The Effects of Insecticide Treated Netting on Male-Female Interactions in Red Flour Beetles

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Abstract

Courtship behaviors take place before and after copulation for the purpose of stimulating the female (Evardsson & Arnqvist 2000). Females will mate with different males within minutes of the first copulation (Pai &Yan 2003), with males showing a preference for virgin females (Lewis & Iannini 1995). Red flour beetles (*Tribolium castaneum*) show a decrease in progeny output following exposure to insecticide treated netting (Scheff et al. *in prep*). To assess whether females exposed to insecticide treated netting had decreased courtship attempts, virgin females were placed with a single male to accurately determine the number of mating pair interactions. Courtship behavior and mating pair interactions were recorded to determine if the number of mating pair interactions will decrease after exposure to insecticide treated netting. Our results indicate no significant difference between the number of interactions between females exposed to control netting or insecticide treated netting. However, with females exposed to insecticide treated netting the duration of interactions increased and more interactions were initiated by males. These results suggest that the decline in progeny output may be due to reallocation of reproductive resources following exposure to insecticide treated netting and not due to decreased numbers of mating attempts.

Purpose

The purpose of this research is to determine if exposure to insecticide treated netting affects mating pair interactions and courtship behavior in red flour beetles (*Tribolium castaneum*).

Questions and Hypotheses

<u>Question</u>: Does exposure to insecticide treated netting affect mating pair interactions and courtship behavior in red flour beetles (*Tribolium castaneum*)?

<u>Hypothesis</u>: The number of mating pair interactions in red flour beetles will decrease after exposure to insecticide treated netting.

Study System

The red flour beetle, or *Tribolium castaneum*, is a common stored-product pest of grain and cereal products. It is in the order Coleoptera and family Tenebrionidae. An adult red flour beetle is approximately 3-4 mm long with a reddish-brown, oval-shaped body (Figure 1, left). Larvae are slender and yellowish-white in color (Figure 1, right). Adults can live five to eight-month and females can lay between 300-400 eggs. The larvae and pupae stages of red flour beetles can cause significant damage to grains. Adults feed on the germ of the cereal and contaminate a wide variety of crops such as oats, barley, walnuts, maize, sorghum, wheat, and dried stored products. They are present in most tropical and subtropical countries and can fly over small distances (Horticulture and Home Pest News).

Figure 1. Red flour beetle adult (left). Red flour beetle larva, pupa, and adult beetle. Photo Source: https://hortnews.extension.iastate.edu/red-flour-beetle

Methods and Experimental Design

Red flour beetle pupae were sexed, separated, and placed in an incubator for two weeks (Figure 2A). Female beetles were chilled and marked with a silver dot on their thorax before being exposed to either the control netting (non-treated) or the insecticide treating netting for 10 minutes (Figure 2B and C). One female and one untreated male were then placed in a petri dish "arena" with a filter paper liner. Interactions were video recorded for thirty minutes (Figure 2D-G) and the time interaction occurred, the number of interactions, time and duration of copulation were recorded.

To begin analyzing the data, we assessed if there was any difference in the average number of interactions between females and males for females exposed to control netting for each thirty-minute video. A generalized linear mixed model (*proc glimmix*) in SAS was used to do this. Next, we analyzed the duration of interactions in seconds between females exposed to control and females exposed to treated netting using *proc glimmix* as well. For post-hoc tests, there were several long interactions due to copulation events, so we dropped them from analysis and focused on unsuccessful interactions. Finally, we examined the difference for in who initiated each interaction. *Proc logistic* was used to determine if there was a difference in male, female, both, or neither for interaction initiation between treated and control netting.

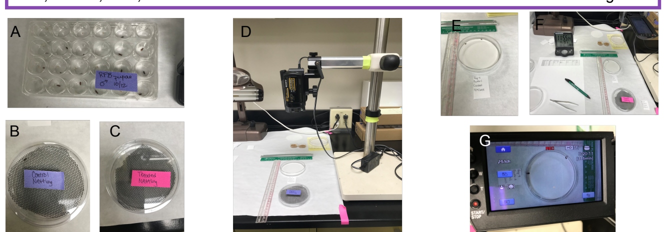

Figure 2. A)Males and females were held in 24-well plates as pupae until they emerged as adults. B)Control netting (untreated) and C) insecticide treated netting. D)Video recording set-up with arena with filter paper and Sony Handycam Recorder. E-F)Close-up view of the arena with ruler to calibrate scale if needed. G)Picture of arena set-up through the video camera.

Results

There is no significant difference between the average number of interactions for females exposed to treated netting and those exposed to control netting (p=0.49) or in the duration of interactions when all interactions are compared (p=0.31; Figure 3, left). However, when copulation events are removed, there is a significant difference in duration of interactions (p=0.016), with interactions of treated females having interactions up to 50 seconds longer than the control (Figure 3, right). There is a difference in who initiates interactions in females that are exposed to treated netting and those that are exposed to control netting (p=0.0092). When females are exposed to treated netting, the number of interactions initiated by males increases and the number of interactions initiated by both decreases (Figure 4).

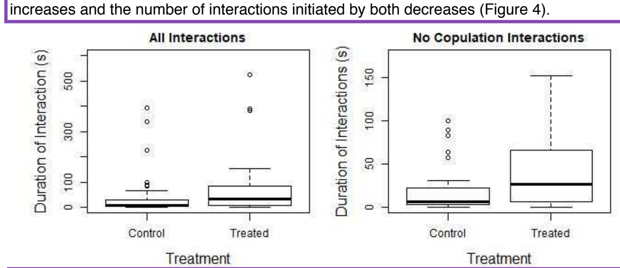

Figure 3. Box plots of the duration of interactions for all interactions (left) and interactions where no copulation occurred (right). Copulation occurred in a total of 7 interactions, 4 in control and 3 in treated female interactions. The duration of interactions was significantly different between treatments when copulation events were not included (right).

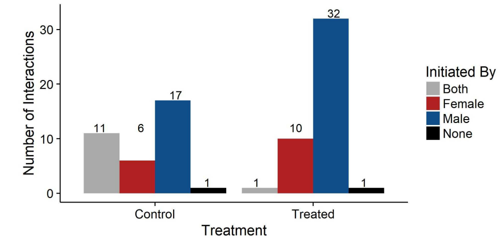

Figure 4. Summary of who initiated each interaction for females treated with insecticide treated netting or those exposed to untreated netting.

Conclusions

My original hypothesis stated the number of mating pair interactions would decrease when females were exposed to insecticide treated netting. This hypothesis was not supported, with mating pair interactions increasing when females were exposed to insecticide treating netting. This data causes us to reevaluate the how effective the insecticide is in inhibiting mating in red flour beetles. However, the number of interactions initiated at the same time by males and females decreased, with the number of interactions initiated by males increased. When the females were exposed to insecticide treated netting, the females may be experiencing negative physiological effects but the males still initiated interactions with females regardless of insecticide exposure.

Future Directions

To continue this experiment, I would gather more data to determine if the slight increase in interactions initiated by females exposed to insecticide treated netting is biologically significant. In this experiment, it is not, but with more data collected the increase could become more prominent. In addition, another experiment could be conducted with the males being exposed to the insecticide treating netting as opposed to the females. Also, I would conduct an experiment with both male and female red flour beetles exposed to the insecticide treated netting. In an environmental setting, there is nothing to prevent both male and females from being exposed to the netting. By exposing both beetles, the results could be significantly different.

References

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