Larvae of Trogoderma respond behaviorally to whole body extracts

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Abstract

Behavioral responses to semiochemicals by *Trogoderma* (Coleoptera: Dermestidae) stored product pests were assayed in a small arena. Hexane extracts were obtained from Khapra beetle, *Trogoderma granarium*, warehouse beetle, *Trogoderma variable*, and the larger cabinet beetle *Trogoderma inclusum* that were killed by being frozen for 48 hours at -20° C. These extracts were analyzed using gas chromatography coupled with mass spectrometry (GC/MS), and it was confirmed that they contain several cuticular hydrocarbons, fatty acids and sterols. Two choice experiments were performed inside Petri dish arenas, with filter paper fully covering the bottom surfaces. Two smaller 3cm filter papers were placed on opposite ends within each arena. Each of the smaller papers were folded three times in parallel to present a corrugated surface that the insects could move underneath if they chose. In each case, one paper had a 100µl aliquot of one of the extracts, and the other 100µl of hexane as a control. 10 late instar larvae of the same species as the treatment extract were placed in the arena and allowed to acclimate overnight in a dark room. For all three species, it was found that larvae were more likely to be found on the side of the Petri dish with the hexane control rather than the conspecific larval extract. They were also more likely to be on or near the smaller corrugated filter paper treated with the control as opposed to the filter paper treated with the larval extract. Thus repellency of the conspecific extract was demonstrated at that particular dose. Further assays using different doses of the raw extracts and their individual chemical components are planned. The use of these semiochemicals in novel management strategies will be considered.

Keywords: Aggregation, behavior, khapra beetle, management, pheromone

Introduction

The khapra beetle (KB), *Trogoderma granarium* is a serious pest of stored products and is the only stored products pest that is currently quarantined in the United States. KB larvae feed on a wide range of dry food products of plant and animal origin including cereal grains, dried fish and museum specimens (Hagstrum et al. 2013). It currently has an extensive distribution throughout warm and arid regions of Eurasia and Africa. It is a quarantine pest in the United States, which has a history of interception at ports and successful eradications of various scales, at specific locations (Armitage, 1958, Myers and Hagstrum 2012). Preventing establishment of the khapra beetle in the US is crucial to maintaining access of products to export markets. Various kairomone attractants have been developed for monitoring the species. Additionally a pheromone produced by adult females is used in current APHIS-PPQ monitoring efforts (Barak, 1989).

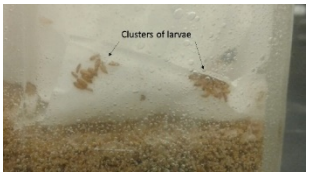

Fig. 1. KB larvae assembling in clusters on paper placed in a laboratory colony jar.

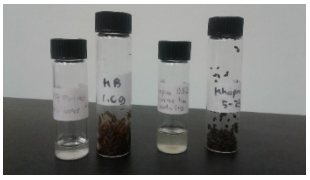

Fig. 2. Hexane extracted from KB larvae. The extract is contained in the hexane in vial on the left, which was removed from the one on the right containing the dead larvae.

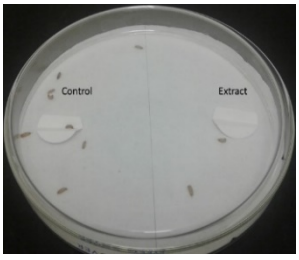

Fig. 3. Bioassay showing that in this instance, KB larvae preferred to remain on the left side of the arena, but without substantial clustering on the smaller treatment paper.

The possibility that pheromones affect the movement of larvae has not been investigated. Any such chemicals potentially could be incorporated into novel management strategies. For larvae, it was shown some time ago that carbon dioxide and certain food odors can be attractive, while many short chain (3-7 carbon) alcohols and organic acids can be repellant (Spangler 1965). Currently the adult pheromone and host associated kairomones are used in traps. However, it is readily observed that KB larvae, as well as those of other Trogoderma species will assemble in clusters, even on non-food sources such as papers placed in laboratory colony jars (Figure 1). The question of whether there may be semiochemicals that mediate this particular behavior has not been investigated. In this study, this possibility was researched in KB, as well as two related species, the warehouse beetle (WB), *Trogoderma variable*, and larger cabinet beetle (LCB), *Trogoderma inclusum*. We included the additional species because it is of interest to what degree such behaviors are common in the genus, particularly since several species share the same adult pheromone.

Materials and Methods

Hexane extracts were obtained from late instar larvae and adult that were killed by being frozen for 48 hours at -20° C (Figure 2). Extracts were made at a ratio of 4ml of hexane/ 1 g of larvae of each species. These extracts have been analyzed using gas chromatography coupled with mass spectrometry (GC/MS), indicating the presence of a number of compounds with fragmentation patterns that are consistent with those of cuticular hydrocarbons. Previously published research confirms that similarly prepared extracts of khapra beetle larvae using dichloromethane rather than hexane contained a number of cuticular hydrocarbons (Maliński et al., 1986). Furthermore adults also have a similar hydrocarbon profile (Dubis et al., 1987). In our newly prepared extracts, there were no noticeable differences among the traces for any of the different species. Furthermore each contained a region of several compounds indicative of hydrocarbons, all at similar retention times. The identities of such compounds in our extracts have not been confirmed, but there is little reason to believe they are different from the chemicals described in the literature. There were also a number of other compounds in the extracts, including particular fatty acids and sterols. These compounds also may potentially affect behavior.

For assessing whether the extracts can influence the behavior of the larvae, two choice experiments were performed in static air enclosures. Inside of 15cm glass petri dish arenas, a filter paper was placed that fully covered the bottom surface (Figure 3). Within each of these arenas, two smaller 3cm filter papers were placed, which were used for presenting chemical treatments and providing a possible clustering surface. To encourage clustering, each of the papers were folded three times in parallel to present a corrugated surface. For each experimental replicate that was performed in an arena, one of the smaller corrugated papers received a 100µl aliquot of one of the extracts, and the other smaller paper 100 µl of hexane as a control. This was a dose of roughly five larval equivalents. Ten late instar larvae were placed in the arena and allowed to acclimate overnight in a dark room.

Long-chain hydrocarbons have very low volatility, and if behaviorally significant, generally will function as close range pheromone attractants. Thus the static air environment of the Petri dishes is not different from the context within which the larvae are likely to respond to such signals. The dose of five larval equivalents was selected because it would represent the chemical equivalent of an existing cluster of larvae.

Results

For all three species, the larvae were more likely to be found on the side of the Petri dish with the hexane control, while less likely to be on the side with the conspecific larval extract. A total of 36 replicates were performed for each species and the percentages of larvae on the control side of the arenas were 63% for KB, 72% for WB, and 71% for LCB. Most of these larvae were not found on or under the folded control or treated filter papers. Thus 66% of KB, 65% of WB, and 69% of LCB, were found in other parts of the arena. When not clustered on the folded papers, several were often

clustered in other locations. Among those that were on the clustered papers, 80% of KB, 76% of WB, and 75% of LCB, were on the control paper, versus the treated one. Thus the conspecific extract was repelling the assembly of the larvae of all three species. The attraction that was expected given the observations of clustering behavior did not occur.

Discussion

In considering these results, it should be noted that the dose applied was greater than the equivalent of a single larvae, and thus the concentration of the chemicals may indicate a biologically unrealistic situation. Furthermore, it is not clear yet whether the cuticular hydrocarbons are producing this reaction or if some of the other compounds in the extracts, such as the fatty acids and sterols may be causing repulsion. It is possible that production of these same compounds, or others were elicited by the stress of the insects being frozen to kill them before the extraction. Any such a compound would thus function as an alarm pheromone that repels other insects from joining with and aggregating near other larvae that are distressed.

Whatever the causal factor of the repellency may be, understanding the mechanism may provide a product useful for the management of KB. For example it may be possible to incorporate such a compound into treatments of products or their packaging in a way that repels the larvae to protect the products. There is also the possibility that a repellent compound could perhaps be used in push-pull trapping strategies.

Additionally, it may also be worth revisiting the idea of whether clustering can occur if perhaps only the cuticular hydrocarbon portions of the extracts are used. If there is another compound causing repellency, it could be masking the effects of attractive compounds. There are also potential dosage issues with any behaviorally active compound. It could be that repellent compounds are attractive at other doses. Thus much additional work will be needed to fully utilize the capabilities of the behaviorally active components of such extracts. However, it is promising that at this stage in our investigations, behavioral activity has been clearly demonstrated.

Another final consideration may be whether adults have similar responses to such compounds. We did attempt to assess the response of adults to such extracts in the assay described above. However, it became clear very quickly that the adult insects were highly mobile and did not settle in clusters like the larva. Many were actively crossing between the sides of the Petri dish as they were being evaluated at the end of the overnight period. Thus, the evaluation of the adult behavior with respect to these extracts may require a different assay. For example, a small scale wind tunnel, or two-choice olfactometer with moving air, may be more applicable.

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