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 (Barak 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-dimethyldecanal (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 dimethyldecanal 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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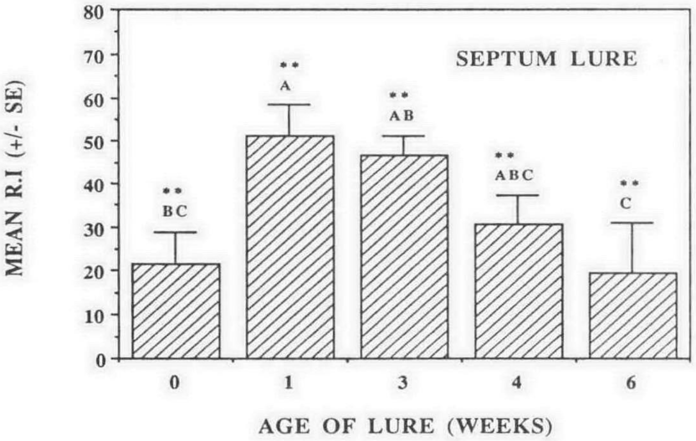

Fig. 1. Response of *T. castaneum* to rubber septum lures in two-choice pitfall bioassays. Histogram shows mean response index and standard error ($N=10$). The response index (RI) was calculated as $RI = (T-C/Tot) \times 100$, for which T is the number responding to the treatment, C is the number responding to the control, and Tot is the total number of insects released. Mean RIs followed by different letters are significantly different ($P<0.05$, ANOVA and LSD). Significant difference between treatment and control within each age class are indicated as ** ($P<0.01$).

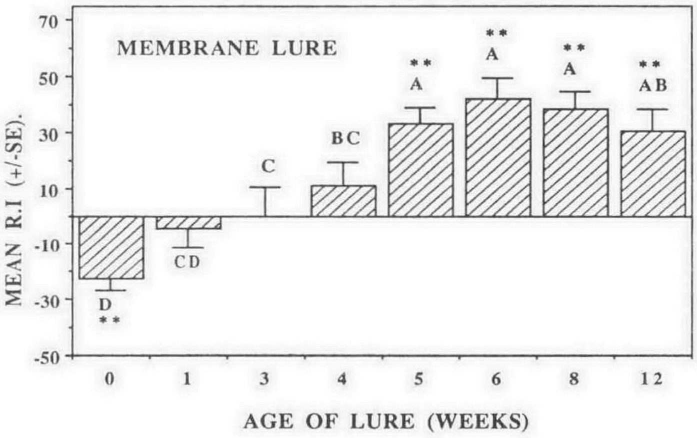

Fig. 2. Response of *T. castaneum* to membrane lures in a series of two-choice pitfall bioassay. Histograms show mean response index (RI, see above) and standard error ($N=10$). Mean RIs followed by different letters are significantly different ($P<0.05$, ANOVA and LSD). Significant difference between treatment and control within each age class are indicated as ** ($P<0.01$).

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 punchouts. The two bottom circular plates fit the 4 cm square × 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 $4 \times 10 \times 1$ cm, constructed primarily from hard paper. Responding insects enter ramps on each end of the trap, climb the ramp to the edge of a $4 \times 4 \times 1$ 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 onto 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-track') 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 $92 \times 92 \times 9$ 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

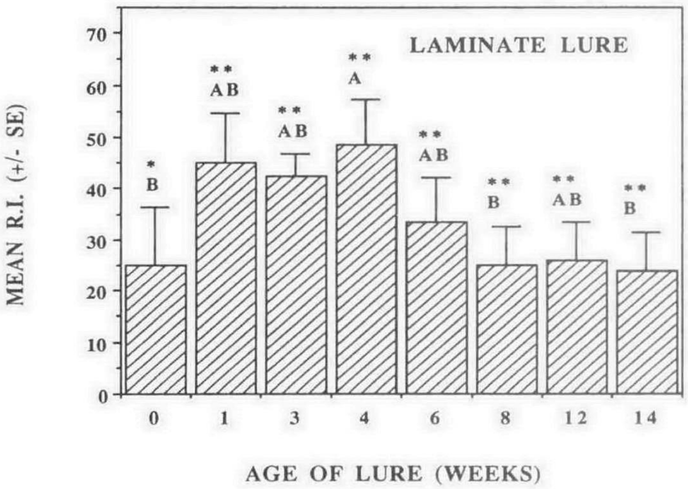

Fig. 3. Response of *T. castaneum* to laminate lures in a two-choice pitfall bioassay. Histograms show mean response index (RI, see above) and standard error (N=10). Mean RIs followed by different letters are significantly different ($P<0.05$, ANOVA and LSD). Significant difference between treatment and control within each age class are indicated as ** ($P<0.01$) and * ($P<0.05$).

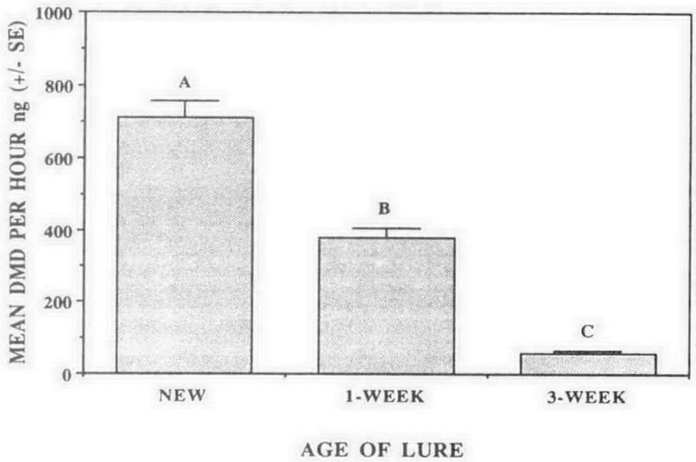

Fig. 4. Amounts of pheromone released by laminate lures at different ages. Volatiles from lures were collected on super-Q and analysed with quantitative GC. Mean release rates with different letters are significantly different ($P<0.05$, ANOVA and LSD).

minimum of 15 cm from a side and 30 cm from another trap. Eight replicates of each pair-wise comparison were performed. One-week-old, mixed sex beetles were counted and kept without food for 30 minutes before the experiments. An inverted glass funnel of 2 cm diam. was placed in the centre of the tray arena and the test beetles were released into it. Fifty insects were kept confined under the inverted glass funnel for 15 minutes and then were released into the arena by lifting the glass funnel. After releasing the beetles in the arena the trays were covered with a screen to prevent any insect escape by flight. Bioassays were conducted for 20 hours in complete darkness at 27°C and 60 ± 10% r.h., after which the number of beetles caught in each trap was determined.

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 storgard design with the modified storgard 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 storgard and the rounded storgard. 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.

Exp. #	Traps tested	Mean % caught (±SE)[a]	Difference[b]
1	Storgard vs	11.50 + 2.38	**
	modified storgard	26.00 + 2.85	
2	Modified storgard	20.50 + 3.46	NS
	vs rounded storgard	24.50 + 3.89	
3	Flit-Trak	23.00 + 2.93	*
	vs rounded storgard	11.50 + 1.68	
4	Fuji	26.00 + 4.44	**
	vs rounded storgard	15.50 + 2.35	
5	Fuji	25.50 + 3.44	**
	vs Flit-Trak	12.50 + 2.60	

[a] Per cent of beetles caught in each trap after release of 50 beetles.
[b] Differences between traps determined by Student's t-tests on arcsin transformed percentages; **, $P<0.01$; *, $P<0.05$; NS, $P>0.05$; N=8.

gard traps (Table 1, Expts 3 and 4). When Fuji and Flit-Trak traps were compared in the same experiments the Fuji trap captured significantly more beetles (Table 1, Expt 5). Thus, of the two ramp style traps tested, it appears that the Fuji trap, which employs a straight cardboard ramp at each end, is more effective at trapping *T. castaneum* than the Flit-Trak trap, which employs a circular plastic ramp.

It is clear from these studies that formulation and release rate of pheromone is very important for response of *T. castaneum*. Most commercial pheromone lures tested elicited poor responses when first removed from their packages, but yielded positive responses by beetles after some period of aging. This 'burst' effect of slow release pheromone devices is commonly noted (McDonough 1991). A large amount of pheromone is presumably released early in the life of the device as the transfer matrix becomes saturated with pheromone and release equilibrates. Our studies also indicate that trap design can be very important in capture of *T. castaneum*. The success of the ramp-pitfall designs may relate directly to a natural tendency of beetles to be negatively geotropic (Mullen 1992). Additionally, ramp traps may allow for more unimpeded release of pheromone from the lure compared to cardboard traps, in which there is not a direct path for escape of the pheromone from the lure to the outside of the trap. These studies provide a framework for future studies that should address efficacy of lures and traps in field situations.

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