Structural fumigation efficacy against *Tribolium castaneum* in flour mills

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Abstract

Structural fumigations of food processing plants to manage stored-product insects have been a major component of pest management programs, but limited information on field efficacy is available. Efficacy, based on pheromone trapping data, consists of initial reduction in captures after treatment and recovery in trap captures over time after treatment (i.e., rebound). Patterns of *Tribolium castaneum* reduction and rebound were evaluated after 21 fumigations in two flour mills. Influence of time of year fumigation occurred, environmental conditions, and impact of other pest management tactics on efficacy was determined as well. Information generated can be used to guide fumigation decisions, including the development of risk thresholds for levels of pheromone trap captures.

Keywords: *Tribolium castaneum,* Fumigation, Flour mills, Efficacy, Methyl bromide

1. Introduction

It is challenging to evaluate the effectiveness of pest management tactics in food facilities such as mills, processing plants, and warehouses. Pest infestations are difficult to identify and sample because they are spatially and temporally patchy and typically in cryptic locations (Campbell, 2006). Pheromone trapping programs are widely used to determine temporal and spatial patterns of pest populations in food facilities, but since these traps primarily capture dispersing individuals trap captures are not necessarily related to actual population density and distribution (Arbogast and Mankin, 1999). To evaluate field efficacy, bioassay insects can be placed in facilities in areas where insects are thought to be located, but bioassay efficacy does not necessarily reflect the impact on the hidden pest population (Fields, 2007). Evaluation of efficacy is also complicated by the difficulty in replicating treatments given the many differences among facilities and within a facility through time, and in isolating the effects of single pest management tactics given the many other ongoing operations in commercial food facilities that can also impact pest populations. Small-scale laboratory studies that can be replicated and controlled more easily often do not adequately simulate the spatial and temporal patterns of exposure to treatments that occur under more real world conditions (Toews et al., 2009).

As a consequence of these issues, pest management in the food industry often relies on calendar-based application of pesticides or other control tactics rather than using monitoring information to guide management decisions. The lack of information on efficacy has also hampered the adoption of new technologies and more effective integrated pest management (IPM) programs. A major management tool for stored-product insects in food processing plants has been periodic structural fumigation with methyl bromide, although there has been little published research on its effectiveness in the field (Fields and White, 2002). Use of methyl bromide as a structural and commodity fumigant is being phased out worldwide under the 1987 Montreal Protocol on Substances that Deplete the Ozone Layer (UNEP, 2000). The phase-out has triggered new research into the efficacy of methyl bromide and alternative treatments for food facilities (e.g., Campbell et al., 2002; Roesli et al., 2003; Campbell and Arbogast, 2004; Toews et al., 2006; Fields, 2007; Small, 2007). However, it is often difficult to draw firm conclusions from this research given the challenges of evaluating efficacy as described above and the limited number of replicate treatments involved in each study. To move forward, it will be necessary to develop larger data sets for commercial food facilities from which general patterns in efficacy can be determined and the impact of different factors on treatment efficacy evaluated.

Assessment of treatment efficacy in a food facility using pheromone traps has two components: immediate reduction in insect captures and rebound in captures over time following treatment. There are a number of factors that can influence efficacy, in addition to the characteristics of the treatment itself, including pest population abundance and distribution, environmental conditions during and after treatment, other management tactics being conducted concurrently. Here we summarize research which is presented more fully in Campbell et al. (2010a,b) that used data sets generated by long-term monitoring of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), in two commercial mills to evaluate the impact of fumigation on the number of beetles trapped and how pest abundance, environmental conditions, and changes in management programs impact both the immediate reduction and rebound of beetle captures. Using these data, we also developed beetle capture thresholds that might be used to assess the risk of rapid increases in abundance, as measured using pheromone traps.

2. Materials and methods

Mill #1 was a five-floor flour mill in which eleven structural fumigations were performed (nine with methyl bromide and two with sulfuryl fluoride (ProFume, Dow AgroSciences, Indianapolis, IN, USA)) between July 2002 and December 2008. Methyl bromide concentration ranged from 20 g/m^3 to 26 g/m^3 for 24 h and the two sulfuryl fluoride concentrations were 32 g/m^3, 19 h exposure, and 111 g/m^3, 18 h exposure. An improved management program was instituted after November 2004, which included the integration of regular aerosol applications of either 1% or 3% synergized pyrethrins (Entech Fog-10 or Entech Fog-30, Entech Systems, Kenner, LA, USA) (1.0 mL/m^3) and methoprene (Diacon II, Wellmark International, Schaumburg, IL, USA) (0.01 mL/m^3) at 2-4 week intervals, with enhanced sanitation and targeted insecticide treatment or cleanup of hot spots (located by trapping). This change provided a unique opportunity to evaluate changes in pest populations achieved by aerosol applications and sanitation over a period of several years as well as its impact on fumigation efficacy. Mill #2 was a five-floor wheat processing facility which underwent twelve structural fumigations between March 2003 and December 2008. This mill was fumigated twice a year with methyl bromide at a typical concentration of 24 g/m^3 and ~20 h exposure time. Both mills were in the same geographic area and had a pest management and sanitation program in place throughout the study.

Tribolium castaneum was monitored using Dome traps baited with pheromone lures for *Tribolium* spp. and a kairomone attractant (Trécé Inc. Adair, OK, USA). There were 55 trapping locations in Mill #1 and 32 in Mill #2. Traps were checked approximately every two weeks and the mean number of beetles captured per trap per standardized two week period (beetles/trap/period) and the proportion of the traps that captured one or more beetles per standardized two week period (i.e., probability of capture) were calculated.

Air temperature on each floor of Mill #1 and Mill #2 was recorded hourly using data loggers (HOBO® H8 family, Onset Computer Corp., Pocasset, MA, USA) placed 1.5 m above the floor and used to calculate mean daily temperatures inside each mill. Outside daily air temperatures were obtained from local weather stations. Mean temperatures during fumigation were calculated using hourly temperature data collected during the period when the fumigation was performed.

3. Results and discussion

3.1. Seasonal patterns

Mean number captured and probability of capture of *T. castaneum* varied considerably among sampling periods at both mills, but generally tended to increase over time, unless a fumigation occurred between monitoring periods. There was an average increase of 52.7±8.2% (n=286) in mean *T. castaneum* capture and 24.8±4.7% (n=285) in probability of capture between monitoring periods when no fumigation was performed, and in Mill #1 neither mean trap capture (General Linear Models (GLM) Procedure: F=2.24; d.f.=1,153; P=0.14) nor probability of capture (GLM: F=1.73; d.f.=1,153; P=0.19) was changed significantly by institution of the enhanced IPM program. This pattern of relatively consistent increase over time differs from other species in these mills, such as *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), the Indian meal moth, and *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), the warehouse beetle, that followed seasonal patterns of increase and decrease in abundance (Campbell et al., 2002; Toews et al., 2006).

Favorable interior temperatures for *T. castaneum* development and fumigation effectiveness were maintained most of the year, but there was seasonal variation in temperature. Temperature inside the mill tended to follow outside temperatures during the warm season (April to September), although it was always warmer than outside, and to stabilize at a temperature warmer than outside during the cool season (October to March). For example, at mill #1 the mean temperature in the warm season was 29.6±0.1°C inside and 21.0±0.2°C outside (8.3±0.1°C warmer) and in the cool season it was 24.0±0.1°C inside and 5.3±0.2°C outside (18.5±0.2°C warmer). The mean air relative humidity inside Mill #1 was relatively low year round at 32±16%. The change in mean interior temperature between seasons could impact population growth rates (Sokoloff, 1974), and the differences between interior and exterior temperatures could impact immigration.

3.2. Initial reduction following fumigation

Mean number of *T. castaneum* captured per trap following fumigation was reduced by 84.6±4.6% (n=23 fumigations): an average of 11.4±3.5 beetles/trap/period captured in the period immediately before compared to 0.8±0.2 beetles/trap/period in the period immediately after fumigation. Fumigation reduced the probability of capture by 70.9±5.1%: an average probability of capture of 0.58±0.07 and 0.20±0.05 for the monitoring periods immediately before and after fumigation, respectively.

Capture of adult *Tribolium* in traps immediately after fumigation has been reported previously (Campbell and Arbogast, 2004, Toews et al., 2006, Small, 2007) and could result from survival within structure and/or movement into structure after treatment. If survival inside the mill were the primary mechanism behind the presence of adult beetles in traps immediately after fumigation, then we hypothesize that the number of beetles captured after fumigation should be positively correlated with the number present before fumigation. And, the number of beetles per trap and probability of capture after fumigation were positively correlated with the mean number captured and the probability of capture immediately before fumigation (Pearson Correlations, $P>0.05$). If movement into structure after fumigation was the primary cause of adult beetle capture immediately after fumigation, then we hypothesize that trap captures after fumigation should be greater after warm season fumigations and less after cool season fumigations. Fumigations were sorted into two seasons: spring (April - June) (n=9) and fall (October – December) (n=11) periods: which differed in outside temperatures (11.8±1.8°C during fall and 18.9±1.2°C during spring fumigations (GLM: $F=8.90$; d.f.=1,16; $P=0.001$)), but not in inside temperature (24.4±0.6°C; GLM: $F=0.03$; d.f.=1,16; $P=0.86$). There was no difference between seasons in reduction immediately after fumigation in either mean number of beetles captured (GLM: $F=2.86$; d.f.=1,18; $P=0.11$) or probability of capture (GLM: $F=0.59$; d.f.=1,18; $P=0.45$). There was also no difference between the spring and fall fumigations in the mean number of beetles captured (GLM: $F=0.05$; d.f.=1,18; $P=0.82$) or the probability of capture (GLM: $F=0.34$; d.f.=1,18; $P=0.56$) immediately after a fumigation. These findings do not support the hypothesis that the presence of beetles in these mills after fumigation is due to immigration, as has been observed for other stored product species (Campbell and Arbogast, 2004); rather it involves survival of treatment within the mills.

3.3. Rebound after treatment

Rebound refers to the recovery or increase in trap captures following a reduction due to application of a treatment, and in this particular analysis refers to time to reach a particular capture threshold. Rebound results from individuals surviving treatment, including eggs and early instars not detectable in pheromone traps immediately after treatment, individuals that immigrated into facility, and the progeny of the survivors and immigrants; as modulated by environmental conditions and management tactics. Rebound rate in mean trap capture and probability of capture varied considerably among the fumigations and no linear or non-linear regression model significantly fit the combined data from all fumigations. Therefore, we developed threshold values and analyzed the time to reach the first monitoring period that matched or exceeded those thresholds as a measure of rebound. The mean trap capture threshold used was 2.5 beetles/trap/period, which corresponded to the median value immediately prior to fumigation in these mills. Time to rebound to the mean trap capture threshold was 174±33 d (n=21; censored observations=8), which is a biased mean since it is likely shorter than the actual rebound time because some times mills were fumigated prior to reaching threshold (i.e., censored observation). It took a significantly longer time to reach the threshold after fall fumigations than spring fumigations (survival analysis using Kaplan-Meier log-rank test, $Z=4.122$, $P=0.042$): 248±50 d (n=9, censored events=5) compared to 104±21 d (n=9, censored events=3). The probability of capture threshold was 0.50 of the traps with captures of one or more beetles, which also corresponded to the median value prior to fumigation. For rebound to the probability of capture threshold, the mean time was 120±21 d (n=21, censored observations=4) and there was not a significant difference between seasons (Kaplan-Meier analysis: Z=3.752, P=0.05).

Rebound patterns measured in this study were highly variable, which probably reflects the impact of these many diverse factors on population growth and also that pheromone traps are imperfectly correlated with actual population levels. Clearly, management practices, including timing of fumigation, can impact rebound pattern, and reducing the rate of population increase can reduce the need or frequency of fumigation or other structural treatments.

3.4. Impact of management program on fumigation efficacy

Improvement of management practices at Mill #1, which started in the fall of 2004, provided an opportunity to evaluate the impact on trap captures and fumigation efficacy over a period of four years. After change in management strategy, there was a nine-fold reduction in mean *T. castaneum* capture and two-fold reduction in probability of capture in the mill. In Mill #2, which did not have the same change in management program, there were no differences in these measures over the same time periods. Reduction in mean trap capture immediately after fumigation was not significantly effected by change in management program (92.2±2.8% before versus 91.2±4.0% after management change (GLM: F=0.04; d.f.=1,9; P=0.84)). However, probability of capture immediately after fumigation was significantly effected by change in management (46.2±9.3% before versus 82.8±9.3% after change, GLM: F=7.59; d.f.=1,9; P=0.02).

After the pest management changes it took longer to rebound to the mean capture threshold (Kaplan-Meier analysis: Z =4.874, P=0.03); 49±15 d (n=5, censored events=0) before and 246±71 d (n=5, censored events=2) after change. It also took longer to rebound to the probability of capture threshold (Kaplan-Meier analysis: Z =5.801, P=0.02); 38±14 d (n=5, censored events=0) before and 165±46 d (n=5, censored events=0) after change. A consequence of these changes in pest abundance was that the number of fumigations was reduced from two or more per year before the management change to one per year afterward. The single fumigation per year after change in management program was in the fall.

Reduced rebound time following fumigation under the improved program could be explained in part by a smaller starting population following fumigation, reduced population growth rate due to lower interior temperatures and reduced immigration due to lower exterior temperatures associated with fall fumigations, or because the management program is increasing the mortality rate within the population. There was a negative correlation between mean beetle capture immediately after fumigation and time to rebound to mean trap capture threshold (Pearson Correlation: r=-0.626, P<0.01, n=21) and probability of capture threshold (Pearson Correlation: r =-0.596, P<0.01, n=21). This means that rebound to threshold took longer with decreasing numbers of individuals surviving the fumigation, and at Mill #1 mean trap capture after fumigation was lower following the change in management program compared to before. It is difficult to separate the effect of changing the time of year the fumigation is performed, which as described above impacts rebound rate, from the impact of the change in management program because only one fall fumigation occurred prior to the management change in Mill #1. However, we can analyze percentage change in mean beetle capture between sequential monitoring periods by both season and before and after change in IPM program. The overall GLM model was marginally significant for change in mean trap capture (F=2.70; d.f.=1,146; P=0.05) and season was a significant factor (P=0.02), but change in management and the interaction between season and change in management tactics were not significant (P>0.05). Sorting data by season, the change in mean trap capture between monitoring periods was 23.0±9.0% (n=79) in the cool season and 66.1±15.8% (n=71) in the warm season monitoring periods. For the proportion of traps with captures, the overall GLM model was not significant (F=0.81; d.f.=1146; P=0.49). This analysis suggests that while improving IPM program likely has important consequences on pest populations, changing the season fumigations are performed may have the largest single impact on rebound rate. Further evaluation using population models and before and after comparisons from other locations may provide more insight into the relative importance of the IPM program changes.

3.5. Development of risk thresholds

Theoretically, unchecked *T. castaneum* populations will grow exponentially and rebound in beetle capture data was frequently consistent with this pattern, although with a considerable amount of variation and with increases frequently truncated due to fumigations. There are currently no standardized pest management action thresholds for food facilities, but if a pest management program can keep *T. castaneum* trap captures in the relatively flat portion of the exponential curve there should be reduced risk of large increases in beetle captures in subsequent monitoring periods.

We first tested for a correlation between mean beetle capture and the increase in trap captures in the next monitoring period, but this was not significantly correlated (Pearson Correlation: r=0.08, *P*=0.15, n=292). This is likely because as mean trap capture increases it is more likely that interventions will increase – e.g., fumigation, insecticide applications, sanitation – resulting in slower rates of increase or decreases. Next, we tested for a correlation between change from the previous monitoring period compared to the current mean trap capture, which did have a significant positive correlation (Pearson Correlation: r=0.69, *P*<0.01, n=290). Finally, we used the rebound threshold values developed earlier and calculated the changes in trap captures above and below these thresholds. Below the mean trap capture of 2.5 beetles/trap/period, the increase in mean trap capture in the next monitoring period was 0.34±0.08 (n=202), but above this threshold the increase in mean trap capture in the next monitoring period was five times this amount (1.76±0.85 (n=90)), although the difference was not significantly different (Mann-Whitney Rank Sum Test: *U*=8746.5, *P*=0.61). Focusing just on intervals where beetle captures increased, the degree of increase was significantly greater above the 2.5 beetles/trap/period threshold (5.4±1.2, n=51) than below (0.9±0.2, n=119) (Mann-Whitney Rank Sum Test, U=1185.0, P<0.01). Similar results were obtained when analyzed using the probability of capture threshold. Preliminary analysis of a pooled dataset collected from a total of 12 wheat or rice mills gave similar results, which lends support to this approach, although further evaluation is still needed.

The rate of population increase is something that can be managed in food facilities such as flour mills: e.g., reduce population growth rate through increased mortality (sanitation and insecticide use), reduction in availability of food patches (sanitation and structural modification), reduced ability to colonize (exclusion and insecticide use), and reduced indoor temperatures (slower development rates). Pheromone based monitoring data can be used to evaluate the effectiveness of a management program, but it has been unclear how to use this data effectively. The mean trap capture and probability of capture thresholds appear to provide a good starting point for a risk threshold for mills. Given the limits of modern milling operations, flexible management targets based on risk thresholds may be more useful than traditional action thresholds based on responding when pest levels reach a certain point, as developed for other systems. In Mill #1 the enhanced pest management program and the shift in time of year that fumigations were performed resulted in trap captures exceeding the risk thresholds less frequently. The end result was less frequent fumigations and the suggestion that they might be reduced even further in frequency. This approach holds potential for improving management programs, since it is relatively simple to calculate, can be used to evaluate success of current program, and can be easily adapted to a given facility type and its management goals by adjusting the target threshold up or down based on criteria developed by management.

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