

# Detection of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) Larvae in Different Host Trees and Tissues by Automated Analyses of Sound-Impulse Frequency and Temporal Patterns

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**ABSTRACT** *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), an invasive pest quarantined in the United States, is difficult to detect because the larvae feed unseen inside trees. Acoustic technology has potential for reducing costs and hazards of tree inspection, but development of practical methods for acoustic detection requires the solution of technical problems involving transmission of resonant frequencies in wood and high background noise levels in the urban environments where most infestations have occurred. A study was conducted to characterize sounds from larvae of different ages in cambium, sapwood, and heartwood of bolts from three host tree species. Larval sounds in all of the tested trees and tissues consisted primarily of trains of brief, 3–10-ms impulses. There were no major differences in the spectral or temporal pattern characteristics of signals produced by larvae of different ages in each tissue, but larval sounds in sapwood often had fewer spectral peaks than sounds in cambium and heartwood. A large fraction, but not all background sounds could be discriminated from larval sounds by automated spectral analyses. In 3-min recordings from infested bolts, trains containing impulses in patterns called bursts occurred frequently, featuring 7–49 impulses separated by small intervals. Bursts were rarely detected in uninfested bolts. The occurrence of bursts was found to predict infestations more accurately than previously used automated spectral analyses alone. Bursts and other features of sounds that are identifiable by automated techniques may ultimately lead to improved pest detection applications and new insight into pest behavior.

**KEY WORDS** quarantine, eradication, invasive species

Larvae of the Asian longhorn beetle, *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae), feed internally in trunks and branches of maple (*Acer* spp.), willow (*Salix* spp.), poplar (*Populus* spp.), and other hardwoods in China and Korea (Haack et al. 1997). The range of *A. glabripennis* in China extends across climatic zones that corresponds to North American locations between southern Mexico and southern Canada (Yan 1985); consequently, this pest has considerable potential for permanent establishment in North America if incidentally transported individuals are not eradicated soon after introduction. In addition to their potential harm to the lumber, maple syrup, tourism, and hardwood tree nursery industries, *A. glabripennis* infestations have the potential to eliminate up to 35% of the current canopy cover in North Amer-

ica (1.2 billion trees), with losses of \$669 billion (Nowak et al. 2001).

Infested trees have been reported in areas of New York City and Long Island, NY (1996), Chicago, IL (1998), Jersey City, NJ (2002), Toronto, Canada (2003), and Carteret, NJ (2004) (Smith et al. 2004), and recently in Linden, NJ (2006) (Mastro et al. 2007), Prall Island, NY, and Staten Island, NY (2007) (Muccio 2007). In addition, adult *A. glabripennis* were discovered in Sacramento, CA (June 2005), putting at risk various tree species in the western United States (Ric et al. 2007). Quarantines have been established around areas of known infestation, and host trees within infested areas are individually inspected for signs of attack (e.g., oviposition pits and emergence holes) by using binoculars, hydraulic lifts and tree climbers (Ric et al. 2007). These approaches are time-consuming and expensive. Thus, current eradication programs have adopted the practice of removing all host trees within the infested areas, as well as within a 400–800-m radius of the infested areas, thereby resulting in the removal of >40,000 and >25,000 high-value landscape shade trees from the quarantined areas in the United States and Canada, respectively (Mastro et al. 2007, Markham 2007). There is an urgent need for detection methods that target *A. glabripennis* infesta-

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tions reliably and spare uninfested host trees in quarantined areas.

Acoustic technology has potential for reducing the expense and dangers involved in tree inspection, but there are difficult technical challenges to overcome in developing methods that reliably identify sounds made by larvae in different tree tissues and structures in a noisy urban environment. Variations in the compositional and structural characteristics of the wood, including the density, elasticity, and hardness (Cramer et al. 1988, Hambric 2006) can distort transmission of signals through the tree to the detecting sensors, particularly in small structures or near external edges (Evans et al. 2005). Internal noises caused by physiological processes (e.g., Fukuda et al. 2007) or externally induced vibrations or impacts can produce brief sounds that are difficult to distinguish from insect sounds. In addition, the weak signals produced by moving and feeding larvae can be masked by high-amplitude traffic and other background noise. Nevertheless, acoustic recordings from insects in trees often reveal signals with spectral and temporal features that make them distinctive and easily detectable (Mankin et al. 2002, and references therein). An improved automated analysis focused specifically on these features might identify *A. glabripennis* larval sounds even in the presence of considerable signal distortion and background noise.

Until now, automated acoustic analyses of sounds produced by hidden insect infestations have focused primarily on spectral aspects of the insect-produced signals (Mankin et al. 2000, 2001, 2007). Temporal patterns were considered by Zhang et al. (2003a) in classifying larval sounds as snaps, rustles, or repeated pulses, and it has been observed that different stored product insects produce distinctly different sound patterns (Andrieu and Fleurat-Lessard 1990, Mankin et al. 1997), but such features have not been applied for automated discrimination of insect sounds from background noise. In studies that classified signals through spectral analysis, infestations were identified by matching the spectra of individual, 3–10-ms sound impulses against spectral averages (profiles) constructed from records of externally validated insect sounds or background noises (Mankin et al. 2000, 2001). A spectral profile is constructed as an average power spectrum, calculated by first performing fast Fourier transformations (FFTs) (e.g., Press et al. 1990) on discretely sampled impulses, and then averaging the spectrum levels (i.e., spectrum amplitudes; Beranek and Ver 1992) over all recorded impulses at each FFT frequency. To capture spectral features common to all impulses of a specific type, the profile is constructed from a recorded period judged by experienced listeners to contain multiple impulses of the type being profiled (Mankin et al. 2007). In this study, experienced listeners who assessed various characteristics of signals in this study included the five authors of the report, as well as Everett Foreman and Betty Weaver in the Acknowledgments. Spectral profile analysis alone is sufficient to distinguish insect sounds from background noise successfully in many subter-

**Table 1.** Environment of bolts prepared for acoustic recordings at different moisture levels

Treatment	Wood moisture level		
	Dry	Intermediate	Saturated
Temp (°C)	23.9–27.8	20–24	20–24
RH (%)	45–55	75–85	75–85
Top of bolt waxed with paraffin	No	Yes	Yes
Bottom of bolt submersed in tap water	No	Yes	Yes
Duration before larval insertion (d)	14–18	1–5	7–11

anean environments because the insect sounds contain energy primarily above 0.5 kHz, whereas the background noise contains energy primarily below 0.2 kHz (Mankin et al. 2000, 2007). Preliminary surveys of background noise in wood indicated, however, that substantial energy often was present at frequencies above 0.5 kHz, which could reduce the accuracy of automated spectral profile analyses. A more powerful method was needed to reliably discriminate insect sounds from background noise in wood.

A study was conducted under the hypothesis that distinctive spectral and temporal features in *A. glabripennis* larval sounds can be combined to construct improved indicators (Mankin et al. 2007) for automated discrimination of infested from uninfested trees in urban environments. To develop an understanding of the range of conditions under which this might be accomplished, recordings of sounds from individual, first to fifth instars of *A. glabripennis* in bolts of *Salix nigra* Marshall (black willow), *Acer rubrum* L. (red maple), and *Robinia pseudoacacia* L. (black locust) trees were collected over a range of temperatures (10–32.2°C) and moisture conditions (31.7–72.2%), and then they were analyzed to automate identification of distinct types of sounds that occurred frequently when a larva was present but only rarely when absent.

## Materials and Methods

**Insects and Tree Bolts.** *A. glabripennis* larvae were obtained from a colony in quarantine at the USDA-ARS Beneficial Insects Introduction Research Unit, Newark, DE, maintained as described in Dubois et al. (2002). Acoustic records were collected from first to fifth instars placed individually into cambium (20 samples), sapwood (18 samples), or heartwood (17 samples) in 31-cm-long by 12–21-cm-diameter bolts cut from trunks or branches of 14 black willow, 25 red maple, and 16 black locust trees grown in the Blackbird State Forest, Delaware. These three host trees contain wood of relatively low, intermediate, and high density, respectively. The bolts were cut 1–18 d before testing and then were held in different temperature and moisture environments for different periods to obtain a range of wood moisture conditions (Table 1). Nine of the analyzed records were collected at ≈10°C, 38 at ≈21.1°C, and eight at ≈32.2°C. The numbers of

each instar tested were five first instars, six second instars, 17 third instars, 10 fourth instars, and 17 fifth instars.

**Recording Arenas and Signal Collection.** In preparing each bolt for recording, a 0.64-cm-diameter, 2.54-cm-long stainless steel screw was inserted midway down the length of the bolt,  $\approx 2.5$  cm from where the larva was to be placed (see below). The screw served as a waveguide for a piezoelectric sensor (Oyster 723, Schaller Electronics, Postbauer-Heng, Germany) that was attached to it with guitar putty. To construct a feeding arena in cambium, a mallet and chisel were used to cut three perpendicular, 2.54-cm incisions making a hinged flap of outer and inner bark, with care taken to avoid cutting into the xylem. The flap hinge was parallel to the cross-section, 15.24 cm from the top of the bolt. To construct a feeding arena in sapwood, a drill press was used to construct a 1.27-cm-diameter tunnel in the outer sapwood on the day the bolt was cut. A rubber stopper was inserted into the tunnel to conserve tissue moisture. The tunnel extended down 12.7 cm from the top of the bolt; its center was 1.27 cm from the edge of the cambium. A similar procedure was used to construct a feeding arena in heartwood, except that the center of the drilled tunnel was 0.25 cm from the pith of the bolt.

Before recording larval sounds in cambium, the top of the bark flap was pried open with a chisel, and an individual first, second, or third instar was inserted with flexible forceps. The larva was set 2.54 cm directly above the horizontal plane of the sensor waveguide screw. For larval sounds in sapwood or heartwood, the predrilled starter hole was extended 0.5–1 mm, exposing fresh tissue, and a third, fourth, or fifth instar was dropped in. Dry sawdust then was dropped into the hole and gently packed with a 0.63-cm dowel to make a  $\approx 1.27$ -cm-long plug. The head capsule of each larva was measured before it was placed into the bolt. The infested bolt was set vertically in a 117- by 78.7- by 60.3-cm environmental chamber (model I-30BLL, Percival Scientific, Inc., Perry, IA), and maintained at 10, 21.1, or 32.2°C. The chamber was lined with 7.6-cm-thick foam for temperature and sound insulation.

A 20-min record of acoustic signals was collected within 1–7 d after the infested bolt was placed in the environmental chamber. Signals detected by the sensor were amplified (model ENC1485, Encore Electrical Corp., Saratoga Springs, NY) and stored on an audio recorder (model TCD-D10 Pro II, Sony, Tokyo, Japan). Immediately afterward, the larva was carefully removed from the cambium with forceps, or from the sapwood or heartwood with a suction aspirator, and an additional 5-min period of background noise signals was recorded from the disinfested bolt. The recordings were subsequently converted to .wav files, digitized at 44.1 kHz (Sound Blaster Live, Creative Labs, Milpitas, CA), and saved on compact disks. The environmental chamber was placed in a foam-insulated plywood box to reduce background noise, but otherwise, no special noise reduction precautions were taken; consequently, background noise was detected frequently in recordings from both infested and dis-

infested bolts (see below). Temperatures inside the bolt were measured at 30-s intervals using a data logger (HOBO, Onset Corp., Pocasset, MA) with a stainless steel probe.

**Tissue Moisture Measurements.** Samples for tissue moisture measurements were taken from each bolt after recording was completed. For cambium moisture measurements, a 3.5–4-cm square of bark was removed from the middle of the bolt with a mallet and 2.54-cm chisel. A utility knife was used to cut the inner bark (cambium) from the outer bark. The cambium was immediately transferred using forceps to a glass petri dish and covered with the lid. The lid was opened only long enough to add more tissue to the dish, in an effort to prevent moisture loss from the sample. This procedure was repeated until 8–10 g of cambium was collected. The petri dish and sample were then weighed to obtain the wet weight of the sample (sample weight = dish and sample weight – dish weight). For sapwood moisture measurements, a 7–8-cm slab was cut from the middle of the bolt with a chainsaw. A 1.3-cm square was marked on the face of the cut slab. The square was set 1 cm from the drilled tunnel that had contained the larva, within the identical growth rings as the tunnel. An ax and hammer were used to split the slab at the four marks, resulting in a volume  $\approx 1.3$  by 1.3 by 7–8 cm. A similar procedure was used to obtain the heartwood moisture sample. A 7–8-cm slab was removed from midway down the bolt. A 1.3-cm square was marked on the slab, as described above, and the slab was split. In this case, the drilled tunnel was in the heartwood, and care was taken to avoid the pith tissue when marking and splitting the slab. Immediately after removal, samples were weighed, dried in a drying oven, at 102–108°C for 48–96 h, and then reweighed. Percentage of moisture was calculated as  $100 \text{ (wet weight} - \text{dry weight) / wet weight}$ .

**Signal Processing.** The full 25-min record from each examined bolt was inspected using Raven 1.2 software (Cornell Lab of Ornithology, Ithaca, NY) or a speech analysis system (CSL-4300B, Kay Elemetrics, Lincoln Park, NJ) that enabled playback of audio simultaneously with signal amplitude (oscillogram) and frequency–time (spectrogram) displays. A 3-min period was saved from the 20-min record of signals with the larva present, preferably containing all or most of the different types of sounds detectable in the full record, but without overwhelming background noise. A second 3-min period was saved from the 5-min record of signals after larval excision. The signals were band-pass filtered between 0.2 and 5 kHz, after which their spectral and temporal pattern features were analyzed using a custom-written signal processing program, DAVIS (Digitize, Analyze, and Visualize Insect Sounds; Mankin 1994, Mankin et al. 2000). Because most of the energy in insect sounds occurred between 1.5 and 4 kHz (see below), filtering out of signals <0.2 and >5 kHz enabled easier observation of the weakest insect sounds in both the oscillogram and the spectrogram.

**Sound-Impulse Classification.** To perform automated classification of signals, the DAVIS program conducted a series of analyses that identified and timed occurrences of particular features within a user-specified signal period. The process was controlled by an impulse-identification subroutine (IIS) that examined consecutive digitized samples from the beginning to the end of the specified period, and branched temporarily to an FFT-analysis subroutine (FFTS) whenever the signal magnitude exceeded a preset amplitude threshold,  $T_a$ , just above the highest background noise level (see Amplitude Thresholds). The FFTS searched for the time of highest amplitude, i.e., the peak time, within the next 256 samples (6 ms), and calculated a power spectrum with the peak time at the center of a 512-sample spectrum interval,  $I_{FFT}$ .

The next stage of processing depended on whether the program had been set by the user to construct a spectral profile (Mankin et al. 2000, 2001, 2007) or to classify an impulse using preexisting spectral profiles. In the spectral profile processing branch, the power spectrum of the first impulse encountered was accepted as the initial spectral profile, but for subsequent impulses, the power spectrum of the current impulse was matched against the existing spectral profile (Mankin et al. 2000, 2001). The impulse was accepted as valid if the average deviation of the impulse spectrum from the spectral profile was less than a preset threshold,  $T_s$ , where the average was calculated as the mean of the squared differences between the impulse spectrum level and the profile spectrum level at each FFT frequency between 0.2 and 4 kHz. The setting used in this study was determined from comparisons of classifications obtained with higher and lower thresholds. Thresholds set too low cause rejection of many valid impulses, and thresholds set too high cause incorrect acceptance of many noise impulses. If the impulse was accepted, the spectral profile was recalculated as the mean spectrum level at each FFT frequency, based on the total number of valid impulses that had been processed in the analysis period. The spectral profile subroutine then branched to a second impulse identification subroutine that examined the next 6 ms ( $0.5 I_{FFT}$ ), beginning at the sample after the last sample in the preceding  $I_{FFT}$ . If the signal rose again above  $T_a$  in this interval, the signal in the new interval was considered to be a continuation of the current impulse, and the program reentered the FFTS (see above). If the signal failed to rise above  $T_a$  during the  $0.5 I_{FFT}$  interval, the impulse was considered to have ended and the program branched back to the original impulse-identification subroutine (IIS) (see above).

When the program was set to process impulses without constructing a profile, the impulse spectrum was compared against one or more previously constructed spectral profiles. As in the spectral profile construction subroutine, the impulse was accepted as valid if the average deviation of the impulse spectrum from the spectral profile was less than  $T_s$ . If more than one profile was tested, as in this study, the profile from

which the average deviation was lowest was selected as the closest match if the average was less than  $T_s$ .

The valid sound impulses were timed by DAVIS, which set their beginnings at the times when the signals first rose  $>T_a$  and their endings at the times when the signal last exceeded  $T_a$ . The impulse times were saved in a spreadsheet for further analysis. The numbers of impulses used in constructing the spectral profiles in this study are listed in Results and Discussion.

**Impulse Trains and Bursts.** Because experienced listeners often grouped together impulses as a single distinct sound, we performed a similar, automated process in DAVIS, identifying and timing groups (trains) of impulses with interpulse intervals less than a preset duration,  $I_i$ . For this study,  $I_i$  was set at 250 ms, based on auditory assessments which usually distinguished larger intervals as separate sounds. The beginning of a train was set as the beginning of the first impulse after a period where the interpulse interval was  $>I_i$ , and the ending was set as the end of the last impulse whose interpulse interval was  $<I_i$ . The beginning and ending times of impulse trains, and the number of impulses per train,  $r_p$ , also were stored in a spreadsheet to consider whether they provided information useful for identifying infested bolts.

Experienced listeners frequently classified extremely short or long impulse trains as potential noise rather than potentially valid insect sounds. Preliminary analyses suggested that a valid sound usually contained seven or more, but  $<50$  impulses. This subgroup of impulse trains were designated as bursts in the automated analyses, and they were tested for the capacity to serve as indices of infestation, as discussed in the Results and Discussion.

**Amplitude Thresholds.** Inspection of the oscillograms and spectrograms in the files used to generate spectral profiles suggested that the timing of the beginning and endings of impulses and impulse trains depended strongly on both the mean level of background noise and the setting for  $T_a$ , particularly when the sound was of low amplitude. Setting  $T_a$  too high would lead to undercounting of impulses and incorrect durations of impulse trains. Setting  $T_a$  too low could cause overcounting of impulses, however, because the effectiveness of the spectral profiles in rejecting background sounds decreases as the signal amplitude decreases. Consequently, to correctly track the temporal patterns of impulse trains by automated techniques, it would be preferable to adjust  $T_a$  whenever the background noise changed significantly. To accomplish such adjustments, we performed a moving-threshold analysis. The program calculated the root mean square of consecutive 0.186-s (8192-sample) intervals to obtain a moving average of the background noise amplitude in each interval. The  $T_a$  was reset for each of these signal intervals by multiplying the root mean square by a user-settable factor, based on the results of comparing several different values in five files with different types of background noise.

A side effect of holding  $T_a$  close to the background noise level was an increase in the total number of

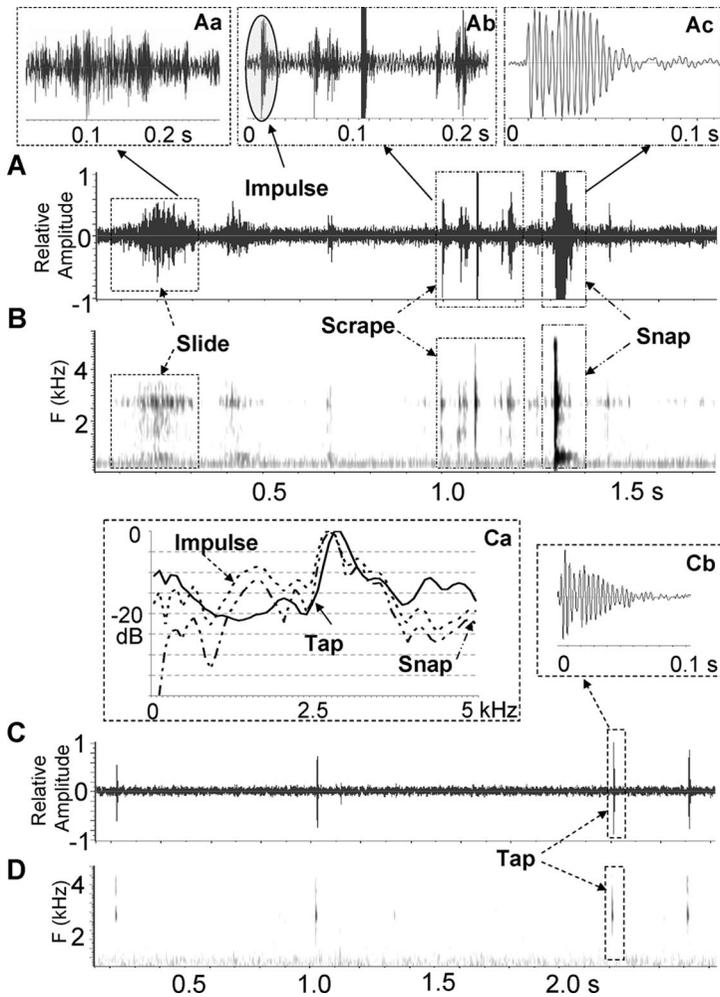


Fig. 1. Oscillogram (A) and spectrogram (B) of 1.8 s recorded from an infested bolt, and oscillogram (C) and spectrogram (D) of 2.4 s recorded from a disinfested bolt; insets show expanded views of a slide (Aa), a scrape (Ab), a snap (Ac), and a tap (Cb); shaded oval in Ab contains example of an impulse; and inset Ca shows examples of mean spectra from impulses, snaps and taps, with vertical scale in dB relative to maximum spectrum level, and horizontal scale in kilohertz. Areas of darker shading in spectrograms indicate higher relative energy at those frequencies ( $F$  in kilohertz) and times (in seconds).

impulses that were incorrectly classified as valid. The results below confirmed, however, that the impulse-train analysis benefited more from the enhanced capability to identify and process weak, insect-produced impulses than it was harmed by the increased rates of false-positive classifications.

### Results and Discussion

The records from infested and subsequently disinfested bolts of black willow, red maple, and black locust cambium, sapwood, and heartwood contained discrete, 3–10-ms impulses with amplitudes ranging from background levels up to  $\approx 2$  orders of magnitude higher, similar to insect sound impulses that had been observed previously in wood and other fibrous structures (Mankin et al. 2000, 2002). The impulses occurred either singly or in trains with short interpulse

intervals. On playback, particular combinations of impulses in infested bolts were consistently categorized as distinctive sounds by experienced listeners. Examples of three distinctive sounds recorded from a red maple bolt containing a third instar are shown in Fig. 1A and B. The most prominent impulse in the record is a high-amplitude snap, expanded in inset Ac. Although the snap has considerably greater amplitude than the impulses preceding it, its spectrum is similar to those of the lower amplitude impulses, with high energy between 1.5 and 4 kHz (Fig. 1B and 1Ca). The two impulse trains denoted as a slide and a scrape, expanded in insets Aa and Ab, respectively, sounded like an object sliding over a smooth surface and then scraping over a rough surface. A difference between the two sounds can also be seen in the spectrogram (Fig. 1B); the impulses in the slide are less distinct than those in the scrape. All of the impulses in the Fig.

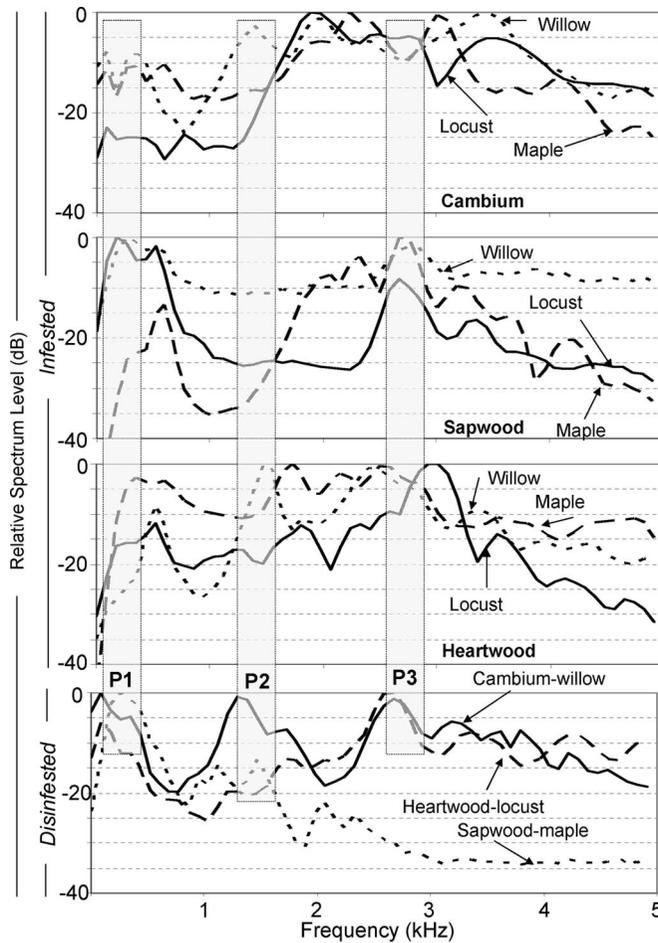


Fig. 2. Mean spectra of impulses detected in cambium, sapwood and heartwood of black willow (willow), red maple (maple), and black locust (locust) bolts infested with third-instars, compared with examples of mean spectra of background noise impulses detected after disinfestation. Shaded boxes (P1–P3) indicate frequencies where impulses from both infested and disinfested bolts contained relatively high energy. Environmental conditions and numbers of impulses in spectral profiles,  $n_{imp}$ , are listed in Table 2.

1 record were considered to be valid insect-produced signals based on their spectral characteristics, described in the next section.

Examples of a fourth type of sound detected in both infested and disinfested bolts are denoted as taps in Fig. 1C and D. Two characteristics that distinguish the taps in Fig. 1C from the impulses in the slide, scrape, and snap in Fig. 1A are the relatively short durations of the taps, which had only a few occurrences of multiple impulses, and their relatively low proportion of signal between 0.6 and 1.8 kHz (Fig. 1Ca and D). The production mechanism remains unknown, but to the ear, the taps were reminiscent of short, perpendicular impacts, in contrast to a longer duration, sliding or scraping movement that might be attributed to larval activity.

In  $\approx 25\%$  of records from disinfested bolts (see sections below), impulses occurred that were even more difficult than the taps shown in Fig. 1C and D to classify as background noise by using either spectral

profile analysis or listener assessment. This difficulty confirmed our initial concern that additional signal processing tools might be needed to offset uncertainties caused by the resonant characteristics of wood structures (Cremer et al. 1988, Hambric 2006). If signals like those in Fig. 1A and B above could be classified easily as distinctive sounds by an experienced listener, they might have underlying spectral and temporal pattern features (e.g., Cavaco and Lewicki 2007) that could facilitate automated identification and classification (Nakasone and Beck 2001).

To develop an approach for conducting such classifications, we first considered in detail the spectral and temporal characteristics of sounds in 15 records that spanned a range of instars, tree species, tree tissues, and environmental conditions (see next section). Automated procedures were applied to identify insect sound impulses, impulse trains, and bursts in this initial data set. In the final phase of development (see Burst Rates as Indicators of Infestation), the au-

**Table 2.** Treatment environments of infested (Inf.) and subsequently disinfested (Dis.) bolts yielding spectral profiles in Figs. 2–3, with  $n_{imp}$ , numbers of impulses in spectral profile,<sup>a</sup> numbers of impulse trains,<sup>b</sup> and numbers of bursts<sup>c</sup> in 3-min record

Spectral profile label in fig. panel		°C	% moisture	$n_{imp}$	No. trains		No. bursts	
Infested	Disinfested				Inf.	Dis.	Inf.	Dis.
Fig. 2								
Cambium locust		21.1	66.3	350	87	25	121	0
Cambium maple		21.1	52.8	42	162	25	70	0
Cambium willow	Cambium-willow	21.1	68.7	313	185	43	81	0
Heartwood locust	Heartwood-locust	10	N.A. <sup>d</sup>	17	71	0	8	0
Heartwood maple		32.2	46.7	94	212	59	103	0
Heartwood willow		32.2	51.4	170	202	13	98	0
Sapwood locust		10	32.7	45	91	4	2	0
Sapwood maple	Sapwood-maple	32.2	49.9	94	162	31	72	0
Sapwood willow		10	60.4	57	25	0	5	0
Fig. 3								
Cambium first instar		32.2	52.2	115	204	86	32	1
Cambium second instar		32.2	37.8	226	224	73	109	0
Cambium third instar	Cambium	32.2	48.8	201	202	55	111	0
Sapwood third instar	Sapwood	21.1	44.8	87	171	5	79	0
Sapwood fourth instar		21.1	39.2	1113	242	58	66	0
Sapwood fifth instar		21.1	46.7	115	102	2	14	0

<sup>a</sup> Impulses are discrete identifiable signal segments beginning when the signal first rose above a threshold amplitude ( $T_a$ ) above the highest background noise level and ending when the signal last exceeds  $T_a$  (see Materials and Methods). A spectral profile is the average power spectrum of a series of  $n_{imp}$  valid impulses.

<sup>b</sup> Impulse trains are groups of impulses with a interpulse intervals <250 ms, distinguishable as distinct sounds.

<sup>c</sup> Bursts are trains of impulses with >6 but <50 impulses in the train.

<sup>d</sup> Not available.

tomated procedures were extended to process a larger set of 100 records from infested and disinfested bolts to establish proof of principle.

**Spectral Profiles of First–Fifth Instars in Different Trees and Tissues.** Examples of spectral profiles of sounds produced by individual third instars in recordings from cambium, sapwood, and heartwood of black willow, red maple, and black locust bolts are shown in Fig. 2 and Table 2, infested. These profiles are similar in many respects to profiles of signals by individual first–third instars in recordings from cambium, and by individual third–fifth instars in sapwood of red maple bolts, examples of which are shown in Fig. 3, Infested. The bolt temperature and moisture conditions, and the numbers of impulses in the spectral profiles from each infested bolt,  $n_{imp}$ , are listed in Table 2, along with numbers of impulse trains and bursts.

In comparisons among instars and tissues, there was possibly a slight reduction in spectral energy of first and second instars relative to older instars >3 kHz. For all instars, there was often a larger contribution of signal near 0.5 kHz in sapwood than in cambium, and a peak in relative energy between 2.5 and 2.8 kHz was more consistent in sapwood than in heartwood or cambium. The rates of impulse trains varied considerably within and among the recordings considered in this part of the study, but there was a noticeable trend that impulse-train rate tended to increase with temperature, similar to what had been observed previously with subterranean insects (Zhang et al. 2003a). None of the observed variations are large in comparison with the variations commonly seen in the literature (Mankin et al. 2000, 2001, 2007), which suggests that a small number of spectral profiles could be used to cover the full range of tree species, tissues, tem-

peratures, moisture levels, and insect instars in this study.

To contrast with records from infested bolts, spectral profiles of signals recorded after larvae were removed from black willow cambium, red maple sapwood, and black locust heartwood are shown in Fig. 2 *Disinfested*, and spectral profiles of signals recorded after third instars were removed from cambium and sapwood of two red maple bolts are shown in Fig. 3, *Disinfested*. Many of the profiles from both infested and disinfested bolts have significant energy in the ranges of 0.05–0.35, 1.3–1.6, and 2.5–2.8 kHz (see shaded boxes P1–P3 overlying Fig. 2 and boxes P1 and P3 in Fig. 3). Only one of the background noise profiles (Fig. 2, *Disinfested* sapwood-maple) resembles profiles that are commonly observed in recordings of subterranean background noise (Mankin et al. 2000, 2001, 2007). The observed differences in the *Disinfested* panels of Figs. 2–3 may be due partly to differences in bolt temperature or moisture, but the most parsimonious hypothesis for the differences is that they reflect, in part, the spectral variations in the different external sounds or vibrations that were detected in these periods, the differences in the reactions of the wood fibers to those different excitations (Hambric 2006), and the differences between the attenuation of high-frequency signals in wood and soil (Mankin et al. 2000).

Because there were no strong differences among the spectral profiles from different tree species, tree tissues, and insect ages, our initial approach to the problem of automating the *A. glabripennis* spectral profile matching analysis was to construct nine profiles from all combinations of cambium, sapwood, and heartwood of infested black willow, red maple, and

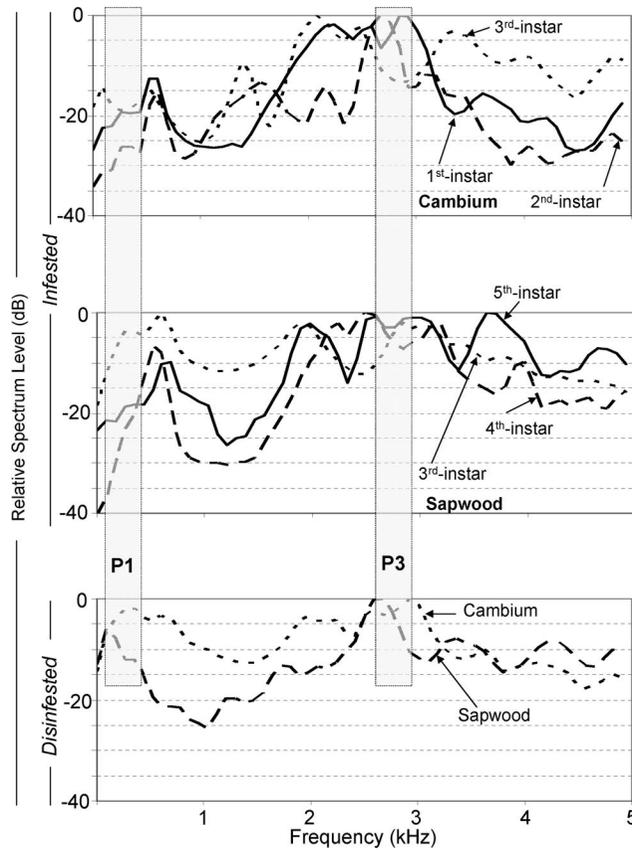


Fig. 3. Mean spectra of impulses detected in cambium of red maple bolts infested with first-third instars, and sapwood infested with third-fifth instars, compared with examples of mean spectra of background noise impulses detected after larval removal. Shaded boxes (P1 and P3) indicate frequencies where impulses from both infested and disinfested bolts contained relatively high energy. Environmental conditions and numbers of impulses in spectral profiles,  $n_{imp}$ , are listed in Table 2.

black locust bolts and then survey a sample of randomly selected records to identify profiles that matched frequently to the power spectra of individual impulses. The environmental conditions and numbers of impulses in the spectral profiles,  $n_{imp}$ , are listed in the first six columns of Table 3. The three profiles (Fig. 4) that matched most frequently to the sounds in the randomly selected files were selected to be profiles for automated analyses of temporal patterns (see next section). Similarly, nine 3-min periods recorded from separate bolts after larvae had been removed were analyzed to consider the range of background sounds encountered. For automated analyses, we identified three profiles (Fig. 5) that most frequently matched background sounds in 20 additional, randomly selected recordings.

Inspection of the three most often matched insect spectral profiles and background spectral profiles suggests that the two sets capture signals with different spectral characteristics. The profile in Fig. 4A captures signals primarily with relatively high energy between 1.3 and 1.6 kHz, the range in box P2 of Fig. 2, whereas the profile in Fig. 4B captures signals primarily with two peaks, one below 0.5 kHz, corresponding to the range in box P1 in Fig. 2, and the other between 2.5 and

Table 3. Treatment environments of infested and disinfested bolts yielding spectral profiles in Figs. 4–5, with  $n_{imp}$ , numbers of impulses in spectral profile, numbers of impulse trains,<sup>a</sup> and numbers of bursts<sup>a</sup> in 3-min record

Instar	Tree	Tissue	%			No. trains	No. bursts
			°C	Moisture	$n_{imp}$		
Infested 1	Maple	Cambium	21.1	57.1	244	129	59
3 <sup>b</sup>	Locust	Cambium	21.1	60.3	110	142	47
4 <sup>b</sup>	Maple	Heartwood	21.1	35.6	93	57	30
3	Maple	Cambium	32.2	48.8	328	71	24
4	Maple	Sapwood	21.1	52.4	155	9	8
2	Maple	Cambium	20.8	55.3	151	18	5
3 <sup>b</sup>	Willow	Sapwood	21.1	31.6	55	119	4
1	Locust	Cambium	21.1	72.2	68	56	3
4	Willow	Sapwood	21.1	65.5	58	30	2
Disinfested	Willow	Sapwood	21.1	35.3	329	157	35
—	Maple	Sapwood	10	N.A. <sup>c</sup>	378	32	12
—	Maple	Cambium	21.1	57.1	82	4	0
—	Willow	Sapwood	21.1	31.5	132	8	0
—	Maple	Heartwood	21.1	38.5	36	8	0
— <sup>d</sup>	Maple	Cambium	20.8	55.3	53	11	0
— <sup>d</sup>	Willow	Sapwood	21.1	65.5	28	12	0
— <sup>d</sup>	Locust	Heartwood	21.1	29.5	304	12	0
— <sup>d</sup>	Locust	Cambium	21.1	72.2	109	37	0

<sup>a</sup> See Table 2 for definitions of impulse train and burst.

<sup>b</sup> Profile shown in Fig. 4.

<sup>c</sup> Not available.

<sup>d</sup> Profile shown in Fig. 5.

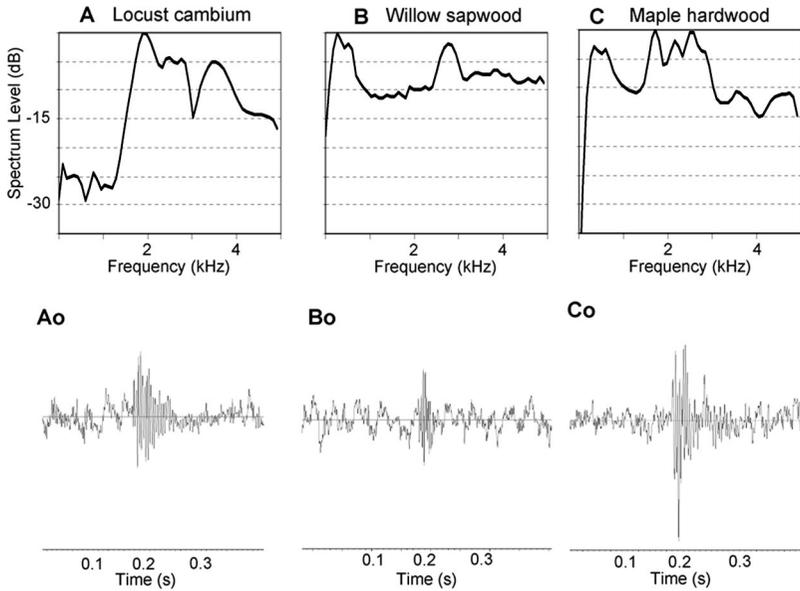


Fig. 4. Spectral profiles of insect sound impulses used for automated categorizing of valid and invalid sound impulses. Examples of impulses that closely matched profiles A, B, and C are shown in oscillograms Ao, Bo, and Co, respectively. Environmental conditions and numbers of impulses in spectral profiles,  $n_{imp}$ , are listed in Table 3.

2.8 kHz, the range in box P3. The profile in Fig. 4C captures other signals with energy below 0.5 kHz and higher relative energy at an intermediate frequency between 1.5 and 2.5 kHz. In contrast, the three background spectral profiles in Fig. 5 capture signals primarily with relatively high energy below 0.5 kHz, with the profile in Fig. 5B also capturing highly broadband noise with increased energy at lower frequencies. However, except for profile 5B, the background pro-

files failed to screen out occasional signals with high-frequency components in the records from disinfested bolts. Additional tools were needed to reliably screen out high-frequency background noise impulses.

**Impulse-Train Temporal Patterns.** A preliminary test using the three insect-sound (Fig. 4) and background-noise (Fig. 5) spectral profiles to classify impulses and quantify impulse trains and bursts (i.e., impulse trains with  $>6$  but  $<50$  impulses) was con-

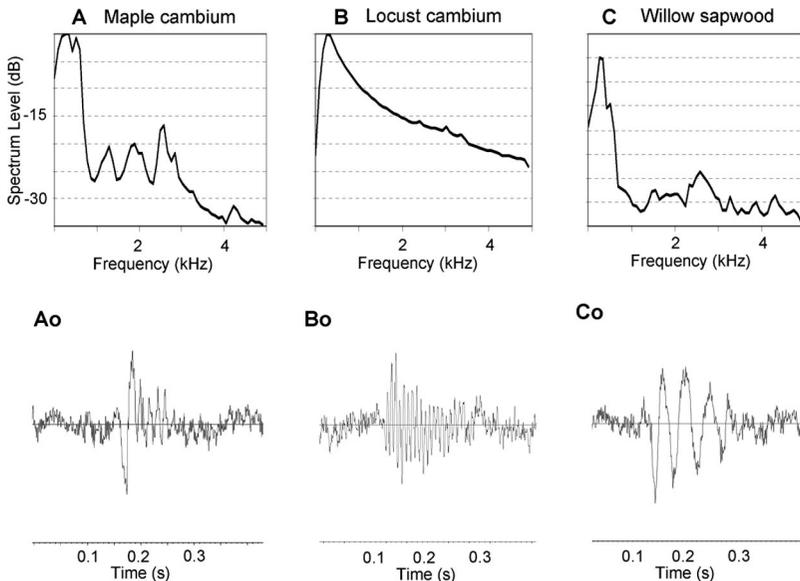


Fig. 5. Spectral profiles of background sound impulses used for automated categorizing of valid and invalid sound impulses. Examples of background sound impulses that closely matched profiles A, B, and C are shown in oscillograms Ao, Bo, and Co, respectively. Environmental conditions and numbers of impulses in spectral profiles,  $n_{imp}$ , are listed in Table 3.

ducted on the 15 recordings that had been used to consider the effects of tree species, tree tissue, and larval age (see No. trains and No. bursts columns in Table 2). Only impulses classified as valid by the spectral profiles were included in the impulse train and bursts measurements. Records from all of the infested bolts contained at least one burst (mean  $64.73 \pm 10.9$ ), but records from all but one of the disinfested bolts contained no bursts (mean  $0.07 \pm 0.07$ ) (Table 2). These differences also were reflected in the audio playbacks. Typically, the longer pulse trains were identified by listeners as scrapes or slides, or possibly, movements associated with pushing frass out of the feeding chambers. Such pulse trains occurred primarily in the infested rather than disinfested bolts. Shorter pulse trains were more difficult for listeners to interpret as particular behaviors and less likely to be assessed as insect sounds at all. In a few cases, long trains with 50 or more impulses occurred, but these usually were considered likely to have embedded background noise or to consist entirely of periods of background noise.

We tested the possibility of using bursts to construct indicators of infestation likelihood, setting the likelihood as low if no bursts occurred and high if more than one burst occurred during a 3-min interval. The distributions of burst numbers in 3-min intervals recorded from infested and disinfested bolts were significantly different (i.e., not independent of the acoustic assessment) in a contingency test ( $\chi^2 = 23.0$ ,  $df = 2$ ,  $P < 0.001$ ).

The mean rate of impulse trains was greater in infested than disinfested bolts (mean no. trains per min =  $52.04 \pm 5.59$  for infested,  $21.58 \pm 3.62$  for disinfested bolts;  $t = 6.93$ ,  $df = 14$ ,  $P < 0.001$ ); consequently, it seemed feasible to use the rate of valid impulse trains also to construct indicators for infestation likelihood (Brandhorst-Hubbard et al. 2001; Mankin et al. 2001, 2007). The indicator value for likelihood of infestation was set to low if the rate was  $<16.67$  trains per min (50 per 3-min interval), and it was set to high if the rate was  $>33.33$  trains per min. Using these indicator values, the distributions of impulse train rates in infested and disinfested bolts were significantly different in a contingency test ( $\chi^2 = 6.93$ ,  $df = 2$ ,  $P < 0.05$ ).

**Burst Rates as Indicators of Infestation.** The utility of bursts for quantifying infestation likelihood was confirmed in a larger scale comparison of numbers of impulse trains and bursts in 50 infested bolts that were reexamined after disinfestation. The distributions of both the numbers of bursts and the numbers of impulse trains in 3-min periods recorded from infested and disinfested bolts were significantly different in the expanded test (Table 4), with  $\chi^2 = 35.84$ ,  $df = 2$ ,  $P < 0.001$  for burst rate and  $\chi^2 = 14.67$ ,  $df = 2$ ,  $P < 0.001$  for impulse rate. A more direct assessment of the acoustic detectability of the larvae was obtained by calculating the accuracy, sensitivity, and specificity of the acoustic measurements, by using the terminology commonly used for assessing sampling errors (Venette et al. 2002, Zhang et al. 2003b). For this calculation, we considered bolts rated at high and medium likelihoods

**Table 4.** Numbers of infested and disinfested bolts assessed at low, medium, or high likelihoods of infestation by sound-impulse and burst rate indicators

Likelihood of infestation	Sound-impulse rate		Burst rate	
	Disinfested	Infested	Disinfested	Infested
Low	45	28	37	8
Medium	3	13	4	5
High	2	9	9	37

Threshold values for burst rate indicators of infestation likelihood were as follows: 0 bursts per 3-min interval, low; 1, medium; and  $>1$ , high. Threshold values for sound-impulse rate indicators of infestation were as follows:  $<50$  per 3-min interval, low;  $\geq 50$  but  $\leq 100$ , medium; and  $>100$ , high (see Impulse-Train Temporal Patterns above).

of infestation to be positive, and those rated low to be negative, whereas true and false referred to the correctness of the rating. The accuracy then is the proportion of correct results (true positive + true negative) / (true positive + true negative + false positive + false negative). The sensitivity is the proportion of infested bolts that are correctly identified (true positive / (true positive + false negative)), and the specificity is the proportion of uninfested bolts that are correctly identified (true negative / (false positive + true negative)).

Under this assessment of sampling error, the burst rate provided a more accurate and sensitive measure of infestation likelihood than the impulse rate, but it was slightly less specific (Table 5). Although either method could be used to estimate the likelihood of infestation, the burst rate method is better at correct identification of infested bolts, but the use of the sound impulse rate makes fewer relative errors in misidentifying uninfested bolts as positive.

It was of interest to us that no bursts were detected in the 3-min records analyzed from eight infested bolts in the study (Table 4) because such a result would lead to incorrect, false-negative classifications of those bolts. We reprocessed these original files, analyzing the full 20-min record to determine whether sampling over periods  $>3$  min would lead to improved accuracy. Five of the eight 20-min records did contain bursts, confirming that accuracy could be improved by increasing the sampling duration. However, it is not

**Table 5.** Accuracy, sensitivity, and specificity of larval impulse-train and burst rates indicators for assessments of infestation likelihood

Measure of detection validity <sup>b</sup>	Indicator of infestation likelihood <sup>a</sup>	
	Impulse-train rate	Burst rate
Accuracy	0.67	0.79
Sensitivity	0.44	0.84
Specificity	0.9	0.74

<sup>a</sup> Bolts rated at high and medium likelihoods of infestation were classified as positive, and low as negative.

<sup>b</sup> Accuracy = (true positive + true negative) / (true positive + true negative + false positive + false negative); sensitivity = true positive / (true positive + false negative); specificity = true negative / (false positive + true negative).

certain that the resultant benefit of increasing the accuracy from 79 to 84% would have been worth the cost of recording and analysis of an additional 17-min period. Additional studies would be required to identify the best trade-off between sampling duration and larval detectability in different urban environments.

Additional studies in urban environments also would be of value in assessing interference from background noises with amplitudes or temporal patterns that could mask larval sound-impulse bursts. Although these are relatively large insects, *A. glabripennis* may be difficult to detect during periods of high wind or high traffic noise, as has been observed in acoustic detection studies of other insects in field (Mankin and Lapointe 2003, Mankin et al. 2007) and urban (Mankin et al. 2002) environments, because the high amplitudes of these noises overwhelm the amplitudes of the larval sounds. Background noise levels also affect the distances over which larvae can be detected. In the laboratory, termite sounds have been detected over distances of 1.8 m or longer along the length of a plank (Mankin et al. 2002); comparable experiments have not yet been conducted in with *A. glabripennis* in urban trees.

Given that the number of impulses in a burst can be counted either by relatively simple signal processing procedures or by observation of oscillograms, the use of burst rate to augment assessments of infestation likelihood is an easily implemented analysis for acoustic detection of insects that frequently perform scraping, sliding, frass movement, and other repeated behaviors. Not all insects perform such behaviors, so it will always be important to measure rates of individual valid sound impulses, snaps, and impulse trains shorter than seven impulses per train, but multiple examples of such behaviors have already been documented for stored-product insects (Andrieu and Fleurat-Lessard 1990, Mankin et al. 1997) and subterranean insects (Zhang et al. 2003a). These and other features of insect sounds, many yet to be identified, may ultimately lead not only to improved applications for detection of important, hidden insect pests but also to new insight into insect behavior.

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