

## Thermal Treatments to Increase Acoustic Detectability of *Sitophilus oryzae* (Coleoptera: Curculionidae) in Stored Grain

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**ABSTRACT** Hidden infestations of stored-product insect larvae are detected most rapidly by acoustic techniques when the larvae are highly active. Larval activity is periodic, however, and it tends to decrease after the larvae are disturbed or cooled. Because of the practical need for rapid inspection of grain at commercial elevators, several heat treatments were tested as potential methods of increasing larval activity and improving the speed and reliability of acoustic detection under adverse conditions. Samples of grain infested with 4th instars of *Sitophilus oryzae* (L.) were exposed to different radiant and convective heat treatments after they had been conditioned at 11°C, 17°C, or room temperature for 12–24 h. Relative activity levels were evaluated over periods of 0–12 h based on the mean levels in a 15-min interval, 2 h after the beginning of a trial. In comparisons among treatments with precooled larvae, relative activity levels 5–10 min after brief heat pulses were 2–30 times higher than activity levels in precooled controls exposed only to ambient temperatures (25°C). After 15–25 min, the relative activity levels of these heated larvae remained 2–5 times higher than those of the ambient controls. Brief movement disturbances inhibited activity for ≈20 min at any temperature. These results suggest that, in general, larval detectability is enhanced if cool grain samples are warmed and all samples are left undisturbed for 15–20 min before inspection.

**KEY WORDS** *Sitophilus oryzae*, acoustic detection, stored products, grain

THE RELIABILITY OF measurements by automated systems that acoustically monitor stored product insect infestations (e.g., Shuman et al. 1993, 1997; Fleurat-Lessard et al. 1994; Hagstrum et al. 1996; Au 1997) can be affected adversely by natural periodicity in insect activity and quiescence induced by unfavorable temperatures or mechanical disturbance. This problem was considered by Vick et al. (1988), who listened to *Sitophilus oryzae* (L.) larvae developing in kernels of grain. Sounds were detected in 70–90% of random 5-min listening intervals beginning 13 d after oviposition and ending at pupation. Shuman et al. (1993) detected 4th-instar *S. oryzae* in 70% of 9-min tests with an automated detection system designed to count the number of larvae in a 1-kg grain sample. They hypothesized that the undetected larvae were not producing sounds at a rate sufficient for acoustic detection at densities of 1–3 larvae per kilogram. Extending the trials to 30 min, Weaver et al. (1996, 1997) detected *S. oryzae* larvae in 86% of trials with a similar acoustic system. The need to decrease listening intervals and increase the proportion of detected insects led to a search for treatments that would increase larval activity during a detection trial.

Temperature is an easily manipulatable factor that has the potential to increase insect activity and optimize acoustic detectability of adults and larvae. Hagstrum and Flinn (1993) found that adults of several stored-product insect species had increased rates of movement and sound production as the temperature increased from 17.5 to 30°C. Grain inspectors make use

of this effect by placing samples under an infrared lamp. The heat and disturbance elicit evasive movement in adults, making them more visible (Mankin et al. 1996). Similar lamps are frequently used in Berlese funnels and related devices (Edwards 1991).

The effects of temperature on the growth and development of stored-product insect larvae have been well documented (Birch 1945, Stinner et al. 1974, Flinn et al. 1997), and the existence of such effects supports the potential of thermal treatment as a method to increase larval activity. Direct effects of temperature on feeding rates (Matsuki et al. 1994) and sound production (Shade et al. 1990, Au 1997) also have been reported. In the latter study, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) larvae were more likely to be acoustically detected if infested cotton bolls were warmed to 38°C before sensing.

The ideal temperature for grain storage is lower than the 25–28°C temperatures typical of acoustic detection studies in the laboratory, and commonly used storage practices such as aeration often reduce temperatures below levels favorable for growth of insects and mold (Burgess and Burrell 1964, Loschiavo 1985, Noyes et al. 1995, Maier et al. 1996). Thermal treatments could be helpful for increasing the acoustic detectability of larvae in grain samples from cool storage environments.

Another potentially confounding difference between the environment of grain samples in an acoustic detection laboratory and samples in a commercial

grain elevator is the amount of handling immediately before a detection trial. Some insect larvae respond to mechanical disturbance by stridulating or moving evasively (Minnich 1936, Masters 1979, Roces and Manrique 1996), but other insects become quiescent (Moore and Williams 1990). The additional sound generated by evasive behavior or stridulation could enhance acoustic detectability. However, the behavioral responses of internally feeding stored-product insects to disturbance have not been investigated formally. Preliminary studies with 1–10 *S. oryzae* larvae in 1-kg samples suggested that larvae may become briefly quiescent after the incidental disturbance of being placed in an acoustic detection chamber.

The purpose of this study was to identify and evaluate rapid conditioning treatments to increase the activity of larvae in grain samples under inspection at a commercial grain elevator. The focus was on the effects of mechanical disturbance incidental to the collection and handling of samples being inspected, and on the potential benefits of heat treatments similar to those already in common use for detection of adult insects.

### Materials and Methods

**Insect Rearing and Handling.** Tests were done on 300-ml (260 mg) samples of soft red winter wheat, *Triticum aestivum* L., infested with 4th instars of *S. oryzae* (mean age, 22 d; range, 18–28 d). The samples were obtained from a laboratory colony cultured at  $26 \pm 1^\circ\text{C}$  and  $54 \pm 5\%$  RH with a photoperiod of 14:10 (L:D) h, near the optimal conditions of  $28^\circ\text{C}$  and 50% RH found by Birch (1945). Counts of the number of adults emerging from each sample,  $\approx 500$ , indicated that the density was  $\approx 2,000$  larvae per kilogram of wheat.

**Sample Treatments.** To obtain different combinations of treatments, samples were first cooled to 17 or  $11^\circ\text{C}$  in an incubator for  $>24$  h, or kept under ambient conditions (*Ambnt*) at room temperature,  $ITrmT = 25 \pm 1^\circ\text{C}$ . All samples were then exposed to 1 of 3 thermal treatments (see below)—radiant heat (*Infrd*), convective heat (*Cnvct*), or ambient conditions (*Ambnt*). The samples were mechanically disturbed, either by stirring (*Stir*) or fanning (*Fan*), as described below. Immediately after the mechanical disturbance, samples were placed in an acoustic measurement chamber and the temperatures and rates of sound production were monitored for 2–12 h (see *Acoustic System*). All of the samples that had been preconditioned under ambient conditions also were monitored in the acoustic chamber for a 2-h period immediately before treatment to enable comparisons of the activity just before treatment with the activity 2 h after treatment. To refer to different treatments in this report, we list the treatment type followed by the initial treatment temperature (*ITrmT*), specify the initial temperature at the start of the trial (*ITrIT*) if the treatment results in a temperature change, and then note the type of disturbance in parentheses. Under this nomenclature, a convective heat treatment

changing the temperature of a precooled sample from 11 to  $31^\circ\text{C}$  is labeled *Cnvct11->31 (Fan)*, and a room temperature, stirred control is labeled *Ambnt25 (Stir)*.

For radiant heat treatments, a grain sample was placed under a 125-W infrared lamp in a moisture balance (model 26680; CSC Scientific, Fairfax, VA). The moisture balance housing shielded the grain from extraneous temperature fluctuations. A thermistor probe (RD-TEMP model 3366; Omega, Stamford, CT), set near the center of the grain mass ( $\approx 2$  cm thick), was connected to a data logger that recorded the temperature at 12-s intervals. The infrared lamps were similar to those used in Grain Inspection, Stockyard, and Packers Administration offices to detect and increase the activity of adult insects in a grain sample (Mankin et al. 1996, 1997a). The mean temperature at the beginning of the trial was 29, 31, and  $34^\circ\text{C}$ , respectively, for heating durations of 60, 90, and 120 s. The 3 infrared heat treatments, *Infrd25->29 (Stir)*, *Infrd25->31 (Stir)*, and *Infrd25->34 (Stir)* were compared with a control, *Ambnt25 (Stir)*, exposed only to ambient temperatures.

After preliminary experiments, we decided not to do radiant heat tests with cooled grain because of concerns that exposures long enough to warm all the grain to room temperature would overheat larvae in kernels at the grain surface,  $\approx 1$  cm below the lamp. Although a convective heat system is more complicated and generates more mechanical disturbance than an infrared lamp, the heat is distributed more uniformly through the grain sample.

For treatments with convective heat, the bottom of a 2-liter plastic drink bottle was replaced with wire mesh screen. After grain was loaded through a funnel, the neck was covered with screen and the bottle was inverted on a ring stand. The neck was connected to compressed air at  $2.4 \times 10^5$  Pa (35 psi), and a heat fan, set on low, blew air at  $60^\circ\text{C}$  into the bottle for 40 s while the grain was agitated. The heating element was turned off for tests with ambient air convection. Fanning was used in 3 different treatments as follows: (1) an ambient control, *Ambnt25 (Fan)*; (2) precooled grain at  $11^\circ\text{C}$  exposed to fanning at ambient conditions that increased *ITrIT* to  $17^\circ\text{C}$ , *Ambnt11->17 (Fan)*; and (3) precooled grain at  $11^\circ\text{C}$  exposed to heated fanning that increased *ITrIT* to  $31^\circ\text{C}$ , *Cnvct11->31 (Fan)*.

Because grain is a poor heat conductor, all samples not exposed to convection were stirred with a spatula to equilibrate the temperature before they were placed in the acoustic detection chamber to start a trial. This treatment also served to standardize the amount of handling immediately before the start of a trial. The stirring procedure was to turn the spatula clockwise through the sample 3 times, then counterclockwise 3 times, repeating 5 times. The temperature in the center of the samples was monitored continuously with the thermistor probe after the samples were placed in the acoustic detection chamber. There were 2 stirred, precooled treatments, *Ambnt11 (Stir)* and *Ambnt17 (Stir)*, and an ambient control treatment, *Ambnt25 (Stir)*.

**Acoustic System.** The acoustic detection chamber was a polyvinyl chloride cylinder, 7.62 cm in diameter by 6.67 cm long. A hole was cut for a piezoelectric microphone (Shuman et al. 1993) midway between the ends. During the acoustic detection trials, the chamber was placed inside a lead box inside an anechoic chamber (Mankin et al. 1996, 1997b). Signals from the microphone were amplified 80 dB (model 2610; Brüel and Kjær [B&K], Nærum, Denmark) and bandpass filtered between 3 and 6 kHz (model 3100; Krohn-Hite, Avon, MA). A programmable counter (model 5316A; Hewlett-Packard, Palo Alto, CA) counted the number of occurrences where signal levels exceeded a 0.15-V threshold set to eliminate effects of background noise (Webb et al. 1988).

The counter was controlled by a Quick-Basic program that saved the number of counts every 12 s (Webb et al. 1988). The thermistor system was controlled with Omega Boxcar software on a separate computer. Counter time and thermistor time were synchronized by alignment of the internal personal computer clocks. Trials with precooled insects were run for 12 h after removal from the incubator. Trials at ambient conditions were run as 2 separate tests. The samples were first placed in the anechoic chamber and monitored for 2 h. Then the disturbance or thermal treatments were performed, and the samples were monitored for an additional 2 h. Only the 2nd test was included as a trial, but the 1st and 2nd tests were compared for quality control. The beginning of a trial,  $t_T = 0$ , was specified as the time when a stirred or fanned sample was initially placed in the anechoic sound detection chamber and the count recording program was started.

**Statistical Analysis.** Activity counts and temperatures were analyzed using SAS (SAS Institute 1988). Regressions of mean activity rate on time were analyzed with PROC NLIN (nonlinear) and PROC GLM (linear analysis). Because the mean activity rates varied significantly among samples and among trials (because of differences in insect numbers, ages, and natural periodicity), the sample *count rates* (counts per minute) were normalized by dividing by the mean rate during  $105 \text{ min} < t_T < 120 \text{ min}$  after treatment, hereafter termed *finalrate*. There were  $\approx 500$  larvae per sample (replicate), but an unknown number of larvae were within range of the acoustic detector in any given trial. Random variation in the insect density near the detector undoubtedly contributed to the sample activity rate variance, as it also would in grain elevator samples. This trial-to-trial variation was eliminated in comparisons of *relative activity* = *count rate* / *finalrate* among treatments.

## Results

All of the treatments caused temporary reductions in *S. oryzae* larval activity (Fig. 1) and some had effects that continued through 12-h trials. The pattern of responses depended on the initial treatment temperature, *ITrmtT*. With larvae initially at 25°C room temperature, the activity rates recovered quickly and sta-

bilized within 45–60 min. This pattern occurred for disturbance-alone treatments [*Ambnt25 (Stir)* and *Ambnt25 (Fan)* in Fig. 1A] and for infrared heat treatments (Fig. 1B). With precooled larvae, however, activity rates failed to stabilize within 2 h for ambient [*Ambnt17 (Stir)* and *Ambnt11 (Stir)*, Fig. 1A] or convectively heated (Fig. 1C) treatments. The 2 different response patterns are considered in more detail further below.

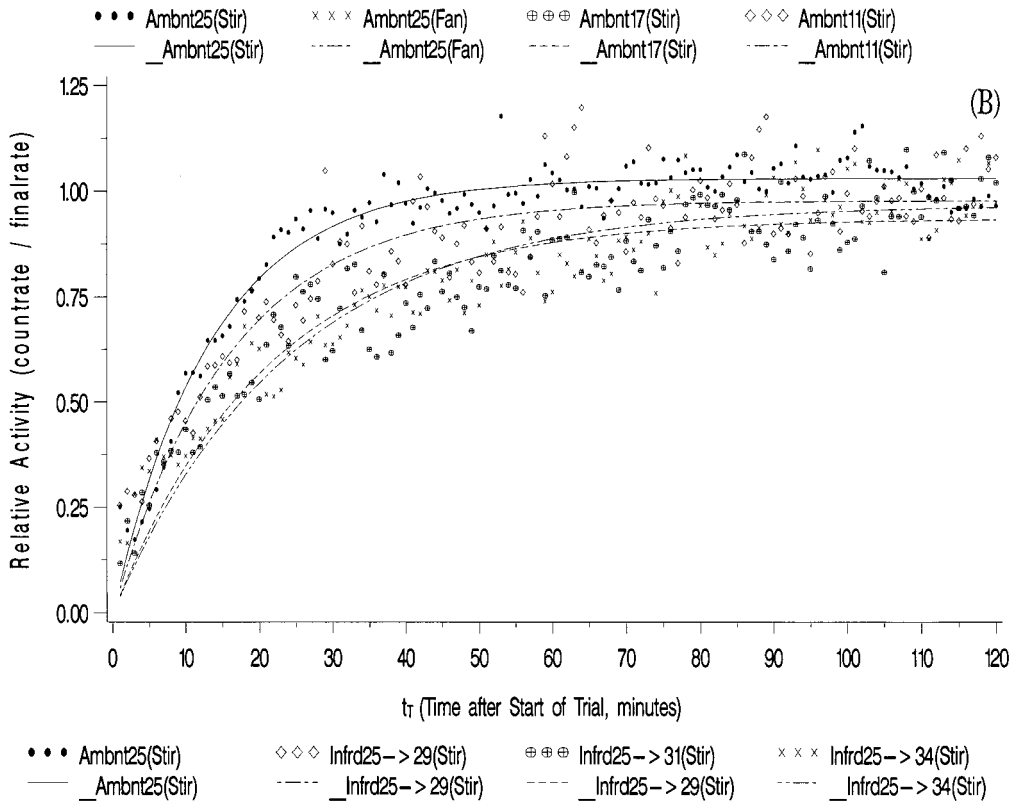
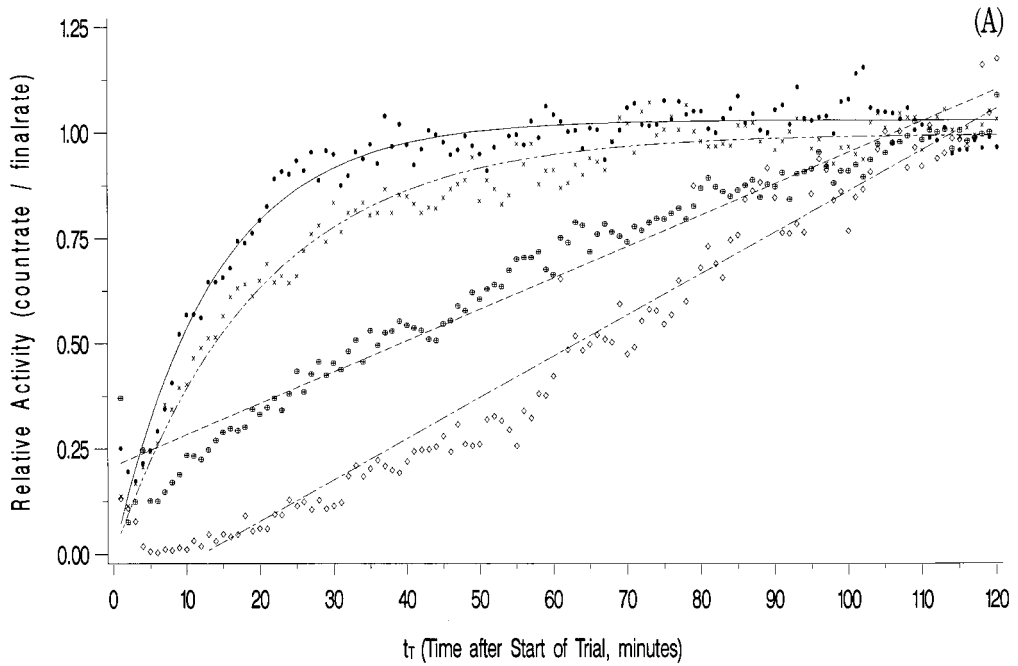
The short- and long-term effects of heat treatments should be considered within the context of the temperature changes during the trials (Fig. 2). The mean temperature in the center of the grain sample increased rapidly after a radiant heat treatment and then slowly approached room temperature. The 60-s infrared pulse elevated the temperature 4°C, and the 90- and 120-s pulses elevated it 6 and 9°C, respectively. The temperatures decreased rapidly, but remained  $>0.5^\circ\text{C}$  above room temperature for the duration of the 2-h trials. Unheated fanning increased the temperature of the 11°C grain by 6°C within 5 min, and heated fanning increased the temperature by 20°C. The temperature then remained above 25°C for  $>2$  h.

### Larval Activity Within 10 min After Treatment.

Because of the practical need for a rapid inspection process, the effects of different treatments on a period of *initial activity* (5–10 min after the beginning of the trial) were considered in detail (Table 1). The larvae in the 60-s infrared lamp treatment recovered more quickly from disturbance than those in other treatments, but there was little practical difference among levels of *initial activity* in the infrared lamp treatments and ambient control (38–42% of *finalrate* for the 1st 4 treatments in Table 1). Recovery from the fanning disturbance was slower at room temperature [*Ambnt25 (Fan)*] than recovery from stirring [*Ambnt25 (Stir)*], but larvae in precooled samples recovered most rapidly after heat fan treatment [*Cwct11->31 (Fan)*]. These results indicate that brief heat treatments can significantly increase the acoustic detectability of cooled insects within 5–10 min, and they have no negative impact on detectability of insects already at room temperature.

### Larval Activity Longer than 10 Minutes After Treatment.

In contrast to treatments at room temperature, precooled insects exposed to gradual warming or convective heat were delayed in reaching peak activity levels. After 0.5 h, the activity levels of samples precooled to 11°C [*Ambnt11 (Stir)* in Fig. 1A] were only 20% of the *finalrate* level at 2 h. The activity levels of samples precooled to 1°C were only 50% of *finalrate* [*Ambnt17 (Stir)* in Fig. 1A] after 0.5 h. Part of the delay in recovery of the activity in precooled, ambient treatments to peak levels was caused by the insulating properties of grain. The unheated samples had not yet reached room temperature after 2 h (Fig. 2A). Physiological processes also may have contributed to the observed pattern, however. The activity levels of convectively heated samples [*Cwct11->31 (Fan)*, Fig. 1C] recovered slowly even though the samples remained above room temperature (Fig. 2C).



Figs. 1A and B.

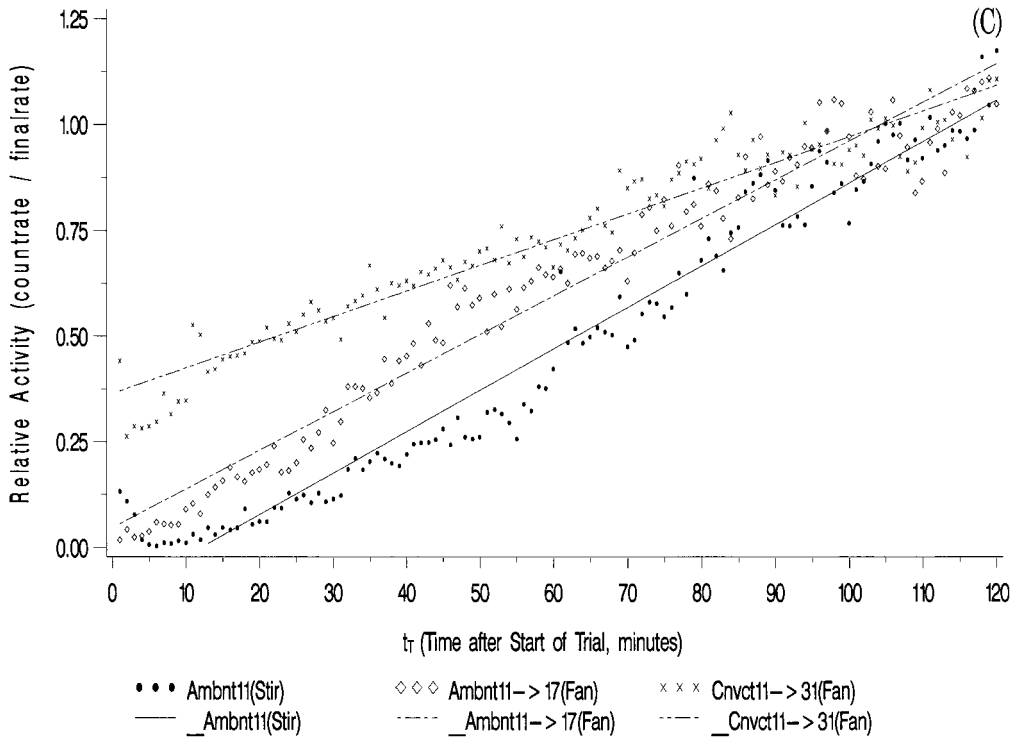


Fig. 1. Relative activity of 4th instars of *S. oryzae* after heat treatments and handling. Terms:  $t_T$  (time after start of trial), min; *count rate* (rate of sounds counted at time,  $t_T$ ), counts per minute; *final rate* (mean *count rate* during [105 min <  $t_T$  < 120 min]), counts per minute; *relative activity* (*count rate*/*final rate*), dimensionless; *ITrIT* (temperature at  $t_T=0$ ), °C. (A) Recovery of activity after fanning or stirring at different initial temperatures. (B) Recovery after infrared heating to different temperatures. (C) Recovery of precooled samples after fanning.

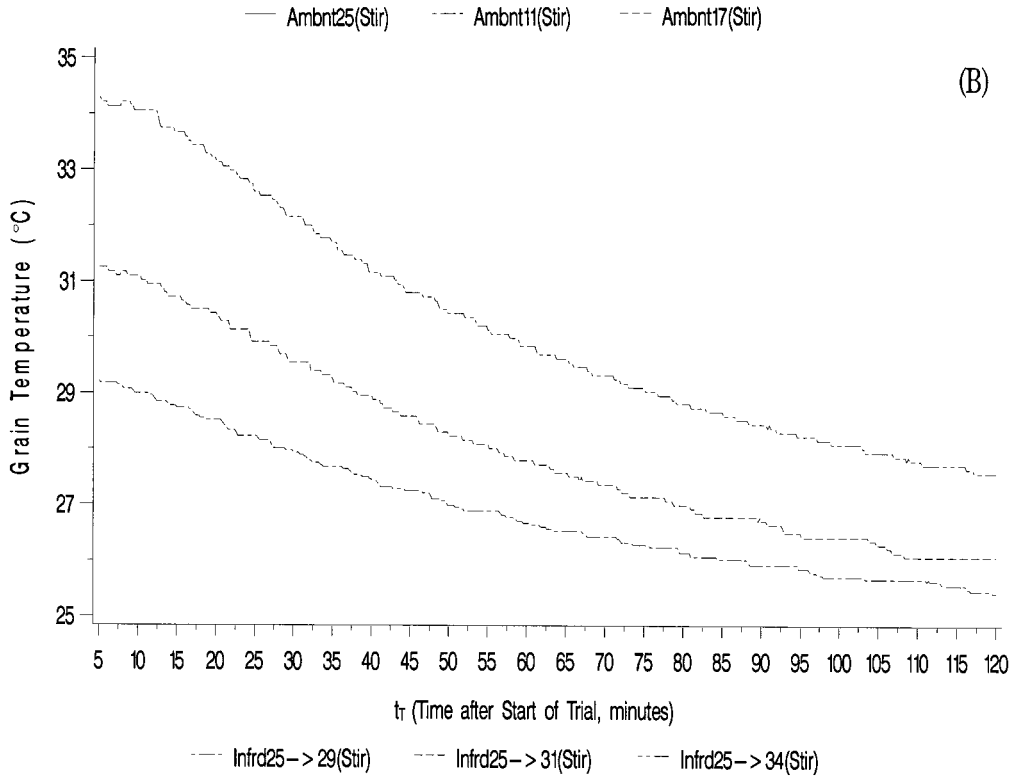
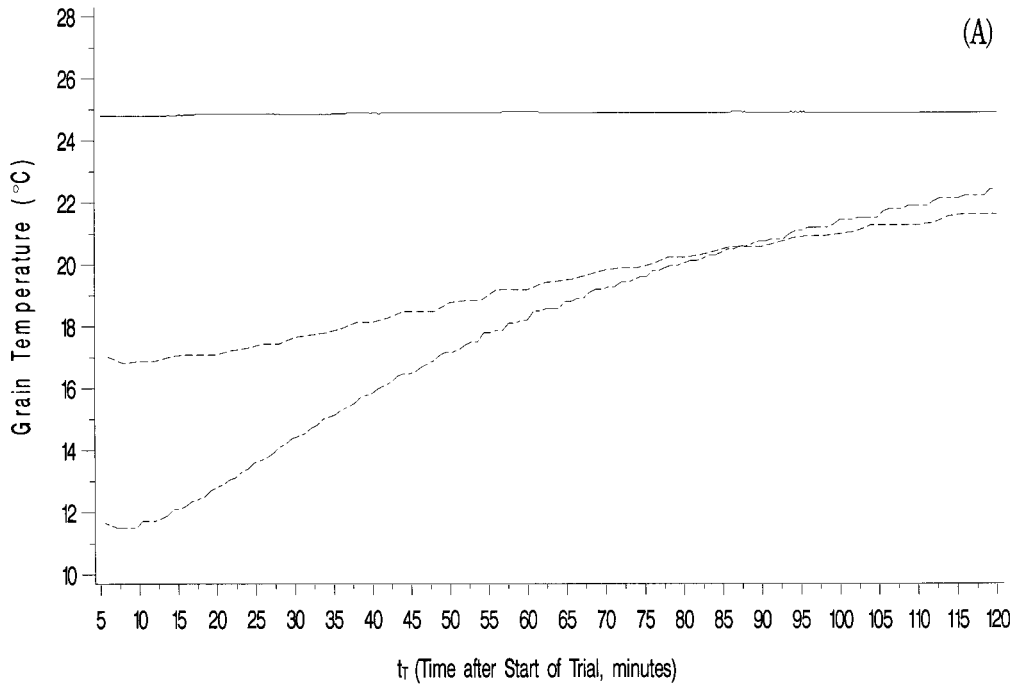
Heating did not strongly affect samples initially at room temperature (Fig. 1B), but increased the *relative activity* of larvae in samples precooled to 11°C (Fig. 1C). Within 0.1 h after convective heating to 31°C, activity levels recovered to 30% of *final rate* [*Cnvct11->31 (Fan)* in Fig. 2C], and within 0.5 h they recovered to 55% of *final rate*. This effect can be seen in the regression equations describing the pattern of relative activity for precooled samples (Table 2). There was a trend for the regression intercept to increase and the slope to decrease as the initial trial temperature (*ITrIT*) increased because the initial activity increased relative to the *final rate*.

Over a 10-h period beginning 2 h after the start of the trial, the activity of precooled insects increased by a factor of 1.1–1.9 over the *startrate* levels at 2 h (Fig. 3). The significance of the long-term effect is shown in Table 3, which lists the slopes and intercepts of regression equations for *relative activity* between 2 and 12 h after the start of the trial. If the treatments had no long-term effect, the intercepts would have the value  $A = 1$  and the slope,  $B = 0$ . However, the regression equations for the treatments in Table 3 all have significant slopes of  $15\text{--}100 \times 10^{-5} \text{ min}^{-1}$ , suggesting that the treatment effects continued for over much of the 12-h period after the beginning of the trials. Note also the large scatter of points in Fig. 3. This

reflects the high variability in sound production rates observed from individual *S. oryzae* larvae.

**Time Course of Disturbance Effects.** The pattern of response to disturbance by larvae initially at room temperature suggested that the recovery from disturbance was exponential and could be characterized by a half-time,  $t_H$ , the time to reach half of the maximal activity. Accordingly, regression equations were fit to exponential functions of the form *relative activity*( $t_p$ ) =  $A_M [1 - \exp(-t_p/t_R)]$ , where  $t_p = t_T/120$  min,  $A_M$  is the maximal activity,  $t_R$  is the recovery time, and *relative activity*( $t_p$ ) = (*count rate* at time,  $t_p$ )/*final rate*. Parameters estimated by PROC NLIN (SAS Institute 1988) are listed in Table 4. The half-time,  $t_H$ , the time to achieve half-maximal activity ( $0.5A_M$ ), is  $\ln(2) (t_R)$  (120 min). All the half-times for treatments initially at room temperature clustered in the range of 100–15 min, which suggests that the pattern of activity in Fig. 1B is a generalized response by *S. oryzae* larvae to brief disturbance.

Except for the 1st 5–10 min after a disturbance, activity rates of radiantly heated samples initially at room temperature increased less rapidly than those of unheated controls, as indicated by the lower magnitudes of  $1/t_R$  (i.e., higher magnitudes of recovery time) in Table 4. However, the radiant treatments had only minor effects on activity rates after 1 h (Table 5).



Figs. 2A and B.

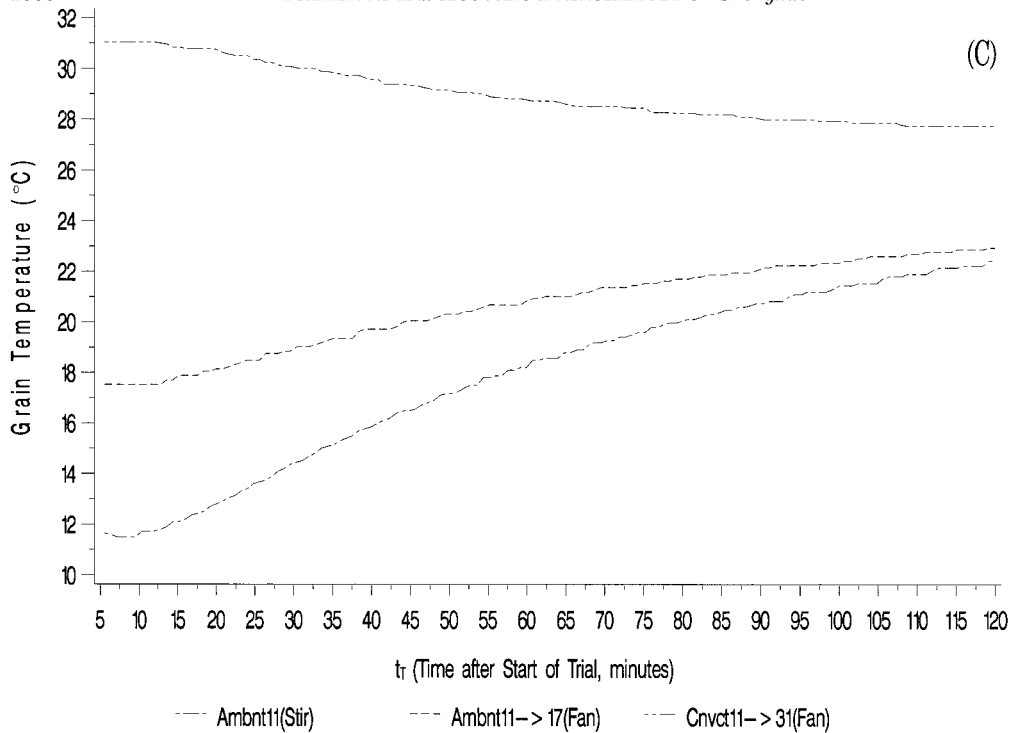


Fig. 2. Pattern of temperature changes in grain samples after different heat treatments and disturbance. Terms:  $t_T$  (time after start of trial), min;  $ITrIT$  (temperature at  $t_T = 0$ ), °C. (A) Stirred, unheated samples. (B) Stirred samples heated to 29, 31, or 34°C by infrared lamp. (C) Stirred or fanned samples precooled to 11°C.

The slope of the activity rate between 1 and 2 h was not significantly different from 0 in the *Ambnt25 (Stir)* and the *Infrd25->29 (Stir)* treatments. The slope was statistically different from 0 for the *Infrd25->31 (Stir)* and *Infrd25->34 (Stir)* heat treatments, but even the highest slope was only 0.29% per minute, a total of 20% during the 2nd h.

Table 1. Effects of disturbance, chilling, and heat treatments on initial activity of 4th instars of *S. oryzae*

Treatment	No. reps.	Initial activity, mean ± SE
<i>Infrd25-&gt;29 (Stir)</i>	40	0.424a ± 0.007
<i>Ambnt25 (Stir)</i>	120	0.392b ± 0.007
<i>Infrd25-&gt;34 (Stir)</i>	20	0.376b ± 0.005
<i>Infrd25-&gt;31 (Stir)</i>	20	0.376b ± 0.002
<i>Ambnt25 (Fan)</i>	36	0.339c ± 0.008
<i>Cnvt11-&gt;31 (Fan)</i>	40	0.331c ± 0.004
<i>Ambnt17 (Stir)</i>	116	0.159d ± 0.002
<i>Ambnt11-&gt;17 (Fan)</i>	20	0.056e ± 0.005
<i>Ambnt11 (Stir)</i>	24	0.010f ± 0.001

Mean values of initial activity followed by same letter are not significantly different by Waller-Duncan *K*-ratio *t*-test (*K*-ratio = 100,  $P < 0.05$ , residual ms error = 0.003).  $t_T$  (time after start of trial), min; *countrate* (rate of sounds counted at time,  $t_T$ ), counts per minute; *finalrate* (mean *countrate* during [105 min <  $t_T$  < 120 min]), counts per minute; *relative activity* (*countrate*/*finalrate*), dimensionless; *initial activity* (mean *relative activity* during [5 min <  $t_T$  < 10 min]), dimensionless; *ITrIT* (temperature at the start of treatment), °C.

Discussion

**Behavioral Basis of Modified Sound Production Rates.** Several short- and long-term effects of disturbance or heat have the potential to alter acoustically detectable movement of larvae inside grain kernels. The brief cessation of movement by *S. oryzae* larvae in all treatments except heated fanning [*Cnvt11->31 (Fan)* in Fig. 1C] is 1 of 2 typically observed responses to disturbance by larvae (Minnich 1936, Pearson 1988). The primary acoustic effect of the disturbances in these tests was a temporary reduction of sound

Table 2. Estimated parameters and correlations with initial trial temperature (*ITrIT*) in regressions of activity by 4th instars of *S. oryzae* on time

Treatment	A	B	<i>ITrIT</i> <sup>a</sup>	Residual ms error
<i>Ambnt11 (Stir)</i>	-0.12 ± 0.01	1.18 ± 0.02	11	0.03
<i>Ambnt11-&gt;17 (Fan)</i>	0.05 ± 0.01	1.10 ± 0.02	17	0.02
<i>Ambnt17 (Stir)</i>	0.21 ± 0.001	0.89 ± 0.02	17	0.07
<i>Cnvt11-&gt;31 (Fan)</i>	0.36 ± 0.01	0.73 ± 0.02	31	0.04

Equation parameters: *relative activity* =  $A + B t_p$ , with  $t_T$  (time after start of trial), min;  $t_p$  ( $t_T/120$  min), dimensionless; *countrate* (rate of sounds counted at time,  $t_T$ ), counts per minute; *finalrate* (mean *countrate* during [105 min <  $t_T$  < 120 min]), counts per minute; *relative activity* and A (*countrate*/*finalrate*), dimensionless; B,  $\text{min}^{-1}$ ; *ITrIT*, (temperature at  $t_T = 0$ ), °C; *ITrIT*, (temperature at start of treatment), °C.

<sup>a</sup> Note the correlation between increased temperature at the start of the trial (*ITrIT*), increase in intercept (A), and decrease in slope (B).

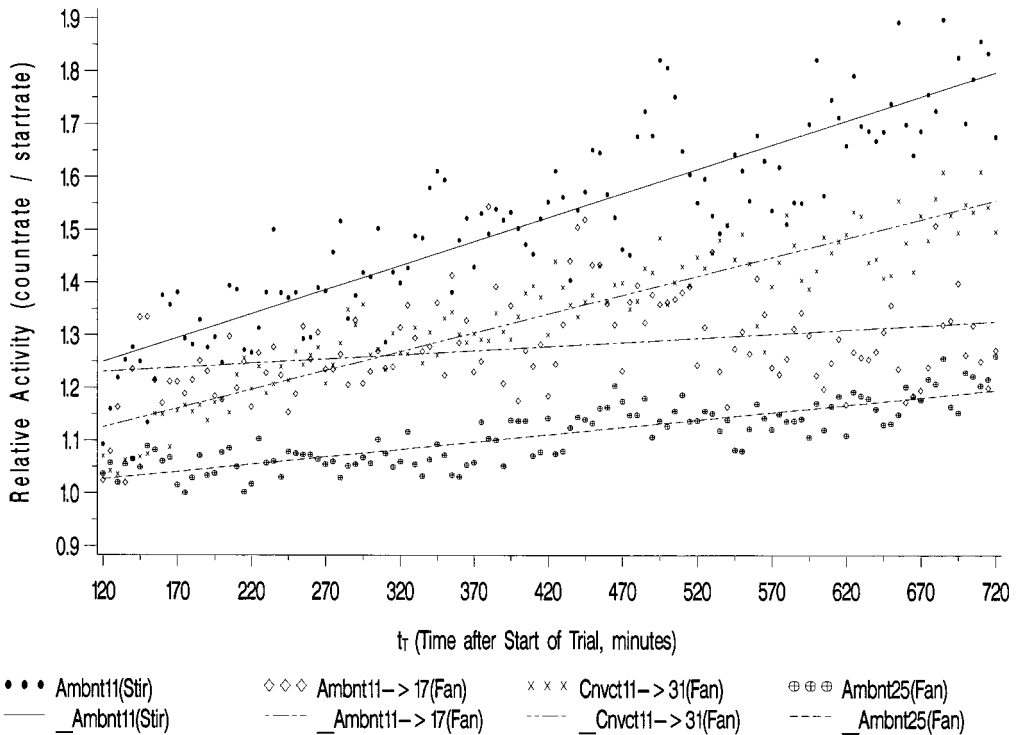


Fig. 3. Long-term (2-12 h) changes in activity levels of 4th-instar *S. oryzae*. Terms:  $t_T$  (time after start of trial), min; *countrate* (rate of sounds counted at time,  $t_T$ ), counts/min; *startrate* (mean *countrate* during [105 min <  $t_T$  < 120 min]), counts per minute; *relative activity* (*countrate*/*startrate*), dimensionless; *ITrIT* (temperature at  $t_T = 0$ ), °C.

rather than a behavior beneficial for acoustic detection. It remains to be determined whether larvae of other internally feeding stored-product insect pests respond to disturbance in the same way as *S. oryzae*.

The longer-term (2–12 h) effect of temperature on sound production by *S. oryzae* larvae (Table 3; Fig. 3) may be caused by the generally observed temperature dependence of larval growth rate and development rate (Cotton and Wilbur 1982, Honěk 1996). The feeding rate of *Nematus calais* Kirby larvae, for example, is ~6 times greater at 24 than at 6°C (Matsuki et al. 1994). Ryoo and Cho (1988) found that *S. oryzae* emerged in

55 d at 20°C but in only 28 d at 28°C. An increased rate of feeding at temperatures optimal for development is probably the main cause of the higher rate of sound production at 5–10 h than at 1 h for samples that were precooled to 11 or 17°C.

**Effects of Heat and Disturbance on Acoustic Detectability of Larvae.** Disturbance caused a greater decrease in *S. oryzae* larval activity for longer than the period of 1–3 min estimated from preliminary experiments (Shuman et al. 1993). Although the effect of disturbance is likely to be reduced by habituation, there may be some potential for the use of tumbling or other mechanical disturbances to reduce or eliminate feeding or oviposition activity, particularly in small-scale applications (Quentin et al. 1991).

Exposure to heat increased activity levels of larvae in precooled samples by a factor of 2–5 for short periods after treatment [*Ambnt11 (Stir)* compared with *Cnvc11->31 (Fan)* at 20 min in Fig. 1C], but it had no effect on larvae initially at optimal temperatures for development. The temperature in a grain bin is likely to be near optimal for insect development and sound production during summer (25–28°C; Cotton and Wilbur 1982, Loschiavo 1985, Noyes et al. 1995), but too low in winter and early spring (<15°C), particularly after aeration. Under such conditions, larvae are more likely to be detected after waiting periods of 0.5–1.0 h or after exposure to heat.

Table 3. Estimated parameters for test of hypothesis that specified treatments had no long-term effects on relative activity of 4th instars of *S. oryzae*

Treatment	A	B	No. reps.	Residual ms error
<i>Ambnt11 (Stir)</i>	1.14 ± 0.02	91.36 ± 4.86	6	0.007
<i>Ambnt11-&gt;17 (Fan)</i>	1.21 ± 0.03	15.85 ± 4.50	5	0.007
<i>Cnvc11-&gt;31 (Fan)</i>	1.04 ± 0.01	71.73 ± 2.57	10	0.002
<i>Ambnt25 (Fan)</i>	0.99 ± 0.01	28.07 ± 1.69	9	0.001

Regression equation: *relative activity* = A + B  $t_T$ , with  $t_T$  (time after start of trial), min; *countrate* (rate of sounds counted at time,  $t_T$ ), counts per minute; *finalrate* (mean *countrate* during [105 min <  $t_T$  < 120 min]), counts per minute; *relative activity* and A (*countrate*/*finalrate*), dimensionless; B,  $10^{-5} \text{ min}^{-1}$ ; *ITrIT* (temperature at the start of treatment), °C. Note: A = 1 and B = 0 are expected for null hypothesis of no long-term effect.



**Table 4.** Estimated parameters in equations describing effects of disturbance and heat treatments on relative activity of 4th instars of *S. oryzae* initially at room temperature ( $ITrmtT = 25$ )

Treatment	$A_M$	$1/t_R$	Half-time ( $\tau_H$ )	No. reps.	Residual ms error
<i>Ambnt25 (Stir)</i>	1.03 ± 0.005	8.80 ± 0.24	9.45	30	0.06
<i>Infrd25→29 (Stir)</i>	0.98 ± 0.01	7.44 ± 0.53	11.12	10	0.12
<i>Infrd25→31 (Stir)</i>	0.94 ± 0.01	5.59 ± 0.30	14.87	5	0.03
<i>Infrd25→34 (Stir)</i>	0.96 ± 0.01	4.96 ± 0.24	16.76	5	0.03
<i>Ambnt25 (Fan)</i>	1.00 ± 0.006	6.02 ± 0.17	13.82	9	0.02

Regression equation:  $relative\ activity = A_M (1 - \exp(-t_p/t_R))$ ; with  $t_T$  (time after start of trial), min;  $countrate$  (rate of sounds counted at time,  $t_T$ ), counts per minute;  $finalrate$  (mean  $countrate$  during [105 min <  $t_T$  < 120 min]), counts per minute;  $relative\ activity$  ( $countrate/finalrate$ ), dimensionless;  $t_p = t_T/120$  min, dimensionless;  $A_M$  (maximum relative activity level), dimensionless;  $t_R$  (normalized recovery time), dimensionless;  $\tau_H$  (time for recovery to 50% of maximal relative activity), min.

The range of half-times in Table 4, and comparisons of the activity rate patterns in Fig. 1, suggest that acoustic monitoring is enhanced further by postponing inspection for 15–20 min after loading the sample into the acoustic test chamber. Monitoring can begin within 1 min after the grain is placed in the sample container (Shuman et al. 1993). However, the observed short-term reduction in larval activity after a disturbance may increase the probability that an *S. oryzae* or other internally feeding larva will not be detected initially.

If practical considerations permit, there are other reasons for extending the duration of acoustic sampling. Any mechanical or thermal disturbance of grain results in a period of settling during which the grain produces sounds that are difficult to distinguish from low-level insect sounds (Mankin et al. 1996; 1997a, b). Our preliminary tests suggest that such sounds decline to negligible levels within 20 min after the disturbance.

The enhanced effects of warming samples and briefly delaying the start of a trial would be most significant when few larvae are present in the grain sample, because the probability that at least 1 larva is active increases as the number of larvae increases. However, a low larval density is typically the case in inspections at a commercial grain elevator where most samples are expected to be uninfested. Our results

suggest that enhancement of acoustic detectability could be provided in a grain elevator inspection office by use of infrared lamps or heat fans.

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**Table 5.** Estimated parameters in test of hypothesis that radiant heat treatments had no long-term effect on relative activity of 4th instars of *S. oryzae*

Treatment	A	B	$P(T > t \text{ for } H_0: B = 0)^a$	Residual ms error
<i>Ambnt25 (Stir)</i>	1.05 ± 0.03	-0.000 ± 0.0003	0.4870	0.05
<i>Infrd25→29 (Stir)</i>	0.95 ± 0.09	0.0004 ± 0.0009	0.6595	0.16
<i>Infrd25→31 (Stir)</i>	0.70 ± 0.06	0.0026 ± 0.0007	0.0001	0.04
<i>Infrd25→34 (Stir)</i>	0.68 ± 0.05	0.0029 ± 0.0005	0.0001	0.03

Regression equation:  $relative\ activity = A + B t_T$ ; with  $t_T$  (time after start of trial), min;  $countrate$  (rate of sounds counted at time,  $t_T$ ), counts per minute;  $finalrate$  (mean  $countrate$  during [105 min <  $t_T$  < 120 min]), counts per minute;  $relative\ activity$  and A ( $countrate/finalrate$ ), dimensionless; B,  $min^{-1}$ .

<sup>a</sup> Probability of a larger value of Student  $t$  in test of hypothesis that  $B = 0$ .

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