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An assessment of re-randomization methods in bark beetle (Scolytidae) trapping bioassays

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Abstract

1 Numerous studies have explored the role of semiochemicals in the behaviour of bark beetles (Scolytidae).

2 Multiple-funnel traps are often used to elucidate these behavioural responses. Sufficient sample sizes are obtained by using large numbers of traps to which treatments are randomly assigned once, or by frequent collection of trap catches and subsequent re-randomization of treatments.

3 Recently, there has been some debate about the potential for trap contamination to occur when semiochemical treatments (baits), and not trap-treatment units (traps and baits), are re-randomized among existing traps. Due to the volatility of many semiochemicals, small levels of contamination could potentially confound results.

4 A literature survey was conducted to determine the frequency of re-randomizing semiochemical treatments (baits) vs. trap-treatment units (traps and baits) in scolytid trapping bioassays. An experiment was then conducted to determine whether differences in the response of *Dendroctonus brevicomis* LeConte to attractant-baited traps exist between the two methods.

5 The majority of papers examined reported use of a large number of fixed replicates (traps) rather than re-randomization of treatments at frequent intervals. Seventy-five percent of papers for which re-randomization methods could be determined reported relocation of semiochemical treatments (baits) only.

6 No significant differences in trap catch were observed among multiple-funnel traps aged with *D. brevcomis* baits (Phero Tech Inc., Canada) for 0, 30 and 90 days, suggesting that contamination did not influence the results.

7 It is concluded that re-randomizing baits is a viable cost-effective option to re-randomizing trap and bait units.

Keywords Bark beetles, *Dendroctonus brevicomis*, experimental design, pheromone, randomization, semiochemicals, Scolytidae, trapping.

Introduction

The Scolytidae are a large and diverse group of insects consisting of approximately 6000 species worldwide (Wood, 1982). Most species feed on the phloem and cambium, or xylem tissue of woody plants. The biology of the most economically important species has been well described. Numerous studies have explored the role of aggregation pheromones and host volatiles in the colonization of host trees, the role of anti-aggregation pheromones and nonhost volatiles in regulation of attack rates, density and within-tree spacing and the use of kairomones by natural enemies and competitors in prey location and host finding. These have led to advances in bark beetle management through the forecasting of infestation trends (Billings, 1988), the development of containment methods (Borden *et al.*, 1983; Gray & Borden, 1989), stand protection and spot suppression (Gibson *et al.*, 1991; Shea *et al.*, 1992; Shore *et al.*, 1992; Ross & Daterman, 1994, 1995; Clarke *et al.*, 1999; Progar, 2003) and mass trapping efforts

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(Räty *et al.*, 1995; Shea & Neustein, 1995). Baited traps are commonly used to determine the response of bark beetles to semiochemicals in the field. Multiple-funnel traps (Lindgren, 1983) are preferable for uses that require large numbers of traps, long-distance transportation, or frequent relocation of individual trap-treatment units (McCravy *et al.*, 2000).

In most experiments, completely randomized (CRD) or randomized complete block designs (RCBD) are used (Reeve & Strom, 2004). In a CRD, the data are viewed as if they were random samples from a normal distribution. Each treatment is randomly assigned among all sample units (traps). Randomized complete block designs allow the investigator to reduce the residual error in an experiment by removing variability due to known and controllable nuisance variables. In bark beetle trapping bioassays, these variables are usually based on site or temporal factors. Each treatment is randomly assigned among the number of sample units within each block, and not the entire sample population as in the CRD (Montgomery, 1991). The Latin square design also utilizes the blocking principle, but allows for the control of two nuisance variables (such as time and site factors). The row and column of each square represents the two levels of restriction on randomization. Each treatment occurs only once in each row and column (e.g. date and trapping location) (Montgomery, 1991).

The response variable is the number of insects (bark beetles or associates) caught in each trap per unit time. In CRD and RCBD, sufficient sample sizes are obtained by using large numbers of traps to which treatments are randomly assigned once, or by frequent collection of trap catches, and then subsequent re-randomization of treatments on a daily or weekly basis. The latter is often carried out to correct for Poisson-distributed responses, which are common in scolytid trapping studies (Sun *et al.*, 2004). Large numbers of replicates reduce the influence of positional effects because some trap locations collect few beetles regardless of treatment. The Latin square design ensures that each treatment will occupy each possible position in the trapping array, but the total number of observations (and thus replicates) is limited by the number of treatments (t^2) unless multiple Latin squares are used.

There are many reasons why re-randomization may be desirable in bark beetle trapping bioassays. Traps and baits are often expensive, and investigators may not be able to afford the large numbers required to obtain sufficient replication over space. Space may be limited due to restrictions concerning accessibility, political and social constraints, or infestation (population) size. Attractive treatments that are placed in a single location over lengthy periods of time may cause spillover (i.e. a phenomenon whereby beetles apparently attracted to baited traps infest adjacent trees), resulting in undesirable tree mortality and potentially confounding effects. In addition, frequent re-randomization allows for collection of large numbers of replicates in a relatively short period of time. Re-randomization should not be confused with multiple collections from a single trap over time. The later violates assumptions of the analysis of variance (ANOVA) if collections are erroneously considered true replicates (Montgomery, 1991). In this situation (and in the absence of re-randomization), the total number of beetles collected over time, regardless of the number of collections, is the appropriate value for that replicate. Experimental designs that initially randomize treatments, which are then rotated in some predetermined fashion after each 'replicate', similarly violate assumptions of ANOVA.

When treatments are re-randomized, a choice is made between relocating semiochemical treatments (hereafter referred to as baits) or entire trap-treatment units (hereafter referred to as traps and baits) among replicate locations. Recently, there has been some debate about the potential for trap contamination to occur when baits, and not traps and baits, are re-randomized among traps (2003 Southern Forest Insect Work Conference, New Orleans, Louisiana, USA). Semiochemical residues may adhere to the trap, or volatiles may impregnate trap materials, both of which could potentially confound results. To reduce potential contamination, some authors use new traps and re-randomize trap and bait units when conducting trapping bioassays, such as those for the western pine beetle, *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae).

Dendroctonus brevicomis is a major cause of ponderosa pine, *Pinus ponderosa* Dougl. ex. Laws., mortality in the western U.S.A. Under certain conditions, the beetle can aggressively attack and kill apparently healthy trees of all ages and size classes (Miller & Keen, 1960). Female *D. brevicomis* produce (+)-exo-brevicomin whereas males produce frontalin. Both pheromones elicit an aggregation response (Wood *et al.*, 1976; Browne *et al.*, 1979). Myrcene, a host monoterpene of *P. ponderosa*, enhances the response in both laboratory and field bioassays (Bedard *et al.*, 1969). The objectives of the present study were: (i) to review and determine the frequency of each re-randomization method in three journals where relevant bark beetle studies are published; (ii) to determine the response of *D. brevicomis* to aged attractant-baited traps as a model to estimate the importance of each re-randomization method; and (iii) to estimate the amount of time required to implement each method.

Materials and methods

Literature review

A survey was conducted to determine the frequency of papers reporting trapping bioassays that investigated the chemical ecology of scolytid species in *Agricultural and Forest Entomology*, *Environmental Entomology* and *Journal of Chemical Ecology*, 2000–04. The survey was limited to these journals and dates to keep the number of papers reviewed manageable in number, and to limit descriptions to current trends in the literature. For each relevant paper, an attempt was made to determine the experimental design and method of re-randomization when applicable.

Trapping experiment

A trapping bioassay was conducted at McCloud Flats, Shasta-Trinity National Forest, Siskiyou Co., California (41.35°N, 121.95°W; 1150 m elevation) during 10–21 June

2004. The stand was dominated by large diameter *P. ponderosa* (mean ± SEM d.b.h. = 35.8 ± 3.6 cm) growing on soils of volcanic origin. Mean stand density was 31.5 m^2 of basal area per ha of which 95.5% was *P. ponderosa* with the remainder represented mainly by incense cedar, *Calocedrus decurrens* (Torr.) Florin and white fir, *Abies concolor* (Gond. and Glend.) Hildebr.

Twelve trap locations, each separated by 25–30 m, were selected adjacent to a forest road. Each trap location was randomly assigned one of four treatments: (i) new, previously unused, 16-unit multiple-funnel trap (Lindgren, 1983; Phero Tech Inc., Delta, British Columbia, Canada); (ii) new, previously unused, 16-unit multiple-funnel trap aged with one *D. brevicomis* bait [Phero Tech Inc.; 3.0 mg/24 h frontalin (racemic), 3.0 mg/24 h exo-brevicomin (racemic), 18.0 mg/24 h myrcene] for 30 days; (iii) new, previously unused, 16-unit multiple-funnel trap aged with one *D. brevicomis* bait (replaced after 45 days) for 90 days; and (iv) new, previously unused, 16-unit multiple-funnel trap baited with one *D. brevicomis* bait. Baits were attached to the inside of the eighth funnel with a small binder clip. Traps were aged in direct sunlight under field conditions, and then sealed in individual plastic bags (113.6 L; Glad Products Company, Oakland, California) during transportation to the field. Baits were removed from treatments (ii) and (iii) just prior to field deployment. Traps were hung on 3-m metal poles with collection cups 80–100 cm above the ground. A 3 × 3-cm time-released insecticidal Prozap Pest Strip (2,2-dichlorovinyl dimethyl phosphate; Loveland Industries Inc., Greely, Colorado) was placed in the collection cup to kill arriving insects and reduce damage or loss by invertebrate predators. Samples were collected and treatments re-randomized daily between 06.30 and 10.00 h to avoid disturbing traps during periods of peak flight activity (Fettig *et al.*, 2004). Catches were immediately transported to the laboratory for storage and analysis. Specimens were tallied and identified using available keys (Wood, 1982) and voucher specimens.

The experimental design was completely randomized with four treatments and 33 replicates per treatment. A test of normality was performed, and appropriate transformations were used when the data deviated significantly from a normal distribution (Sokal & Rohlf, 1995). A two-way ANOVA was performed on the number of *D. brevicomis* caught per trap per day (SigmaStat Version 2.0, SPSS Inc., Chicago, Illinois). If a significant treatment effect was detected, Tukey's multiple comparison test (Tukey's HSD) was used for separation of treatment means.

Cost simulations

The amount of time required to re-randomize baits alone and traps and baits was recorded for 36 traps to which 12 single-device semiochemical treatments (three replicates each) were randomly assigned. Trap locations were spaced 30 m apart along a 750-m long transect at McCloud Flats, Shasta-Trinity National Forest, California. Traps were hung on 3-m metal poles with collection cups 80–100 cm above the ground. The same individual (S.R.M.) conducted each of the eight simulations.

Results

Literature review

A total of 19 papers met the criterion of reporting trapping bioassays that investigated the chemical ecology of scolytid species (Table 1). *Environmental Entomology* contained the largest number of papers (47%). Sixty-nine percent of studies reported use of randomized complete block designs. Completely randomized and Latin square designs were equally represented (11%). The majority of papers (47%) reported the use of a large number of traps to which treatments were randomly assigned once at the beginning of each experiment rather than re-randomizing at frequent intervals. In several of these studies, additional experiments were conducted immediately after the initial experiment using the same traps and trapping locations. It is possible that similar levels of contamination could occur during these studies. Several papers (26%) did not adequately describe the experimental design in a manner sufficient to allow determination of the method of re-randomization. One paper (Zhang, 2003) reported use of an experimental design in which treatments were initially randomized and then rotated after each 'replicate', which violates assumptions of ANOVA. Four papers reported re-randomization on the basis of semiochemical treatments, whereas only one study reported the re-randomization of trap and bait units (Table 1).

Trapping experiment

A total of 5489 *D. brevicomis* were captured in multiple-funnel traps over the 11-day period. There was a significant treatment–gender interaction ($F_{3,256} = 6.92$; $P < 0.0002$). A test of normality was performed, and square root transformations were used because the data deviated significantly from a normal distribution. A significant treatment effect was observed ($F_{3,256} = 237.84$; $P < 0.0001$). Overall, the ratio of males to females was 0.52, but this difference was not statistically significant ($t = -1.898$. d.f. = 262; $P = 0.059$). Significantly more male and female *D. brevicomis* were captured in attractant-baited traps than any other treatment (Table 2).

Table 1 Frequency of experimental designs and randomization methods reported in 19 papers in *Agricultural and Forest Entomology, Environmental Entomology* and *Journal of Chemical Ecology* during scolytid trapping bioassays, 2000–04

Criterion evaluated	Number of occurrences
Experimental designs	
Completely randomized	2
Randomized complete block	13
Latin square	2
Split plot	1
Factorial	1
Randomization methodology	
Fixed replicates, no re-randomization	9
Re-randomization, semiochemicals only	4
Re-randomization, traps and semiochemicals	1
Not reported	5

Table 2 Mean ± SEM daily catch of western pine beetle, *Dendroctonus brevicomis* LeConte, in attractant-baited multiple-funnel traps, McCloud Flats, Shasta-Trinity National Forest, California, 10–21 June 2004

Treatment[a]	n[b]	Males (mean ± SEM[c])	Females (mean ± SEM[c])
90 days	33	0.18 ± 0.10[a]	0.30 ± 0.17[a]
30 days	33	0.67 ± 0.32[a]	0.42 ± 0.19[a]
0 days	33	0.82 ± 0.53[a]	0.94 ± 0.57[a]
Bait	33	55.52 ± 9.79[b]	107.59 ± 18.22[b]

[a]90, 30 and 0 days, traps aged with *D. brevicomis* bait (Phero Tech Inc.) for the specified period; Bait, contains *D. brevicomis* bait.
[b]Number of replicates per treatment.
[c]Means followed by the same letter are not statistically different ($P > 0.05$; Tukey's HSD).

Ninety-eight percent of all captures occurred in these traps. Few individuals were collected in traps aged with *D. brevicomis* baits. There were no significant differences in trap catch among 0, 30 and 90-day treatments (Table 2).

Cost simulations

There was a substantial difference in the amount of time required to re-randomize baits vs. trap and bait units (46.3 ± 2.2 vs. 172.8 ± 2.8 min, respectively). By limiting re-randomization to baits alone, a 73% reduction in time commitment was observed.

Discussion

The *D. brevicomis* bait [Phero Tech Inc.; 3.0 mg/24 h frontalin (racemic), 3.0 mg/24 h exo-brevicomin (racemic), 18.0 mg/24 h myrcene] is effective for capturing large numbers of *D. brevicomis* (Table 2), and for initiating *D. brevicomis* attack on apparently healthy *P. ponderosa* (Haverty *et al.*, 1998), and often on trees adjacent to these baited trees (C. J. Fettig, personal observation). The original hypothesis was that multiple funnel traps containing this potent bait for lengthy periods of time (30 and 90 days) would be more attractive to *D. brevicomis* than unbaited traps (0 days). It is possible that the plastics from which these traps are constructed could become impregnated with bait volatiles, or that semiochemicals beading on the external membrane of the associated release device could come in direct contact with, and adhere to, the trap (funnel) wall. Such a phenomenon could potentially confound results during trapping bioassays.

The data obtained indicate that this phenomenon is of little concern, as no difference in trap catches was found among multiple-funnel traps that contained *D. brevicomis* baits for 0, 30 and 90 days before field deployment. Large numbers of beetles were captured in attractant-baited traps suggesting that sufficient numbers of beetles were in the vicinity of these traps to elicit this effect should it occur (Table 2). It is concluded that re-randomizing baits alone is a viable option to re-randomizing trap and bait units based on the model used in the present study. This technique will result in a considerable time and cost savings. Users of other trap forms, such as slot traps (Niemeyer, 1985), which may be more cumbersome than multiple-funnel traps (McCravy *et al.*, 2000), should realize even larger savings in time when re-randomizing baits only. If studies are conducted over lengthy periods of time (i.e. an entire field season), the release rate of individual semiochemical components will probably be reduced during later replicates and could potentially confound results. To eliminate this concern, all semiochemicals should be replaced on a regular basis, or when significant amounts of liquid have eluted through the membrane.

Caution is warranted in that these results may be limited to the scolytid and semiochemical blend used in the present model. However, the results may have broader implications for bark beetle trapping studies involving closely-related species and semiochemicals of a similar structure and origin.

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References

Bedard, W.D., Tilden, P.E., Wood, D.L., Silverstein, R.M., Brownlee, R.G. & Rodin, J.O. (1969) Western pine beetle: field response to its sex pheromone and a synergistic host terpene, myrcene. *Science*, **164**, 1284–1285.

Billings, R.F. (1988) Forecasting southern pine beetle infestation trends with pheromone traps. *Integrated Control of Scolytid Bark Beetles* (ed. by T.L. Payne and H. Saarenmaa), pp. 295–306. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Borden, J.H., Conn, J.E., Friskie, L.M., Scott, B.E., Chong, L.J., Pierce, H.D. Jr & Oehlschlager, A.C. (1983) Semiochemicals for the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae), in British Columbia: baited-tree studies. *Canadian Journal of Forest Research*, **13**, 325–333.

Browne, L.E., Wood, D.L., Bedard, W.D., Silverstein, R.M. & West, J.R. (1979) Quantitative estimates of the western pine beetle attractive pheromone components, exo-brevicomin, frontalin, and myrcene in nature. *Journal of Chemical Ecology*, **5**, 397–414.

Clarke, S.R., Salom, S.M., Billings, R.F., Berisford, C.W., Upton, W.W., McClellan, Q.C. & Dalusky, M.J. (1999) A scentsible approach to controlling southern pine beetles – two new tactics using verbenone. *Southern Journal of Applied Forestry*, **97**, 26–31.

Fettig, C.J., Shea, P.J. & Borys, R.R. (2004) Diurnal flight pattern of *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae) in northern California. *Journal of Entomological Science*, **39**, 453–456.

Gibson, K.E., Schmitz, R.F., Amman, G.D. & Oakes, R.D. (1991) *Mountain Pine Beetle Response to Different Verbenone Dosages in Pine Stands of Western Montana.* Research Paper 444. US Department of Agriculture. Forest Service, Intermountain Region, Ogden, Utah.

Gray, D.R. & Borden, J.H. (1989) Containment and concentration of mountain pine beetle (Coleoptera: Scolytidae) infestations with semiochemicals: validation by sampling of baited and surrounding zones. *Journal of Economic Entomology*, **93**, 1399–1405.

Haverty, M.I., Shea, P.J., Hoffman, J.T., Wenz, J.M. & Gibson, K.E. (1998) *Effectiveness of Esfenvalerate, Cyfluthrin, and Carbaryl in Protecting Individual Lodgepole Pines and Ponderosa Pines From Attack by Dendroctonus spp*. Research Paper 237. US Department of Agriculture Forest Service, Pacific Southwest Research Station, Albany, California.

Lindgren, B.S. (1983) A multiple funnel trap for scolytid beetles (Coleoptera). *The Canadian Entomologist*, **115**, 299–302.

McCravy, K.W., Nowak, J.T., Douce, G.K. & Berisford, C.W. (2000) Evaluation of multiple-funnel and slot traps for collection of southern pine bark beetles and predators. *Journal of Entomological Science*, **35**, 77–82.

Miller, J.M. & Keen, F.P. (1960) *Biology and Control of the Western Pine Beetle*. Misc. Publication 800, US Department of Agriculture, Forest Service, Washington, District of Columbia.

Montgomery, D.C. (1991) *Design and Analysis of Experiments*, 3rd edn. John Wiley & Sons, Inc., U.K.

Niemeyer, H. (1985) Field response of *Ips typographus* L. (Col., Scolytidae) to different trap structures and white versus black flight barriers. *Journal of Applied Entomology*, **99**, 44–51.

Progar, R.A. (2003) Verbenone reduces mountain pine beetle attack in lodgepole pine. *Western Journal of Applied Forestry*, **18**, 229–232.

Räty, L., Drumont, A., De Windt, N. & Grégoire, J.C. (1995) Mass trapping of the spruce bark beetle *Ips typographus* L.: traps or trap trees? *Forest Ecology and Management*, **78**, 191–205.

Reeve, J.D. & Strom, B.L. (2004) Statistical problems encountered in trapping studies of scolytids and associated insects. *Journal of Chemical Ecology*, **30**, 1575–1590.

Ross, D.W. & Daterman, G.E. (1994) Reduction of Douglas-fir beetle infestation of high risk stands by antiaggregation and aggregation pheromones. *Canadian Journal of Forest Research*, **24**, 2184–2190.

Ross, D.W. & Daterman, G.E. (1995) Response of *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae) and *Thanasimus undatulus* (Coleoptera: Cleridae) to traps with different semiochemicals. *Journal of Economic Entomology*, **88**, 106–111.

Shea, P.J., McGregor, M.D. & Daterman, G.D. (1992) Aerial application of verbenone reduces attack of lodgepole pine by mountain pine beetle. *Canadian Journal of Forest Research*, **22**, 436–441.

Shea, P.J. & Neustein, M. (1995) Protection of a rare stand of Torrey pine from *Ips paraconfusus*. *Application of Semiochemicals for Management of Bark Beetle Infestations – Proceedings of an Informal Conference*. (ed. by S. M. Salom and K. R. Hobson) General Technical Report 318. US Department of Agriculture Forest Service, Intermountain Research Station, Ogden, Utah.

Shore, T.L., Safranyik, L. & Lindgren, B.S. (1992) The response of mountain pine beetle (*Dendroctonus ponderosae*) to lodgepole pine trees baited with verbenone and exo-brevicomin *Journal of Chemical Ecology*, **18**, 533–541.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry*, 3rd edn. WH Freeman, New York, New York.

Sun, J., Miao, Z., Zhang, Z., Zhang, Z. & Gillette, N.E. (2004) Red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Scolytidae), response to host semiochemicals in China. *Environmental Entomology*, **33**, 206–212.

Wood, S.L. (1982) The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae). A taxonomic monograph. *Great Basin Naturalist Memoirs*, **6**, 1359.

Wood, D.L., Browne, L.E., Ewing, B. *et al*. (1976) Western pine beetle: specificity among enantiomers of male and female components and attractant pheromone. *Science*, **192**, 896–898.

Zhang, Q.H. (2003) Interruption of aggregation pheromone in *Ips typographus* (L.) (Col. Scolytidae) by non-host bark volatiles. *Agricultural and Forest Entomology*, **5**, 145–153.

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