the centre of the arena after 30 min acclimatization.

Experiments (Exp.) 1 – 8 tested the response of males (Exps. 1, 5), females (Exps. 2, 6), nymphs (Exps. 3, 7), , and a mixed group consisting of two males, one female and two 3rd instars (Exp. 4), or consisting of 5 males, 5 females and 10 3rd or 4th instars (Exp. 8) to played-back sonic signals previously recorded from 'calling' males during 120 min of sound acquisition. The played-back stimulus was looped (automatically rerun) during the bioassay period. At the end of the 3- or 24-h experiments (Exp. 1, 2, 4, or 3, 5-8), number of insects on a speaker, or within the quadrant containing a speaker, were recorded as responders. All others were recovered and classed as non-responders.

After completion of three consecutive replicates, interior surfaces of the Plexiglas arena were wiped with a paper towel saturated with a Sparkleen™-solution and aired for 24 h. Numbers of responding insects in bioassays were compared using Fisher's Exact test ($P < 0.05$) (Zar, 1984).

2.3.2 Laboratory bioassay experiments with substrate borne vibration

Experiments 9-11 employed a vertical hickory wood dowel (Fig. 1, B; 5 mm diam., 15 cm tall, 10- cm arm hot-glued at the cross point), and tested tactic responses of climbing WCSBs to played-back vibratory test stimuli or silent controls. Piezoelectric devices were modified by soldering a 18 guage steel wire to the Piezo element. Devices were suspended so that the wire was in contact with one arm of the cross dowel, and recordings were played back as described above. After 30 min acclimatization in a Petri dish, one insect per replicate was placed near the bottom of the vertical dowel in a glass holding tube for each replicate. After 5 minute acclimatization in the glass tube the stopper was released gently and the insect was given the opportunity to exit the tube onto the wooden dowel. If the insect did not leave the glass tube after 30 minutes, it was classed as a non-responder.

Experiments (Exp.) 9 – 11 (Table 1) tested the response of males (Exp. 9), females (Exp. 10), and nymphs (Exp. 11) to played-back vibrational signals during the scotophase. The looped stimulus was played back for up to one hour. Insects reaching and moving >2.5 cm away from the vertical dowel on the cross dowel were classed as responders. The position of the insect was noted at first choice, and two and five minutes after the first choice, Individuals remaining in the glass holding tube after 30 minutes were classed as nonresponders as were individuals that remained on the dowel but did not move within 2.5 cm of either stimulus initially, after 2 minutes or after 5 minutes. Individuals that flew off of the dowel throughout the experiment were also considered non responders for consequent observations.

2.3.3 Field experiments with airborne sound

Experiments 12-14 tested tactic responses of WCSBs to played-back sonic test stimuli or silent controls during the early to late scotophase in the Kalamalka and Sunshine Coast seed orchards in late May (Exp. 12; one trap night) or mid September 2005 (Exp. 13: four trap nights; Exp. 14: three trap nights).

Paired treatment and control piezoelectric devices were suspended 1.5 m above the ground within a wire mesh cage (30 cm^3) with six funnel-type entrances, each cage encasing the distal portion of two opposing pine (*Pinus contorta longitudina*) branches. These traps minimized transference of sonic signals to the branch they are suspended from. Recordings were played back as described above. Insects on or in the trap were recorded.

2.3.4 Field experiment with substrate-borne vibrations

Experiment 15 tested tactic responses of WCSB to played-back vibratory test stimuli or silent controls during the late photo- to early scotophase in mid September 2004. Paired treatment and control piezoelectric devices were suspended so that the wire was in contact with the medial portion of opposing branches about 1m from the ground. Recordings were played back as described above. Previously collected insects were released on the tree trunk below the lowest branch. Insects within 10 cm of a wire were recorded.

3 Results

Male WCSB produced sounds 20 decibels (dB sound pressure level) above the lower threshold of human hearing (0 dB = 20 μP), with two dominate frequencies of 115 +/-10 and 175 +/-15 (Fig. 1), and a distinct temporal pattern by tapping abdomens on substrate (Fig. 1 C). Repeated distinct pulse trains were not observed. There was no evidence that females produce airborne sound to attract mates and no evidence for ultrasonic sound production by either sex. Nymphs were not investigated for vibration induction.

In arena bioassay experiments, males and females preferred played-back sonic signals from males to silent control stimuli (Fig. 2, Exps. 2 and 3), whereas nymphs failed to leave the Petri dish in ten replicates (Fig. 2; Exp. 1). Groups of WCSBs strongly preferred male-produced sonic signals to the silent control (Fig. 2; Exp. 4). With piezoelectric devices for the emission of signals, males and females also preferred played-back sonic signals to silent speakers (Fig. 3; Exps. 5 and 6), nymphs were non-responsive (Fig. 3; Exp. 7), and mixed groups of WCSBs strongly preferred played-back sonic signals.

In the wooden dowel substrate-bound vibration bioassay experiments (Fig. 3; Exp. 9-11) individually tested female adults showed no preference initially, but did show preference for vibratory signals over controls after 2 minutes and after 5 minutes (Fig. 4; Exp.11). Males or nympths showed little preference for vibratory signals either initially, after 2 minutes and after 5 minutes (Fig. 3; Exps. 9, 11). The percentage of individuals that flew off of the dowel during the experiment was 16% for males and 14% for females.

In field experiments, played-back airborne signals from male WCSB were preferred to silent control stimuli (Fig. 4, Exps. 12-14). Similarly, substrate-borne vibrations were preferred to control stimuli (Fig. 4, Exp. 15).

4 Discussion

Our results provide evidence that both air- and substrate-borne acoustic signals are part of the WCSB communication system. Production of substrate-borne acoustic signals by males (Fig. 1C), orientation of males (Exps. 1, 5, 8), females (Exps. 1, 6, 8, 11), and nymphs (Exps. 4, 8) to acoustic speakers, or devices generating male-produced vibratory and airborne acoustic signals, support the conclusion that WCSB use air- and substrate-borne acoustic communication signals. However, nymphs may not be orienting to vibrations or airborne acoustic signals (Exps. 4, 7, 9), but rather to chemical signals from conspecifics (Exp. 4). Based on visual observation during experiments, nymphs appeared to follow males and other nympths but not female conspecifics (Exp. 4: personal observation).

Emission of intraspecific, substrate-borne vibratory communication signals during courtship and mating has been demonstrated in various species of Pentatomorpha (e.g Cokl et al. 2001, Cocroft and Rodriguez 2005). Most of these signals were determined to be close to 100 Hz, which is the optimal signal frequency for the transmission of bending waves in plants (Michelsen et al. 1982). The smaller amplitude fundemental frequency observed in this study (Fig. 1, B) is similar to the optimal signal transmission frequency found for similarly sized *Nezara viridula* (Cokl and Virant-Doberlet 2003.) . Unexpectedly, however, , a larger amplitude higher fundamental frequency is also present. Studies of vibrational transmission have concentrated on small diameter herbaceous plants while this study involved coniferous tree branches. The observed higher frequency may be an adaptation to different frequency filtering and attenuation of vibration frequency in larger woody compared to smaller herbaceous plants.

Use of at least three sensory modalities, including olfaction, substrate-vibration and airborne sound, by WCSB, may be highly adaptive in ensuring successful communication in structurally complex microhabitats like conifer branches. Should prolonged exposure to male-produced aggregation pheromone or habitat volatiles cause adaptation or habituation of the olfactory system, WSCB remain able to respond to substrate-vibration and sonic signals. Transmission through solids can be a more efficient method of communication than through air due to a lower degree of energy attenuation.

Piezoelectric speakers with the appropriate reproductive properties for sound and vibration (flat frequency response between 0.1 and 12500 kHz) proved effective in the field, each withstanding the effects of harsh weather in conifer seed orchards. They also transferred vibratory energy to substrates, and their cost effectiveness compared well with membrane-type loudspeakers.

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Figure Captions

Fig. 1. A. Midrange loudspeaker based recording apparatus for acquiring substrate borne signals.

B. Bioassay device

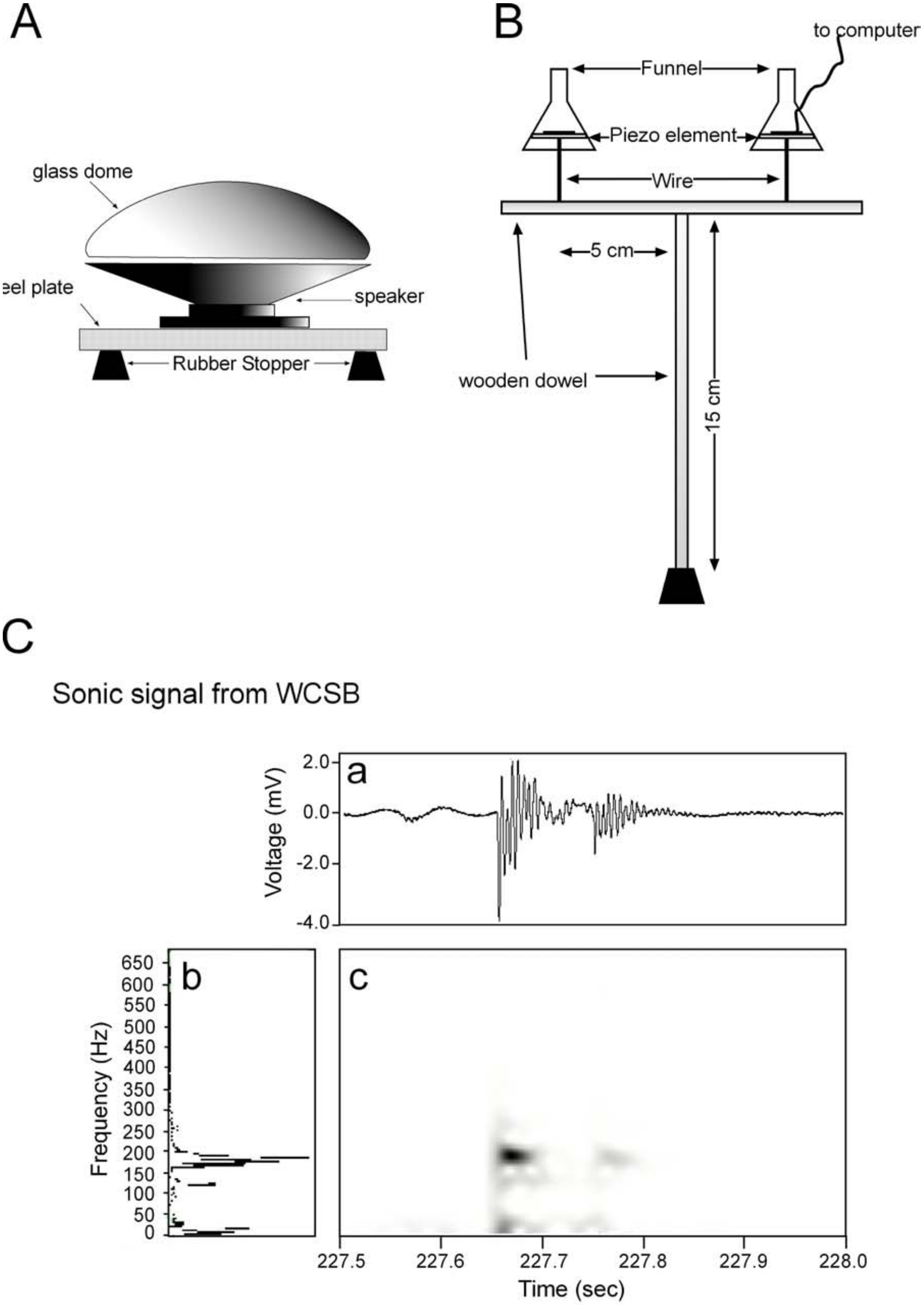
C. Analysis of waveform (a), frequency (b), and time-frequency sound intensity (c) of a substrate-borne sonic signal recorded from a male *Leptoglossus occidentalis*.. The more intense the shading in diagram c, the more intense the frequency component of the signal.

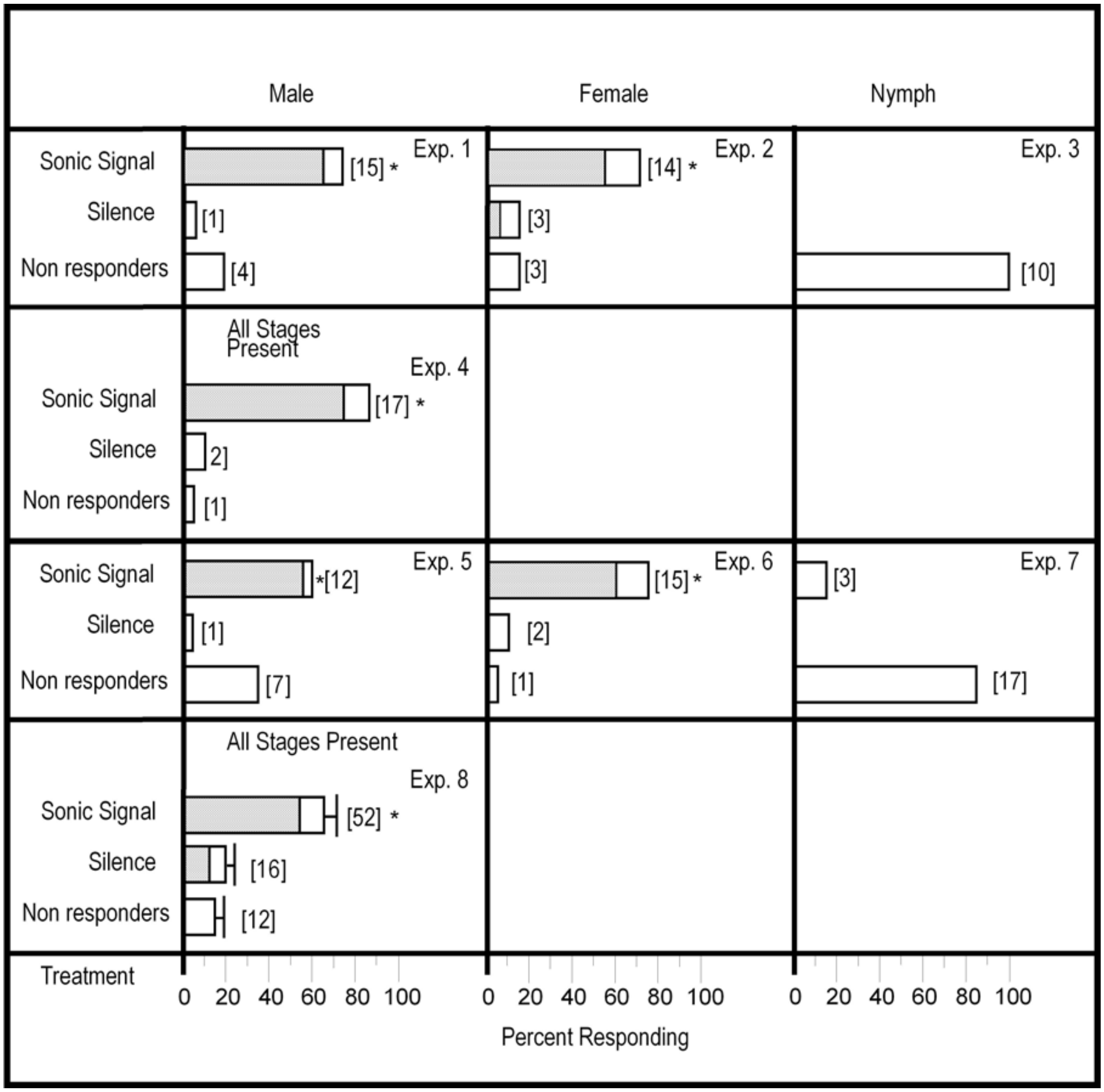
Fig. 2. Arena bioassay experiments using mid-range acoustic speakers (Exps.1-4) showing responses of male (Exp. 1), female (Exp. 2), 3rd instar (Exp. 3), and mixed groups (Exp. 4) of *Leptoglossus occidentalis* to test stimuli . Experiments 5-8 show the responses of male (Exp. 5), female (Exp. 6), 3rd instar (Exp. 7), and mixed groups (Exp. 8) of *Leptoglossus occidentalis* to test stimuli in arena bioassay experiments using piezoelectric devices. Bars represent percent of insects responding to a particular stimulus. For each experiment, an asterisk (*) indicates a significant preference for a particular stimulus [Fisher Exact test (Exps. 1 - 3; $P < 0.05$) Note: Cross hatching on bars represent the number of insects in or on the loudspeaker or piezoelectric device.

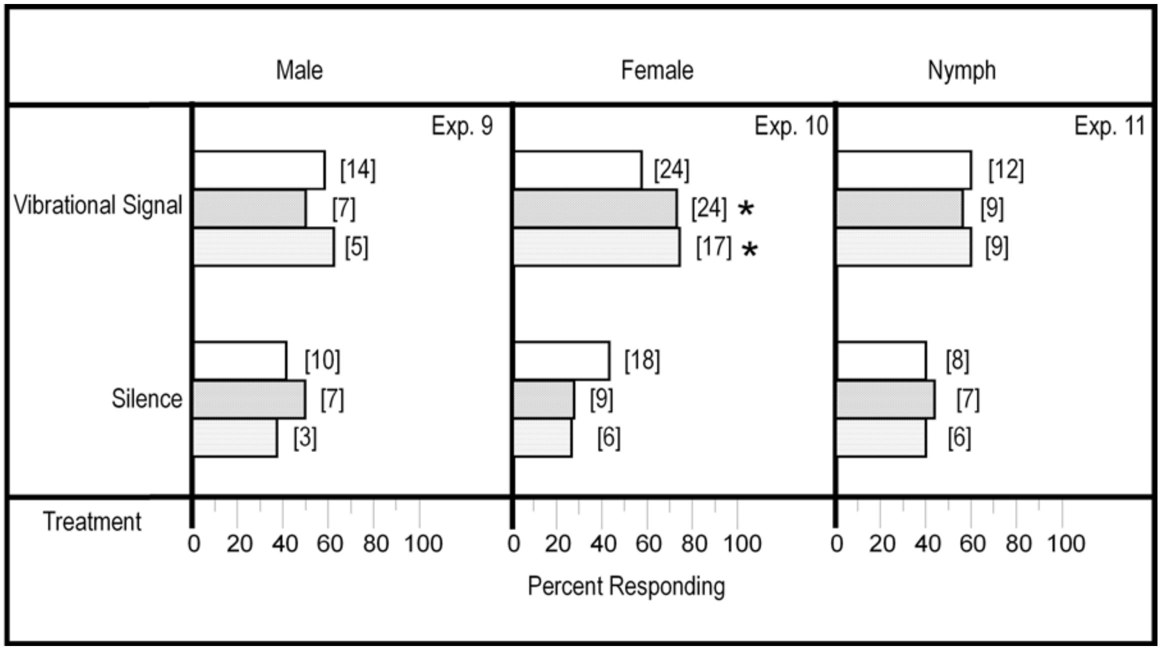
Fig. 3. Responses of male, female (Exps. 9, 10) and 3rd or 4th instar (Exp. 11) *Leptoglossus occidentalis* to vibratory test stimuli in laboratory experiments with

artificial branches and piezoelectric devices. Bars represent percent of insects responding to a particular stimulus. For each experiment, an asterisk (*) indicates a significant preference for a particular stimulus [Fisher Exact test (Exps. 9 - 11; $P < 0.05$)

Fig. 4. Responses of *Leptoglossus occidentalis* to played-back airborne sonic (Exp 12 – 14) or vibratory (Exp 15) stimuli in a conifer seed orchard using piezoelectric devices. Bars represent percent of bugs responding to a particular stimulus. Crosshatched, diagonal, and open bars, respectively, indicate cumulative captures of nymphs, males and females. For each experiment, an asterisk (*) indicates a significant preference for a particular stimulus [Fisher Exact test (Exps. 12-15; $P < 0.05$)


A
glass dome
eel plate
speaker
Rubber Stopper
B
to computer
Funnel
Piezo element
Wire
5 cm
wooden dowel
15 cm
C
Sonic signal from WCSB
a
Voltage (mV)
2.0
0.0
-2.0
-4.0
b
Frequency (Hz)
650
600
550
500
450
400
350
300
250
200
150
100
50
0
c
227.5
227.6
227.7
227.8
227.9
228.0
Time (sec)


Male
Female
Nymph
Sonic Signal
Silence
Non responders
Exp. 1
[15] *
[1]
[4]
Exp. 2
[14] *
[3]
[3]
Exp. 3
[10]
All Stages Present
Exp. 4
[17] *
2]
[1]
Exp. 5
*[12]
[1]
[7]
Exp. 6
[15] *
[2]
[1]
Exp. 7
[3]
[17]
All Stages Present
Exp. 8
[52] *
[16]
[12]
Treatment
0 20 40 60 80 100
0 20 40 60 80 100
0 20 40 60 80 100
Percent Responding


Male
Female
Nymph
Exp. 9
Exp. 10
Exp. 11
Vibrational Signal
Silence
Treatment
[14]
[7]
[5]
[10]
[7]
[3]
[24]
[24] *
[17] *
[18]
[9]
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[9]
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[8]
[7]
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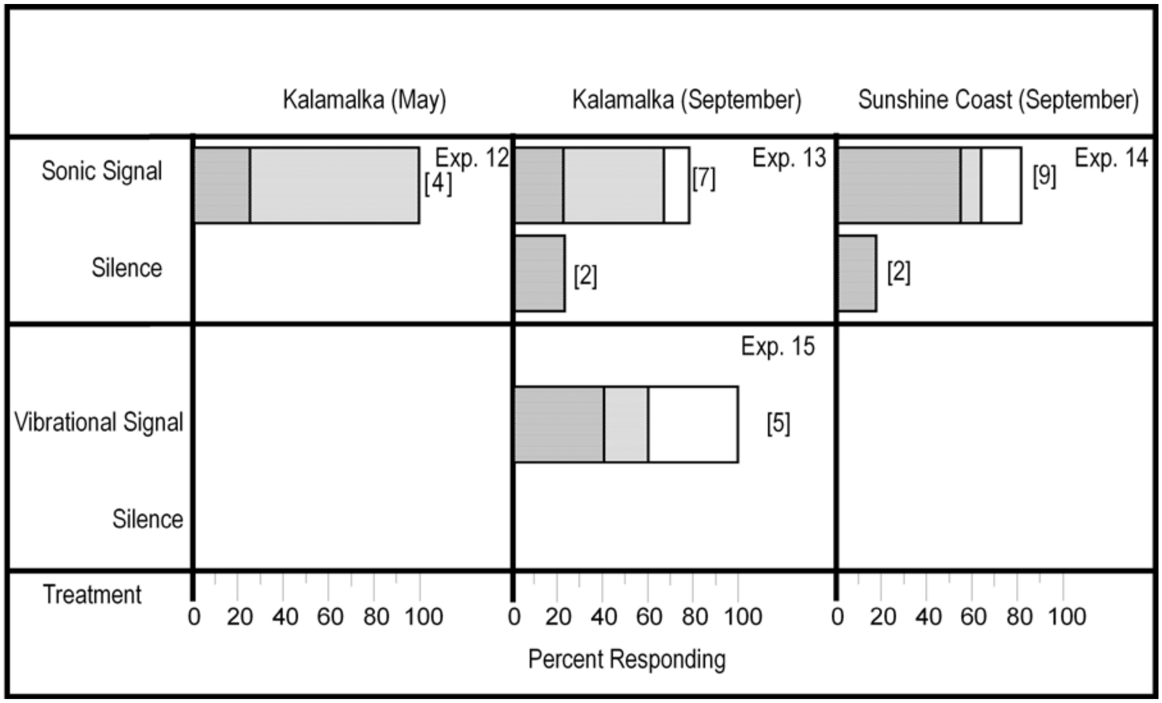
0 20 40 60 80 100
Percent Responding


Kalamalka (May)
Kalamalka (September)
Sunshine Coast (September)
Sonic Signal
Silence
Vibrational Signal
Silence
Treatment
Exp. 12
[4]
Exp. 13
[7]
[2]
Exp. 14
[9]
[2]
Exp. 15
[5]
0 20 40 60 80 100
0 20 40 60 80 100
0 20 40 60 80 100
Percent Responding