Baited pheromone traps track changes in populations of western blackheaded budworm (Lepidoptera: Tortricidae)

V.G. Nealis, P. Silk, R. Turnquist, J. Wu

Abstract—Pheromone lures for eastern and western blackheaded budworms, *Acleris variana* (Fernald) and *A. gloverana* (Walsingham), were synthesized and deployed in traps at locations with decreasing and increasing populations of western blackheaded budworms in British Columbia, Canada. Traps baited with these lures caught comparable numbers of moths at all sites tested in each year. The lures were sensitive to changes in density of budworm populations below observable damage levels, and numbers of moths in traps were strongly correlated with independent estimates of egg densities in the same year. The results confirm the qualitative similarity of the sex pheromones in eastern and western species of blackheaded budworm and demonstrate their utility as a tool for monitoring population trends, including increases in populations to damaging levels.

Résumé—Deux appâts à base de phéromone pour les tordeuses à tête noire de l'épinette de l'est et de l'ouest, *Acleris variana* (Fernald) et *A. gloverana* (Walsingham), ont été préparés et déployés en Colombie-Britannique au Canada, dans des pièges situés au sein de sites positifs où les populations de tordeuses sont soit en baisse, soit en augmentation. Le taux de capture des papillons nocturnes avec ces appâts à chaque site est comparable à ceux des sites testés auparavant. Les appâts ont démontré une sensibilité envers les changements en densité de population de tordeuses inférieure au seuil de détection visuelle de niveaux de dommage, ainsi qu'une forte corrélation entre le taux de capture des pièges et l'estimation indépendante de densité des œufs, à l'intérieur de la même année. Les résultats confirment la similarité qualitative des phéromones sexuelles des tordeuses à tête noire de l'épinette de l'est et de l'ouest, et démontrent leur utilisation comme outil de surveillance des tendances des populations, même jusqu'aux niveaux épidémiques.

Introduction

The western blackheaded budworm, *Acleris gloverana* (Walsingham) (Lepidoptera: Tortricidae), is a native defoliator of conifers in western North America. Periodic outbreaks occur at approximately 12- to 15-year intervals, usually in association with western hemlock, *Tsuga heterophylla* (Raf.) Sarg. (Pinaceae), in the coastal forests of British Columbia (BC), Canada,—on Vancouver Island (Shepherd and Gray 2001), Haida Gwaii (Queen Charlotte Islands), and the adjacent mainland (Garbutt 1992)—and in southeastern Alaska, United States of America (Mask 1992). These outbreaks quickly reach damaging levels but persist only for a year or two in any one location. Less extensive outbreaks are occasionally recorded from interior forests of BC (Unger 1992). The most recent outbreak in coastal BC occurred in the late 1990s and ended in 2001 (Nealis *et al.* 2004).

The western blackheaded budworm was considered conspecific with the eastern blackheaded budworm, *Acleris variana* (Fernald), until Powell (1962) proposed distinct species status. It was assumed that the two species were geographically separated approximately at the continental

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divide, although there has been no systematic work on populations in the Canadian Prairies or the BC interior to confirm boundaries or areas of possible sympatry. The type specimens of eastern and western blackheaded budworms are from the extreme eastern and western portions of their presumed respective ranges (Powell 1962).

Sex pheromones are widely utilized as bait in traps to assess levels of insect populations and may also provide characteristics to distinguish species (Silk and Kuenen 1988). Gries *et al.* (1994) synthesized and described (*E*)-11,13-tetradecadienal (*E*11,13−14:Ald), the major component of the female sex pheromone in *A. variana*. Lures with concentrations between 10 and 100 μg caught the maximum number of male moths. This diene aldehyde was also found to be the major sex-pheromone component for *A. gloverana*, but a concentration of 1000 μg per lure and the addition of 5% (*Z*)-11,13-tetradecadienal (*Z*11,13−14:Ald) as a minor component were required to maximize the catch in western Canada (Gray *et al.* 1996). Little subsequent work has been reported on the relative performance of these lures in the field or the correlation of their catches with other measures of blackheaded budworm populations.

This paper reports a comparison of the two formulations of lures in tracking trends in blackheaded budworm populations in coastal and interior forests of BC during a period of nil to evident defoliation. The relationship between rates of male moth captures in baited pheromone traps and egg densities is also examined to correlate methods of assessing population density useful in predicting future population levels.

Methods

Production of pheromone lures

Adult *A. variana* were reared from collections of larvae in Cape Breton, Nova Scotia, Canada. Gas chromatography (GC)/electroantennography (EAD) of excised female sex pheromone glands using male antennae followed by GC/mass spectrometry (MS) identified *E*11,13−14:Ald as the female sex pheromone by comparing retention time and electron-ionization (EI) mass spectra with those for authentic synthetic material. Moths used in the analyses were 2−6 days post eclosion, and EAD analyses were done 2−3 h into the scotophase of a 16L:8D photoperiod. EAD responses were seen at a single GC peak, and the antennae of male *A. variana* confirmed that *E*11,13−14:Ald was the only EAD-active component in the sex-pheromone glands of female *A. variana*.

Small quantities (400–500 mg) of the primary sex-pheromone component were synthesized using the method of Nesbitt *et al.* (1973) with refinements by Yamada *et al.* (1983), using alkylation of 3-sulfolene as the key step. We introduced further refinements of the synthetic route. Briefly, the hydroxy function of 10-bromodecanol (1) was protected by tetrahydropyranylation using niobium (V) chloride as the Lewis acid of choice and dihydropyran in dichloromethane giving a $>$ 95% yield of protected product. Alkylation of the sulfolene anion (produced by base treatment with hexamethyldisilazide in tetrahydrofuran/hexamethylphosphoramide) with the protected electrophile was achieved at low yield (approximately 10%). This product was subsequently thermally desulfonylated in a closed vessel with sodium bicarbonate to give (*E*)-1-(2-tetrahydropyanoxy)-11.13-tetradecadiene (*E*11,13−14:OTHP) and 5% (*Z*)-1-(2-tetrahydropyanoxy)-11,13-tetradecadiene (*Z*11,13−14:OTHP), where it was subsequently deprotected with pyridinium *p*-toluene sulfonate to give the primary alcohol. Oxidation to the aldehyde was achieved at fair yield (approximately 50%) with pyridinium chlorochromate to give the desired product by GC-MS of 95% *E*11,13−14:Ald and 5% *Z*11, 13−14:Ald, which was then purified on silica gel ($>$ 98%) and stored in the dark in hexane to slow decomposition (oxidation and polymerization). The EI-mass spectrum of the product matched published spectra (Gries *et al.* 1994; El-Sayed 2010). The material was then stored frozen and preserved with butylated hydroxyl toluene (BHT) with a 1% solution in hexane for production of trap baits. All lures contained a 95:5 (*E*):(*Z*) mixture of the geometric isomers of 11,13-tetradecadienal.

Red rubber septa were used as the optimal trap-bait releaser because this method is

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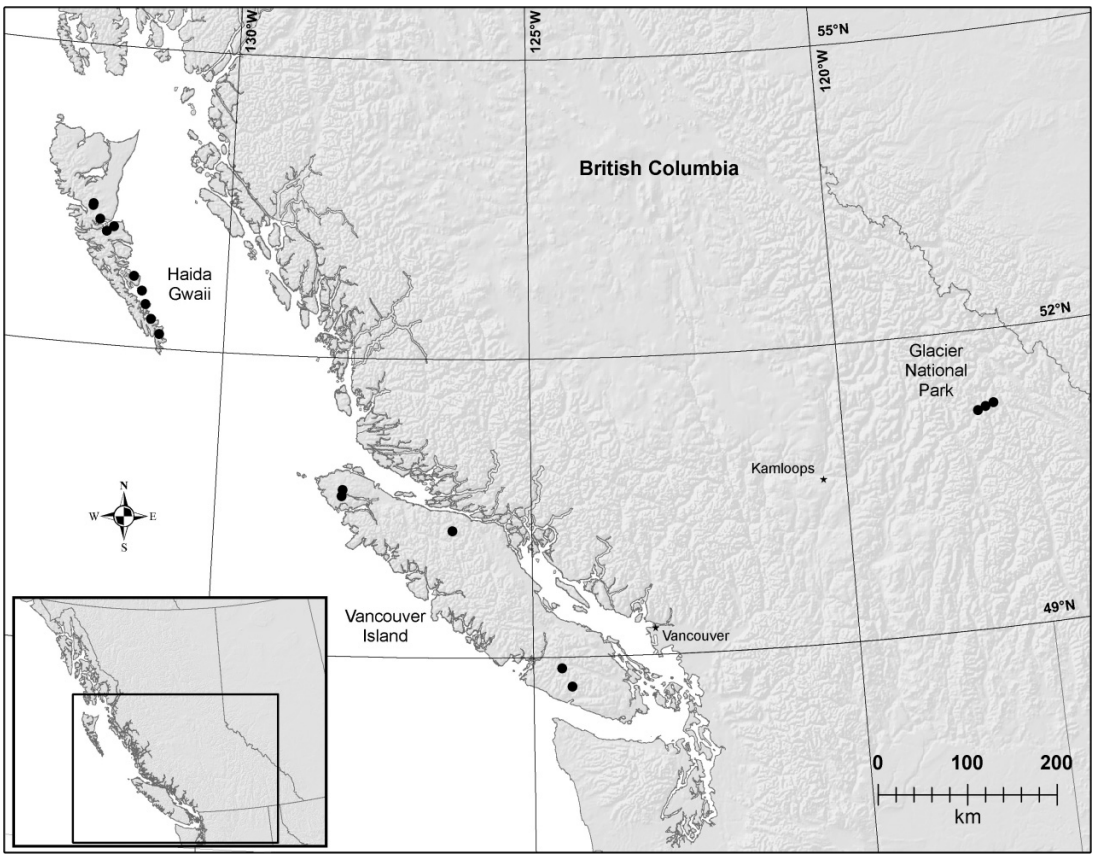
Fig. 1. Locations of pheromone traps and egg sampling for western blackheaded budworm, *Acleris gloverana*, in three regions of British Columbia.



reliable for aldehydes (*e.g.*, those of other Tortricidae, such as species of *Choristoneura* Lederer (Silk *et al.* 1980) and *Croesia semipurpurana* (Kearfott) (Silk *et al.* 1997)). These materials are subject to oxidation/polymerization, so BHT was used as the antioxidant to stabilize the active ingredient.

Deployment of baited pheromone traps

Rubber septa loaded with pheromone (and BHT) were attached with a straight pin to the apex of a triangular trap constructed from a 2 L waxed-cardboard drink container that was open at both ends. The three inside surfaces of the trap measured approximately 850 cm^2 and were coated with a thin layer of Tanglefoot®. Traps were hung from western hemlock branches approximately 2 m above the ground. In 2005, five traps baited with the 10 μg *A. variana* lure plus one blank trap were deployed at 25 m intervals along either a straight or U-shaped transect at each trapping location (Fig. 1). The blank trap was placed at a random location along that transect. The concentration of the *A. variana* lure was increased to 100 μg in 2006–2008; this concentration would be expected to catch approximately three times as many moths as the 10 μg lure used in 2005 (Gray *et al.* 1996). Four traps containing the 100 μg *A. variana* lure and four traps containing a 1000 μg *A. gloverana* lure plus two blank traps were deployed at each site, with the lure types alternating along the transect and blank traps positioned randomly in the series. In 2009, four 1000 μg *A. gloverana* lures only and a single blank were deployed at each site.

Each year, traps were deployed in early August and collected in mid to late October.

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All trap contents were identified and counted. Our previous experience suggested that for *A. gloverana*, these sticky traps are saturated at approximately 350 moths per trap, so exact counts greater than that may have a downward bias.

Analysis was carried out at the site level. To account for the occasional lost trap, the mean number of male moths per baited trap at each site in each year was the measure used for analysis. The *A. gloverana* and *A. variana* lure types were compared in 2006, 2007, and 2008 using a paired *t* test with the null hypothesis of no difference between the numbers of males captured in traps baited with the two lure types. Moth counts were transformed to common logarithms ($\log_{10}$) before analysis and normality of residuals was tested with the Anderson−Darling (A–D) statistic. The calculated $\log_{10}$ mean difference was back-transformed in the results. A summary of temporal trends was produced by pooling sites within three broad and widely separated regions: Haida Gwaii and Vancouver Island (coastal) and Glacier National Park (interior) (Fig. 1).

Egg sampling

Blackheaded budworm moths lay eggs singly on the underside of host foliage in the late summer and autumn (Shepherd and Gray 1990*b*). These eggs overwinter and hatch in the spring. At the time of trap retrieval in the autumn, we removed two 45 cm long branch tips from the midcrown (2−4 m above the ground) of each of 10 trees per site, using extendable pole pruners. Branches were bagged and shipped to the laboratory, where their fresh mass was recorded. Eggs were extracted by washing the foliage in boiling water, followed by vacuum-extracting the effluent to filter paper (Condrashoff 1967). A comparison of this method with direct visual examination of the foliage by experienced survey technicians confirmed the superiority of the extraction method. Egg densities are expressed as the number of eggs per kilogram of fresh foliage to decrease known sample bias caused by the uneven distribution of foliage in the tree crown (Shepherd and Gray 1990*b*). The estimate of egg density was compared with the corresponding density of moths caught in traps baited with the *A. gloverana* lure. Additional data relating egg densities to moth catches from Table 1 of Koot (1997)[2] were included to increase the sample size. For regression of egg density on moth density for all sites following $\log_{10}$-transformation of both variables, Minitab® was employed. Normality of residuals was tested by the A–D statistic.

Results

Only 23 male *A. gloverana* were caught in 136 blank traps deployed between 2006 and 2009, 19 of them in Haida Gwaii in 2009 when blackheaded budworm populations surged to defoliating levels. Thus, there appears to be no attraction of moths to the traps themselves and catches in baited traps can be assumed to be a function of the relative attractiveness of the pheromone lure and local population density of blackheaded budworm.

There was no overall difference in the size of trap catches between the 100 μg *A. variana* and 1000 μg *A. gloverana* lures (difference = 1.05 (1.07) moths per trap (mean, with SEM in parentheses); $t = -0.74$, $P = 0.46$; A–D = 0.17, $P = 0.92$; $n = 54$) in 2006−2008 (Fig. 2). Either lure could be used, since the two lures trapped *A. gloverana* with equal efficacy.

Pheromone-trap catches appear to be responsive indicators of population trends, even at the relatively low population densities in our study (Fig. 2). In Glacier National Park, blackheaded budworm populations were high enough to cause visible defoliation in 2004 (900 ha) and 2005 (2350 ha) (Roke and Unger 2005), but subsequently decreased (Roke and Shand 2006) and remained low until the end of our study. This pattern is reflected in the size of the pheromone-trap captures in that region (Fig. 2A). Note that the 10 μg *A. variana* lure was used in 2005. According to Figure 5 of Gray *et al.* (1996), the 100 μg lure should catch about three times as many moths per trap as the 10 μg lure. It

[2] P. Koot. 1997. Forest Health Network Pest Report on Special Projects, Queen Charlotte Islands, 1997. Unpublished report prepared for the South Moresby Forest Replacement Account, Canadian Forest Service, Natural Resources Canada,Victoria, British Columbia.

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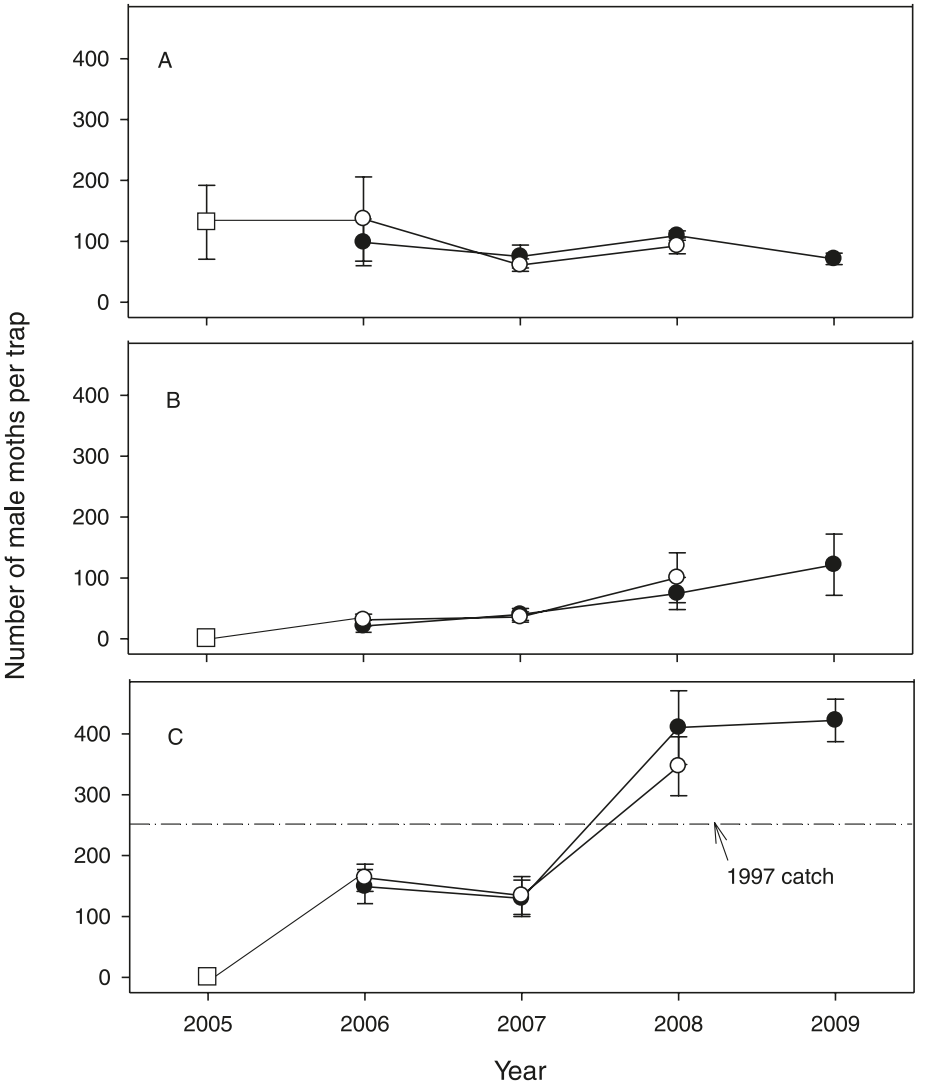
Fig. 2. Numbers (mean ± SE) of male western blackheaded budworm, *Acleris gloverana*, caught in pheromone traps baited with 10 µg *A. variana* (□, 2005 only), 100 µg *A. variana* (○, 2006−2008), or 1000 µg *A. gloverana* (●, 2006-2009) lures at several sites in three regions of British Columbia: Glacier National Park (interior) (A), Vancouver Island (B), and Haida Gwaii (C; the broken line is based on the mean catch in defoliated areas reported in Koot (1997))[2].



follows that if the 100 µg lure used in 2006−2009 had been used in 2005, we would have expected three times as many moths (3 × 131.3 = 393.9) per trap in that year, when defoliation was observed in the region. By comparison, coastal blackheaded budworm populations were expected to be increasing because the last outbreak subsided in 2001, and this was also reflected in the pheromone-trapping results. On Vancouver Island, the size of trap catches indicated a slowly increasing population that was not yet large enough to produce observable defoliation (Fig. 2B), and no defoliation was observed when traps were retrieved in any year. Trap catches increased more rapidly on northern Vancouver Island than on southern Vancouver Island (2009 catches: 173.5 (36.8) *vs.* 35.7 (9.4)

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(mean, with SEM in parentheses), respectively). On Haida Gwaii, however, populations increased from very low to more than 400 insects per trap in 2009 (Fig. 2C), which is greater than the average number of moths per trap observed in 1997 (Fig. 2C) (Koot 1997[2]), the year before substantial defoliation was observed in the region (Nealis *et al.* 2004). In 2009, nearly 14 000 ha of defoliated trees were observed on Haida Gwaii for the first time since 2001 (British Columbia Ministry of Forests and Range 2010). Once again, note that in 2005 the 10 μg *A. variana* lure was used and so blackheaded budworm population densities were probably not actually zero, but were certainly much lower than in subsequent years.

There was a positive relationship between the mean number of eggs per kilogram of fresh foliage (E) and the mean number of moths caught in traps (M) baited with the 1000 μg *A. gloverana* lure in the same year (Fig. 3).

$$E = -2.92(0.94) + 2.21(0.44)M \; (F_{1,17} = 24.7, P = 0.001, R^2 = 0.57; \text{A–D} = 0.21, P = 0.83)$$

Note that the standard errors of the coefficients are relatively large and, on the logarithmic scale, would result in broad confidence intervals around the predicted mean. Nonetheless, given that the range of egg densities sampled in this study resulted in nil to light defoliation at most in the following year (Shepherd and Gray 1990*a*; Koot 1997[2]), the value of this clear relationship between moth densities and egg densities is greatest when it is needed most, *i.e.*, as insect populations are increasing from endemic to damaging levels. Note that the values shown in Figure 3 are all within the confidence intervals for the regression when more than 150 moths per trap were caught. This is the range at which prediction is most critical: these catch sizes indicate a population density approaching that at which visible defoliation may be imminent and egg sampling should be introduced. There are no other sampling methods efficient enough to detect this change. Where population densities are high enough that traps may become saturated, as was becoming the case in some trap locations on Haida Gwaii in 2009 (Fig. 2C), shifting to a nonsaturating trap design might be desirable. However, the system used here is inexpensive and easy to deploy and has the predictive capacity to track otherwise undetectable increases in populations when local population density is below the observable damage threshold. When moth densities exceed approximately 250 per trap and (or) egg densities exceed 200 per kilogram of foliage (approximately 5 eggs per 45 cm long branch tip), the high likelihood of an impending outbreak and the need for management decisions are clear. Maintenance of a network of nonsaturating pheromone traps during the relatively brief outbreak phase of this insect may not result in more refined predictive capacity that is worth the initial or ongoing investment.

Conclusion

There was no difference in attractiveness between pheromone traps baited with the 100 μg *A. variana* or 1000 μg *A. gloverana* lures to western blackheaded budworms over a large area, and a change in population density of at least two orders of magnitude. Similar results were obtained when either lure was used to trap eastern blackheaded budworms in eastern Canada (P. Silk and G. Thurston, unpublished data). The practical implication of these results is that it might be possible to use either lure to monitor western blackheaded budworm populations, although it would be prudent to continue with the specific lure until more operational testing is completed.

These results also confirm that the *A. gloverana–A. variana* complex (Powell 1962) has few obvious isolating mechanisms, including pheromone specificity, to support the separation of the eastern and western species. A single transcontinental species with longitudinal clines in host preferences and limited morphological variation may be an equally viable taxonomic view. The type specimens of *A. gloverana* and *A. variana* were collected from the extreme margins of this transcontinental range, and little comparative work has been done on any characters from populations in between (Powell 1962). Geographic variation in pheromone response has been described recently for another well-known forest insect with a transcontinental range (Grant *et al.* 2009). Similar ambiguity in transcontinental populations of *Pissodes strobi*

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Fig. 3. Relationship between the mean number of western blackheaded budworm, *Acleris gloverana*, eggs per kilogram of fresh foliage (E) and the mean number of moths caught per trap baited with the *A. gloverana* lure (M): $E = -2.92 + 2.21M$ ($F_{1,17} = 24.7$, $P < 0.001$, $R^2 = 0.57$), including 95% confidence intervals for the regression. Each data point represents egg and moth densities at one site in one year.

(Peck) (Coleoptera: Curculionidae), which, like blackheaded budworms, varies in its host preferences from east to west, was resolved by genetic analysis (Langor and Sperling 1995) and this approach may be required in order to shed further light on the taxonomy of the blackheaded budworm complex.

In conclusion, baited pheromone traps are a useful operational tool to efficiently track changes in density of blackheaded budworm populations in the field when population levels are very low but increasing to outbreak levels. Approximate correlations between trap catch and defoliation level are now available, and the correlation between moth catch and subsequent egg density is very strong. The importance of this tool is underscored by the nature of blackheaded budworm outbreaks, which reach damaging levels quickly, cause severe defoliation for only a year or two, and subside equally quickly (Nealis *et al.* 2004). Pheromone monitoring is ideally suited for such fast-cycling defoliator populations, as it provides advance information on impending outbreaks and allows the implementation of management options that might reduce damage (Shepherd 1994). For example, thinning juvenile stands of coastal hemlock increases their susceptibility to damaging defoliation (Nealis *et al.* 2004), and timing these silvicultural prescriptions relative to the likelihood of a new outbreak as revealed by pheromone-trap captures would be one way of integrating forest practices with pest risk reduction. Similarly, when forest communities have advance notice of outbreaks, protection programs can be implemented more effectively and allow sufficient time for consultation and planning.

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