

1-1-2012

Area-wide application of verbenone-releasing flakes reduces mortality of whitebark pine *Pinus albicaulis* caused by the mountain pine beetle *Dendroctonus ponderosae*

Nancy E. Gillette

USDA Forest Service, ngillette@fs.fed.us

E. Matthew Hansen

USDA Forest Service, matthansen@fs.fed.us

Constance J. Mehmel

USDA Forest Service, cmehmel@fs.fed.us

Sylvia R. Mori

USDA Forest Service, smori@fs.fed.us

Jeffrey N. Webster

Webster Forestry Consulting

See next page for additional authors

Follow this and additional works at: <http://digitalcommons.unl.edu/usdafsfacpub>

Gillette, Nancy E.; Hansen, E. Matthew; Mehmel, Constance J.; Mori, Sylvia R.; Webster, Jeffrey N.; Erbilgin, Nadir; and Wood, David L., "Area-wide application of verbenone-releasing flakes reduces mortality of whitebark pine *Pinus albicaulis* caused by the mountain pine beetle *Dendroctonus ponderosae*" (2012). *USDA Forest Service / UNL Faculty Publications*. Paper 225.
<http://digitalcommons.unl.edu/usdafsfacpub/225>

This Article is brought to you for free and open access by the USDA Forest Service -- National Agroforestry Center at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in USDA Forest Service / UNL Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Nancy E. Gillette, E. Matthew Hansen, Constance J. Mehmel, Sylvia R. Mori, Jeffrey N. Webster, Nadir Erbilgin, and David L. Wood

Area-wide application of verbenone-releasing flakes reduces mortality of whitebark pine *Pinus albicaulis* caused by the mountain pine beetle *Dendroctonus ponderosae*

Nancy E. Gillette*, E. Matthew Hansen†, Constance J. Mehmelt‡, Sylvia R. Mori*, Jeffrey N. Webster§, Nadir Erbilgin¶ and David L. Wood**

*USDA Forest Service, PSW Research Station, Albany, CA 94710, U.S.A., †USDA Forest Service, Rocky Mountain Research Station, Logan, UT 84321, U.S.A., ‡USDA Forest Service, Forest Health Protection, Wenatchee, WA 98801, U.S.A., §Webster Forestry Consulting, Redding, CA 96002, U.S.A., ¶Department of Renewable Resources, University of Alberta, Edmonton, AB T6G 2E3, Canada, and **Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, U.S.A.

- Abstract**
- 1 DISRUPT Micro-Flake Verbenone Bark Beetle Anti-Aggregant flakes (Hercon Environmental, Inc., Emigsville, Pennsylvania) were applied in two large-scale tests to assess their efficacy for protecting whitebark pine *Pinus albicaulis* Engelm. from attack by mountain pine beetle *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytinae) (MPB). At two locations, five plots of equivalent size and stand structure served as untreated controls. All plots had early- to mid-outbreak beetle populations (i.e. 7.1–29.2 attacked trees/ha). Verbenone was applied at 370 g/ha in both studies. Intercept traps baited with MPB aggregation pheromone were placed near the corners of each plot after the treatment in order to monitor beetle flight within the plots. Trap catches were collected at 7- to 14-day intervals, and assessments were made at the end of the season of stand structure, stand composition and MPB attack rate for the current and previous years.
 - 2 Applications of verbenone flakes significantly reduced the numbers of beetles trapped in treated plots compared with controls at both sites by approximately 50% at the first collection date.
 - 3 The applications also significantly reduced the proportion of trees attacked in both Wyoming and Washington using the proportion of trees attacked the previous year as a covariate in the model for analysis of current year attack rates; in both sites, the reduction was $\geq 50\%$.
 - 4 The flake formulation of verbenone appears to have promise for area-wide treatment by aerial application when aiming to control the mountain pine beetle in whitebark pine forests.

Keywords Aerial application, anti-aggregation pheromones, bark beetles, behavioural chemicals, Coleoptera, pheromones, Scolytinae, semiochemicals.

Introduction

The mountain pine beetle *Dendroctonus ponderosa* Hopkins (Coleoptera: Scolytinae) (MPB) is the most damaging insect pest of several pine species, including ponderosa pine *Pinus ponderosa* ex P. & C. Lawson and lodgepole pine *Pinus contorta* Douglas ex Loudon throughout most of their ranges in

North America (Furniss & Carolin, 1977; Wood *et al.*, 2003). It has recently become even more widespread and severe in high-elevation whitebark pine *Pinus albicaulis* Engelm. stands throughout the western U.S.A. (Keane, 2001; Perkins & Roberts, 2003; Gibson, 2006). Gibson (2006) reported that beetle-caused mortality of whitebark pine in the Greater Yellowstone Area reached record highs in 2005, exceeding the tree mortality for any previous year for which records exist. Whitebark pine, a candidate for listing as an endangered species (Federal Register, 2011), is a crucial resource for wildlife and

Correspondence: Nancy E. Gillette; Tel.: +1 510 559 6474; fax: +1 510 559 6440; e-mail: ngillette@fs.fed.us

serves as an important food source for Clark's nutcrackers, red squirrels and grizzly bears (Tomback *et al.*, 2001). MPB typically erupts in large, episodic outbreaks and it is predicted that a warming climate will favour MPB expansion into higher elevation alpine stands where whitebark pine predominates (Logan & Powell, 2001; Krist *et al.*, 2007; Kurz *et al.*, 2008).

Verbenone (4,6,6-trimethylbicyclo(3.1) hept-3-en-2-one) is a semiochemical with broad behavioural activity across the genus *Dendroctonus* (Borden, 1997). When released at high rates, it interrupts beetle response to conspecifics, host trees or traps baited with aggregation pheromone in almost all of the scolytid species that have been tested, with the exception of *Conophthorus ponderosae* (Coleoptera: Scolytinae) (Rappaport *et al.*, 2000) so there is little risk of inducing secondary pest outbreaks with area-wide applications. More recently, verbenone pouches containing 7.5 g of active ingredient (ConTech International, Canada), have been shown to reduce losses of both whitebark pine and lodgepole pine to MPB (Kegley *et al.*, 2003; Kegley & Gibson, 2004, 2009; Bentz *et al.*, 2005). These pouches must be attached to the tree trunks at a height of approximately 3 m (ConTech International, Delta, BC, Canada). The remote and steep character of stands that compose most of the remaining component of whitebark pine, however, makes such hand application impractical and expensive, so the use of pouches is limited to smaller and more accessible stands. In addition, results from some previous tests of verbenone pouches for control of MPB in lodgepole pine have been equivocal (Progar, 2005; Bentz *et al.*, 2005), prompting us to seek a new release device with potentially more favourable release characteristics and greater ease of application in remote and rugged terrain, including the option of aerial application.

Other pheromone release systems have been developed and tested for control of forest insect pests, including polymer bead formulations (McGregor *et al.*, 1984; Shea *et al.*, 1992), although they did not provide consistent efficacy (Holsten *et al.*, 2000). Pheromone-releasing laminated flakes, on the other hand, have been used successfully for decades in the USDA Forest Service 'Slow-the-Spread' program to control the gypsy moth *Lymantria dispar* (Sharov *et al.*, 2002) in suburban areas. This release device, composed of a three-layer plastic laminate with semi-permeable top and bottom and a pheromone-releasing inner layer, is currently registered by the U.S. Environmental Protection Agency and has undergone the environmental and human health assessments required for biopesticides in the U.S.A. Laboratory testing has shown that these flakes release for a longer period than earlier bead formulations (Holsten *et al.*, 2000; N. Starnier, Hercon Environmental, personal communication), perhaps because each laminated flake is essentially a reservoir with a relatively small surface area for release (i.e. the edges of the flakes), unlike the beads, which had a coating of the active ingredient that is released from the surface. An earlier coated-bead formulation of verbenone showed initial promise for mitigating MPB damage to *P. contorta* (Shea *et al.*, 1992) but subsequent tests failed to confirm its efficacy (Holsten *et al.*, 2000), perhaps because of insufficient longevity of release of the pheromone.

Previous studies have shown the efficacy of verbenone-releasing flakes for the protection of lodgepole pine from attack by MPB (Gillette *et al.*, 2006, 2009a) and of

methylcyclohexenone-releasing flakes for the protection of Douglas-fir (*Pseudotsuga menziesii*) from attack by the Douglas-fir beetle *Dendroctonus pseudotsugae* (Gillette *et al.*, 2009b). Accordingly, we hypothesized that it might be equally effective in protecting whitebark pine stands threatened by MPB outbreaks. Whitebark pine could be even more susceptible to MPB than lodgepole pine, however, because its xylem resin contains, on average, 10-fold more myrcene and terpinolene than that of lodgepole pines (Smith, 2000). These two monoterpenes substantially synergize the attraction of MPB to its aggregation pheromone (Borden *et al.*, 2008) and so it is necessary to demonstrate the efficacy of verbenone-releasing flakes for protection of this tree species. Additionally, although the product is registered with the U.S. Environmental Protection Agency for applications in forest ecosystems, it is useful for forest managers to obtain efficacy data in support of its use for this endangered pine species.

Materials and methods

Verbenone formulation

Verbenone-releasing flakes, 3.2 × 3.2 mm square ('Disrupt' Microflake Verbenone; Hercon Environmental, Inc., Emigsville, Pennsylvania), were formulated to contain approximately 15% verbenone in a central layer of plastisol bounded by two thin layers of polymer laminate. Thus, 1 kg of flakes contained approximately 150 g of verbenone. The release rate of verbenone from these flakes is dependent on temperature and humidity, although laboratory oven tests indicated that biologically relevant levels of verbenone release for more than 42 days under constant high temperature (30 °C). Earlier tests of the same formulation targeting *Dendroctonus brevicomis* LeConte showed interruption of trap catch for 10 weeks after application (N. Erbilgin, D. R. Owen, J. N. Webster & D. L. Wood, unpublished data), indicating release of significant amounts of verbenone over the primary flight period of MPB. Bioassays (beetle trapping studies) indicated much longer longevity under field conditions, probably because of diurnal temperature fluctuations and shading of many of the flakes by understory vegetation (N. E. Gillette & D. L. Wood, unpublished data).

Study site and experimental design: Wyoming

Ten 4.0-ha plots containing predominantly whitebark pine, located on the Bridger-Teton and Shoshone National Forests, Wyoming, were established in the Moccasin Basin area (43°42'N; 110°03'W), approximately 10 km southeast of Togwotee Pass. Moccasin Basin was chosen because of its road access and high levels of MPB activity in whitebark pine, which was intended to present a strong challenge to the treatments. Because local whitebark pine stands are often < 4.0 ha in size and are often delineated by meadows or nonhost conifers, core measurement plots of 2.0 ha in size were nested within the 4.0-ha treated plots. Plots were located with a minimum spacing of 200 m between any plots. On-the-ground inspections were conducted to assure predominance of mature whitebark pine and adequate current MPB infestations to challenge the treatments. Ten plots were delineated as square blocks, using cardinal directions, and five of these were randomly assigned

to receive the pheromone treatment with the remaining five left as untreated controls.

Study site and experimental design: Washington

This study was conducted on the Chelan Ranger District of the Wenatchee National Forest (120°17'44.156''W; 48°6'18.953''N) in a stand consisting of primarily whitebark pine but with an admixture of lodgepole and ponderosa pines. We selected 10 4.0-ha plots, at least 400 m apart, with similar stand stocking levels and existing rates of MPB infestation. We then randomly assigned the pheromone treatment to half of the plots, reserving the remaining half as untreated controls. A core plot of 2.0 ha was established in the centre of each of the 10 plots to measure stand structure and treatment effects (beetle flight and rate of attack on trees) while minimizing potential edge effects.

Verbenone applications

In both Wyoming and Washington, aerial applications were made without stickers or liquids because previous studies have demonstrated good efficacy without such tank additives (Gillette *et al.*, 2009a, b). Furthermore, this type of dry application results in considerable savings because of reduced weight and volume of product and more efficient loading of tanks. With a dry application, the flakes fall through the understory vegetation to the forest floor.

Verbenone flakes were applied in Wyoming on 28–29 June 2005, at the rate of 370 g active ingredient/ha by a five-person crew using broadcast spreaders with slot augers calibrated to dispense evenly and at the desired rate. This application rate is equivalent to approximately 9.7 flakes/m². To minimize potential edge effects, verbenone flakes were applied to the entire 4.0 ha block with a 2.0-ha central core plot used for host mortality and stand structure metrics. Evenness and precision of application were assessed by placing four pieces of cardboard (1 m²) at random per plot, each sprayed with a tacky substance to catch dispersing flakes; flakes were counted to confirm evenness of application and calibrate dispensers. We were unable to assess whether beetle flight had begun at the time of application because of limited site access early in the season, although subsequent monitoring trap catches indicate that beetle flight was well underway within 2 weeks after application.

Application in Washington was made on 5 June 2007 from a Bell 47-G3B2A turbine helicopter (Bell Helicopter Textron Inc., Fort Worth, Texas) equipped with two dispensing pods (described above), each fitted with slot augers feeding a hydraulic spinner to achieve even distribution of flakes. The airspeed during application was 72.5 km/h. Evenness and precision of application were determined as in Wyoming. Analysis of beetle catches in monitoring traps suggests that beetle flight had not yet begun at the time of application.

Beetle flight, stand structure and beetle attack rate measurements

At both study sites, Intercept panel traps (Advanced Pheromone Technologies, Marylhurst, Oregon) were installed in the core

plots immediately after the verbenone applications aiming to monitor beetle flight within treated and untreated plots. In Wyoming, we installed two traps at randomly selected corners of the core plots, whereas in Washington, we installed four traps: one at each corner of the core plots. In an effort to avoid the potential confounding effect of inducing MPB attack on nearby whitebark pines by the baited traps (and the consequent release of natural beetle aggregation pheromone by attacking beetles), the traps were suspended on nonhost trees or shrubs as far away from hosts as possible. The traps were baited with MPB aggregation pheromone, a three-part blend of *trans*-verbenol, *exo*-brevicommin and myrcene (ConTech International, Inc., Canada). To reduce the release rates of the semiochemicals and the associated potential for beetle attack on nearby host trees, bait components were placed in a semi-permeable zip-lock sandwich bag with half the surface area covered with polyethylene terephthalate tape. Collection cups attached to the trap bottoms contained Vaportape (Hercon Environmental, Inc.) insecticide-releasing strips to reduce losses of responding MPB to predators. In Wyoming, trapped insects were collected only twice (14 and 28 July 2005) during the season because of difficulty of access. In Washington, trap catches were collected on a weekly basis for 10 weeks after application. Trapped insects were shipped to the Wood Laboratory at the University of California, Berkeley, California, for identification to species level; voucher specimens were submitted to the Essig Museum of Entomology, Berkeley, California.

Stand characteristics, including post-treatment beetle attack and host mortality, were measured in Wyoming at each 2-ha core plot on 12–14 September 2005. All live trees \geq 10 cm diameter at breast height (DBH) were tallied by species and measured using Biltmore sticks. Subsequent to the year 2000, MPB in Idaho, Wyoming and Montana have been observed with a range of life cycles: univoltine, semivoltine and fractional voltinism (e.g. one generation in 14 months) (Bentz & Schen-Langenheim, 2007). Attacked trees were therefore classified by year of attack (current year: pitch tubes and boring dust on otherwise green trees, immature brood; previous year: needles ranging from pale green to brilliant orange, often with mature brood; older: needles ranging from dull orange to fallen, no live brood) and type of attack (mass, strip, or pitch-out). Per-hectare attack rates were calculated for all host trees attacked by MPB in 2004 and 2005.

In Washington, stand characteristics, including post-treatment beetle attack and host mortality, were measured on 25–26 September, 2007, at each 2-ha core plot as in Wyoming. All live trees \geq 15 cm DBH were tallied by species and measured using Biltmore sticks. Per-hectare attack rates (characterized by type of attack: mass, strip, or pitch-out) were calculated for all host trees attacked by MPB in 2006 and 2007.

Statistical analysis

In general, we have found it useful to include a covariate that estimates beetle populations in our models because background beetle population levels play a key role in current-year attack rates. Indeed, North American bark beetle researchers typically use the ratio of 'green-attacked : red-attacked' trees to evaluate

bark beetle attack trends (Wulder *et al.*, 2008). In other words, the attack rate in the previous year ('red-attacked' trees, whose needles have died and turned red) is used to estimate beetle attack rates in the previous year and 'green-attacked' trees compose the group of trees attacked in the current year. For these two studies, therefore, we used the proportion of stems/ha mass-attacked by beetles the previous year, including all host species present in the stands, as a surrogate for beetle pressure during the year that treatments were applied. For both Wyoming and Washington tests, we used the proportions of whitebark pines/ha that were mass-attacked as the response variables. Mass-attacked trees are presumed killed (although a very few may survive) and so this response variable was taken as a measure of tree mortality, which can take many months to be expressed.

For the Wyoming test, an over-dispersed Poisson regression from the family of generalized linear models (McCulloch & Searle, 2001) was used to model the response (i.e. proportion of whitebark pines mass-attacked in 2005) versus treatment, using the proportion of all host pine trees (whitebark and lodgepole pines) mass-attacked in 2004 as an explanatory variable or covariate (Gillette *et al.*, 2009a). The two treatment levels (treated and control) were statistically compared with the likelihood ratio test. The SAS Genmod procedure (SAS, version 9.2; SAS Institute, Cary, North Carolina) was used for the estimation and comparison tests. For the Washington test, the same over-dispersed Poisson Regression was used to model the proportion of whitebark pines mass-attacked in 2007 versus treatment, using the proportion of all pine trees (whitebark, lodgepole and ponderosa pines) that were mass-attacked in 2006 as an explanatory variable. The two treatment levels (treated and control) were statistically compared as in the Wyoming study.

A generalized linear model for over-dispersed Poisson was also used to model the number of beetles per trap placed at the corners of each site aiming to compare the two treatment levels at each of the two 2-week periods in Wyoming, and averaged over 10 sampling periods (27 July 2007 to 15 September 2007) in Washington, using the likelihood ratio test to assess treatment differences. The SAS GENMOD procedure was used for the estimation and comparison tests.

Results

Wyoming

Verbenone flakes significantly reduced the numbers of beetles trapped at 2 and 4 weeks after treatment ($P = 0.002$) (Fig. 1), with a mean of 229 beetles/trap in treated plots versus 512 beetles/trap in control plots at the first sampling interval. Regarding host mortality measures, the model without a covariate (i.e. the proportion of stems mass-attacked during 2004) found no significant difference between treated and control plots ($P = 0.14$) (Table 1). In the model that included the covariate, however, both the previous-year infestation rate and the treatment effect were statistically significant ($P = 0.007$ and $P = 0.021$, respectively) (Fig. 2 and Table 2). The overall mean numbers of stems mass-attacked/ha in the control and treated plots were 26.5 (out of 333.9 whitebark pines/ha)

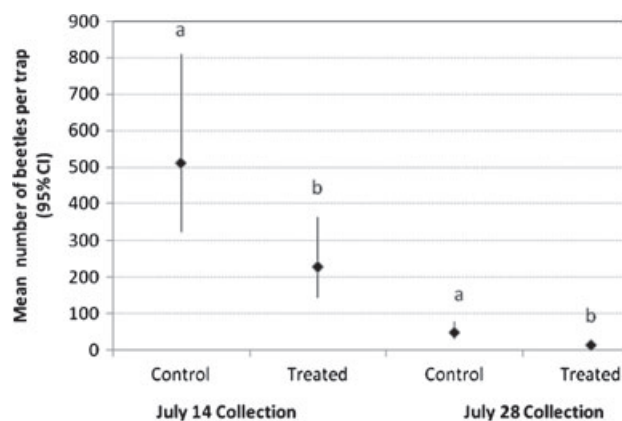


Figure 1 Mean number of mountain pine beetle beetles trapped during each 2-week sampling interval, with 95% confidence interval (CI): Wyoming, 2005. For each period, means with the same lowercase letter are not significantly different at experiment-wise error rate $\alpha = 0.05$ using the likelihood ratio test with Bonferroni adjustment.

and 16.4/ha (out of 383.2 whitebark pines/ha), respectively (Table 1), and the overall mean basal areas attacked in control and treated plots were 3.7 m²/ha (out of 27.0 m²/ha whitebark pine basal area) and 2 m²/ha (out of 27.5 m²/ha whitebark pine basal area), respectively (Table 1).

Washington

Verbenone flakes significantly reduced the numbers of beetles trapped at 10 sampling dates after treatment ($P = 0.008$) (Fig. 3), with a mean of 3005 beetles/trap in controls versus 1672 beetles/trap in treated plots for the entire 10-week sampling period. For the model with just treatment effect (no covariates), the treatments also significantly reduced the proportion of trees attacked ($P = 0.02$) (Table 3). When the proportion of trees mass-attacked in the previous year was included as a covariate, both the previous-year infestation rate and the treatment effect were statistically significant ($P = 0.031$ and $P < 0.0001$, respectively) (Fig. 4 and Table 2). The overall mean numbers of stems mass-attacked/ha in the control and treated plots were 29.2 (out of 194.7 whitebark pines/ha) and 7.0/ha (out of 89.5 whitebark pines/ha), respectively (Table 3). The overall mean basal areas attacked in the control and treated plots were 1.2 m²/ha (out of 7.4 m²/ha whitebark pine basal area) and 0.6 m²/ha (out of 4.7 m²/ha whitebark pine basal area), respectively (Table 3).

Discussion

It is clear from the beetle trap catch results (Figs 1 and 3) that both the simulated aerial application in Wyoming and the helicopter application in Washington successfully reduced MPB numbers in treated stands during the primary period of beetle flight, which coincides with the attack and aggregation phases of MPB host colonization. At peak beetle flight, the number of beetles in treated plots was less than half that in control plots at both sites. It is also evident that the interruption of

Table 1 Stand structure characteristics of whitebark pines (*Pinus albicaulis*) and associated trees, and pre- and post-treatment attack rates in treated and control plots, Wyoming, 2005

	Mean ± SE total basal area (m ² /ha)	Mean ± SE <i>Pinus albicaulis</i> basal area (m ² /ha)	Mean ± SE <i>Pinus albicaulis</i> DBH (cm) ^a	Mean ± SE number of stems/ha (all tree species)	Mean ± SE number <i>Pinus albicaulis</i> stems/ha	Mean ± SE number <i>Pinus contorta</i> and <i>Pinus ponderosa</i> stems/ha	Mean ± SE trees/ha mass attacked, 2004	Mean ± SE trees/ha attacked, 2005 ^b	Mean ± SE basal area/ha of trees mass-attacked, 2005
Control	43.3 ± 5.2*	27.0 ± 4.9*	15.4 ± 1.6*	614.1 ± 70.0*	333.9 ± 71.1*	27.4 ± 13.2*	14.5 ± 4.2*	26.5 ± 5.2*	3.7 ± 0.7*
Treated	42.9 ± 1.5*	27.5 ± 3.6*	16.6 ± 1.4*	624.3 ± 52.7*	383.2 ± 68.4*	8.0 ± 2.0*	16.4 ± 4.1*	16.4 ± 6.5*	2.0 ± 0.8*
Ratio, control/treated	1.00	0.98	0.93	0.98	0.87	3.4	0.88	1.62	1.85

^aDBH, diameter at breast height. *Not significantly different at experiment-wise error rate $\alpha = 0.05$ (maximum likelihood ratio test).

^bThe comparison is tested for a model without the explanatory variable, proportion of mass-attacked trees in the previous-year, including that covariate yields a significant treatment effect.

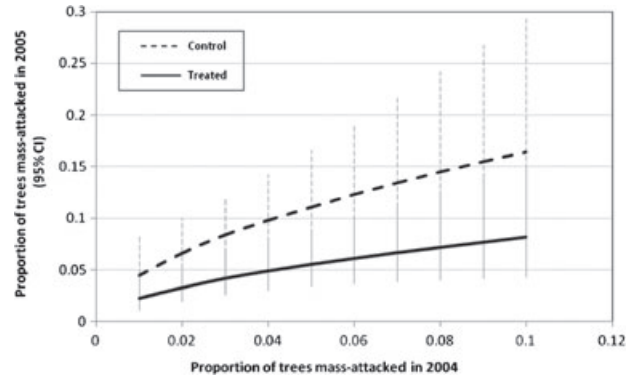


Figure 2 Proportion of whitebark pine (*Pinus albicaulis*) trees attacked in 2005 as a function of the proportion of trees attacked the previous year (green-attacked : red-attacked ratio): Wyoming. CI, confidence interval.

MPB aggregation within treated plots resulted in a significantly lower level of attack on whitebark pines at both sites (Figs 2 and 4). Collectively, these findings suggest that verbenone-releasing flakes can be useful for protecting stands in remote or steep terrain, either from the ground or from aircraft. Although we did not completely eliminate beetle-induced tree mortality with this approach, the technique clearly reduced beetle-caused mortality by over 50% compared with untreated stands, even with high background beetle populations.

A decade of research has largely confirmed the efficacy of verbenone pouch release devices (Gillette & Munson, 2009), which must be applied by hand from the ground level. Efficacy was especially good where beetle populations were not extremely high or stands were not overstocked (Progar, 2003, 2005; Borden *et al.*, 2006). For example, Bentz *et al.* (2005) found that deployment of verbenone pouches in lodgepole and whitebark pine stands significantly reduced the rate of attack by mountain pine beetle for up to three consecutive years, although they reported that some treated plots, particularly those with large emerging beetle populations, showed higher attack rates than controls. In these cases, higher concentrations of verbenone (Miller *et al.*, 1995) or the use of a combined 'push-pull' strategy employing baited traps (Cook *et al.*, 2007) may have been needed to overcome the effect of high levels of tree stress (i.e. drought and/or high beetle populations).

Treatments using other types of hand-applied anti-attractant pouches (Borden *et al.*, 2003) or attractant-baited traps in trap-out and concentration approaches (Borden *et al.*, 2006) may be promising for small, high-value stands, although these are too labour intensive to be used over large areas. Furthermore, beetle flight in many high-elevation whitebark stands begins when roads in many areas are impassable, making alternatives such as an aerially applied treatment highly desirable for achieving timely applications early in the season.

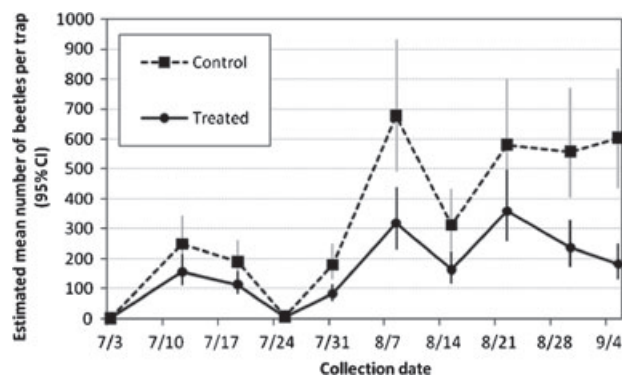
In our tests in Wyoming and Washington, a significantly smaller proportion of trees was attacked in stands treated with verbenone flakes than in control stands, with attack rates being reduced to less than half even with the relatively low application rate of 370 g/ha (for regression coefficients for current-year attack rates, see Table 2). For comparison, a verbenone risk assessment prepared at the request of the US Department

Table 2 Estimates of the regression coefficients for previous year attack rates and treatment comparisons, Wyoming, 2005 and Washington 2007

Site and year	Parameter	Estimate	Lower 95% CI	Upper 95% CI	Wald chi-square value	<i>p</i> -value
Wyoming 2005	2004 attack coefficient	1.76	1.17	2.65	7.30	0.007
	Ratio, control/treated	2.02	1.11	3.67	5.30	0.021
Washington 2007	2006 attack coefficient	1.11	1.01	1.23	4.66	0.031
	Ratio, control/treated	2.30	1.59	3.32	19.41	<0.0001

of Agriculture (Syracuse Environmental Research Associates, Inc., 2000) assumed application rates of 800–1200 g verbenone/ha using pouch release devices. It is clear, both intuitively and from our results (Figs 2 and 4), that higher beetle populations present a greater challenge, and it is therefore advisable to use higher application rates when beetle abundance is high. The flake formulation could be applied when beetle populations have erupted, although it could also be applied preemptively when stands are expected to be vulnerable because they are stressed by drought, thinning, fire or disease. Such an approach might be useful both with aerial application in larger landscapes and with ground applications employing fertilizer spreaders and/or paint-ball applicators in special-use sites such as seed collection areas, campgrounds and administrative sites. For example, ecologically important western white pine species such as limber pine (*Pinus flexilis*) and whitebark pine are currently threatened by both MPB and white pine blister rust (*Cronartium ribicola* J. C. Fischer) (Tomback *et al.*, 2001), and programmes are in place to collect seeds from disease-resistant individuals of these species (Schoettle & Sniezko, 2007). Disease-resistant seed sources are very important for the maintenance of these species in western forest ecosystems, although attacks by MPB have further compromised the survival of these pine species. A pheromone-based approach for protecting these valuable resources is therefore much desired. Additionally, we expect that larger treated areas might achieve better efficacy because of the smaller edge effect with larger pheromone-treated plots. Although questions have been raised regarding the possibility that treatments may simply herd beetles to adjacent areas, causing higher mortality outside of treated plots, we have never found this to be the case in the decade that we have been conducting this type of field testing (J. N. Webster, personal observations). This question does warrant further research, and plans are underway for a large scale test (using transects flanking treated plots) aiming to test the hypothesis that beetles repelled from treated stands do not concentrate at other locations outside the treated area. Although this type of application is somewhat costly, the use of aircraft results in rapid application that creates economies of scale because hundreds of hectares can easily be treated in a single morning.

Trends in climate change (Breshears *et al.*, 2005; Kurz *et al.*, 2008) and forest stand conditions (Hessburg *et al.*, 2000) suggest a continuing need for area-wide treatment for bark beetle management. Although we recognize that silvicultural prescriptions to minimize stand susceptibility to MPB are the most durable, long-term solution to this problem (Wood *et al.*, 1985; Amman *et al.*, 1998; Fettig *et al.*, 2007), the thinning of stands is time-consuming and is sometimes contraindicated by management objectives, especially on public lands. Insect pheromones, which can reach the target pest more effectively

**Figure 3** Mean number of mountain pine beetles trapped during 10 sampling periods (27 July to 15 September 2007), with 95% confidence interval (CI): Washington, 2007.

than contact insecticides, often have the further advantage of low toxicity toward nontarget organisms, including other insects, and especially natural enemy complexes (Erbilgin *et al.*, 2007; Gillette & Munson, 2009).

Aerial verbenone applications may prove useful for a rapid response to MPB outbreaks in periods after prolonged drought, wildfire and silvicultural treatments such as thinning when stands are temporarily vulnerable to beetle attack. They may also be useful for protecting old-growth pine stands that are susceptible to bark beetle attack but must be managed at higher than optimal basal areas to provide valuable habitat for endangered wildlife species. This approach requires the development and validation of species-appropriate pheromone blends (Fettig *et al.*, 2005) and so careful testing is required to establish efficacy for each beetle species and perhaps each beetle/host combination.

Nevertheless, the consequent research and development costs are likely to be offset by savings gained by reduced wildland fuels, enhanced resource values and a reduced need for the removal of hazardous, beetle-killed trees. Some past pheromone-based approaches have failed to protect pines at very high beetle populations (Bentz *et al.*, 2005; Progar, 2005), although, in the present study, reductions in tree mortality averaging 50% were achieved even with mortality in untreated plots in the range 5–25% (Figs 2 and 4). For example, background beetle abundance increased dramatically in Washington from 2006 to 2007, with tree mortality escalating in control plots from 2.7 to 29.2 trees/ha (Table 3), whereas in treated plots, the increase was far less, from 2.1 to 7.2 trees/ha (Fig. 4 and Table 3). Similarly, in Wyoming, the rate of tree mortality in untreated stands almost doubled from 2004 to 2005 (from 14.5 to 26.5 stems/ha) (Table 1) but remained constant

Table 3 Stand structure characteristics of whitebark pines (*Pinus albicaulis*) and associated trees, and pre- and post-treatment attack rates in treated and control plots, Washington, 2007

	Mean ± SE total basal area (m ² /ha)	Mean ± SE <i>Pinus albicaulis</i> basal area (m ² /ha)	Mean ± SE <i>Pinus albicaulis</i> DBH (cm) ^a	Mean ± SE <i>Pinus</i> stems/ha (all tree species)	Mean ± SE <i>Pinus albicaulis</i> stems/ha	Mean ± SE number <i>Pinus ponderosa</i> stems/ha	Mean ± SE <i>Pinus contorta</i> and <i>Pinus ponderosa</i> stems/ha	Mean ± SE trees/ha mass attacked, 2006	Mean ± SE trees/ha mass attacked, 2007 ^b	Mean ± SE basal area/ha mass attacked, 2007
Control	21.7 ± 4.5*	7.4 ± 1.9*	21.1 ± 1.0*	448.3 ± 129.5*	194.7 ± 52.7*	50.4 ± 25*	2.7 ± 2.7*	29.2 ± 11.5*	1.2 ± 0.5*	
Treated	15.0 ± 3.3*	4.7 ± 1.0*	23.0 ± 1.4*	255.9 ± 67.8*	89.5 ± 16.0†	36.6 ± 23*	2.1 ± 1.0*	7.0 ± 1.2†	0.6 ± 0.1*	
Ratio	1.45	1.57	0.92	1.75	2.18	1.38	1.29	4.17	2.00	

^aDBH, diameter at breast height. *Means followed by the same symbol within the same column are not significantly different at experiment-wise error rate $\alpha = 0.05$ (maximum likelihood ratio test).
^bThe comparison is tested for a model without the proportion of trees that were mass-attacked in the previous-year covariate.

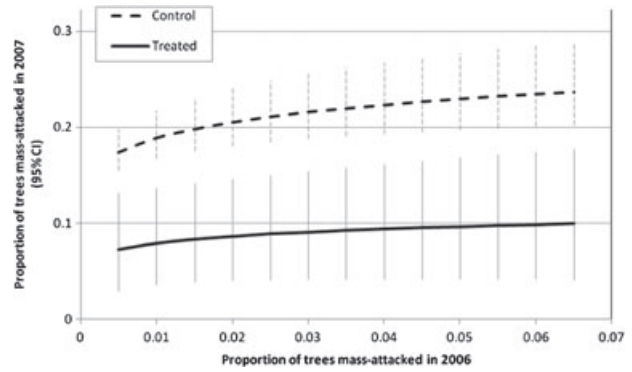


Figure 4 Proportion of whitebark pine (*Pinus albicaulis*) trees attacked in 2007 as a function of the proportion of trees attacked the previous year (green-attacked : red-attacked ratio); Washington. CI, confidence interval.

in the pheromone-treated stands (Fig. 2 and Table 1). We plan to also assess other semiochemicals such as nonhost volatiles that have been shown to be effective for other bark beetle species (Jakuš *et al.*, 2003; Zhang *et al.*, 2004; Fettig *et al.*, 2005; Schiebe *et al.*, 2011) for addition to verbenone, in the hope of achieving greater efficacy.

We strongly recommend that pheromone-based approaches be used as part of a broader integrated pest management strategy that includes the removal of infested trees, where feasible, and the use of silvicultural treatments to minimize competition (Fettig *et al.*, 2007). The demonstrated efficacy of verbenone-releasing flakes for MPB control offers hope for rapid, area-wide treatments in the face of explosive, widespread bark beetle outbreaks. These treatments should be applied before the outbreaks escalate dramatically because they are generally most effective at lower beetle populations (Carroll, 2007), although it is encouraging that we have shown repeated efficacy of this approach at multiple sites and with this and other MPB hosts (Gillette *et al.*, 2009a), even with high beetle populations. Future research should focus on ways of optimizing the efficacy of this pheromone-based technique by testing higher application rates and combining it with silvicultural methods, sanitation and other pest management approaches. Finally, the newly-developed biodegradable flake formulations should also be considered for testing, especially on public lands where nonbiodegradable formulations may be controversial.

Acknowledgements

These studies were funded by grants from the Forest Service Pesticide Impact Assessment Program, administered by USDA Forest Service, Forest Health Protection. We thank John D. Stein (retired, USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV) for helpful advice; the Bridger-Teton, Shoshone, and Wenatchee National Forests for access to plots; Darci Carlson, Roy Magelssen, Amanda St Ivany, Jamie Dinkins, Coleman Worthen and Calah Seese for assistance in the field; and Kristina Bischel for identifying and tallying insects in the Wood Laboratory, University

of California, Berkeley. Dan Miller (USDA Forest Service, Southern Research Station, Athens, GA) and Brice McPherson (University of California, Berkeley, CA) provided helpful reviews. The use of trade names and identification of firms or corporations does not constitute an official endorsement or approval by the U.S. Government of any product or service to the exclusion of others that may be suitable. Funding for this research was provided by the USDA Forest Service. The USDA is an equal opportunity provider and employer.

References

- Amman, G.D. & Logan, J.A. (1998) Silvicultural control of mountain pine beetle: prescriptions and the influence of microclimate. *American Entomologist*, **44**, 166–177.
- Bentz, B.J. & Schen-Langenheim, G. (2007) The mountain pine beetle and the whitebark pine waltz: has the music changed? *Whitebark Pine: A Pacific Coast Perspective*, R6-NR-FHP-2007-01, pp. 43–50. USDA Forest Service, Portland, Oregon.
- Bentz, B.J., Kegley, S., Gibson, K. & Their, R. (2005) A test of high-dose verbenone for stand-level protection of lodgepole and whitebark pine from mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) attacks. *Journal of Economic Entomology*, **98**, 1614–1624.
- Borden, J.H. (1997) Disruption of semiochemical-mediated aggregation in bark beetles. *Insect Pheromone Research: New Directions* (ed. by R. T. Cardé and A. K. Minks), pp. 421–438. Chapman & Hall, New York, New York.
- Borden, J.H., Chong, L.J., Earle, T.J. & Huber, D.P.W. (2003) Protection of lodgepole pine from attack by the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae) using high doses of verbenone in combination with nonhost bark volatiles. *The Forestry Chronicle*, **79**, 685–691.
- Borden, J.H., Birmingham, A.L. & Burleigh, J.S. (2006) Evaluation of the push–pull tactic against the mountain pine beetle using verbenone and non-host volatiles in combination with pheromone-baited trees. *The Forestry Chronicle*, **82**, 579–590.
- Borden, J.H., Pureswaran, D.S. & Lafontain, J.P. (2008) Synergistic blends of monoterpenes for aggregation pheromones of the mountain pine beetle (Coleoptera: Curculionidae). *Journal of Economic Entomology*, **101**, 1266–1275.
- Breshears, D.D., Cobb, N.S., Rich, P.M. et al. (2005) Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 15,144–15,148.
- Carroll, A.L. (2007) The mountain pine beetle, *Dendroctonus ponderosae*, in western North America: potential for area-wide integrated management. *Area-Wide Control of Insect Pests: From Research to Field Implementation* (ed. by M. J. B. Vreysen, A. S. Robinson and J. Hendrichs), pp. 297–307. Pacific Forestry Centre, Canadian Forest Service, Canada.
- Cook, S.M., Khan, Z.R. & Pickett, J.A. (2007) The use of push–pull strategies in integrated pest management. *Annual Review of Entomology*, **52**, 375–400.
- Erbilgin, N., Gillette, N.E., Mori, S.R., Stein, J.D., Owen, D.R. & Wood, D.L. (2007) Acetophenone as an anti-attractant for the western pine beetle, *Dendroctonus brevicomis* LeConte (Coleoptera: Scolytidae). *Journal of Chemical Ecology*, **33**, 817–823.
- Federal Register (2011) Proposed Rules, Tuesday, July 19, 2011. Federal Register **76**, 42631. Available online at: <https://www.federalregister.gov/articles/2011/07/19/2011-17943/endangered-and-threatened-wildlife-and-plants-12-month-finding-on-a-petition-to-list-pinus>. Federal Register/Vol. 76, No. 138/Tuesday, 19 July 2011/Proposed Rules **42631**.
- Fettig, C.J., McKelvey, S.R. & Huber, D.P.W. (2005) Nonhost angiosperm volatiles and verbenone disrupt response of western pine beetle, *Dendroctonus brevicomis* (Coleoptera: Scolytidae), to attractant-baited traps. *Journal of Economic Entomology*, **98**, 2041–2048.
- Fettig, C.J., Klepzig, K.D., Billings, R.F., Munson, A.S., Nebeker, T.E., Negron, J.F. & Nowak, J.T. (2007) The effectiveness of vegetation management practices for prevention and control of bark beetle infestations in coniferous forests of the western and southern United States. *Forest Ecology and Management*, **238**, 24–53.
- Furniss, R.L. & Carolin, V.M. (1977) *Western Forest Insects*. USDA Forest Service Miscellaneous Publication 273. USDA Forest Service, Washington, District of Columbia.
- Gibson, K. (2006) *Mountain Pine Beetle Conditions in Whitebark Pine Stands in the Greater Yellowstone Ecosystem, 2006*. Forest Health Protection Report Numbered Report 06-03. Northern Region, USDA Forest Service, Missoula, Montana.
- Gillette, N.E. & Munson, A.S. (2009) Semiochemical sabotage: behavioral chemicals for protection of Western conifers from bark beetles. *The Western Bark Beetle Research Group: A Unique Collaboration with Forest Health Protection – Proceedings of a Symposium at the 2007 Society of American Foresters Conference* (ed. by J. L. Hayes and J. E. Lundquist Compilers), pp. 85–109, 134 p. U.S. Department of Agriculture, Forest Service, Pacific, Northwest Research Station, Portland, Oregon. General Technical Report PNW-GTR-784.
- Gillette, N.E., Stein, J.D., Owen, D.R., Webster, J.N., Fiddler, G.O., Mori, S.R. & Wood, D.L. (2006) Verbenone-releasing flakes protect individual *Pinus contorta* trees from attack by *Dendroctonus ponderosae* and *Dendroctonus valens* (Coleoptera: Curculionidae, Scolytinae). *Agricultural and Forest Entomology*, **8**, 243–251.
- Gillette, N.E., Erbilgin, N., Webster, J.N. et al. (2009a) Aerially applied verbenone-releasing laminated flakes protect *Pinus contorta* stands from attack by *Dendroctonus ponderosae* in California and Idaho. *Forest Ecology and Management*, **257**, 1405–1412.
- Gillette, N.E., Mehmehl, C.J., Webster, J.N., Mori, S.R., Erbilgin, N., Wood, D.L. & Stein, J.D. (2009b) Aerially applied methylcyclohexenone-releasing laminated flakes protect *Pseudotsuga menziesii* stands from attack by *Dendroctonus pseudotsugae*. *Forest Ecology and Management*, **257**, 1231–1236.
- Hessburg, P.F., Smith, B.G., Slater, R.B., Ottman, R.D. & Alvarado, E. (2000) Recent changes (1930s–1990s) in spatial patterns of interior northwest forests, USA. *Forest Ecology and Management*, **136**, 53–83.
- Holsten, E.H., Webb, W., Shea, P.J. & Werner, W.A. (2000) *Release Rates of Methylcyclohexenone and Verbenone from Bubblecap and Bead Releasers Under Field Conditions Suitable for the Management of Bark Beetles in California, Oregon, and Alaska*. USDA Forest Service Research Paper PNW-RP-544. USDA Forest Service, Portland, Oregon.
- Jakuš, R., Schlyter, F., Zhang, Q.-H. et al. (2003) Overview of development of anti-attractant based technology for spruce protection against *Ips typographus*: from past failures to future success. *Journal of Pest Science*, **76**, 89–99.
- Keane, R.E. (2001) Can the fire-dependent whitebark pine be saved? *Fire Management Today*, **61**, 17–20.
- Kegley, S. & Gibson, K. (2004) *Protecting Whitebark Pine Trees from Mountain Pine Beetle Attack Using Verbenone*. Forest Health Protection Report No FHP 04–8. Northern Region, USDA Forest Service, Missoula, Montana.
- Kegley, S. & Gibson, K. (2009) *Individual–Tree Tests of Verbenone and Green-Leaf Volatiles to Protect Lodgepole, Whitebark and Ponderosa Pines, 2004–2007*. Forest Health Protection Numbered Report 09-03. USDA Forest Service, Forest Health Protection, Missoula, Montana.

- Kegley, S., Gibson, K., Schwandt, J. & Marsden, M. (2003) *A Test of Verbenone to Protect Individual Whitebark Pine from Mountain Pine Beetle Attack*. USDA Forest Service, Forest Health Protection Report No FHP 03–9. Northern Region, USDA Forest Service, Missoula, Montana.
- Krist, F.J. Jr, Sapio, F.J. & Tkacz, B.M. (2007) *Mapping Risk from Forest Insects and Diseases*. USDA Forest Service, Forest Health Protection, Fort Collins, Colorado.
- Kurz, W.A., Stinson, G., Rampley, G.J., Dymond, C.C. & Neilson, E.T. (2008) Risk of natural disturbance makes future contribution of Canada's forests to the global carbon cycle highly uncertain. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 1551–1555.
- Logan, J.A. & Powell, J.A. (2001) Ghost forests, global warming, and the mountain pine beetle (Coleoptera: Scolytidae). *American Entomologist*, **47**, 160–173.
- McCulloch, C.E. & Searle, S.R. (2001) *Generalized, Linear, and Mixed Models*. John Wiley & Sons, Inc, New York, New York.
- McGregor, M.D., Furniss, M.M., Oaks, R.D., Gibson, K.E. & Meyer, H.E. (1984) MCH pheromone for preventing Douglas-fir beetle infestation in windthrown trees. *Journal of Forestry*, **82**, 613–616.
- Miller, D.R., Borden, J.H. & Lindgren, B.S. (1995) Verbenone: dose-dependent interruption of pheromone-based attraction of three sympatric species of pine bark beetles (Coleoptera: Scolytidae). *Environmental Entomology*, **24**, 692–696.
- Perkins, D.L. & Roberts, D.W. (2003) Predictive models of whitebark pine mortality from mountain pine beetle. *Forest Ecology and Management*, **174**, 495–510.
- Progar, R.A. (2003) Verbenone reduces mountain pine beetle attack in lodgepole pine. *Western Journal of Applied Forestry*, **18**, 229–232.
- Progar, R.A. (2005) Five-year operational trial of verbenone to deter mountain pine beetle (*Dendroctonus ponderosae*; Coleoptera: Scolytidae) attack of lodgepole pine (*Pinus contorta*). *Environmental Entomology*, **34**, 1402–1407.
- Rappaport, N.G., Stein, J.D., del Rio Mora, A.A., de Groot, P., DeBarr, G. & Mori, S. (2000) Responses of *Conophthorus* spp. (Coleoptera: Scolytidae) to behavioral chemicals in field trials: a transcontinental perspective. *Canadian Entomologist*, **132**, 925–937.
- Schiebe, C., Blaženec, M., Jakuš, R., Unelius, C.R. & Schlyter, F. (2011) Semiochemical diversity diverts bark beetle attacks from Norway spruce edges. *Journal of Applied Entomology*, **135**, 726–737.
- Schoettle, A.W. & Sniezko, R.A. (2007) Proactive intervention to sustain high-elevation pine ecosystems threatened by white pine blister rust. *Journal of Forest Research*, **12**, 3327–3336.
- Sharov, A.A., Leonard, D., Liebhold, A.M., Roberts, E.A. & Dickerson, W. (2002) 'Slow the spread': a national program to contain the gypsy moth. *Journal of Forestry*, **100**, 30–35.
- Shea, P.J., McGregor, M.D.G.E. & Daterman, G.E. (1992) Aerial application of verbenone reduces attack of lodgepole pine by mountain pine beetle. *Canadian Journal of Forest Research*, **22**, 436–441.
- Smith, R.H. (2000) *Xylem Monoterpenes of Pines: Distribution, Variation, Genetics, Function*. General Technical Report PSW CTR-177. Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture, Albany, California.
- Syracuse Environmental Research Associates, Inc. (2000) *Verbenone Human Health and Ecological Risk Assessment*. Final Report submitted to Animal and Plant Health Inspection Service (APHIS) Biotechnology, Biologics and Environmental Protection Environmental Analysis and Documentation. United States Department of Agriculture, Riverdale, Maryland.
- Tomback, D.F., Arno, S.F. & Keane, R.E. (2001) *Whitebark Pine Communities, Ecology and Restoration*. Island Press, Washington, District of Columbia.
- Wood, D.L., Stark, R.W., Waters, W.W., Bedard, W.D. & Cobb Jr., F.W. (1985) Treatment tactics and strategies. *Integrated Pest Management in Pine-Bark Beetle Ecosystems* (ed. by W.W. Waters, R.W. Stark and D.L. Wood), pp. 121–140. John Wiley and Sons Inc., New York, New York.
- Wood, D.L., Koerber, T.W., Scharpf, R.F. & Storer, A.J. (2003) *Pests of the Native California Conifers*. University of California Press, Berkeley, California.
- Wulder, M., White, J., Coops, N. & Butson, C. (2008) Multi-temporal analysis of high spatial resolution imagery for disturbance monitoring. *Remote Sensing of Environment*, **112**, 2729–2740.
- Zhang, Q.-H. & Schlyter, F. (2004) Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles. *Agricultural and Forest Entomology*, **6**, 1–19.

Accepted 25 February 2012

First published online 11 June 2012