Long-Term Monitoring of *Tribolium castaneum* in Two Flour Mills: Seasonal Patterns and Impact of Fumigation

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ABSTRACT Data from long-term *Tribolium castaneum* (Herbst) pheromone trapping programs in two flour mills was used to evaluate the impact of structural fumigations ($n = 23$) on pest populations. The two mills differed in mean number of beetles captured and proportion of traps with captures of one or more beetles, but in one of the mills the mean number of beetles captured was reduced after implementing a more intensive integrated pest management program. Mean number of beetles per trap and proportion of traps with captures increased by 52.7 ± 8.2 and 24.8 ± 4.7% from one monitoring period to the next but decreased by 84.6 ± 4.6 and 71.0 ± 5.1% when fumigation occurred between periods, respectively. Mean number of beetles per trap and proportion of traps with captures immediately after fumigation were both positively correlated with number captured per trap and proportion of traps with captures in the monitoring period immediately before fumigation. Mean daily air temperature inside the mill fluctuated with the season, and although always warmer than the outside temperature, the relative difference varied with season. Relationship between inside and outside temperature could be explained well by an exponential equation with the parameters a = 20.43, b = 2.25, and c = −15.24 ($r^2 = 0.6983$, which is 94% of the maximum r^2 obtainable). Although outside temperature differed between spring and fall fumigations, inside temperature and reduction in beetle captures was not affected by season. A better understanding of pest populations and the impact of structural treatments within commercial food facilities is critical for improving the management of pest populations and for the adoption of methyl bromide alternatives.

KEY WORDS *Tribolium castaneum*, flour mill, fumigation, methyl bromide, monitoring

Flour mills are facilities that process whole kernels of wheat, *Triticum aestivum* L., into different fractions through a series of breaking, shifting, and rolling steps to produce flour and other products. The red flour beetle, *Tribolium castaneum* (Herbst), and confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), are the major pest species of mills worldwide. They are difficult to monitor and manage, in part because they can exploit hidden refugia where food material accumulates (e.g., in equipment, ledges, wall voids, cracks, and crevices) and move from there into the product either as it is being milled or stored as finished product. Because of these characteristics, the milling industry has relied on periodic structural treatments such as fumigation or heating that have the ability to penetrate into refugia to eliminate or reduce infestations (Bell 2000, Fields and White 2002). Because of limited monitoring information, lack of economic thresholds, and limited flexibility in treatment timing, structural treatments have typically been performed on a calendar basis.

Methyl bromide has been the predominant structural fumigant used to manage *Tribolium* and other pest species in flour mills, but it is an ozone-depleting substance and its use is being phased out worldwide under the 1987 Montreal Protocol on Substances that Deplete the Ozone Layer (Fields and White 2002). Except for quarantine and preshipment treatments, methyl bromide use in the United States has continued to a limited extent through allowances under the critical use exemptions (CUE) process. The CUE process has enabled the continued use of methyl bromide in flour mills to allow additional time to find technically and economically viable alternatives. Methyl bromide in laboratory studies is highly effective, because it rapidly kills all stages of many pest species (Bell 1988). However, published data on its field efficacy is limited (Campbell and Arbogast 2004, Toews et al. 2006, Small 2007). Lack of information on the effectiveness of

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methyl bromide fumigation and alternative pest control methods in mills has hampered the adoption of alternatives and limited optimization of structural treatments.

Alternatives to methyl bromide structural fumigations include sulfuryl fluoride, heat, and a combination of integrated pest management (IPM) tactics. Sulfuryl fluoride (ProFume, Dow AgroSciences LLC, Indianapolis IN) is an alternative structural fumigant with physical properties similar to those of methyl bromide (Cryer 2008, Chayaprasert et al. 2009) and is becoming more widely used as a structural fumigant for stored-product insects. Like methyl bromide, there is limited published research on its efficacy against natural infestations in mills (Small 2007). Heat treatments have been used for many years and involve heating the structure above the lethal temperature of the insects and holding this temperature long enough for the heat to penetrate into the structure (Fields and White 2002, Beckett et al. 2007). Sanitation, insecticide sprays and aerosols, sealing doors and windows, temperature management, product rotation, and other tactics are commonly used enhanced IPM techniques that can reduce the need to perform a structural treatment.

Evaluating fumigation efficacy in commercial food facilities is challenging for a variety of reasons, e.g., cryptic habitats exploited by pests make monitoring density imprecise; survival within treatment area and recolonization from untreated areas can both occur and are difficult to separate; and variation in facility type, building structure, geographic location, environmental conditions inside and outside, pest population density and distribution, and other ongoing management tactics can all impact efficacy. The variation among mills and temporal variation within a mill makes it difficult to replicate structural treatments. To incorporate these sources of variation into analysis and determine their relative importance in terms of efficacy, data should be collected from a large number of fumigations at both multiple locations and multiple times at the same location.

In this study, we evaluate the results of a long-term *T. castaneum* monitoring project from two flour mills in the same geographic area where fumigations were routinely performed. Over the course of this monitoring project a total of 23 fumigations were performed, typically in the spring and the fall, which provides a unique opportunity to evaluate temporal patterns in *T. castaneum* populations, as measured using pheromone/kairomone trapping; the impact of fumigations on pest populations; and the influence of season on beetle captures in traps and fumigation efficacy. This analysis incorporates some data originally included in Campbell and Arbogast (2004) and Toews et al. (2006), but here we include 15 additional fumigations and four to five additional years of continuous monitoring data. We use trends in beetle capture to estimate population trends in pest populations with the caveat that capture in traps may not accurately estimate pest population density in the structure. First, the general patterns in beetle capture, between the two mills and using the combined data from both mills, is evaluated to determine trends in capture in the absence of fumigation. Second, change in beetle captures after fumigation is used as a measure of fumigation efficacy. The impact of temperature and season on both types of data also were assessed. We analyzed two measures of pest population abundance and distribution derived from the trapping data: the mean beetle capture which gives us an estimation of the number of individuals present and the proportion of traps with captures of one or more beetles that provides a measure of the how widely distributed the beetles are within the mill (Toews et al. 2006). Campbell et al. (2010) evaluates rebound of *T. castaneum* populations after fumigation at these mills.

Materials and Methods

Flour Mills. Mill 1 was a flour mill ($\approx$4,531 m^3) with five floors, $\approx$179 m^2 per floor, and was attached to an elevator with bulk grain silos and a packaging/warehouse building. On the property surrounding the mill were office and receiving buildings, sheds, bunker storage, and another grain elevator. This mill was monitored continuously between July 2002 and December 2008. During this period, 11 structural fumigations were performed, with 10 complete interfumigation periods of monitoring data. Nine fumigations were with methyl bromide and two were with sulfuryl fluoride (Table 1). The packaging/warehouse building was usually fumigated at the same time (data not shown). Other pest management tactics were performed as part of an IPM program during the monitoring period, including targeted aerosol applications with pyrethrins (handheld aerosol applicator), and spray applications of cyfluthrin (Tempo SC Ultra, Bayer Corp., Kansas City, MO) (11.8 ml/3.8 liter) and bifenthrin (Talstar One, FMC Corp., Philadelphia PA) (14.8 ml/3.8 liter). Approximately halfway through the monitoring period (November 2004), the IPM program was improved by adding regular aerosol treatments with 1 or 3% synergized pyrethrins (Entech Fog-10 or Entech Fog-30, Entech Systems, Kenner, LA) (29.6 ml/28.3 m^3) and methoprene (Diacon II, Wellmark International, Schaumburg, IL) (3.0 ml/283.2 m^3), enhanced sanitation, and targeted sanitation and residual insecticide application in areas where pheromone trap captures were elevated.

Mill 2 was a wheat processing mill ($\approx$11,242 m^3) with five floors, each $\approx$240 m^2, in area, with two attached structures, a warehouse and a building for producing a grain-based product. An office, receiving buildings, and an animal feed mill (operational during part of the study) also were located on the property. The flour mill was monitored continuously between March 2003 and December 2008. During this period, 12 structural fumigations were performed, with 11 complete interfumigation periods of monitoring data. Mill 2 was fumigated twice a year with methyl bromide—in spring and fall (Table 2). The warehouse building and other parts of the structure were not fumigated but were typically treated with dichlorvos (VAP-20 with CO_2, Chem-Tech, Des Moines, IA) or

Table 1. Fumigations performed at mill 1 and their impact on the trap captures of *T. castaneum*

Date	Treatment[a] gas type (application rate (/m^3), ≈ exposure time)	Inside air temp (°C)[b] (min/x̄/max)	Outside air temp (°C)[b] (min/x̄/max)	Wind speed (km/h) (x̄ ± SEM)	No. captured before fumigation (x̄ ± SEM)	No. captured after fumigation (x̄ ± SEM)	Reduction in beetle captures (%) after fumigation	Proportion traps with captures before fumigation	Proportion traps with captures after fumigation	Reduction (%) in proportion after fumigation
13 July 2002	MB (20 g, 24 h)	na	15/22/29	12 ± 1	11.9 ± 1.5	2.1 ± 0.4	83	0.96	0.70	27
16 Nov. 2002	MB (na[c])	na	−2/6/14	13 ± 1	7.8 ± 1.0	0.2 ± 0.1	97	0.85	0.22	75
28 June 2003	MB (20 g, 24 h)	26/29/32	14/22/30	18 ± 1	24.6 ± 2.7	3.8 ± 0.7	84	0.98	0.71	28
23 Aug. 2003	MB (na)	32/35/39	22/30/39	14 ± 1	31.1 ± 5.0	1.1 ± 0.2	96	0.96	0.47	51
19 June 2004	SF[d] (32 g, 19 h)	19/23/24	12/14/18	15 ± 1	45.9 ± 14.5	0.7 ± 0.2	98	1.00	0.29	71
4 Sept. 2004	MB (na)	na	20/27/33	31 ± 1	60.8 ± 8.1	2.8 ± 0.4	95	1.00	0.74	25
11 June 2005	SF (111 g, 18 h)	18/25/29	16/21/27	12 ± 1	3.8 ± 2.1	0.2 ± 0.1	96	0.53	0.04	93
12 Nov. 2005	MB (24 g, 18 h)	14/22/27	10/16/20	22 ± 2	1.6 ± 0.4	0.2 ± 0.0	88	0.38	0.20	47
4 Nov. 2006	MB (24 g, 18 h)	19/26/33	4/10/17	10 ± 1	0.1 ± 0.1	0.0 ± 0.0	100	0.05	0.00	100
3 Nov. 2007	MB (24 g, 18 h)	18/23/31	−5/6/17	14 ± 1	2.5 ± 0.3	0.1 ± 0.0	95	0.87	0.09	89
15 Nov. 2008	MB (26 g, 18 h)	22/23/23	−1/3/8	18 ± 1	1.0 ± 0.2	0.2 ± 0.2	77	0.45	0.05	85
x̄ ± SEM		26 ± 1	16 ± 3	16 ± 2	17.4 ± 6.2	1.0 ± 0.4	92 ± 2	0.73 ± 0.1	0.32 ± 0.09	63 ± 8

[a] Methyl bromide (MB) or sulfuryl fluoride (SF) applied at a given rate and approximate exposure time (start shooting gas to begin aeration).
[b] Data collected hourly for length of the fumigation, temperature data inside obtained from data loggers, and outside temperature and wind speed from local weather station.
[c] Fumigation service report on rate and exposure time not available.
[d] Sulfuryl fluoride target concn/time (CT) of 428 g-h/m^3, average CT achieved of 427 g-h/m^3 (averaged across floors), and half loss time of 15.6 h (averaged across floors).

Table 2. Fumigations performed at mill 2 and their impact on the trap captures of *T. castaneum*

Date	Treatment[a]	Inside air temp (°C)[b] (min/x̄/max)	Outside air temp (°C)[b] (min/x̄/max)	Wind speed (km/h) (x̄ ± SEM)	No. captured before fumigation (x̄ ± SEM)	No. captured after fumigation (x̄ ± SEM)	Reduction in beetle captures (%) after fumigation[c]	Proportion traps with captures before fumigation	Proportion traps with captures after fumigation	Reduction (%) in proportion after fumigation
21 May 2003	MB	20/21/21	9/17/24	15 ± 1	0.5 ± 0.3	0.2 ± 0.2	51	0.17	0.05	68
7 Oct. 2003	MB	25/26/27	13/20/27	27 ± 1	0.5 ± 0.3	0.1 ± 0.1	89	0.13	0.04	68
20 April 2004	MB	na	7/16/24	17 ± 2	0.1 ± 0.1	0.0 ± 0.0	100	0.04	0.00	100
11 Oct. 2004	MB	22/23/23	9/13/17	14 ± 1	34.4 ± 17.6	4.3 ± 4.1	87	0.83	0.17	80
4 April 2005	MB	19/20/21	9/19/28	13 ± 1	3.3 ± 1.1	0.3 ± 0.3	91	0.48	0.04	92
16 Oct. 2005	MB	26/27/28	12/20/27	20 ± 1	12.7 ± 3.0	0.9 ± 0.4	93	0.81	0.40	50
10 April 2006	MB	21/23/24	na	39 ± 2	0.2 ± 0.1	0.5 ± 0.3	0	0.23	0.11	50
13 Nov. 2006[d]	MB	21/23/23	−2/7/12	11 ± 1	2.3 ± 0.7	0.4 ± 0.3	84	0.58	0.08	87
1 May 2007	MB	25/27/29	11/18/23	16 ± 1	2.2 ± 0.7	0.6 ± 0.4	71	0.46	0.19	58
7 Nov. 2007	MB	21/24/27	1/11/19	15 ± 2	11.8 ± 2.7	0.3 ± 0.3	98	0.88	0.04	96
24 June 2008[e]	MB	28/29/31	16/24/30	17 ± 1	1.6 ± 0.6	0.3 ± 0.3	83	0.46	0.04	92
4 Nov. 2008	MB	25/27/28	12/18/23	39 ± 2	0.5 ± 0.2	0.0 ± 0.0	100	0.27	0	100
x̄ ± SEM		24 ± 1	17 ± 1	20 ± 3	5.8 ± 2.9	0.7 ± 0.3	78 ± 8	0.44 ± 0.08	0.10 ± 0.03	78 ± 6

[a] Methyl bromide (MB) applied at rate of 24 g/m^3 and ≈20-h exposure time (start shooting gas to begin aeration) for all fumigations for which service report information is available.
[b] Data collected hourly for length of the fumigation, temp data inside obtained from data loggers and outside temp and wind speed from local weather station.
[c] If trap captures after are higher than before fumigation, presented as reduction of zero, rather than using negative numbers.
[d] Traps remained in mill during fumigation, so for this comparison it is the last full monitoring period before the fumigation compared with the first full monitoring period after the fumigation, with monitoring period spanning the fumigation excluded.
[e] Mill was shut down for 3 mo before the fumigation and an extensive sanitation program was conducted before the fumigation.

pyrethrin aerosol insecticides at the same time as the mill was fumigated. The IPM program at the facility included sanitation, regular spray applications of 0.05% cyfluthrin (Tempo SC Ultra), and occasional aerosol treatments with dichlorvos and pyrethrins.

Insect Monitoring Program. Red flour beetles inside the mill were monitored using pitfall traps placed on the floor (Dome traps, Trécé Inc., Adair, OK) containing pheromone lures (Trécé Inc.) for *Tribolium* spp. [*T. castaneum* and *Tribolium confusum* (Duval) (Coleoptera: Tenebrionidae)] and food oil attractant (Trécé Inc.). At mill 1, pheromone lures for *Trogoderma* spp. [*T. variabile* and *Trogoderma granarium* Everts (Coleoptera: Dermestidae)] also were placed in traps. Although traps captured a range of stored-product pest species, only data for *T. castaneum* are presented because it was the primary pest of the mills and the species most clearly established within the structures (Campbell and Arbogast 2004; Toews et al. 2006).

There were 11 trapping locations on each floor of mill 1, for 55 locations in total. Nine of the locations on each floor were roughly equidistant from each other along the outer walls and two were placed near walls, pillars or pieces of equipment in the interior space. In mill 2, there were 32 traps in total in the mill positioned along perimeter walls or under equipment, with five traps each on the first, second and fourth floors and six traps each on third and fifth floors. At both facilities, additional monitoring was conducted in other structures and using different trap types and pheromone lures for other species, but only data on *T. castaneum* captured in Dome traps are reported here.

Traps at both mills were typically serviced every 2 wk and insects either removed from the traps or the traps replaced, and the insects captured were identified and counted in the laboratory. Pheromone lures were replaced every 2 mo. The length of the trapping intervals sometimes varied, usually due to the scheduling of fumigations, but all capture data have been standardized to a 2-wk trapping interval to facilitate comparison. From these data, two measures of *T. castaneum* prevalence were used: the average number of beetles captured per trap per standardized 2-wk period (beetles per trap per period) and the proportion of the traps that captured one or more beetles per standardized 2-wk period.

Temperature Monitoring. At both mills, inside temperatures on each floor of the mill were recorded hourly using data loggers (HOBO H8 family, Onset Computer Corp., Pocasset, MA) situated 1.5 m above the floor. The hourly temperature data from the data loggers was averaged and then used to calculate mean daily temperatures for each mill. Outside daily mean, maximum, and minimum temperatures were obtained from weather stations located within five miles of the mills. The daily means are hereafter referred to simply as inside and outside temperatures. The mean temperatures during fumigation were calculated using the hourly temperature data collected during the period when the fumigation was performed.

Statistical Analysis. General Linear model Procedure (GLM) and Pearson Correlations were performed using SAS version 9 software (SAS Institute, Cary, NC). Data are presented as mean ± SEM. Proportional data were arcsine square root transformed before analysis, but untransformed data are presented.

Results

Comparison of Beetle Captures at Two Mills. To evaluate the baseline differences in abundance and distribution of *T. castaneum* between the two mill locations, mean beetle capture and proportion of traps with captures were determined for the time periods during which the two monitoring programs overlapped (from March 2003 to December 2008). Mean number captured during a monitoring period was greater in mill 1 (4.5 ± 0.7, ranging from 0 to 61 beetles per trap per period; $n = 151$) compared with mill 2 (2.6 ± 0.4, ranging from 0 to 34 beetles per trap per period; $n = 147$) ($F = 4.72$; df = 1,296; $P = 0.0307$). The mean proportion of the traps that captured one or more *T. castaneum* was also greater in mill 1 than mill 2; 0.49 ± 0.03 (ranging from 0.00 to 1.00) at mill 1 and 0.33 ± 0.02 (ranging from 0.00 to 0.96) at mill 2 ($F = 21.35$; df = 1,296; $P < 0.0001$). Focusing just on the time period after the implementation of enhanced IPM at mill 1 (November 2004), the mean number of *T. castaneum* captured was less in mill 1 (1.2 ± 0.1 beetles per trap per period; $n = 106$) than in mill 2, which maintained a relatively unchanged mean capture number (3.1 ± 0.4 beetles per trap per period; $n = 104$) ($F = 16.76$; df = 1,208; $P < 0.0001$), and the proportion of traps with captures was not different between the mills: 0.35 ± 0.03 and 0.39 ± 0.03 for mills 1 and 2, respectively ($F = 1.23$; df = 1,208; $P = 0.2682$).

T. castaneum captures (mean number captured and proportion of traps with captures) at both mills fluctuated considerably over the course of this monitoring program, but visual assessment indicates that fumigation events rather than seasonal changes had the greatest impact on the populations (Fig. 1 and 2). To statistically evaluate this pattern, the combined data from both mills was used and the average change in number captured between two sequential monitoring periods was calculated. There was an increase of 52.7 ± 8.2% ($n = 286$) in the absence of a fumigation between monitoring periods and a decrease of 84.6 ± 4.6% ($n = 23$) when a fumigation occurred ($F = 19.96$; df = 1,307; $P < 0.0001$). The average change in the proportion of traps with captures between two sequential monitoring periods increased by 24.8 ± 4.7% ($n = 285$) in the absence of a fumigation and decreased by 71.0 ± 5.1% ($n = 23$) when a fumigation occurred ($F = 33.23$; df = 1,306; $P < 0.0001$). The two mills did not differ in the average percentage of change in the mean number of beetles captured per trap between sequential monitoring periods ($F = 1.14$; df = 1,284; $P = 0.2859$) (mill 1: increase of 44.7 ± 9.2%; mill 2: increase of 62.2 ± 14.1%) or the proportion of traps with captures ($F = 2.29$; df = 1,283; $P = 0.1315$) (mill 1: increase of 18.3 ± 4.7%; mill 2: increase of 32.5 ± 8.5%). Comparing

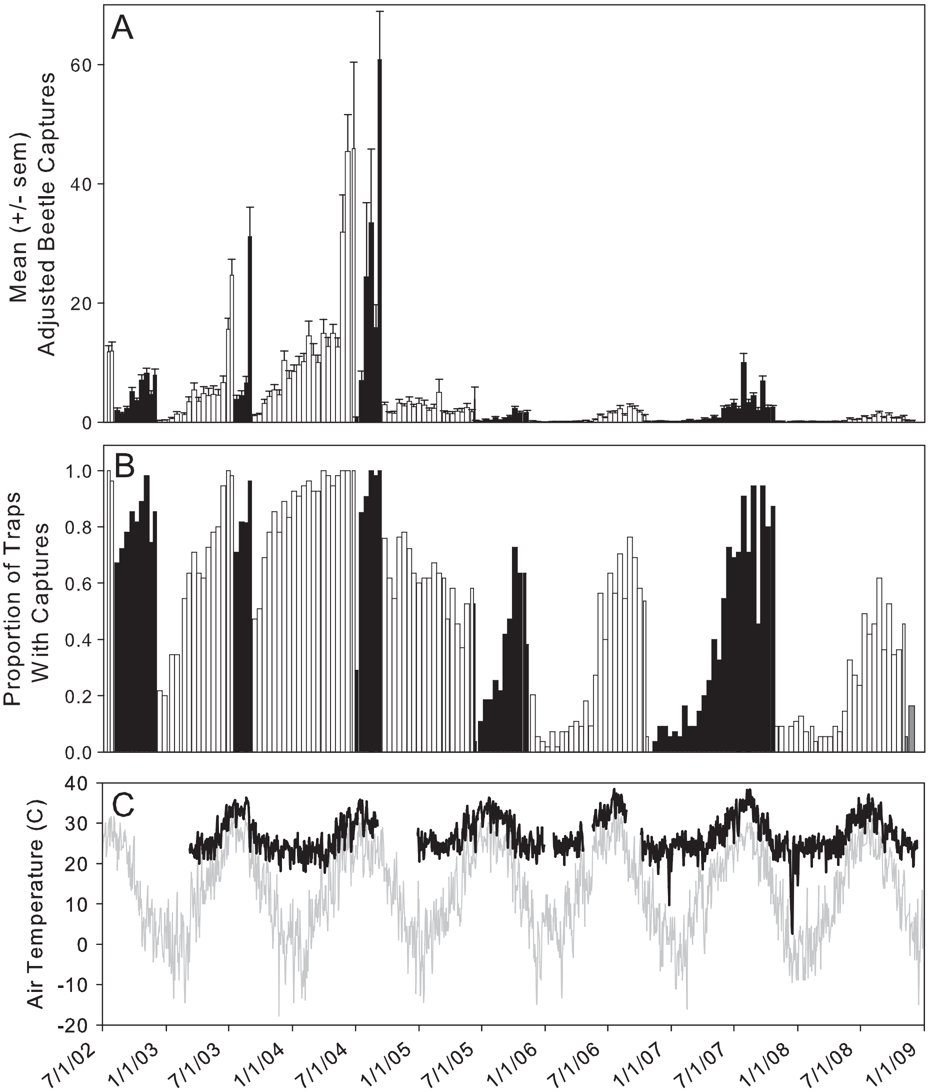

Fig. 1. Red flour beetle captures (mean beetle capture [A] and proportion of traps with capture of one or more adults [B]) and the indoor and outdoor daily average air temperatures (C) at mill 1. Black and white colored bars in A and B are used to indicate periods between fumigations. In C, the black line is the daily mean temperature inside the mill and the gray line is the daily mean temperature outside the mill.

before and after the enhanced pest management program in mill 1, there was no significant difference in the percentage of change in mean beetle captures ($F = 2.24$; df = 1,153; $P = 0.1369$) or proportion of traps with captures ($F = 1.73$; df = 1,153; $P = 0.1902$); 63.7 ± 20.0% increase before ($n = 53$) and 34.8 ± 9.3% increase after ($n = 102$) for change in mean beetle capture and 9.6 ± 4.2% increase before and 22.7 ± 6.8% increase after for change in proportion of traps with captures.

Temperature and Beetle Capture. Temperature recorded inside and outside the mill showed a more or less regular pattern of seasonal fluctuation in which inside temperature was always higher than that outside (Figs. 1C and 2C), but the difference was less during the warm season (April–September) than during the cool season (October–March). Both mills had similar inside temperatures ($F = 3.80$; df = 1, 3,949; $P = 0.0512$) and outside temperatures ($F = 1.89$; df = 1, 4,247; $P = 0.1696$) (Table 3). At mill 1, the difference between inside and outside temperature was 8.3 ± 0.1°C in the warm season and 18.5 ± 0.2°C in the cool season. The corresponding values for mill 2 were 10.0 ± 0.1 and 19.2 ± 0.2°C. The relationship between inside and outside temperatures for the combined mills fit an exponential equation with the parameters a = 20.43, b = 2.25, and c = −15.24 ($r^2 = 0.6983$, which is 94% of the maximum r^2 obtainable) (Fig. 3A). Mill temperature was relatively stable at cooler outside temperatures (<10°C), with lower then expected out-

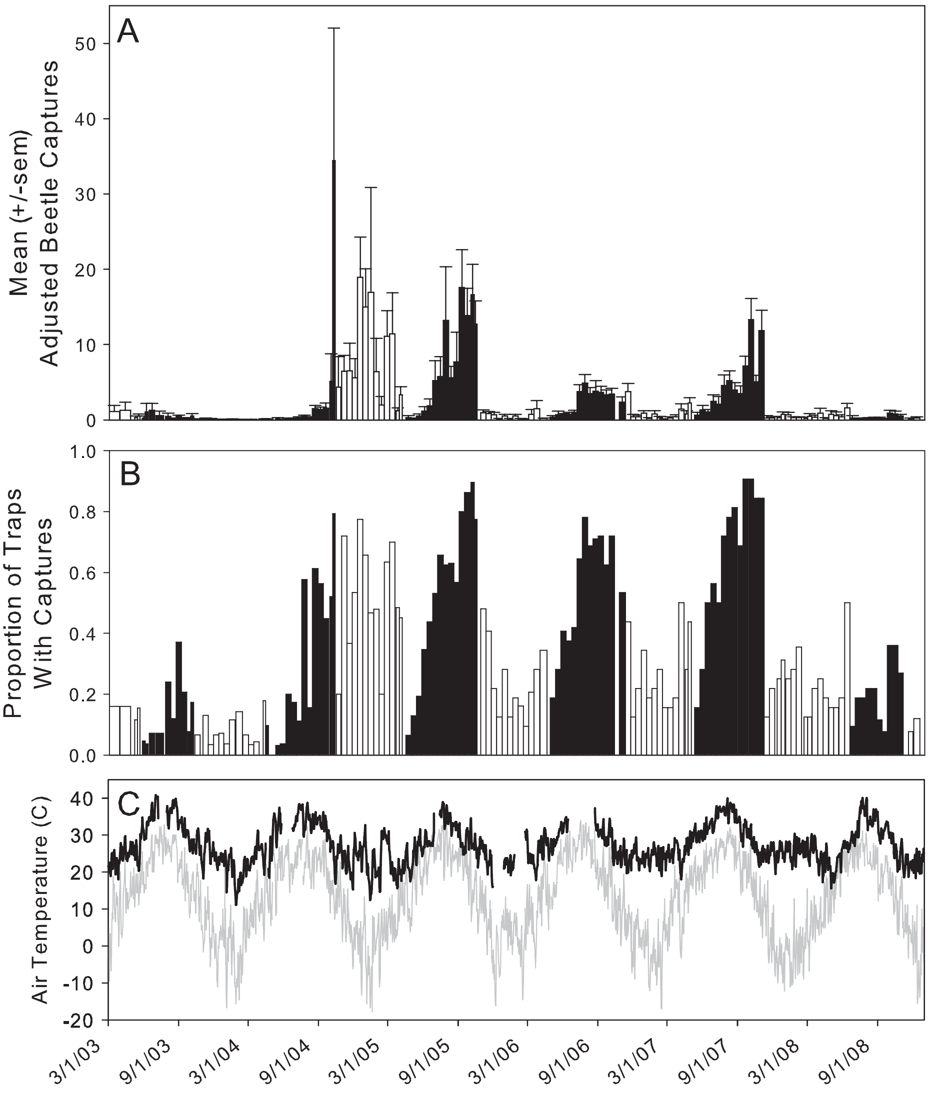

Fig. 2. Red flour beetle captures (mean beetle capture [A] and proportion of traps with capture of one or more adults [B]) and the indoor and outdoor daily average air temperatures (C) at mill 2. Black and white colored bars are used to indicate periods between fumigations. In C, the black line is the daily mean temperature inside the mill and the gray line is the daily mean temperature outside the mill.

Table 3. Daily average air temperature (Celsius) for the two mills used in monitoring project[a]

Mill	Location	Both seasons	Warm season[b]	Cool season[c]
1	Outside	13.4 ± 0.2 (2116) −17.8 to 33.3	21.0 ± 0.2 (1092) −2.2 to 33.3	5.3 ± 0.2 (1024) −17.8 to 27.2
	Inside	26.8 ± 0.1 (1997) 2.6–38.5	29.6 ± 0.1 (975) 9.9–38.5	24.0 ± 0.1 (962) 2.6–32.4
2	Outside	12.9 ± 0.2 (2133) −17.8 to 33.9	20.9 ± 0.2 (1084) −2.8 to 33.9	4.7 ± 0.2 (1049) −17.8 to 27.8
	Inside	27.2 ± 0.1 (1957) 11.1–40.8	30.3 ± 0.2 (961) 15.6–40.8	24.2 ± 0.1 (996) 11.1–34.5

[a] Data presented as mean ± SEM (n) temperature in the first row and range from low to high temperature in the second row.
[b] Warm season from April to September.
[c] Cool season from October to March.

liers occurring during short periods when the mills were shut down, but mill temperature increased with outside temperature at warmer outside temperatures (>10°C). The average relative humidity inside was 32 ± 16 and 29 ± 12% in mills 1 and 2, respectively.

There were some significant correlations between beetle capture and temperature (Fig. 3). The proportion of traps with captures was correlated with outside temperature in both mill 1 (n = 168; r = 0.3309, $P <$ 0.0001) and mill 2 (n = 134; r = 0.3180, P = 0.0002). In mill 1, the proportion of traps with captures was also correlated with the daily mean inside temperature (n = 140; r = 0.2249, P = 0.0075) but not in mill 2 (n = 67; r = 0.0111, P = 0.9287). Mean beetle capture was correlated with outside temperature in mill 1 (n = 168;

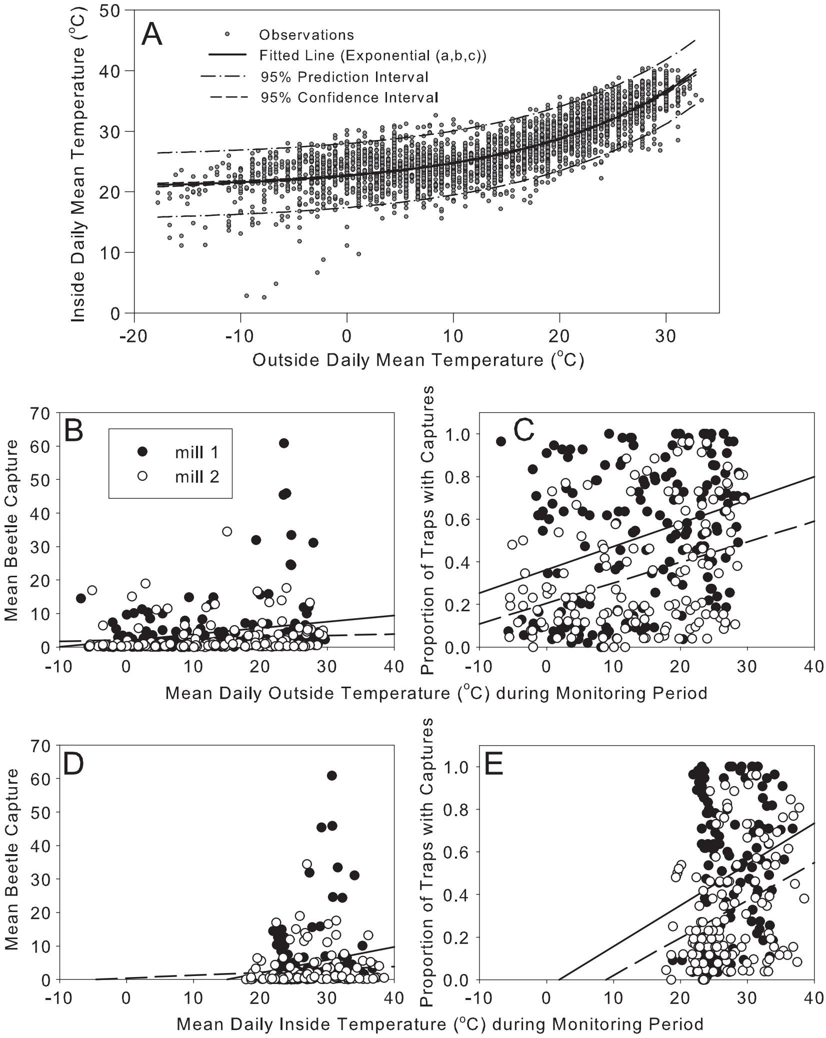

Fig. 3. Relationship (exponential functions with values a = 20.43, b = 2.25, and c = −15.24 [r^2 = 0.6983]) between inside and outside daily mean temperature for the combined data from both mills (A); relationships between red flour beetle mean beetle capture (B) and proportion of traps with captures of one or more individuals (C) and mean daily outside temperature during the monitoring period over which the traps were placed in the mills; and the relationships between mean beetle capture (D) and proportion of traps with captures (E) and mean daily inside temperature during the monitoring period. Solid regression line is for mill 1 data and dashed regression line is for mill 2 data.

r = 0.2150, P = 0.0051) but not in mill 2 (n = 134; r = 0.0835, P = 0.3375). There was no correlation between mean beetle capture and indoor temperature in either mill (mill 1: n = 140; r = 0.1617; P = 0.0563 and mill 2: n = 67; r = 0.0633, P = 0.6105).

Fumigation Efficacy: Reduction in Beetle Captures. Reduction in mean beetle capture and proportion of traps with captures related with each fumigation and the average for each mill are shown in Tables 1 and 2. For the combined fumigations from both mills (n = 23), there was an 84.6 ± 4.6% reduction in beetles per trap per period after fumigation. The two mills did not differ from each other in the percentage of decrease in mean *T. castaneum* capture after fumigation (F = 1.42; df = 1,21; P = 0.2464). The average beetle capture before (F = 2.98; df = 1,21; P = 0.0991) and after (F = 2.14; df = 1,21; P = 0.1579) fumigation was not different between mills. For the combined mills, 11.4 ± 3.5 beetles per trap per period were captured in the period immediately before fumigation compared with 0.8 ± 0.2 beetles per trap per period immediately after. Across both mills, there were only three fumigations when no adults were recovered in the pheromone traps during the monitoring period that immediately followed fumigation. The mean number of beetles per trap per period immediately after fumigation was significantly correlated with mean number captured (n = 23; r = 0.681, P = 0.0003) and proportion of traps with captures (n = 23; r = 0.575, P = 0.0041) in the monitoring period immediately before fumigation.

For combined mills (n = 23), there was a 70.9 ± 5.1% reduction in the proportion of traps with captures after fumigation. Percentage of reduction in proportion of traps with captures after fumigation was not different between the mills (F = 1.42; df = 1,21; P = 0.2464), but mill 1 had a higher proportion of traps with captures immediately before (F = 5.00; df = 1,21; P = 0.0363) and after (F = 6.16; df = 1,21; P = 0.0216) fumigation than mill 2. The mean proportion of traps with captures was 0.58 ± 0.07 and 0.20 ± 0.05 for the monitoring periods immediately before and after fumigation, respectively. There was a significant positive correlation between the proportion of traps with captures immediately after fumigation and both the mean number before fumigation (n = 23; r = 0.688, P = 0.0003) and proportion of traps with captures before fumigation (n = 23; r = 0.706, P = 0.0002).

Most fumigations used methyl bromide, but two sulfuryl fluoride fumigations were performed in the spring at mill 1: one fumigation at a low rate and the other fumigation at 3 times this rate (Table 1). The two sulfuryl fluoride fumigations gave 98 and 96% reductions in mean beetle capture, respectively, which was similar to methyl bromide fumigations (84 ± 5%; n = 21) overall and with just those performed in the spring (69 ± 13%; n = 7). A similar pattern was found for the reduction in proportion of traps with captures after fumigation: 71 and 93% reduction for the low and high rate sulfuryl fluoride fumigations, respectively, compared with 70 ± 5% (n = 21) and 70 ± 10% (n = 7) for the methyl bromide total and spring fumigations, respectively. Due to lack of replication, formal comparisons are not possible but the general trends justify the combining of two different fumigants in the overall analysis.

To determine whether there were differences in temperature and fumigation efficacy with time of year the fumigation was performed, fumigations were sorted into spring (April–June) (n = 9) and fall (October–December) (n = 11) periods. Summer (July–September) (n = 3) fumigations were not included in this analysis because they only occurred at one of the mills, had limited replication, and were not typical times when fumigations are scheduled for these mills. There was no difference between the mills in the inside and outside temperatures during fumigation for either season (P > 0.05). For the combined mills, outside temperature during fumigation differed between the two seasons (F = 8.90; df = 1,16; P = 0.0083); fall fumigations (11.8 ± 1.8°C) had lower outside temperatures than spring fumigations (18.9 ± 1.2°C). However, indoor temperatures during fumigations did not differ between spring (24.6 ± 1.2°C) and fall (24.4 ± 0.6°C) (F = 0.03; df = 1,16; P = 0.8625).

There was no difference between the seasons in reduction in the mean number of beetles captured (F = 2.86; df = 1,18; P = 0.1083) or the proportion of traps with beetles (F = 0.59; df = 1,18; P = 0.4528) after a fumigation. The mean reduction in beetle captures was 91.6 ± 2.2 and 74.9 ± 10.8%, and mean reduction in proportion of traps with captures was 79.7 ± 5.5 and 72.4 ± 8.04% for the fall and spring fumigations, respectively. There was no difference in the reduction in beetle captures between the mills for either spring or fall fumigations (P > 0.05). The beetle captures immediately after fumigation were evaluated by season as well because this is an estimate of the founding population that can contribute to population rebound. There was no difference in the mean number of beetles captured (F = 0.05; df = 1,18; P = 0.8226) or the proportion of traps with captures (F = 0.34; df = 1,18; P = 0.5649) immediately after a fumigation between the spring and fall fumigations.

Analysis of the relationship between beetle capture and temperatures inside and outside the mill during fumigation (Tables 1 and 2) showed no significant correlation between minimum, mean, or maximum temperature inside the mill and the percentage of reduction in mean beetle capture (P > 0.05). However, there were significant correlations between minimum (n = 22; r = −0.525, P = 0.0121), mean (n = 22; r = −0.521, P = 0.0130), and maximum (n = 22; r = −0.484, P = 0.0226) temperature outside the mill and percentage of reduction in the proportion of traps with captures. The percentage of reduction in the proportion of traps with captures tended to be less with fumigation at warmer outside temperatures than at lower outside temperatures.

Discussion

All of the fumigations were considered successful in that the fumigants reached target concentrations and were held for the target exposure times, but there was considerable variation in both the percentage of reduction in *T. castaneum* captures per trap and proportion of traps with captures after the fumigation. The two mills differed structurally and in their pest management programs, and although they differed in the average beetle captures and proportion of traps with captures, they did not on average differ from each other immediately before fumigation, nor in the reduction in beetle captures after fumigation. The average reduction in beetles per trap per period after fumigation was 84.6 ± 4.6%, and only rarely were no adults captured in the monitoring period immediately after fumigation. There was considerable variation in the average number of beetles captured before treatment, with some fumigations occurring at relatively low levels where structural treatments would perhaps not be warranted.

Capture of adult *Tribolium* in traps in the period immediately after methyl bromide or sulfuryl fluoride fumigation has been reported previously (Campbell and Arbogast 2004, Toews et al. 2006, Small 2007), even though adults are among the more susceptible stages (Hole 1981). Small (2007) found >90% reduction in average beetle captures of *Tribolium* spp. 2 wk after treatment in four mills fumigated with either methyl bromide or sulfuryl fluoride. Results of only two sulfuryl fluoride fumigations are reported here, and each used a different rate, so ability to draw conclusions is limited. However, the results in terms of their initial impact on the beetle captures were consistent with the methyl bromide fumigations at these mills and with the findings of Small (2007). However, the egg stage is more resistant to sulfuryl fluoride and the results of egg survival would not become apparent until >2 wk after treatment. Although the number of fumigations at each mill provides a good indication of the average impact of the treatments at these locations and are consistent with other studies, further research at other mill locations is needed to confirm the generality of the findings.

Presence of adult *T. castaneum* in traps within the mill immediately after fumigation could result from two nonmutually exclusive general mechanisms: survival of treatment within structure or movement into structure after treatment. Insect survival within mill could indicate either a failure of the fumigation to reach target gas concentration and time (CT) in the mill as a whole (not supported by fumigation reports) or in certain microhabitats within the mill or that the target CT was insufficient to cause 100% mortality in the tested populations. Spatial variation in CT achieved combined with variation in temperature within structures may have generated conditions where survival could occur. Also, some hidden refugia containing *T. castaneum* may be more difficult for the gas to penetrate into either because they are sealed off from the gas or because sufficient food material was present (e.g., inside a wall void, holding tank for bulk product) to limit the ability of the gas to penetrate or to allow for increased adult survival. The presence of food material will increase survival of adult *T. castaneum* after exposure to certain contact and aerosol insecticides (Arthur 2000, Arthur and Campbell 2007). The capture of adults within 2 wk of treatment suggests that the exposed stages that survived would have to be adults, pupae, or possibly late instars. Bell et al. (1988) reported that the pupa was the stage most resistant to methyl bromide. Adults are typically more sensitive to fumigants, so survival of the adults within the mill would indicate that a very low CT was achieved in some areas, although given the greater tolerance of pupae it could be that adults captured within traps immediately after fumigation were newly emerged. CTs providing control in the lab are lower than those targeted or measured in the mills studied here, but measurements were not taken in the hidden areas where lower CTs might have occurred. Although there is not much evidence for resistance or tolerance of methyl bromide by *Tribolium* species, there has been little recent research on the status within flour mills. Hole (1981) reported that *T. castaneum* strains collected from around the world varied considerably in susceptibility to methyl bromide when treated at a low dosage, but Rajendran (1992) reported that resistance to methyl bromide increased only slightly in laboratory selection experiments. An early survey in the United States found no variation in susceptibility to methyl bromide among field strains of *T. castaneum* (Lindgren and Vincent 1965). Thus, CTs achieved in certain microhabitats may have been insufficient to provide complete mortality either because target CT was not achieved and/or target CT was insufficient.

Beetles captured in a mill immediately after fumigation also may have colonized the mill from areas not fumigated, either from other parts of the facility (beetles being driven from the building and returning afterward or resident subpopulations that already occurred in these areas) or from off site and may have been physically moved into the building through human activity (bringing in infested grain or other products/equipment) or active immigration. Dispersal inside and outside food facilities and the ability to immigrate into facilities from outside has been shown to be important for some stored-product species (Campbell et al. 2002, Campbell and Arbogast 2004, Campbell and Mullen 2004), but *T. castaneum* was not recovered in large numbers outside of the mill 1 (Campbell and Arbogast 2004; J.F.C., unpublished data). Small (2007) reported *Tribolium* spp. recovered on the roof of one mill after treatment, suggesting that populations in untreated areas may be present on site. Graham (1970), in evaluating the reinfestation of bagged maize, *Zea mays* L., stores fumigated with methyl bromide, concluded that enough individuals escaped from under the tarp during fumigation and persisted in untreated areas of the building to cause rapid recolonization. Also, captures in traps generally increase after sanitation procedures (Roesli et al. 2003), such as occurs before fumigation, so this disturbance may have driven some insects into areas that were not treated, and this also could contribute to recolonization.

To begin evaluating the relative importance of pest survival within the mill versus immigration from outside during initial recovery of adults after fumigation, we can make some predictions of expected patterns and compare them to results obtained in this study. If immigration into mill from outside sources was the primary factor, then we predict that reduction in beetle captures after fumigation would be significantly affected by season because cooler outside temperatures should reduce immigration, especially by active dispersal. However, we did not detect a significant seasonal impact on the reduction in beetle captures after fumigation, although in some cases outside temperatures were correlated with inside beetle captures. Also, if survival of individuals inside the mill was the primary mechanism, then the number of beetles captured after fumigation would be positively related with the number present before treatment. In our analysis, the number of beetles per trap and the proportion of traps with captures after the fumigation were correlated with the mean number captured and the proportion of traps with captures immediately before fumigation. These patterns suggest that presence of beetles after fumigation is related more to the survival of the treatment by the population already present within the structure than by rapid replacement from outside sources as has been observed for other stored product species (Campbell and Arbogast 2004), although further research focused on this question is needed. Regardless of the mechanism behind the presence of adults in traps soon after fumigation, our results indicate that because fumigants provide no residual activity the process of population rebound is beginning immediately and management tactics focused on reducing the rate of rebound are likely to be critical in reducing the rate of population increase and time before a subsequent fumigation is required.

Information on temperature within commercial mills is lacking because monitoring programs do not usually include temperature information, and mill operations do not typically record this type of information. This type of information is critical for development of predictive mathematical population models for flour mills. Because temperature data are not available for most mills, temperature information from nearby weather stations will be very useful in estimating temperatures inside the mill for the purpose of developing population models and expert systems for the milling industry. The observed relationship between inside and outside temperatures indicated that a base temperature is maintained in the winter, regardless of outside temperature, and that during the summer temperatures inside tracked those outside but were always warmer (≈8°C warmer than outside temperature). Dyte (1965) found that temperature in three mills in England was related to heat production during milling and seasonal weather conditions, with temperatures inside equipment 6–7°C higher than in the air above the equipment. Additional data from other locations is needed to determine whether the trends observed here hold up in other geographic areas and other structures, but results do suggest the potential of this approach for making predictions about inside temperature.

Temperature variation can play a role in trapping *T. castaneum* within a mill. First, temperature affects the rate of development and thus the rate of population growth (Sokoloff 1974), which in turn would be expected to affect beetle captures. Generally, conditions within the mills were favorable for *T. castaneum* development throughout the year although differences in temperature would influence the rate of development. Average daily temperatures were within the range at which development can be completed (development from egg to adult can occur between 22°C and 40°C, with rate increasing with temperature; Howe 1965) for 92 and 83% of the days and in the optimal range (32–35°C; Howe 1965) for 18 and 11% of the days, for mills 1 and 2, respectively. *Tribolium* spp. have the ability to move to preferred temperature conditions within the mill (Graham 1958, Jian et al. 2005), and there is considerable temperature variation within a mill (J.F.C., unpublished data), so it is unknown what the specific environmental conditions were for the different subpopulations within the mill. Second, temperature can impact beetle capture efficiency, because beetle mobility is correlated with temperature (Surtees 1965) and the volatility of the attractants in the traps also is related to temperature. Temperature is also likely to influence the rate of immigration by beetles from outside sources. The temperature variation observed inside the mills seems insufficient to have had a large enough impact on beetle mobility to explain temporal patterns of beetle capture, and generally there were no relationships between daily temperatures and beetle captures over a monitoring period. Significant correlations between outside temperature and inside beetle captures occurred in some cases (Fig. 3), but generally relationships were not very strong; attempts to fit regression models to the data resulted in very low r^2 values. Changes in outside temperatures could influence immigration, but might cause differences in the spatial patterns of temperature and range of temperatures inside the mill and thus might indirectly influence inside beetle captures.

The temperatures inside the mills and the difference between inside and outside temperature can impact fumigation both in terms of gas loss from buildings (half-loss time) (Estes 1965, Chayaprasert et al. 2009) and mortality caused by gas concentration (Kenaga 1957). Fumigation effectiveness increases with temperature, but there was no significant correlation between inside temperature and reduction in beetle captures found in this study, probably because the range of temperatures was insufficient to detect an impact, although immigration of new individuals and variation in other environmental conditions also could confound detection of any potential impact. The average temperature inside during the fumigations was 26 and 24°C, for mills 1 and 2, respectively, and no fumigations were conducted outside recommended temperatures. Although there was considerable variation in outside temperatures and difference between inside and outside temperatures with season, there was no significant impact of reduction in either mean beetle capture or the proportion of traps with captures after fumigation. So although there is potential for environmental conditions to impact fumigation efficacy as measured using pheromone trapping, no major influence was found in this study.

Flour mills vary considerably in their size, physical layout, structural features, geographic location, and management programs, which makes replication difficult. The repeated fumigations in these two mills provide a unique opportunity to evaluate the impact of fumigation on *T. castaneum* populations while controlling some of the variance associated with different mills. Our results indicate that fumigation has a major impact on beetle capture of *T. castaneum*, which should be correlated with the impact on the hidden population in the mill but that the overall population within the mills is able to persist over time even with the frequent bottlenecks associated with the regular fumigations. Although 100% mortality rates are obtained in laboratory studies and in fumigation chambers, this is unlikely under the complex situations associated with structural fumigation. The presence of beetles within 2 wk after fumigation indicates that the process of population rebound begins immediately after treatment. Further evaluation of fumigations from other locations to determine why beetles are found immediately after treatment, and how postfumigation founder population and rate of population increase can be better managed is needed to facilitate the reduction in the need to resort to methyl bromide fumigations and structural treatments in general.

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