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A REFEREED PAPER

LISTENING TO THE LARVAE: ACOUSTIC DETECTION OF *DIAPREPES ABBREVIATUS* (L.)

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Abstract. ***Diaprepes abbreviatus* (L.) is an important pest of citrus trees in Florida and the Caribbean. The larvae feed underground on the root systems, reducing productivity and facilitating invasion by root pathogens, including *Phytophthora* spp. Field studies to survey or control larval populations typically involve labor-intensive, destructive excavation of root systems. However, nondestructive, portable instruments are now available that can detect sounds made by insects moving and feeding underground. Several different instruments have been tested successfully for detection of subterranean *D. abbreviatus* larvae and other insects, but many questions remain about the use and reliability of acoustic detection tools in specific insect-detection applications. This report describes recent experiments with currently available acoustic systems to assess the detectability and interpretability of sounds produced by *D. abbreviatus* larvae and other organisms in root systems of individual trees in citrus groves. It was confirmed that such instruments could successfully predict the presence or absence of subterranean insects under individual trees. However, the instruments do not provide a comprehensive picture of the distribution of sounds (or insects) around a tree unless multiple samples are recorded at ca. 10-cm spacings within the root system. The rates of sounds detected from a subterranean insect can vary considerably at different times, depending on its patterns of behavioral activity. The rates also can vary considerably at different positions within a sensor's detection range, depending on the types of sound produced and the presence of roots or stones between the insect and the sensor.**

The development of improved methods for detecting and monitoring *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) has been a long-standing priority for citrus growers and regulatory agencies (*Diaprepes* Task Force, 1995; Nigg et al., 1999). The larvae feed on the root systems of citrus trees (Beavers and Selhime, 1975), directly damaging them or facilitating invasion by root pathogens, including *Phytophthora* spp. (Rogers et al., 1996).

Until now, direct detection of the larvae usually has involved labor-intensive excavation and inspection of root systems (McCoy et al., 2003). However, new acoustic technologies are showing potential as rapid, nondestructive tools for surveying subterranean insect infestations (Mankin et al., 2000). Several different microphone and accelerome-

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ter systems have been used successfully to detect sounds generated by subterranean larvae in citrus groves (Mankin et al., 2001), forage fields (Brandhorst-Hubbard et al., 2001), and nursery containers (Mankin and Fisher, 2002a). The success of these initial studies led recently to the development of user-friendly instrumentation designed specifically for insect detection applications (Mankin and Fisher, 2002b).

Although considerable progress has been made in developing such instruments as insect detection tools, many unanswered questions remain about the interpretability of measurements and the distances over which insects can be detected. Such uncertainties are due partly to the physical structure of soil. Like stored grain (Shuman et al., 1997), soil is not acoustically homogeneous. The presence of different soil textures, intervening roots, or stones can considerably alter the temporal and spectral qualities of an insect-generated sound over distances of a few cm. In addition, sounds produced at the same location may be transmitted over considerably different distances if they are produced with different amplitudes and spectral profiles (Mankin et al., 2000). As the distance between a sound source and a sensor increases, uncertainty about the original spectral profile and location of the source increases considerably more rapidly in soil than in air. We conducted a study in a citrus grove infested with *D. abbreviatus* in September, 2002, to gain further insights into the detectability and interpretability of acoustic signals from subterranean larvae in field environments.

Materials and Methods

Acoustic Systems. Two portable acoustic systems were used in this field study. The primary system, described in more detail in Mankin et al. (2000, 2001) and Mankin and Fisher (2002a), included an accelerometer and charge amplifier (0-80 dB gain), a dual-channel digital audio tape recorder, and a stereo headphone. The spectral range of the accelerometer system was ca. 0-8 kHz. The accelerometer was attached to a 30-cm long, 0.6-cm-diameter steel probe, pushed into the soil at an angle to pass near the crown of the citrus tree roots. The amplified signal was monitored through the headphones and passed to the recorder for further analysis (see *Signal Analysis and Assessment of Infestation Likelihood*).

A second acoustic system, custom-designed for insect detection applications (Mankin and Fisher, 2002b), included a sensor-preamplifier module attached to a 20-cm-long, 0.6-cm-diameter probe (Model SP-1, Acoustic Emission Consulting, Inc. [AEC], Sacramento, Calif.). The preamplifier supplied 40-dB-gain between 1 and 50 kHz. The module was shielded to reduce airborne background noise, and the reduced sensitivity at frequencies <1 kHz eliminated much of the remaining noise, which usually has peak frequencies below 400 Hz (Mankin et al., 2000). The SP-1 sensor was attached to a Model AED-2000 (AEC, Sacramento, Calif.) amplifier unit providing a programmable, 0-60-dB additional gain. The amplifier had an output for oscilloscopes or recorders, a headphone port, a serial port for computer logging and signal display, and a front-panel display of signal intensity and sound pulse counts.

Recording and Infestation Verification Procedures. Tests were conducted with the accelerometer and SP-1 probes during September 25-26, 2002, in an experimental grove of 15-yr-old 'Minneola' tangelo trees (*Citrus paradisi* Macf. × *C. reticulata* Blanco) on ×639 rootstock (*C. reticulata* Blanco × *Poncirus trifoliata* (L.) Raf.) at the IFAS Indian River Research and Education Center, Ft. Pierce, Fla. The grove was on double beds on Winder sand depressional soil (hyperthermic Typic Glossaqualfs). In an initial survey to locate sites with infestations that could be studied in depth, a >3-min period was recorded from one or more probes inserted into the soil underneath 10 separate trees. The probes were positioned within 10 cm of the trunk and pointed towards the crown.

To consider the distribution of sounds around an entire tree, recordings were done at 10 additional positions around Tree No. 9, identified in the initial survey as one of the most active trees (see *Signal Analysis and Assessment of Infestation Likelihood*). Several of these measurements were made by simultaneously feeding the output from the accelerometer amplifier and the AED-2000 into separate channels of the dual recorder, enabling the same sounds to be compared instantaneously at multiple positions.

Just before excavation on the second day, the tops of Trees No. 6,7, 9, and 10 were cut off at ca 10-cm height and simultaneous recordings were obtained from probes inserted into the trunk and in the soil. The tops were removed to reduce interference from wind noise (Mankin et al., 2000, 2002) and facilitate direct comparison of the acoustic signals from the trunk and the soil probes. Loud sounds were detected both in the soil and in the trunk at Tree No. 6. For an in depth analysis, we first recorded three consecutive 3-min intervals with the SP-1 probe inserted into the trunk and the accelerometer probe in the soil at a single position, 3 cm from the trunk. The signals were fed simultaneously into separate channels of the recorder. A fourth interval then was recorded with the SP-1 probe moved to a point 3 cm from the trunk and 3 cm from the accelerometer probe.

After the recordings were completed on the second day, the tested trees were excavated and the root systems were examined. The numbers of *D. abbreviatus* larvae and other sound-producing organisms recovered from the root systems were noted for comparison with the acoustic assessments of infestation likelihood (see next section).

Signal Analysis and Assessment of Infestation Likelihood. The recorded signals were digitized in the laboratory and quantitatively analyzed using a custom-written signal processing system (Mankin et al., 2000, 2001). When high levels of background noise sometimes interfered with analysis of a complete 3-min recording, we evaluated shorter, contiguous sections; however, the file was discarded if we could not find an analyzable section of at least 30-s.

Moving and feeding *D. abbreviatus* grubs produce 2-5-ms clicks and scraping sounds that experienced listeners and computer programs identify as grub sound pulses (Mankin et al., 2001; see also http://cmave.usda.ufl.edu/~rmankin/soundlibrary.html). These distinctive pulses are easily distinguishable from wind noise, bird calls, or engine noise, but cannot be distinguished easily from sounds made by mole crickets (*Scapteriscus* sp.) and other subterranean insects often found in citrus groves (Mankin et al., 2000). In the rest of this report, all such sounds were designated as grub pulses, although some of them were produced by *Scapteriscus* (see Table 1). The rate of grub sound pulses can be used as a guide to assess the likelihood that a tree is infested. In a previous study of *D. abbreviatus* (Mankin et al., 2001), the likelihood of infestation was scaled as *low* for pulse rates ≤2/min, *medium* for rates between 2 and 20/min, and *high* for rates > 20/min. We adopted the same likelihood scale in this study.

Table 1. Numbers of subterranean organisms recovered and rates of grub sound pulses detected by accelerometer under tangelo trees in Diaprepes root weevil-infested grove.

Tree No.	No. *D. abbreviatus*	No. other insects	No. grub-sound pulses/min
1	3	1 (*Scapteriscus* sp.)	7
2	3		0
6[z]	0	1 (*Scapteriscus* sp.)	see Table 2
7[z]	0		0
9[z]	3	1 (*Dermaptera* sp.)	see Table 3
10[z]	0		0

[z]After subterranean recordings were completed, the top of this tree was removed and recordings were made from a probe inserted into the top of the stump.

Table 2. Rates of grub sound pulses in three consecutive 3-min intervals with the accelerometer (ACC) at a single position, 3 cm from the trunk of Tree No. 6, recorded simultaneously with the SP-1 probe inserted into the trunk, followed by one recording with SP-1 in soil, 3 cm from ACC.

	No. grub sound pulses/min		dB[z]	
Test No.	ACC	SP-1	ACC	SP-1
6.1	127.3	21.1	18.0	10.2
6.2	212.2	86.7	20.3	16.4
6.3	236.3	232.0	20.7	20.6
6.4	243.7	115.0	20.8	17.6

[z]No. grub sound pulses/min transformed into decibel scale using: dB = 10 $\log_{10}$ (pulse rate/T_d), where T_d = 2 pulses/min (see *Comparisons among SP-1 and Accelerometer Sensors*).

Results and Discussion

Accelerometer probes under Trees No. 1, 6, and 9 detected signals with 2-5 ms durations and spectral peaks between 600 and 2000 Hz that are typical of *D. abbreviatus* grub sound pulses (Mankin et al., 2001). The likelihood of pest infestation was rated *medium* at Tree No. 1, where sounds were detected at a low rate of 7/min (Table 1). The likelihood was rated *high* at Tree No. 6 and No. 9, where rates were >20/min in many recordings (columns 2-3 in Table 2 and column 5 in Table 3). The distribution of actual infestations is compared with the distribution of predicted infestations in Table 4. In this case, the mole cricket at Tree No. 6 was counted as a pest because mole crickets are considered harmful to root systems. The relationship between computer-rated likelihood and the observed infestation was statistically significant (χ^2 = 6.43, 2 df, $P < 0.05$). The results were similar to those in Mankin et al. (2001), where highly active insects were quickly detected but the absence of sound could indicate either that no insect was present or that no insect was active.

Comparisons among SP-1 and Accelerometer Sensors. From previous experience, we expected that different sounds produced by *D. abbreviatus* larvae might be detected differentially by the SP-1 and accelerometer systems, depending on the spectral patterns of the sounds and the distance between the probe and larva. Grub sound pulses have highly variable amplitudes and spectral patterns, partly because different feeding and movement activities generate sounds with different spectral profiles (Mankin et al., 2000, 2001). Root clipping and scraping sounds, for example, might be detected most easily by a SP-1 probe inserted into the trunk. These sounds have high frequency components that travel well in wood (see Mankin et al. 2002 and references therein), and SP-1 probes are differentially sensitive to high frequencies. Low-frequency, low-amplitude sliding movements might be detected most easily by the accelerometer because it is highly sensitive to signals <1 kHz that travel well in soil (Mankin et al., 2000). However, many insect sounds contain both high- and low-frequency components that might be detected by both sensors, especially if the insect were nearby and the signals were of high amplitude.

We confirmed in a series of recordings at Tree No. 6 that both sensors could detect the same nearby, high-amplitude sound source. On day 2, we detected frequent pulses at the first position tested with the accelerometer probe, ca. 3 cm from the trunk. The high-amplitude of the signal indicated that an insect was nearby, next to the trunk. We took three consecutive, 3-min simultaneous recordings with the accelerometer at its original position and the SP-1 probe in the trunk. A fourth simultaneous measurement was taken immediately afterward, with the SP-1 probe in the soil, 3 cm from the accelerometer and the trunk (Table 2). When the tree was extract-

Table 3. Detection rates of grub sound pulses recorded with accelerometer (ACC) or SP-1 probes at multiple positions under Tree No. 9.

Test No.	Position[z]	Probe	Recorded duration (sec)	No. grub sound pulses/min	dB[y]
9.1	P1	ACC	122	8.9	6.5
9.2	P2	ACC	180	6.3	5.0
9.3	P3	ACC	180	12.3	7.9
9.4	*P3*[x]	ACC	86	5.6	4.5
9.5, 9.6	P4, P5	ACC	180	0.0	—
9.7	P6	ACC	180	6.0	4.8
9.8	P7	SP-1	180	48.0	13.8
9.9	*P7*[x]	SP-1	172	89.7	16.5
9.10	P8	ACC	60	48.0	13.8
9.11	*P8*[x]	ACC	60	9.0	6.5
9.12	P9	ACC	180	0.0	—
9.13	Trunk	SP-1	180	108.0	17.3

[z]See Fig. 1 for details of spacing around tree.
[y]See Table 2.
[x]Second recording obtained ca. 3 min after the previous recording at same site.

Table 4. Numbers of uninfested and (*D. abbreviatus* or *Scapteriscus*) pest-infested trees rated at different likelihoods of pest infestation by computer analysis of grub sound pulse rates.

Computer-rated infestation likelihood[z]	No. uninfested trees	No. infested trees
Low	6	1
Medium	0	2
High	0	2

[z]Basis of computer-rated infestation likelihood: *low*, ≤2 grub pulses/min; *medium*, 20 ≤ rate > 2 grub pulses/min; *high*, >20 grub pulses/min.

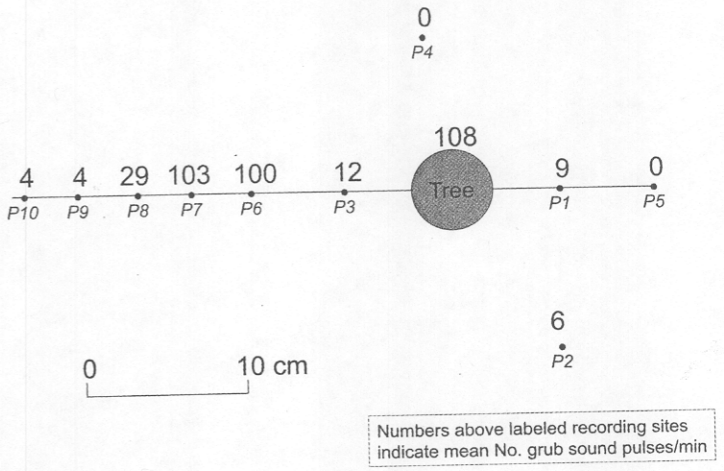

Fig. 1. Mean rates of detection of grub sound pulses recorded from different positions with accelerometer or SP-1 probe in soil underneath citrus tree or inserted into trunk (see Table 3).

ed and the roots examined, the sound source was found to be a single mole cricket, (see sample posted at http://cmave.usda.ufl.edu/~rmankin/molecricket4-sept02.wav). Mole cricket sounds cannot yet be reliably distinguished from *D. abbreviatus* (Fig. 1).

As expected, the measurements with the two probes provided essentially equivalent results in the paired recordings. The mean rate of sounds detected by the SP-1 probe, 113.7 ± 44.1 grub pulses per min, was less than the rate detected by the accelerometer, 204.9 ± 26.7 grub pulses per min, but the difference was not statistically significant ($t = 1.76$, df = 4, $P = 0.14$). Because the sample variability was large and standard errors were not homogeneous, it was convenient to transform the results using a decibel scale based on the threshold distinguishing *low* from *medium* infestation likelihood, $T_d = 2$ grub sounds/min (Mankin et al., 2001). The dB-transformed values are listed in the last two columns of Table 2. Considering that the difference between 244 and 115 pulses per min is probably less relevant behaviorally than the difference between 127 and 21 pulses per min, the transformed values are easier to interpret.

Comparisons among Closely Spaced Sensor Positions. In multiple recordings under Tree No. 9, grub sound pulses were detected at eight of ten positions (Fig. 1), and the rate of sound production varied from background levels up to 234 grub pulses per min (Test No. 9.16 in Table 5). The high rates of sounds detected at positions, *P6* and *P7*, suggests that at least one of the four recovered insects (see Table 1) was located between them. As at Tree No. 6, the rates varied considerably in consecutive recordings at the same position (Tests No. 9.8-9.9, and 9.16-9.17 at *P7*, Tests No. 9.10-9.11 at *P8*, and Tests No. 9.12 and 9.17 at *P9*). Consequently, the decibel values listed in the last two columns may be more relevant for temporal comparisons than the untransformed rates. High variability also was observed in the rates of sounds detected from closely spaced sensors. In Test No. 9.15 of Table 5, for example, sounds detected at high rates were barely detectable in a simultaneous recording at a probe 11 cm away. Such variability can be of utility in locating individual insects, but to map out a comprehensive distribution of the locations and sound patterns of all the insects around a tree would be more time- and labor-intensive than is usually feasible.

The high spatial and temporal variability of the sound rates that we have observed in this and related studies currently limits their utility for quantitative analysis of subterranean insect behavior. It is not realistic to expect that many field studies will be performed with multiple recordings from probes spaced 10-cm apart throughout the root systems of multiple trees. In the future, however, we hope to develop improved sound classification methods that may help distinguish among different larval behaviors, and improve the capability to distinguish among different species. In that case, measurement of the rates of specific types of sound may provide additional information that cannot be easily be obtained solely from the unadjusted total rate of grub sound pulses. Even without such improvements, the recent development of more portable, user-friendly instrumentation has increased the opportunity of researchers and grove managers to use acoustic technology in a variety of new *D. abbreviatus* detection applications.

Table 5. Detection rates of grub sound pulses in simultaneous recordings with accelerometer (ACC) and SP-1 probes at multiple positions under Tree No. 9.

	Sensor		Probe Position			No. pulses/min		dB[y]	
Test No.	Probe 1	Probe 2	Probe 1	Probe 2	Dist[z]. (cm)	Probe 1	Probe 2	Probe 1	Probe 2
9.14	ACC	ACC	P3	P6	6	5.0	285.7	4.0	21.5
9.15	SP-1	ACC	P7	P6	4	40.7	8.0	16.1	6.0
9.16	SP-1	ACC	P7	P10	11	234.0	6.0	20.7	4.8
9.17	ACC	ACC	P9	P10	4	8.0	1.3	4.8	—

[z]Distance between probes in cm.
[y]See Table 2.

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A REFEREED PAPER

TRIFLOXYSULFURON-SODIUM – A POSSIBLE NEW HERBICIDE FOR WEED CONTROL IN CITRUS

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Abstract. **Bioefficacy studies of trifloxysulfuron-sodium, a new sulfonylurea herbicide, were conducted with and without surfactant and compared with glyphosate (Rodeo) and halosulfuron-methyl (Permit). In general, trifloxysulfuron-sodium was not effective without a surfactant, except the highest rate (31.5 g a.i./ha). Application of trifloxysulfuron-sodium with adjuvants (nonionic – X-77, organosilicone – L-77 or oil – MSO) significantly increased herbicide efficacy. Application of glyphosate at 500 g a.i./ha increased weed mortality significantly by providing 51%, 65%, 83%, and 88% control of yellow nutsedge (*Cyperus esculentus* L.), guineagrass (*Panicum maximum* Jacq.), redroot pigweed (*Amaranthus retroflexus* L.), and hairy Spanish needles (*Bidens bipinnata* L.), respectively. With the exception of guineagrass, increasing dosage of halosulfuron (8.75 to 35 g a.i./ha) did not influence yellow nutsedge, redroot pigweed, or Spanish needles. Trifloxysulfuron-sodium, even at 7.5 g a.i./ha, was comparable with the highest rates of glyphosate (500 g a.i./ha) or halosulfuron (35 g a.i./ha). Trifloxysulfuron-sodium at 30 g a.i./ha provided maximum control (≥86%) of all the test weed species. In subsequent studies, application of trifloxysulfuron-sodium to three different citrus rootstocks resulted in significant phytotoxic effects to the primary stem in the form of necrotic leaves and further growth was stopped. However, upon pruning the necrotic tissue, lateral growth arose from the trimmed point followed by normal rootstock growth.**

The environment is subjected to a greater risk when high rates of pesticides are used for pest control. In addition, repetitive use of a single active ingredient over time increases chances of resistance development in the target pests. Glyphosate, which has been used worldwide for more than 20 years (Bradshaw et al., 1997) and accounts for 11% of the worldwide sales of herbicide (Powles et al., 1997) is a good example. Continuous use of glyphosate has resulted in the appearance of resistant weed populations of rigid ryegrass (*Lolium rigidum* Goud.) (Holt et al., 1993; Powles and Holtum, 1994; Powles et al., 1998).

Trifloxysulfuron-sodium [N-(4,6-Dimethoxy-2-pyrimidinyl)-3-(2,2,2-trifluoroethoxy)-pyridin-2-sulfonamide sodium salt] is a new broad-spectrum, low-rate technology herbicide for over-the-top post-emergence application, developed for use in sugarcane and cotton (Rawls et al., 2000). This sulfonylurea herbicide, with the proposed common name of trifloxysulfuron sodium, has been field tested as a 75% water-dispersible granule (WDG) for the past several years in North America, Africa, and Asia under the code name trifloxysulfu-

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