

Assessing trap and lure effectiveness for the monitoring of *Sirex noctilio*

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Abstract

1. Lure-baited traps are an important tool for monitoring the spread and establishment of the Sirex woodwasp *Sirex noctilio*. The utility of these traps, however, is limited in areas with low wasp populations due to the reliance on a plant volatile (kairomone) lure in the absence of an identified pheromone. Knowledge of the optimal trap type and deployment strategy is also lacking.
2. We tested the effectiveness of a putative pheromone in baited traps, by means of a series of field trials in South Africa over a three-year period. We also examined the influence of lure type, trap type and trap height on capture success.
3. The pheromone was found to be ineffective as an attractant under South African field conditions for both male and female wasps. Lure type, trap type and trap height were found to have little to no effect on female wasp catch. Given moderately strong responses to the blend in wind tunnel and laboratory conditions, we suggest possible aspects of the biology and life history of *S. noctilio* that may influence lure effectiveness.
4. The traditional black intercept panel traps with kairomone lure remains the best trap for *S. noctilio*, at least where populations are high.

Keywords: pheromone, kairomone, insect detection and monitoring, intercept panel traps

Introduction

Accurate information on the abundance and spread of alien invasive species is required to inform management decisions and to predict and minimize potential detrimental effects to natural and human landscapes. For alien invasive forest insect species, traps baited with either tree stress volatiles or synthetic pheromones that closely mimic those produced and detected by the insects themselves are among the most effective tools for monitoring spread and establishment (Brockerhoff *et al.*, 2006; Witzgall *et al.*, 2010; Nadel *et al.*, 2012). Trap design and deployment strategy is often target insect-specific, with myriad variables of trap shape, size, color, position and timing of deployment being critical to success. Successful examples of forest insect pests whose spread has been monitored with lure-based traps include the red turpentine beetle *Dendroctonus valens* (Erbilgin *et al.*, 2008), the emerald ash borer *Agrilus planipennis* (Crook & Mastro, 2010), gypsy moth *Lymantria dispar* (Sharov *et al.*, 1997), and pine processionary moth *Thaumetopoea pityocampa* (Athanassiou *et al.*, 2007), among many others. However, despite the importance of lure-based traps for monitoring spread and establishment of forest insect pests, this tool is not available or well developed for some important invasive species.

The Sirex woodwasp *Sirex noctilio* is one such invasive insect pest for which lure-based traps for monitoring requires further development. Native to Eurasia, this insect has been accidentally introduced to many parts of the southern hemisphere and, more recently, to the USA and Canada (Hoebeke *et al.*, 2005; de Groot *et al.*, 2006; Hurley *et al.*, 2007). Females of the Sirex woodwasp attack primarily stressed or weakened *Pinus* trees, injecting a phytotoxic mucus together with inoculum of an obligate fungal symbiont (*Amylostereum areolatum*) resulting in rapid tree death, usually within a few months (Talbot, 1977; Bordeaux & Dean, 2012). In the southern

hemisphere, where *S. noctilio* was first detected in the 1900s, it has caused widespread mortality and considerable economic losses to non-native pine forestry (Hurley *et al.*, 2007). Though it remains a significant threat to pine production in many areas, losses incurred due to established *S. noctilio* populations have been offset by management strategies, specifically silvicultural methods to reduce stress in the stands and the introduction of various biological control agents. Accurate knowledge of the current and changing distribution of *S. noctilio* is crucial to ensuring appropriate management. This applies particularly to North America, northeastern Australia, Chile and other areas where *S. noctilio* is still spreading to new areas (Carnegie *et al.*, 2005; Beeche *et al.*, 2012; Dodds & de Groot, 2012), and where early detection in these new areas can assist management strategies.

Various methods have been used to detect and monitor populations of *S. noctilio* as it has spread through its invaded range. Ground and aerial surveys have been widely used in Australia, South America and South Africa (Carnegie *et al.*, 2008; Beeche *et al.*, 2012; Hurley *et al.*, 2012; Klasmer *et al.*, 2012) but are time consuming and costly when conducted on a large scale (Bashford & Madden, 2012). More recently satellite imagery has been used to monitor *S. noctilio* populations (Ismail & Mutanga, 2011) but this approach depends on differences in spectral reflectance correlated with tree stress/decline and is not specifically linked to infestation by *S. noctilio*. In addition, aerial methods are inefficient at detecting low density populations of *S. noctilio* where infestations are largely in sub-dominant trees obscured by the canopy when viewed from above.

Other approaches used to monitor and detect *S. noctilio* include the use of herbicide or mechanically-killed trap trees (Madden, 1971) as well as traps baited with kairomone lures based on plant stress volatiles (Bashford, 2008). The trap tree

approach is successful at detecting *S. noctilio* at low densities and can be co-utilized to introduce the parasitic nematode, *D. siricidicola* (Bedding & Iede, 2006), but this method is expensive and highly labor intensive. Traps baited with kairomone lures have experienced variable success that appears to be linked to differences in population densities throughout the wasp's invasive range. For example, in South Africa where *S. noctilio* populations are high, plant volatile lures are reasonably successful while in mixed stands of North America where wasp densities are comparatively low, traps appear to be less efficient (Dodds & de Groot, 2012). In areas with native pine, by-catch of native siricids and other bark and wood-feeding insects is also a concern.

In 2010, extensive GC-EAD investigations and subsequent laboratory assays identified a putative male-produced aggregation pheromone with the potential for use as a field attractant for *S. noctilio*. The compounds (major component: (*Z*)-3-decenol; minor components: (*Z*)-4-decenol and (*E,E*)-2,-4-decadienal) induced a significant positive response in both males and females using Y-tube olfactometers and a wind tunnel (Cooperband *et al.*, 2012). The goal of this study was to test the effectiveness of a synthetic pheromone lure developed incrementally over three years by Cooperband *et al.* (2012). In the first year of field trials, only the main pheromone component had been discovered, and the presence of minor components was still being investigated. This study was carried out in three consecutive years (2010-12) in pulp plantations of *Pinus patula* in South Africa, largely due to the consistently high densities of wasps and knowledge of the distribution, abundance and phenology of the insect in this area. In 2011 and 2012, we also varied lure type, trap type and height in the canopy in an effort to maximize capture rate, in part based on results from 2010.

Methods

Field sites and overall general approach

Trap trials were established over a three-year period from 2010 to 2012. In each year, the trap trial was established at three different sites near Waterval Boven in Mpumalanga province (S25.74 E29.83). The trap trial in 2010 included an additional three sites near Mthata in the Eastern Cape province (S30.54 E28.91). Each site was located in 10-15 year old plantations of *Pinus patula* and separated by between 10 and 50 km. In each year a randomized complete block design was used, but the number and type of treatments differed between years (Table 1). In all years, the plant stress volatile (herein “kairomone”) lure known to be effective as an attractant to *S. noctilio* in South African plantations was used as a positive control and was used in conjunction with the putative pheromone. Additionally, we included trap blanks (no chemical component) to control for the visual effect of the different trap types together with the possibilities of random intercepts as wasps fly through the stands.

Year by year experimental design

For the 2010 trial, each site contained 14 treatments with five replicate traps per treatment. Black intercept panel traps (70 total per site, 420 total) were hung on trees at a height of 2.5 m in a 7 x 10 grid, maintaining a distance of 20 m between traps. Treatments included the major pheromone component ((Z)-3-decenol, identified by Cooperband et al., (2012)) at four concentrations (0; 0.3; 3; 30 mg), a suspected minor compound at that time (nonanoic acid), present at 1% of the concentration of the major component, or absent, and the kairomone present (at 30 mg) or absent (Table

1). When the major pheromone component (*Z*)-3-decenol was at 0 mg, nonanoic acid was also absent. Rubber septa were used as the release devices for the two-compound blend. The kairomone lure was the lure developed in previous collaborative trials with USDA-APHIS and consisted of a blend of (+)-alpha-pinene (12.5 %) (-)-alpha-pinene (12.5 %), (-)-beta-pinene (25.0 %), (+)-3-carene (30.0 %), (+)-camphene (5.0%), beta-myrcene (10.0%), (+)-limonene (2.5 %) and (-)-limonene (2.5%). This is the same lure used in the South African National Sirex Monitoring program (Insect Science (Pty) Ltd, Tzaneen, South Africa). Plastic bottles (clear bottle 15 ml in length and 650 micron thickness, in polyethylene bag) were used as the release device for the kairomone lure.

Each site in the 2011 trial contained 16 treatments, with five replicates of each treatment. Black intercept panel traps (80 total per site, 240 total) were hung on trees at a height of 2.5 m in an 8 x 10 grid, maintaining a distance of 20 m between traps. Treatments consisted of the new three-component pheromone lure, with (*Z*)-3-decenol, (*Z*)-4-decenol, and (*E,E*)-2-4-decadienal at 100:1:1, respectively (Cooperband *et al.*, 2012), at three dosages (0; 0.1; 1 mg); presence and absence of the same kairomone lure used in the 2010 trial; two trap designs (panel and clear jar); and two trap heights (2.5 m and 8 m above ground level; Table 1).

The new pheromone lures used in the 2011 trial consisted of the same major component as that used in the 2010 trial, but excluded nonanoic acid, and instead included two new potential minor components that were identified in the Otis Laboratory (USDA-APHIS) in 2011 using GC-EAD, GC-MS and Y-tube bioassays (Cooperband *et al.*, 2012). The two trap designs tested were traditional black intercept panel traps and a new clear jar trap design, constructed from rectangular plastic jars (64-oz. PVC Pinch Grip-It Jars, US Plastic Corp., Lima, OH, USA), (Sarvary *et al.*,

2014). The clear jar design was developed in response to the observation that although both sexes were attracted to pheromone lures in wind tunnel experiments, females flew into the targets made of black panel material, whereas males were able to avoid or land on the targets made of black panel material, potentially explaining why males were rarely trapped in collection buckets in field trials (Cooperband *et al.*, 2012). Prototypes were developed at APHIS and showed promise in wind tunnel experiments in catching *S. noctilio* males (Sarvary *et al.*, 2014). Given logistical and budget constraints, all combinations of lures and trap height were used only for the black intercept panel traps. We excluded the 0.1 mg dosage of the pheromone lure and the low trap height for the newly designed clear jar traps (Table 1).

The 2012 trap trial contained one 60-trap site with 12 treatments and two 40-trap sites with eight treatments. Each treatment was replicated five times giving a total of 140 traps. Intercept panel traps were hung on trees at a height of 2.5 m in a 6 x 10 grid or 4 x 10 grid, maintaining 20 m between traps. Treatments for the 60-trap site included the same 3-component pheromone blend used in the 2011 trial, but with three lure types and dosages (septa lure 1 mg, Flexlure 2 mg, Flexlure 8 mg) (Flexlure, Contech Enterprises Inc., Victoria, BC, Canada), presence and absence of kairomone lure, and intercept trap color (black and clear). Clear intercept traps were only used with the septa lure and kairomone and only at a single site, due to the limited numbers of clear traps available. The same treatments were used in the 40-trap sites, excluding the treatments with the clear intercept traps.

In the three years of field studies, the major pheromone component was synthesized by Dr. Tappey Jones (Virginia Military Institute, USA) as described in Cooperband *et al.* (2012), and this process resulted in the presence of a small amount of the (*E*)-3-decenol isomer. In the third year, the (*Z*)-3-decenol was sent to Contech

to attempt further purification. In 2010, 2011, and 2012, the amount of (*E*)-3-decenol in 100 parts of (*Z*)-3-decenol used in lures was: 0.6%, 0.8%, and 0.3%, respectively.

Data collection

The monitoring period of the traps included the peak emergence of *S. noctilio* in the trial areas, based on historical data. For the 2010 trial, the traps were monitored for five weeks, from the week of 8-12 November to the week of 6-10 December. For the 2011 and 2012 trials, the traps were monitored for six weeks, from the weeks of 24-28 October to 28 November – 2 December in 2011 and 5-9 November to 10-14 December in 2012. Traps were checked weekly. Wasps were counted, sexed and removed from traps, and the buckets re-filled with soapy water. Lures were replaced at the end of the third week of the monitoring period, based on the recommendation of the supplier.

Data analysis

Mean female trap catch across the sampling weeks were analyzed using separate ANOVA models for each year. The dependent variable(s) were $\log(x+1)$ transformed for 2010 and 2011 data and square root transformed in the 2012 data to conform to model assumptions of normality and homogeneity of variance. Due to very low male catches, only data from female catches were analyzed statistically. Preliminary tests in each year revealed the absence of a block effect and so block was discarded from reported models. We also performed a repeated measures ANOVA on weekly time series data. Results from the repeated measures analysis were qualitatively similar to

those based on mean trap catch. For simplicity, all trends reported are based on mean catch across weeks.

For the 2010 data, since the design was slightly unbalanced (i.e., the minor component was not tested in the absence of the major component), we tested the contribution of the minor component using a subset of data comprising a fully factorial experiment (i.e., by removing trap data from treatments where the major component was absent). We also considered the effects of region (north v. south) and site as a nested factor within region. In 2011 data, clear jar trap catch was not included in the statistical analyses due to negligible capture rates (10 wasps across all sites and weeks). For the 2012 trap trial, clear intercept panels traps were placed only in a single site and were analyzed using trap type (clear v. black), kairomone (presence/absence), and lure (septa v. none) in a subsetted $2 \times 2 \times 2$ factorial design. No transformation was necessary in this subset of the data. The black intercept panel trap data were treated separately as a $3 \times 2 \times 4$ factorial ANOVA. All statistical analyses were performed in R (R Core Team, 2013).

Results

A total of 12 464 *S. noctilio* were caught in the traps over the three years of trap trials (3423 in 2010, 2212 in 2011 and 6827 in 2012). Of these, only 161 were males, representing 1.3 % of the total wasps caught. This number is especially low considering the male bias in the population (measured as 6.5:1 male:female ratio in the region in 2012; unpublished data).

In the 2010 trial, trap catch varied significantly by site (nested within region; $F_{4,380} = 191.2$; $P < 0.0001$) but not region ($F_{1,4} = 0.14$; $P = 0.73$). Among the lure

combinations tested, wasps showed by far the strongest response to the kairomone lure ($F_{1,380} = 495.4$; $P < 0.0001$; Figure 1), irrespective of pheromone treatment. This effect was consistent both within and across weeks. The major pheromone component showed no significant main effect on wasp catch ($F_{3,405} = 1.97$; $P = 0.12$) in the 2010 trial. The major pheromone component did interact marginally with the kairomone lure ($F_{3,405} = 2.6$; $P = 0.05$); trap catch increased more or less linearly from 0-30 mg concentration, but only in the absence of the kairomone. Planned linear contrasts revealed that where the kairomone was absent, the major pheromone component was no more attractive than the control at a concentration of 0.3 mg ($t_{405} = 1.19$; $P = 0.12$), but that lures containing 3 mg and 30 mg attracted more wasps than the lures without the major pheromone component ($t_{405} = 1.88$; $P = 0.03$ and $t_{405} = 2.38$; $P = 0.009$ respectively). However, the biological significance of these differences was questionable (least squares means for the 0, 0.3, 3.0, and 30.0 mg treatments: 0.24, 0.35, 0.46 and 0.53 wasps per trap per week respectively, versus 1.57, 1.96, 1.95 and 1.58 wasps in the presence of kairomone). The nonanoic acid component did not appear to influence lure attractiveness ($F_{1,405} = 0.003$; $P = 0.96$).

In 2011, mean wasp catch varied by site ($F_{2,144} = 106.7$; $p < 0.0001$), with two sites yielding the majority of the wasps collected in this trial (1108 females and 11 males, and 917 females and five males respectively). Wasp catch peaked in weeks four and five (14-25 November) at these two sites (554 and 397 wasps respectively). The clear jar traps were ineffective under field experimental conditions, with only 10 females and no males caught in the 60 traps over the six weeks. For intercept panel traps, female wasp catches showed by far the strongest response to the kairomone lure ($F_{1,144} = 294.5$; $P < 0.0001$; Figure 2), with trap catches significantly higher when the kairomone was present. Pheromone concentration showed no significant influence on

female catch ($F_{2,144} = 2.2$; $P = 0.10$). Trap height on the other hand did significantly influence capture rates ($F_{1,144} = 4.5$; $P = 0.04$); traps placed at 8m attracted more females than those placed at 2m, though differences overall were minor (1200 and 986 wasps respectively across sites and panel trap treatments). Trap height also interacted with site ($F_{2,144} = 8.3$, $p < 0.001$) and kairomone lure ($F_{1,144} = 8.1$; $P = 0.005$), but biological differences were minor. Ten males were collected in the higher traps versus 6 in the low.

For the 2012 trial, trap type influenced female wasp catch ($F_{1,32}=20.5$, $p<0.0001$; Figure 3) as did kairomone lures ($F_{1,32}=35.9$, $p<0.0001$; Figure 3), with black traps and kairomone lures attracting significantly more wasps. There was also a significant trap type by kairomone lure interaction ($F_{1,32}=16.6$, $p=0.0002$) with kairomone lures strongly improving trap catch, but only in the black intercept panel traps. The presence or absence of the septa lures had no effect on trap catch at this site ($F_{1,32}=0.07$, $p=0.79$). For black intercept panel traps across all three sites, we found a significant effect of site ($F_{2,96}=16.5$, $p<0.0001$) and kairomone lures ($F_{1,96}=103.9$, $p<0.0001$) on mean female trap catch across weeks (Figure 4). There was no significant effect of pheromone lure as a main effect ($F_{3,96}=1.4$, $p<0.25$), nor did the pheromone lure interact significantly with any other treatment. These results were equivalent in all weeks.

Discussion

This study investigated the efficacy of a novel synthetic pheromone to trap *S. noctilio* along with other factors hypothesized to influence baited trap performance, namely trap design, transparency and placement in the canopy. This was

accomplished by means of a series of replicated trapping trials over a three-year period. Results clearly indicate that the pheromone lures were ineffective at attracting *S. noctilio* under South African field conditions and with the trap designs employed. Kairomone lures were confirmed as effective attractants. Factors such as pheromone lure concentration, panel transparency and placement in the canopy had little or no effect on wasp captures. The clear jar traps were entirely ineffective. When baited with the kairomone lures, black trap color greatly enhanced trap capture compared to clear traps.

There are several potential explanations for the apparent lack of pheromone lure attractiveness in our trials. Firstly, it is possible that the identity and/or ratios of the major and minor components are incorrect or additional components are required. This is particularly relevant for 2010, prior to the discovery of the two minor components. It is possible that the amount of (*E*)-3-decenol, a known inhibitor when added at 1% (Cooperband *et al.*, 2012), could have interfered with attraction in 2011 when it was present at 0.8% of the major compound. However, given the positive electroantennographic response and positive orientation behavior in choice assays using North American wasps and the same lures (Cooperband *et al.*, 2012; Sarvary *et al.*, 2014), other hypotheses warrant consideration.

Alternate hypotheses to explain the ineffectiveness of the pheromone lure include that pheromones and/or pheromone receptors are different in southern hemisphere *S. noctilio* populations, which originated from a distinct introduction relative to North American populations (Boissin *et al.*, 2012). GC-EAD work to evaluate this possibility is currently in progress. Innate behavior of male wasps also could have limited male trap catch. Males were observed in wind tunnel experiments to land on pheromone-baited targets instead of bumping into them (Cooperband, pers obs.), and

were captured by the pheromone-baited clear jar traps in wind tunnel studies, where they were also found to be strongly positively phototactic (Sarvary *et al.*, 2014). The phototactic behavior may explain why alternative trap designs to circumvent this potential problem were likewise unsuccessful at capturing males in pine plantations at heights of 2.3 and 8 m, which are well below the canopy. Finally, with regards to females, pheromone lure attractiveness may be dependent on the age and mating status, which, given the mating behavior and life history of *S. noctilio*, is likely to complicate the use of pheromone traps.

Pheromone production, perception and behavioral response can be influenced by factors such as insect age and mating status (Barrozo *et al.*, 2011). Due to the apparently strong phototactic response of wasps (Sarvary *et al.*, 2014), emerging male and female *S. noctilio* fly to the tree canopy where they form mating swarms (Morgan & Stewart, 1966; Madden, 1988). If this behavior overrides response to chemical volatiles, wasps may leave the understory irrespective of the pheromones present. Post-mating, males may continue to be positively phototactic and seek additional mates above the canopy, and females are likely to respond most strongly to host volatiles and visual cues (Madden, 1968; Sarvary *et al.*, 2014). While we have little empirical data to support this hypothesis to date, over 70% of spermatheca dissected from trapped females contained sperm (A.L. Wooding, unpublished data), consistent with the idea that behaviour and timing may influence trap catch. Anecdotally, males were present in large numbers at the times of peak capture, but by and large were found on the forest floor, presumably having spent the bulk of their energetic reserves. More direct measurements of the context dependence of attraction of pheromones in the lab or field enclosures could shed more light on these questions.

The influence of trap height, trap design and trap color was also investigated as it was suggested that these might increase wasp catch, specifically male wasps. Such factors have been recorded to significantly influence trap catch in other forest insects of economic importance, including the oak processionary moth *Thaumetopoea processionea* (Williams *et al.*, 2013), pine moth *Thaumetopoea pityocampa* (Athanossiou *et al.*, 2007), emerald ash borer *A. planipennis* (Ryall *et al.*, 2012), and generally for cerambycid beetles and siricid wasps (Morewood *et al.*, 2002; Graham *et al.*, 2012). However, the influence of these factors appears to be highly specific to the target species (Brar *et al.*, 2012). In the current study, the marginally higher trap efficiency at 8 m *versus* 2 m is not large enough to warrant the additional expense and complexity of placing and monitoring traps at mid-canopy.

In summary, traditional black intercept panel traps with kairomone (plant volatile) lures in use in South Africa since 2004 remain the best choice for trapping studies, at least where populations are high. The tested pheromone blend was not an effective lure to catch *S. noctilio* males or females with the current trapping or release system, nor did it synergize the kairomone lure. Placing traps higher in the tree may slightly increase wasp catch, but given the logistical challenge and costs associated with placing and monitoring at ~8m, this approach does not appear to be worthwhile. Improving trap design may be necessary to increase catch of male wasps, and further testing on the context-dependency of the attractiveness of synthesized pheromones to *S. noctilio*, together with an integrated understanding of wasp biology are warranted.

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Table 1. Summary of the treatments tested for the trap trial over the three-year period.

Each treatment was replicated five times at each site.

Year	Treatment	Levels		
2010	Major component	4	0; 0.3; 3; 30 mg	
	Minor component	2	Y/N	
	Plant volatile	2	Y/N	
2011	Panel traps	Pheromone lure	3	0; 0.1; 1 mg
		Plant volatile lure	2	Yes / No
		Trap height	2	Low / High
	Bottle traps	Pheromone lure	2	0; 1 mg
		Plant volatile lure	2	Yes / No
		Trap height	1	High only
2012	Clear traps	Pheromone lure	1	Septa 1 mg
		Plant volatile lure	2	Yes / No

Figure 1. Mean trap capture of female *S. noctilio* from 2010 trap trial by major component ((*Z*)-3-decenol), concentration, presence v. absence of 1% nonanoic acid (triangles v. squares) and presence v. absence of kairomone lure (closed symbols, solid lines v. open symbol, dashed lines). Error bars are standard error.

Figure 2. Mean trap capture of female *S. noctilio* for 2011 trap trial by treatment. Black bars correspond to the absence of 3-component pheromone blend, and grey and white bars to low and high (0.1 and 1.0 mg) concentrations respectively. Clear jar traps were only tested at two sites, at zero and high pheromome concentration and at 8m meters above ground level (high), and caught a total of 10 wasps during the course of the experiment.

Figure 3. Comparision of the number of female *S. noctilio* caught between black and clear panel traps for the 2012 trap trial.

Figure 4. Comparison of the number of *S. noctilio* wasps caught by lure type and kairomone lure presence (light bars) or absence (dark bars) in the 2012 trap trial. Flex-L and Flex-H refer to “Flex lures” (Contech Enterprises Inc., Victoria, BC, Canada) infused with 2 mg and 8 mg of pheromone blend, respectively, compared to the 1 mg septa.

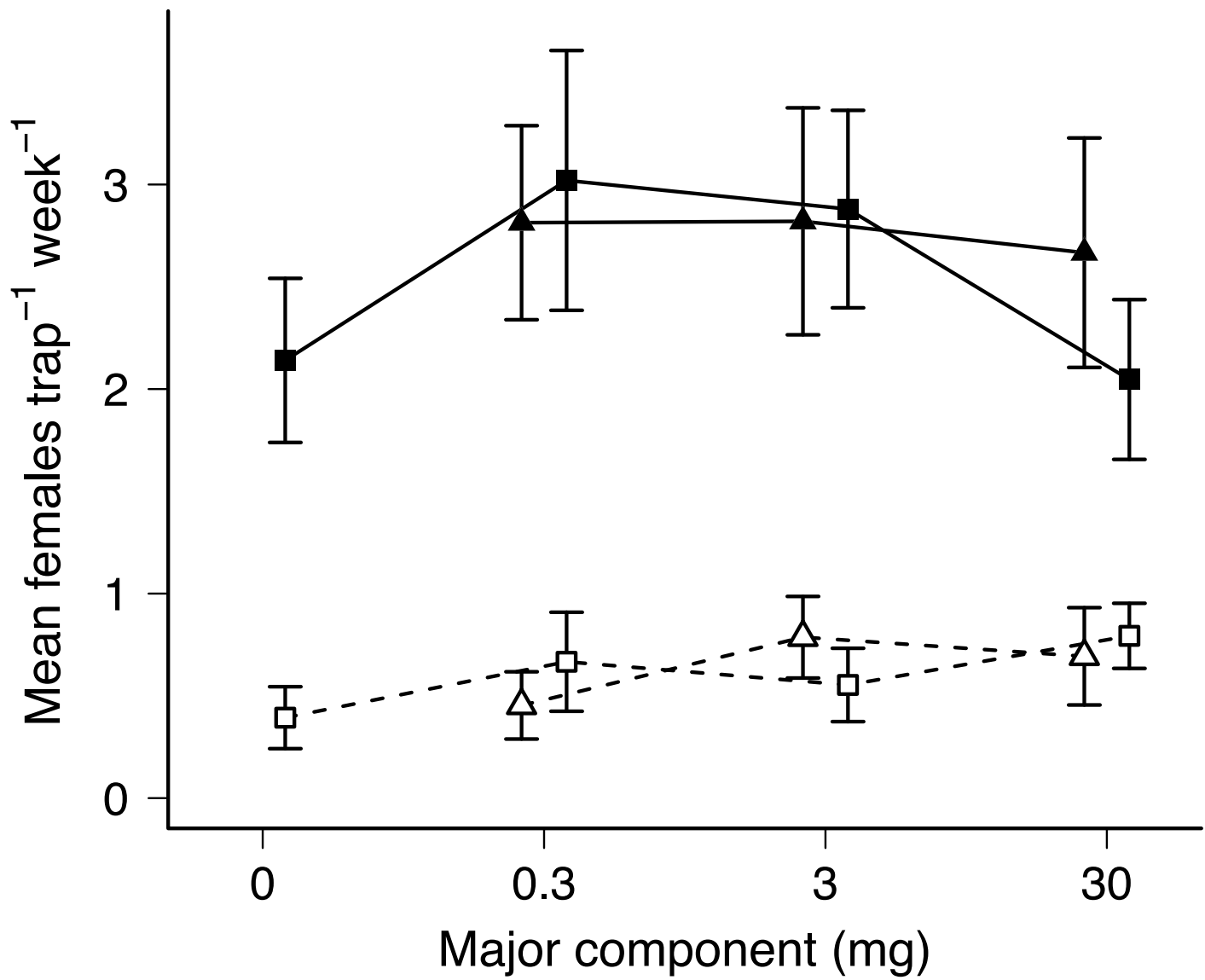


Figure 2.

