INCREASE IN ACOUSTIC DETECTABILITY OF *PLODIA INTERPUNCTELLA* (LEPIDOPTERA: PYRALIDAE) LARVAE IN STORED PRODUCTS AFTER ELECTRICAL STIMULATION

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Modern computer and electronic technology has enabled the development of powerful acoustic methods for detection of hidden insect infestations in stored products (e.g., Shade et al. 1990, Shuman et al. 1993, 1997; Pittendrigh et al. 1997), wood (Scheffrahn et al. 1993, 1997), and soil (Mankin et al. 2001). Despite their power, the utility of acoustic tools for detecting insect infestations is limited under conditions of reduced activity such as low temperatures (Maier et al. 1996), molting or pupation (Vick et al. 1988, Pittendrigh et al. 1997), and after disturbance, depending on the species (Mankin et al. 1999, Miyatake 2001). Even under optimal temperatures, stored product insect larvae are quiet in 10-30% of 5-minute monitoring periods during growth phases (Vick et al. 1988), and quiescent periods can extend from 3-5 minutes after a disturbance (Miyatake 2001) to >8 h during molting phases (Pittendrigh et al. 1997). The reliability of acoustic surveillance would be improved if effective excitatory stimuli could be applied to inactive insects just before monitoring, particularly if the excitation increased the loudness of the sounds produced.

One potential use of acoustic technology that remains largely undeveloped is nondestructive surveying for insect infestations in packaged goods. *Plodia interpunctella* (Hübner) is a major pest of packaged goods in warehouses and retail stores (Arbogast et al. 2000) and early detection of larvae in packages could reduce high-value product losses and eliminate the buildup of populations. However, the weak sounds produced by 3rd-4th instar (5-15 mg) *P. interpunctella* larvae are difficult to detect through multiple layers of protective packaging. The detectability of these larvae could be improved if artificial stimuli were available to increase the rate and loudness of larval sounds during acoustic monitoring periods.

Treatments used previously to stimulate insect activity and increase their visual or acoustic detectability include heat (Shade et al. 1990, Hagstrum and Flinn 1993, Au 1997, Mankin et al. 1999), disturbance, usually of highly mobile species (Minnich 1936, Masters 1979, Roces and Manrique 1996), and electrical stimulation (Vander Meer et al. 1999). Heat has been used in Berlese funnels and related devices (e.g., Edwards 1991) but its activating effects are indirect and may take hours to develop. Electrical stimulation is used to control activity of farm animals but rarely of insects.

In a high-school student research project conducted by Brett Miller, 4th-instar *P. interpunctella* larvae were monitored to determine if commercially available electrical stimulation tools increased larval activity levels and acoustic detectability. Individual larvae were placed on dog food biscuits 1-2 days before testing and kept in separate containers. Stimulation was applied by placing an infested biscuit between the two prongs of an electric prod (Model Sabre Six, Hot-Shot Products, Inc., Savage, MN) (9 kV, 19 mA) and then briefly toggling the actuator. The prongs were 3.5 cm apart and the biscuits were ~2.5 cm wide. For control tests, the prod was inactivated by removing its battery pack.

Acoustic monitoring was performed by placing each infested biscuit on a 4.5-cm-diameter piezoelectric disk (MuRata Erie model PKM28-2AO, Smyrna, GA) (see Mankin et al. 2000). Sounds generated by moving and feeding larvae were monitored for 180-s periods at 24-26°C in an acoustically shielded chamber (Mankin et al. 1996). On 3 different weeks (blocks), recordings were obtained from larvae in 20 biscuits treated with an active prod and 20 biscuits treated with an inactive prod (120 larvae altogether). Monitoring was done immediately before the biscuit was placed between the electrodes, immediately after the activation treatment, and 10 minutes later to consider the duration of stimulatory effects.

The acoustic signal collection and analysis procedures were similar to those described in Mankin et al. (2001). A noise threshold was set at a level that eliminated low-level, extraneous sounds. At this threshold, no sound pulses were registered in control tests with uninfested biscuits, with or without electrical stimulation. A spectral profile of *P. interpunctella* sounds was calculated from averages of 20-50 pulses collected during a noise-free period of recording, and signals above the noise threshold that matched this profile were counted as larval sound pulses using a custom-written computer program, DAVIS (Mankin 1994). Because this study was conducted in an acoustically shielded anechoic chamber, the recordings contained minimal noise and almost all of the sound pulses matched the larval profile. Background noise is prevalent in field studies but insect profiles can be used to screen out all but a small percentage of background noises (Mankin et al. 2000, 2001).

A treatment that stimulates increased activity is of most benefit for acoustic detectability when

TABLE 1. COMPARISON OF MEAN ACTIVITY RATES OF *P. INTERPUNCTELLA* LARVAE BEFORE, IMMEDIATELY AFTER, AND 10 MINUTES AFTER TREATMENTS WITH ACTUATED OR INACTIVE ELECTRICAL PROD.

Measurement period	Mean activity rate (pulses/s) ± standard error in treatments with[1]		
	Inactive prod (N = 60)	Actuated prod (N = 60)	Actuated prod (Low initial activity)[2] (N = 23)
Before treatment	2.33 ± 0.37 a	2.03 ± 0.43 a	0.07 ± 0.02 a
Immediately after	2.19 ± 0.28 a	4.09 ± 0.76 b	1.17 ± 0.36 b
10 minutes after	2.60 ± 0.41 a	1.70 ± 0.33 a	0.15 ± 0.05 a

[1]Mean activity rates followed by the same letter in the same column are not significantly different using the Waller-Duncan K-Ratio test (K-Ratio = 100, $\alpha = 0.05$) (SAS Institute 1988).
[2]Biscuits in which the larval activity rate before treatment was below threshold of 0.33 pulses/s (Mankin et al. 2001) for *high* likelihood of infestation.

the insects surveyed are inactive before treatment. Previous acoustic surveys of hidden infestations have found that the likelihood of correctly identifying an infested sample increases in proportion to the rate of insect sound pulses (Mankin et al. 2001, Brandhorst-Hubbard et al. 2001). In Mankin et al. (2001), for example, the likelihood of infestation was rated *high* if the insect sound activity rate in a sample exceeded 0.33 pulses/s and *low* if the rate was below a background noise level of 0.033/s. When the field-test samples were verified by visual inspection, insects were confirmed in all samples rated *high* and 36% of samples rated *low*. Detection of insects in the *low*-rated samples would have benefited from a treatment that increased activity levels above 0.33 pulses/s. Consequently, a question of interest in this study was whether a stimulatory treatment could increase the activity levels of a quiet sample sufficiently to change its rating from *low* to *high* likelihood of infestation.

Analysis of variance (SAS Proc ANOVA, SAS Institute 1988) of the rates of sounds produced by *P. interpunctella* larvae before, immediately after, and 10 minutes after electrical stimulation (Table 1) revealed significant increases in activity rates immediately after treatment ($\alpha = 0.05$). The significance of the increased activity was even greater for the 21 electrical stimulation tests where the initial activity was below 0.33 pulses/s ($F = 8.59$; df = 2, 60; $P = 0.0005$; minimum significant difference = 0.56 pulses/s) than for the complete set of stimulation tests ($F = 5.78$; df = 2, 177; $P = 0.0037$; minimum significant difference = 1.5 pulses/s). In these tests, originally below the threshold for *high* likelihood of infestation, the activity rate increased above threshold to 1.17 pulses/s. In all cases, the activity rates returned to pre-treatment levels within 10 minutes. Equivalent treatment without electrical activation produced no significant differences (F = 0.33; df = 2, 177; P = 0.69). Anticipated modifications of this technique that extend its scale to enable larger sample sizes and packaging layers have considerable potential for increasing the reliability of acoustic surveys for *P. interpunctella* in high-value packaged goods.

SUMMARY

P. interpunctella larvae are important pests of stored products but they are difficult to detect by nondestructive acoustic methods through several layers of packaging. In this study, electrical stimulation was considered as a potential method for increasing the activity levels of *P. interpunctella* larvae, thereby increasing their acoustic detectability. Individual dog biscuits containing 4th-instar larvae were treated with a large-animal electric prod and acoustically monitored. The level of activity increased temporarily by a factor >2 and the effect was greater for larvae that were initially least active. The success of these tests indicates that electrical stimulation has potential as a method of improving the reliability of nondestructive acoustic surveys of stored product insects in packaged goods.

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