cis-Verbenol: An aggregation pheromone for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae)

DANIEL R. MILLER
CENTRE FOR PEST MANAGEMENT
DEPARTMENT OF BIOLOGICAL SCIENCES
SIMON FRASER UNIVERSITY
BURNABY, BRITISH COLUMBIA V5A 1S6 CANADA
AND
JEAN P. LAFONTAINE
PHERO TECH INC. 7572 PROGRESS WAY, RR 5
DELTA, BRITISH COLUMBIA
V4G 1E9 CANADA

ABSTRACT

cis-Verbenol increased catches of male mountain pine beetles, *Dendroctonus ponderosae* Hopkins, to multiple-funnel traps baited with myrcene and *exo*-brevicomin. *cis*-Verbenol had no effect on the response of males to traps baited with myrcene, *exo*-brevicomin and *trans*-verbenol. In contrast, *cis*-verbenol increased catches of female *D. ponderosae* to semiochemical-baited traps irrespective of the absence or presence of *trans*-verbenol. Our results demonstrate that *cis*-verbenol is an aggregation pheromone for *D. ponderosae* and that the combination of *cis*- and *trans*-verbenol elicits sex-specific responses.

Additional keywords: *trans*-verbenol, myrcene, *exo*-brevicomin, sex-specificity.

INTRODUCTION

Various studies on the use of semiochemicals by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), including some that were specifically aimed at determining the effect of *trans*-verbenol, inadvertantly used *trans*-verbenol contaminated with 6-20 % *cis*-verbenol (Pitman 1971; Billings *et al.* 1976; Ryker and Rudinsky 1982; Borden *et al.* 1983; Conn *et al.* 1983). The role of *trans*-verbenol as a pheromone for *D. ponderosae* has subsequently been verified with chemical purities >97% (Ryker and Rudinsky 1982; Libbey *et al.* 1985; Borden *et al.* 1987).

However, no attempt has been made to discern the role of *cis*-verbenol in the chemical ecology of *D. ponderosae* and interpretation of studies using contaminated *trans*-verbenol must therefore be suspect. The issue is of economical importance since *D. ponderosae* is a major pest of lodgepole pine in the Pacific northwest (Safranyik *et al.* 1974; Furniss and Carolin 1980). Semiochemical-based manipulation of *D. ponderosae* has become a major component of lodgepole pine silviculture in British Columbia (Borden and Lacey 1985) Due to production costs, the tree bait most commonly used against *D. ponderosae* contains a 13:87 mix of *cis*- and *trans*-verbenol (Phero Tech Inc., Delta BC), together with myrcene and *exo*-brevicomin. *Dendroctonus ponderosae* uses myrcene as a kairomone (Billings *et al.* 1976; Conn *et al.* 1983; Libbey *et al.* 1985; Borden *et al.* 1987) and both enantiomers of *exo*-brevicomin as male-produced pheromones (Pitman *et al.* 1969; Rudinsky *et al.* 1974; Pitman *et al.* 1978; Borden *et al.* 1983; Libbey *et al.* 1985; Borden *et al.* 1987).

Our objective was to demonstrate that *cis*-verbenol is an aggregation pheromone for *D. ponderosae* in stands of lodgepole pine. *cis*-Verbenol is produced by *D. ponderosae* (Pitman *et al.* 1969; Hughes 1973; Ryker and Rudinsky 1982; Libbey *et al.* 1985; Pierce *et al.* 1987; Hunt *et al.* 1989). Antennae of both sexes of *D. ponderosae* are sensitive to *cis*- and *trans*-verbenol equally (Whitehead 1986; Whitehead *et al.* 1989). It is quite probable, therefore, that *cis*-verbenol is a pheromone for *D. ponderosae*. We tested the two following hypotheses: 1) *cis*-verbenol should increase attraction of *D. ponderosae* to traps baited with myrcene and *exo*-brevicomin; and 2) *cis*- and *trans*-verbenol should have an additive effect on the attraction of *D. ponderosae*.

MATERIALS AND METHODS

cis-Verbenol was prepared by reduction of *S*-(-)-verbenone by the alkoxy-substituted lithium aluminum hydride method (Brown and Deck 1965) and concentrated *in vacuo*. *trans*-Verbenol was prepared by subjecting *cis*-verbenol to acid-catalysed isomerisation at 23 °C (Cooper *et al.* 1967) and purified by flash chromatography on silica gel (Still *et al.* 1978). Chemical and optical purities of verbenols were determined by split, capillary, gas chromatography (Hewlett Packard HP 5890 using a 30-m X 0.32-mm ID DB-1 fused silica column), before and after derivatisation to acetyl lactate diastereomers (Slessor *et al.* 1985). The chemical purities of *cis*- and *trans*-verbenol were 97 and 99%, respectively, while the chiral ratios were identical at 83% *S*-(-): 17% *R*-(+).

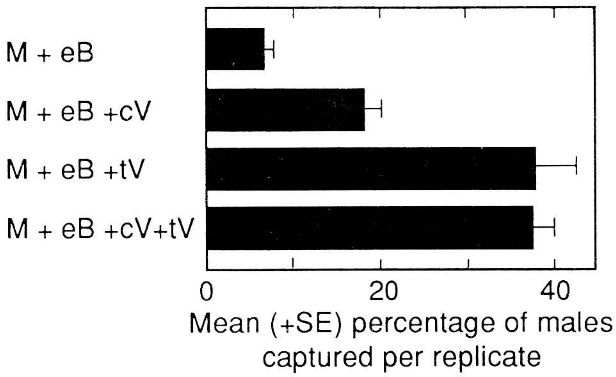
Verbenol lures consisted of polyethylene, bubble-cap devices (Phero Tech Inc., Delta BC) containing 1,3-butanediol solutions of *cis*- and *trans*-verbenol, respectively. The release rates of *cis*- and *trans*-verbenol were approximately 3.38 and 2.58 mg/day at 24-27 °C (determined by weight loss). Phero Tech Inc. (Delta BC) supplied the following additional lures: 1) (±)-*exo*-brevicomin (chemical purity, 98%) laminar lures ; and 2) ß-myrcene (chemical purity, 98%) in closed, polyethylene, screw-cap bottles (15 mL). The release rates of *exo*-brevicomin and myrcene were approximately 0.01 and 281 mg/day, respectively, at 24 °C (determined by collection of volatiles on Porapak-Q for *exo*-brevicomin and by weight loss for myrcene).

Forty, 8-unit, multiple-funnel traps (Lindgren 1983) (Phero Tech Inc., Delta BC) were set in 10 replicate grids of 2 x 2 in stands of mature lodgepole pine near Princeton BC. Grids were spaced at least 100 m apart, and traps were set 10-15 m apart within each replicate. Each trap was suspended between trees by rope so that the top funnel of the trap was 1.3-1.5 m above ground. No trap was within 2 m of any tree. All traps were set during the period of 2 to 26 September 1989. Treatments were randomly assigned within each replicate. The control treatment consisted of myrcene and *exo*-brevicomin while the remainder consisted of myrcene, *exo*-brevicomin and one of the following: 1) *cis*-verbenol; 2) *trans*-verbenol; 3) *cis*- and *trans*-verbenol. Sexes in subsamples (N=20) of tured *D. ponderosae* were determined by dissection and internal examination of genitalia.

The data were analysed using the SAS statistical package ver. 5.0 (SAS Institute Inc., Cary NC). For each sex, trap catch data, transformed by ln(Y+1), were subjected to 3-way ANOVA, using replicate, *cis*-verbenol, *trans*-verbenol, and the interaction of *cis*- and *trans*-verbenol, as model factors. Two orthogonal contrasts were performed on each data set, comparing catches in traps baited with myrcene and *exo*-brevicomin against catches to traps baited with myrcene, *exo*-brevicomin and *cis*-verbenol and catches to the combination of myrcene, *exo*-brevicomin and *trans*-verbenol against catches to traps baited with all four components.

RESULTS AND DISCUSSION

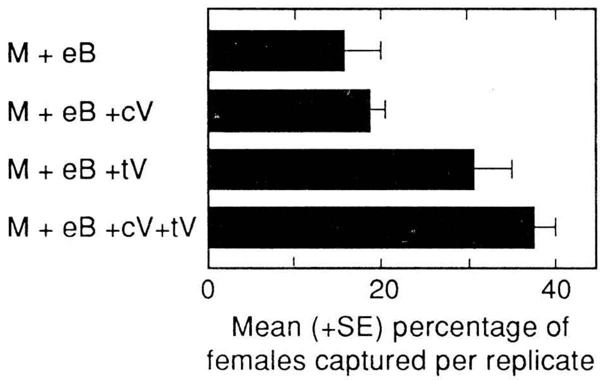
Both *cis*- and *trans*-verbenol significantly increased the catches of *D. ponderosae* to semiochemical-baited funnel traps (Figs. 1 and 2), although the effect of *cis*-verbenol on females was only weakly significant. There was a significant interaction between *cis*- and *trans*-verbenol on the capture of male *D. ponderosae* (Fig. 1). Catches of males in traps baited with myrcene, *exo*-brevicomin and *trans*-verbenol were not significantly different from catches in traps baited with all four components (orthogonal contrast, $F(1,35)$, $P=0.750$). Catches of males in traps baited with myrcene, *exo*-brevicomin and *cis*-verbenol were significantly higher than those in traps baited with myrcene and *exo*-brevicomin alone (orthogonal contrast, $F(1,35)$, $P=0.004$). In contrast, there was no interaction between *cis*- and *trans*-verbenol on the catches of female *D. ponderosae* (Fig. 2).



ANOVA

Source	df	P
A	9	0.651
B	1	<0.001
C	1	0.058
B*C	1	0.018
Error	26	

Figure 1. The effects of *cis*- (cV) and *trans*-verbenol (tV) on the attraction of male *D. ponderosae* to multiple-funnel traps baited with myrcene (M) and *exo*-brevicomin (eB) near Princeton BC from 2 to 26 September 1989 (n=10). Data were transformed by ln(Y+1) and subjected to ANOVA using the following model factors: replicate (A), *cis*-verbenol treatment (B), *trans*-verbenol treatment (C), and the interaction between *cis*- and *trans*-verbenol treatments (B*C). The total number of males caught was 2303.



ANOVA

Source	df	P
A	9	0.001
B	1	< 0.001
C	1	0.089
B*C	1	0.984
Error	26	

Figure 2. The effects of *cis*- (cV) and *trans*-verbenol (tV) on the attraction of female *D. ponderosae* to mutiple-funnel traps baited with myrcene (M) and *exo*-brevicomin (eB) near Princeton BC from 2 to 26 September 1989 (n=10). Data were transformed by ln(Y+1) and subjected to ANOVA using the following model factors: replicate (A), *cis*-verbenol treatment (B), *trans*-verbenol treatment (C), and the interaction between *cis*- and *trans*-verbenol treatments (B*C). The total number of females caught was 5303.

Our results demonstrate that *cis*-verbenol is an aggregation pheromone for *D. ponderosae*. It is produced by female *D. ponderosae* (Pitman *et al.* 1969; Hughes 1973; Ryker and Rudinsky 1982; Libbey *et al.* 1985; Pierce *et al.* 1987) and is attractive to both sexes (Figs. 1 and 2). Interpretations of results from previous studies that used *trans*-verbenol, with chemical purities less than 97%, should consider the effect of *cis*-verbenol, and the possible interactions of *cis*-verbenol with other treatments.

Our results further show that *cis*- and *trans*-verbenol have an additive effect on the attraction of female *D. ponderosae* but not on male *D. ponderosae*. The interaction between the verbenols resulted in sex-specific responses; males and females responded differently to *cis*- and *trans*-verbenol separately than to the combination of verbenols (Figs. 1 and 2). These sex-specific responses may be a function of either the presence of both verbenols together or of the ratio of verbenols. The ratio of *cis*- and *trans*-verbenol following autoxidation of α-pinene ranges from 29:71 to 20:80 (Hunt *et al.* 1989). Axenically-reared *D. ponderosae* produce verbenol with a *cis*:*trans* ratio of 14:86 when exposed to α-pinene odors while wild beetles produce a ratio of 2:98 (Hunt *et al.* 1989).

Regardless of the mode of specificity, both *cis*- and *trans*-verbenol are required to maximise the attraction of female *D. ponderosae*. Since females initiate attacks on trees (Safranyik *et al.* 1974), it is critical that tree baits used in silvicultural practices to control populations of *D. ponderosae* contain both pheromones . Fortunately the bait currently employed operationally for controlling *D. ponderosae* contains both *cis*- and *trans*-verbenol at a ratio of 13:87 (Phero Tech Inc., Delta BC), well within the range of observed production ratios.

ACKNOWLEDGEMENTS

We thank T.C. Baker, J.H. Borden, B.S. Lindgren and three anonymous reviewers for critical reviews of the manuscript. Assistance in the manufacture of release devices was kindly provided by Phero Tech Inc., Delta BC. Voucher specimens of *D. ponderosae* have been deposited at the Entomology Museum at Simon Fraser University, Burnaby BC. This research was supported in part by the Natural Sciences and Engineering Research Council of Canada (Operating Grant No. A3881 and Strategic Grant No. G1611), and the Science Council of British Columbia [Grant No. 1(RC 14-16)].

REFERENCES

Billings, R.F., R.I. Gara and B.F. Hrutfiord. 1976. Influence of ponderosa pine resin volatiles on the response of *Dendroctonus ponderosae* to synthetic *trans*-verbenol. Environ. Entomol. 5: 171-179.

Borden, J.H. and T.E. Lacey. 1985. Semiochemical-based manipulation of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins: A component of lodgepole pine silviculture in the Merritt timber supply area of British Columbia. Z. ang. Ent. 99: 139-145.

Borden, J.H., J.E. Conn, L.M. Friskie, B.E. Scott, L.J. Chong, H.D. Pierce, Jr., and A.C. Oehlschlager. 1983. Semiochemicals for the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae), in British Columbia: Baited tree studies. Can. J. For. Res. 13: 325-333.

Borden, J.H., L.C. Ryker, L.J. Chong, H.D. Pierce, Jr., B.D. Johnston and A.C. Oehlschlager. 1987. Response of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae), to five semiochemicals in British Columbia lodgepole pine forests. Can. J. For. Res. 17: 118-128.

Brown, H.C. and H.R. Deck. 1965. Selective reductions. VIII. The stereochemistry of reduction of cyclic and bicyclic ketones by the alkoxy-substituted lithium aluminum hydrides. J. Am. Chem. Soc. 87: 5620-5624.

Conn, J.E., J.H. Borden, B.E. Scott, L.M. Friskie, H.D. Pierce, Jr., and A.C. Oehlschlager. 1983. Semiochemicals for the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae) in British Columbia: Field trapping studies. Can. J. For. Res. 13: 320-324.

Cooper, M.A., J.R. Salmon and D. Whittaker. 1967. Stereochemistry of the verbenols. J. Chem. Soc. (B) 1967: 1259-1261.

Furniss, R.L. and V.M. Carolin. 1980. Western forest insects. U.S.D.A. For. Serv. Misc. Publ. 1339.

Hughes, P.R. 1973. Effect of α-pinene on *trans*-verbenol synthesis in *Dendroctonus ponderosae* Hopk. Naturwiss. 60: 261-262.

Hunt, D.W.A., J.H. Borden, B.S. Lindgren and G.Gries. 1989. The role of autoxidation of pinene in the production of pheromones of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Can. J. For. Res. 19: 1275-1282.

Libbey, L.M., L.C. Ryker and K.L. Yandell. 1985. Laboratory and field studies of volatiles released by *Dendroctonus ponderosae* Hopkins (Coleoptera, Scolytidae). Z. ang. Ent. 100: 381-392.

Lindgren, B.S. 1983. A multiple-funnel trap for scolytid beetles. Can. Ent. 115: 299-302.

Pierce, H.D., Jr., J.E. Conn, A.C. Oehlschlager and J.H. Borden. 1987. Monoterpene metabolism in female mountain pine beetles, *Dendroctonus ponderosae* Hopkins, attacking ponderosa pine. J. Chem. Ecol. 13: 1455-1480.

Pitman, G.B. 1971. *trans*-Verbenol and alpha-pinene: Their utility in manipulation of the mountain pine beetle. J. Econ. Ent. 64: 426-430.

Pitman, G.B., M.W. Stock and R.C. Knight. 1978. Pheromone application in mountain pine beetle - lodgepole pine management: Theory and practice, pp. 165-173. *In* A.A. Berryman, G.D. Amman and R.W. Stark (eds.). Theory and practice of mountain pine beetle management in lodgepole pine forests. College of Forest Resources, University of Idaho, Moscow ID.

Pitman, G.B., J.P. Vité, G.W. Kinzer and A.F. Fentiman, Jr. 1969. Specificity of population-aggregating pheromones in Dendroctonus. J. Insect Physiol. 15: 363-366.

Rudinsky, J.A., M.E. Morgan, L.M. Libbey and T.B. Putnam. 1974. Antiaggregation-rivalry pheromone of the mountain pine beetle. Environ. Entomol. 3: 90-98.

Ryker, L.C. and J.A. Rudinsky. 1982. Field bioassay of *exo-* and *endo-*brevicomin with *Dendroctonus ponderosae* in lodgepole pine. J. Chem. Ecol. 8: 701-707.

Safranyik, L., D.M. Shrimpton and H.S. Whitney. 1974. Management of lodgepole pine to reduce losses from the mountain pine beetle. Forestry Canada For. Tech. Rep. 1.

Slessor, K.N., G.G.S. King, D.R. Miller, M.L. Winston and T.L. Cutforth. 1985. Determination of chirality of alcohol or latent alcohol semiochemicals in individual insects. J. Chem. Ecol. 11: 1659-1667.

Still, W.C., M. Kahn and A. Mitra. 1978. Rapid chromatographic technique for preparative separations with moderate resolution. J. Org. Chem. 43: 2923-2925.

Whitehead, A.T. 1986. Electroantennogram responses by mountain pine beetles, *Dendroctonus ponderosae* Hopkins, exposed to selected semiochemicals. J. Chem. Ecol. 12: 1603-1621.

Whitehead, A.T., D.T. Scott, R.F. Schmitz and K. Mori. 1989. Electroantennograms by mountain pine beetles, *Dendroctonus ponderosae* Hopkins, exposed to selected chiral semiochemicals. J. Chem. Ecol. 15: 2089-2099.

Melanchra picta (Harris) (Lepidoptera: Noctuidae), a cutworm new to British Columbia

SHEILA M. FITZPATRICK and JAMES T. TROUBRIDGE[1]

AGRICULTURE CANADA RESEARCH STATION,
6660 N.W. MARINE DRIVE, VANCOUVER, B. C., CANADA V6T 1X2

1. ORDER OF AUTHORS DECIDED BY TOSS OF A COIN.

Here we report the occurrence of the zebra caterpillar, *Melanchra picta* (Harris) as a minor pest of commercial cranberries, *Vaccinium macrocarpon* Ait., in Langley and Pitt Meadows, British Columbia, during the summer of 1991. In the field, zebra caterpillars ate the growing tips of cranberry runners and uprights. In the laboratory, larvae preferred succulent cranberry tissue, consuming mature leaves only if no new growth remained. In the field and laboratory, larvae also consumed the foliage of dicotyledonous weeds such as cutleaf blackberry, *Rubus laciniatus* Willd., western St. John's wort, *Hypericum formosum* Humboldt, marsh St. John's wort, *Triadenum virginicum* L., and Watson's willow herb, *Epilobium watsonii* Barbey. In eastern Canada, zebra caterpillars have been reported to feed on a wide variety of fruit, vegetable, and leguminous forage crops (Beirne, 1971).

Early records of zebra caterpillar infestations in British Columbia (Cockle, 1911; Middleton, 1913) actually referred to *Mamestra canadensis* Smith, now considered a synonym of *Lacanobia nevadae* (Grote). In Canada, the zebra caterpillar, *M. picta*, occurs from the Atlantic coast, west to the foothills of the Rocky Mountains, whereas in the U.S.A. its range extends further west into California, Oregon, and Washington. There are no specimens of *M. picta* from B.C. in the Canadian National Collection, the Royal British Columbia Museum, or the Spencer Collection, University of British Columbia, nor does *M. picta* appear on lists of B. C. fauna (*e.g.* Llewellyn Jones, 1951). Recent reports of *M. picta* on strawberries, *Fragaria* x *ananassa* Duch., in 1981, highbush blueberries, *Vaccinium corymbosum L.*, in 1983 (Belton, 1988), and corn, *Zea mays* L., in 1990 (Philip, 1991) in B. C. probably refer to this species. Since the zebra caterpillar has previously been found very close to the B.C. border in Washington State, (Tonasket, 40 km south of Osoyoos, B.C.; Puyallup, 55 km south of Seattle), we believe that its presence in B. C. represents a recent range extension rather than an introduction.