Oviposition response of spruce budworm (Lepidoptera: Tortricidae) to host terpenes and green-leaf volatiles

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Abstract—A dual-choice behavioral bioassay and gas chromatography – electroantennogram detection (GC–EAD) were used to determine the effect of host terpenes and nonhost green-leaf volatiles (GLVs) on the oviposition preference of the spruce budworm, *Choristoneura fumiferana* (Clemens). Some emphasis was placed on assessing the ability of females to distinguish between enantiomers of chiral monoterpenes because (+)-α-pinene but not (–)-α-pinene or (±)-α-pinene had been shown previously to promote oviposition. Headspace volatiles from white spruce, *Picea glauca* (Moench) Voss (Pinaceae), and balsam fir, *Abies balsamea* (L.) Mill. (Pinaceae), were sampled using solid-phase microextraction and identified by gas chromatography – mass spectrometry with the aid of a chiral column. Females deposited significantly more egg masses on filter paper substrate treated with host monoterpenes than on controls. Contrary to expectation, substrates treated with several GLVs were also preferred over the controls. None of the GLVs or terpenes was deterrent. Females showed no significant ability in either the behavioral or the GC–EAD bioassays to distinguish between enantiomers of selected chiral monoterpenes, including α-pinene, in contrast to earlier findings. We conclude that host terpenes serve as general rather than host-specific oviposition stimuli for spruce budworm.

Résumé—Un bioessai comportemental à deux choix et une technique GC–EAD (chromatographie en phase gazeuse et détection électro-antenno-graphique) nous ont servi à déterminer les effets des terpènes de l'hôte et des substances volatiles des feuilles vertes (GLV) ne provenant pas de l'hôte sur les préférences de ponte de la tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* (Clemens). Nous avons, de façon particulière, déterminé la capacité des femelles à distinguer entre les énantiomères des monoterpènes chiraux, puisqu'on a démontré antérieurement que la (+)-α-pinène favorise la ponte, ce qui n'est pas le cas de la (–)-α-pinène, ni de la (±)-α-pinène. Nous avons échantillonné à l'aide de SPME (micro-extraction en phase solide) et identifié par chromatographie en phase gazeuse et par spectrométrie de masse à l'aide d'une colonne chirale les substances volatiles dans l'espace supérieur immédiat émises par l'épinette blanche, *Picea glauca* (Moench) Voss (Pinaceae) et le sapin baumier, *Abies balsamea* (L.) Mill. (Pinaceae). Les femelles pondent significativement plus de masses d'oeufs sur un substrat de papier filtre traité avec les monoterpènes de l'hôte que sur les témoins. Contrairement à notre prévision, les substrats traités avec différents GLV sont aussi préférés aux témoins. Aucun des GLV et des terpènes n'est inhibiteur. Contrairement à des résultats antérieurs, les femelles ne montrent aucune aptitude significative, tant dans les tests comportementaux que dans les tests GC–EAD, à distinguer entre les énantiomères des monoterpènes chiraux sélectionnés, y compris de la α-pinène. Nous concluons que les terpènes de l'hôte servent de stimulus généraux de la ponte chez la tordeuse de bourgeons de l'épinette, plutôt que de stimulus spécifiques à l'hôte.

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Introduction

Terpenes are the characteristic volatiles emitted by most conifers. Considerable indirect evidence has accumulated linking the presence or absence of specific monoterpenes as potential host-finding or host-recognition cues, as oviposition stimuli, or as host resistance factors for lepidopteran pests of conifers (Leather 1987, 1996; Valterova *et al.* 1995; Jactel *et al.* 1996; Sadof and Grant 1997; Tiberi *et al.* 1999; Rocchini *et al.* 2000; Syed *et al.* 2003; Zhang *et al.* 2003; and Asaro *et al.* 2004, among others). However, direct experimental evidence that specific host monoterpenes elicit these behavioral effects from adult forest lepidopterans is limited to only a few species, including two pyralids, *Dioryctria amatella* (Hulst) (Fatzinger and Merkel 1985; Hanula *et al.* 1985) and *D. abietivorella* (Grote) (Shu *et al.* 1997), a noctuid, *Panolis flammea* (Denis and Schiffermüller) (Leather 1987), a notodontid, the pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Tiberi *et al.* 1999), and a tortricid, the spruce budworm, *Choristoneura fumiferana* (Clemens) (Städler 1974).

The spruce budworm (SBW) is the major defoliator of spruce, *Picea* spp. (Pinaceae), and balsam fir, *Abies balsamea* (L.) Mill. (Pinaceae), forests across North America. Females deposit egg masses on foliage needles and, when given a choice, prefer white spruce, *Picea glauca* (Moench), over balsam fir (Grant 2006). Both physical and chemical host cues influence their oviposition behavior (Wilson and Bean 1963; Städler 1974; Renwick and Radke 1982; Rivet and Albert 1990; Grant and Langevin 1994, 1995; Banga *et al.* 2003; Grant 2006). With regard to chemical cues, Städler (1974) showed that SBW preferred to oviposit on paper substrates treated with host terpenes (+)-α-pinene or (–)-β-pinene rather than on the controls, but substrates treated with (–)-α-pinene, also a host terpene, or racemic α-pinene were not preferred. None of the other host terpenes from balsam fir or other hosts was investigated. Nonetheless, these results suggested that female preference is influenced by specific host monoterpenes and that SBW females discriminate between enantiomers of at least one chiral host monoterpene, α-pinene. There is little other direct evidence for this ability in other adult lepidopterans attacking conifers even though a large proportion of conifer monoterpenes are chiral. In gas chromatography – electroantennogram detection (GC–EAD) bioassays of the pine processionary moth with host terpenes, Zhang *et al.* (2003) found that (–)-limonene consistently elicited antennal responses, whereas (+)-limonene did not, but no behavioral data were reported to indicate the importance of this difference. Tiberi *et al.* (1999), however, reported that application of (+)-limonene to host trees inhibited oviposition by this moth, while (–)-limonene appeared to promote it. Other GC–EAD studies involving moth pests of conifers did not examine the effects of chirality of host terpenes (Syed *et al.* 2003; Asaro *et al.* 2004). In the case of lepidopteran pests of agricultural crops, there is both electrophysiological and behavioral evidence that some moth species can distinguish between enantiomers of chiral host terpenes (Mozuraitis *et al.* 2002; Stranden *et al.* 2003; Hull *et al.* 2004).

In view of the limited host terpenes tested by Städler (1974), the objective of our study was to determine whether additional host terpenes function as oviposition stimuli for the spruce budworm. Emphasis was placed on assessing the ability of mated females to differentiate between enantiomers of chiral monoterpenes in behavioral and GC–EAD bioassays. In addition, we included some green-leaf volatiles (GLVs) as test stimuli. Several studies of insects attacking conifers have shown that nonhost volatiles, such as GLVs, can interrupt their host-finding response (Dickens *et al.* 1992; Byers *et al.* 2000; Poland and Haack 2000, among others). We speculated that GLVs as characteristic signals from deciduous nonhosts might deter or repel ovipositing SBW and hence included them in this study to evaluate this possibility.

Materials and methods

Identification of host terpenes

Eight trees each of balsam fir and white spruce located near Sault Ste. Marie, Ontario, were selected for *in situ* sampling of foliage head space with the solid-phase microextraction (SPME) technique. White spruce was sampled on 19 July 2000 and balsam fir was sampled on 4 July 2001. A 40 cm × 20 cm bag made from a sheet of Tedlar® (Richmond Aircraft Products, Norwald, California) was carefully slipped over a branch of each tree so as not to dislodge foliage needles and held in place at the base with a clip (Turgeon *et al.* 1998). The bag was

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allowed to equilibrate for 20 min before a conditioned SPME fiber (100 μm, polydimethylsiloxane) (Supelco, Bellefonte, Pennsylvania) was inserted into the bag (avoiding contact with the foliage) and allowed to sample the enclosed atmosphere for 30 min. The fiber was retracted and returned to the laboratory for analysis, usually on the same day. If analysis was delayed until the next day, the fiber was stored in a freezer (–19 °C). Gas chromatography – mass spectrometry (GC–MS) analysis was conducted on an HP 5989 instrument (Hewlett-Packard) equipped with a 30 m Rt-βDEXsm™ chiral column (Restek Corp., Bellefonte, Pennsylvania). The GC oven program started at 60 °C, held for 1 min, increased at 4 °C/min to 200 °C, and held for 10 min. The on-column injector temperature was 250 °C. Retention times were compared with authentic standards and mass spectra were compared with the Wiley Registry™ and NIST mass spectral libraries.

Oviposition bioassay

Insects were obtained from a long-established laboratory colony maintained at the Canadian Forest Service laboratory, Sault Ste. Marie, Ontario. Larvae were reared on artificial diet but mated females were provided with fresh balsam fir foliage for oviposition (Grisdale 1984). To produce mated females for bioassay, newly emerged females and 1-day-old males were placed in a screen cage 5 h before the end of the photophase of a 16L:8D light cycle, in a well-ventilated room held at 25 °C and 50%–55% RH. Twenty pairs of moths *in copula* were collected 2–3 h later and placed in a bioassay cage (32 cm high × 30 cm wide × 20 cm deep) made of aluminum screening. Each cage had a removable screen top, which was fitted on the underside with two 15 cm × 4 cm strips of Whatman No. 1 filter paper fixed about 10 cm apart in the center with narrow strips of sticky tape. The paper strips served as oviposition substrates. Each strip had three 6 mm diameter holes evenly spaced along the center line to allow delivery of chemical stimuli into the cage (see below). Their location on the undersurface took advantage of the female's preference for depositing egg masses on the underside of horizontal surfaces (Grant and Langevin 1994) and was key to the success of this bioassay. Females began ovipositing during the first photophase after mating and continued during the second photophase. Egg masses on the filter paper strips were counted as a measure of female preference for the substrates after the second oviposition period, about 48–50 h after the start of the bioassay (Grant and Langevin 1994).

Chemical stimuli were emitted from open, 2 mL polyethylene vial closures (caps) (No. 60975d-3, Kimball) packed with a wad of surgical cotton batting. Typically, 100 μL of neat monoterpene or sesquiterpene, or 200 μL of a candidate GLV, was pipetted onto the cotton batting of each of the three treatment caps, which were then inverted on the top of the cage and over the holes in one of the filter paper strips on the underside of the top. When compounds were tested against a control, three additional caps filled with untreated cotton batting were inverted over the holes of the other filter paper strip. The positions of the control and treated substrates were alternated between replicate trials (n = 5) of a test chemical. As most chemicals were used neat, no solvent was used for the control except for bioassays involving camphene and camphor. These terpenes were not liquid at room temperature and hence were dissolved in hexane to produce 5 and 2.5 mol/L solutions, respectively; 125 or 250 μL, respectively, of these solutions was deposited into the treatment caps. Comparable volumes of hexane were added to the control caps. To determine the females' response in the absence of chemical cues, a replicated (n = 5) experiment was performed in which both substrates were untreated (*i.e.*, the caps lacked chemicals).

We first tested the females' response to the enantiomers of four commercially available chiral monoterpenes, α-pinene, β-pinene, limonene, and camphene; each enantiomer was tested individually against the blank control. We then tested the enantiomeric pairs of the four chiral host monoterpenes competitively against each other to determine whether there was a female preference for one enantiomer over the other. The comparisons were (+)-α-pinene *vs.* (–)-α-pinene, (+)-β-pinene *vs.* (–)-β-pinene, (+)-limonene *vs.* (–)-limonene, and (+)-camphene *vs.* (–)-camphene. One set of caps was treated with 100 μL of the (+) enantiomer and inverted over one of the paper substrates, while the second set of caps was treated with an equal amount of the corresponding (–) enantiomer and inverted over the other substrate. Other pairs of enantiomers that might have been tested were either not available or too costly for the behavioral bioassays, which required

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Table 1. List of sources and purity of test compounds used in the oviposition and GC–EAD bioassays.

Compound	Purity	Source*
Monoterpenes		
(+)-α-Pinene	99% (97% ee)	Aldrich
(–)-α-Pinene	99% (97% ee)	Aldrich
(±)-α-Pinene	98%	Aldrich
(+)-β-Pinene	98%	Aldrich
(–)-β-Pinene	99% (97% ee)	Aldrich
(–)-Limonene	96%	Aldrich
(+)-Limonene	97% (98% ee)	Aldrich
(+)-Camphene	80% (technical grade)	Aldrich
(–)-Camphene	80% (technical grade)	Aldrich
β-Myrcene	90%	Sigma
(+)-Car-3-ene	99% (97% ee)	Fluka
(±)-Linalool	97%	Fluka
(+)-Camphor	99%	Sigma
(–)-Camphor	99%	Aldrich
(+)-Borneol	98%	Fluka
(–)-Borneol	99%	Fluka
(+)-α-Terpineol	99%	Fluka
(–)-α-Terpineol	99%	Fluka
(+)-Bornyl acetate	99%	Fluka
(–)-Bornyl acetate	97%	Aldrich
Sesquiterpenes		
α-Humulene	Unknown	Sigma
(–)-*trans*-Caryophyllene	Unknown	Sigma
α-Farnesene (mixture of isomers)	Unknown	Bedoukian
Green-leaf volatiles		
Hexanal	98%	Aldrich
Hexanol	99%	Sigma
Hexyl acetate	99%	Aldrich
(*E*)-2-Hexenal	98%	Aldrich
(*E*)-2-Hexenol	Unknown	Bedoukian
(*Z*)-3-Hexenol	99%	Aldrich
(*E*)-3-Hexenyl acetate	Unknown	Bedoukian

*Compounds from Aldrich, Sigma, and Fluka were ordered from their respective catalogues through Sigma-Aldrich Canada Ltd., Oakville, Ontario. Bedoukian compounds were ordered from Bedoukian Research Inc., Danbury, Connecticut, USA. ee, enantiomeric excess.

relatively large amounts of each chemical. We then tested other commercially available host monoterpenes and a few sesquiterpenes against the blank control. Finally, we tested seven common GLVs as nonhost stimuli, including saturated and unsaturated aldehydes, alcohols, and acetates.

The commercial sources and purity of the terpenes and GLVs used in the behavioral and GC–EAD bioassays are listed in Table 1. With a few exceptions, the selection of monoterpenes was based on their identification in the volatile emissions of balsam fir or white spruce foliage (see Table 2). Test compounds were recently purchased and opened just prior to their initial bioassay.

To gauge the volatility of representative test stimuli, an additional three caps of (±)-α-pinene, (+)-β-pinene, myrcene, (+)-limonene, (*E*)-2-hexenal, and (*E*)-2-hexenol were prepared

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and weighed periodically for up to 55 h to measure weight loss and provide an estimate of the release rates.

GC–EAD bioassays

GC–EAD bioassays were performed to determine the responsiveness of female antennae to enantiomers of chiral monoterpenes. Separate solutions of (+) and (–) enantiomers of the following monoterpenes were combined in pentane (100 ng/μL of each compound): α-pinene, β-pinene, limonene, camphor, borneol, α-terpineol, and bornyl acetate. Borneol and α-terpineol were not detected in our host volatiles but they have been found in steam distillates of balsam fir foliage (Hunt and von Rudloff 1974) and hence were included in these tests. Solutions of (+)-camphene and (–)-camphene (both 80% technical grade) were also prepared and tested separately because of the high level of impurities associated with these compounds.

One microlitre of a test solution was injected into a Varian 3400 gas chromatograph fitted with a nonpolar HP-1 capillary column (25 m × 0.2 mm i.d.) (Hewlett-Packard) with helium as the carrier gas. The GC temperature program started at 40 °C, held for 1 min, increased at 10 °C/min to 160 °C, held for 10 min, and then increased at 25 °C/min to 190 °C and held for 5 min. The column effluent was split 1:1, with one part going to the flame ionization detector of the GC and the other going through a heated (205 °C) transfer line (Syntech, Hilversum, the Netherlands) into a humidified airstream (300 mL/min) directed at an excised antenna from a mated, 1–2-day-old female. The cut ends of the antenna were inserted into small droplets of electrode gel (Signa Gel, Parker Laboratories, New Jersey) and Ag/AgCl glass electrodes filled with saline were inserted into the gel. The electrodes were connected to a Syntech portable INR-2 amplifier and to a personal computer loaded with Syntech GC–EAD software (version 2.2) for recording and analyzing the GC–EADs. Each of the four test solutions was tested separately against five antennae.

Data analysis

The results of each oviposition experiment (replicated bioassay of a chemical) were analyzed with the paired *t* test (Zar 1984) to determine statistical significance ($P \leq 0.05$). Comparisons of the proportions of egg masses deposited on substrates treated with two different test compounds from separate bioassays were analyzed with a test for two proportions (Zar 1984). GC–EAD responses to the enantiomers of the seven chiral monoterpenes were $\log_{10}$ transformed and analyzed by a two-factor ANOVA, with terpenes and enantiomers as the factors (Zar 1984). In the release rate experiment, weight loss was analyzed by linear regression.

Table 2. Relative proportions of the major terpenes identified in foliage emissions of white spruce (n = 8) and balsam fir (n = 8), obtained by static sampling of headspace with the solid-phase microextraction (100 μm polydimethylsiloxane fiber) technique.

	% composition (±SE)	
Compound	White spruce	Balsam fir
(–)-α-Pinene	5.39±0.97	3.62±0.68
(+)-α-Pinene	6.30±1.6	1.87±0.40
(–)-Camphene	nd*	4.09±0.66
(+)-Camphene	1.03±0.44	nd*
Myrcene	5.23±1.75	1.20±0.28
Sabinene†	1.20±0.43	nd*
(+)-β-Pinene	0.25±0.25	0.22±0.11
(–)-β-Pinene	5.72±1.17	30.70±4.34
(+)-Carene	6.35±3.86	10.52±2.62
(–)-Limonene	6.18±1.41	2.48±0.79
(+)-Limonene	0.42±0.42	nd*
1,8-Cineole	1.25±0.73	nd*
β-Phellandrene†	1.39±0.68	5.28±0.77
γ-Terpinolene	0.96±0.33	0.12±0.12
Linalool†	1.34±0.53	nd*
α-Terpinolene	2.01±0.62	0.24±0.24
(+)-Camphor	4.35±1.17	nd*
Bornyl acetate†	5.56±1.24	9.17±1.43
Sesquiterpenes	13.25±4.98	1.16±0.40
Other	31.56±6.37	29.33±8.04

*nd, not detected in extracts.
†Enantiomers of these chiral monoterpenes were not resolved.

Results

Identification of host terpenes

The major terpene volatiles identified in the headspace of balsam fir and white spruce foliage are summarized in Table 2. Ten of the monoterpenes were chiral, including the following, which were common to both tree species: (+)- and (–)-α-pinene, (+)- and (–)-β-pinene, (+)-3-carene, (–)-limonene, bornyl acetate, and β-phellandrene. Enantiomers of the latter two

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compounds were not identified because authentic standards were lacking at the time of analysis. Not common to both species were (+)-camphene, (+)-camphor, (+)-limonene, sabinene, and linalool (enantiomers of the latter two were not identified), which were found in white spruce but not balsam fir volatiles, and (–)-camphene, which appeared in balsam fir but not white spruce volatiles. In addition, other substantial differences between the two hosts were apparent. For example, (–)-β-pinene was the predominant and characteristic terpene in balsam fir volatiles, whereas it was considerably less abundant in white spruce volatiles. Sesquiterpenes were proportionally much more abundant in white spruce than in balsam fir volatiles. The sesquiterpenes identified in the white spruce headspace were α-humulene, (*E*)-β-farnesene, and α-farnesene, while *trans*-caryophyllene was identified in balsam fir volatiles. Additional sesquiterpenes were emitted by both species but their identification was hampered by the lack of commercially available authentic standards for comparison.

The proportion of foliage volatiles made up by other compounds (non-terpenes and unidentified compounds) was 32% and 29% for white spruce and balsam fir, respectively (Table 2). About 13.5% of the non-terpene compounds in the white spruce volatiles consisted of octanol, decanol, decanal, pentadecane, and heptadecane, while 9.1% of the non-terpene volatiles from balsam fir were aliphatic aldehydes (decanal, dodecanal, and tetradecanal). When some of the GLVs were found to elicit a positive oviposition response from SBW, we re-examined the GC–MS data, looking for GLVs. Hexanal was the only GLV detected. It was found in all SPME samples of balsam fir but not those of white spruce. It represented, on average, less than 5% of the (–)-α-pinene peak area in balsam fir.

Oviposition bioassays

Under field conditions, female moths normally oviposit where they emerge and hence do not move much until they have laid several egg masses (Sanders and Lucuik 1975). In our bioassays, however, female moths were surprisingly mobile before and during oviposition. When introduced into the bioassay cage and while still *in copula*, most females (dragging a male behind them) walked readily from their initial location at the bottom of the cage up the sides of the cage to rest near the top. During the subsequent oviposition periods, which occurred during the latter half of the next photophase, females walked vigorously upside down between the two oviposition substrates, stopping occasionally to oviposit on them. In contrast to the males, no females were observed flying.

A comparison of the oviposition responses to the corresponding (+) and (–) enantiomers of α-pinene, β-pinene, limonene, and camphene, tested individually against the blank control (Fig. 1), indicated that both enantiomers of each of these chiral monoterpenes were behaviorally active and preferred over the control. There was no indication that (–)-α-pinene was inactive, as Städler (1974) had found. In the competitive bioassays where females were given a choice between corresponding enantiomers of a chiral monoterpene, no female preference for one enantiomer over another was observed for each of the chiral terpenes (Fig. 2).

In bioassays involving the remaining host monoterpenes, females preferred the treated substrate over the control in all cases including (±)-α-pinene (Table 3). Ratios of egg masses on treated *vs.* control substrates for all 14 monoterpenes tested individually (Fig. 1, Table 3) varied widely, from 1.8:1 for (–)-limonene and (+)-camphene to 5.2:1 for (+)-camphor, suggesting possible differences in stimulating effectiveness among the compounds. In tests with the sesquiterpenes, (–)-*trans*-caryophyllene and the mixture of farnesenes were preferred over the controls, whereas α-humulene had no effect on oviposition (Table 3).

The total number of egg masses produced in the monoterpene bioassays was relatively constant (mean 119 ± 4.6, $n = 14$), although some monoterpenes such as (–)-bornyl acetate, (+)-camphor, and the enantiomers of limonene appeared to increase production of egg masses (Table 3, Fig. 1). In all cases, egg masses were deposited more or less evenly over the treatment substrate rather than focused at the points of emission beneath the caps containing the test compounds. In the absence of monoterpenes (*i.e.*, both substrates untreated), egg masses were still deposited but fewer appeared to be produced (Table 3). Although we did not measure egg mass size in this study, there was no evident effect of monoterpenes on the size of egg masses. In a previous study, egg mass size was measured but no effect was produced by

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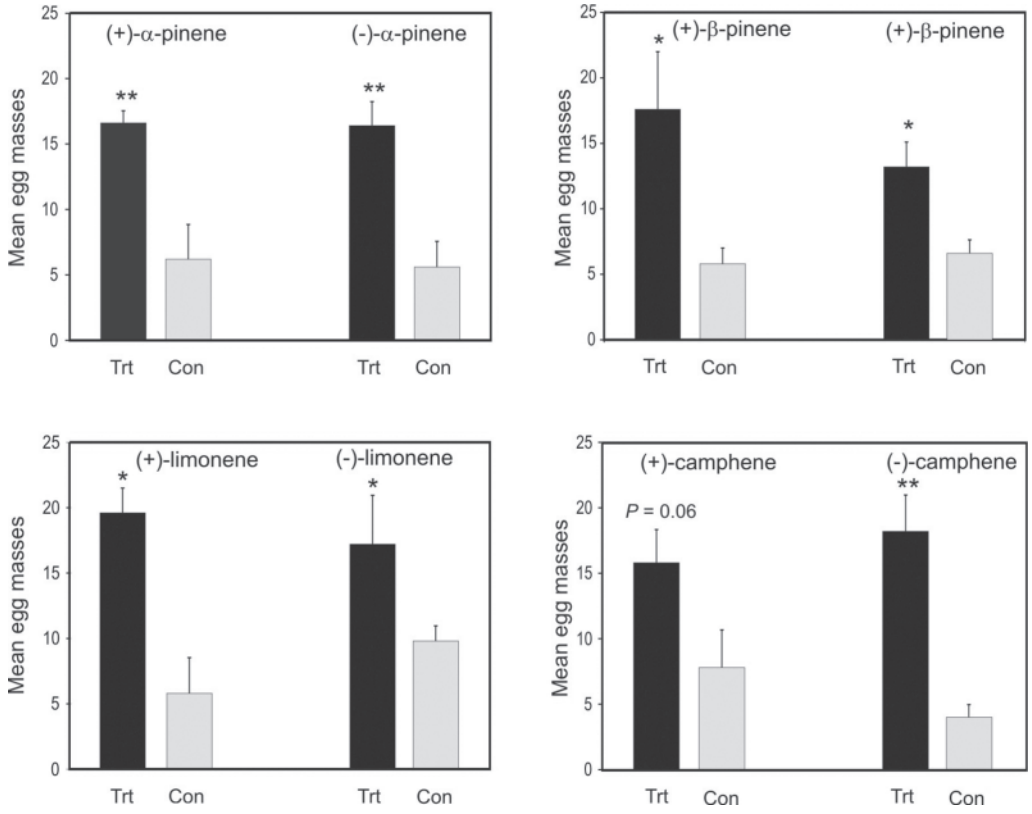
Fig. 1. Preference of spruce budworm (*Choristoneura fumiferana*) for filter paper oviposition substrates treated with enantiomers of chiral monoterpenes against a blank control in individual bioassays. An asterisk over a treatment (Trt) column indicates a significant difference from the control (Con) (*, $P = 0.05$; **, $P = 0.01$; paired t test, $n = 5$). Error bars = SE.



host extracts when compared with the control (Grant and Langevin 1994).

Contrary to expectation, none of the GLVs had a negative (repellent or deterrent) effect on ovipositing females (Table 4). Indeed, females preferred substrates treated with four of the seven GLVs over the controls. Among all the compounds tested, the response to hexanal resulted in the largest ratio of egg masses on the treated substrate relative to the control (8.4:1); however, this effect was not significantly greater than that of the more stimulating monoterpenes, such as (+)-camphor ($P = 0.18$, comparison of 2 proportions) or racemic α-pinene ($P = 0.13$).

In the weight loss study of the four monoterpenes and two GLVs, the major portion of each test stimulus, with the exception of (*E*)-2-hexenol, decreased exponentially over the first 18 h or so. At this point, their release rates became linear. (*E*)-2-Hexenol decreased linearly from the beginning. A linear regression of the weight loss from 18 to 53 h (the oviposition period) provided an estimate of the release rates for the test stimuli, as follows: (±)-α-pinene, 43 µg/h ($y = –0.043x + 4.47$, $r^2 = 0.94$); (+)-β-pinene, 75 µg/h ($y = –0.075x + 8.58$, $r^2 = 0.98$); myrcene, 63 µg/h ($y = –0.063x + 8.63$, $r^2 = 0.94$); (+)-limonene, 141 µg/h ($y = –0.141x + 8.89$, $r^2 = 0.94$); (*E*)-2-hexenal, 181 µg/h ($y = –0.181x + 88.38$, $r^2 = 0.93$); (*E*)-2-hexenol, 2580 µg/h ($y = –2.580x + 156.9$, $r^2 = 0.99$). The slope for each equation was greater than zero, $P > 0.05$.

GC–EAD bioassays

Both enantiomers of the eight chiral monoterpenes tested (which included the four chiral monoterpenes evaluated in the oviposition bioassays) elicited detectable EAD responses from the antennae of mated females (Figs. 3A, 3B). There was no significant difference in the response between the corresponding (+) and (–)

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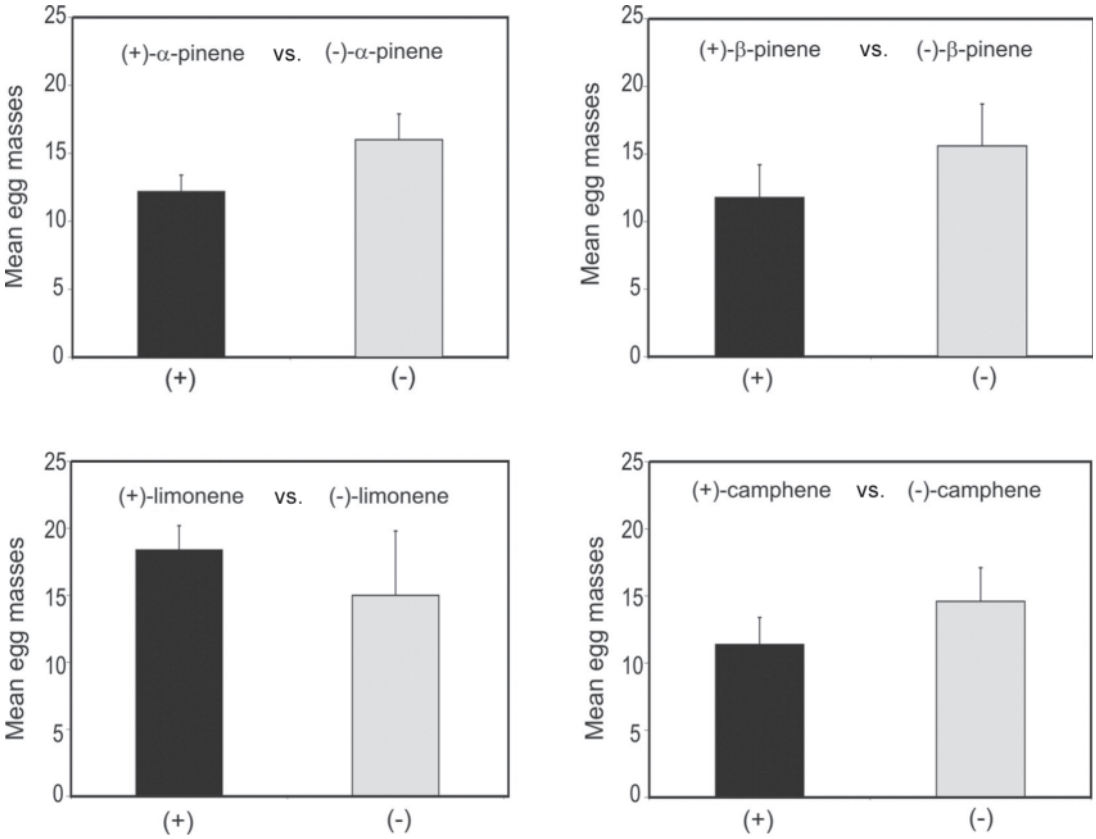
Fig. 2. Preference of spruce budworm for filter paper oviposition substrates treated with the (+) and (–) enantiomers of chiral monoterpenes in competitive bioassays. No significant difference was observed between enantiomers in all four cases ($P > 0.05$, paired t test, $n = 5$). Error bars = SE.



enantiomers of the seven combined monoterpenes ($F_{1,56} = 3.34$, $P = 0.07$) (Fig. 3A) or between the enantiomers of camphene, which were tested separately (paired t test, $P = 0.50$). On the other hand, there were some differences in stimulating effectiveness among the seven monoterpenes ($F_{6,56} = 10.24$, $P < 0.001$) (Fig. 3A), but there was no interaction between the enantiomer and monoterpene factors ($F_{6,56} = 0.32$, $P = 0.92$). The weak EAD response to (–)-β-pinene contrasted with the quantitative prominence of this monoterpene in balsam fir volatiles (Table 2). A similar lack of correspondence between quantity of host monoterpene and the EAD response was observed by Zhang *et al.* (2003).

Discussion

An assumption of this study was that female SBW use specific foliage terpenes, particularly enantiomers of chiral monoterpenes (Städler 1974), as oviposition and possibly host-recognition cues. The bioassays clearly show that with the exceptions of (+)-camphene and α-humulene, all of the terpenes tested were behaviorally active and promoted oviposition on the treated substrates. Although there may be differences in the magnitude of the oviposition responses to the various terpenes, overall their effect was similar and appeared to be nonspecific in nature.

The results also show that female SBW do not distinguish behaviorally between enantiomers of chiral host monoterpenes (at least the ones tested), including (+)-α-pinene and (–)-α-pinene. They also responded to racemic α-pinene, and there was no indication that either enantiomer was inhibitory. These results contrast with those of Städler (1974), who found that SBW females preferred paper substrates treated with (+)-α-pinene but not with (–)-α-pinene or racemic α-pinene. The different results obtained

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Table 3. Oviposition response of spruce budworm (*Choristoneura fumiferana*) to host terpenes in a dual-choice oviposition bioassay.

	Mean* (±SE) no. of egg masses				
Treatment	Treated (T)	Control (C)	Ratio (T/C)	$P^{\dagger}$	Total no. of egg masses
Monoterpenes					
(±)-α-Pinene	18.0±3.5	3.8±1.0	4.7	0.02	109
β-Myrcene	19.0±3.0	4.4±1.0	4.3	0.005	117
(+)-3-Carene	15.8±1.7	5.6±2.3	2.8	0.004	107
(+)-Camphor	22.0±4.0	4.2±1.2	5.2	0.009	131
(–)-Bornyl acetate	26.6±6.4	6.8±3.3	3.9	0.005	167
(±)-Linalool	17.8±4.6	4.4±1.5	4.1	0.02	111
Sesquiterpenes					
α-Humulene	8.4±2.4	2.8±0.5	3.0	0.13	56
(–)-*trans*-Caryophylene	11.2±1.9	5.0±1.6	2.2	0.02	81
α-Farnesene (mixture)	18.0±2.0	4.8±2.2	3.8	0.01	114
Control					
Blank *vs.* blank	9.8±4.0	8.2±3.0	1.2	0.29	90

*Mean of 5 replicates.
†Paired *t* test.

Table 4. Oviposition response of spruce budworm to green-leaf volatiles (GLVs) in a dual-choice oviposition bioassay.

	Mean* (±SE) no. of egg masses				
Treatment	Treated (T)	Control (C)	Ratio (T/C)	$P^{\dagger}$	Total no. of egg masses
Hexanal	26.8±4.1	3.2±0.8	8.4	0.005	150
Hexanol	10.8±2.1	14.8±3.4	0.7	0.45	128
Hexyl acetate	15.4±2.7	10.2±1.4	1.5	0.15	128
(*E*)-2-Hexenal	25.8±4.4	5.4±1.6	4.8	0.02	156
(E)-2-Hexenol	13.4±2.8	7.0±1.7	1.9	0.03	102
(Z)-3-Hexenol	12.8±1.3	5.8±1.1	2.2	0.001	93
(E)-3-Hexenyl acetate	12.0±2.4	9.6±2.7	1.3	0.51	108

*Mean of 5 replicates.
†Paired *t* test.

in the two studies might be explained by differences in bioassay methods. Possibly an important difference was the position of the oviposition substrates. Our substrates were located at the top of the oviposition cage, where females prefer to oviposit (Grant and Langevin 1994), rather than on the bottom of the cage, as in Städler's study. We found in preliminary tests that oviposition substrates on the bottom, on the sides, or hanging in the middle of our bioassay cages received few egg masses and were ineffective for bioassay purposes. Other differences in the physical nature of the substrates in the two bioassays exist and may be a contributing factor. Otherwise, the two assay methods were fundamentally similar. Both used comparable and relatively large (milligram) quantities of the test compounds on a paper substrate. We found that a large initial quantity of test compound was necessary to compensate for its rapid exponential loss due to volatility before oviposition began approximately 20 h later. The release rates of the monoterpenes at that point were relatively constant and not excessive. For example, they were comparable to rates found to attract noctuid moths to floral odors (Meagher 2002). We used fresh chemicals to avoid any changes with aging, such as

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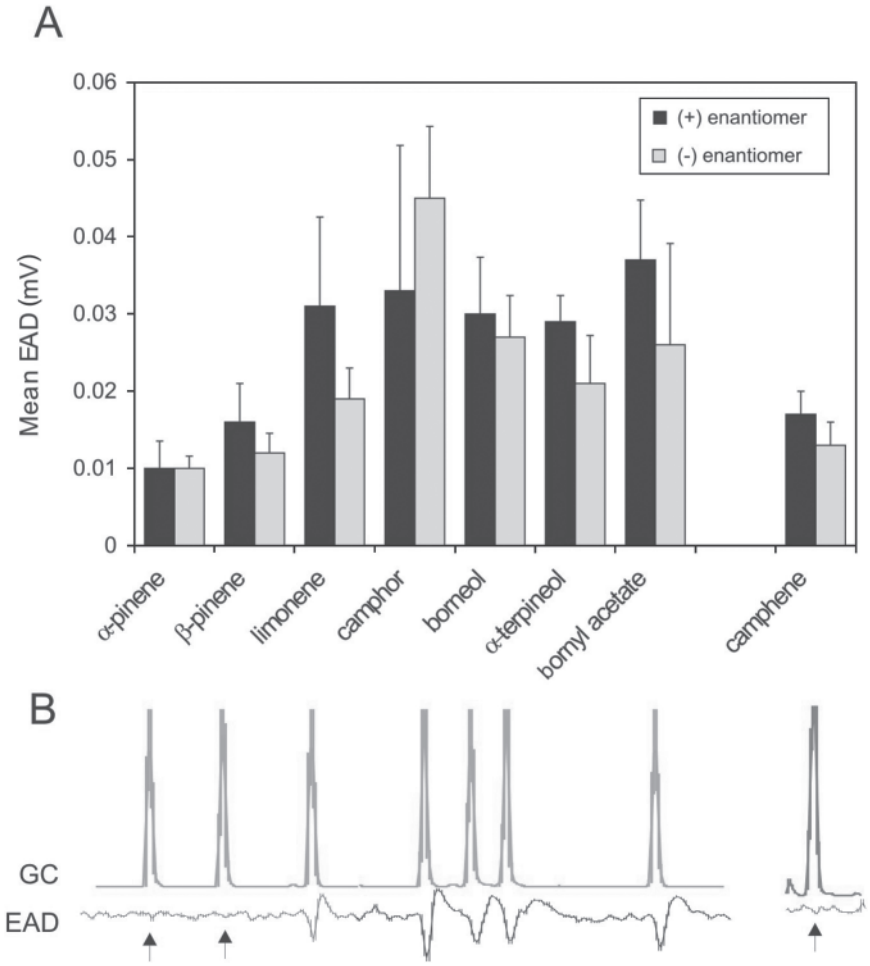
Fig. 3. (A) Mean (±SE) antennal responses (n = 5) from mated female spruce budworm to (+) and (–) enantiomers of eight chiral monoterpenes, assessed by gas chromatography – electroantennogram detection (GC–EAD). There was no significant difference ($P < 0.05$) between responses to the corresponding enantiomers of the chiral monoterpenes. (B) GC–EAD traces in response to a pentane solution containing 100 ng each of the (–) enantiomers of the following chiral monoterpenes: α-pinene, β-pinene, limonene, camphor, borneol, α-terpineol, and bornyl acetate, respectively. (–)-Camphene (technical grade) was injected separately because of its high level of impurities. Arrows point to small EADs.



oxidation. Both studies also used laboratory-reared insects. Our colony females were provided with host foliage for oviposition, so it was reasonable to expect that the test females maintained normal oviposition behavior. The mobility they displayed during oviposition in our experiments, in contrast to feral females (Sanders and Lucuik 1975), may reflect the fact that the experiments started with females that had been disturbed for mating purposes and then introduced into the cages while still *in copula*. Also, they were not in contact with host foliage, which may inhibit movement.

Our GC–EAD results were consistent with the results of our behavioral bioassays. Unlike the GC–EAD study of the pine processionary moth (Zhang *et al.* 2003), our study showed no indication of a differential sensory response by female SBW to (+) and (–) enantiomers of the eight host terpenes that we tested, including the four chiral monoterpenes used in the behavioral bioassays. Relatively high doses (50 ng) of the monoterpenes were required to consistently elicit detectable responses in the GC–EAD bioassay, and high doses (microgram amounts) were also required in electroantennogram

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bioassays to elicit detectable responses (unpublished data), suggesting that female antennal receptors were not particularly sensitive to these compounds. Because atmospheric levels of terpenes in coniferous forest are high, and females generally do not fly before completing several bouts of oviposition (Sanders and Lucuik 1975), high sensory sensitivity to these compounds may be unnecessary.

Contrary to our expectations, none of the GLVs tested in our bioassays had a negative effect on SBW oviposition, whereas several of them elicited significant positive responses. We discovered that one GLV, hexanal, was produced by balsam fir, although at low levels. This may account for its effect on oviposition and possibly that of the related (*E*)-2-hexenal, although the latter was not detected in host volatiles. The emission of hexanal from one of the spruce budworm's hosts, however, would not account for the female's response to the two unsaturated alcohol GLVs.

We conclude that female stimulation by specific host terpenes is an unlikely prerequisite for oviposition by SBW because a wide array of host stimuli elicited oviposition. In previous experiments, females also showed no apparent preference for specific contact chemical cues from host foliage (Grant and Langevin 1994). They oviposited readily on paper substrates treated with surface extracts of coniferous host foliage or with extracts of nonhost foliage of deciduous trees. Indeed, chemical cues do not appear to be essential for SBW oviposition, as it occurred in the absence of chemical stimuli in our bioassays. Thus, host terpenes and other host chemical cues most likely serve as general, nonspecific stimuli that promote oviposition rather than act as key oviposition or host-recognition cues. However, volatile host chemicals might aid host finding once females have produced several egg masses and begin to fly or migrate.

Physical stimuli also play a role in the oviposition behavior of SBW (Wilson and Bean 1963; Städler 1974; Renwick and Radke 1982; Grant and Langevin 1994; Grant 2006). Recent experiments have shown that the spatial arrangement of foliage needles on twigs (foliage architecture) independent of chemical host cues accounts for the female's preference for white spruce over balsam fir (Grant 2006).

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