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Too close for comfort: effect of trap spacing distance and pattern on statistical inference of behavioral choice tests in the field

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Abstract

Behavioral choice tests provide a powerful and commonly used technique for evaluating the biological activity of chemical signals. Despite the widespread application of this approach, relatively few studies have evaluated a key assumption, that is, relative independence among treatments. Previous work has demonstrated that both the number of choices and their physical arrangement can affect the results of choice tests with leaf-feeding insects in laboratory assays. Here, we consider another spatial component, the distance between treatments, in a field assay, using a bark beetle as our model. We used three geometries of trap arrangements, two spacing levels, and both 'low activity' lures and a 'high activity' lure in our behavioral assays. We found that proximity to an attractive treatment generated unexpectedly high trap catches at relatively non-attractive treatments, even in the presence of a uniform treatment effect and relatively constant insect population size. Increases in traps baited with 'low activity' lures proximate to a highly attractive treatment ranged from 4 to 7× the catch observed in configurations with traps spaced wider apart. Moreover, even in the absence of 'high activity' lures, a lure catching less than one insect per day on average could obscure the effect of a control trap at proximate spacing. In our example, a spacing distance of 15 m appears to provide independence among traps used to sample the bark beetle *Ips pini* (Say) (Coleoptera: Curculionidae: Scolytinae), but 3 m does not. Our broader intent is to provide a useful approach to the design and evaluation of behavioral choice experiments in the field.

Introduction

Behavioral choice tests provide one of the most effective and commonly used approaches for evaluating the relative attractiveness of various stimuli. They have been used for a wide variety of behaviors, types of stimuli, and taxonomic groups, and under a wide array of field and laboratory conditions. Despite the widespread use of behavioral choice tests, the underlying assumption of independence between choices remains an ongoing area of research (Horton, 1995; Raffa et al., 2002).

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Several papers have addressed specific experimental conditions that can bias the results of behavioral choice tests (Marquis & Braker, 1987; Raffa & Frazier, 1988; Jones & Coleman, 1988). For example, in a previous laboratory study on the analysis of multiple choice assays, Raffa et al. (2002) observed that both the number of treatments and their arrangement can skew results. They also noted that an experimenter's decision on how to address spatial considerations as the number of choices increases tends to differ between laboratory and field studies. Whether for reasons of expediency, habit, or precedence, laboratory tests typically keep the size of the arena constant and hence vary distances between treatments, whereas field experiments typically retain a constant distance between treatments and hence expand the arena. Both have ramifications for statistical analysis. In the former, variable distance is a confounding factor. In the latter, there is an implicit assumption of equivalent population distributions across the sampling universe.

In field assays, even if the assumption that there is a fixed (and available) population size across the sampling universe holds true, the radius of attraction for a chemical may only be known post-hoc. For example, interference among traps may occur when the effects of one lure obscure the effects of another. Whether a push (i.e., repulsive) or pull (i.e., attractive) effect, the ensuing bias may alter the distribution of insects among traps. Consequences of trap interference are varied, ranging from altered parameters in dispersal studies (Byers, 1999) to violation of statistical assumptions, which may or may not be serious (Horton, 1995). Trap interference in behavioral choice assays, could, for example, alter distribution of insects among traps and obscure a treatment effect where one does, in fact, exist (Type II error), or create the appearance of a treatment effect where one does not occur (Type I error). Many choice studies interested in the bioactivity of various compounds classically test for an overall treatment effect; that is, does the average insect catch vary between lures (Reeve & Strom, 2004).

In standard bioassays consisting of an experimental configuration of different lures and controls that is replicated multiple times, the classic F-test testing treatment means can detect overall treatment effects and facilitate post-hoc means comparisons but cannot easily detect lure bias. Indeed, beyond operational considerations, it is difficult to know how serious the challenge of establishing trap independence may be for most choice studies. To formally test trap bias, the experimenter needs to vary trap spacing and configurations.

The purpose of this experiment was to investigate various numbers, arrangements, and strengths of lures to test whether a lure with high biological activity can obscure subtle effects of weaker lures in the presence of treatment effects. We conducted this experiment with natural insect populations responding to known attractants.

Materials and methods

Model system

The pine engraver, *Ips pini* (Say) (Coleoptera: Curculionidae: Scolytinae), colonizes the phloem tissue of conifers. As with most other bark beetles, adults fly from the trees in which they developed, locate new hosts, and emit aggregation pheromones that facilitate group colonization. These pheromones are attractive to both sexes. In Wisconsin (USA), males produce racemic ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) plus lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one). Ipsdienol is attractive by itself, in a dose-dependent fashion (Miller et al., 2003). Lanierone elicits no attraction by itself, but synergizes the attractiveness of ipsdienol to *I. pini* (Teale et al., 1991; Seybold et al., 1992).

Site location and trapping system

The experiment was conducted in a mature red pine [*Pinus resinosa* Aiton (Pinaceae)] plantation in Columbia County (WI, USA). Beetles were sampled using 12-unit funnel traps (Lindgren, 1983). We baited each trap with a bubble-cap lure of 50(+)/50(−) ipsdienol containing 0, 0.2, 2, 20 mg, corresponding to release rates of 0, 1, 10, 100 μg per day at 25 °C, plus a similar bubble-cap lure of 20 mg lanierone with each level of ipsdienol (release rate 100 mg per day at 25 °C) (Pherotech, Delta, BC, Canada). Traps were suspended from ropes between trees at 3 m above the ground. A 3 × 2 cm piece of No Pest Strip (Loveland Industries, Greeley, CO, USA) was placed in each collection cup to prevent destruction of contents by predators.

Experimental design

Each block within the plantation contained two, three, or four traps, each with a spacing of either 3 or 15 m, for a total of six pheromone concentration and spacing combinations (Figure 1). The traps for the two-, three-, and four-choice assays were configured in a line, triangle, and square, respectively, and treatments were assigned using a random number table. For the square, the spacing refers to distance between adjacent corners. Each treatment, that is, a pheromone × spacing combination, was replicated four times, for a total of 24 blocks. Each block was 45 × 45 m. Blocks were separated by a minimum of 30 m.

We sampled the traps every 4 days, from 27 July to 28 August 2004, for a total of nine sampling dates. The lures were re-randomized within each block at each collection. Insects were identified according to Wood (1982).

Statistical analysis

The effect of spacing and the number of treatments on the total trap catch of *I. pini* was examined using ANOVA in a linear mixed-effects model, where block was incorporated as a random effect. Separate ANOVAs examining the effect of pheromone treatments on total average catch were fit to subsets of data reflecting the different geometric configurations of traps (line, triangle, square; Figure 1). Because we were interested in how experimental inference about lures with low biological activity changed with the number

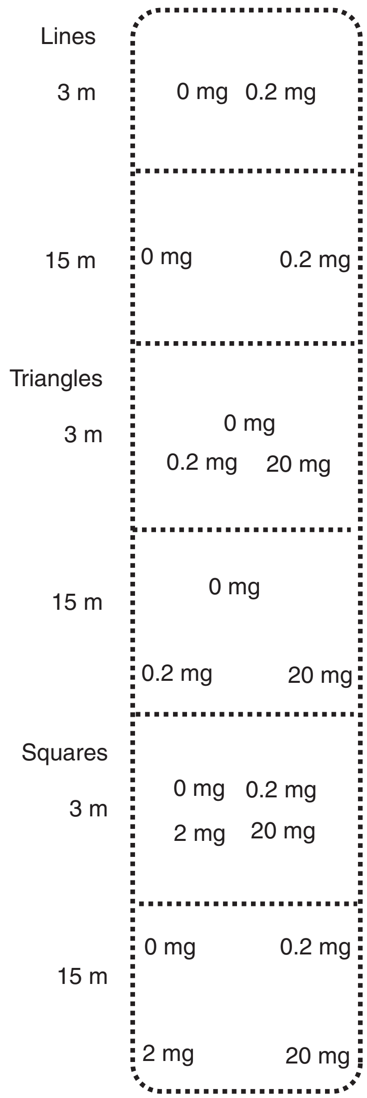

Figure 1 Schematic arrangement of the six different configurations of spacing, trap-distance, and pheromone treatments deployed to sample *Ips pini*. Note that the blocks are not to scale (each block measures 45 × 45 m, separated by a minimum of 30 m). Each block is replicated four times throughout the plantation, for a total of 24 blocks. All treatments include 20 mg lanierone.

of traps and trap pattern, these ANOVAs were parameterized as cell-means models that compare average catches for each treatment with 0 (Pinheiro & Bates, 2002). Finally, focusing only on the 2-mg lure, we examined whether the distribution of insects among traps changed with trap arrangement by examining the effect of trap spacing and arrangement on captures of *I. pini* for that lure. All data analysis was performed in R (Ihaka & Gentleman, 1996; R Development Core Team, 2009).

Results

A total of 7 338 *I. pini*, including 3 856 females and 3 482 males, were collected over the nine time periods. The number of *I. pini* caught during each time period varied with weather, as in previous studies (Aukema et al., 2005). In general, the numbers of insects caught during the first four time periods were almost double the numbers captured in later time periods. Other insect species were not caught in large enough numbers to justify statistical analysis.

The number of beetles caught in traps with 0, 0.2, and 2 mg ipsdienol was very low, averaging only a single insect or fewer in each trap for each collection period (Figure 2). The 20 mg ipsdienol lure was highly attractive, however, with an average catch of 50 *I. pini* each collection period.

Does spacing and number of treatments affect overall trap catch?

We investigated this question for both the 'high activity' blocks, that is, those containing the 20-mg lure (the triangle and square trap arrangements in Figure 1), and the 'low activity' blocks, that is, those without the 20-mg lure (the single line arrangements in Figure 1). For the 'high activity' blocks, neither the number of treatments in different geometric configurations nor their spacing affected the total capture of *I. pini* ($F_{1,12}$ = 2.70, P = 0.13 and $F_{1,12}$ = 0.69, P = 0.42, respectively; configuration*spacing interaction term not significant: $F_{1,12}$ = 0.17, P = 0.68). Each block captured approximately the same number of insects, likely because of the presence of a single 20-mg lure (Figure 1). Similarly, for blocks without the 20-mg lure where traps were arranged in a line (Figure 1), there was no difference in total capture over a block when the traps were spaced at 3 vs. 15 m ($F_{1,6}$ = 0.02, P = 0.91). The number of insects in the sample universe was quite consistent across blocks.

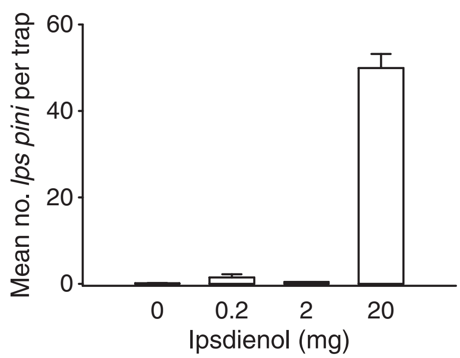

Figure 2 Effect of ipsdienol concentration on mean (+ SE) number of *Ips pini* collected per collection period. All treatments include a lanierone lure.

Does the distribution of insects among traps within a block change, although the total number across a given arrangement is constant?

As spacing did not affect overall trap capture across blocks, we restricted analysis to potential treatment effects for each of the different geometric arrangements. For all geometric arrangements, there was always a treatment effect ($P<0.05$), although there was sometimes a treatment*spacing interaction. For this reason, we display mean catch per trap per day for each configuration and spacing category in Table 1. For each arrangement, the lure with the highest release rate always captured the most insects. This effect occurred whether the lure with the highest release rate was the 20-mg lure (i.e., triangle, square arrangements) or the 2-mg lure (line arrangement). Traps baited without a lure (i.e., the control) always captured the fewest insects.

Despite this pattern of consistency for catches associated with the strongest and non-baited traps, trap catches with 'low activity' lures (0.2 and 2 mg) demonstrate that trap spacing and arrangement affect the distribution of insects among traps. In a square arrangement, the low number of insects (0.14) captured in traps baited with 0.2-mg lures is not significantly different from 0 when the traps are spaced at 15-m distances. However, when the traps are spaced closer together at 3 m, the catch in traps baited with 0.2-mg lures increases by a factor of 7× and is now significantly greater than 0 (0.97). A similar pattern occurs with the 2-mg trap in the triangle arrangement. At 15-m spacing, only 0.12 insects are caught per trap, which is not significantly different from 0. When the traps are positioned closer to the more attractive 20-mg lures at 3-m spacing, 4× more are captured, with an average of 0.51 insects per trap. Again, this number is statistically greater than 0.

When catches from traps baited with the 2-mg lure are compared not only within the triangle arrangements, but also across the stand, we find that traps with the 2-mg lures captured more insects, on average, when located in the triangle and the square configurations, than in the line configuration ($F_{1,18} = 16.27$, $P = 0.0008$ and $F_{1,18} = 9.19$, $P = 0.0072$, respectively, for each comparison). Of course, both the triangle and square configurations contained the 20-mg lure associated with increased attraction (Figures 1 and 2).

Although an interference effect in the 0.2- and 2-mg lures is likely exacerbated by the more potent 20-mg lure at close (3 m) spacings (Table 1), it appears that subtle redistribution of insect populations among traps occurs even in the absence of highly attractive lures. Traps aligned in a pair arrangement with only a control and 'low activity' 2-mg lure show a small but significant treatment effect

Table 1 Effect of trap arrangement and release rate on trap catch of *Ips pini* in central Wisconsin (USA), 27 July–28 August, 2004

		Treatment effect						
Configuration	Spacing	F	d.f.	P	Lure (mg ipsdienol)	Mean catch[1] (*Ips* per trap day^{-1})	Different from 0?[2]	P
Square	Distant (15 m)	782.54	4,137	<0.0001	0 (control)	0.06	No	0.40
					0.2	0.14	No	0.05
					2	0.25	Yes	0.0014
					20	44.47	Yes	<0.0001
	Close (3 m)	240.78	4,137	<0.0001	0 (control)	0.25	No	0.08
					0.2	0.97	Yes	<0.0001
					2	0.39	Yes	0.0107
					20	41.89	Yes	<0.0001
Triangle	Distant (15 m)	288.51	3,101	<0.0001	0 (control)	0.02	No	0.87
					2	0.12	No	0.31
					20	25.64	Yes	<0.0001
	Close (3 m)	312.93	3,101	<0.0001	0 (control)	0.19	No	0.27
					2	0.51	Yes	0.010
					20	38.62	Yes	<0.0001
Line	Distant (15 m)	3.73	2,67	0.029	0 (control)	0.00	No	1.0
					2	0.08	Yes	0.0093
	Close (3 m)	1.37	2,67	0.26	0 (control)	0.02	No	0.54
					2	0.05	No	0.11

All treatments contained 20 mg lanierone.
[1]Means from cell-means model fit.
[2]t-test of each coefficient from ANOVA.

when the traps are widely spaced at 15-m distances. In this arrangement, the baited trap catches 0.08 insects per trap per day, which is statistically different from 0 (Table 1). The control trap did not catch any insects in any of the similar blocks. However, the treatment effect disappears when the traps are positioned closer together at 3 m, as insects in the vicinity of the baited traps may be inadvertently captured in a control trap.

Discussion

Our results indicate that spacing distance can interact with the attractiveness of semiochemicals to affect trap catch, and hence can affect our interpretation of the bioactivity of various compounds and concentrations. In our example, this effect was manifested in the form of higher numbers of insects caught in a relatively non-attractive treatment. This by-catch was likely pulled into the area by a more attractive lure. Such trap interference suggests that it is possible to overestimate the minimum active concentration in behavioral assays. This masking may occur despite the presence of an overall treatment effect, which is typically the focal point of the study. In systems where organisms are trapped with high efficiency, it is possible that a similar effect might manifest itself as an underestimation of a minimum active concentration. This might occur if more potent lures act as a sink, pulling insects away from less attractive lures.

In the present study, detection of interference created by a 'high activity' lure was simplified by a uniform sampling universe; that is, the same total numbers of insects were captured in the different blocks regardless of internal trap spacing. This is not always the case, however, as larger spacings may actually increase total catch (Paiva, 1982; Östrand & Anderbrant, 2003). In such cases, detection of interference could be more difficult if more insects were captured without necessarily changing their relative distributions among traps, especially if the bias is consistent.

Information on interaction distances among chemical signals has practical applications for sampling pest insects, in addition to basic studies of chemical ecology. For example, Bentz (2006) observed that trap catches of mountain pine beetles (*Dendroctonus ponderosae* Hopkins) were reduced during periods of peak beetle emergence and suggested that quantitative monitoring is impeded by interference from natural attacks. Likewise, Elkinton & Cardé (1988) estimated that interference among pheromone traps can occur at 80 m when sampling gypsy moth, *Lymantria dispar* (L.), populations. Determining proper spacing for any study will depend on experimental objectives (e.g., behavioral bioassay, trap-out, etc.), effective sampling range of the trap (Schlyter, 1992; Turchin & Odendaal, 1996), attractive radius of the insect (Byers et al., 1989; Byers, 1999), and complex meteorological conditions that cannot necessarily be controlled (Fares et al., 1980; Wedding et al., 1995; Murlis et al., 2000; Bisignanesi & Borgas, 2007).

The detection of interference among treatments has received fruitful research in the field of crop science, where competition among cultivars of varying heights in neighboring plots may obscure variety comparisons in yield trials (Kempton, 1997; Clarke et al., 1998). Unfortunately, many methods to contend with interplot interference in this discipline are not directly applicable to insect trapping studies. For example, suggestions include harvesting centre rows and leaving border rows as buffers (David et al., 2001), or allocating rows of neighboring plots across buffers of varying distances to test for interference effects (Gomez & Gomez, 1984). In insect trapping studies, the trap is frequently the experimental unit, analogous to a cultivar plot. The analogous subunit to a row within the plot does not exist, as traps cannot be subdivided. Nevertheless, there is mutual agreement among disciplines that the general protocol of spacing out treatments to encourage independence, and explicit testing for such independence, are good experimental practice.

These results provide a general approach for testing the effect of spacing distance on trap catch in behavioral choice tests conducted in the field. However, our interpretation is limited by the simplicity of our experimental design, in which all treatments were either relatively attractive or unattractive. Future experiments should include a range of bioactivities, to further evaluate the underlying assumptions of behavioral choice tests.

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