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TEMPORAL AND SPECTRAL FEATURES OF SOUNDS OF WOOD-BORING BEETLE LARVAE: IDENTIFIABLE PATTERNS OF ACTIVITY ENABLE IMPROVED DISCRIMINATION FROM BACKGROUND NOISE

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ABSTRACT

Rhynchophorus (curculionid) larvae produce economic damage to ornamental and date palm crops that could be mitigated significantly by early detection and treatment. Acoustic technology enables early detection, but often it is difficult to distinguish insect sounds from background noise containing energy at the resonant frequencies of stiff, fibrous structures in trees and other plants. Tests were conducted with currently available acoustic instrumentation and software to assess the capability of these methods to discriminate curculionid, cerambycid, and buprestid larval sounds from background noise in woody structures. An approach to the discrimination problem is to monitor the temporal patterns of the 3-10-ms sound impulses produced by locomotory and feeding activities. Playback and computer analyses of larval sounds revealed trains of impulses separated by intervals of less than 500 ms that experienced listeners frequently use as indications of potential insect sounds. Further analyses identified a subgroup of trains, denoted as bursts, containing > 6 and < 200 impulses, which occurred frequently when larvae were present but only rarely when larvae were absent. The incorporation of bursts into the analysis process significantly improved the capability to distinguish sounds produced by beetle larvae from background noise when these insects were hidden in stiff, fibrous structures, and likely will be of assistance also in other applications where consistent activity patterns of hidden pests can be identified.

Key Words: *Rhynchophorus ferrugineus*, *Monochamus titillator*, *Agrilus dozieri*, acoustic detection

RESUMEN

Las larvas de *Rhynchophorus* (Curculionidae) producen daño económico a los cultivos ornamentales y a la palmera dactilífera que puede ser mitigado significativamente por la detección temprana y tratamiento. La tecnología acústica permite una detección temprana, pero a menudo es difícil distinguir los sonidos hechos por los insectos del ruido de fondo que contiene energía a las frecuencias resonancia de las estructuras fibrosas presentes en los árboles y otras plantas. Se realizaron pruebas con instrumentos acústicos actuales disponibles y programas de computadora para evaluar la habilidad de estos métodos para discriminar los sonidos de larvas de las familias Curculionidae, Cerambycidae y Buprestidae del ruido de fondo de las estructuras de la madera. Un estrategia al problema de discriminación es hacer un monitoreo de los patrones temporales de los impulsos de sonido de 3-10 ms (milisegundos) producidos por las actividades de locomoción y alimentación. El análisis de los sonidos de las larvas recolectados por la computadora y el repaso de la grabación de sonido reveló una serie de impulsos separados por intervalos de menos de 500 ms que los oyentes con experiencia usan frecuentemente como indicadores de sonidos potenciales de insectos. Los análisis subsiguientes identificaron un subgrupo de series, denotados como brotes repentinos de > 6 y < 200 impulsos, que ocurrió con frecuencia cuando las larvas estaban presentes pero raramente cuando las larvas estaban ausentes. La incorporación de los brotes repentinos en el proceso de análisis mejoró significativamente la habilidad para distinguir los sonidos producidos por larvas de escarabajos del ruido de fondo cuando estos insectos estaban escondidos en estructuras duras y fibrosas, y probablemente esta incorporación será de ayudar en otras aplicaciones donde los patrones de actividad consistentes con plagas escondidas pueden ser identificados.

The *Rhynchophorus* palm weevils (Coleoptera: Curculionidae) are important worldwide pests of palm trees, Arecaceae (Palmae) spp., including economically important coconut (*Cocos nucifera* L.), date (*Phoenix dactylifera* L.), and ornamental palms (Murphy & Briscoe 1999). The American palm weevil, *R. palmarum* L., is destructive in the Neotropics (Giblin-Davis 1993, Oehlschlager et al. 2002), and the palmetto weevil (PTW), *R. cruentatus* (F.), in southern Florida. The red palm weevil (RPW), *R. ferrugineus* (Olivier), has caused losses of up to 10-25% or more in coconut palm plantations in Asia and the Middle East (Murphy & Briscoe 1999). Currently, RPW is spreading in European Mediterranean countries, endangering picturesque landscapes attractive to tourists.

Adult females of these species lay eggs in softer or protected areas of the trees, including in wounds in the trunks of established trees, at the base of the palm leaves at tree crowns, and adjacent to offshoots. The young larvae penetrate into the trunk, creating cavities and tunnels (Giblin-Davis 2001). Several generations may develop within a single tree. Infested trees suffer from reduced productivity, and heavy infestations result in collapsed trees, leading occasionally to total loss of a date palm crop (Blumberg et al. 2001).

Adult populations of RPW, PTW, and *R. palmarum* can be efficiently monitored using pheromone based traps (Soroker et al., 2005; Oeshlager et al., 1993, 1995 for RPW and PTW, respectively). However, these methods are unsuitable for quarantine inspections of planting material. The larvae are large, but their cryptic tunneling behavior makes their direct detection by visual inspection impossible. Consequently, infested planting material is often transported to a new location before the first detectable symptoms of infestation appear. There is a need for direct, rapid and accurate techniques for examination of transported planting material and suspected trees.

The chewing and locomotory activities of palm weevil larvae produce distinct sounds, and heavy infestations can often be heard by humans (Giblin-Davis 2001). However, severe damage to palm tissue already has occurred by the time the larval sounds can be detected without electronic assistance. Preliminary studies conducted by Mizrach et al. (2003), Hetzroni et al. (2004a, b), and Soroker et al. (2004, 2006) demonstrated that sensitive microphones and dedicated amplifiers enable detection of the movement and feeding sounds of RPW larvae in palm shoots and trees. They characterized the sounds as short impulses with strong energy between 2 and 6 kHz, but confirmed that certain types of background noise could interfere with identification of the larval signal.

The experiments and signal processing analyses reported here were conducted to gain experience with available acoustic technology and to evaluate potential methods for improving the ca-

pability to discriminate sounds of beetle larvae hidden in fibrous plants from incidental background noises.

MATERIALS AND METHODS

Insects and Recording Procedures

Sounds produced in 6 two-year-old potted date palms infested with RPW larvae were compared with sounds in 3 uninfested controls. Infestation was conducted by inserting a 3rd-4th-instar (85.7 ± 31.6 mg) into a 6-mm-diam. hole bored into the trunk. Sounds of larval activity were recorded weekly for 10 weeks for 1-2-min periods, once per week starting one week after infestation, with a microphone and amplifier (Larven Lauscher, NIR-Service, Bad Vilbel, Germany) attached to each tree (Soroker et al. 2004). In addition, laboratory experiments were conducted to examine patterns of insect feeding and movement sounds. Second and third instars (20-30 mg) were introduced into 28-33-mm-diam. stalks of sugarcane (*Saccharum officinarum* L), cut into lengths of 60-80 mm, on which they fed readily. The larvae were inserted into holes drilled parallel to the fibers. A 1-2-min record was obtained from each stalk 62-69 d after egg-laying. The larvae were weighed immediately after the recordings had been obtained, and ranged in weight from 0.17 to 1.7 g (mean \pm SE, 0.76 ± 0.156 g). Recordings also were obtained from uninfested sugarcane stalks.

For comparisons with other tree-boring insects, sounds of pine sawyer (PSW) larvae, *Monochamus titillator* (F.), were collected for 1-2-min periods on several different days from a pine bolt with the aid of an accelerometer system (Mankin et al. 2000, 2002). The ages of the larvae were unknown, but 4 adults emerged over a 1-month period, beginning about 2 weeks after the recording. Sounds of 2 buprestid (BUP) larvae, *Agilus dozieri* Fisher, were collected similarly from an oak tree branch about 2 weeks before the adults emerged, 3 d apart. The experiments with PSW and BUP were conducted in a laboratory with low levels of background noise. In all the experiments, subjective evaluation of larvae activity was conducted by listening to the sounds with a headset while recording (usually by an author). Recordings were digitized at 44.1 kHz on a computer and saved in .wav format.

Signal Processing

Digitized signals were analyzed with Raven 1.2 software (Cornell Lab of Ornithology, Ithaca, NY) and other dedicated signal sampling software developed in Matlab (MathWorks, Inc., Natick, MA), or with a customized software program, DAVIS (Mankin 1994; Mankin et al. 2000). Signals were band-pass filtered between 0.2 and 12

kHz to eliminate low-frequency background noise. Fast Fourier Transforms were calculated on 256-point time-slices of the waveforms with a Hamming window, and spectrograms were calculated from sections with 90 per cent overlap. Mean spectra (profiles) used for characterizing and identifying putative insect sound impulses were calculated based on 512-point time slices centered on the peak of each impulse in a section of recording independently verified to contain insect sounds without background noise (see Mankin 1994; Mankin et al. 2000, 2007).

In playback and oscillographic analyses of these signals, it became evident that a considerable fraction of the larval sound impulses were of low amplitude, barely above the peak background noise levels. Consequently, it was necessary to systematically adjust the signal processing amplitude threshold, T_a (Mankin et al. 2000), to maintain its level just above background. This was accomplished by calculating the root mean square of consecutive samples in 0.186-s (8192-point) time slices to estimate the peak to peak background noise in each interval. The T_a was reset for each time slice by multiplying the root mean square by a user-settable factor (3.25 in these tests), which held T_a just above the local peak background noise.

The DAVIS program identified and timed groups (trains) of impulses with interpulse intervals less than a preset duration, I_p , storing the beginning and end times of these trains in a spreadsheet along with the numbers of impulses in each train. Impulses that failed to match one or more specified insect sound profiles were discarded and not entered in the spreadsheet. The records contained sometimes as few as 30 or as many as 10,000 impulses, of which about 5%-70% were discarded, depending on the background noise levels. The beginning of a train was set as the beginning of the first valid impulse after a period where the interpulse interval was $> I_p$, and the end was set as the end of the last valid impulse whose interpulse interval was $< I_p$. We analyzed the signals in this study using the setting, $I_p = 500$ ms.

RESULTS AND DISCUSSION

Larval Sound-Impulse Characteristics

Examples of sounds produced by moving and feeding *R. ferrugineus*, *A. dozieri*, and *M. titillator* larvae in a palm tree, oak branch, and pine bolt, respectively, are shown in Fig. 1. Signals produced by RPW in sugarcane were similar in temporal pattern to those in palm. The beetle larvae produced variable-amplitude, 3-10-ms impulses with strong energy between 0.4-8 kHz (see spectrograms A_s , B_s , and C_s in Fig. 1). The signals extend to higher frequencies than the 0.5-1.8 kHz range of sounds produced in soil because wood

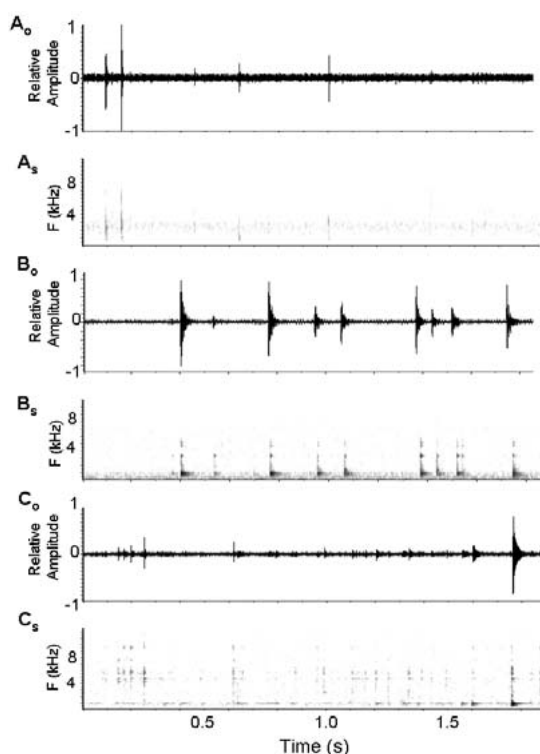


Fig. 1. Oscillograms, and spectrograms of several sound impulses produced by a (A_o , A_s) RPW, (B_o , B_s) BUP, and (C_o , C_s) PSW larvae; in the spectrograms, a darker/lighter color indicates frequencies of greatest/least energy.

has a lower attenuation coefficient than soil at high frequencies (Mankin et al. 2000). Other differences between the transmission of sound in a fibrous structure and soil include resonances that are influenced by the dimensions, mass, and stiffness of the structure (Cremer et al. 1988; Evans et al. 2005; Hambric 2006). Signals with energy at a resonance frequency will transmit farther in structures than nonresonant signals, and the long-distance transmission of background noises with components at resonant frequencies can interfere with detection of weak, insect-produced signals. One such resonance can be seen as a continuous, faint band near 2.5 kHz in the RPW spectrogram (Fig. 1 A_s), and similar resonances can be seen near 3 kHz in the BUP spectrogram (Fig. 1 B_s) and near 4.6 kHz in the cerambycid spectrogram (Fig. 1 C_s).

The effects of resonance are observable not only in the background noise spectra but also in the spectra of larval sound impulses (Fig. 2A-B). The examples of RPW sounds recorded from separate palms have peaks near 1.1 and 2.6 kHz in Fig. 2A. The sounds recorded from sugarcane have similar peaks. However, an example of palm

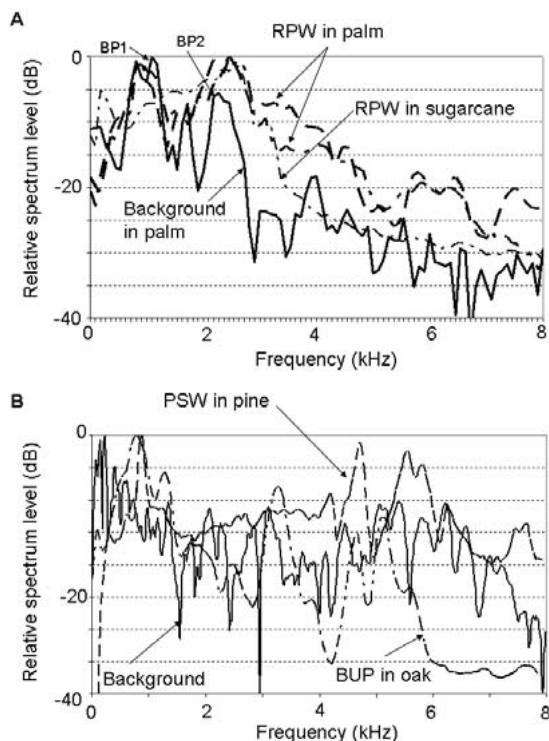


Fig. 2. Mean spectra of sounds recorded during a 1-min period from larval infestations: (A) RPW in palm trees (dashed line, $n = 303$; dash-dot-dot line, 348 impulses) and in sugarcane (dash-dot line, $n = 139$), and (B) BUP in oak branch (dash-dot line, $n = 142$) and PSW in pine bolt (dashed line, $n = 131$). Example spectrum of background noise is shown in (A) palm tree and (B) oak branch (solid lines), with background peak at 1 kHz marked as BP1, and peak at 2.4 kHz marked as BP2.

tree background noise also has peaks very close to these values, at 1 (marked at BP1 in Fig. 2A) and 2.4 kHz (marked at BP2); consequently, the energy at these frequencies is not due necessarily to the larvae alone. This result is unlike what is typically found for background noise in soil or air (Mankin 1994; Mankin et al. 2000; Mankin & Benshemesh 2006), where the energy of most background noise was found to decrease rapidly above 0.2 kHz. In previous studies with soil insects, the relatively low energy of background noise at frequencies above 0.5 kHz enabled construction of insect sound profiles and background noise profiles that could be used to identify individual sound impulses as insect sounds or background noises (Mankin et al. 2000, 2001, 2007).

For RPW in palm trees, there are differences in the spectra of larval and background noise between 3.4 and 6 kHz (Hetzroni et al. 2004a, b, see also Fig. 2A) that can be used to distinguish RPW sounds from background noise. Such differences were used to correctly classify ca. 90% of sounds

produced in infested palm trees (Hetzroni et al. 2004a, b). However, the examples of BUP spectra and background noise in oak branches present a more complicated result. In Fig. 2B, the BUP signal has peaks near 4.7 and 5.5 kHz, but there also are large peaks near 4.5 and 5.4 kHz in background sounds recorded from an uninfested oak branch. Other examples have been observed, particularly in urban environments, where background noise contains peaks of high energy above 0.5 kHz; which provides impetus to develop another method in addition to spectral profile analysis to help distinguish insect sounds from background noises.

Larval Sound Temporal Patterns

An additional method of interest for discriminating insect sounds from background noise is the identification of repetitive patterns that may occur when the larvae move inside their tunnels or scrape the wood fibers during feeding or tunneling activity. An example of such a pattern is seen in Fig. 3, adapted from Hetzroni et al. (2004a, b). A group of repeated impulses appear in Fig. 3Aa that are clearly distinguishable from the unpatterned impulses that were recorded from the uninfested palm (Fig. 3Bb). The existence of identifiable, repeated patterns in records of insect sounds has been noted previously (Andrieu & Fleurat-Lessard 1990; Mankin et al. 1997; Zhang et al. 2003).

Unlike the patterned sounds produced during insect communication (e.g. Walker et al. 2003), the RPW larval sound patterns examined in this study, as well as those produced by the BUP and PSW larvae, were consistent over only short periods of time. The most consistent pattern was not the series of high-amplitude impulses seen in Fig. 3A, but rather a mixture of low- and high-amplitude impulses that occurred in well-defined trains with short interpulse intervals, separated by 0.5-s or longer intervals between trains. Experienced listeners usually recognized these trains as distinct sounds, and frequently recognized them as characteristic of insect sounds.

The numbers of impulses per train varied considerably over the records in the palm tree study (1-99 impulses) and even greater in the sugarcane tests (1 - 239 impulses). Playbacks of records suggested that trains with > 6 but < 200 impulses were most recognizable to experienced listeners as insect movement and feeding activity. Generally, background noises in records from uninfested wood or sugarcane had fewer than 7 impulses per train. Consequently, we separated out a particular class of impulse trains, those with > 6 but < 200 impulses separated by intervals < 500 ms, as potential predictors of insect infestation, denoting them as bursts.

The distributions of impulse trains and bursts in six recordings from different insects are shown in Table 1, and examples of several impulse trains

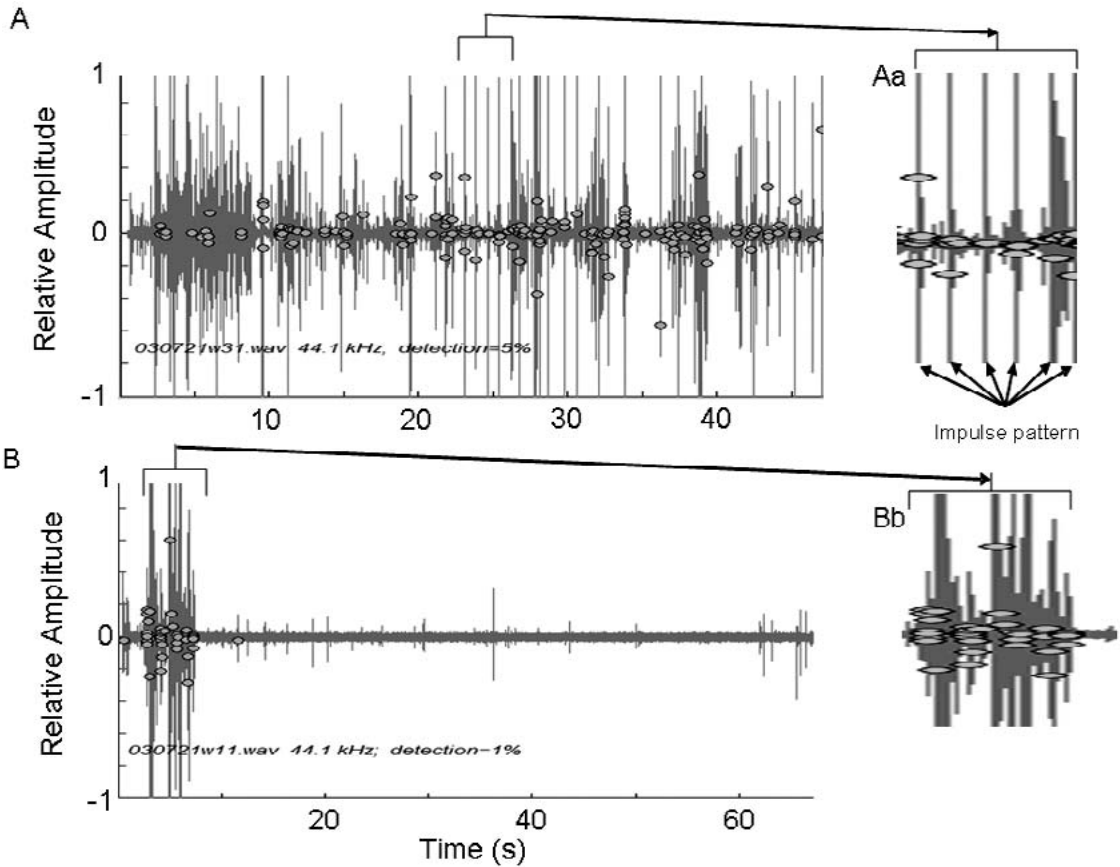


Fig. 3. Acoustic identification of RPW larval activity recorded from an infested (A) and uninfested (B) palm trees. Continuous line indicate original signal and shaded ovals indicate positive identification of potential larvae activity. Expanded view (Aa) show regularly timed impulses recorded from infested palm, and expanded view (Bb) shows irregularly timed impulses recorded from uninfested palm.

denoted as bursts are shown in Fig. 4. Most of the computer-identified bursts in the 4 examples, denoted by the numbered, shaded areas, correspond to groups of low- and high-amplitude impulses separated by periods with only sparsely occurring impulses. In Fig. 4A1, however, almost all of the impulses are low-amplitude, which suggests that the use of burst analysis as a method to augment profile analysis will be most successful in tests where sensors can detect very weak signals near the background noise threshold.

Use of Impulse-Train and Burst Analyses to Assess Infestation Likelihood

An important goal of insect acoustic detection is to discriminate whether or not individually tested samples contain infestations. A successful approach has been to construct indices of infestation based on quantitative and/or qualitative characteristics of detected sounds, and assess the likelihood of infestation using discretized indicators, e. g., low, medium, and high likelihoods of infesta-

tion based on lower and upper thresholds of the indices (Mankin et al. 2007). In 1 example, recording sites in soil were classified at a low likelihood of white grub, *Phyllophaga crinita* (Burmeister), infestation if 2 or fewer sounds (impulse-trains) per min were detected that matched white grub spectral profiles and at high likelihood if greater than

TABLE 1. NUMBERS OF IMPULSE TRAINS AND BURSTS DETECTED IN 1-MIN RECORDINGS FROM LISTED SOURCE.

Source	No. trains	No. bursts ¹
RPW in palm	55	15
RPW in palm	13	12
BUP in oak branch	29	5
BUP in oak branch	28	3
PSW in pine bolt	6	6
PSW in pine bolt	6	5

¹No. impulse trains with > 6 and < 200 impulses with intervals < 500 ms.

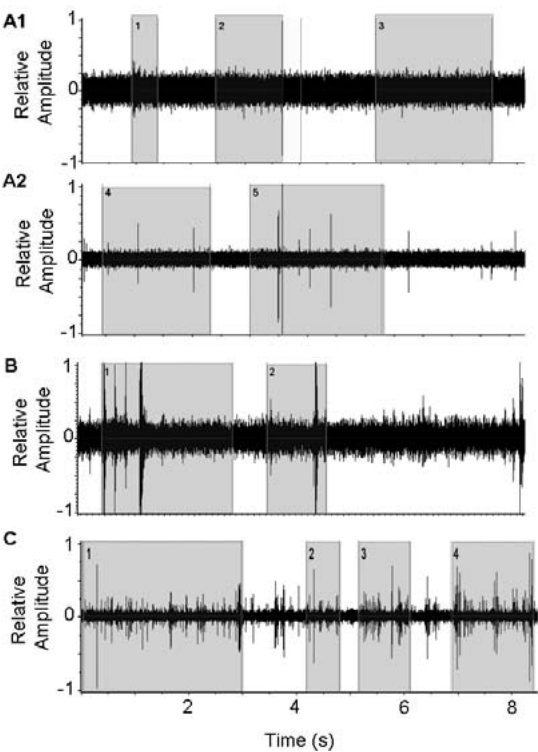


Fig. 4. Examples of impulse trains identified by computer as potential larval sound bursts (indicated by numbering and shading) in records from (A1) and (A2) RPW in palm, (B) BUP in oak branch, and (C) PSW in pine bolt.

20 impulse-trains per min were detected (Mankin et al. 2007). Here we considered a similar use of impulse-train bursts to assess RPW infestations in the laboratory tests with sugarcane.

Initial analyses of records from infested and uninfested sugarcane indicated that the RPW-in-palm and RPW-in-sugarcane spectral profiles in Fig. 2 readily classified background signals as

noise with DAVIS and correctly identified signals produced in infested sugarcane as being produced by RPW. To consider the distribution of impulse-train bursts in infested and uninfested sugarcane, we analyzed one 50-s segment recorded from each of 21 different infested and 4 uninfested stalks, discarding sounds that did not closely match the profiles (Mankin et al. 2007, 2000). The results are summarized in Table 2, where the complete data set is separated into 4 categories, 1 for recordings in uninfested sugarcane, and 3 for records in infested sugarcane where the numbers of bursts ranged from 7 to 17, 4 to 6, and 0 to 3, respectively, and the mean larval weights, numbers of trains, and numbers of impulses per train are listed for each burst category. The correlation the numbers of bursts and the larval weight was not significant (Spearman $\rho = 0.41$, $P = 0.08$ for null hypothesis of $\rho = 0$) (Proc CORR, SAS Institute 2004a). Likewise, the correlation between numbers of impulse trains and larval weight correlation was not significant (Spearman $\rho = 0.06$, $P = 0.79$ for null hypothesis of $\rho = 0$) (Proc CORR, SAS Institute 2004a).

The observed distribution of bursts suggested that the likelihoods of infestation (Mankin et al. 2007) in these tests could be set at: Low, $n_b < 1$; Medium, $1 \leq n_b < 3$; High, $n_b \geq 3$, where n_b is No. bursts per 50-s record. Based on these criteria, 2 of the 4 records from uninfested sugarcane had Low likelihood of infestation, and 2 had Medium likelihood of infestation, while 2, 1, and 18 of 21 records from infested sugarcane had Low, Medium, and High likelihoods of infestation, respectively. These 2 distributions were significantly different under the Wilcoxon Two-sample Exact Test, with sum of scores = 17.0 and $P = 0.0025$ (Proc NPAR1WAY, SAS Institute, 2004b). Although additional testing will be necessary to establish the practical efficacy of incorporating burst analysis into acoustic surveys of RPW in palm trees and offshoots, these results indicate that it is possible, using already existing signal processing software, to extract previously unmea-

TABLE 2. COMPARISONS OF MEAN LARVAL WEIGHTS, NO. IMPULSE TRAINS, AND NO. IMPULSES PER TRAIN FOR RECORDINGS IN WHICH LISTED NOS. BURSTS WERE DETECTED IN 50-S PERIOD.

Range of nos. bursts ¹	Mean \pm SE		
	Larval wt. (g) (n_v)	No. trains (n_t)	No. imp. / train
7-17	0.98 \pm 0.19 (9)	20.10 \pm 2.45 (10)	15.00 \pm 5.74
4-6	0.69 \pm 0.39 (7)	14.14 \pm 5.59 (7)	40.10 \pm 17.27
0-3	0.27 \pm 0.05 (3)	1.25 \pm 0.75 (4)	47.80 \pm 44.36
	Uninfested ²	6.5 \pm 1.71 (4)	5.58 \pm 2.93

¹Records with Nos. bursts in listed range, where a burst is an impulse train with > 6 and < 200 impulses: (n_v), No. records in mean for larval weight in listed burst range; (n_t), No. records in means for trains and impulses per train in listed burst range. Weights not available for 2 records.

²In 4 recordings from uninfested sugarcane, 2 had 0 bursts, and 2 had 1 burst.

sured information from acoustic recordings that can assist the process of discriminating sounds produced by hidden insect infestations from background noise. The burst analysis method appears to be robust in that it can be applied to signals from multiple species of insects recorded from multiple species of trees. Experienced listeners will always have superior capabilities to identify spectral and temporal patterns that assist in discrimination of insect sounds from background noise, but better understanding of the behaviors that produce the detected sounds and better understanding of the specific methods used by listeners in identifying signals likely will lead to continued improvements in automated acoustic detection methods.

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