Responses of *Tribolium castaneum* to different pheromone lures and traps in the laboratory

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**Abstract**

Three different pheromone lure designs and three different trap designs were tested with *Tribolium castaneum* for behavioural response in the laboratory. Two-choice pitfall bioassays were used for lure studies in which beetles oriented to one of two holes in the floor, below which were placed stimulus or control materials. The test arena for trap studies was a large tray with one layer of wheat grains on the floor. Traps were placed randomly within the arena. All lures were least effective when new due to high release rate of pheromone. The membrane/reservoir design elicited very low beetle responses when newly removed from the package, but after five to six weeks of aging/aeration these baits elicited high response (50–60%). The rubber septum was attractive in weeks one to three but declined in activity thereafter. Laminated baits were consistently attractive from one to three weeks (60–65% response). Laminated baits were thus used for trap comparisons. Trapping studies compared corrugated cardboard/pitfall, circular inclined ramp pitfall, and rectangular inclined ramp pitfall trap designs. The rectangular inclined ramp pitfall trap was most effective and caught 25% of released beetles compared to 10% and 4% in cardboard and circular traps, respectively.

**Introduction**

Pheromones are effective monitoring tools for a number of stored-product pests (Burkholder and Ma 1985). Pheromone-based monitoring and detection requires that synthetic pheromone must be formulated into a controlled release device and used in a trap that is effective, durable, and serviceable. Optimum designs for both the trap and the controlled release device are primary objectives for pest management (Barat et al. 1990). Several designs for controlled release pheromone dispensers have been used for different insect species, each one giving characteristic release for specific pheromones (McDonough 1991). Traps of various designs have been developed for stored-product insects, but their efficacy for specific insect species has not been fully investigated.

Despite the early identification of 4,8-dimethyldecal (DMD) as the aggregation pheromone for *Tribolium castaneum* (Suzuki 1980), limited work has been reported on the use of the pheromone in trapping systems. Barak and Burkholder (1984) describe a trap made of corrugated cardboard with an oil-filled pitfall cup that was effective for several beetle species. They show that *T. castaneum* was caught in this trap, and eventually this design was patented and commercialised (Barak and Burkholder 1984). Controlled release pheromone lures provided with the cardboard traps are simply rubber septa impregnated with synthetic pheromone. Mullen (1992) reported a new trap design for *T. castaneum* and compared it with the cardboard trap and two other designs. The objectives of this study were to (a) evaluate the activity and longevity of various controlled dimethyldecal release devices that employed three different release designs, and (b) test the effectiveness of several trap designs.

**Methods and Materials**

**Insects**

A laboratory colony of *Tribolium castaneum*, established in 1990 from beetles collected on a farm in Dane County, Wisconsin, was used in all experiments. Beetles were reared on a mixture of whole wheat flour and brewer’s yeast (95:5, v:v) in a growth chamber maintained at 27 ± 1°C, 60% r.h. and a photoperiod of 16:8 (L:D) hours. Parent beetles were sifted from cultures one week after inoculation, and new adult progeny were removed for bioassay five to seven days after emergence.

**Lure formulations and bioassay**

Commercially produced lures described below were all formulated with the 4(R), 8(R,S) isomeric blend of DMD at >90% purity. The three general lure designs studied differed in their formulation and mechanism of release. These designs will be referred to as ‘septum’, ‘membrane’, and ‘laminate’ lures throughout this report. Septum lures were provided by Trécé Inc., Salinas, CA, and were composed simply of a red rubber septum (sleeve stopper type), 9.1 × 18.8 mm, onto which a solution of DMD had been applied (loading rate not provided). The pheromone is thus soaked into the rubber and is released slowly over time as a function of the rubber matrix. Membrane lures were from Consep Membranes Inc., Bend, OR, and contained DMD in a reservoir between an impermeable backing material and a plastic membrane through which the pheromone evaporated slowly as a function of membrane characteristics. Membrane lures were flattened rectangular devices that had a circular releasing surface and a reservoir loaded with 8.0 mg of DMD. Laminate lures, provided by Hercon Environmental Co., Emigsburg, PA, were a ‘sandwich’ design in which 1.0 mg of DMD was formulated into a PVC reservoir and placed between an impermeable Mylar bottom and a permeable PVC top piece. Pheromone release from the laminate lure is primarily through the top permeable layer, and its rate is a function of film thickness, film composition, and total lure dimension (i.e. area of release surface). Replicate samples of the lure designs were subjected to bioassay for activity at different ages (one to two-week intervals) following their removal from the packages. Maximum ages of lures tested were six weeks for septum lures, 12 weeks for

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membrane lures, and 14 weeks for laminate lures. Individually identified lures were aged in a fume hood at room temperature between bioassays. A two-choice pitfall bioassay, in which beetles oriented to one of two holes in the floor of a steel can arena below which were placed stimulus or control materials (described by Phillips et al. 1993), was used to evaluate all lures in this study. For all bioassays the test lure was placed in the treatment dish and the control dish was left empty. Twenty adult T. castaneum were released in each can arena and given two hours to respond; ten replicates of each lure and age class were deployed.

Quantification of DMD released from laminate lures

Release of pheromone from laminate lures over time was determined by collecting volatiles from lures and subjecting these to quantitative GC analysis. Aeration chambers, collection procedures, and quantitative GC analyses were the same as those reported for live beetles by Hussain et al. (these proceedings). Five lures were aerated for one hour immediately after removal from their packages. After aeration the lures were aged in a fume hood and analysed again.

Trap designs

Three different trap designs were obtained from commercial suppliers, and two modifications of one of the designs was made, yielding the following five designs.

1. Storgard traps from Trécé Inc. (Salinas, CA), were made of corrugated cardboard and are 9 cm on a side when folded. Three out of four folds are punched out to fit in a small cup. Corrugations are oriented diagonally across each section and most flutes lead to the cup that contains oil (described by Barak and Burkholder 1984).

2. The modified storgard used the fundamental storgard, but had a bigger cup of 4 cm square and 1 cm high. Additionally, the orientation of the corrugations was changed from diagonal to perpendicular in relation to the trap sides, and the direction of corrugations was alternated for adjacent layers.

3. A second modification of the above traps, the rounded storgard trap, consisted of four circular, 4.5 cm diameter plates of corrugated cardboard. All four plates had 4 cm square punctures. The two bottom circular plates fit the 4 cm square x 1 cm high cup and were mounted on a circular cardboard of the same size. The other two were used as a cover and punchout openings led into the cup. The two top and two bottom corrugated plates were glued together to make two main top and bottom pieces. The circular corrugated plates were placed on each other so that orientation of the corrugations was perpendicular.

4. The Fuji Trap (Japan Tobacco Co.) was a rectangular ramp-and-pitfall design, approximately 4×10×1 cm, constructed primarily from hard paper. Responding insects enter ramps on each end of the trap, climb to the ramp to the edge of a 4×4×1 plastic cup. The opening for the cup is covered with cloth netting, onto which beetles crawl, but from which they eventually lose footing and fall into an oil-soaked pad. The whole trap is covered with a plastic rectangular sleeve that provides support to hang a lure.

5. The Trécé ‘Flit-Trak’ (pronounced ‘flight-trak’) trap was also a ramp-and-pitfall design and consisted of a wide-mouth plastic cup and an octagonal paper lid and base. The cup is of an inverted cone shape that is 10 cm diam. at the bottom, and from which a ramp rises at a 50° angle to a 5 cm diameter top. The ramp had a rough surface to facilitate insect crawling whereas the top edge was smooth so that insects would fall into the cup. The lure was glued to the upper portion of the lid.

Trap bioassays

All trap experiments were conducted in a 92×92×9 cm steel tray with one layer of whole wheat grains in the arena. A layer of grains provided footing as well as a natural environment to the responding beetles. The sides of the trays were coated with liquid Teflon to prevent insect escape. All trap experiments used laminate lures that had been aged 9–12 days in a fume hood. All pitfall traps contained 0.5 mL oil in their reservoirs. The oil was provided by Trécé Inc. and was a mixture of grain and mineral oils. Trécé oil presumably enhanced the attraction of beetles and served to kill the trapped beetles by suffocation. Only two types of traps were tested at a time, and each was placed randomly in the arena a
Results and Discussion

All commercial lure designs elicited significant attraction by *T. castaneum* under certain conditions, but response varied greatly with design and age of the lure. All of the lures exhibited low activity when first removed from their packages (0 week), and typically elicited maximum response at one or more weeks of age. Rubber septum lures (Fig. 1) showed highest activity at one week of age, and yielded similar responses at three and four weeks of age. Significantly lower responses to rubber septum lures were recorded at six weeks of age, and these were similar to the initial low response to new lures. Responses to the membrane lures were characteristically low when lures were first taken from the packages, and then showed increased activity with age. The membrane lure design tested here (Fig. 2) significantly repelled beetles when new and elicited no significant responses on weeks one, three and four, but were significantly attractive during later test periods. Membrane lures possessing different release characteristics were evaluated by Hussain (1993) and elicited responses similar to those reported here. Responses of *T. castaneum* to laminate lures revealed similar patterns over time as those found for membrane and septum lures (Fig. 3).

All ages of the laminate lure design tested (labelled ‘slow’ by the manufacturer) elicited significant attraction of *T. castaneum* compared to blank controls. Lowest responses to laminate lures were at week 0 and on weeks 6–14, and highest responses occurred during weeks 1–4 (Fig. 3). Additional designs of laminate lures examined by Hussain (1993) yielded less desirable response characteristics than the designs tested here. Amounts of DMD released over time from laminate lures used here were determined by GC analysis of collected volatiles (Fig. 4). It is clear that the new lures (0 week) that elicited low behavioural response were releasing much higher levels of pheromone than the older lures that were attractive to beetles.

Responses of *T. castaneum* to controlled release formulations of DMD are similar to what was observed for response to different doses of synthetic pheromone on filter paper discs (Hussain 1993). For that dose/response study, attraction was typically low at low doses, increased to some optimum level with increased doses, then decreased sharply at very high doses. The lure studies presented here reflect the same pattern as that for dose/response, but in reverse. Brand new lures (0 week) were presumably releasing at very high levels (e.g. Fig. 4), and thus were repellent, neutral, or slightly attractive. As release rate decreases with time the level of pheromone approaches an optimum level and attraction is highest. As release rate slows even more with age, attractive response diminishes. Loss of activity from DMD lures in these studies could also be caused by oxidation of the aldehyde group on the pheromone molecule. The ideal controlled release device should emit pheromone at a constant rate over a specified period of time (Roelofs 1979). Chemical analyses of volatiles from the laminate lure designs (Fig. 4) indicates release rate was not stable over the three-week test period, and behavioural responses of beetles reflect this variation in pheromone release.

A series of two-choice experiments were conducted with the five different traps in large tray arenas (Table 1). Initial experiments compared the original stogard design with the modified stogard design (bigger square cup, corrugations perpendicular to sides) and determined that the modified design captured significantly more beetles. A subsequent experiment (Expt 2, Table 1) determined that there was no difference in response of beetles to the square modified stogard and the rounded stogard. The Flit-Trak traps and Fuji traps each caught significantly more beetles than the Rounded Stor-
Table 1. Responses of *Tribolium castaneum* to various trap designs in large tray arena bioassays.

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Traps tested</th>
<th>Mean % caught (±SE)*</th>
<th>Differenceb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Storgard vs</td>
<td>11.50 ± 2.38</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>modified storgard</td>
<td>26.00 ± 2.85</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Modified storgard</td>
<td>20.50 ± 3.46</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>vs rounded storgard</td>
<td>24.50 ± 3.89</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Flit-Trak</td>
<td>23.00 ± 2.93</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>vs rounded storgard</td>
<td>11.50 ± 1.68</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Fuji</td>
<td>26.00 ± 4.44</td>
<td>**</td>
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<tr>
<td></td>
<td>vs rounded storgard</td>
<td>15.50 ± 2.35</td>
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<tr>
<td>5</td>
<td>Fuji</td>
<td>25.50 ± 3.44</td>
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<tr>
<td></td>
<td>vs Flit-Trak</td>
<td>12.50 ± 2.60</td>
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</table>

*Per cent of beetles caught in each trap after release of 50 beetles.

bDifferences between traps determined by Student's t-tests on arcsin transformed percentages; **, P<0.01; *, P<0.05; NS, P>0.05; N=8.

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References


