Tribolium castaneum (Coleoptera: Tenebrionidae) Associated With Rice Mills: Fumigation Efficacy and Population Rebound

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ABSTRACT The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is the most important stored-product insect pest infesting rice (*Oryza sativa* L.) mills in the United States. Due to the phasing out of methyl bromide in accordance with the 1987 Montreal Protocol, the efficacy of alternative fumigants in controlling flour beetles in mill structures must be evaluated. Long-term trapping data sets (2–6 yr) of *T. castaneum* in and around seven rice mills were analyzed to assess the efficacy of sulfuryl fluoride fumigation ($n = 25$). Fumigation efficacy was evaluated as the percentage reduction in mean trap captures of adults and proportion of traps capturing at least one adult beetle. Beetle trap captures fluctuated seasonally, with increased captures during the warmer months, June–September, that dropped off during the cooler months, October–March. Fumigations resulted in a 66 ± 6% (mean ± SE) reduction in mean trap captures within mills and a 52 ± 6% reduction in the proportion of traps capturing at least one adult beetle. Lengths of time for captures to reach prefumigation levels, or rebound rates, were variable, and adult capture levels inside were most influenced by seasonal temperature changes. Temperatures inside mills followed those outside the mill closely, and a significant positive relationship between outside temperatures and trap captures was observed. Inside and outside trap captures exhibited a significant, positive relationship, but fumigations consistently led to reductions in beetle captures outside of mills, highlighting the interconnectedness of populations located inside and outside mill structures.

KEY WORDS red flour beetle, fumigation, sulfuryl fluoride, pheromone trap, monitoring

Rice (*Oryza sativa* L.) cultivation in the United States is restricted to ≈1.2 million ha in Arkansas, California, Louisiana, Mississippi, Missouri, and Texas (Snyder and Slaton 2001). In spite of the small area devoted to rice production, it accounts for ≈11% of world rice exports (Childs and Burdett 2000), making rice production and processing important components of U.S. agriculture. In the United States, there are 53 rice mills with an estimated annual production of >6.1 million metric tons (Richardson and Outlaw 2010). The rice milling process begins with the removal of the inedible rice hull from rough rice, resulting in brown rice that retains the bran layers. Brown rice can be further processed by removing the bran layers, yielding clean (i.e., milled, white) rice. Depending on the facility, additional processing steps also may be involved, including parboiling and milling into flour.

Insects can cause economic losses because of their ability to infest rough and milled rice and the milling by-product material that accumulates in structures and equipment where rice is processed. Mills receive rough rice and store it in large bins or warehouses at or near the milling facility. Insects infesting rough rice are internal feeders which develop inside intact rice kernels [e.g., *Sitophilus oryzae* (L.), rice weevil; *Sitotroga cerealella* (Olivier), Angoumois grain moth; and *Rhyzopertha dominica* (F.), lesser grain borer] and external feeders that primarily exploit partially and completely milled rice, milling by-products (hull, germ, bran, and broken kernels), and damaged rice kernels (Mutters and Thompson 2009). The primary insect pests of rice milling buildings are *Tribolium confusum* Jacquelin du Val, the confused flour beetle, and *Tribolium castaneum* (Herbst), the red flour beetle (Coleoptera: Tenebrionidae). Packaged products stored in warehouses and accumulations of food material in equipment and building structures are also vulnerable to infestation. Insect movement from rough rice storage may serve as a source of insect infestations within mill and warehouse structures (Campbell 2008).

T. confusum and *T. castaneum* can use brown rice, processed rice, broken kernels, rice bran, dust, and debris, resulting from the milling process, but they tend to develop more quickly and have a higher fecundity on brown rice and rice bran (McGaughey 1970, 1974; Imura 1991; Via 1991). Thus, extent of rice

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Table 1. Monitoring information for rice mills

Mill	Volume, m^3	Monitoring dates	No. trapping periods	Days per trapping period, mean ± SD (range)	No. traps (inside[a]/outside[b])
CA1	11,900	12 May 2005–26 Dec. 2007	52	18 ± 8 (3–42)	19/0[c], 30/5, 31/5[d]
CA2	76,700	22 July 2005–18 Feb. 2011	108	19 ± 6 (11–42)	30/5 or 31/5[e]
CA3	64,400	5 May 2005–15 Feb. 2011	113	19 ± 6 (11–42)	20/2[f], 28/7, 30/6[e]
TX1	152,900	19 Oct. 2005–31 Jan. 2007	18	26 ± 11 (14–56)	30/5
TX2	113,300	19 Oct. 2005–31 Jan. 2007	18	26 ± 11 (14–56)	30/5
LA1	5,100	6 June 2007–15 Sept. 2010	47	25 ± 9 (11–51)	18/11
TX3	6,000	6 Jan. 2009–27 Jan. 2011	43	17 ± 4 (13–28)	12/8

[a] Located inside the mill structure.
[b] Located outside, near mill structure.
[c] Trap number and location for first trapping period, 12–28 May 2005.
[d] Trap number and location after 13 April 2007.
[e] Trap number and location after 6 April 2007.
[f] Trap number and location for first trapping period, 5–20 May 2005.

milling, products produced, and types of by-products created at a mill likely affect beetle population growth and the need for flour beetle management. Economic damage is caused by direct loss of milling yield due to feeding; rejection of product due to infestation; cost of management tactics such as fumigation and insecticide fogging; contamination by quinones secreted by beetles in heavy infestations (Hodges et al. 1996; Mutters and Thompson 2009); and perhaps most significantly, loss of consumer trust in the company, product, or both. Managing these pests in rice mills is complicated by the difficulty in assessing actual pest population density and the difficulty in applying treatments directly to populations in cryptic refugia.

Pest management programs in rice mills rely on a combination of tactics including sanitation, residual and aerosol insecticides, and fumigants. Pheromone baited traps are an important source of information on pest density and can be used to evaluate treatment efficacy (Campbell et al. 2010a,b). However, because pheromone traps only capture dispersing adults they indirectly estimate pest abundance. Furthermore, the relationship between capture in traps and level of infestation can be affected by other pest management tactics, especially application of residual insecticides, level of sanitation (Toews et al. 2005, 2009), and amount of immigration (Campbell and Arbogast 2004).

Fumigation of milling structures is an important component of pest management of *T. confusum* and *T. castaneum* in many rice mills. Fumigations are infrequently used because they are disruptive to milling operations because they often take ≥24 h to perform, and adding set-up time, aeration of the building, and cleanup, mills may cease operations for up to 2–3 d. By using a calendar-based fumigation schedule, treatments can be planned over holidays or other times when production within the mill is slowed or stopped. Considering that fumigations are so costly, both in loss of production and in actual cost of the application, it is critical to ensure that treatments are effective. In the past, methyl bromide was the principal fumigant used in structural fumigations (Fields and White 2002). However, methyl bromide was identified as an ozone-depleting substance under the 1987 Montreal Protocol on Substances that Deplete the Ozone Layer, leading to an agreement among developed countries to phase out its use (Fields and White 2002). Due to a lack of effective alternatives, the use of methyl bromide in the United States has continued in flour and rice milling facilities under the critical use exemptions process. Sulfuryl fluoride is an alternative fumigant that has been adopted by some rice mills. However, assessment of the efficacy of sulfuryl fluoride fumigation in food facilities is limited and restricted to flour mills (Small 2007; Tsai et al. 2011).

We used pheromone trapping at seven rice mills located in California, Texas, and Louisiana to quantify populations of *T. confusum* and *T. castaneum* occurring within and around mill facilities and to assess the efficacy of commercial sulfuryl fluoride structural fumigations in terms of both initial reduction in captures and rebound in captures over time after treatment. Because temperature has an important impact on pest population growth and fumigation effectiveness, temperature was measured and we explored the relationship between captures and temperature both inside and outside the mills.

Materials and Methods

Rice Mills. Populations of *T. confusum* and *T. castaneum* were monitored in seven rice mills between 2005 and 2011. The mills are located in two U.S. regions: mills CA1, CA2, and CA3 are located in northern California (CA), and mills TX1, TX2, TX3, and LA1 in Texas (TX) or Louisiana (LA). The mills were grouped this way because each region represented discrete rice growing areas with different climatic conditions that may have caused differences in fumigation efficacy and population rebound. Mills varied in construction material (i.e., concrete, timber, metal, or a combination of these materials), size, and number of floors (Table 1).

Although fumigations were the primary focus of our analysis, it is important to note that other practices such as sanitation, insecticide fogging, and perimeter insecticide sprays were used concurrently as part of an integrated pest management program. The type, frequency, and efficacy of these additional tactics varied among mills. Although it was not expected that these tactics significantly affected immediate reduction in pests after fumigation, they could affect rebound rates.

Monitoring *T. confusum* and *T. castaneum*. Personnel at each mill serviced traps independently, so lengths of trapping periods varied (Table 1). We set the standard trapping period at 14 d and corrected data to reflect this standard trapping period for ease of comparison. Between 19 and 36 traps were placed at various locations inside and outside each mill (Table 1). A change in trap number and location occurred at mills CA1, CA2, and CA3 due to a change in shipping method, allowing an expanded number of traps.

Traps (Storgard The DOME Trap, Trécé Incorporated, Adair, OK) consisted of two interlocking plastic pieces: a top, in which up to three pheromone lures could be attached, and a bottom, a circular ramp with a central pitfall trap containing a 3.5-cm-diameter filter paper saturated with kairomone, an oil-based food attractant (Storgard oil, Trécé Incorporated). Beetles were attracted to the trap by the *Tribolium* spp. aggregation pheromone (Storgard Cap, Trécé Incorporated, Adair, OK) and the food attractant. Traps in their original configuration (unmodified traps) were used throughout the monitoring period at mills TX1 and TX2 and until March 2007 at mills CA1, CA2, and CA3. Entire traps were sent to the mills in advance of the trap change, and fresh food attractant and, if needed, new pheromone lures were added to clean traps that replaced traps removed from the mill. Entire traps were placed individually in plastic bags and shipped back to the laboratory at Kansas State University for processing.

Modified traps were used at mills CA1, CA2, and CA3, after March 2007, and for the entire monitoring period at mills LA1 and TX3. Modified traps were made by gluing a rivet to the bottom of the pitfall portion of the trap and attaching the pheromone lure to the top of the rivet. A hole was punched in the center of filter papers to accommodate the rivet. This placed the pheromone in roughly the same location, suspended above the kairomones-treated filter paper, as an unmodified trap but enabled trap bottoms fully loaded with pheromone lures and food attractant to be shipped to and from the mills. This reduced the labor and time required to service traps at the mills.

To limit trap loss, modified traps were attached to a metal base designed to keep traps in place. The metal base consisted of a perforated metal plate (15.5 by 15.5 cm) onto which two 1.5-cm-diameter washers and a rotating metal clip were attached. The trap lid edges were slid under the washers and the clip rotated to either lock the trap in place on the metal plate or allow it to be removed for replacement. At the conclusion of a trapping period, traps were removed from the metal base and bottoms of the traps switched with second set of trap bottoms with fresh food attractant and, if necessary, new pheromone lures. Bottoms were shipped back to the laboratory at the Center for Grain and Animal Health Research for processing. Bottoms were placed in a wooden frame designed to hold the traps in place and prevent sample loss during shipment.

For both trap types, pheromone lures were replaced approximately every 8 wk. For modified traps, two sets of pheromone lures were rotated between shipments and were stored in a freezer between placements, with only total time in the mills, not time in freezer, used for calculating replacement time. Once in the laboratory, insects were removed from the trap and all *T. confusum* and *T. castaneum* adults were enumerated.

Fumigations. Mills were fumigated two to six times during the monitoring period; except mill TX3 that was not fumigated (Table 2). All fumigations were with sulfuryl fluoride (SF) (ProFume, Dow AgroSciences, Indianapolis, IN) with the exception of the final fumigation at mill, LA1, with methyl bromide (MB) (Meth-O-Gas 100, Great Lakes Chemical Corporation, West Lafayette, IN). For each SF fumigation, the fumigation service providers used a software program (ProFume Fumiguide, Dow AgroSciences) that calculated the proper dosage based on the size of the space to be fumigated, type of fumigation (i.e., a structure or a commodity), length of the fumigation, pest species and life stages being targeted, half-loss time (HLT), and temperature. Specific treatment information for all fumigations included in the analysis was not available. Fumigations for which information was available (two SF fumigations at each CA mill) had target accumulated SF dosage of 340–500 g-h/m^3 and observed accumulated dosages of 350–674 g-h/m^3. Observed HLTs ranged from 11 to 37 h. The MB fumigation at mill LA1 was performed with a target dosage of 5.7 kg-h (0.68 kg/28 m^3) for a total of 612 kg of MB. Treatment dates, interior temperatures if available, and wind speed during fumigations are presented in Table 2.

Monitoring Temperature. Temperatures inside mills CA2, CA3, LA1, and TX3 were monitored beginning in December 2008 for mills CA2 and CA3, November 2008 for mill LA1, and March 2009 for mill TX3. Small data loggers (SmartButton Data Logger, ACR Systems Inc., Surrey, BC, Canada) were attached to the bottom of three traps sent to each mill. In mill CA2, data loggers were placed on the first and fifth floors of the mill and in a warehouse. In mill CA3, data loggers were placed in the basement and on the second and seventh floors of the mill. In mill LA1, data loggers were placed on the first and fifth floors of the mill and in a processed rice storage warehouse. In mill TX3, the data loggers were placed in the mill, dryer elevator tunnel, and elevator tunnel. When traps were shipped back to the laboratory, data loggers were removed, temperature data downloaded, and loggers returned to the trap. Superfluous temperature data logged before traps being placed in the mill and after traps were removed from the mill were excluded from the data set based on shipping and receiving dates. Only temperature data collected from loggers placed inside the mill are included. Outside temperatures and wind speeds for each mill were obtained from local weather stations near each mill (www.wunderground.com). For both inside and outside temperatures, the daily min/mean/max are presented.

Table 2. Sulfuryl fluoride fumigation information and reduction in mean no. of *T. castaneum* adults captured and proportion of traps with capture

Mill	Fumigation date	Inside temp (°C) (min/mean/max)	Outside temp (°C) (min/mean/max)	Wind speed (km/h) (max/mean)	Mean capture before fumigation	Mean capture after fumigation	Reduction in captures[a] (%)	Proportion of traps with captures before fumigation	Proportion of traps with captures after fumigation	Reduction in proportion[a] (%)
CA1	28 May 2005		13/19/26	34/24	1.8 ± 1.0	0.1 ± 0.1	92	0.28	0.10	64
	2 Sept. 2005		13/23/33	23/16	9.8 ± 5.1	6.7 ± 2.7	32	0.53	0.67	0
	26 May 2006		11/17/24	29/14	0.4 ± 0.2	0.9 ± 0.5	0	0.23	0.36	0
	1 Sept. 2006		14/24/36	16/6	3.7 ± 1.1	0.2 ± 0.1	94	0.65	0.14	79
	24 May 2007	NA/27/NA	13/22/32	19/11	0.2 ± 0.1	0.0 ± 0.0	100	0.13	0.00	100
	31 Aug. 2007		23/30/39	26/13	0.6 ± 0.3	0.0 ± 0.0	94	0.29	0.03	89
CA2	5 Aug. 2005		17/28/39	16/6	4.3 ± 1.3	0.3 ± 0.1	92	0.72	0.28	61
	18 Aug. 2006		14/23/32	19/10	9.5 ± 2.6	2.8 ± 1.3	71	0.85	0.80	6
	21 July 2007	NA/29/NA	16/24/33	29/13	1.2 ± 0.2	0.2 ± 0.2	80	0.63	0.10	84
	25 July 2008		14/25/36	19/5	4.3 ± 1.2	0.3 ± 0.1	94	0.85	0.23	73
	13 Aug. 2009	22/27/31	18/27/36	27/6	3.2 ± 0.7	0.5 ± 0.1	85	0.72	0.35	51
	20 Aug. 2010	20/24/28	13/23/32	24/10	0.5 ± 0.2	0.2 ± 0.1	68	0.31	0.13	58
CA3	21 May 2005		10/18/24	23/8	2.0 ± 0.7	0.4 ± 0.2	81	0.60	0.26	56
	3 Aug. 2006		14/24/34	26/11	13.7 ± 3.0	0.6 ± 0.2	96	0.82	0.30	64
	14 July 2007	NA/27/NA	14/24/33	26/13	1.3 ± 0.4	0.2 ± 0.1	83	0.50	0.20	60
	8 Aug. 2008		13/21/29	27/16	2.1 ± 1.1	1.2 ± 1.1	65	0.37	0.17	55
	7 Aug. 2009	21/23/25	13/22/29	23/6	2.3 ± 1.3	1.0 ± 0.7	59	0.46	0.30	35
	12 Aug. 2010	18/23/29	12/22/32	24/6	1.5 ± 0.5	0.6 ± 0.4	58	0.50	0.30	40
TX1	11 Nov. 2005		19/22/26	16/6	0.9 ± 0.3	0.7 ± 0.7	25	0.40	0.08	80
	15 April 2006		21/24/29	40/19	0.3 ± 0.1	0.3 ± 0.1	11	0.44	0.33	24
	4 July 2006		24/27/29	23/6	0.8 ± 0.2	0.5 ± 0.1	35	0.57	0.52	8
TX2	11 Nov. 2005		19/22/26	16/6	5.2 ± 1.6	0.2 ± 0.1	96	0.72	0.19	74
	15 April 2006		21/24/29	40/19	0.7 ± 0.3	0.3 ± 0.2	60	0.46	0.20	56
	4 July 2006		24/27/29	23/6	1.8 ± 0.5	0.8 ± 0.2	57	0.79	0.43	46
LA1	13 June 2008	NA/33/NA	23/28/33	26/8	1.0 ± 0.3	0.5 ± 0.2	46	0.71	0.50	30
	24 July 2009[b]	27/29/33	23/28/33	11/2	1.3 ± 0.6	0.6 ± 0.3	54	0.53	0.28	48
Mean ± SEM[c]					2.9 ± 0.7	0.8 ± 0.3	66 ± 6	0.54 ± 0.04	0.28 ± 0.04	52 ± 6

NA, not available.
[a] When there was an increase in beetle capture or proportion of traps with captures after a fumigation, percentage reduction was zero.
[b] Fumigation with methyl bromide.
[c] Means do not include methyl bromide fumigation at mill LA1.

Statistical Analysis. *T. confusum* and *T. castaneum* populations within the mills were quantified in two ways: beetle capture per trap and number of traps with one or more beetles. Beetle capture data were expressed as the mean number of *T. confusum* or *T. castaneum* per trap inside or outside the mill (hereafter mean inside or outside trap captures) corrected to reflect the standard trapping period. Traps capturing one or more beetles was expressed as the proportion of inside or outside traps capturing at least one beetle over the sampling period (hereafter the proportion of inside or outside traps with captures).

Efficacy of SF fumigations was measured as percentage reduction in mean inside trap captures and the percentage reduction in the proportion of inside traps with captures. The efficacy of the MB fumigation was not analyzed due to the lack of replication. These measures of efficacy were compared among mills using analysis of variance (ANOVA) (PROC GLM, SAS 9.2, SAS Institute, Cary, NC). The same measures of fumigation efficacy were compared between regions, CA and TX/LA, by using a Student's *t*-test (PROC TTEST, SAS 9.2, SAS Institute). To fulfill the normality assumption, the arcsine square-root transformation was applied to percentage reduction in mean beetles per inside trap. Pearson's correlation coefficients were calculated to explore the relationship of fumigation efficacy and rate of rebound with maximum, mean, and minimum temperatures and maximum and mean wind speed during fumigations (PROC CORR, SAS 9.2, SAS Institute). Pearson's correlation coefficients also were calculated to explore the relationship between fumigation efficacy and mean captures per inside trap during the sampling period immediately preceding treatment (PROC CORR, SAS 9.2, SAS Institute).

Time-to-event analysis was used to assess the rate of rebound of beetle populations after fumigation. We used the thresholds established by Campbell et al. (2010b) for *Tribolium* in flour mills of 2.5 beetles per trap per 14 d and 50% of traps capturing at least one beetle. These thresholds were the median beetles per inside trap and proportion of traps with captures during sampling periods immediately before fumigations in two midwestern flour mills (Campbell et al. 2010b). Because the median beetles per inside trap and proportion of traps with captures in rice mills was 1.6 and 53%, respectively, time-to-event analyses also were performed using these thresholds. Number of days postfumigation until each of these thresholds was met or exceeded was determined and used in the time-to-event analysis (Kaplan–Meier single group survival analysis, SigmaPlot 12.2, Systat Software, Chicago, IL). To determine whether mill region affected rebound rate, we also performed a time-to-event analysis comparing rebound rates between the CA and TX/LA regions (Kaplan–Meier log-rank survival analysis, SigmaPlot 12.2, Systat Software). Cases where the threshold was not reached before another fumigation or before the conclusion of monitoring were censored. Pearson's correlation coefficients were calculated to determine whether mean inside trap captures immediately before fumigation was related to the rate of rebound (PROC CORR, SAS 9.2, SAS Institute).

We used the mean daily inside and outside temperatures to evaluate the relationship between beetle captures and temperature. We performed linear regressions to determine whether increased beetle captures outside the mill led to increased captures inside the mill, and whether changes in outside temperature led to changes in mean inside beetle capture or in proportion of traps with captures (PROC GLM, SAS 9.2, SAS Institute). Linear regression also was used to determine the extent to which outside temperatures were related to inside temperatures (mills CA2, CA3, LA1, and TX3). Mill was included as a factor in the regressions, so that if a significant relationship between the variables was found, the nature and strength of the relationship could be compared among mills by comparing slope estimates among mills (CONTRAST statement, PROC GLM, SAS 9.2, SAS Institute). To compensate for multiple comparisons, we used a Bonferroni correction, reducing the critical *P* value from 0.05 to 0.0024 for the regressions of mean inside captures on mean outside captures, mean inside captures on outside temperature, and proportion of traps with captures on outside temperature and to 0.0083 for the regression of inside temperature on outside temperature. Data are presented as untransformed means ± SEM, unless otherwise noted.

Results

T. castaneum was captured in traps at all the mills; however, *T. confusum* was captured at only four of the mills: CA1 (0.40% *T. confusum*), CA2 (0.07% *T. confusum*), CA3 (1.08% *T. confusum*), and LA1 (0.03% *T. confusum*). Most of the *T. confusum* individuals were captured at mill CA3, 100 total individuals, whereas at mills CA1, CA2, and LA1 only 13 individuals in total were captured. Because so few *T. confusum* were captured, we focused our analysis on trap captures of *T. castaneum* of which 26,600 total individuals were captured.

Mean inside trap captures followed a distinctly seasonal pattern in most of the mills, with highest captures recorded in the warm months, and dropped off as outdoor temperatures cooled (Fig. 1). This trend was most apparent in mills monitored for several years, i.e., mills CA1, CA2, CA3, LA1, and TX3, but it was less obvious in mills TX1 and TX2 that were monitored for just over 1 yr (Fig. 1). Proportion of inside traps with captures followed a similar trend (Fig. 2). The mean change in trap capture between two consecutive sampling periods was close to zero, −0.0 ± 0.1 beetles per trap, indicating that no clear trends or patterns were observed. Similarly, the mean change in proportion of traps capturing at least one beetle was close to zero, −0.00 ± 0.01.

In total, 25 SF fumigations were performed during the monitoring periods at the six treated mills. Fumigations resulted in a 66 ± 6% reduction in beetles captured per trap, from 2.9 ± 0.7 per trap before SF fumigation, to 0.8 ± 0.3 per trap in the sampling period

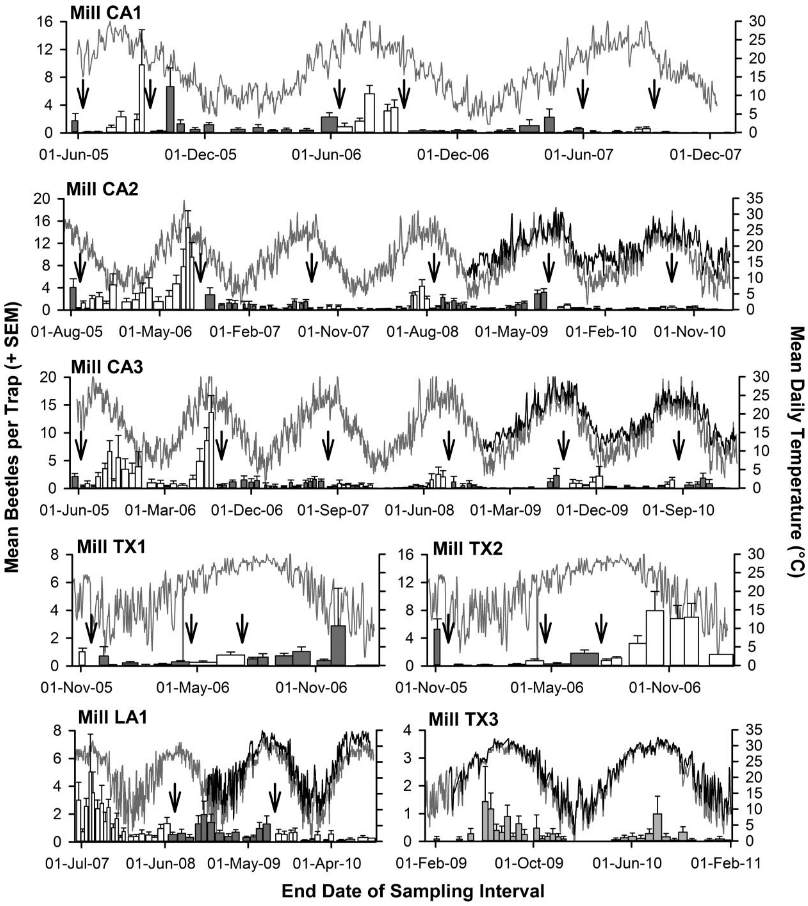

Fig. 1. Mean (+ SEM) *T. castaneum* per inside trap (bar graphs) with outside (gray line) and inside (black line) temperatures (°C) for the seven mills. Inside temperatures were only available for part of the monitoring period for mills CA2, CA3, LA1, and TX3. Arrows and change in color of bars indicate a fumigation. Mill TX3 was not fumigated while being monitored.

after fumigation (Table 2). Fumigation efficacy, as measured by the percentage reduction in mean inside trap captures, did not significantly vary by mill ($F = 1.63$; df = 5, 19; $P = 0.2012$). The impact of region on reduction in capture was marginally nonsignificant ($t = 1.94$, df = 23, $P = 0.0646$), with reductions in captures of 73 ± 6% for CA mills and of 47 ± 11% for TX/LA mills. Fumigations resulted in a 52 ± 6% reduction in proportion of traps capturing at least one beetle during the monitoring period. Before fumigation, the proportion of traps with captures was 0.54 ± 0.04, which was reduced to 0.28 ± 0.04 after fumigation (Table 2). Reduction in proportion of traps with captures did not significantly vary among mills ($F = 0.31$; df = 5, 19; $P = 0.9024$) or between regions ($t = 0.69$, df = 23, $P = 0.4970$). Reduction in beetle capture and proportion of traps with captures were not significantly correlated with mean or minimum temperature or maximum or mean wind speed during fumigation. Reduction in proportion of traps with captures was not correlated with maximum temperature; however, reduction in beetle capture was significantly, positively correlated with maximum temperature during fumigation ($\rho = 0.477$, $n = 25$, $P = 0.0160$). Fumigation efficacy, reduction in trap capture and reduction in proportion of traps with captures, were not significantly correlated with prefumigation trap captures.

Rebound in trap captures and in proportion of traps with captures after fumigation in CA mills seemed to be influenced by seasonal temperature fluctuations. Regardless of the date of fumigation, there is a consistent reduction in both beetles per trap and proportion of traps with captures at the CA mills from January to March, with a corresponding increase from May to September (Fig. 3a and c). This same pattern was observed in TX/LA mills, but the trend was less clearly evident (Fig. 3b and d).

After SF fumigation, the threshold of 2.5 beetles per inside trap, the median beetles per trap observed in flour mills, was reached after 270 ± 31 d. However, of the 25 fumigations, only 11 reached this threshold before the next fumigation or the end of the monitoring period (Fig. 4a). Time to reach 1.6 beetles per inside trap, the median beetles per trap immediately

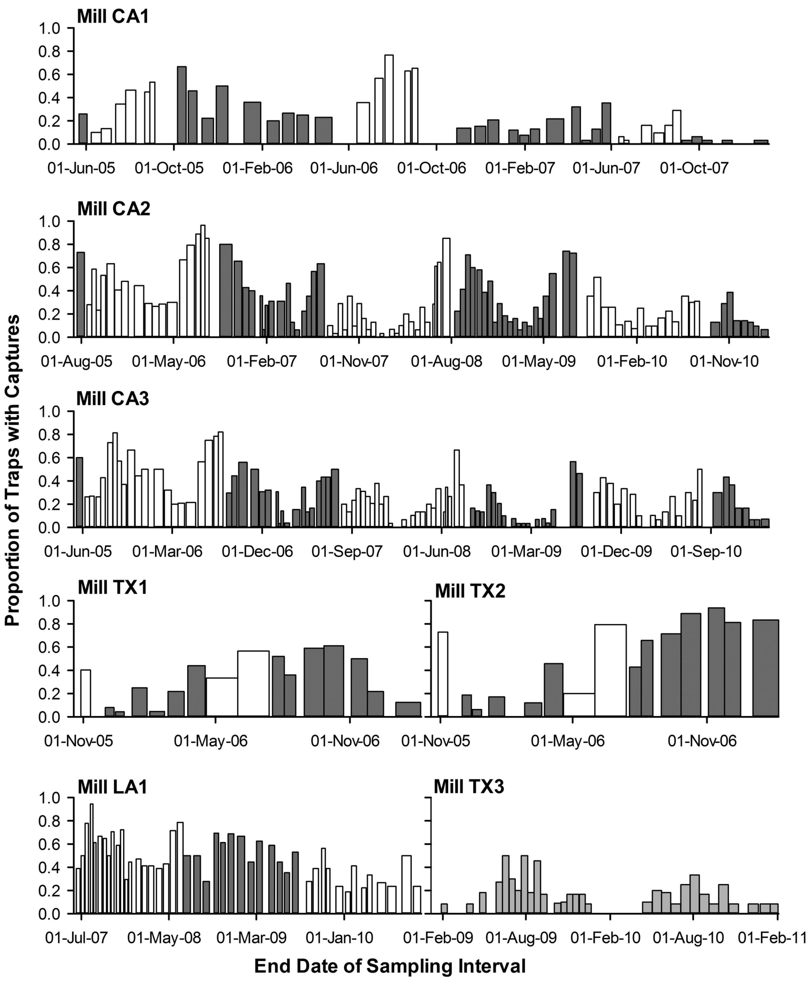

Fig. 2. Proportion of inside traps capturing at least one *T. castaneum* during a monitoring period for the seven mills. Change in color of bars indicates a fumigation. Mill TX3 was not fumigated while being monitored.

before fumigation in the mills was 189 ± 28 d, with 18 of the 25 fumigation events reaching this threshold before the next fumigation or termination of monitoring (Fig. 4b). Time to reach the threshold of 50% of traps capturing at least one beetle, median before fumigation in flour mills, was shorter, only 183 ± 30 d, and was reached after 18 of the 25 fumigations (Fig. 4c). It took 202 ± 31 d to reach the threshold of 53% of traps capturing at least one beetle, the prefumigation median in the rice mills and 16 of the 25 fumigations reached this threshold before the next fumigation or the end of the monitoring period (Fig. 4d). The time to reach these thresholds did not significantly vary between mill regions ($P > 0.05$). Rate of rebound to the prefumigation median of 53% of traps capturing at least one beetle was significantly, positively correlated with minimum temperature during fumigation ($\rho = 0.713$, $n = 16$, $P = 0.0019$). Rebound rate was not significantly correlated with prefumigation trap captures or with maximum temperature, mean temperature, or wind speed during fumigation.

One fumigation at mill LA1 was with MB rather than SF (Table 2). Efficacy of the two fumigants could not be compared directly due to the lack of replication. However, the MB fumigation resulted in reductions in trap capture and proportion of traps with captures that were similar to the mean for the SF fumigations and to the earlier SF fumigation at mill LA1 (Table 2). The pattern of rebound after the MB fumigation was also similar to that of the SF fumigations at the TX/LA mills (Fig. 3).

There was a significant, positive relationship between mean captures per trap inside mills and mean captures per trap outside mills (all mills combined: $r^2 = 0.439$, df = 394; MSE = 1.86; $P < 0.0001$; Fig. 5). Thus, increased captures in traps located outside the mill was associated with increased captures inside the mill. Both the intercept and slope of the regression line were significantly different from zero (intercept: $F = 9.53$, df = 7, $P < 0.0001$; slope: $F = 37.19$, df = 7, $P < 0.0001$). Contrasts comparing the slope of the regression lines among mills indicated that mill TX2 had a significantly greater slope, 1.822 ± 0.413, than mills

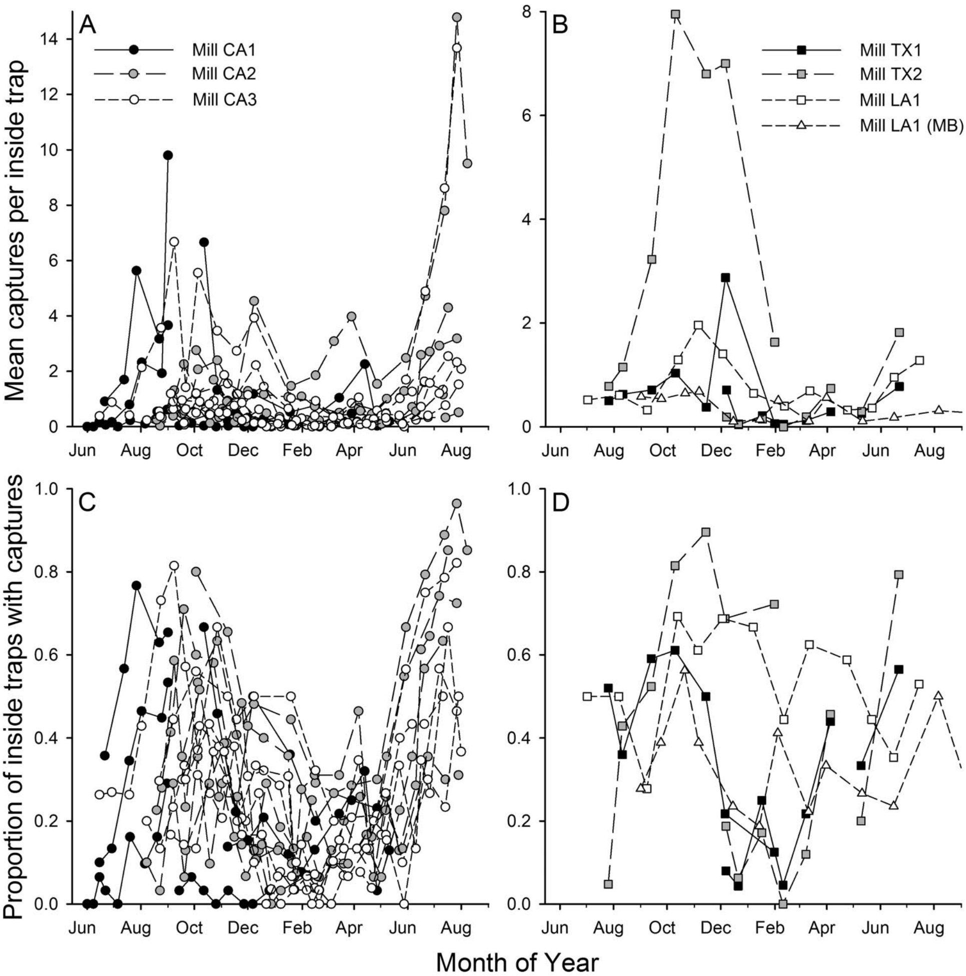

Fig. 3. Rebound by month of the year of *T. castaneum* captured per trap (A and B) and proportion of traps with captures (C and D) after fumigation at CA mills (A and C) and TX/LA mills (B and D).

CA1 (slope = 0.147 ± 0.017, $P < 0.0001$), CA2 (slope = 0.074 ± 0.012, $P < 0.0001$), CA3 (slope = 0.153 ± 0.014, $P < 0.0001$), TX1 (slope = −0.056 ± 0.268, $P = 0.0002$), and LA1 (slope = 0.123 ± 0.068, $P < 0.0001$) (Fig. 3). Mills CA1 (P = 0.0005) and CA3 ($P < 0.0001$) had significantly greater slopes than mill CA2 (Fig. 5). The slope of the regression line for mill TX3 was not significantly different from that of any other mill, and mills TX1 and TX3 were the only mills with negative slopes; but the negative slopes were not significantly different from zero ($P > 0.05$) (Fig. 5) indicating no relationship between inside and outside captures.

Outside temperature and mean beetle capture per inside trap had a significant, positive relationship ($r^2 = 0.199$, df = 395; MSE = 2.64; $P < 0.0001$; Fig. 6), indicating that as outdoor temperatures increased, trap captures inside the mill also increased. The intercept was not significantly different from zero (F = 1.59, df = 7, P = 0.1362); however, the slope of the regression line was significantly different from zero ($F = 9.72$, df = 7, $P < 0.0001$). Contrasts comparing the slopes of the regression lines did not indicate significant differences in estimated slope among the mills. Outside temperature and proportion of inside traps with captures also had a significant, positive relationship ($r^2 = 0.426$, df = 395; MSE = 0.03; $P < 0.0001$) and contrasts indicated estimated slopes of regression lines significantly varied among mills. Mill CA2 had a significantly greater slope, 0.0234 ± 0.0029, than mill LA1, 0.0067 ± 0.0040 (P = 0.0007). All other mills had similar slope estimates.

In those mills where inside temperature was monitored, inside and outside temperatures were similar, regardless of time of year (Fig. 1). Linear regression indicated that the relationship was significant and positive ($r^2 = 0.934$, df = 2896; MSE = 2.67; $P < 0.0001$; Fig. 7). Contrasts indicated the estimated slopes of regression lines varied among mills, with mills LA1 and TX3 having significantly larger slopes 0.8830 ± 0.008 and 0.8847 ± 0.008, respectively, than mills CA2 and CA3, 0.7368 ± 0.009 and 0.6434 ± 0.009, respectively ($P < 0.0001$ for all comparisons). In addition, slope for

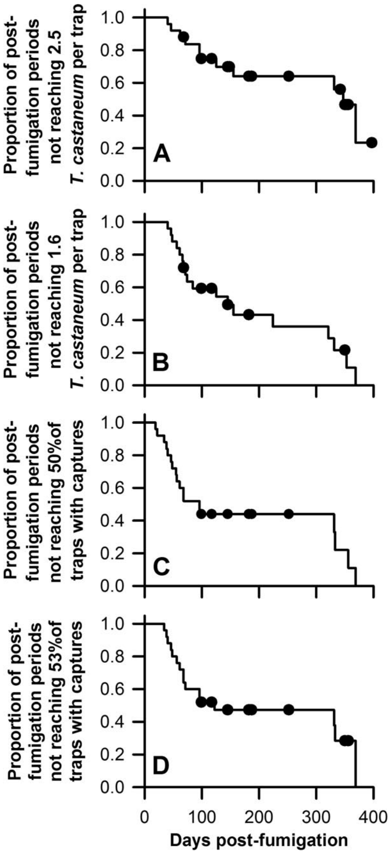

Fig. 4. Time-to-event curves for rebound to threshold of 2.5 *T. castaneum* per trap (A), 1.6 *T. castaneum* per trap (B), 50% of traps capturing at least one *T. castaneum* (C), and 53% of traps capturing at least one *T. castaneum* (D) over a standardized 14-d sampling interval. Data from the six mills undergoing fumigations during the monitoring period were combined. Closed circles indicate censored events (the next fumigation occurred before reaching the threshold or monitoring was terminated before reaching the threshold).

mill CA2 was significantly greater than that of mill CA3 ($P < 0.0001$). The large r^2 values and slopes close to one indicate that temperatures inside the mill follow those outside the mill closely, but that CA2 and CA3 tended to stay warmer at cooler outside temperatures than LA1 and TX3.

Discussion

Seasonality and Efficacy of Fumigation. Trends in *T. castaneum* captures inside the seven rice mills followed seasonal patterns in which captures and proportion of traps capturing at least one beetle increased throughout spring, peaked in summer months, and then dropped off during the colder winter months. This is a different seasonal pattern than observed in trap captures in midwestern flour mills; beetle captures in flour mills tended to increase, albeit at different rates, throughout the year (Campbell et al. 2010a). In the flour mills, fumigations appeared to have a stronger impact than seasonality on rebound, whereas in rice mills season seems to have a stronger influence on beetle captures than time after fumigation. Seasonality may have a weaker influence on beetle captures in flour mills because flour mill interiors tended to be buffered from extreme outside conditions that resulted in a nonlinear relationship between inside and outside temperature (Campbell et al. 2010a). Rice mills did not exhibit similar buffering of indoor temperatures (Fig. 7), the possible causes of which are discussed below; thus, beetle populations would have been exposed to climatic extremes.

Fumigations in the flour mills were more effective at reducing trap captures with a mean reduction of 85 ± 5% (Campbell et al. 2010a) at mills in the midwestern United States and 95 ± 1% at mills in the United Kingdom (Small 2007), both higher than the mean reduction observed in rice mills of 66 ± 6% (Table 2). Differences in efficacy of fumigations might have been caused by differences in fumigant used; rice mills were fumigated with SF, whereas flour mills were

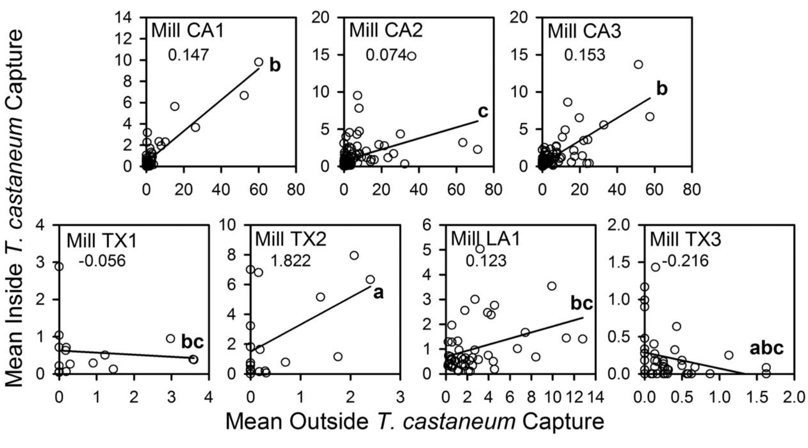

Fig. 5. Relationship between mean *T. castaneum* capture in traps located inside the mill and outside the mill. Estimated slope of regression lines included below panel label. Different letters after the regression line indicate the difference in the estimate of the slope of the regression line is significant.

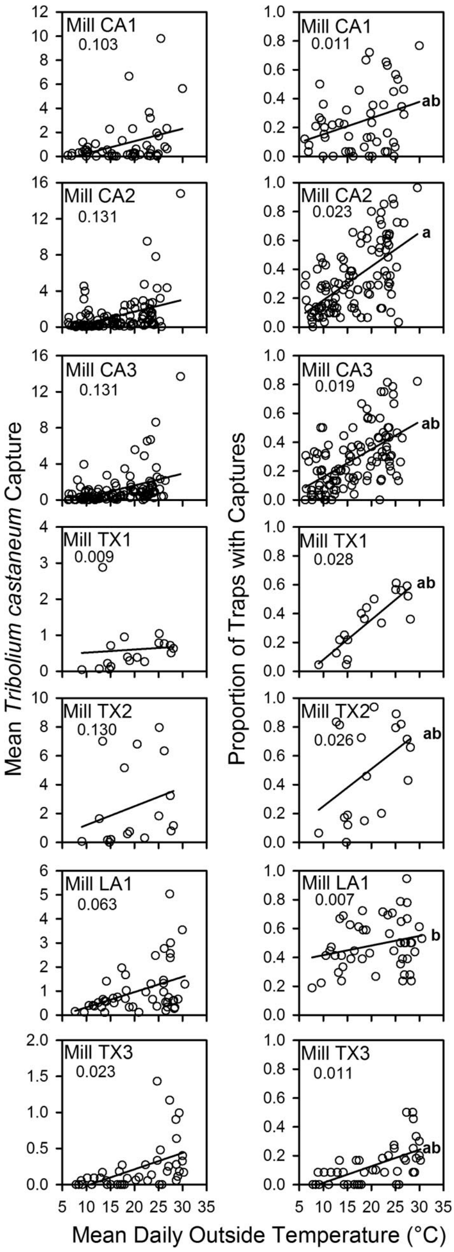

Fig. 6. Relationship between mean daily outside temperature (°C) and mean inside *T. castaneum* capture (left column of panels) and proportion of inside traps capturing at least one *T. castaneum* (right column of panels). Estimated slopes of regression lines included below panel label. Slopes did not significantly vary among mills for mean *T. castaneum* capture. For proportion of traps with captures, regression lines followed by different letters indicate a significant difference between estimated slopes of the regression lines.

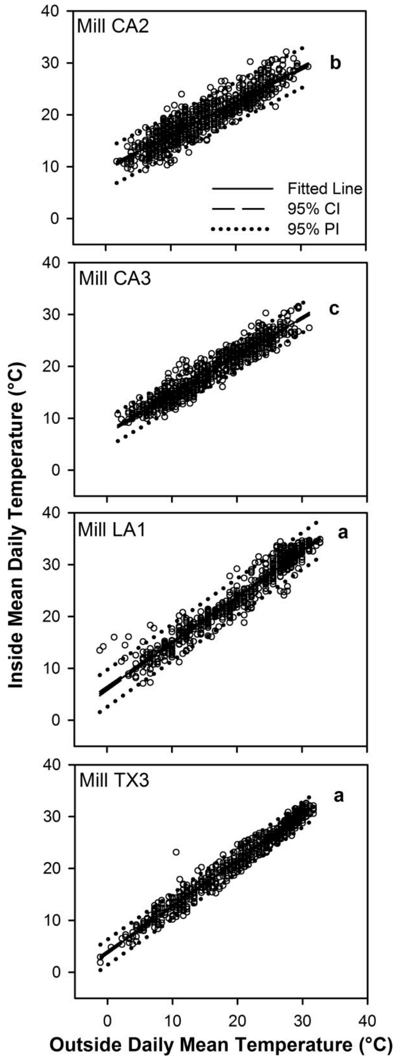

Fig. 7. Relationship between inside and outside temperatures (°C) in those mills where inside temperatures were monitored. Regression lines followed by different letters indicate significant differences between estimated slopes of regression lines.

fumigated with MB (Campbell et al. 2010a; Table 2). However, when SF and MB fumigations were compared directly in wheat (*Triticum aestivum* L.) flour mills, efficacy of SF and MB fumigations measured as reduction in captures immediately after treatment have been comparable (Small 2007, Tsai et al. 2011). Multiple studies have reported that higher doses of SF are required to control the *Tribolium* spp. egg stage compared with other beetle developmental stages (Bell et al. 1999, Hartzer et al. 2010). However, trap captures in the sampling period immediately after fumigation will initially reflect mortality of adults and pupae, as larvae and eggs would not have become adults by this time and therefore would not have been captured in traps. Thus, if the dosage of SF was not high enough to cause egg mortality, *T. castaneum* egg survival would not become apparent for weeks or months after fumigation, depending on temperature and food quality.

There are two likely reasons why observed fumigation efficacy was lower in the rice mills compared with similar treatments in flour mills. First, lower beetle densities were observed at the rice mills before fumigation, 2.9 ± 0.7 beetles per trap (Table 2), compared with prefumigation captures at the flour mills of 17.4 ± 6.2 and 5.8 ± 2.9 (Campbell et al. 2010a). Because beetle captures at the rice mills were frequently low (i.e., less than two beetles per trap) before fumigation, reducing captures even further would not necessarily be expected (Table 2). At such low rates of capture, capture rates were likely difficult to quantify accurately and stochastic events may have had a larger influence on captures, both of which would obscure detection of a reduction in beetle capture. Furthermore, at Midwestern flour mills, when captures before fumigation were less than two per trap, captures were reduced by 76 ± 11%; however, when prefumigation captures were more than two per trap, captures were reduced by 91 ± 2% (Campbell et al. 2010a).

Second, efficacy may have been lower for these rice mills because evidence suggests that beetle populations were not restricted to the mill, in contrast to populations in flour mills. A significant positive relationship between inside and outside trap captures in five of the seven mills and higher beetle captures outside the mill compared with inside the mill in four of the mills supports the conclusion that *T. castaneum* populations may move freely between indoor and outdoor habitats (Fig. 5; Campbell and Arbogast 2004; Campbell et al. 2010a). Thus, these nonfumigated outdoor areas could serve as a source for colonization of the mills after fumigation, lowering the apparent efficacy. These two factors alone and in combination might explain the lower immediate reduction in capture after fumigation.

Although nonsignificant, the difference in efficacy of fumigation between the two rice growing regions showed a trend of higher efficacy in the CA mills, 73 ± 6% reduction in trap capture, compared with the TX/LA mills, 47 ± 11% reduction in trap capture (Table 2). Many factors could have contributed to this disparity, including differences in building size or structure, SF dosage, weather conditions, differences in pest management strategies, or a combination. For a fumigation to be effective, the fumigant must be maintained at the appropriate concentration for the required length of time to achieve the prescribed accumulated dosage within the structure to kill the target pest. Differences in treatment methodology, mill structure, or availability of refugia may have reduced the accumulated dosage to which beetles were exposed, possibly contributing to differences in efficacy observed between the two regions. Although a comparison of achieved accumulated dosages and HLTs between regions, among mills, and among fumigation events might help to explain the observed differences in efficacy, we do not have enough specific treatment data to make such comparisons. However, we do know that all SF fumigations were made according to the label (i.e., the Fumiguide calculation); thus, our conclusions in regard to the efficacy of the SF fumigations are based on the label rate and not a specific accumulated dosage. Number of SF fumigations performed might also have been a factor explaining differences between regions, although this is difficult to quantify. Our data set indicated a larger number of consecutive treatments with SF in the CA mills than at the TX/LA mills. The higher level of experience using this fumigant might have led to more effective SF fumigations in the CA facilities, especially considering that at the TX/LA mills some treatments represent first experience with this fumigant. Thus, there are many factors related to treatment with the potential to affect fumigation efficacy which were not measured during fumigations included in our analyses. It is possible that a combination of these factors contributed to the relatively large, though nonsignificant, differences in fumigation efficacy between regions.

Weather conditions during fumigation, such as temperature and wind speed, also have the potential to affect efficacy of fumigation. Increasing the temperature from 25 to 30°C reduced the required accumulated dosage of SF to kill *T. castaneum* eggs from 1,700 g-h/m^3 to 1,150 g-h/m^3 (Bell et al. 1999). Thus, at lower temperatures, it may be difficult to reach the required accumulated dosage to kill all the life stages of *T. castaneum.* In the rice mills studied, maximum daily temperature exhibited a significant, positive correlation with reduction in beetle capture after fumigation. This supports the positive relationship between efficacy of fumigation and temperature. High wind speeds may also increase the rate of SF loss within fumigated structures which may lead to reduced accumulated dosages and reductions in efficacy. However, we did not detect any significant relationships between maximum or mean wind speeds and fumigation efficacy or rate of rebound in this study.

Population Rebound After Fumigation. The rate at which beetle populations rebound to prefumigation levels is an additional measure of fumigation efficacy. Rebound rate depends on beetle survival after fumigation, beetle immigration and emigration during and/or after fumigation, mill temperature, and additional management tactics used in the mill. Increased beetle mortality, prevention of beetle immigration and emigration, cooler temperatures, and use of additional management such as sanitation can all slow the rebound rate. We used thresholds of 1.6 and 2.5 beetles captured per trap, thresholds that refer to median beetles captured per trap during the prefumigation sampling periods for rice and flour mills (Campbell et al. 2010b), respectively, and 53% and 50% of traps with captures, values referring to median proportion of traps with captures during the prefumigation sampling periods for rice and flour mills (Campbell et al. 2010b), respectively, to analyze rebound rates. Rebound rates at the rice mills were highly variable, ranging from 19 d to 369 d, depending on the threshold applied (Fig. 4). In addition, in multiple instances beetle capture did not reach the threshold before the next fumigation (Fig. 4). Although these thresholds are somewhat arbitrary in that they are not based on balancing economic loss due to product contamination with loss due to the cost of treatment, they are useful in assessing rebound rate and in comparing rebound rates between mill types.

Rebound in flour mills occurred more rapidly than in rice mills, which may be due to higher prefumigation beetle densities in flour mills, identified as a factor related to rebound rate (Fig. 4; Campbell et al. 2010b). Warmer temperatures observed in flour mills, particularly over the winter, also may have contributed to the more rapid rebound. Furthermore, rebound in flour mills was more consistent in that *T. castaneum* captures were sharply reduced immediately after fumigation and then steadily increased until the next fumigation. These trends were particularly evident when season of fumigation was taken into account (Campbell and Arbogast 2004; Campbell et al. 2010b). The same trends were not observed in rice mills as fumigations did not always lead to sharp declines in beetle captures nor was rebound consistent over time (Figs. 1 and 2). Small (2007) monitored *T. confusum* populations for up to 84 d postfumigation, and for both SF and MB fumigations, beetle populations did not rebound to prefumigation densities within this time frame.

Relationships between temperatures outside and inside of mills varied between rice mills in California, Texas, and Louisiana and Midwest flour mills (Fig. 7; Campbell et al. 2010a). Inside temperatures in rice mills were more similar to outdoor temperatures and this disparity was most pronounced during the winter, whereas flour mills tended to follow outside temperatures only during the warm season. Temperatures in rice mills are not as buffered from outside conditions (Fig. 7), and rice mills do not seem to be heated during the cooler months. In addition, the machinery used to mill rice may produce less heat than in flour mills because of a reliance on belts and gravity rather than the pneumatics and motorized equipment used in flour mills and the reduced amount of processing of the grain that occurs. In addition, many rice mills are typically under negative building air pressure. All three of these differences could contribute to the differences in temperature fluctuations between rice and wheat flour mills. Flour mill interior temperatures during the cool season were generally stable due to heating of the mills and maintained temperatures that support beetle development year-round (Campbell et al. 2010a). In warm season, flour mill temperatures tended to be warmer than outside, whereas in rice mills inside and outside temperatures were more similar. Cooler temperatures inside rice mills would have directly affected population growth inside mills and also indirectly affected indoor populations via a corresponding reduction in outside activity and movement of individuals into mills; both may have contributed to the increased time to rebound observed.

Food quantity and quality also may have caused beetle captures in flour mills to rebound more quickly than in rice mills. The amount of fine material produced when grinding grain into flour would be expected to be larger, allowing for larger accumulations of food material in flour mills compared with rice mills. However, this is a factor that still needs to be directly measured. *T. castaneum* development is also delayed on rice products when compared with wheat milling by-products (Imura 1991). Via (1991) found that two strains of *T. castaneum* tended to develop more quickly and reach larger population sizes on wheat flour compared with rice flour, although the improvement was nonsignificant for either strain. When reared on rice, flour beetle fecundity was reduced as the extent of milling was increased (McGaughey 1974). Rice mills producing primarily polished white rice will have less of the preferred brown rice readily available as a food source. Over several generations such small increases in development time or reductions in fecundity could contribute to the lower beetle densities and slower rebound rates observed in the rice mills (Throne 1989).

Interestingly, at the flour mills, the rate of beetle rebound was significantly affected by the season during which the fumigation was performed, with rebound occurring most rapidly after summer fumigations and least rapidly after fall fumigations (Campbell et al. 2010a). In rice mills, however, the season during which fumigation was performed did not seem to influence the rate of rebound. Most of the rice mill fumigations took place during the summer (62%), with fewer in the spring (23%) and fall (15%) (Table 2). With so few fumigations represented for the spring and fall, there was probably not enough replication to make an accurate comparison in rebound rate among the seasons at the rice mills. With additional rebound data from spring and fall fumigations in rice mills, a seasonal trend may emerge, which would be expected considering the strong impact of seasonality on trap captures (Fig. 1).

Inside Versus Outside *T. castaneum* Capture. A significant, positive relationship between mean inside trap captures and mean outside trap captures was observed, with higher mean captures in traps located outside of four of the seven rice mills (Fig. 5). It is difficult to assess whether beetles captured outside the mill were moving into or out of the mill. Higher beetle capture outside of the mill would suggest movement into the mill at CA1, CA2, CA3, and LA1. Two of the Texas mills, TX1 and TX3, had a negative relationship between inside and outside mean trap capture and TX2 had higher trap captures inside the mill than outside the mill, suggesting that at these mills, emigration from the mill may have been occurring (Fig. 5). However, for all fumigated mills, outside beetle capture was consistently reduced in the sampling period immediately after fumigation implying that fumigated areas served as a population source for outdoor populations and fumigation eliminated or reduced this source. Thus, although we cannot conclusively determine whether beetles were moving primarily into or out of the mill, we can conclude that beetle populations inside and outside the mill were interconnected. Similar monitoring at flour mills showed lower beetle captures outside the mill than inside the mill, suggesting that beetle populations were likely established in and mostly confined to the mill (Campbell and Arbogast 2004). The presence of adult beetles shortly after fumigation at the flour mills was likely not due to beetle movement into mills from outside, but rather due to survival of fumigation or movement from nonfumigated areas within the mill (Campbell et al. 2010a).

Role of Temperature. Temperature is an important consideration for pest management in rice mills because beetles require ambient temperatures above certain minima to develop and to disperse. Cooler temperatures slow beetle population expansion (Erdman 1964); thereby slowing the rate of population rebound after a fumigation. Inside trap captures and proportion of traps with captures tended to increase as outside temperature increased in all seven mills (Fig. 6). This trend would be expected considering the strong impact of seasonality on beetle captures. Generally, the relationship between proportion of traps with captures and outside temperature was stronger than between mean inside trap captures and outside temperature. This is not surprising, because as outside temperatures increased, so did inside temperatures (Fig. 7), that likely led to an increase in beetle activity. More rapid beetle development and population expansion, coupled with increased movement at higher temperatures would be expected to cause increased beetle contact with traps. As beetles migrated to new habitat patches more often, a larger proportion of traps were likely to catch at least one beetle. The positive relationship between temperature and mean beetles per inside trap was weaker probably due to wide variations in trap captures among trap locations, possibly masking more significant trends (Fig. 6).

The relationship between inside and outside temperatures at rice mills was very close. The regression of inside temperatures on outside temperatures yielded high r^2 values and slopes close to 1, indicating that a 1°C change in outside temperature led to a nearly identical change in temperature inside these mills (Fig. 7). Mills LA1 and TX3 had significantly larger estimated slopes of the regression line when inside mean temperature was regressed on outside temperature (Fig. 7). Thus, an increase in outside temperature at these mills resulted in a larger increase in inside temperature than an identical change at the CA2 or CA3 mills. This difference could have been due to differences in the structure of the buildings. The TX3 mill is a metal and wooden structure making it difficult to seal for fumigation, which is why it was not fumigated during the monitoring period.

T. castaneum cease embryonic development at temperatures below 17.5°C (Howe 1956); thus, *T. castaneum* population growth at temperatures below this threshold would not occur. At the midwestern flour mills, inside temperatures rarely fell below this minimum over the 7 yr of the study (Campbell et al. 2010a). However, at the four rice mills where indoor temperatures were monitored, mean daily temperatures frequently fell below 17°C and, during the winter, remained below this threshold for extended periods (Fig. 1).

Future Directions. We have used trap captures and proportion of traps capturing at least one beetle as a measure of *T. castaneum* population density; however, it is unknown how pheromone trap captures are related to actual insect densities, further complicating the assessment of fumigation efficacy. Adult beetle captures in traps were used in this study to compare infestations among different facilities, inside and outside a facility, and within a specific facility over time. However, because this is an indirect sampling method capturing dispersing individuals, the data are potentially prone to influence by differences in landscape and abiotic conditions among locations. Variation in probability of capturing insects may result in captures not being related to population trends and may affect treatment efficacy evaluations at the detriment to managing pest infestations. Research exploring how beetle captures vary among locations within and between food processing facilities and the relationships between captures in traps and other metrics of pest abundance would improve the utility of trapping data and help validate the trends reported here.

Although the ability to relate trap capture to actual pest densities would allow the development of economic thresholds, this is unlikely to be achieved considering the difficulty in quantifying definite densities within structures. Controlled experimentation expanding the understanding of factors that influence insect trap capture would help mill personnel determine whether changes in capture indicate changes in actual density or are caused by other confounding factors. Such an increased understanding of pest dynamics has the potential to prevent unnecessary fumigations. With the phasing out of MB, any reduction in fumigation frequency will ease the transition to alternative pest management tactics.

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