Acoustic Estimation of Infestations and Population Densities of White Grubs (Coleoptera: Scarabaeidae) in Turfgrass

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ABSTRACT Incidental sounds produced by *Phyllophaga crinita* (Burmeister) and *Cyclocephala lurida* (Bland) (Coleoptera: Scarabaeidae) white grubs were monitored with single- and multiple-sensor acoustic detection systems in turf fields and golf course fairways in Texas. The maximum detection range of an individual acoustic sensor was measured in a greenhouse as approximately the area enclosed in a 26.5-cm-diameter perimeter (552 cm^2). A single-sensor acoustic system was used to rate the likelihood of white grub infestation at monitored sites, and a four-sensor array was used to count the numbers of white grubs at sites where infestations were identified. White grub population densities were acoustically estimated by dividing the estimated numbers of white grubs by the area of the detection range. For comparisons with acoustic monitoring methods, infestations were assessed also by examining 10-cm-diameter soil cores collected with a standard golf cup-cutter. Both acoustic and cup-cutter assessments of infestation and estimates of white grub population densities were verified by excavation and sifting of the soil around the sensors after each site was monitored. The single-sensor acoustic method was more successful in assessing infestations at a recording site than was the cup-cutter method, possibly because the detection range was larger than the area of the soil core. White grubs were recovered from >90% of monitored sites rated at medium or high likelihood of infestation. Infestations were successfully identified at 23 of the 24 sites where white grubs were recovered at densities >50/m^2, the threshold for economic damage. The four-sensor array yielded the most accurate estimates of the numbers of white grubs in the detection range, enabling reliable, nondestructive estimation of white grub population densities. However, tests with the array took longer and were more difficult to perform than tests with the single sensor.

KEY WORDS Coleoptera, Scarabaeidae, sound, remote sensing, turfgrass

ROOT-FEEDING WHITE GRUBS, particularly *Phyllophaga* spp. (May beetles, June beetles, or Junebugs) and *Cyclocephala lurida* (Bland) (southern masked chafers), are important pests of turfgrass in Texas (Crocker 1982, Crocker et al. 1996). Determining the location of white grub infestations and estimating their population densities traditionally involves the digging and inspection of multiple soil samples (Burrage and Gyrisco 1954, Villani and Wright 1990). Because this procedure is laborious and destructive, other potentially useful detection methods are under investigation, including radiography (Villani et al. 2002) and sound.

Acoustic technology is well established as a tool for locating hidden seismic targets underground (Chen and Willemann 2001) and underwater objects or organisms (Davis and Pitre 1995). Although the sounds that insects produce incidentally in movement and feeding activities are usually low in intensity, numerous researchers have overcome this technical challenge and developed acoustic systems for insect detection. For decades, acoustic monitoring has been used to detect insects (Brain 1924, Adams et al. 1953, Vick et al. 1988) and estimate their population densities in stored grain (Hagstrum et al. 1988, Mankin et al. 1997, Shuman et al. 1997). Hickling et al. (1994, 2000) used microphones to estimate numbers of pink bollworm in cotton bolls. Ultrasonic detectors have been used to detect and estimate populations of termites in wooden structures (Fujii et al. 1990, Scheffrahn et al. 1993, Lemaster et al. 1997). Recently, acoustic systems were developed for detection of subterranean insects in nursery containers (Mankin and Fisher 2002) and field environments (Mankin et al. 2000, 2001, Brandhorst-Hubbard et al. 2001). Such

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systems have considerable potential for use as tools for estimation of subterranean insect population densities.

Mathematically, acoustic estimation of white grub densities is accomplished by estimating the detection range of the sensor, estimating the number of separate sources in the detection range, and then dividing the number by the range. White grubs commonly are found only in the top 5–20 cm of soil; consequently, the range can be defined in terms of area rather than volume. However, estimation of the number of sound sources in a volume or area of soil is inherently less precise than estimation in air or water (Mankin et al. 2000, and references therein). Soil is much more heterogeneous than air or water, and the rate of sound attenuation in soil can vary greatly over distances of a few centimeters. Because the rate of attenuation is proportional to frequency, different sounds produced by the same insect may have considerably different detection ranges if they contain different frequencies. In addition, it is difficult to count the number of separate sound sources unless the sounds can be distinguished as coming from separate locations (Shuman et al. 1993, 1997). On average, the number of insects at a recording site is proportional to the rate of insect sound pulses, but in any single recording, the rate is not a reliable predictor of population density (Mankin et al. 2001).

The purpose of this study was to assess the performance of different acoustic methods for estimating white grub population density. We measured the range over which currently available acoustic sensors detect white grub sounds and field-tested a multisensor array designed to facilitate enumeration of separate sound sources, validating the results using standard digging and sifting techniques.

Materials and Methods

Monitoring Arenas and Acoustic Equipment. Acoustic monitoring and soil core sampling of white grub populations were performed in fields at the Texas A&M University (TAMU) Research and Education Center, Dallas County, Dallas, TX and in fairways at the Pecan Hollow Golf Course, Collin County, Plano, TX. Single-sensor acoustic systems and four-sensor arrays were used in different tests at both locations. Activity was recorded on a dual-channel digital audio tape recorder (DAT; PCM-M1, Sony, New York, NY), usually for 3 min at each monitoring site. If sounds were produced at a high rate, recording durations were as short as 1 min because a listener could easily assess that the site had a high likelihood of infestation (see below). If the rate of sounds was low, durations were extended up to 15 min to enable assessment of greater numbers of sounds.

The single-sensor acoustic system was constructed by the National Center for Physical Acoustics at the University of Mississippi, Oxford, MS (Hickling et al. 2000). The primary component was an electret microphone sensor mounted in a modified 4.5-cm-diameter stethoscope head (4-cm-diameter diaphragm). To facilitate insertion into the soil, the sensor mounting was set into the 5-cm-wide face of a 15-cm-long, wedge-shaped metal probe (1.9-cm maximum thickness). The diaphragm of the sensor mounting was flush with and midway down the front face of the probe. To provide some attenuation of airborne background noise, a cast-iron cover (26.5 cm in diameter, 552 cm^2 in area by 5 cm in height) was welded to the upper end of the probe. When the probe was fully inserted into the ground, the rim of the cover rested on the ground surface and the top of the sensor mounting was $\approx$7 mm below the soil surface.

The multisensor array consisted of a single-sensor system with three additional stethoscope-mounted sensors placed around the rim of the sensor cover (see Materials and Methods: *Sensor Array Estimation of Population Densities*).

Signal Analysis and Grub Sound Pulse Profiles. During monitoring sessions, the acoustic sensors were connected individually to custom-built amplifiers that fed to the DAT. When signals were monitored by the multisensor array, pairs of sensors were connected to the left and right DAT channels simultaneously. Stereo headphones (Optimus PRO 40, Tandy Corp., Fort Worth, TX) attached to the DAT were used for listener assessment of the white grub activity as well as signal storage for laboratory analyses. Recordings were played back in the laboratory, where individual sounds were digitized (Mankin 1994) and/or displayed on a digital oscilloscope (model TDB 210, Tektronix, Inc. Wilsonville, OR). Individual sound pulses were counted as "grub sound pulses" or background noises based on temporal and spectral similarity to previously verified grub sound pulses (Mankin et al. 2001). The grub sound identification and discrimination process relied on prior findings that subterranean insect movement and feeding sound pulses are shorter, lower in intensity, and different in temporal pattern from most interfering background noises (Brandhorst-Hubbard et al. 2001; Mankin et al. 2000, 2001).

The sounds made by particular white grub species in soil are difficult to distinguish from sounds of closely related species, but distinctive differences in amplitude, frequency, and temporal pattern have been identified in laboratory analyses of white grub, earthworm, and typical background sounds (Mankin et al. 2000). The spectral patterns (profiles) of sounds produced by white grubs and many other insect larvae have peaks between 600 and 2,000 Hz, a range easily discernible by ear or observable on an oscilloscope or a spectrogram. Criteria based on these spectral and temporal features have been developed that enable a computer or an experienced listener to distinguish grub sound pulses from background noises with longer durations and lower frequencies (Mankin et al. 2000).

Sensor Detection Range. For tests to estimate the detection range of the single-sensor acoustic system, third instars of *Phyllophaga crinita* Burmeister (Coleoptera: Scarabaeidae) were collected from the golf course. The larvae were kept individually in sand-filled plastic cups (4 cm in diameter by 4 cm in height) and fed on pieces of ripe sweet potato, *Ipomoea batatas* (L.) Lam (Convolvulaceae), until they were tested. The testing arena was a raised bed (3.7- by 2.6-m area, 0.2 m in height) filled with sandy loam soil, framed with lumber, and covered with an established irrigated turf of St. Augustine grass, *Stenotaphrum secundatum* (L.) Persoon (Poaceae), in a greenhouse at TAMU. St. Augustine is a commonly used turfgrass in the southern United States and is susceptible to white grub infestation (Merchant and Crocker 1999).

In 54 recordings with 25 different larvae, the single-sensor probe was inserted into the bed at a predetermined site. A few (3–30) minutes before the recording, a healthy, active third instar was positioned 10–20 cm from the insertion point. The grub was set 1–8 cm below the surface along one of five different directions (0°, 45°, 90°, 135°, and 180°) with respect to the perpendicular to the sensor face. Presence or absence of grub sound pulses was noted for each recording along with the larva's distance from the probe, depth in the soil, and the angle with respect to the sensor face. In several tests, the probe was pulled out of the soil and reinserted 2 cm closer to the larva in the same orientation if the larva could not be detected initially. The larva was recovered and its position rechecked after the recording session ended. The larvae did not move significant distances from their original positions during these tests.

Single-Sensor Assessment of the Likelihood of Infestation. Acoustic monitoring and recording were conducted with the single-sensor system at 26 TAMU field sites and 42 golf course fairway sites. Two different acoustic methods were used to rate the likelihood of white grub infestation at each site on a 3-category rating scale similar to that described in Mankin et al. (2001) and Mankin and Fisher (2002). The results of both methods were assigned a rating on the same categorical scale, low, medium, and high likelihood of infestation, to enable cross-comparisons of predictions. For a rapid, qualitative assessment, listeners with headphones rated the likelihood of infestation as low if there were no subterranean sounds or only a few, faint grub sound pulses, easily lost in the background noise. The likelihood was medium if sporadic or moderately frequent but faint grub sound pulses were heard, sometimes obscured by background noises. The likelihood was high when frequent grub sound pulses were heard, possibly from multiple larvae, easily distinguished from background. For a quantitative assessment, ratings of low, medium, or high likelihood of infestation were assigned after the recording sessions using signal processing analyses similar to those in Mankin et al. (2001). The ratings assigned in the laboratory were based on the rates of grub sound pulses detected at white grub-infested and -uninfested sites (see below).

Sensor-Array Estimation of Population Densities. An array of four sensors was used to estimate numbers of white grubs at 25 separate recording sites at TAMU and 10 at the golf course. To perform a monitoring test, a single-sensor system was inserted into the ground and a 7-cm-deep cut was made with a knife in the soil

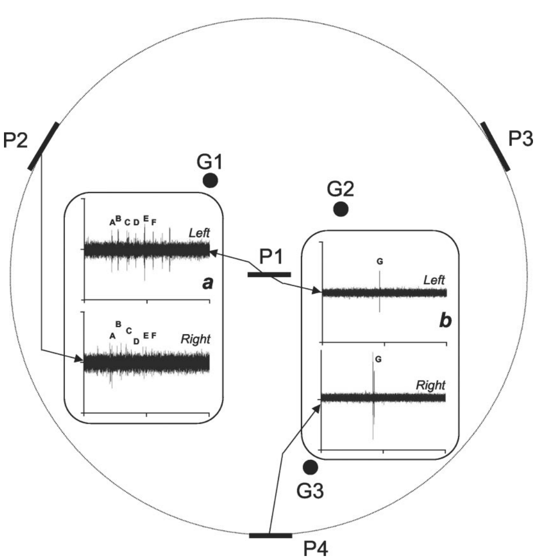

Fig. 1. Diagram of acoustic array for estimating numbers of white grubs under sensor cover. P1-P4 show positions of the sensors around the rim of the cover (dashed line). G1-G3 show positions of three third instars recovered after paired dual-channel DAT recordings were obtained from P1-P2, P1-P3, P1-P4, P2-P3, P2-P4, and P3-P4. Inset (a) shows oscillograms of sound pulses, A–F, recorded simultaneously at P1 (left) and P2 (right). Inset (b) shows oscillograms of sound pulse, G, recorded simultaneously at P1 (left) and P4 (right). Each oscillogram is 4 s in duration.

along the cover perimeter. Three peripheral sensors were set into the cut, with the heads facing the central probe (Fig. 1). The sensor faces were pushed against the soil to make firm contact and the backs were covered with a piece of sponge to reduce environmental noise.

Equipment limitations did not permit simultaneous monitoring of all four sensors. Two sensors were connected at a time to left and right channels of the DAT recorder, and their signals were recorded and monitored with headphones for 1–3 min. In cases where white grub sounds were clearly audible, only 1 min was recorded. Analysis of the amplitudes and spectral patterns of sound pulses and comparisons of arrival times of pulses at different sensors were used to infer the numbers of separate sound sources (see Materials and Methods: *Acoustic Estimation of Insect Population Densities*).

Cup-Cutter and Excavation Sampling Procedures. The acoustic measurements were verified by two methods commonly used for sampling soil insect populations. First, a 15-cm-deep soil core was excavated next to the face of the sensor using a 10-cm-diameter cup-cutter (78.5 cm^2). The numbers of different organisms and the instars of different white grub species in the soil core were noted. After the cup-cutter core was examined, the soil was excavated and examined out to the rim of the sensor cover and down to ≈20-cm depth. At most sites, the white grubs were recovered within a depth of ≈10 cm.

Table 1. Numbers of white grubs and other invertebrates recovered in field sites at TAMU in relation to detection rates of grub sound pulses and listener ratings of infestation likelihoods

Sample no.	Soil temp. (°C)	No. invertebrates under sensor cover				Detection rate (grub sound pulses/min)	Listener rating[c]
		White grubs[a]		Earthworms	Total of grub sound producers[b]		
		Inside cup	Outside cup				
6-8-3	26.5	1	3	2	4	3.0	Medium
5-20-4[d]	24	1	2	7	3	9.2	High
6-15-3	24.7	1	2	4	3	0.3	Medium
5-24-3[e]	22.5	1	1	1	2	11.0	High
5-21-4[f]	25	0	2	0	2	4.0	Medium
5-24-2[c]	21.4	1	1	0	2	1.7	Medium
6-7-2	27.5	0	1	10	1	22.0	High
5-24-5[e]	23.3	0	1	3	1	9.7	High
5-21-2[f]	24	0	1	3	1	8.3	High
5-21-1[f]	24	0	1	5	1	7.3	High
5-20-3[d]	24	0	1	8	1	3.8	Medium
5-21-3[f]	24.5	0	1	0	1	3.0	Medium
6-8-5	26.5	0	1	0	1	1.7	Low
5-24-1[e]	21	0	0	0	0	3.3	Medium
6-7-3	27.5	0	0	4	0	2.7	Low
6-8-4	26.5	0	0	0	0	1.7	Low
6-7-4	27.5	0	0	0	0	1.3	Low
5-24-4[e]	23.5	0	0	0	0	0.7	Low
5-20-2[e]	24	0	0	12	0	0.2	Low
5-20-1[d]	24	0	0	15	0	0	Low
6-7-1	27.5	0	0	5	0	0	Low
6-15-1	24.7	0	0	5	0	0	Low
6-15-2	24.7	0	0	4	0	0	Low
6-8-1	26.5	0	0	2	0	0	Low
6-8-2	26.5	0	0	0	0	0	Low
6-15-4	24.7	0	0	0	0	0	Low
Sums		5	18	90	23		
Means	24.9					3.7	

[a] *P. crinita* 3rd instars unless otherwise noted.
[b] This number includes the total number of white grubs either in the cup-cutter core sample or under the sensor cover but outside the core, and excludes earthworms. Sites containing at least one white grub were classified as infested.
[c] Ratings scale described in Materials and Methods: *Single Sensor Assessment of Likelihood of Infestation.*
[d] Soil moisture level was 19.3%, and recording interval was 5 min.
[e] Soil moisture was 18.4%.
[f] Soil moisture was 18.2%.
[g] *P. crinita* 1st instar.

During the monitoring sessions, soil temperature was taken with a digital thermometer (450-ATF, OMEGA, Stamford, CT) at a depth of 3 cm. Soil moisture was determined at several sites by extracting a 22-mm-diameter soil core. The top 3 cm of the soil sample was discarded, and the residual core was used to produce a 200-g soil sample, which then was kiln-dried and reweighed.

Results and Discussion

Sound pulses produced by white grubs were readily detected and identified at the TAMU (Table 1) and golf course (Table 2) field locations. In both tables, the last three columns list for each recording site the numbers of organisms capable of producing grub sound pulses, compared with the measured rates of grub sound pulses and the listener assessments of infestation likelihood. A site was classified as infested if an organism capable of producing grub sound pulses was recovered from the excavated sample. At TAMU (Table 1), all 23 grub sound pulse producers were third instars of *P. crinita,* either from the cup-cutter core or the remaining area under the sensor cover. Ninety earthworms (Lumbricidae) were recovered and noted, but they were not included in the grub sound producer totals because their low-frequency, long-duration signals usually can be distinguished from grub sound pulses (e.g., site 5-20-1; Mankin et al. 2000). At the golf course (Table 2), the 159 grub sound producers included first, second, and third instars of *P. crinita* larvae, *C. lurida* larvae, and a few other coleopteran and lepidopteran larvae. Eleven earthworms and a colony of ants were noted but were not included in the grub sound pulse producer totals. Consequently, in this experiment, all sites classified as infested contained at least one white grub.

The mean density of white grub infestation was higher at the golf course (63.4 grubs per square meter) than at TAMU (16.0 grubs per square meter). The infestation densities exceeded the threshold for treatment of white grub infestations (50 grubs per square meter) (Merchant and Crocker 1999) at 21 of the 42 golf course recording sites.

Although the rate of insect sound production was proportional to temperature in previous studies (Hagstrum 1993, Mankin et al. 1997), the regression of grub sound pulse rate on soil temperature was not statisti-

Table 2. Numbers of white grubs and other invertebrates recovered at golf course sites in relation to detection rate of grub sound pulses and listener ratings of infestation likelihoods

Sample date and no.	Soil temp. and moisture		No. invertebrates under sensor cover: *P. crinita*[a] Inside (a) or outside cup (b)				Other: grub sound producers[b] or not[c] *(d)*		Total of grub sound producers	Detection rate (grub sound pulses/min)	Listener rating[d]
				b							
	°C	%	a	1st	2nd	3rd	c	d	(a + b + c)		
7-12-2	28	18.7	5[e]	3	10	0	0	0	18	17.7	High
8-26-4	27	14.8	1	0	7	4	0	0	12	5.3	Medium
8-26-1	27	14.8	4	0	0	7	0	0	11	10.7	High
7-12-1	28	18.7	0	2	6	0	2[f]	0	10	18.0	High
9-15-5	22	21.4	0	0	0	9	0	1	9	11.6[g]	High
8-26-3	27	14.8	0	0	1	7	0	0	8	18.7	High
8-26-2	27	14.8	2	0	0	5	0	0	7	3.7	Medium
7-20-1	30	16.5	0	3	2	0	1[f]	0	6	8.3	Medium
8-26-5	27	14.8	0	0	1	5	0	0	6	6.0	Medium
7-19-1	28	16.1	3[h]	3	0	0	0	0	6	2.3	low
8-31-1	26	13.9	1	0	0	4	0	0	5	18.7	High
9-1-3	25	9.8	0	0	0	0	5[i]	0	5	10.7	High
9-14-1	26	16.9	1	0	0	4	0	0	5	6.7[g]	Medium
9-15-1	22	21.7	1	0	0	4	0	0	5	5.3[g]	Medium
9-1-1	25	9.8	0	0	0	0	4[i]	0	4	14.3	High
8-31-4	26	13.9	1[i]	0	0	2	1[j]	0	4	10.0	High
9-15-2	22	21.7	2	0	0	2	0	0	4	6.0[g]	High
9-3-2	26	11.6	2	0	0	0	2[i]	0	4	5.3	Medium
9-3-3	26	11.6	2[i]	0	0	0	2[i]	0	4	3.0	Medium
8-31-3	26	13.9	0	0	0	3	0	0	3	13.7	High
9-2-2	27	11.2	0	0	0	3	0	0	3	9.1	High
7-20-3	30	16.5	0	0	1	0	1[k]	0	2	13.7	High
9-3-4	26	11.6	0	0	0	1	1[i]	0	2	12	High
7-20-2	30	16.5	0	0	2	0	0	0	2	8.7	Medium
9-1-5	25	9.8	0	0	0	0	2[l]	2[l]	2	8.0	High
8-31-5	26	13.9	0	0	0	2	0	0	2	6.0	Medium
9-2-1	27	11.2	0	0	2	0	0	1[m]	2	2	Low
7-15-1	26	20.1	2[n]	0	0	0	0	0	2	0.7	Medium
8-31-2	26	13.9	0	0	0	1	0	4	1	12.7	High
9-1-2	25	9.8	0	0	0	0	1[j]	0	1	6.0	Medium
9-2-3	27	14.3	0	0	1	0	0	0	1	5	Medium
7-19-2	28	16.1	0	1	0	0	0	0	1	3.3[o]	Medium
9-1-4	25	9.8	0	0	0	0	1[i]	0	1	1.7	Low
7-15-2	26	20.1	0	1	0	0	0	0	1	1.0	Low
9-3-5	27	11.6	0	0	0	0	0	0	0	7.1	Medium
9-15-4	22	19.0	0	0	0	0	0	1	0	2.9[g]	Medium
9-15-3	22	19.0	0	0	0	0	0	1	0	1.7[g]	Low
9-3-1	26	11.6	0	0	0	0	0	1	0	1	Low
9-14-3	26	16.9	0	0	0	0	0	0	0	0.5[g]	Low
7-7-2	28	12.4	0	0	0	0	0	0	0	0.3	Low
9-14-2	26	16.9	0	0	0	0	0	0	0	0.2[g]	Low
7-7-1	28	12.4	0	0	0	0	0	0	0	0	Low
Sums			27	13	33	63	23	11	159		
Means	26	14.9								7.1	

[a]Unless otherwise noted, *P. crinita:*(a) 3rd instars found in cup-cutter soil core or (b) 1st, 2nd, or 3rd instars found under sensor cover but outside of soil core.

[b]These included other coleopteran and lepidopteran larvae that produce signals with grub-sound profiles (see Materials and Methods: *Signal Analysis and Grub-Sound Profiles*). Sites containing at least one organism in category a, b, or c were classified as infested.

[c]Primarily lumbricid and formicid spp., which produce low-amplitude, low-frequency signals, distinguishable from grub sound pulses.

[d]Rating scale described in Materials and Methods: *Single-Sensor Assessment of the Likelihood of Infestation.*

[e]Two 1st and three 2nd instar of *P. crinita.*

[f]Curculionid larvae.

[g]Fifteen-minute recording duration.

[h]1st instar of *P. crinita.*

[i]2nd instar *C. lurida.*

[j]3rd instar of *C. lurida.*

[k]2nd instar of *Spodoptera frugiperda* (Smith).

[l]One 2nd instar of *C. lurida*, two lumbricid spp., and one curculionid larva.

[m]Many small formicid workers.

[n]One 1st and one 2nd instar of *P. crinita.*

[o]Twelve-minute recording of persistent, faint sounds.

cally significant (PROC GLM, SAS Institute 1988). However, temperature was not controlled in this study. The 8°C (22–30°C) range was small compared with studies where the effect had been demonstrated. Similarly, there were no statistically significant relationships identified between soil moisture levels and

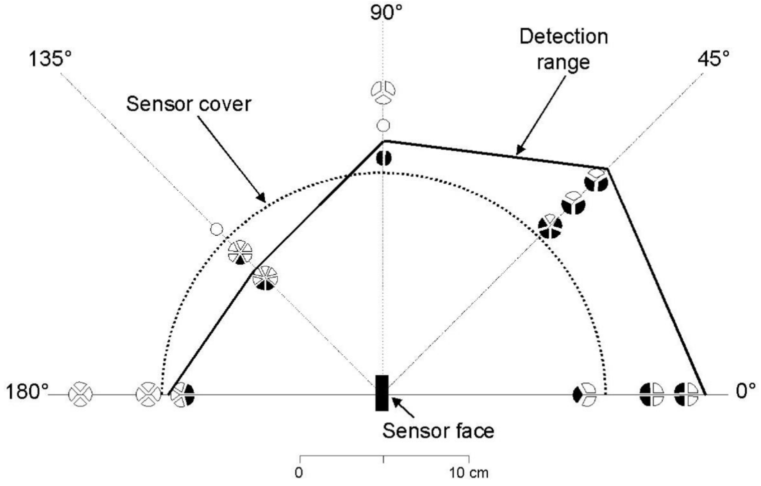

Fig. 2. Maximum detection distances of sounds produced by third instars positioned at different angles from sensor face. The number of white grubs tested at each position is indicated by the number of sections in each circle, with detected insects indicated in black. Solid line segments indicate approximate detection range and the dashed arc indicates the rim of the sensor cover. For simplicity, only one-half of the bilaterally symmetrical range is shown.

the numbers of white grubs or the rates of grub sound pulses, but the range of soil moistures varied only from 9.8 to 21.4%.

If white grubs produced sound pulses of uniform intensity in a uniform medium, the pulses would decrease in amplitude in proportion to the square of the distance from the sensor, and the rate of detectable pulses would be constant at all positions in the detection range (Mankin et al. 2000). The rate of grub sound pulses at each recording site would be proportional to the number of white grubs in the range (Mankin et al. 2001). However, different white grubs have different weights and behavior patterns, and white grub sound pulses can vary considerably in rate and amplitude independently of the distance between grub and sensor. Consequently, we did not expect to find a strict proportionality between the rate of grub sound pulses and the numbers of white grubs recovered at each site. Indeed, several anomalous results are indicated in Tables 1 and 2, with the sites sorted in order of high to low numbers of grub sound producers. Innate differences in activity levels may have contributed to unexpected results at sites 6-7-2 and 6-15-3 of Table 1, for example, and 7-19-1 and 8-31-2 of Table 2. At site 6-7-2, a single larva produced sound pulses at a high rate, but three larvae barely produced enough grub sound pulses for detection in 6-15-3. In Table 2, a single white grub at 8-31-2 produced sounds at a higher rate than six white grubs in 7-19-1. In general, however, the sites with the highest rates of grub sound pulses and the highest ratings of infestation likelihood tended to have the greatest numbers of white grubs. At TAMU, listeners rated the likelihood of infestation as medium or high at 13 of 26 recording sites. White grubs were recovered at 12 (92%) of these sites. Similarly, listeners rated the likelihood of infestation as medium or high at 36 of 44 golf course sites, and white grubs were recovered from 34 (94%) of these sites. The >90% correspondence between the acoustic ratings of infestation likelihood and the observed presence of white grubs suggests that the white grubs could be detected out to the sensor cover perimeter with good reliability under the conditions of this experiment.

Sensor Detection Range. The detection range measurements in the greenhouse arena provided additional support of the above-mentioned results that third instars were reliably detected inside the perimeter of the sensor cover. The maximum detection distance varied with the angle between the larva and the perpendicular to the sensor face (Fig. 2). The sensor was most sensitive to sounds coming from positions in front of the sensor face. At 0–45° from the perpendicular, 56% of the white grubs tested were detected at 16 or 18 cm from the probe. At 90°, no white grubs were detected beyond 14 cm. At 135–180°, no white grubs were detected at 14 cm, and even at 10–12 cm, the percentage detected rose only to 28%. Based on these measurements, the detection range was approximately an oblong spheroid with one axis that extended from 16 to 18 cm in front of the sensor face to 10–12 cm behind it.

It should be noted that, although the above-mentioned range estimated with third instars in a greenhouse bed could be defined more precisely by measuring additional samples, any range thus obtained is only partially applicable to other environmental conditions. Many environmental factors, such as the ambient noise level, the presence of relatively similar sounds produced by other soil animals, and soil temperature, moisture, type, structure, and level of compaction can affect the range of a sensor (Mankin et al. 2000). The strengths of the grub sound pulses and their detection ranges depend on the sizes of the larvae and the vigor of their movements.

Given all these uncertainties, we found it convenient to approximate the sensor detection range as a

Table 3. Numbers of TAMU recording sites assessed at different likelihoods of infestation by grub sound-pulse rate, listening, and cup-cutter rating methods

Likelihood	Grub sound pulse rate		Listening		Cup-cutter	
	Uninfested	Infested	Uninfested	Infested	Uninfested	Infested
Low	11	3	12	1	13	8
Medium	2	7	1	6		
High	0	3	0	6	0	5

Grub sound pulse rate, listening, and cup-cutter ratings of infestation likelihood defined in Results: *Quantitative Acoustic Rating of Infestation Likelihood.* Infested sites contained ≥1 white grub in excavation under sensor cover.

20-cm-deep, 26.5-cm-diameter cylinder underneath the sensor cover. This was approximately the volume excavated and sifted when the white grub numbers and positions were verified after acoustic sampling (see Materials and Methods: *Cup-Cutter and Excavation Sampling Procedures*). If the range was to be specified as an area rather than a volume, it was most conveniently approximated as the area underneath the sensor cover.

Quantitative Acoustic Rating of the Likelihood of Infestation. An important application of acoustic detection systems for subterranean insects is in nondestructive surveying of infestations. Two acoustically based methods used successfully in previous studies were evaluated to nondestructively rate the likelihood that a monitored site was infested. Mankin et al. (2001) and Mankin and Fisher (2002) (see Materials and Methods: *Single-Sensor Assessment of Likelihood of Infestation*) adopted a listening scale that provided a rapid, qualitative assessment. Mankin et al. (2001) and Brandhorst-Hubbard et al. (2001) used a quantitative scale based on the rate of grub sound pulses detected at a recording site (see below). For a third method of assessing the likelihood of infestation, we used a two-category scale based on the cup-cutter samples extracted before the site was excavated. On this scale, the likelihood of infestation at a recording site was rated low if no white grubs were recovered in the cup and high if one or more white grubs were recovered in the cup.

In developing the grub sound pulse rate classifications, we assigned ratings based on the overall mean rates at infested and uninfested sites in Tables 1 and 2, similar to the procedures used in Mankin et al. (2001). Grub sound pulses were detected at a mean rate of (7.9 ± 0.8)/min when white grubs were recovered ($n = 47$), but the mean rate was only (1.1 ± 0.4)/min when white grubs were absent ($n = 21$). We set an upper limit of 1.9/min (mean + 2 SE) for a low likelihood of infestation, and a lower limit of 9.5/min (mean + 2 SE) for a high likelihood of infestation. Recording sites that had rates between these two limits were assigned a medium likelihood of infestation.

The distributions of grub sound pulse-rate, listener ratings, and cup-cutter ratings were compared across infested and uninfested sites at TAMU (Table 3) and the golf course (Table 4). At both locations, the occurrence of 10 or more grub sound pulses per minute was a reliable predictor that the site contained a grub sound producer. The sites with three or more white grubs are of particular interest because three white grubs per 0.0552 m^2 (the area under the sensor cover) is equivalent to the 50 grub per square meter threshold for white grub economic damage (Merchant and Crocker 1999). Of 24 sites at TAMU and the golf course where three or more white grubs were recovered, 23 sites were rated at a medium or high likelihood of infestation by listeners and by acoustic classification. The acoustic system reliably targeted areas where white grub management treatments were most necessary.

The distributions of low, medium, and high likelihoods of infestation in Tables 3 and 4 were significantly different between infested and uninfested sites in assessments by using grub sound pulse rate, listening, and cup-cutter rating methods (Table 5). All three rating methods provided statistically significant relationships at both locations, but the grub sound pulse rate and listener methods were more accurate than the cup-cutter method. The difference in the area sampled (552 cm^2 under the cover compared with 78.5 cm^2 for the cup cutter) contributed to the improved ratings of the grub sound pulse rate and listener methods. Because subterranean insects usually are not randomly distributed (Southwood 1966, Guppy and Harcourt 1970, Villani and Wright 1990), the variance per unit area decreases as the sampling area increases (Burrage and Gyrisco 1954, Venette et al. 2002).

Table 4. Numbers of golf-course recording sites assessed at different likelihoods of infestation by grub sound pulse rate, listening, and cup-cutter rating methods

Likelihood	Grub sound-pulse rate		Listening		Cup-cutter	
	Uninfested	Infested	Uninfested	Infested	Uninfested	Infested
Low	6	3	6	4	8	21
Medium	2	18	2	14		
High	0	13	0	16	0	13

Grub sound pulse rate, listening, and cup-cutter ratings of infestation likelihood defined in Results: *Quantitative Acoustic Rating of Infestation Likelihood.* Infested sites contained ≥1 white grub in excavation under sensor cover.

Table 5. Comparisons of statistical significance of relationships between grub sound-pulse rate, listening, and cup-cutter ratings and actual white grub infestations at TAMU and golf course monitoring sites

Infestation likelihood rating method	TAMU		Golf course	
	χ^2	df	χ^2	df
Grub sound-pulse rate	10.3**	2	17.4***	2
Listening	18.9***	2	15.1***	2
Cup-cutter	6.2*	1	4.4*	1

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.005$.

A measure of the overall accuracy of the acoustic assessments is the fraction of correct positive and negative predictions, i.e., accuracy = (true positive + true negative) / (true positive + true negative + false positive + false negative). Two other common measures of detection errors are sensitivity = true positive/ (true positive + false negative), and specificity = true negative/ (false positive + true negative) (Venette et al. 2002). The grub sound pulse rate and listening rating scales permit two different measures of accuracy, sensitivity, and specificity, depending on the relative importance of false negative and false positive predictions.

If it were most important to identify an infestation, even if a few uninfested sites were misidentified, the sites with medium and high likelihood of infestation should be classified as positive predictions, with sites at low likelihood classified as negative predictions (presence-biased classification). However, if incorrect prediction of an infestation would be costly, the sites with medium and low likelihood of infestation should be classified as negative predictions (absence-biased classification). The accuracies for the two different classification schemes are compared for the TAMU and golf course sites in Table 6. The results suggest that the single-sensor acoustic system has greater accuracy and sensitivity for presence-biased than for absence-biased classification. Both methods of classification provided greater sensitivity than the cup-cutter method (cup cutter sensitivity = 0.38 at both TAMU and golf course). The presence-biased method provided greater accuracy than the cup cutter method (cup cutter accuracy = 0.5 at TAMU and 0.69 at golf course). For the detection of rare individuals, high sensitivity is often more valuable than high specificity (Venette et al. 2002). The cup-cutter method had a specificity of 1.0 at both locations, like the absence-biased acoustic classification method.

At TAMU, 23% of the sampled sites contained multiple white grubs, and at the golf course, 68% contained multiple white grubs. Although the single-sensor system is effective in identifying infestations, it does not provide a reliable estimate of the number of white grubs in areas of high infestation. We calculated a regression of white grub counts on grub sound pulse rate from the combined set of single-sensor measurements in Tables 1 and 2 by using PROC GLM (SAS Institute 1988). The model was number of grubs = A + B (sound pulse rate). Although the regression was statistically significant ($F = 27.42$; df = 1, 66; $P < 0.001$, residual mean square error = 548.43, $r^2 = 0.29$), with A = 3.49 ± 0.72 and B = 0.89 ± 0.17, it was not very accurate. It estimated, for example, that all the TAMU recording sites contained at least three white grubs, but half contained none. Clearly, multiple sensors are needed to spatially distinguish the individual sound sources and enable counting of separate sound sources.

Acoustic Estimation of Insect Population Densities. The use of four sensors greatly improved the capability of the acoustic system to predict the number of insects at a recording site. With paired sensors amplified equally, a listener could use differences in the relative strengths and arrival times of signals to help distinguish among multiple larvae in the same way as for sound sources above ground. Aural impressions were supplemented by oscilloscope playback and digital signal processing of the dual-channel DAT recordings (e.g., pulses A–F in Fig. 1a, see also B.4.c-d at cmave.usda.ufl.edu/~rmankin/soundlibrary.html). The amplitudes of the sound pulses are larger in the recording from sensor, P1 (Fig. 1a, left), than in the recording from P2 (Fig. 1a, right). This is consistent with a hypothesis that these sounds were produced by grub, G1, found between P1 and P2 but closer to P1. Similarly the amplitude of pulse, G, is larger in the recording from P4 (Fig. 1b, right) than in the recording from P1 (Fig. 1b, left), which suggests it came from G3, found between P1 and P4 but closer to P4. A total

Table 6. Accuracy, sensitivity, and specificity of grub sound pulse rate and listening assessments of white grub infestations at TAMU and golf course monitoring sites based on presence and absence-biased classifications

Measure of detection validity[a]	Classification							
	Presence-biased[b]				Absence-biased[c]			
	Sound pulse rate		Listening		Sound pulse rate		Listening	
	TAMU	Fairway	TAMU	Fairway	TAMU	Fairway	TAMU	Fairway
Accuracy	0.81	0.84	0.92	0.82	0.62	0.48	0.73	0.55
Sensitivity	0.77	0.91	0.92	0.88	0.23	0.38	0.46	0.47
Specificity	0.85	0.75	0.92	0.75	1.0	1.0	1.0	1.0

[a]Sites rated at high and medium likelihoods of white grub infestation classified as positive, and low as negative.
[b]Sites rated at high likelihood of white grub infestation classified as positive, and medium and low classified as negative.
[c]Accuracy = (true positive + true negative) / (true positive + true negative + false positive + false negative); sensitivity = true positive/ (true positive + false negative), and specificity = true negative/ (false positive + true negative).

of three grubs were recovered at this site, the same total inferred from paired comparisons of recordings from P1-P2, P1-P3, P1-P4, P2-P3, P2-P4, and P3-P4.

In 35 tests with four sensors, with counts ranging from zero to five grubs per site, the actual white grub counts matched the predicted counts in 22 tests. The site shown in Fig. 1, for example, was found to contain three white grubs, the same as the predicted number. The actual and predicted counts differed by one white grub in nine tests, and by as many as two white grubs in only four tests. The actual and predicted means were not significantly different in a *t*-test with paired comparisons (actual mean = 49.6 grubs per square meter, predicted = 50.2 grubs per square meter, $t = 0.20$, df = 34, $P > 0.84$).

Unfortunately, with our equipment, the four-sensor array was a much slower method than either the single-sensor or the cup-cutter method. The necessity of installing four sensors properly and assessing combinations of signals two sensors at a time was time-consuming in comparison to measurements with a single sensor or to digging and sifting of the soil. Future investigations may benefit from having a sensor array designed for multiple sensors rather than as an improvisation from what was originally designed to be a single-sensor platform, and from having fully multichannel hardware for efficient handling of signals from all sensors simultaneously.

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