THE PAN-PACIFIC ENTOMOLOGIST
84(1):51–56, (2008)

Scientific Note

An improved synthetic attractant for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae), in northeastern California

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins 1902, is found in pine forests throughout the western U.S., north to northern British Columbia and Alberta, Canada and south to Mexico. It causes high levels of pine mortality throughout its range. Hosts include many species of *Pinus* (Pinaceae); in northern California, *D. ponderosae* is a pest of pine types dominated by *P. ponderosa* Douglas ex Lawson et C.Lawson, *P. contorta* Douglas ex Loudon, *P. monticola* Douglas ex D. Don, and in mixed conifer types where *P. lambertiana* Douglas is the principal host (Struble 1945). The extensive geographic range of *D. ponderosae*, and the variability in environments in which it occurs, create challenges for developing effective semiochemical tools for reducing beetle-caused tree mortality. Nonrandom genetic variation occurs among populations of *D. ponderosae* and has been attributed to both geography and host differences (Stock & Amman 1980, Sturgeon & Mitton 1986, Langor & Spence 1991, Kelley et al. 2000, Mock et al. 2007). Variation has also been observed in *D. ponderosae* responses to host-produced pheromone synergists (Pitman & Vité 1969, Billings et al. 1976, Libbey et al. 1985, Miller & Lindgren 2000), leading to uncertainties in semiochemical deployment among locations and forest types.

Semiochemical attractants (lures) can be used efficiently only when their effectiveness in different locations and host types is understood. For more than 20 years the standard lure for *D. ponderosae* has consisted of three components: two pheromones, *trans*-verbenol and *exo*-brevicomin, and myrcene, a host-based monoterpene synergist (Borden & Lacey 1985). This lure has generally caught greater numbers of *D. ponderosae* than other combinations of host compounds; however, most comparisons have been limited to British Columbia (e.g., Borden et al. 1987, Pureswaran & Borden 2005).

In western U.S., uncertainty remains about the relative effect of individual monoterpenes for synergizing aggregation pheromones of *D. ponderosae*. This is especially true for terpinolene, which is commonly found as a minor component of oleoresin in hosts of *D. ponderosae* (e.g., Smith 2000), and has been found to catch similar numbers of beetles to myrcene in some forest types (Billings et al. 1976). Terpinolene appears to be less ubiquitous in western pines than myrcene, but in California is frequently present in the oleoresins of both *P. ponderosa* and *P. lambertiana* among others (Smith 2000, S. L. Smith et al. unpublished data). Because the effectiveness of terpinolene as a pheromone synergist for *D. ponderosae* has varied (Billings et al. 1976, Conn et al. 1983), its utility for particular applications (geographic regions and forest types) must be evaluated before it can be effectively used.

The objective of this study was to evaluate the effect of terpinolene as a component of synthetic *D. ponderosae* lures in northeastern California. We compared three monoterpene treatments, always deployed with *trans*-verbenol and

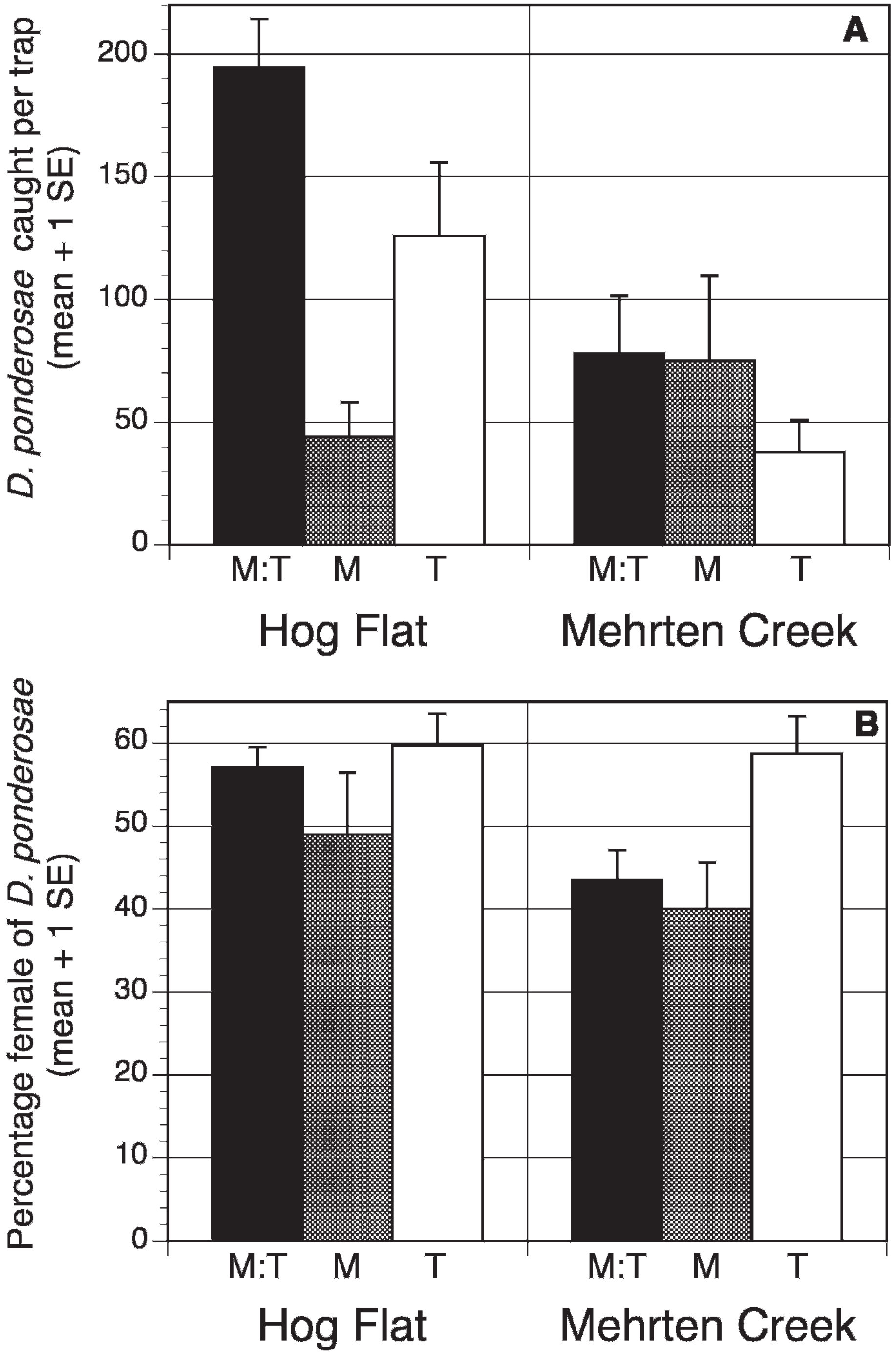

Figure 1. Mean number (A) and mean percentage of females (B) of *D. ponderosae* caught in multiple-funnel traps at two sites in northern California during summer 2006. Hog Flat (25 May to 23 June 2006, Lassen NF) was east side pine type dominated by *Pinus ponderosa* (Ponderosa Pine Series) while Mehrten Creek (20 June to 8 August 2006, Eldorado NF) was Mixed Conifer-Pine

exo-brevicomin, in two different environments (sites) for their ability to attract *D. ponderosae* to traps. Myrcene alone, terpinolene alone, or a 1:1 mixture of myrcene and terpinolene were evaluated. One site was near Susanville, CA (Hog Flat, Lassen NF) and the other was southwest of Lake Tahoe (Mehrten Creek, Eldorado NF). The Hog Flat site was predominately *P. ponderosa* (Ponderosa Pine Series, CALVEG 1981) with an elevation of ca. 1300 m, while the Mehrten Creek site was Mixed Conifer-Pine Series (CALVEG 1981) with an elevation of ca. 1675 m and a large component of sugar pine, *P. lambertiana*. The sites were about 320 km apart. Monoterpenes were evaporated from identical devices (one per trap) with an estimated release rate of about 175 mg/d @ 20°C (Synergy Semiochemicals Corp., Burnaby, BC, Canada).

At each site, lure treatments were randomly assigned to 12-unit multiple-funnel traps (Lindgren 1983) separated by at least 0.1 mi to assure their independence as experimental units (Shea et al. 1984). Traps were deployed at the Hog Flat site (n = 15 traps) from 25 May to 23 June 2006 and at the Mehrten Creek site (n = 20 traps) from 20 June to 8 August 2006. Traps and treatments were left in-place after deployment. Beetles were collected weekly. Collection cups contained a piece of No Pest Strip (Dichlorvos, 18.6%, Hot Shot, St. Louis, MO) to kill captured insects. Collections were frozen until counting and identification of sex were completed. Sums of collected *D. ponderosae* were determined, per trap, and means compared among treatments (Fig. 1). Data from two traps at Mehrten Creek were deleted from analyses because infested trees were found near them shortly after deployment. In addition, one trap from the Hog Flat site was eliminated from analysis of sex-ratio because it contained a single individual. Collected *D. ponderosae* (n = *3013*) and *D. brevicomis* LeConte 1876 (n = *129*) were separated by observing differences in elytral setae (*D. ponderosae* more variable and longer; R. Borys, Forest Service, PSW, personal communication) and the declevity in the frons (less apparent in *D. ponderosae*). Sex of each intact *D. ponderosae* was determined by observing the dorsal margin of the penultimate abdominal segment (Lyon 1958). About six percent of captured *D. ponderosae* were not sufficiently intact to determine their sex. Voucher specimens were deposited in the insect collection housed at USFS, SRS-RWU-4552, 2500 Shreveport Highway, Pineville, LA 71360.

Statistical analyses were done using R (V. 2.5.0, R Development Core Team 2007). The sum of *D. ponderosae* caught in each trap was used as the response variable in an analysis of variance (ANOVA) model to evaluate treatment effects on total catch. Percentage of females caught was similarly used as the response variable to evaluate treatment effects on sex-ratio. In all cases, each trap was considered an independent replicate. The ANOVA model consisted of two main effect factors (site and treatment) and their interaction. Site is a complex variable that includes potential differences in beetle populations and how they respond to semiochemicals between our locations (e.g., from host, elevation, timing). If the interaction proved to be non-

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Series with a significant overstory component of *P. lambertiana*. All traps were baited with standard attractant pheromones for *D. ponderosae*, *trans*-verbenol and *exo*-brevicomin. Monoterpene synergists were varied and consisted of a 1:1 mixture of myrcene and terpinolene (M:T, black bars), myrcene alone (M, gray bars), or terpinolene alone (T, white bars). Error bars denote one standard error of the mean (SE).

significant ($P < 0.20$), it was dropped and the model rerun. Sums of *D. ponderosae* caught per trap were transformed by their square root; inspection of distributions and model residuals indicated that transformed data better met the assumptions of parametric ANOVA. Transformations were deemed unnecessary for percentage female data. Means were subjected to family-wise evaluation using the Westfall correction for the number of pairwise comparisons ($P < 0.05$; R, MULTCOMP package).

Analysis of variance models provided a significant result for both response variables (F = 4.03, df = 5,27, $P < 0.007$ for mean catch per trap and $F = 4.6$, df = 3,28, $P < 0.009$ for mean percentage females). Site explained a significant amount of observed variation in mean trap catch (F = 6.46, df = 1,27, $P < 0.017$), as did monoterpene treatment (F = 3.39, df = 2,27, $P < 0.05$) and their interaction (F = 3.45, df = 2,27, $P < 0.05$). The multiple comparison procedure showed that traps deployed with myrcene as the lone monoterpene (mean ± 1 SEM) (57.8 ± 16.9) caught significantly fewer *D. ponderosae* than the myrcene:terpinolene blend (119.6 ± 22.5; $P < 0.04$). Terpinolene (81.9 ± 21.2) caught marginally fewer *D. ponderosae* than the 1:1 blend ($P < 0.09$). The difference between terpinolene alone and myrcene alone was not significant ($P < 0.40$). Although there was a significant interaction between site and monoterpene treatment, traps with 1:1 mixture of terpinolene and myrcene caught the most *D. ponderosae* at both sites (Fig. 1A). Rankings below this most attractive blend, however, varied with site. At our northernmost site (Hog Flat, Ponderosa Pine Series), *D. ponderosae* captures with terpinolene alone were higher than those with myrcene alone. At our more southern site (Mehrten Creek, Mixed Conifer-Pine Series), myrcene was better than terpinolene at synergizing the pheromone blend. This reinforces earlier reports of response instabilities among monoterpenes used to attract *D. ponderosae* to pheromone-baited traps (Billings et al. 1976). Additional experiments are necessary to elucidate causes of these differences (e.g., site, host, time of year) and to optimize lures for total catch of *D. ponderosae*.

Monoterpene treatments also significantly affected sex-ratio of the *D. ponderosae* captured (F = 4.65, df = 2,28, $P < 0.02$). Site again provided a significant main effect (F = 4.61, df = 1,28, $P < 0.04$); however, the interaction between monoterpene treatment and site was not significant for *D. ponderosae* sex-ratio (F = 0.95, df = 2,26, $P < 0.40$). Consequently, this effect was removed from the model prior to final analysis. Lures with terpinolene alone caught the greatest percentage of female *D. ponderosae* (mean ± 1 SEM) (59.2 ± 2.8%). This was significantly greater than the percentage captured by either myrcene alone (45.0 ± 4.8%; $P < 0.02$) or the 1:1 blend (48.7 ± 3.0%; $P < 0.05$, Fig. 1). Percentage of females captured in the 1:1 blend of myrcene to terpinolene and myrcene alone were not different ($P < 0.28$).

Standard, three-component synthetic lures for trapping *D. ponderosae* consist of *exo*-brevicomin, *trans*-verbenol and myrcene. Averaged across two sites in northern California, this lure caught about 48% of the most attractive semiochemical combination that we tested and was the least attractive of our three treatments (Fig. 1). Our mixture of myrcene and terpinolene was most attractive at both sites, despite the second- and third- best component combinations trading ranks. Optimizing the blend of myrcene and terpinolene will require additional studies, but we recommend a 1:1 blend of myrcene:terpinolene be used in northeastern California when attempting to attract *D. ponderosae*.

Terpinolene deployed without myrcene attracted significantly more females, which are the pioneering (first-attacking) sex in *Dendroctonus*. This reinforces Billings et al. (1976) and suggests that the effectiveness of blended myrcene and terpinolene (1:1) may be improved by evaluating different ratios of each monoterpene for their ability to increase total trap catch and that of females. Current experiments are assessing optimal blends for *D. ponderosae* in multiple environments.

Identification of an improved attractant for *D. ponderosae* will advance our ability to detect and monitor its presence, and will aid in the development of semiochemical tools for reducing its negative impacts. To be most effective, lures must be competitive with natural semiochemical sources (i.e., beetles attacking trees). To date this level of attraction has not been realized, but each improved lure has value. In research, semiochemical attractants are frequently used to evaluate behavioral deterrents and insecticides, both of which rely on attracting beetles to an area to adequately challenge treatments of interest (e.g., Shea et al. 1984). Our results suggest that application of *D. ponderosae* lures with myrcene and terpinolene (1:1) can improve the rigor of future experiments.

Acknowledgments

We thank B. Parpart, J. Parpart, H. Reed, K. Reed, S. Walters (Forest Service, SRS, Pineville, LA), D. Cluck, E. Frolli, D. Luna, R. Mahnka and L. Yazzie (Forest Service, R5-FHP, Susanville, CA) for technical assistance. A. Mangini (Forest Service, R8-FHP, Pineville, LA), J. Reeve (Department of Zoology, Southern Illinois University, Carbondale, IL) and K. Gibson (Forest Service, R1-FHP, Missoula, MT) graciously provided comments to improve the manuscript. We also thank the Eagle Lake Ranger District, Lassen NF, and the Amador Ranger District, Eldorado NF for their cooperation in providing sites.

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Received 1 August 2007; Accepted 22 November 2007. Publication date 12 June 2008.