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Short Communication

# Spatial Displacement of a Lure Component Can Reduce Catches of Two Nontarget Species During Spring Monitoring of Southern Pine Beetle

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## Abstract

Local outbreak risk for the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae), is forecast with a trapping survey conducted every spring throughout the southeastern United States. Traps baited with pine odors and components of the *D. frontalis* aggregation pheromone are used to obtain abundance estimates of both this species and its clerid predator *Thanasimus dubius* (F.) (Coleoptera: Cleridae); these data are entered into a predictive model that estimates outbreak risk. An attractant synergist for *D. frontalis*, *endo*-brevicomin, has recently been included in the survey lure, but it can have the unintended effect of attracting nontarget species *Hylesinus pruinosus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) and *Enoclerus nigripes* (Say) (Coleoptera: Cleridae) which, due to their sometimes large numbers and general similarity in appearance to the target species, could complicate sorting and counting of trap catches. Analysis of bycatch data from a previously-published, 31-mo trapping study in Mississippi indicated that displacement of the *endo*-brevicomin releaser 6 m from the trap largely eliminated catches of the nontarget species *H. pruinosus* and *E. nigripes* while not reducing catches of the target species *D. frontalis* and *T. dubius*. Our analysis demonstrates that interspecific differences in spatial responses to attractive semiochemicals can be used to improve insect trap selectivity. Both nontarget beetle species were captured in highest numbers during late winter/early spring, coinciding with the *D. frontalis* survey.

**Key words:** *Hylesinus pruinosus*, *Enoclerus nigripes*, *Dendroctonus frontalis*, *Thanasimus dubius*, bycatch

The southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Curculionidae) is a native pest species that causes significant economic losses to pine forests in the southeastern United States (Price et al. 1998). Adult *D. frontalis* use an aggregation pheromone to organize mass attacks that can overwhelm the defenses of healthy host trees (Sullivan 2011). They then feed and reproduce in the phloem of the host which inevitably dies as a result of colonization. Beetles disperse in the spring and, when population densities are sufficient, may initiate localized infestations or 'spots' that consist of patches of adjacent, infested trees. Infestations may grow through the spring and summer, but this growth may be suppressed through felling of infested and adjacent trees (Billings 2011). *D. frontalis* outbreaks occur periodically and may last for several years (Turchin et al. 1991, Birt 2011).

Outbreaks may not be apparent until summer when flagging of foliage of killed trees becomes visible (Billings and Kibbe 1978). Therefore, every spring a network of traps is established throughout the southeastern United States to detect whether outbreak-level populations of *D. frontalis* exist and to make forecasts regarding the abundance of infestations which may require control during the following summer (Billings and Upton 2010, Billings 2011). Until this year (2017), traps have been baited with an aggregation pheromone component (frontalin) and host volatiles (pine turpentine or a mixture of *alpha*- and *beta*-pinene); this combination attracts both *D. frontalis* and a major predator, *Thanasimus dubius* (F.) (Coleoptera: Cleridae). The numbers of *D. frontalis*, as well as the proportion of *T. dubius* trapped, are entered into a model used to predict population levels and infestation trends later in the year (Billings 2011). The ability to predict the severity of an outbreak months in advance allows forest pest managers to more effectively plan for spot detection and suppression activities.

The male-produced pheromone component *endo*-brevicomin is a potent synergist of the combination of frontalin and host compounds in attracting *D. frontalis* to traps located outside of beetle infestations (Sullivan et al. 2007). Furthermore, the degree of catch enhancement is similar or increased when an *endo*-brevicomin lure is displaced a few meters from (as opposed to being placed directly on) a trap baited with the other lure components (Moreno et al. 2008, Sullivan and Mori 2009). The capacity of *endo*-brevicomin to act as an attractive synergist for *D. frontalis* has led to the decision to include it in the lure combination used for the annual spring survey of *D. frontalis* (John T. Nowak, personal communication).

A sympatric species of *D. frontalis*, the ash bark beetle, *Hylesinus pruinosus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), employs (+)-*endo*-brevicomin as an aggregation pheromone component and is strongly attracted to lures with either racemic or (+)-*endo*-brevicomin (Shepherd et al. 2010). Similarly, the sympatric bark beetle predator *Enoclerus nigripes* (Say) (Coleoptera: Cleridae) is attracted to *endo*-brevicomin, presumably utilizing this compound as a host-location kairomone. Neither species appears to be attracted significantly to frontalin and host odors (authors' unpublished data). These nontarget species superficially resemble *D. frontalis* and *T. dubius*, respectively, and their presence could confound the identification and counting of beetles in survey samples. Such confusion is more likely if the personnel sorting samples are not thoroughly trained/experienced or a microscope is not available to them. Bycatches of *H. pruinosus* and *E. nigripes* might occur if *endo*-brevicomin is included in the *D. frontalis* survey lure, and there are clear benefits in adjusting procedures to reduce bycatches if it can be done without reducing lure efficacy for target species. A previous study conducted by the authors (Sullivan et al. 2016) examined the response of *D. frontalis* and *T. dubius* to traps baited with *endo*-brevicomin devices positioned either on or 6 m distant from monitoring traps during a 31 mo. interval. In the present study, we examined catch data for *H. pruinosus* and *E. nigripes* from the previous study to determine if displacement of the *endo*-brevicomin lure might have benefits in reducing the proportion of these nontarget species in survey traps.

## Materials and Methods

Detailed methods for the trapping experiment are given in Sullivan et al. (2016) and are summarized here. Six 12-unit Lindgren multiple-funnel traps were spaced 450–720 m apart within mixed pine/hardwood forests in the Homochitto National Forest, Mississippi (within 5 km of W91.200, N31.419). Traps were suspended from metal standards with their collection cups approximately 1 m above the ground. Each trap was baited with devices releasing frontalin (>95% purity, racemic; release rate = 1–5 mg/d) and *alpha*-pinene (>95% purity, 25% (+)-enantiomer; release rate = 1–5 g/d), both from Synergy Semiochemicals, Vancouver, British Columbia, Canada. A third lure (racemic *endo*-brevicomin; >95% purity; release rate = 0.25–1.5 mg/d; Synergy Semiochemicals) was either absent, placed directly on the trap, or positioned 6 m east of the trap at 1.5 m height. Release rates of each lure were measured gravimetrically in a fume hood at room temperature (20–24°C) prior to initial deployment and after removal from the trap. We observed a substantial decrease in release over the lifetime of each lure, and this is reflected in the ranges of release rates reported above. Traps were grouped into two lines of three traps, then one of each of the three treatments were assigned randomly to each trap of the group. Treatments were then rotated (by movement of *endo*-brevicomin lure) continuously through the three positions of each group; treatments were reassigned at the time of catch collection. Collections were made approximately biweekly between 6 February 2009 and 6 September 2011. All *D. frontalis*, *T. dubius*, *H. pruinosus*, and *E. nigripes* were sorted and counted.

We analyzed these data to determine whether displacement of the *endo*-brevicomin lure significantly altered the ratios of bycatch species relative to the target species with which they might potentially be confused (i.e., the ratio *H. pruinosus* to *D. frontalis*, and *E. nigripes* to *T. dubius*). Two data subsets were created for *H. pruinosus* and *E. nigripes,* respectively, from the 31 mo of trapping, by including only groups of three consecutive collections (i.e., approx. 6 wk; one complete lure rotation) during which at least one individual was trapped; periods in which no or merely sporadic *H. pruinosus* or *E. nigripes* catch occurred were considered irrelevant to our question of interest. Catches were then averaged within treatment and trap to obtain six averages (one per trap) for each treatment. Mean catches per trap per day were cube root transformed to meet assumptions of normality and homoscedasticity prior to analyses. The ratios of *E. nigripes* to *T. dubius* and *H. pruinosus* to *D. frontalis* were calculated for each trap from the untransformed catches and were log transformed. Transformed mean catches for each species and ratios between species were analyzed for treatment effects with ANOVAs using model factors treatment and trap within group (PROCGLM, SAS 9.4). We calculated all pairwise comparisons of treatment means for each species and nontarget/target species ratios using LSD with Bonferroni correction ($\alpha = 0.05$).

## Results

Both *H. pruinosus* and *E. nigripes* were trapped predominantly in the spring with very few caught during the other seasons (Fig. 1). When data were limited to collections from 6-wk intervals in which at least one nontarget beetle was caught, treatment differences were significant for *D. frontalis* ($F = 79.5$; df = 2,10; $P < 0.0001$), *H. pruinosus* ($F = 38.1$; df = 2,10; $P < 0.0001$), and *E. nigripes* ($F = 30.8$; df = 2,10; $P < 0.0001$). For both nontarget species, catches were significantly higher in traps with *endo*-brevicomin attached directly to them rather than displaced 6 m away, while the reverse was observed for *D. frontalis* (Table 1). Treatment differences were significant for both the ratios of *H. pruinosus* to *D. frontalis* ($F = 101.8$; df = 2,10; $P < 0.0001$), and *E. nigripes* to *T. dubius* ($F = 42.1$; df = 2,10; $P < 0.0001$). Both ratios were significantly higher ($P < 0.001$) for collections from traps with the *endo*-brevicomin device attached directly to them compared to those from traps with the *endo*-brevicomin device positioned 6 m away (Table 1). Highest ratio of *H. pruinosus* to *D. frontalis* in any single trap was 37.1:1 (3,633 *H. pruinosus* and 98 *D. frontalis*; recorded in late March/early April 2010); the highest recorded ratio of *E. nigripes* to *T. dubius* was 1.2:1 (117 *E. nigripes* and 98 *T. dubius;* recorded in late February 2011). Both of these extremes were recorded from traps on which the *endo*-brevicomin lure was attached directly.

## Discussion

Undesired bycatches of two potentially confounding species were reduced to low numbers by removing one lure component a few meters from the trap. Changing the placement of an olfactory lure relative to the trap appears to be a novel method for minimizing bycatches while maintaining levels of target species catches. Other methods have been identified for reducing catches of nontarget species for the purpose of improving sorting efficiency or protecting beneficial insects. These include altering the types or enantiomeric blends of chemicals used (Raffa and Klepzig 1989, Aukema et al. 2000, Panzavolta et al. 2014), and altering the design of traps (e.g., adding a mesh screen to prevent entry) to restrict the size of insects that can enter (Ross and Daterman 1998, Martín et al. 2013). Use of specific trap colors has been shown effective in lowering natural enemy and pollinator trap catches (Weber et al. 2005, Mori and Evenden 2013, Spears et al. 2016).

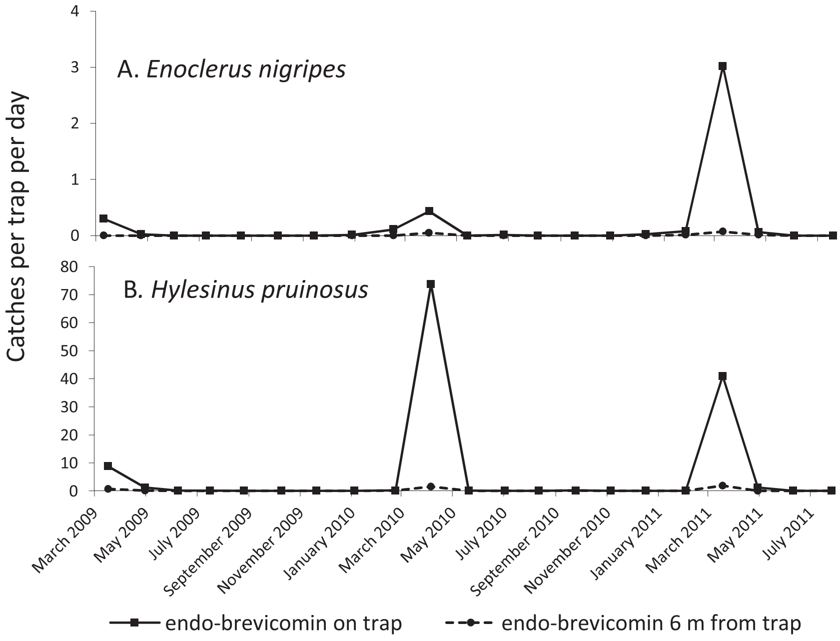

**Fig. 1.** Catches of clerid predator *Enoclerus nigripes* and ash bark beetle *Hylesinus pruinosus* in multiple funnel traps during 31 mo of trapping in the Homochitto National Forest, Mississippi. Traps were baited with frontalin, *alpha*-pinene, and a device releasing *endo*-brevicomin that was located either on the trap or 6 m away. Lure treatments were exchanged among traps so that approximately every 6 wk the treatments were rotated through every trap position. Hence, to balance site effects for trap, mean values shown are the average catches over 6 wk (i.e., a complete rotation). The higher catches of these two species in traps to which the *endo*-brevicomin device was attached directly contrasts with the response of southern pine beetle, *Dendroctonus frontalis* and its clerid predator, *Thanasimus dubius,* caught in the same traps (Sullivan et al. 2016). These latter species either did not discriminate (*T. dubius*) or showed a preference for traps with displaced *endo*-brevicomin (*D. frontalis*), which is a potent attractant synergist for *D. frontalis*.

**Table 1.** Mean[a] (±SE) catch per day and ratios of catches of target and nontarget species in traps baited with attractive lures for *D. frontalis* over a 31-mo interval

| | | *endo*-Brevicomin lure status | | |
|---|---|---|---|---|
| Species | Category | Absent | On trap | 6 m away |
| *E. nigripes* | Nontarget | 0.003 ± 0.002a[b] | 0.462 ± 0.170b | 0.014 ± 0.007a |
| *T. dubius* | Target | 2.980 ± 0.340a | 2.971 ± 0.358a | 3.265 ± 0.511a |
| *E. nigripes*/*T. dubius* | | 0.001 ± 0.001a | 0.185 ± 0.082b | 0.004 ± 0.002a |
| *H. pruinosus* | Nontarget | 0.006 ± 0.003a | 9.708 ± 3.843b | 0.317 ± 0.120a |
| *D. frontalis* | Target | 0.893 ± 0.141a | 3.257 ± 0.236b | 14.867 ± 2.616c |
| *H. pruinosus*/*D. frontalis* | | 0.009 ± 0.004a | 2.957 ± 1.157b | 0.022 ± 0.007a |

[a] Mean catch of each of six traps ($n$ = 6) including averaged catches only from 6-wk intervals in which at least one individual of the nontarget species was trapped (see text).

[b] Means associated with the same letter were not significantly different within species or species combination ($\alpha$ = 0.05; LSD with Bonferroni correction).

It is possible that lowering the release rate of the *endo*-brevicomin device placed directly on the trap would likewise have reduced the proportion of the bycatch species. An *endo*-brevicomin dose-response study (with *endo*-brevicomin devices located on the traps and trapping methodology otherwise similar to the present study; Sullivan 2016, authors' unpublished data) suggested that slightly lowering the release rate of *endo*-brevicomin from than that used in the present study might significantly increase *D. frontalis* and decrease *H. pruinosus* catches. However, in this aforementioned study, at the most attractive release rate of *endo*-brevicomin for *D. frontalis* (approximately 0.16 mg/d), the ratio of trapped *H. pruinosus* to *D. frontalis* was still nearly 1:1. This suggests that lure rate adjustment could not duplicate the bycatch reduction effects of lure displacement.

For both *H. pruinosus* and *E. nigripes,* annual flight peaked in late winter through early spring (February through April) and overlapped with the springtime burst of flight activity of both *D. frontalis* (February through June) and *T. dubius* (February through April) (Sullivan et al. 2016). The period of *H. pruinosus* and *E. nigripes* peak flight also coincides with the period when traps for the annual *D. frontalis* forecasting survey are deployed (the 4 wk following bloom of dogwood, *Cornus florida* L., and, in a recently revised protocol, the bloom of eastern redbud, *Cercis canadensis* L.; John T. Nowak, personal communication).

*endo*-Brevicomin is an attractant synergist for *D. frontalis* that is unusual in its capacity to enhance attraction of this species to sources of attractive lure components (frontalin and host odors) over a radius of at least tens of meters while simultaneously having no tendency to increase attraction to its own point of release (Sullivan and Mori 2009). Thus, it is not necessary for the compound to be released from an attractant-baited trap for *D. frontalis* catches to be enhanced. However, removal of the *endo*-brevicomin device from the trap greatly reduced catches of *H. pruinosus* and *E. nigripes,* suggesting that these species orient close to the source of *endo*-brevicomin which is for them an attractant rather than a synergist (Shepherd et al. 2010). Simultaneously, *T. dubius* does not show behavioral responses to *endo*-brevicomin (Salom et al. 1992, Sullivan et al. 2016), and therefore its placement is irrelevant to this species. Our study demonstrates that interspecific differences in spatial behavior to attractive semiochemicals among insect species can be exploited in order enhance the targeting of particular species.

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