Effect of *Tribolium castaneum* (Coleoptera: Tenebrionidae) Nutritional Environment, Sex, and Mating Status on Response to Commercial Pheromone Traps

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ABSTRACT *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, is an important cosmopolitan pest of stored grains. Commercial traps baited with the synthetic aggregation pheromone 4,8-dimethyldecanal (DMD) are used to monitor *T. castaneum* population densities in storage facilities. However, trap catches may depend on several intrinsic and extrinsic factors. In this study, we explored the effects of beetle nutritional environment, sex, and mating status on the response of *T. castaneum* to commercial Storgard Dome traps. Beetles raised on a low-nutrition diet were 1.7 times more likely to enter DMD-baited traps compared with beetles that were raised on a high-nutrition diet. Although no sex difference in trap response was found, unmated beetles of both sexes were more responsive to DMD than were mated beetles, and this effect was especially pronounced for beetles reared on a low-nutrition diet. These results suggest that estimating *T. castaneum* population densities based on trap catches might be improved by incorporating information about the nutritional quality of infested stored products.

KEY WORDS aggregation pheromone, dimethyldecanal, stored products, trap response

Tribolium flour beetles (Coleoptera: Tenebrionidae) are cosmopolitan pests of stored grains, and they are among the most important insect pests in grain processing and other storage facilities (for review, see Parkin 1954, Sokoloff 1972, Cuperus et al. 1990, Campbell and Arbogast 2004, Rajendran and Devi 2004). In managing stored-product pests, pheromone traps are often used to monitor insect infestations (for review, see Burkholder 1984, Phillips 1997, Rajendran 1999). Several species of *Tribolium* flour beetles are attracted to 4,8-dimethyldecanal (DMD), an aggregation pheromone produced by males and attractive to both sexes (Ryan and O'Ceallachain 1976; Suzuki 1980, 1981). Commercial traps baited with synthetic DMD are effective in detecting the early stages of *Tribolium* spp. infestation, and trap catches generally correlate with infestation levels measured by direct sampling (Campbell and Arbogast 2004, Toews et al. 2005a). However, trap catches may depend on several intrinsic and extrinsic factors. For example, *Tribolium castaneum* (Herbst), the red flour beetle, is patchily distributed within storage facilities, and trap capture is dependent on location of traps and on the presence of external food and shelter (Stejskal 1995, Campbell et al. 2002, Campbell and Arbogast 2004, Toews et al. 2005b). In addition, numerous other extrinsic factors, including temperature, population density, seasonality and trap type, also have been shown to affect trap response by flour beetles (White and Loschiavo 1986; Campbell et al. 2002; Arbogast et al. 2004; Campbell and Arbogast 2004; Toews et al. 2005a, 2005b).

However, responses to external stimuli are mediated by changes in an animal's motivational state, and insect response to pheromone is likely to depend on intrinsic factors affecting motivational state, such as nutritional history and mating status. *Tribolium* flour beetles infest stored products that vary widely in nutritional quality (Sokoloff 1974); yet, to our knowledge, no studies have attempted to assess how different nutritional conditions might affect *Tribolium* spp. response to pheromone-baited traps. Mating status has been shown to alter response to pheromone in other insects (Pureswaran and Borden 2003; for review, see Rafaeli 2005). However, in *T. castaneum*, the only study to compare mated and unmated beetles found no effect of mating status on response to DMD (Obeng-Ofori and Coaker 1990).

Among other factors potentially affecting trap response are behavioral differences between the sexes. Because females contribute most directly to population increases, it is important to know whether there are sex differences in trap response. Based on the observation that virgin females of both *T. castaneum* and *Tribolium confusum* Jacquelin du Val, confused flour beetle, are more strongly attracted to synthetic DMD than are males, some studies have suggested that DMD may have a dual role as an aggregation pheromone as well as a sex attractant for females (Levinson and Mori 1983). In contrast, other studies found that *T. castaneum* males were more attracted to DMD than

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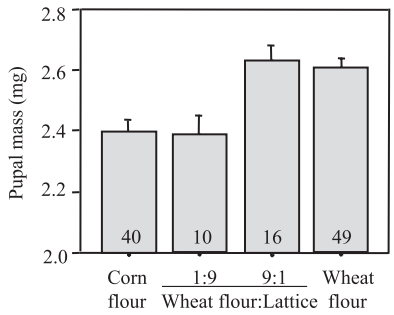

Fig. 1. *T. castaneum* pupal mass (mean + 1 SE) from a preliminary experiment in which larvae were reared on either corn flour, 1:9 wheat flour:Lattice mix, 9:1 wheat flour: Lattice mix, or wheat flour (sample sizes given inside bars).

females (Obeng-Ofori and Coaker 1990). Therefore, further work is needed to determine whether sex differences in attraction to DMD exist in this species.

The current study investigates how food nutritional quality, sex, and mating status of *T. castaneum* flour beetles affect their response to commercial DMD-baited traps. This knowledge may allow better assessment of *Tribolium* population densities in storage facilities containing different kinds of foodstuffs.

Materials and Methods

Manipulating Nutritional Environment. The *T. castaneum* used in our experiments originated from the Berkeley synthetic strain (Lewis and Austad 1990), with cultures maintained on enriched King Arthur flour (Norwich, VT) and kept in a darkened incubator at 29°C and 70% RH. In preliminary experiments to find repeatable methods for manipulating *T. castaneum* nutritional environments, we first raised larvae on corn (*Zea mays* L.) flour (low nutritional quality), wheat (*Triticum aestivum* L.) flour (high nutritional quality), and two levels of a manipulated wheat diet. The latter treatments were designed to avoid the confounding effects of compositional differences among food types. These treatments of artificial high- and low-quality diet were created by mixing enriched King Arthur wheat flour with a non-nutritional filler, Lattice NT200 microcrystalline cellulose (FMC Biopolymer, Philadelphia, PA), at either 1:9 or 9:1. Eggs were placed individually in ≈0.5 g of the respective media, left to develop at 29°C and 70%RH, and weighed upon reaching pupal stage. These data (Fig. 1) indicated that pupal mass was significantly reduced on both corn flour and the 1:9 wheat flour: Lattice diet compared with 9:1 mix or pure wheat flour (analysis of variance [ANOVA]: $F_{3, 111} = 8.28$; $P < 0.0001$). Thus, 9:1 and 1:9 mixtures of wheat flour:Lattice provided a good match to different food qualities normally encountered by *T. castaneum.*

For the main experiment, eggs were collected from stock culture beetles (≈200 unsexed adults) and placed in 100 g of flour for 48 h. Fifteen eggs were placed in 5 g of either high-quality nutritional media (HQ; 9:1 wheat flour:Lattice mix) or low-quality nutritional media (LQ; 1:9 wheat flour:Lattice mix). To obtain mated males and females, eggs were reared until adult beetles eclosed and mated, after which adults were separated by sex based on the presence or absence of sexually dimorphic setiferous glands on male forelegs (Hinton 1942). Unmated males and females were obtained by sexing and separating beetles at the pupal stage. Adults from both mating status treatments were kept in same-sex groups of 15 beetles per 5 g of either HQ or LQ media. In addition to the effects on pupal mass already described (Fig. 1), the effectiveness of our experimental treatments on beetle nutritional condition was confirmed by reduced survival to adulthood (≈50%) and decreased developmental rates in LQ food.

Bioassay of *Tribolium* Trap Response. We used a factorial design to investigate the effects of sex, mating status, and nutritional condition on *Tribolium* trap response. All beetles were assayed within 1 mo posteclosion. Attraction to *T. castaneum* aggregation pheromone was tested using commercial Storgard Dome traps from Trécé, Inc. (Salinas, CA; http://www.trece.com/stgdprod.html). Each dome-shaped, 11.5-cm-diameter trap was baited with the provided pheromone lure (no food oil was used) and placed in the center of a plastic 267- by 190-mm container, the floor of which was covered with paper to increase beetle traction. For each trial, 30–36 beetles were released into each container, which was covered and placed in a dark incubator at 29°C and 70% RH. After 20 h, the number of beetles captured within each trap was recorded. Beetles found on the trap but not in the pitfall receptacle were scored as nonresponding. In 12 trials with beetles from LQ nutritional environment, between one and five nonresponding individuals were found to have died during the bioassay trial, and these beetles were excluded from subsequent analysis. In total, 38 trials were conducted, making up four to five replicates for each of eight treatments, representing all combinations of the three factors under investigation (two levels each of food quality, sex, and mating status). Beetles were not reused, and 1,119 beetles in total were used in these experiments.

Statistical Analyses. We used two different analyses to assess the effects of food quality, sex, and mating status on *T. castaneum* trap response. To examine the effects of the three factors on the mean percentage of beetles responding to pheromone, we used three-way ANOVA with fixed factors. In addition, the effects of these factors on individual behavior were examined using logistic regression to model each beetle's location (in or out of trap) as binary response (JMP 5; SAS Institute, Cary, NC).

Results

Food nutritional quality had highly significant effect on mean percentage of beetles responding to pheromone-baited traps (ANOVA: $F = 29.67$; df = 1, 30; $P < 0.0001$): beetles raised on LQ diet showed a 1.7-fold higher response to pheromone traps compared with beetles from HQ diet (Fig. 2). Neither beetle sex nor mating status influenced mean trap response (sex: $F = 0.441$, df = 1, 30; $P = 0.512$; and mating status: $F = 2.125$;

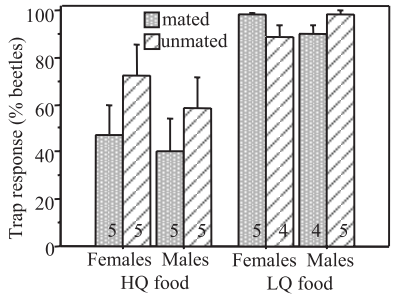

Fig. 2. Percentage (mean + 1SE) of *T. castaneum* adults responding to DMD-baited pheromone traps (Storgard Dome traps) depending on beetles' sex (male or female), mating status (mated or unmated), and food quality (high quality or low quality). Number of replicate trials conducted for each treatment (≈29 beetles per trial) is given inside bars.

df = 1, 30; $P = 0.155$). In addition, no significant interactions were detected between and among the factors (ANOVAs: $F < 2.38$; df = 1, 30; $P > 0.134$).

Logistic regression analysis confirmed the significant effect of food quality on the likelihood that an individual *T. castaneum* would respond to pheromone traps (Table 1). Mating status had a marginally significant effect, with unmated beetles more likely to respond to DMD-baited traps (Table 1). There was also a significant interaction between food and mating status, which reflects an increased effect of mating status on trap response when beetles were raised on HQ diet (Fig. 2). None of the other two-way interactions (mating status by sex, and food quality by sex) affected the likelihood of beetles' responding to pheromone-baited traps.

Discussion

This study found that the response of *T. castaneum* adults to DMD-baited traps depends on the nutritional quality of food they infest: beetles reared in low-quality nutritional environments were 1.7 times more responsive to DMD than beetles reared in high-quality nutritional environments. Although because of their small scale and lack of alternate refugia such experimental studies are likely to yield considerably higher trap capture rates than would be seen in the field, these aspects of study design would not account for the observed nutritional effect. In addition, given the many biological similarities between *T. castaneum* and *T. confusum* (Sokoloff 1974), both species' use of DMD as an aggregation pheromone (Suzuki 1980, 1981), and similar patterns of pheromone response (Levinson and Mori 1983, Obeng-Ofori and Coaker 1990), these findings are also likely to apply to *T. confusum.*

Table 1. Logistic regression modeling the probability of individual *T. castaneum* responding to pheromone traps as a function of beetle's food quality, sex, and mating status

Factors	Coefficient	SE	χ^2	*P* value
Food quality	−1.341	0.105	255.245	<0.0001
Sex	0.089	0.105	0.719	0.396
Mating status	−0.202	0.105	3.706	0.054
Mating × food	−0.223	0.105	4.444	0.035
Mating × sex	0.072	0.078	0.874	0.350
Food × sex	0.111	0.105	1.133	0.287

Trap response is modeled as log odds trapped/nontrapped beetles; coefficients estimate how the likelihood of trap response is altered by changing food quality from low quality to high quality, sex from male to female, and mating status from unmated to mated. Whole model log-likelihood ratio test yielded $\chi^2 = 297.42$ and $P < 0.0001$.

One possible explanation for the greater trap response of beetles reared in low-quality nutritional environments is based on the observation that starved *T. castaneum* produce dramatically reduced quantities of DMD (Hussain et al. 1994). For beetles reared in low-quality nutritional environments, this pheromone might serve as a reliable indicator of a population with greater food availability, and they may thus show a higher response. An alternative explanation could be that starved beetles can climb up trap walls better than well-fed beetles because they are lighter and may be more agile (unpublished data). In another tenebrionid, the lesser mealworm, *Alphitobius diaperinus* (Panzer), beetles showed increased agility and activity during the first week of starvation (Renault et al. 2003). Thus, further studies are needed to determine the exact mechanisms responsible for the observed result that flour beetles reared in low-quality nutritional environments shower higher rates of trap capture.

This finding is consistent with previous studies showing that the presence of external food sources reduces *T. castaneum* capture rates by traps baited with DMD and food oils in mini-warehouses (Toews et al. 2005a), and by traps baited with DMD only in small arenas (Stejskal 1995). Together, these results suggest that greater trap response should be expected if processing or storage facilities contain stored products of low nutritional quality (e.g., corn) compared with stored products of higher nutritional quality (e.g., wheat). This study also may have implications for monitoring *T. castaneum* infestations in processing, warehouse, or retail facilities containing a variety of grain products differing in their nutritional quality. Locating traps around products with lower nutritional quality should facilitate early detection of infestations, because adults emerging from these products have a higher likelihood of being trapped.

Our study did not detect any differences in trap response of *T. castaneum* males and females, suggesting that both sexes are equally attracted to DMD. Previous studies provide contradictory results concerning the relative attraction of *T. castaneum* males and females to DMD. Thus, Levinson and Mori (1983) observed greater response to synthetic DMD by unmated females than by unmated males, whereas Obeng-Ofori and Coaker (1990) found the opposite. We also found that unmated beetles showed higher trap capture rates compared with mated beetles, and this was especially noticeable in the high-quality food treatment. This increased response might be explained by virgin beetles having increased motivation to find mates. However, the study of Obeng-Ofori and Coaker (1990) on *T. castaneum* found no difference

between virgin and mated beetles in their response to synthetic DMD.

In conclusion, our study demonstrates that *T. castaneum* response to DMD-baited traps is dependent on the nutritional quality of the food that beetles infest. Moreover, food quality interacts with mating status to alter beetles' trap response. Finally, *T. castaneum* male and females respond equally to DMD. In trap-based insect monitoring programs, taking the nutritional quality of stored products into account should allow for more accurate assessment of pest population densities.

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