Quantitative Acoustical Detection of Larvae Feeding Inside Kernels of Grain

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ABSTRACT An automated, computer-based electronic acoustic system was developed to quantify infestation of internally feeding larvae in a grain sample using spatial localization of insects in the sample. Localization was determined using arrival times of sounds produced by insect feeding activity as received by an array of acoustic transducers. In a test conducted with 0–3 fourth instars of the rice weevil, *Sitophilus oryzae* (L.), in 1-kg samples of wheat, the system overassessed the number of larvae present in 6% of the trials and underassessed the number of larvae present in 34% of the trials. When Federal Grain Inspection Service (FGIS) standards were applied in evaluating performance, the system was 92% accurate in grading "clean" grain and 64% accurate in grading "infested" grain.

KEY WORDS stored products, sound, detection

THE PRESENCE OF INSECTS in stored grain is a major factor in the determination of quality under current mandated industry standards. Currently, grain inspection involves counting the insects sieved from a defined sample, usually 1 kg. This procedure limits detection to externally feeding larvae and adults. Larvae of two economically important species, the rice weevil, *Sitophilus oryzae* (L.), and lesser grain borer, *Rhyzopertha dominica* (F.), feed inside kernels of grain and are not detected. If adults are not present, because they either have not yet emerged from infested kernels or have been removed by cleaning or other manufacturing processes, grain internally infested may be mistaken for uninfested grain. Current laboratory methods for the detection of internal feeders (X ray, carbon dioxide production, resonance spectroscopy) are costly and time consuming and generally not implemented. There is a need for a rapid, quantitative, and economic method for detecting both adults and larvae of major insect pests of grain.

Detection of insects in fruits and grain by amplifying their feeding and movement sounds was suggested by Brain (1924), but technical difficulties prevented the development of practical systems (Adams et al. 1953, Bailey & McCabe 1965, Street 1971). Recent technological advances (sensitive detectors, suitable band-pass filters, and inexpensive computers) have stimulated studies (Hagstrum 1988; Vick et al. 1988; Hagstrum et al. 1990, 1991) directed at the development of practical acoustic detection systems for stored-product insects. Although the latter studies have demonstrated a strong correlation between the number of insects in a sample and total acoustical activity, it is not sufficiently accurate for the rapid grading of an unreplicated grain sample.

This article describes a system (ALFID [Acoustic Location Fixing Insect Detector]) that was developed, constructed, and evaluated for accurately identifying the number of internally feeding larvae in grain samples. The system operates by determining the number of loci within a sample from which sounds are originating.

Materials and Methods

Operational Principle, Technical Implementation, and Operation. The transit time of a sound is directly proportional to the distance traversed. ALFID incorporates a linear array of acoustic sensors mounted in one wall of a rectangular grain sample container. By identifying the first and second adjacent sensors in the array to receive a particular sound, the location of the sound's source can be inferred to be within a volume bounded by a plane equidistant from the sensors and a parallel plane through the middle of the first sensor. During a defined sampling period, some minimum number of sounds originating from within the same volume indicates the presence of an insect in that volume. This empirically derived minimum number is utilized to reduce the probability of incorrectly identifying ambient and/or grain settling noise as being produced by an insect.

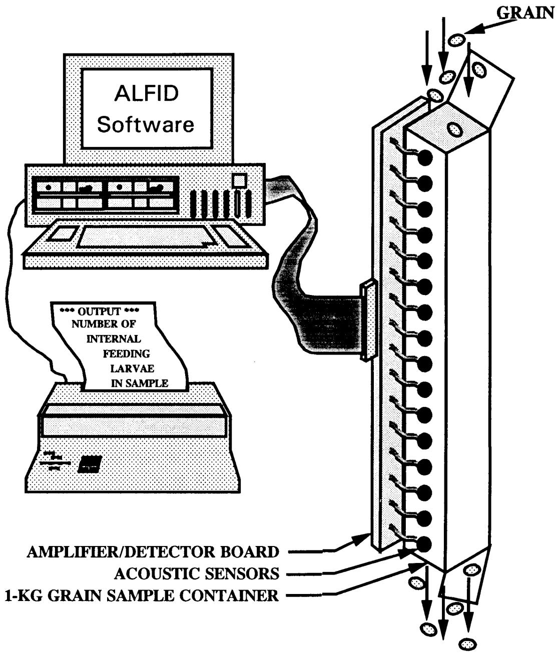

Fig. 1. User's view of the ALFID system in the field, showing its physical implementation. Grain sample container would be housed in a sound attenuation box.

From a user's perspective, the ALFID system (Fig. 1) includes a 1-kg grain sample container (76 by 5 by 4 cm) with a linear array of 16 (for spatial considerations and convenience with 16-bit oriented computers) piezoelectric acoustic sensors (2.8-cm diameter spaced 4.8 cm apart) mounted in one wall (76 by 4 cm) of the container. For field use, the container is oriented vertically to facilitate gravity loading and unloading of grain. A 16-channel electronic circuit board, positioned on an adjacent wall (for electrical noise considerations), locally amplifies (80 dB) and filters (1–10 kHz bandpass) the low-level analog output of each high-impedance sensor. Amplitude threshold detection is then used to delineate the arrival time of any received acoustic signal (Fig. 2). For field use, the grain container should be housed in a suitable sound attenuation box to reduce the effects of ambient noise. A cable from the circuit board connects the 16-channel detector outputs to a remote custom logic circuit implemented with 26 integrated circuit logic chips (gates, latches, and a one-shot from the standard 7400 low-power Schottky series). A computer interface for the custom logic circuit is provided by a commercial digital input/output board installed in a microcomputer that

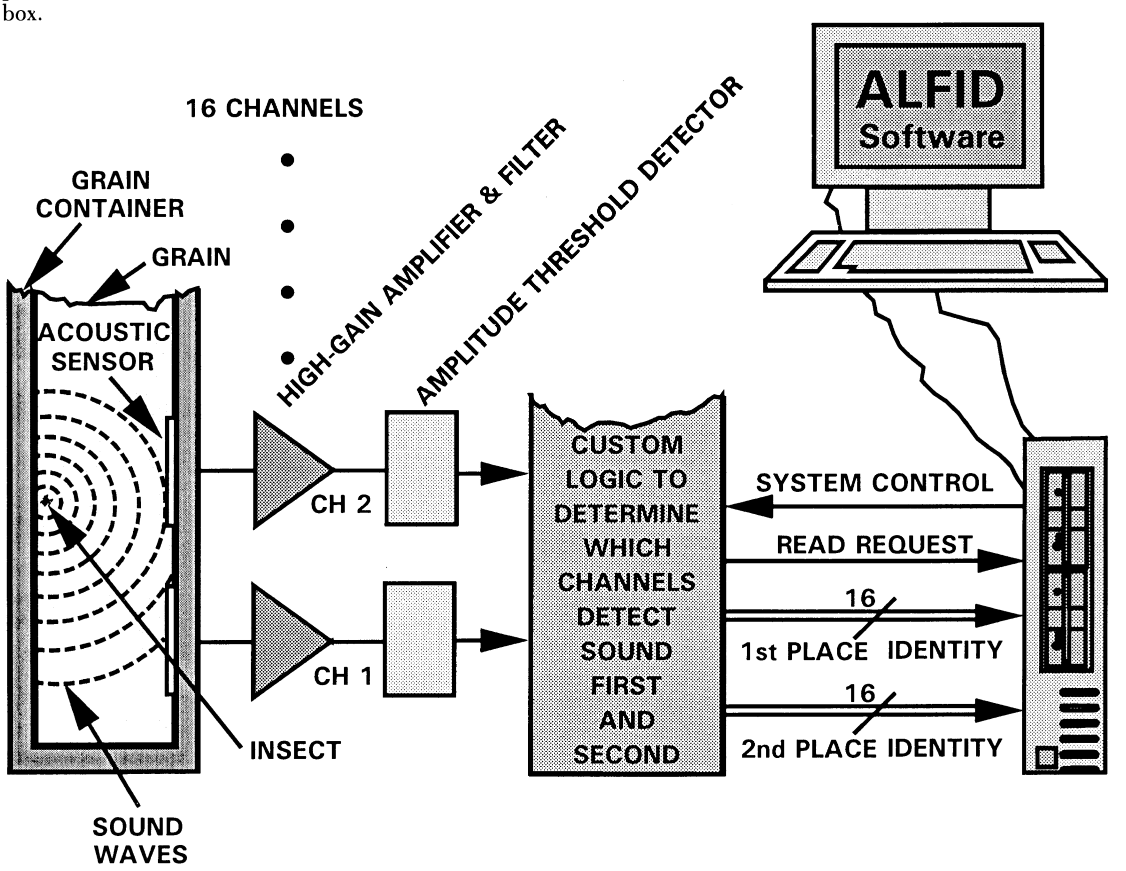

Fig. 2. Functional block diagram of the ALFID system portraying an insect-produced sound.

runs the ALFID software. For field use, the custom logic circuit board could also be mounted inside the case of the computer. The function of the custom logic circuit is to capture (latch) the identity of the first and (possibly) second sensors to receive an acoustic signal, then initiate reading of these identities by the computer. With appropriate timing functions, the circuit also prevents a single acoustic signal from being interpreted as multiple signals. At the end of a specified time interval, the computer summarizes the collected sensor identity data to be analyzed for quantification of the insect infestation in the sample.

Insect Rearing and Handling. *S. oryzae* was used as the test species in this study because larvae feed exclusively within grain kernels. Vick et al. (1988) had shown that larvae produce sounds that are detectable by available piezoelectric sensors. Insects were reared at 25 ± 1°C and 65 ± 5% RH with a photoperiod of 14:10 (L:D) h using methods described by Vick et al. (1988). Individual wheat kernels were taken from cultures that contained fourth instars. One day before use, the presence of a larva within a kernel was determined by listening to individual kernels that were placed on a piezoelectric microphone. Controls (uninfested kernels) were obtained from samples that had been kept at −10°C until 24 h before use.

System Evaluation. The ALFID system evaluation was performed with the grain sample container located in an anechoic chamber. The container was loaded with 1 kg of uninfested wheat 24 h before test trials. Each trial was prepared by inserting treated kernels (infested, control, or both) into the uninfested wheat. For this evaluation the container was oriented horizontally (sensors on the bottom and opened on the top) to facilitate accurate placement of each treated kernel. Placements could be at any of 63 equally spaced deployment positions along a horizontal line 3.3 cm above the centers of the sensors. The 63 deployment positions were located directly above sensors (16 positions), at 1/4 sensor spacing intervals (11.9 mm) between sensors (45 positions), and at a 1/4 sensor spacing interval outside the two end sensors (2 positions).

A double-blind test design was used in which the people who ran the trials did not have any knowledge that could bias their performance. This was accomplished for each trial by having a first person use a random number generator to assign treatments (infested, control, or both) and deployment positions for three color-coded kernels. Then a second person, without knowledge of the treatment color code, positioned them in the grain container for the test.

Three hundred trials were conducted over a 5-wk period. Temperature was 26 ± 2°C. Color-coded kernels were placed in the unit just below the grain surface and 1 min passed before data collection to reduce background noise that resulted from operator movements of the unit. Each trial lasted 9 min, after preliminary tests showed that during this period any larvae present had a high likelihood of producing sounds. The detected sounds were then used to quantify infestation based on the following analysis:

(1) The program read the custom logic circuit for the identity (sensor no.) of the first and (possibly) second sensors to hear a given sound. For example, if a given sound was heard first by sensor 5, then by sensor 4, it was assigned to (i.e., incremented the count of) the 5:4 Sensor Detection Order (SDO) by the ALFID system and was considered possible evidence of an insect located between sensors 4 and 5. If a sound was heard only by sensor 4, it was assigned to the 4: SDO and was considered evidence of an insect somewhere between sensors 3 and 5. If a sound was heard first and second by two nonadjacent sensors, it was considered an error, was not assigned to an SDO (e.g., there was no 4:6 SDO), and was not used in scoring. Thus, with 16 sensors there are 46 possible SDOs to which sounds could be assigned, and many sounds could be assigned to the same SDO. An SDO is not itself a position and should not be confused with the previously discussed 63 kernel deployment positions used in this test.

(2) Background noise was subtracted. In 33 preliminary runs with no insects, background noise resulted in small numbers of sounds being detected and assigned to various SDOs. To compensate for this, all SDOs in the evaluation test (after data collection but before the scoring below) had their counts reduced. A value of 2 was subtracted from the counts of the 16 SDOs involving one sensor (e.g., the 4: SDO) and a value of 1 was subtracted from the counts of the 30 SDOs involving two sensors (e.g., the 5:4 SDO).

(3) Ideally, all the sounds produced by a single insect would be assigned by the ALFID system to the same SDO. However, because of noise and nonuniform sound production and transmission, different sounds originating from activity of one insect could be assigned to different adjoining SDOs. For a number of sounds to be grouped together as having been produced by the presence of a single insect and thus considered a positive score, the algorithm used was:

   (a) if the sounds were assigned to only one SDO, then that SDO count (i.e., the number of sounds assigned to it) had to be >7;

   (b) if the sounds were assigned to two adjoining SDOs (e.g., 4:5 and 5:4 or 4:5 and

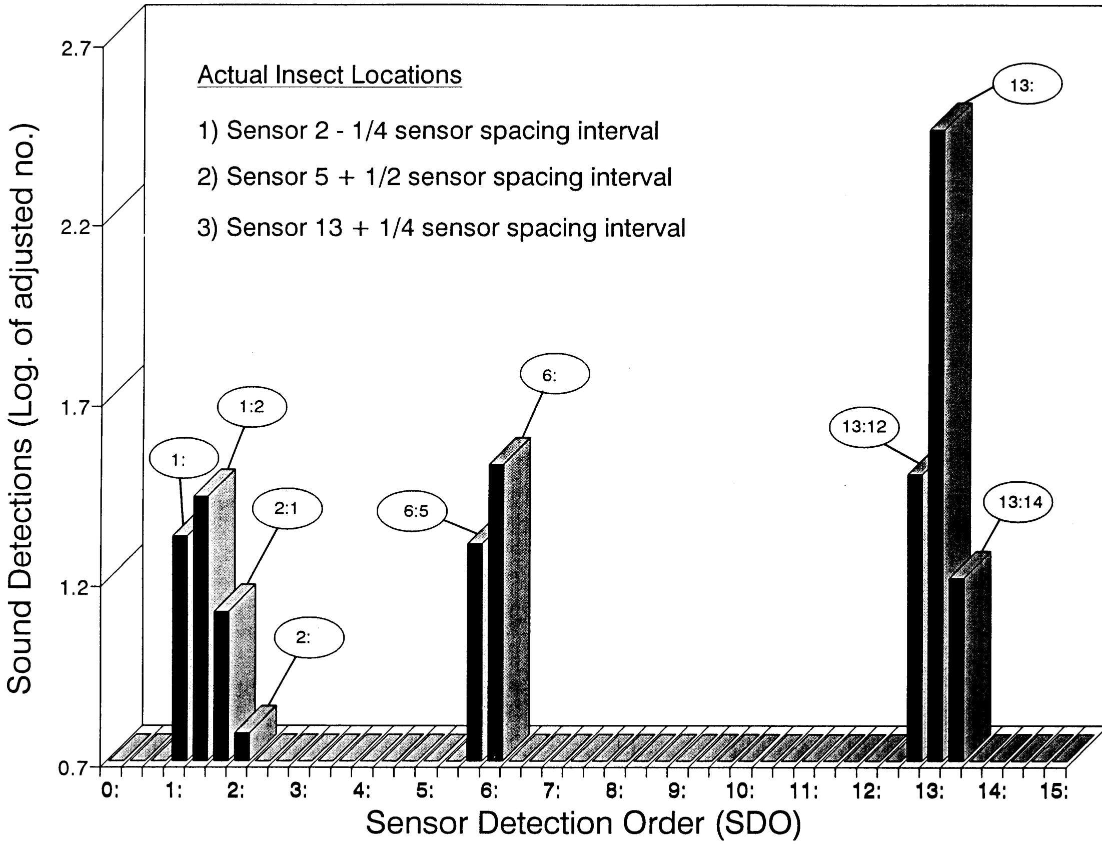

Fig. 3. Results of a representative trial with three insects located as indicated in the figure. Horizontal axis is divided into 46 segments corresponding to all the possible Sensor Detection Orders (SDOs). The unlabeled segments represent SDOs that sounds detected by two adjacent sensors were assigned to (e.g., SDO 1:2). To illustrate the axis pattern, SDOs that had sounds assigned to them in this trial are labeled in balloons. Vertical axis indicates the numbers of sounds assigned to various SDOs after adjusting for background noise.

4:), then both of their counts had to be >3;

(c) if the sounds were assigned to three adjoining SDOs, then two of the three had to be >2;

(d) if the sounds were assigned to four adjoining SDOs, then at least two had to be >2;

(e) if the sounds were assigned to five adjoining SDOs, then at least one had to be >1;

(f) if the sounds were assigned to six or more adjoining SDOs, then it was considered a positive score regardless of the number of sounds assigned to each SDO.

(4) For graphic representation of the data, a value of 3 was added to each corrected SDO count (to obtain a positive integer, see 2 above) to allow plotting on a log scale. Fig. 3 shows an example of a representative trial when three insects were placed in the grain container.

For statistical analyses, data from 50 consecutive trials were pooled and considered to constitute one replication, for a total of six replications and mean and standard error values were calculated. Data from four trials (102, 178, 179, 180) were omitted because temperature fluctuations of >10°C in the anechoic chamber during those runs adversely affected data collection. The data were also examined according to the Federal Grain Inspection Service (FGIS) standards of zero or one insects per kilogram = "clean" grain and more than one insect per kilogram = "infested" grain. Data from trials with zero and one insects for the former and from trials with two and three insects for the latter were pooled.

Results and Discussion

The results of the 296 trials of the ALFID unit are summarized in Table 1. In 90% (n = 34) of the trials in which no insects were present the ALFID system's scoring was correct; 10% (n = 3) of those trials were erroneously scored as having one insect present. No trials were scored as having more than one insect present when there were none. When one insect was present in a

Table 1. Percentage (SEM) distribution of scoring numbers of *S. oryzae* in grain

No. of larvae present	Total no. of trials	Scored as containing indicated no. of larvae[a]			
		0	1	2	3
0	37	90.1 (4.6)	9.9 (4.6)	0.0	0.0
1	95	19.3 (4.6)	69.8 (5.7)	10.9 (3.5)	0.0
2	119	3.2 (1.6)	38.5 (2.9)	55.1 (3.4)	3.3 (2.4)
3	45	0.0	21.9 (7.0)	48.7 (7.4)	29.2 (8.2)

[a] Average of six replications.

trial, it was correctly scored in 70% (n = 66) of the trials; such trials were incorrectly scored as having no insects present in 19% (n = 19) of the trials; false positives (scored as two insects when only one was present) occurred in 11% (n = 10) of the trials; no trials were incorrectly scored as having three insects present when only one was present. When two insects were present, they were correctly scored in 55% (n = 65) of the trials; numbers present were underassessed 42% (n = 50) of the time and overassessed in only 3% (n = 4) of the trials. When three insects were in the unit, they were correctly assessed in 29% (n = 13) of the trials and underassessed 71% (n = 32) of the time.

The percentages of trials in which two or three larvae were present but not detected ([2,0] = 3.2 and [3,0] = 0, Table 1) correspond well with the theoretical probabilities based on results from a single larva (Table 2). However, the percentages of trials in which two or more larvae were detected were lower than predicted from a single larva. This was likely caused by the scoring algorithm, which attempted to avoid overestimating the number of insects present. Overall, only 6% of the trials yielded estimates greater than the numbers of larvae actually present, probably the result of electrical and acoustic noise incorrectly scored as larval presence. A greater error, 34%, was a result of failure to identify larvae that were present, and was because of two causes.

Table 2. Predicted percentage distribution of scoring for more than one larva present based on test results with one larva present

No. of larvae present	Scored as containing indicated no. of larvae[a]				
	0	1	2	3	>3
2	3.7	26.9	52.9	15.2	1.2
3	0.7	7.8	29.4	42.8	19.2

[a] From Table 1, with p(1,0) = probability of 0 detected when 1 is present = 0.193; p(1,1) = 0.698; p(1,2) = 0.109; and P = p(1,0) + p(1,1) + p(1,2) = 1. The predicted distribution when 2 larvae are present is obtained from the expansion of P × P where the probability of a particular score is equated to the appropriate expansion term. Thus p(2,0) = p(1,0) × p(1,0); p(2,1) = 2 × p(1,1) × p(1,0); p(2,2) = p(1,1) × p(1,1) + 2 × p(1,0) × p(1,2); p(2,3) = 2 × p(1,1) × p(1,2); p(2,4) = p(1,2) × p(1,2). The predicted distribution when 3 larvae are present is similarly obtained from the expansion of P × P × P.

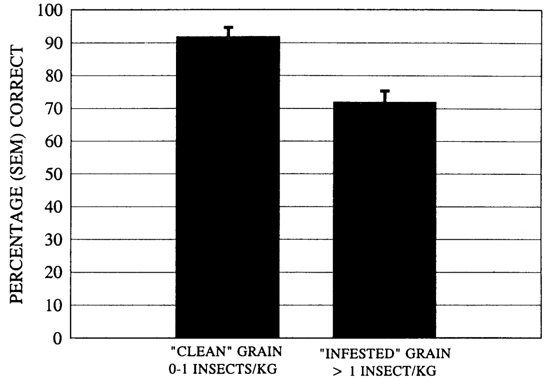

Fig. 4. Percentage of accuracy in determining infestations of *S. oryzae* in grain in terms of Federal Grain Inspection Service standards. Trials in which two or three insects were used and the distance between placed insects was <1.75 sensor spacing intervals are not included.

First, when the randomization procedure for the deployment of infested kernels in the unit was performed, 15% of the trials placed infested kernels <1.75 sensor spacing intervals apart (i.e., less than the empirically determined limit of spatial resolution of the system). Second, about 15% of the insects made no detectable sounds, a value similar to that reported by Vick et al. (1988).

Based on FGIS standards, samples that contained zero or one insects (n = 132) were correctly scored as "clean" in 92% of the trials, with the remainder (8%) incorrectly scored as "infested." Samples with two or three insects ("infested" grain) were correctly assessed 64% of the time (n = 164), with the remainder of the trials being incorrectly scored as "clean." Disregarding trials in which two or three insects were used and the distance between them was <1.75 sensor spacing intervals, the accuracy of the assessment of "infested" grain increases to 72% (Fig. 4). The principal reason that such samples were incorrectly scored probably was the failure of test insects to produce detectable sounds.

These tests demonstrate that ALFID is a potentially useful tool in quantifying infestations of internally feeding larvae that are not detectable by present commercial methods. The tests described in this paper focused on larvae because they are more difficult to detect than adults and provide a new approach to the quantification of insect infestations in stored grain.

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