Potential for combining sex pheromones for the forest tent caterpillar (Lepidoptera: Lasiocampidae) and the large aspen tortrix (Lepidoptera: Tortricidae) within monitoring traps targeting both species

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Trembling aspen, *Populus tremuloides* Michx. (Salicaceae), is the most widely distributed tree species in North America (Perala 1990) and is considered to be an ecologically (Hogg *et al.* 2002) and economically important (Brandt *et al.* 2003) component of the boreal forest. Due to the recently increased economic value of trembling aspen (Brandt *et al.* 2003), the impact of native insect defoliators on tree growth and mortality has become commercially important. Two of the most significant defoliators of trembling aspen throughout its range in Canada are the forest tent caterpillar (FTC), *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), and the large aspen tortrix (LAT), *Choristoneura conflictana* Walker (Lepidoptera: Tortricidae).

Severe defoliation of trembling aspen by FTC in several successive years causes reduced radial growth and branch mortality (Ives and Wong 1988) that can predispose trees to secondary fungal and insect infestation (Hogg *et al.* 2002). Prolonged FTC defoliation is associated with higher levels of weakened, dead, or declining trees (Brandt *et al.* 2003). Feeding damage by LAT larvae is less severe but can reduce trembling aspen vigor and radial growth and cause some crown dieback (Cerezke 1992). Interestingly, outbreak populations of LAT often precede (Ives and Wong 1988) or coincide with (Cerezke 1992) FTC infestations, suggesting that stand suitability may be similar for these two native defoliators of trembling aspen.

The commercial importance of these defoliators warrants the development of sensitive monitoring tools that can determine their spatial and temporal occurrence and predict incipient outbreaks. Sex pheromone baited traps can assist researchers in sampling endemic populations (Schmidt *et al.* 2003) and help to determine the nature of any interaction between these two sympatric species at the stand level. Overlapping distributions of FTC and LAT on their preferred host in Canada provide the opportunity to combine monitoring efforts for both species. Sampling of immature stages as an index of population density has been developed for FTC (Shepherd and Brown 1971; Witter *et al.* 1972; Batzer *et al.* 1995) and, to a lesser extent, for LAT (Henson 1954; Prentice 1955). However, different seasonal phenologies of the immature stages of the two species, as well as different sampling techniques employed for each species, preclude the development of a combined monitoring program targeting immature stages of both species.

Adult LAT moths are active in June and July (Prentice 1955), and the peak activity of FTC moths occurs in July and August (Schmidt and Roland 2003). Due to the overlapping flight activity of these species, it may be possible to develop a monitoring technique based on combined sex pheromones that targets male moths. Various pheromone blends at different doses were recently tested for their attractiveness to FTC at outbreak and non-outbreak population densities (Schmidt *et al.* 2003). A three-component sex pheromone blend released from polyurethane lures captured male FTC in large-volume traps throughout their flight period (Schmidt and Roland 2003). The optimum blend for trapping populations in western Canada consisted of a 100:1:10 ratio of (*Z*,*E*)-5,7-dodecadienal (*Z*5*E*7-12Al), (*Z*,*Z*)-5,7-dodecadienal (*Z*5*Z*7-12Al), and (*Z*)-7-dodecanal (*Z*7-12Al) (Schmidt *et al.* 2003). Sex pheromone baited traps have also been tested for their attractiveness to male LAT. In field trapping studies, (*Z*)-11-tetradecenal (*Z*11-14Al) was shown to be attractive to male LAT throughout its range (Weatherston *et al.* 1978)

Received 24 February 2005. Accepted 23 August 2005.

Table 1. Semiochemical blends used in a pheromone trapping experiment targeting both the forest tent caterpillar (*Malacosoma disstria*; FTC) and the large aspen tortrix (*Choristoneura conflictana*; LAT).

Treatment	Species targeted	Semiochemicals	Quantity in lure (µg)
1	Both	Solvent control	
2	FTC	(*Z*,*E*)-5,7-dodecadienal	100
		(*Z*,*Z*)-5,7-dodecadienal	4
3	Both	(*Z*,*E*)-5,7-dodecadienal	100
		(*Z*,*Z*)-5,7-dodecadienal	4
		(*Z*)-11-tetradecenal	100
4	FTC	(*Z*,*E*)-5,7-dodecadienal	100
		(*Z*,*Z*)-5,7-dodecadienal	4
		(*Z*)-7-dodecanal	10
5	Both	(*Z*,*E*)-5,7-dodecadienal	100
		(*Z*,*Z*)-5,7-dodecadienal	4
		(*Z*)-7-dodecanal	10
		(*Z*)-11-tetradecenal	100
6	LAT	(*Z*)-11-tetradecenal	100

and hypothesized to be the main sex pheromone component for this species (Werner and Weatherston 1980). In subsequent studies, *Z*11-14Al has been demonstrated to be the major pheromone component in female gland extracts and effluvia and is attractive to male LAT throughout their flight period (M.L. Evenden *et al.*, unpublished data).

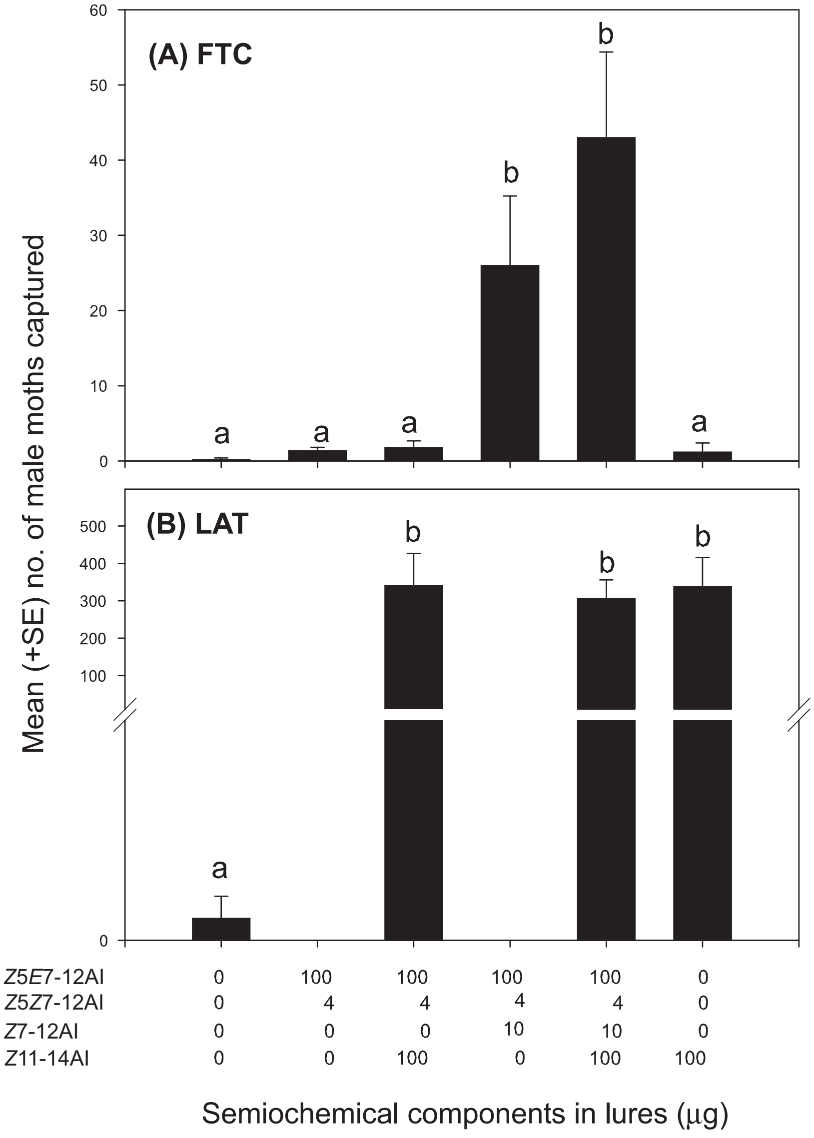
Distantly related moths that do not share similar pheromone components and that can be easily distinguished by the untrained eye are ideal candidates for the development of a combined sex pheromone monitoring system. Here I take the first step in testing this novel approach by combining sex pheromones for FTC and LAT in a single formulation in an attempt to attract male moths of both of these important defoliators of trembling aspen.

Five field sites were established in stands dominated by trembling aspen in the Rocky Mountain foothills of western Alberta between Drayton Valley (53°13′N, 114°59′W) and Rocky Mountain House (52°22′N, 114°55′W). The presence of defoliators at each site was verified by larval sampling at the time of site establishment. At each site, six large-volume, non-sticky Unitraps (Phero Tech Inc., Delta, British Columbia) were hung at head height, separated by 50 m along a linear transect along the edge of the stand. Traps were baited with one of six treatments (Table 1).

FTC pheromone components were synthesized by Norm Avelino (Phero Tech Inc.), and the purity of all compounds tested was analyzed by gas chromatographic analysis. The (*E*,*E*)-5,7-dodecadienal isomer was present in large volumes in treatments 2–5 (~28%) (Table 1) as a by-product of synthesis of the other diene aldehydes. However, the *E*,*E* isomer does not influence the behaviour of FTC males (Chisholm *et al.* 1982) and its mass was not included in that of the blends tested (Table 1). Separation of *Z*5*E*7-12Al and *Z*5*Z*7-12Al was not possible during synthesis, so the tested blends in treatments 2–5 (Table 1) containing 4% *Z*5*Z*7-12Al are intermediate between previously tested attractive blends containing 1% (Schmidt *et al.* 2003) and 10% (Palaniswamy *et al.* 1983) *Z*5*Z*7-12Al. The LAT pheromone component, *Z*11-14Al (93% pure) (Bedoukian, Danbury, Connecticut), was purified by Chris Diaper (Department of Chemistry, University of Alberta) using flash chromatography on silver-nitrate-impregnated silica gel prepared by the addition of a 10% (*w*/*v*) silver nitrate alcoholic solution to silica gel. Purification was followed by gas chromatographic – mass spectrophotometric analysis to ensure 100% purity.

Pheromone components were loaded in ether (FTC components) or hexane (LAT pheromone) in the laboratory onto pre-extracted grey rubber septa (Soxhlet extraction for 48 h using a blend of 5% diethyl ether in methanol; Phero Tech Inc.). Solvent control lures were loaded with 100 µL of hexane. Lures were held in sealed glass jars at –20 °C until transport to field sites

Fig. 1. Mean (+SE) number of (A) forest tent caterpillar (FTC) and (B) large aspen tortrix (LAT) males captured in traps baited with various semiochemical blends. *Z5E*7-12Al, *Z5Z*7-12Al, and *Z*7-12Al are FTC pheromone components and *Z*11-14Al is the major LAT pheromone component. Within each species, bars with different letters are significantly different (randomized block ANOVA; FTC, $F_{5,20} = 34.76$, $P < 0.0001$; LAT, $F_{5,12} = 302.85$, $P < 0.001$; Tukey–Kramer, $P < 0.05$).



in a refrigerated container. Pheromone treatments were assigned to traps randomly. Moth capture was monitored at 1- to 2-week intervals from 10 June to 24 August 2004. Total captures of male moths of each species over the season were log(x + 1) transformed and compared using a randomized block ANOVA with site specified as a random factor (PROC MIXED, SAS® 1996). Treatments in which no male moths were captured were not included in the ANOVA to avoid heterogeneity of variances. Analysis of variance was followed by the Tukey–Kramer procedure (SAS® 1996) to assess male attraction to individual treatments.

Significant trap capture of male FTC was dependent on the presence of the three-component pheromone blend in lures (Fig. 1), as reported previously for FTC populations in western Canada (Schmidt *et al.* 2003). Capture of male FTC was negligible in traps baited with the LAT pheromone alone and did not differ from capture in the solvent control (Fig. 1). Similarly, capture of LAT males was greatest in traps baited with lures containing the major pheromone component of this species, *Z*11-14Al (Fig. 1). There was no capture of LAT males in traps baited with either of the FTC pheromone blends alone, and capture in control traps was minimal (Fig. 1).

Conceivably, there could be several problems associated with dispensing multiple species' pheromones in a single formulation. For the lure to be attractive to more than one species, a combined formulation needs to deliver the correct amount and pheromone component ratio for each species (Weatherston 1990) throughout the activity period. In addition, it is essential to determine that heterospecific pheromone components do not antagonize the pheromone response of the targeted species. In the Lepidoptera, components of species-specific sex pheromones can function as antagonists to heterospecific males (Linn and Roelofs 1995) to reduce competition within the sex pheromone communication channel of sympatric species (Evenden *et al.* 1999) and to prevent interspecific mating attempts between closely related species (Greenfield and Karadinos 1979; Cardé 1986; Linn and Roelofs 1995). In the case of these two native defoliators of trembling aspen, which occur sympatrically and synchronically across their range in Canada, there appears to be either no detection of or no response to heterospecific sex pheromone components. The combination of LAT pheromone with the two- or three-component FTC pheromone blend did not influence capture of males of either species (Fig. 1). Because these species are distantly related and do not share common sex pheromone components, it is unlikely that selection has acted on males to evolve the specialized sensory apparatus needed to detect and respond to heterospecific pheromone components. Production of and response to species-specific pheromone signals appears to provide sufficient refinement of the chemical communication channels of these coexisting species.

These data demonstrate that the biological activity of these two species' pheromones is not influenced by combining the compounds into one lure, which is the first step in demonstrating the potential for a combined sex pheromone lure in monitoring traps targeting both species. Further validation of this system is required to test different doses of the two species' pheromones, the longevity of the formulation, and the attractiveness of the formulation at varying population densities across the overlapping ranges of these defoliators. Research is under way to determine whether trap capture of males of both species is correlated with population density and has predictive capabilities for populations of the next generation.

I thank C. Diaper and J. Vederas (Department of Chemistry, University of Alberta) for purification and analysis of the LAT pheromone and N. Avelino (Phero Tech Inc.) for the synthesis and analysis of the FTC pheromone blends. J. Roland (Department of Biological Sciences, University of Alberta) assisted me with location of field sites. A. Argals and C. Elliott assisted with fieldwork. This work was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada and the Faculty of Science, University of Alberta.

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