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PHEROMONE-FOOD-BAIT TRAP AND ACOUSTIC SURVEYS OF *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: CURCULIONIDAE) IN CURACAO[1]

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ABSTRACT

Pheromone-food-bait trap and acoustic surveys were conducted in Curacao to monitor a recently discovered invasion of *Rhynchophorus ferrugineus* L. (RPW). This pest of economic importance in regions of Asia, the Middle East, and the Mediterranean was not observed in the Americas until 2009. Due to its economic and environmental damage, there is an urgent need to manage or eradicate RPW in Curacao to reduce its impact on the island as well as avoid the possibility of transference to surrounding regions. Studies were conducted to explore methods available for monitoring adults with traps and acoustically assessing larval infestations in trees in the warm, dry but humid Curacao environment - considering also some special challenges of urban conditions, such as increased traffic noise or unwanted human curiosity that could negatively affect monitoring success. Bucket traps baited with 4-methyl-5-nonanol/ 4-methyl-5-nonanone pheromone lure, ethyl acetate and a molasses - ethylene glycol mixture captured RPW adults at consistent rates for ca. 7/d, but the rates of capture were reduced for the 9th to 14th d of a two-week sampling period. The weevils were observed to escape rapidly from dry traps but not from liquid-containing traps. A portable, user-friendly acoustic sensor system enabled identification of larvae in individual infested trees through the use of signal processing analyses that screened out bird and wind noise. This information gained about liquid baits and acoustic differences between background noise and RPW-produced sounds can assist future efforts to monitor, control, or eradicate RPW in Curacao as well as other urban landscapes.

Key Words: Caribbean, invasive species, liquid bait weevil, red palm weevil, signal processing analysis

RESUMEN

Se realizaron sondeos utilizando trampas con cebo de feromonas y comida, y otro acústico en Curazao para monitorear la reciente invasión descubierta de *Rhynchophorus ferrugineus* L, el picudo rojo de las palmeras (PRP). Esta plaga de importancia económica en las regiones de Asia, el Medio Oriente y el Mediterráneo no se observó en las Américas hasta el año 2009. Debido a su daño económico y ambiental, hay una necesidad urgente de controlar o eradicar el PRP en Curazao para reducir su impacto en la isla, así como evitar la posibilidad que se extienda a otras regiones cercanas. Se realizaron estudios para explorar los métodos disponibles para monitorear los adultos con trampas y evaluando acústicamente las infestaciones de larvas en los árboles en el ambiente cálido y seco pero húmedo de Curazao, tomando en cuenta también algunos desafíos especiales de las condiciones urbanas, tales como el mayor nivel del ruido del tráfico y de la inoportuna curiosidad humana que pudiera afectar el éxito del monitoreo. Trampas de cubos cebadas con el señuelo del feromona 4-metil-5-nonanol/4-metil-nonanone, el acetato de etilo y melaza- una mezcla de glicol de etileno capturó los adultos de PRP a tasas consistentes por casi 7 dias, pero la tasa de captura se ha reducido para el dia noveno hasta el dia decimocuarto del periodo de muestreo de dos semanas. Se observaron que los picudos se escaparon rápidamente de las trampas secas, pero no de las trampas que tienen líquidos. Un sistema de sensores acústicos, portátil y fácil de usar permitio la identificación de larvas en árboles individuales infestados mediante el uso de análisis de procesamiento de señales que descaratarон los ruidos hechos por las aves y del viento. Esta información puede ayudar a los futuros esfuerzos para monitorear, controlar o eradicar el PRP en Curazao, así como en otros lugares urbanos.

The red palm weevil (RPW), *Rhynchophorus ferrugineus* L. (Coleoptera: Curculionidae/Rhynchophoridae/Dryophthoridae) is an economically important pest of palm trees in Asia, the Middle East, and the Mediterranean region (Mukhtar et al. 2011), but until recently was not observed in the Americas (Roda et al. 2011). The discovery of RPW in both Curacao and Aruba consequently was of major concern to plant protection authorities and industry because of its potential to cause economic or ecological impact. In the Caribbean region, palms are grown for coconuts, dates, oil and other agricultural purposes, and are important nursery crops. They are also an integral part of the landscape and are ubiquitous in the tourism industry, a major source of revenue in both islands. When palm trees in the hotel resorts are damaged by RPW, the landscape managers usually replace them with fully grown trees at a cost of several thousand dollars each (Roda et al. 2011). Hotel resorts and tourism associations in Curacao therefore are strongly interested in reducing or eliminating RPW populations.

To assist the Curacao Department of Agriculture and the Curacao Hospitality and Tourism Association (CHATA) in monitoring RPW spread and population levels, and to help avoid transference of RPW to other nearby islands or countries, a program to monitor the RPW infestations in Curacao was initiated in Sep 2009 using bucket traps baited with 4-methyl-5-nonanol/ 4-methyl-5-nonanone (an aggregation pheromone; Hallett et al. 1993) and an attractant containing ethyl acetate and a molasses/water/ethylene glycol mixture (Roda et al. 2011). The traps were deployed primarily in urban areas containing palm tree host plants of high value and serviced every 2 weeks.

Additionally, acoustic detection studies were conducted to help identify individual palm trees that needed to be treated or removed due to infestation (Mankin et al. 2011). The early stages of infestations are difficult to detect visually because newly hatched larvae bore into and hide in the trunk tissue (Faleiro 2006). The infestations can be anywhere in the trunk, although the highest likelihood is either close to the ground in heavily irrigated groves, or otherwise near the crown (Faleiro 2006). Infestations near the crown have been acoustically detected from distances of up to 4 m (Mankin et al. 2011). Our previous observations of RPW in Curacao suggested that most infestations would be found in the crown areas, although infestations occasionally were found near the root system.

The Caribbean island urban environment with year-round high heat and humidity, frequent wind, bird song, and high traffic background noise provides unique challenges for both pheromone-food-bait trapping and acoustic surveys. On a small island with limited resources, the costs and availability of bucket trap supplies and the timing of servicing can be major concerns, and in the initial acoustic detection studies conducted in Curacao (Roda et al. 2011; Mankin et al. 2011) a variety of wind and background environmental noise conditions were encountered that restricted the locations and timing recording sessions. This suggested that further acoustic studies would be needed to develop a better understanding of the types of noise that would be encountered and to identify what measures could be taken to reduce the effects of background noise on measurements of RPW infestations.

Here we report on studies that further explored the use of pheromone-food-bait bucket traps to monitor RPW populations in Curacao. We considered the effects of the absence and temporal changes of the trap liquid mixture on RPW behavior and daily trap catches. Additionally, acoustic studies were conducted with a portable, user-friendly instrument designed to filter out low-frequency background noise (Mankin 2011) to assess the likelihood of detecting infestation in an individual tree in a variety of background noise environments, as well as the feasibility of detecting whether a treatment to eliminate an infestation in a tree could be successful.

Materials and Methods

Red Palm Weevil Traps

RPW traps were constructed from white, 13.25-L buckets (Freund Container, Chicago, Ilinois, USA) as described in Roda et al (2011). The traps were baited with 4-methyl-5-nonanol/4-methyl-5-nonanone and ethyl acetate purchased from Chemtica Internacional SA, Heredia, Costa Rica), and 2 L of a readily available mixture of molasses, ethylene glycol, and water. The molasses mixture was prepared by combining 200 ml of molasses (Agarische Kooperatieve Vereniging, Curacao) with 2.5 L ethylene glycol in 17 L of water. The traps were placed on telephone poles or non-host trees at ca. 1.5 m height and ca. 100 m from a *Phoenix* sp. palm. Locations of individual traps were geographically referenced using an eTrex GPS (Garmin, Olathe, Kansas). The traps were monitored daily for 14 d from 10-24 Feb 2011 at 13 different urban locations and again from 24 Feb-10 Mar 2011, when 12 of the original locations were monitored. At the start of each sample period all trap components (e.g. pheromone, ethyl acetate and food bait mixture) were replaced. The number of RPW trapped each d was recorded and all captured RPW were removed. The mean number of RPW trapped per d was analyzed with a repeated measures (split plot) model using the REML (REstricted or REsidual Maximum Likelihood) method (SAS Institute 2008). Locations were treated as a random variable with d (1-14) and the two sample periods repeated within location. Student's t-test was used for separation of the two sample-period means.

Comparisons of Weevil Escapes from Dry and Liquid-Containing Buckets

Laboratory tests were conducted to consider previous observations that RPW escaped quickly from dry but not liquid-containing bucket traps. Five replicates were conducted with 13.25-liter bucket traps and 2-liter plastic cups, first without and then with water. For tests with liquid, 1 liter of water was placed into each 2-liter cup, and two liters into each 13.25-liter bucket In each test, 5 female weevils obtained in the field were introduced into the container and observed until they left the trap or cup or died (approximately 2 wk after introduction).

Acoustic Measurements from Infested and Uninfested Date Palm Trees

To establish further distinctions between RPW signals and environmental background signals, acoustic recordings were obtained immediately below the crown of one date palm (*Phoenix dactylifera* L.) with visual symptoms of infestation (yellowed fronds with observable tunnelling, asymmetrically oriented around crown) and from similar positions below the crowns of 3 date palms exhibiting no visual symptoms of infestation (e.g., without holes or tunnels in leaves or crown, oozing liquid, fermented odor, or other symptoms noted in Faleiro (2006)) at the Exotic Garden Nursery. The trees were in a stand of young date palm trees less than 3 m in height. Information about the recording time and location, wind, and other environmental conditions were documented in notes at the time of recording.

The recording methods were similar to those described in Mankin et al. (2009) and Ulyshen et al. (2011). A nail long enough to extend past the external fronds or a 1.59-mm titanium drill bit was inserted into the palm tree trunk near the crown and a sensor-preamplifier module (model SP-1, Acoustic Emission Consulting, Inc., Sacramento, California) was attached magnetically. The sensor was connected by cable to an AED-2000 amplifier with a headphone output for on-site listener assessment of signals and an output to a digital audio recorder (model HD-P2, Tascam, Montebello, California) for subsequent computer assessments of the spectral and temporal characteristics of the signals (Mankin et al. 2008, 2011). The AED-2000 amplifier filters out signals below 1 kHz, where much of the traffic and wind noise occurs (Mankin et al. 2011), and it has been designed with many user-friendly features to enable rapid training of nonspecialist surveyors. The headphones provide an immediate assessment of infestation likelihood that can be augmented by subsequent computer assessment of whether the signals are detected as bursts (groups) of short impulses typical of insect movement and feeding activity (Mankin 2011).

Results

Spatial and Temporal Patterns of Pheromone Trap Captures

Red palm weevils were captured at 11 of the 13 locations around Curacao (Fig. 1).When RPW were trapped, the total trap catches ranged from 1 weevil/14 d up to 13 weevils/14 d. RPW was found to be present across the entire island. However, the highest numbers of weevils were found in the urban areas of Willemstad where the largest numbers of host plants were observed. There were significantly fewer weevils ($t = 6.24$; $N = 308$; $P = 0.01$) caught in the second sample period, 24 Feb-10 Mar, than in the first period, 10-24 Feb (Fig. 2), but there was no interaction between sample period and d. This suggests that the mean number of RPW flying among trees was lower during the second period. Combining both sampling periods across d, the number of weevils trapped decreased significantly ($F = 3.42$; df = 13, 308; $P < 0.001$) as the food bait mixture aged. The mean number of weevils caught on d 1-7 were significantly higher than the numbers collected on d 9-14. The mean number of weevils caught on day 8 was not different than the means from other d. Daily observations indicated that the level of food bait liquid did not evaporate substantially over the 14 d. However, ferment odors produced by food bait liquid were perceived to increase as the mixture aged.

Evaluation of RPW Capacity to Escape from Dry or Liquid-Containing Traps

All female weevils placed in dry 2- and 13.25-L buckets climbed to the tops of the containers

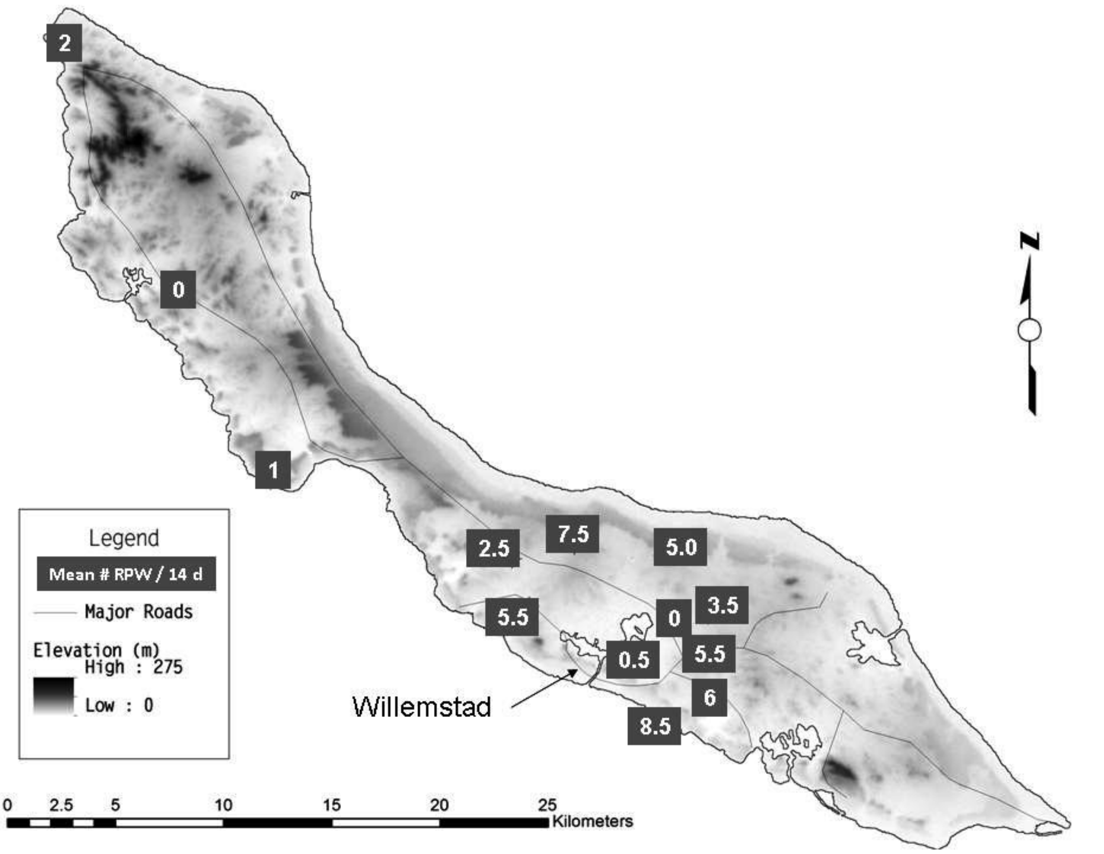

Fig. 1. Mean No. RPW captured in the two trials at each location where traps were placed in Curacao (except at upper NW corner, where the trap disappeared during the second trial and only first trial was used).

within a period ranging from a few seconds to < 5 minutes despite the smooth inner surfaces. Each weevil remained for a few seconds on the lip of the container and then dropped outside the container without attempting to descend by crawling. When water was added to the containers of both types, no RPW succeeded in climbing out. The weevils remained alive for approximately 2 wk, but their behaviors in the water apparently kept them from escaping. Although they were observed frequently to swim to the wall of the bucket, the swimming motions bounced them away from the edge before they could climb onto the container wall. If they encountered another weevil, they attempted to climb over each other but at these water levels they could not reach a position high enough to enable flight and escape.

Comparisons of Acoustic Signals from Infested and Uninfested Date Palm Trees

To accommodate the high levels of background noise associated with wind and bird calls on Curacao, the recordings were analyzed using a three-stage process that first identified periods where listeners detected predominantly a single type of signal, either from larvae, wind, wind-induced leaf-tapping noise, or bird calls. Representative periods were obtained for each type of signal by screening playbacks of all 51 recordings from infested and uninfested palms in Raven 1.3 (Charif et al. 2008). In the second stage of the analysis, a spectral profile of RPW larval sounds from the infested palm was constructed from a 70-s period with 40 impulse bursts (Fig. 3). Spectral profiles also were constructed from a 22-s period of continuous wind, a 21-s period of continuous tapping, and a 120-s period containing 45 bird calls (Fig. 3) recorded from trees which had no visible signs of infestation. Finally, using the profiles in Fig. 3, we passed the signals from all files through a customized program that identified the temporal patterns of sound impulses and discriminated against signals that did not match the typical impulse-burst patterns of insect sounds (Mankin et al. 2009; Mankin 2011). Using this combination of profiles that identified larval impulses and rejected wind, birdcalls, and tapping

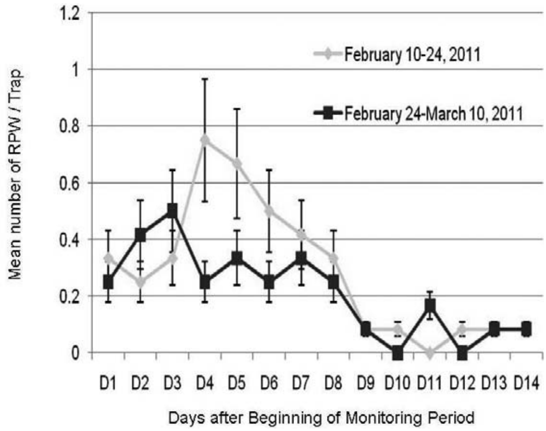

Fig. 2. The mean number (±SE) of red palm weevil adults (male and female) caught from traps located at 12 sites in Curacao

sounds, the analyses identified multiple impulse bursts matching the RPW profile in recordings from the infested palm, as in the example of Fig. 4, but none from the trees lacking visible signs of infestation were present.

The oscillogram and spectrogram of Fig. 4 is typical of the signals found in the recordings that were obtained from the infested palm. The example contains a mixture of signals matching the RPW, wind, and bird song profiles beginning at about 4 s into the recording. Several bird calls then appear, followed by a period of RPW signals. The total rate of production of RPW larval sound bursts in the entire 70-s recording was 0.69 bursts / s, while the rate of bird calls was 0.014 burst / s, and the rate of wind bursts was 0.042 bursts / s. In this recording, the RPW signals are slightly louder than the bird calls and have relatively more signal below 2.5 kHz. The bird song has relatively more signal above 6 kHz, and the wind signals extend from 3-8 kHz.

None of the signals in recording period of Fig. 4 matched the tapping sound profile, possibly because the relatively light wind. Altogether, 11 of 32 separate recordings obtained over a three-day period had relatively high levels of wind and/or noise and 5 had no detectable levels of wind noise. The remainder had intermediate levels of wind noise. The tests were done partly at times and places where wind levels would be higher, e.g., during afternoons and in exposed areas, but it should be noted that wind levels could be reduced if the acoustic tests were conducted well inside the treed area or early in the morning, when wind levels would be low. It was also found that changing the amplification level during different parts of an assessment at a given site enabled listeners to more easily distinguish the RPW sounds from other nontarget noise, because the larvae were present at short range while much of the background noise, including traffic noise, was distant. Changing the amplification level enabled the distant and short-range signals to be differentiated.

Twenty four hours after the recording of the signals in Fig. 3 from the infested date palm tree, the crown was cut open and 9 RPW larvae were removed. An acoustic recording was obtained immediately thereafter, and RPW signals were detected at the rate of 0.15 bursts / s. Wind sounds were detected at the rate of 0.037 bursts / s. The nursery owners applied imidacloprid (Dominion 2L®) at a 7.16 ml/L (v:v) dosage typically used after discovering an infested date palm. Twenty four hours after the treatment we recorded from the tree again. This time, no RPW signals were detected, but wind sounds were detected at the rate of 0.33 bursts / s, and bird sounds were detected at the rate of 0.052 bursts / s.

Discussion

The age of the food bait mixture was found to be an important factor affecting the number of RPW trapped in Curacao. To optimize the number of RPW caught, the molasses based food-bait mixture used in this study should be replaced every 7-8 d. A similar recommendation was reported in the Middle East by Faleiro et al. (2011), who used dates instead of molasses and serviced the traps at 7-d intervals. The reasons for the decreased in attractiveness of the mixture could have resulted from the loss of attractive odors or the increase of unfavorable compounds. In this study, an increase in fermentation odors was perceived with increased age of the mixture. Previous studies have indicated that general fermentation volatiles, such as ethanol appear attractive to *Rhynchophorus* weevils (Hagley 1965; Giblin-Davis et al. 1996; Guarino et al. 2011). The mixture used in Curacao may be producing additional compounds that are overriding the attractive components. A molasses/water mixture was used in Curacao because studies have shown the bait to be equally synergistic as sugarcane and pineapple tissue in attracting *Rhynchophorus* beetles to pheromone-baited traps (Giblin-Davis et al. 1994). In Curacao, molasses is relatively inexpensive and accessible compared to other food baits such as sugar cane, dates or host plant fronds commonly used in RPW trapping systems (Faleiro 2006). Further studies are needed to determine if other food baits, although more costly, may provide continued attraction to the weevils to allow longer duration between servicing traps. Weekly servicing reduces the amount of information lost if a trap is vandalized or removed. However, increasing the time between servicing traps is critical, particularly where human and other resources are limited. Longer service intervals would reduce costs for organizations like the Curacao Hospitality and Tourism Association, for example,

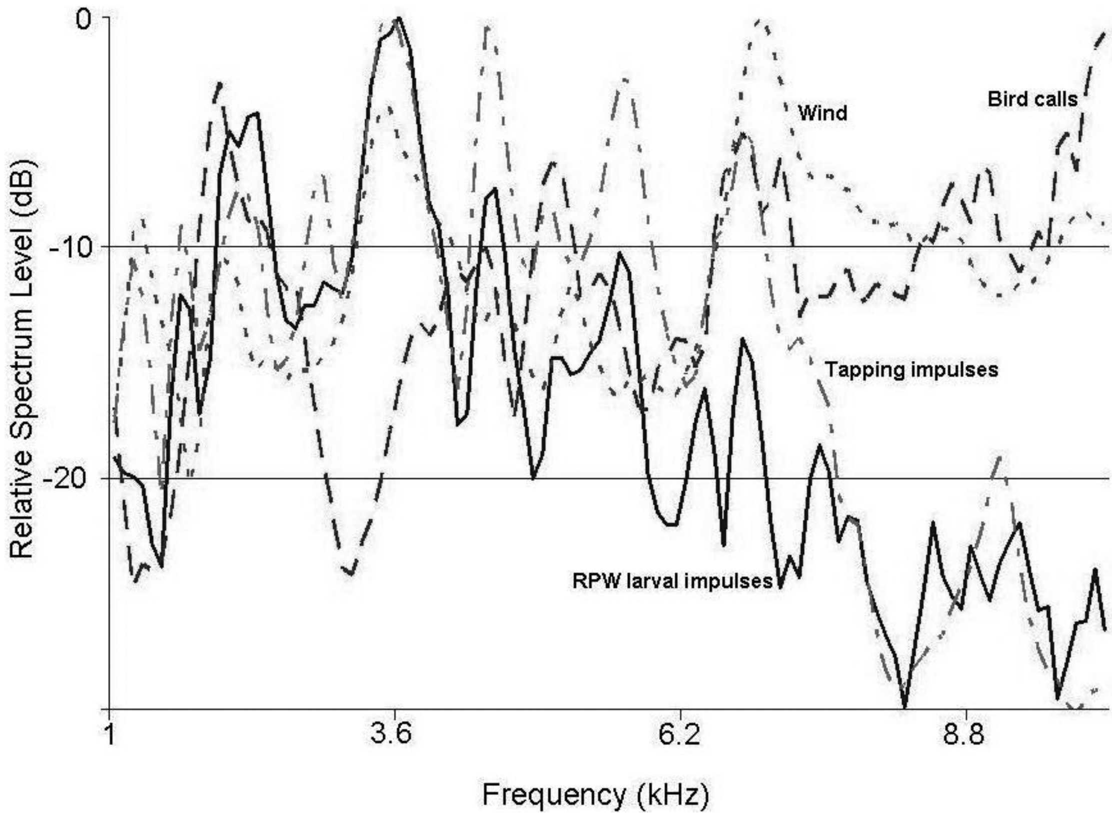

Fig. 3. Spectral profiles of RPW larval impulses (solid line) obtained from palm tree with visible signs of infestation, and wind (dotted line), bird song (dashed line), and wind-induced tapping impulses (dotted line), obtained from palm trees without visible signs of infestation.

which plans to operate a ring of traps to attract weevils away from the tourist areas and reduce influx of weevils from nearby residential areas (Roda et al. 2011).

Contrary to anecdotal suggestions, the results also indicated that *R. ferrugineus* has the capacity to move quickly on smooth surfaces, and confirmed its ability to escape from dry traps. Considering the relatively long time spent in the water before death of adult insects, care should be taken to keep trap liquid levels high. The bucket traps used in Curacao frequently were found dry after 14 d in the field (Roda, personal observation). Optimizing the design of the trap to reduce the evaporation rate of the food bait/water mixture or where physical barriers are placed to prevent the escape of climbing weevils may allow for longer periods between servicing traps.

This and previous studies (Mankin et al. 2008; Potamitis et al. 2009; Mankin et al. 2011 and references therein) indicate that hidden infestations of RPW can be detected acoustically without difficulty in a variety of environmental conditions. However, some of the limits of detectability remain to be determined, including the size of the youngest instars that can be easily detected. In Mankin et al. (2008), spectral differences were found between RPW larval signals and background noises at frequencies between 3.4 and 6 kHz that were helpful in distinguishing the larval sounds from background. In the Curacao study, we considered an expanded range from 3.4 to 10 kHz because the bird calls and wind noise had high frequency components that distinguished them from the larval sound impulses (Figs. 2 and 3). It was more difficult to distinguish wind-induced tapping sounds from larval sounds, partly because both the larval sounds and the leaf-tapping sounds excited some of the same resonance frequencies in the palm tree (Mankin et al. 2011). However, it was possible to discard many bursts of trapping sounds in the computer analyses by excluding bursts with more than 200 impulses per burst (Mankin et al. 2008) because the insects performed their movement or feeding actions for shorter periods of time. Also, listeners who can observe occurrences of wind gusts while simultaneously monitoring signals in the palm tree with headphones can exclude tapping sounds by their context as a part of the extraneous, wind noise background. Consequently, when the

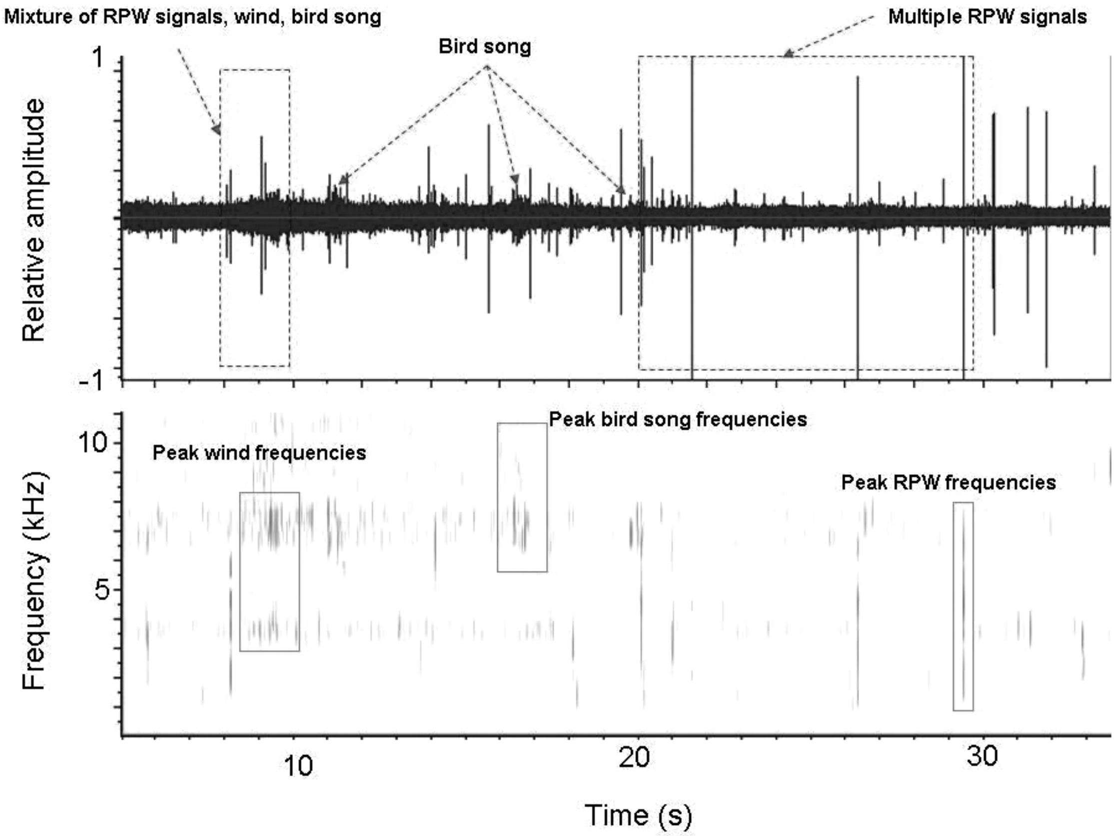

Fig. 4. Oscillogram and spectrogram of a 30-s period of signals recorded from a vibration sensor inserted into a small date palm tree containing visible signs of RPW infestation. Two periods with high levels of RPW activity are marked by dashed rectangles. Other periods with wind and bird song are marked with arrows in the oscillogram.

signal levels of the background noise are not overpowering, it may be possible to distinguish these noises from RPW sounds either by training of the listeners or by using computer analyses that recognize the lower frequency RPW signals in the higher-frequency background. A feature of the AED-2000 that allows rapid changing of amplification levels also assists listeners in distinguishing signals produced near the sensor from those produced further away.

Finally, the result that the RPW signals declined to zero within a day after the imidacloprid treatment suggests that the acoustic methods might be useful for assessment of whether an insecticide treatment was effective. However, additional studies of the time course of decline of larval activity and subsequent larval death would be necessary to establish the efficacy of such assessments

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