

- WALKER, T. J. 1979. Calling crickets (*Anurogryllus arboreus*) over pitfalls: females, males, and predators. Environ. Entomol. 8: 441-443.
- WALKER, T. J. 1982. Sound traps for sampling mole cricket flights (Orthoptera: Gryllotalpidae: *Scapteriscus*). Florida Entomol. 65: 105-110.
- WALKER, T. J., ed. 1984. Mole crickets in Florida. Univ. Florida Agric. Exp. Stn. Bull. 846. 54 pp.
- WALKER, T. J. 1986. Monitoring the flights of field crickets (*Gryllus* spp.) and a tachinid fly (*Euphasiopteryx ochracea*) in north Florida. Florida Entomol. 69: 678-685.
- WALKER, T. J. 1987. Wing dimorphism in *Gryllus rubens* (Orthoptera: Gryllidae). Ann. Entomol. Soc. Am. 80: 547-560.
- WALKER, T. J. 1989. A live trap for monitoring *Euphasiopteryx* and tests with *E. ochracea* (Diptera: Tachinidae). Florida Entomol. 72: (submitted).
- WALKER, T. J., AND G. N. FRITZ. 1983. Migratory and local flights in mole crickets, *Scapteriscus* spp. (Gryllotalpidae). Environ. Entomol. 12: 953-958.
- WALKER, T. J., J. A. REINERT, AND D. J. SCHUSTER. 1983. Geographical variation in flights of mole crickets, *Scapteriscus* spp. (Orthoptera: Gryllotalpidae). Ann. Entomol. Soc. Am. 76: 507-517.
- WEBB, J. C., T. BURK, AND J. SIVINSKI. 1983. Attraction of female Caribbean fruit flies, *Anastrepha suspensa* (Diptera: Tephritidae), to the presence of males and male-produced stimuli in field cages. Ann. Entomol. Soc. Am. 76: 996-998.
- ZUK, M. 1987. Variability in attractiveness of male field crickets, Orthoptera: Gryllidae, to females. Anim. Behav. 35: 1240-1248.

ACOUSTICAL SYSTEM TO DETECT LARVAE IN INFESTED COMMODITIES

J. C. WEBB, D. C. SLAUGHTER AND C. A. LITZKOW
Insect Attractants, Behavior, and Basic Biology Research Laboratory,
Agricultural Research Service, U.S. Department of Agriculture,
Gainesville, Florida 32604

ABSTRACT

An acoustical system to detect larvae in post-harvest commodities is described. The system can detect one-day old fruit fly larvae in grapefruit, mangoes, and other similar fruit and lepidopterous and coleopteran larvae in individual kernels as well as bulk grain. The detectors have been modified so that they can be adjusted to accept fruit of various sizes and shapes. A computer system and software has been developed to monitor the feeding activity of the immature insect for its complete life cycle. The signal is amplified approximately 90 dB and then filtered through appropriate bandpass filters. The variables (trigger levels, time intervals of data collections, and sample rates) are controlled by the computer software. The computer also stores and analyzes the data.

RESUMEN

Se describe un sistema acústico para detectar larvas en productos después de cosechados. El sistema puede detectar larvas de moscas de las frutas de un día de nacida en toronjas, mangos y otras frutas similares, y larvas lepidópteras y coleópteras en granos individuales así como al granel. El detector se ha modificado para que se pueda adaptar para que acepte frutas de varios tamaños y formas. Se ha desarrollado un

sistema de computadoras y de programas para chequear la actividad de cómo comen los insectos inmaduros durante su ciclo de vida. La señal es amplificada aproximadamente 90 dB y filtrada a través de los filtros apropiados de paso-de-bandas. Las variables (niveles disparadores, intervalo de tiempo en la colecta de datos, y la proporción de muestras) son controladas por la computadora. La computadora también guarda y analiza los datos.

Post-harvest commodities are frequently infested with insect larvae, adults or both. As a result of such infestations, enormous quantities of food are lost or rendered unusable each year. Also, because of the quarantine restrictions imposed by many countries against certain insect pests, treatments or detection methods must assure that the affected commodities are insect free. Since the Environmental Protection Agency halted the use of ethylene dibromide (EDB) fumigation as a treatment for many of these commodities, it has been necessary to develop new treatments for the export market. Some of the more common treatments now being studied for fruit are other fumigants, cold treatment, vapor heat treatment, and irradiation. It also has been necessary to develop new detection methods, especially in fruit, to determine the presence of a quarantine pest.

The most common method used to determine the presence of larvae in perishable fruit, (i.e., citrus and mango) and thus to determine the effectiveness of the treatments is to cut the fruit and look for larvae. This method is very labor intensive when large numbers of fruit are involved, and inaccurate especially in detecting first and second instar larvae. The cutting of such fruit also renders it useless.

Several detection methods and devices are currently in use to detect adults and feeding (external and internal) larvae in storage grain. The two most common methods are X-ray (Milner et al. 1950, Fiskus 1972), and carbon dioxide gas analysis (Bruce et al. 1982). Some of the techniques used to detect adult weevils in grain are described by Barak & Hariem (1982) and Barak & Burkholder (1985). Also, adult weevils in grain are detected by sieving the grain through hardware cloth.

Brain (1924) was one of the early scientists to publish on detecting the chewing and moving sounds of insect larvae in agricultural commodities with electromechanical devices. He reported that he could detect apple and quince borers in apple stems and weevils inside grain. Since Brain's work, many scientists have researched this area and each has made major contributions (Adams et al. 1953, Bailey & McCabe 1965, Street 1971, Webb & Landolt 1984, Webb et al. 1988). Unfortunately, early work was plagued by inadequate sensor sensitivity, sensor noise, ambient noise and equipment noise which resulted in poor signal to noise ratio. With the advent of microelectronic equipment, electronic noise and the signal amplification are no longer limiting factors. As in most systems the sensors or detectors are the limiting components.

The objectives of this research were to upgrade and modify the acoustical detection system reported by Webb et al. (1988). These include the development of an adjustable acoustical coupler that can be used on fruit of different size and shape, development of calibration curves for the system and the development of software that can monitor the feeding sounds over the life span of the larvae.

METHODS

ACOUSTICAL DETECTORS

The basic acoustical coupler with one detector is shown in Figure 1. The detector is composed of a 2.54 cm i.d. polyvinylchloride (PVC) pipe with a thin diaphragm mounted

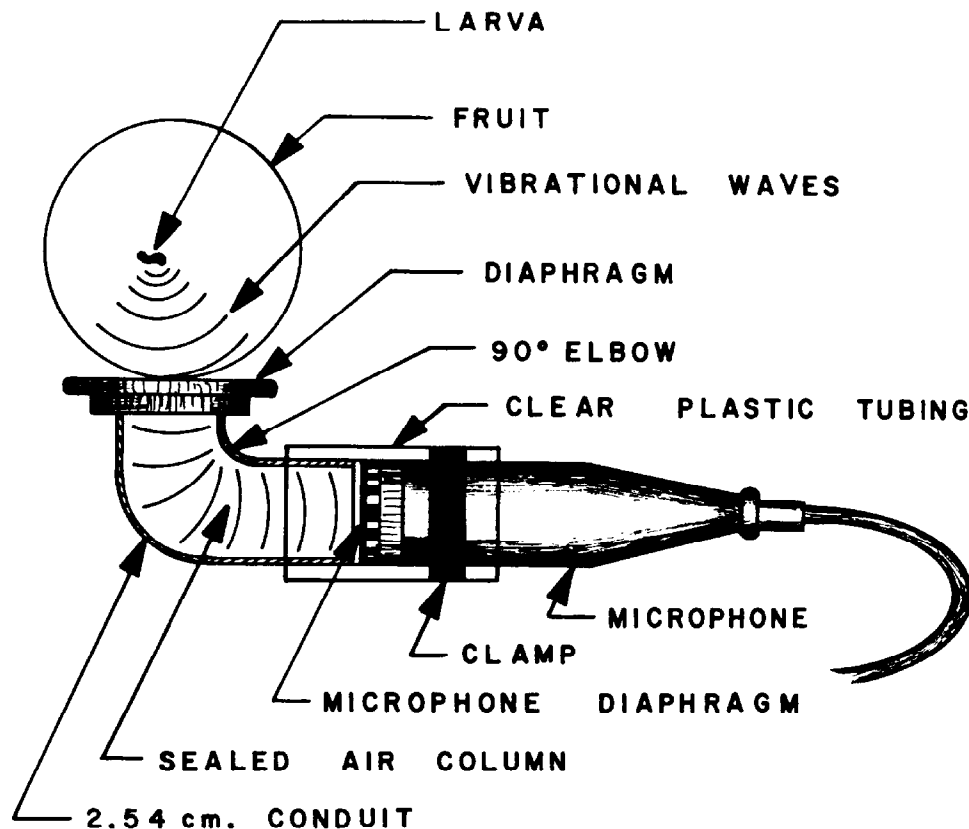


Fig. 1. The basic acoustical coupler with a single diaphragm illustrating the various components

on one end and a microphone on the other. The microphone is inserted into a 2.54 cm flexible plastic tubing that is attached to one end of the pipe and clamped to assure an air-tight seal. The vibrations on the surface of the fruit caused by the feeding larvae are transmitted to the plastic diaphragm causing the diaphragm to vibrate and create standing waves in the PVC pipe (Webb et al. (1988)). To maximize the signal from the detector, the microphone must be tuned to the standing waves. This can be accomplished by carefully sliding the microphone in the pipe until a maximum amplitude is reached. The maximum signal normally consists of a very high amplitude narrow band frequency range when compared to a broad band signal for an untuned system (Figure 2). This basic design of the acoustical coupler can be modified to accommodate more than one diaphragm, thereby, allowing more than one contact point with the commodity which increases the probability of detecting the smaller larvae. An acoustical coupler is defined in this paper as a tuned airtight chamber with one or more plastic diaphragms that can be physically and acoustically coupled to a microphone. A typical acoustical coupler used for round fruit i.e., grapefruit and oranges, is shown in Figure 3 and an acoustical coupler for irregular type fruit i.e., mangoes and papaya, is shown in Figure 4. Ball type swivel sockets have been installed in most acoustical couplers just below the diaphragm to accommodate fruits of different sizes and shapes. The acoustical coupler can be modified to detect larvae in stored grain as shown in Figure 5 (Vick et al 1988). The detector and microphone must be in a relatively quiet environment for most studies; therefore, a lead chamber with a hinged lid was used to house the detector and microphone when additional sound attenuation is needed.

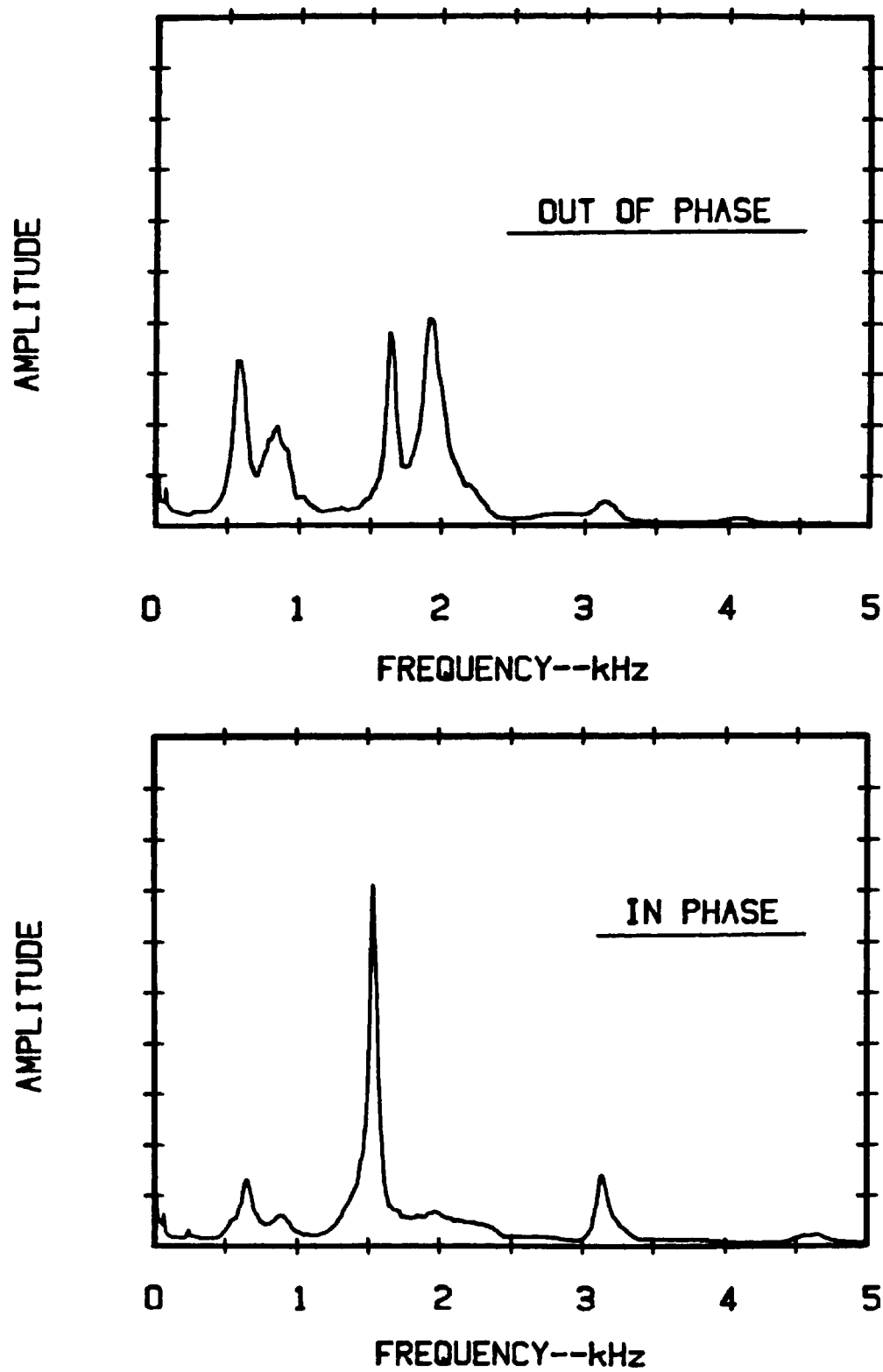


Fig. 2. The output of the system with the signal in phase and out of phase.

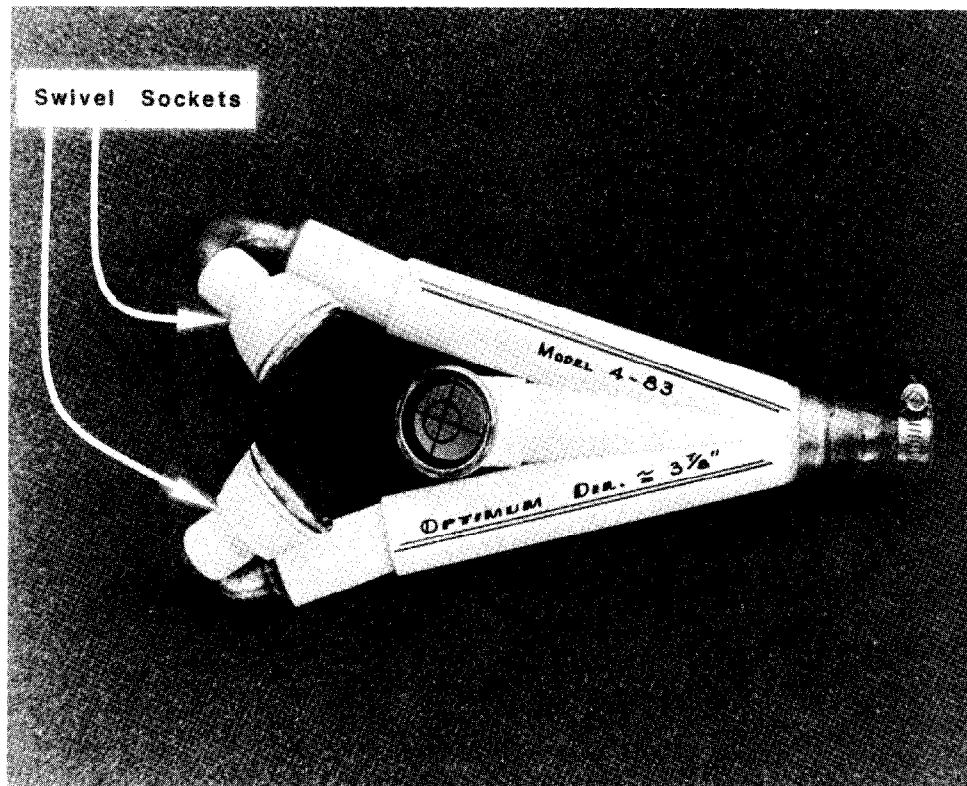


Fig. 3. An acoustical coupler with three adjustable detectors used mainly for round fruit i.e., grapefruit, oranges and apples. Note the swivels are located just under the vertical detectors.

SIGNAL PROCESSING SYSTEM

The equipment used to detect, condition the signal, and monitor the larval sounds are shown in Figure 6. The detection system consists of a sound insulating chamber (to insulate the detector and the commodity being tested from ambient noise), an acoustical coupler and the transducer. The sound insulating chamber can be anything from a commercially available anechoic chamber to a simple plywood box lined with acoustical insulation. The sound insulated chamber is often used in conjunction with a lead cylinder 42 cm long and 25 cm in diameter with a hinged lid located on the side of the cylinder. The lead chamber filters out many of the sounds that are common in the laboratory. A Model 4145 Bruel and Kjaer* (B&K) low noise 2.54 cm diameter condenser microphone was used as the transducer in the acoustic coupler.

The signal processing system is equipped with an 8 channel B&K model 2811 multiplexer for simultaneous monitoring of up to 8 inputs. The multiplexer is equipped with a HP-IB interface bus that makes it possible to be controlled by any IBM compatible computer equipped with an HP-IB interface card. We have written appropriate software for the multiplexer. The signal from the microphone or multiplexer is amplified by a low-noise, high-gain Model 2610 B&K amplifier. The signal amplification ranges from 70 to 90 dB depending upon the signal strength generated by the larvae. The amplifier also provides for subsonic filtering of frequencies below 22.4 Hz and for "A weight" filtering. These filters eliminate extremely low and high frequency noise. A Model 3700 Krohn-Hite* bandpass filter was used to provide additional filtering as well

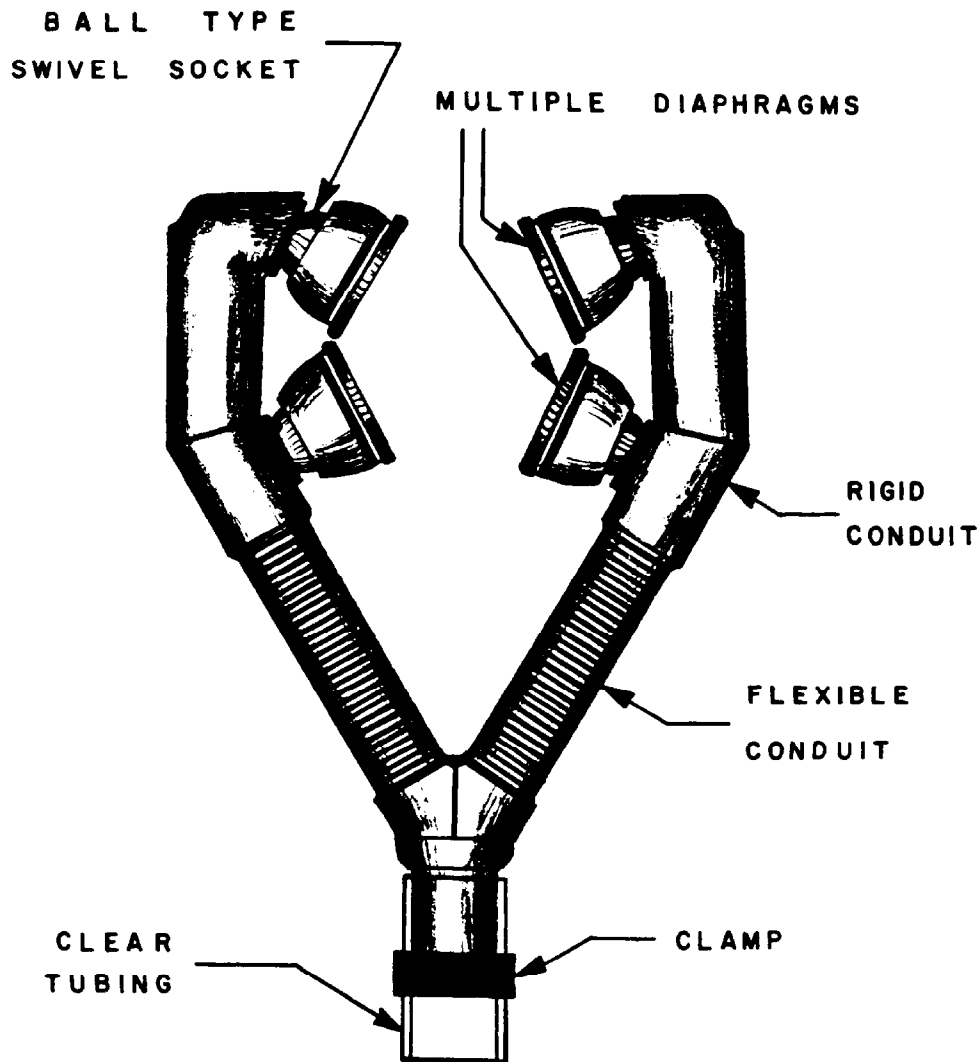


Fig. 4. An acoustical coupler constructed of flexible conduit and four sensors mounted to swivel. This acoustical coupler was designed for irregular fruit i.e., mango and papaya.

as an additional 20 dB gain. The filtered signal is then fed into a general purpose audio amplifier equipped with headphones for audio monitoring, a Model 5111A Tektronix* oscilloscope for visual monitoring and a computer system which controls the A/D converter and counter, collects, stores and analyzes the data. Much of the equipment after the bandpass filter is optional and will depend upon the nature of the measurements desired. For example, only an audio amplifier and earphones are required to detect the presence of larvae, but some type of computer system or data logger is required to monitor the activities over longer periods of time.

COMPUTER SYSTEM

The primary purpose of the computer system is to provide an efficient means of recording, displaying and analyzing the acoustical signals sensed by the detector. This gives, for the first time, a means of continuously monitoring an insect larva's feeding

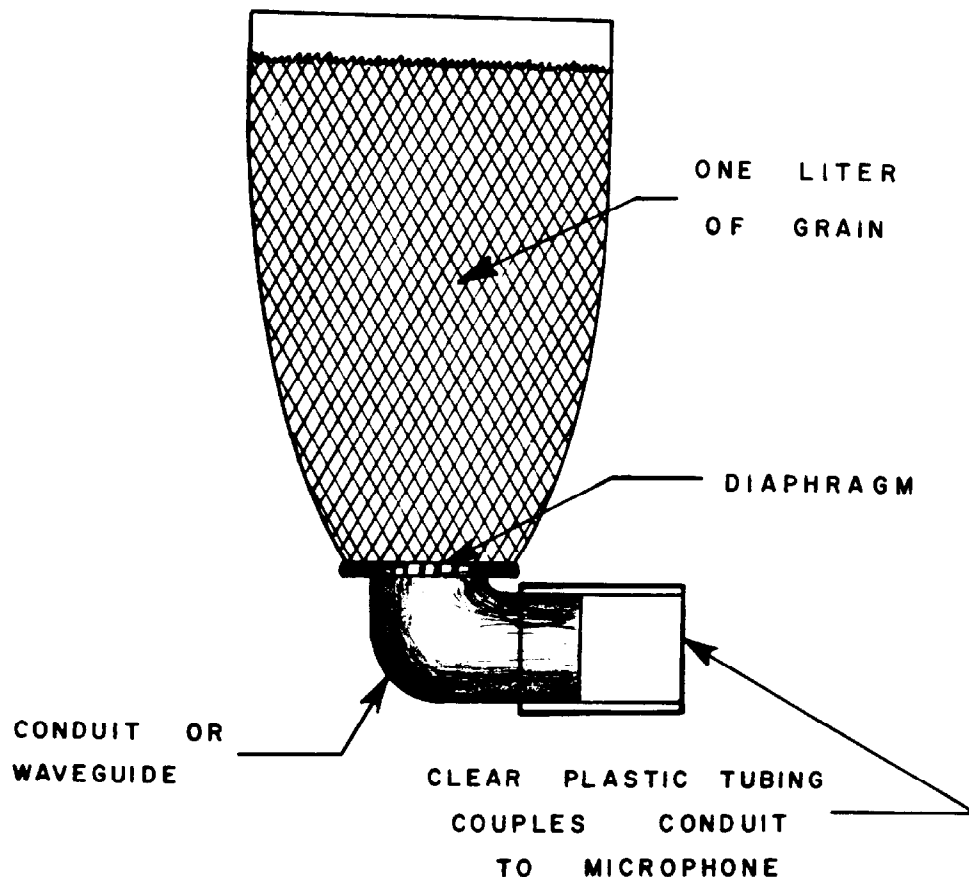


Fig. 5. Acoustical coupler and sample holder for detecting larvae in bulk samples of stored grain. The diaphragm is located in the bottom of the sample holder.

activity throughout its life cycle undisturbed in its natural habitat. The computer system consists of three main components: 1) a personal computer (PC) with at least 640 K bytes to 2.6 M bytes of dynamic random access memory (RAM) (the higher the RAM, the longer the data collection period can be) and a high speed interface bus, such as the IEEE 488-1978 system; 2) an A/D converter and a programmable universal counter with a frequency range of 0 to 10 MHz; and 3) a mass storage device (hard disk).

Specifically this system in our laboratory consists of a Model 3437A Hewlett-Packard (HP) voltmeter (analog to digital, A/D converter) a Model 5316 HP universal counter, and a HP Vectra (IBM compatible) computer with 20 M byte hard disk and a graphics printer (Figure 6). Computer software was developed to control both the A/D converter and the universal counter. The A/D converter was used primarily to measure the amplitude of the voltage when determining the trigger levels of the system and when calibrating the filters. The counter was used to monitor the larval feeding activities by counting the number of voltage spikes in a predetermined time period.

The purpose of the A/D system was to analyze insect activity using sound in the 100 Hz frequency region. The Nyquist sampling theorem requires that the computer sample an A/D converter at more than 200 samples/second to guarantee detection of 100 Hz sounds. This high sampling rate soon creates a data storage problem. For example, if a computer monitored an A/D converter at 200 Hz for a 48-h period, 34.6 million samples would be collected, which far exceeds the capacity of the PC memory. The universal counter was used in place of an A/D converter to alleviate this problem because of its natural data compression capability.

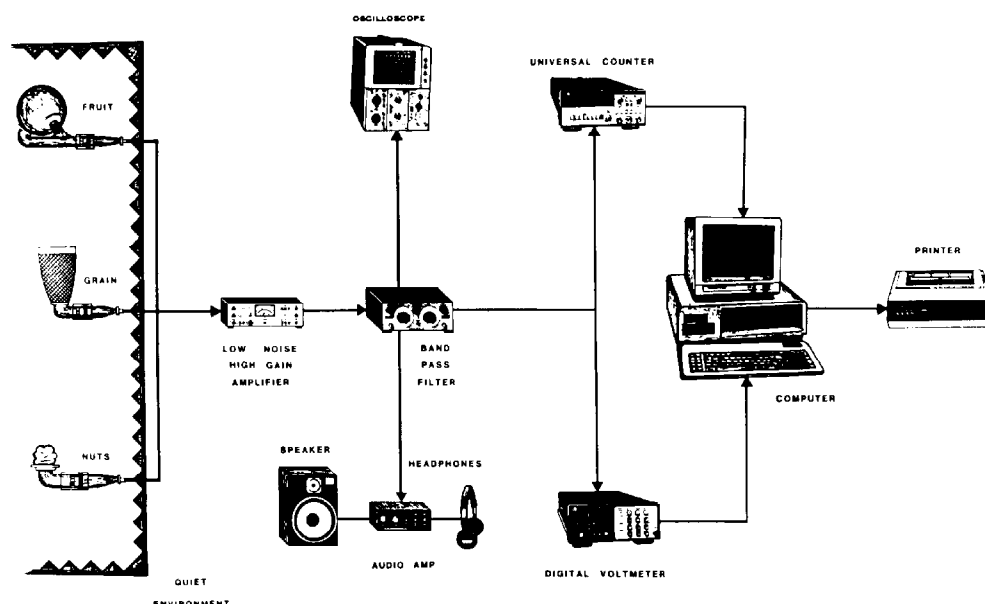


Fig. 6. Pictorial diagram of larval detector and signal processing system.

The function of the programmable universal counter is to monitor continuously an input signal and keep track of the number of times the voltage level exceeds a predetermined threshold level. By substituting a universal counter for the A/D converter the sampling interval can be reduced to a window of time dependent upon the experimental considerations rather than the frequency of the sound emitted by the insect larvae. In comparison, if a particular experiment only required information at a 1-sec resolution over a 48-h period, the computer could sample the counter at 1 Hz (independent of the frequency of sound emitted by the insect larvae) requiring 172.8 thousand samples. The use of a programmable universal counter thus provides a considerable reduction of data (over two orders of magnitude in this example), which not only reduces the storage requirements but reduces the analysis time as well. The primary disadvantage of using a universal counter in place of an A/D converter is that the actual voltage levels (or sound amplitudes in this case) are not recorded. We only know how many times the sound level exceeded the threshold during the selected window of time.

The system's software was developed using the Microsoft QuickBasic language. Basic was chosen as a developmental language because of its ease of use, reduced development time and the availability of a software library for instrument control. The scope of the software was two-fold, first to provide a routine that controlled the universal counter and voltmeter and second, to display data (both in tabular and plotted form) and calculate pertinent statistics.

The software that controls the universal counter permits data to be collected at a resolution ranging from 150 ms to 1 h and to store it in Lotus 1-2-3 readable format. At a resolution of 150 ms the system can monitor signals "continuously" for 7.86 h and at a resolution of 10 seconds, monitoring can be conducted for over 21 days. The term "continuous monitoring" should be tempered with the understanding that between counter measurements the system requires a period of approximately 17 ms to store the measurement in RAM as well as to read and store the time of the measurement and reset the counter. Therefore at a 150 ms resolution, the system is "continuously" monitoring only 90% of the time, but at a 1-sec resolution this increases to 98%.

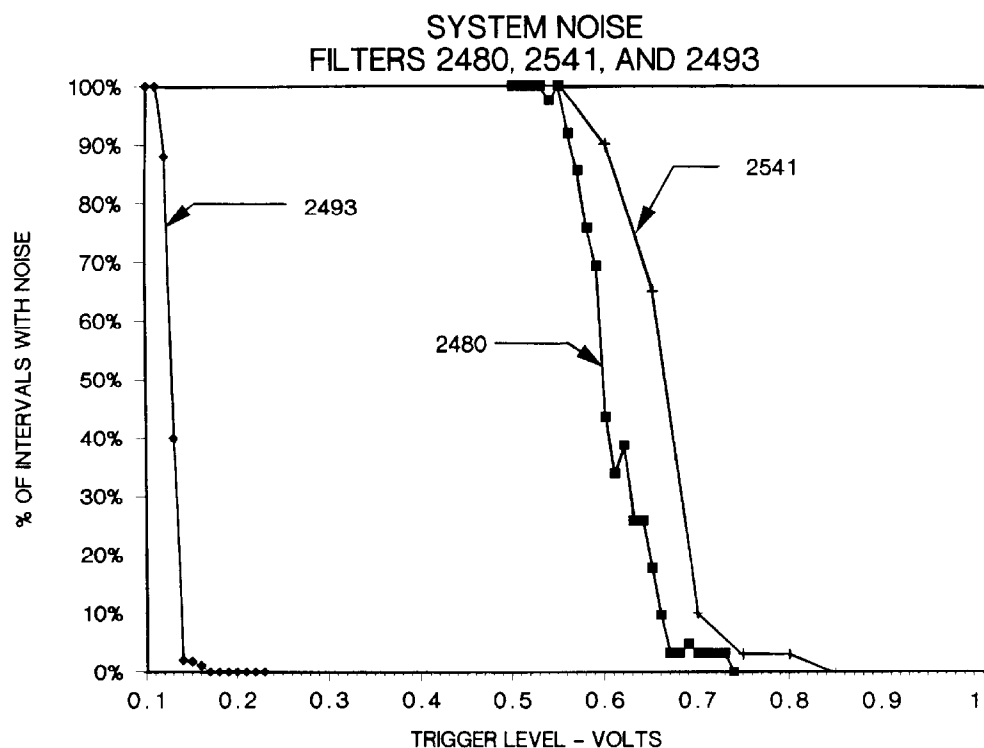


Fig. 7. The performance curves of three Krohn-Hite filters. These filters are identified by serial numbers and the graph shows the percent of system noise voltage spikes generated for a given trigger level.

RESULTS AND DISCUSSION

This detection system can be used to test a variety of commodities for hidden insects. However, one requirement is that the test be conducted in a relatively quiet environment such as an acoustically insulated room or a room with the walls lined with standard acoustical tile or acoustical foam to reduce the ambient noise level. The ambient and the inherent system noise must be determined in order to select the appropriate threshold settings for the counter. It was determined that all components of the various systems in operation could be used interchangeably without effecting the trigger level except the Krohn-Hite filter. The gain varied between filters and this had to be taken into consideration when a system with a different filter was used. Therefore, a calibration curve to determine the trigger level for each filter was developed using the universal counter. The curves were determined by placing a rubber ball filled with water, to simulate the size and weight of a grapefruit, on the detector and monitoring the output of the system. Since the counter was used to collect data for most experiments, the counter was used to collect data for the calibration curves. The counter was set at a gate time of 10 seconds with a total collection time of 1-h, this gave 360 intervals per hour. The minimum trigger level was selected by insuring that each of the 360 intervals contain voltage spikes generated from either ambient or system noise. Then the trigger level was increased in increments of 100 mV until none of the intervals contained spikes. This was the trigger level that was considered to be above the system and ambient noise for a time period of one hour or less. The results from three Kron-Hite filters are shown in Figure 7.

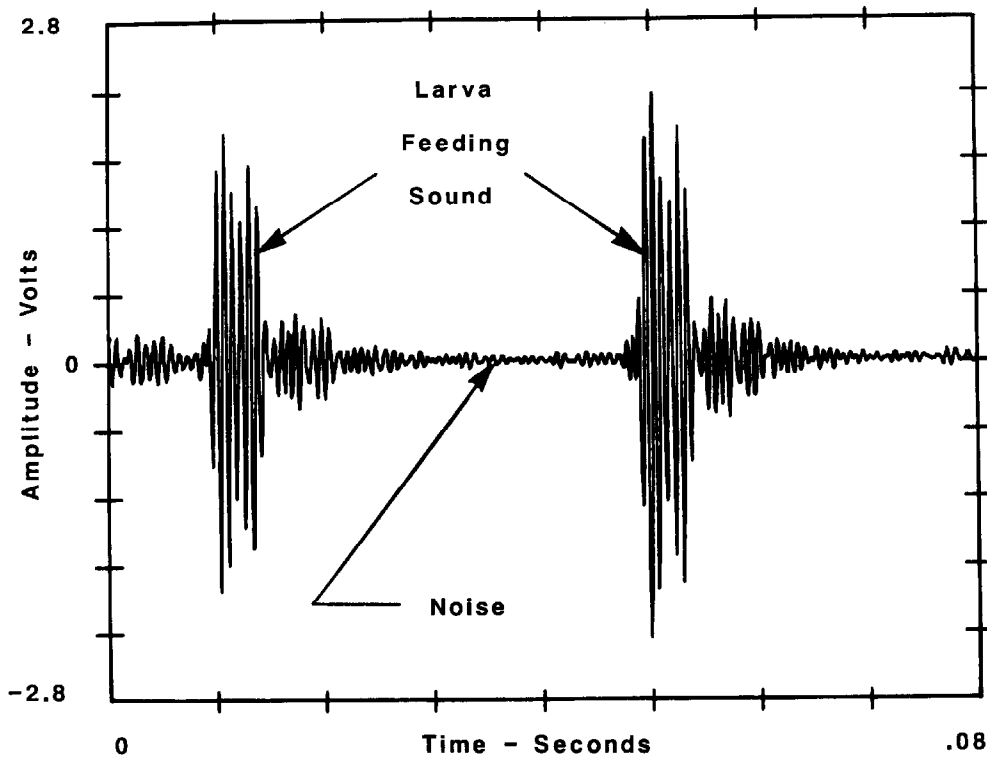


Fig. 8. The amplified and filtered signal of a 2-day-old Caribbean fruit fly larval feeding sound feeding in grapefruit vs background noise.

The trigger level of the A/D converter can be set so that only the voltage of those biologically generated spikes above a predetermined value will be recorded and those below this value will not be measured. Likewise, the trigger level of the counter can be set so that only those spikes that are above a preset voltage level will be counted. Therefore, it is important to know the ambient and system noise level before beginning the measurement of insect feeding sound. A typical example of a filtered and amplified analog signal fed into the A/D converter and the counter from a 2-day-old Caribbean fruit fly (*Anastrepha suspensa* (Loew)) larva in grapefruit is shown in Figure 8. As seen from this figure there is a large signal to noise ratio, therefore, a relatively high trigger level could be selected and still guarantee detection of the feeding sound generated by the larvae. However, as in most biological material not all signals are this large. The peak voltage of the spikes from a one day old larva may range from noise level to several times noise level. But when the larvae reaches 3 to 4 days old, the signal is normally of the magnitude of that shown in Figure 8. Therefore, in order to count the small signals the trigger level may have to be set just above noise level.

The universal counter totals the number of voltage spikes above the threshold level in a given time interval and sends this information to the computer. An example of the summary of the data taken with the counter is shown in Table 1, using a noninfested grapefruit and a grapefruit with one 2-day-old Caribbean fruit fly larva. The data was stored in a Lotus readable format and a plot for both sets of data is shown in Figure 9. Both tests were run for approximately 14-h with a gate time of 10 sec and a trigger level of 0.15 volts. As can be seen from the summary data in Table 1, there were some intervals that contained one or more spikes. If the trigger level were set at a higher value, these spikes could be eliminated. Graphically, these random noise spikes from

TABLE 1. AN EXAMPLE OF THE SUMMARY OF THE DATA SHOWN IN FIGURE 9, COLLECTED WITH THE UNIVERSAL COUNTER.

<u>A noninfested grapefruit</u>	
ID: NONINFESTED GF 2610 30-40 FIL 20DB	1.2-1.7 SOUND HEARD 1-12-87
FILE NAME: A:D701	DATE: 01-21-86
GATE TIME: 10000 msec	TRIGGER LEVEL: .15 volts
MEAN SPIKE/SEC: .0072	STANDARD DEVIATION: .2561 sec
TOTAL SPIKES: 35	TOTAL COLLECTION TIME: 49961 sec
MAXIMUM PERIOD WITHOUT NOISE: 10259.00 sec	
PERIOD OCCURRED AT INTERVAL: 31551.00-41810.00	
MAXIMUM PERIOD WITH NOISE: 1.00 sec	
PERIOD OCCURRED AT INTERVAL: 10550.00-10551.00	
NUMBER OF INTERVALS WITHOUT NOISE: 1846	
NUMBER OF INTERVALS WITH NOISE: 15	
<u>A grapefruit containing one 2-day-old Caribbean fruit fly larva</u>	
ID: INFESTED GF 2610 30-40 FIL 20DB	1.2-1.7 SOUND HEARD 1-12-87
FILE NAME: A:D701	DATE: 01-22-87
GATE TIME : 10000 msec	TRIGGER LEVEL: .15 Volts
STATISTICS FOR DATA BETWEEN 5.15-49966.00 sec	
MEAN SPIKE/SEC: 704.6153	STANDARD DEVIATION: 526.6220 sec
TOTAL SPIKES: 3.42SE + 006	TOTAL COLLECTION TIME: 49966 sec
MAXIMUM PERIOD WITHOUT NOISE: 0.00 sec	
PERIOD OCCURRED AT INTERVAL: 0.00-0.00	
MAXIMUM PERIOD WITH NOISE: 49960.85 sec	
PERIOD OCCURRED AT INTERVAL: 5.15-49966.00	
NUMBER OF INTERVALS WITHOUT NOISE: 0	
NUMBER OF INTERVALS WITH NOISE: 4861	

noninfested fruit (9a) are insignificant compared to the number of intervals showing feeding activity in the grapefruit (9b) with only one 2-day-old Caribbean fruit fly larva presented graphically in Figure 9.

The examples shown here are typical of the feeding sounds of the aribbean fruit fly larvae. It must be emphasized that care should be used to determine the correct threshold level of the system before each test, because proper selection depends upon the gain of the amplifier, the bandpass filter setting and ambient noise level. If the threshold level was set too low, then there is a good chance of detecting false signals, but if the threshold level was set too high, some of the larval sounds may be missed.

When using the A/D converter in measuring the noise or signal voltage it is recommended to use a sampling rate as high as possible, the computer system and software allows a simple rate of up to 500 samples per second. In addition, the noise level calibration must be repeated whenever the detection environment changes.

CONCLUSIONS

In summary, an acoustical sensing system was developed that is capable of detecting the feeding and moving sounds made by early instar larvae in fruit, nuts and grain. The acoustical couplers were modified so they could be adjusted to accept different size and shape fruit. Electronic amplification, filtering, computer monitoring and data analysis

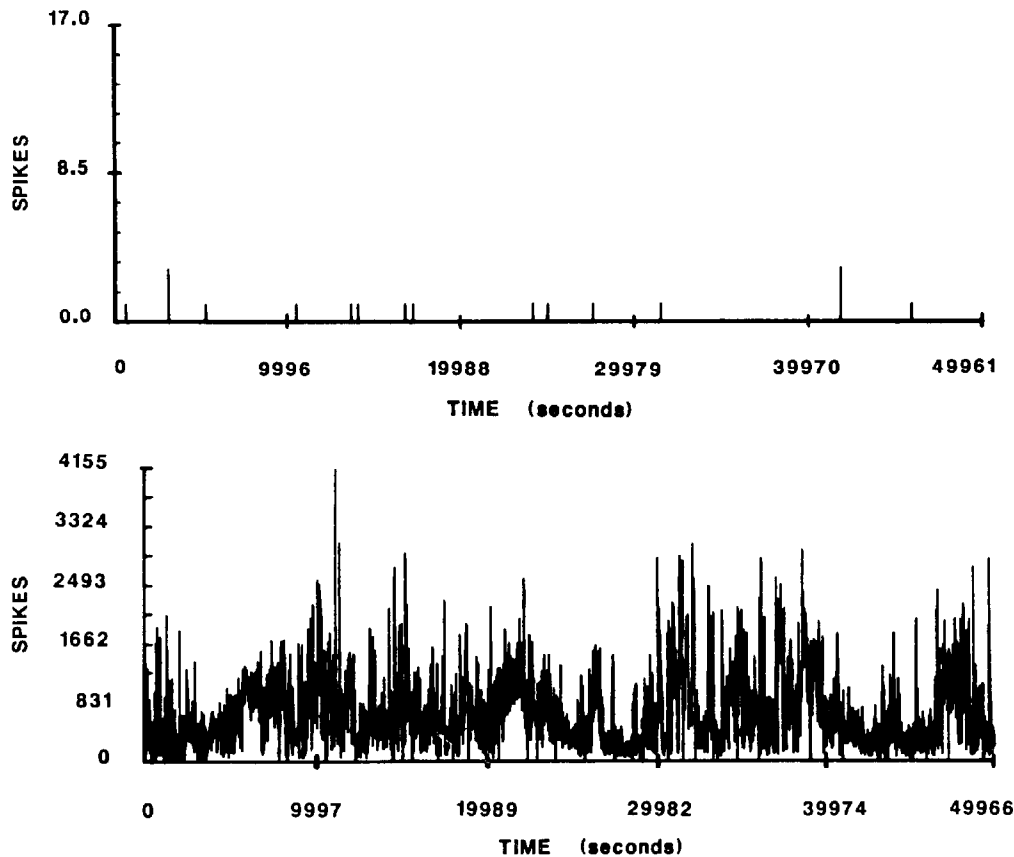


Fig. 9. (a) An example of data taken with the universal counter of a noninfested grapefruit. The vertical lines are noise spikes above background noise, and (b) a grapefruit with one 2-day-old Caribbean fruit fly larvae.

software were developed to process the acoustical signals generated by the feeding sounds of insect larvae. The system has potential in at least two areas. The first is to obtain basic biological information on larval behavioral activities such as time and frequency of feeding, and also the growth rate of insect larvae inside various commodities. Methods to obtain these data were unavailable previously. This information must be known before the detection system can be used effectively in most inspection programs. Therefore, research is being conducted at several USDA research installations across the country to obtain these data and to determine which agricultural commodities and which insects are suited to this method of detection. The system also has great potential for reducing the labor-intensive and destructive task of manually cutting and visually inspecting fruit, which is currently used by USDA commodity inspectors. Research is also under way to determine probability of error in detecting one insect larva in a single grapefruit or orange using a listening time of only 10 sec per fruit.

REFERENCES CITED

- ADAMS R. E., J. E. WOLFE, M. MILNER, AND J. A. SHELLENBERGER. 1953. Aural detection of grain infested internally with insects. *Science* 115: 163-164.
- BAILEY, S. W., AND J. B. MCCABE. 1965. The detection of immature stages of insects within grains of wheat. *J. Stored Prod. Res.* 1: 201-202.

- BARAK, A. V., AND P. K. HARIEM. 1982. Trap detection of stored grain insects in farm-stored, shelled corn. *J Econ. Entomol.* 75: 108-111.
- BARAK, A. V., AND W. E. BURKHOLDER. 1985. A versatile and effective trap for detecting and monitoring stored-product Coleoptera. *Agric. Ecosys. and Environ.* 12: 207-218.
- BRAIN, C. K. 1924. Preliminary note on the adaptation of certain radio principles to insect investigation work. *Ann. Univ. Stellenbosch.* 2: 45-47.
- BRUCE, W. A., M. W. STREET, R. C. SEMPER, AND DAVID FALK. 1982. Detection of hidden insect infestations in wheat by infrared carbon dioxide gas analyses. USDA, ARS Advances in Agricultural Technology. ATT-S.-261: 1-g.
- FESUS, I. 1972. Detection and estimation of internal pest infestation in seeds by application of X-ray techniques. *Bull. Orgn. Eur. Meditirr. Prat. Pl.* 3: 65-76.
- MILNER, M., M. R. LEE, AND R. KATZ. 1950. Application of X-ray technique to the detection of internal insect infestation of grain. *J. Econ. Entomol.* 43: 933-935.
- STREET, M. W. 1971. A method for monitoring of in-kernel insect activity. *J. Georgia Entomol. Soc.* 6: 72-75.
- VICK, K. W., J. C. WEBB, B. A. WEAVER, AND C. LITZKOW. 1988. Sound-detection of stored-product insects that feed inside kernels of grain. *J. Econ Entomol.* 81: 1489-1493.
- WEBB, J. C., AND P. J. LANDOLT. 1984. Detecting insect larvae in fruit by vibrations produced. *J. Environ. Sci. & Health A19:* 367-375.
- WEBB, J. C. C. A. LITZKOW, AND D. C. SLAUGHTER. 1988. A Computerized Acoustical Larval Detection System. *Applied Engr. in Agric.* 4: 268-274.

Swivel Sockets

