Journal of Chemical Ecology, Vol. 29, No. 7, July 2003 (© 2003)

ANTENNAL RESPONSES OF THE TWO HOST RACES OF THE LARCH BUD MOTH, *Zeiraphera diniana*, TO LARCH AND CEMBRAN PINE VOLATILES

Z. SYED,[1] P. M. GUERIN,[1,*] and W. BALTENSWEILER[2]

[1]*Institute of Zoology*
University of Neuchâtel
Rue Emile Argand 11
2007 Neuchâtel, Switzerland

[2]*Blumenbergstrasse 9, 8634 Hombrechtikon, Switzerland*

(Received October 23, 2002; accepted February 13, 2003)

Abstract—The larch bud moth (LBM) *Zeiraphera diniana* Guenée causes defoliation on larch in the Alps at 8- to 10-year intervals, after which populations crash. There are two LBM host races, one on larch and the other on cembran pine. These host races are morphologically indistinguishable as adults but they differ genetically in larval color types. Furthermore, females of each host race produce distinct pheromone blends and show oviposition preferences for their respective hosts. It is not clear to what extent host choice contributes to assortative mating in the LBM. Here, we compare the olfactory sensitivities of the two host races to the odors of fresh foliage of the host plants using the electroantennogram (EAG) technique, and the responses of the two host races to volatiles collected from the two host plants as analyzed by gas-chromatography-linked antennographic detection (GC-EAD). Both sexes of the larch and cembran host races show the same EAG responses to vapors of fresh larch and cembran pine foliage. Fifteen plant volatiles identified as chemostimuli by GC-EAD from larch and cembran pine odors elicited the same antennogram responses from the two host races. However, the GC-EAD analyses indicate that the number and quantity of chemostimuli emanating from each host plant is different. It is, therefore, most probably the array of olfactory receptors responding to the bouquet of volatiles unique to each host plant that underlies the host preferences of the two races. What remains open is the extent to which the similarity of the olfactory systems may contribute to cross-attraction. The fact that LBM individuals with intermediate characteristics between the two host races exist, suggests

* To whom correspondence should be addressed. E-mail: patrick.guerin@unine.ch

0098-0331/03/0700-1691/0

that olfactory perception does not hinder gene flow and contributes to sustained genetic diversity within the species *Z. diniana.*

Key Words—Larch bud moth, *Zeiraphera diniana,* host races, *Pinus cembra, Larix decidua,* plant volatiles, antennogram.

INTRODUCTION

The larch bud moth (LBM) *Zeiraphera diniana* Guenée (Lepidoptera: Tortricidae) is renowned for its regular outbreaks in larch forests in the Alps at 8–10-year intervals, causing conspicuous defoliation (Baltensweiler et al., 1977). Population density climbs in four to five generations to some 20,000-fold at peak density. There are two LBM host races, one feeding on larch (*Larix decidua*) and the other on cembran pine (*Pinus cembra*). Populations on pine also appear to be cyclic (Baltensweiler, unpublished), and one outbreak is reported for Northeastern Asia on *Pinus pumila* (Khomentovsky et al., 1997). Morphologically, these host races are distinguishable only at the fifth instar; larvae on larch are black and those on pine are light yellow-orange. This color polymorphism in the larvae varies during population cycles (Baltensweiler et al., 1977; Baltensweiler, 1993).

The major factors governing reproductive isolation in most sympatric phytophagous insect host races are host fidelity and/or other assortative mating traits such as those governed by pheromones. Host fidelity acts as an effective premating barrier between a wide variety of sympatrically speciating insect species across different insect orders. There are only a few reports on host fidelity in the LBM. One incident of host fidelity is known for the larch host race when moths emigrating from the Engadine Alps were grounded by a cold front over the Lake of Constance and reoriented subsequently to larch trees within the deciduous forest (Baltensweiler and von Salis, 1975). Studies on host alighting preference have revealed a strong preference by the LBM host races to alight on their own host plants, both in laboratory (Bovey and Maksymov, 1959; Drès, 2000) and field experiments (Emelianov et al., 2003). However, "infidelity" has been estimated; the overall probability of the larch race adults to alight on cembran pine is 13%, and the probability that pine race adults will alight on larch is 11% (Emelianov et al., 2003).

Assortative mating mediated by sex pheromones has been hypothesized to play a role in host race maintenance in the LBM (Guerin et al., 1984). The major sex pheromone components of the two races were identified as *E*11-14: Ac and *E*9-12: Ac, with the larch host race producing largely *E*11-14: Ac and the pine race *E*9-12: Ac (Baltensweiler and Priesner, 1988). Females produce the two compounds in their sex pheromone glands in ratios corresponding to the response spectra observed for males (Guerin et al., 1984), and F1 hybrid males show the same electroantennogram (EAG) response amplitudes to the two pheromone

components (Priesner, 1979; Priesner and Baltensweiler, 1987). Using a quartet mate choice design in the laboratory (one male and one female of each of the two races per cage), Drès (2000) estimated the overall degree of hybridization between larch and pine host races to be 28%. Long-range host-associated premating isolation governed by pheromones has recently been tested in the field, suggesting an incomplete premating isolation between the two host races (Emelianov et al., 2001).

What allows the cross-attraction between the host races? Gene flow is estimated to be between 2% and 4% per generation (Emelianov et al., 1995; Drès, 2000). Incomplete specificity of the LBM pheromone system and/or the host plant volatiles could be implicated. Although short- and long-range pheromone attraction has been studied (Drès, 2000; Emelianov et al., 2001), no study has been made on the role of host plant volatiles in the sensory ecology of the LBM. To study this, we compared the antennal responses of the two host races to larch and cembran pine volatiles by using the electroantennogram technique (EAG) (Schneider, 1957) and by gas-chromatography-linked EAG analysis (Arn et al., 1975) of host plant volatiles. In addition, we compared the antennal responses of the two host races to volatiles emanating from larch foliage damaged by larval feeding. Larch needles, even only nibbled at, quickly desiccate in the dry subalpine climate and turn red-brown. Commonly occurring plant volatiles were also tested to assess the broader antennal discrimination capabilities of the two LBM host race antennae. The aim of the study was to determine if the antennal olfactory receptor sensitivity of the LBM host races is selectively tuned to the detection of odors that are associated with their respective host plants.

METHODS AND MATERIALS

Insects. Cembran pine and larch branches infested with LBM late instar larvae were collected at the end of June 1999 and on July 1, 2000, in the Engadine Valley. Larvae were reared on the respective host plants at 20°C, 80% relative humidity (RH), in the laboratory to permit pupation and adult emergence. Moths were sexed after emergence and separated to prevent mating.

EAG Recordings. Recordings were made from excised antennae of the moths. The tip of the antenna was cut to facilitate electrical contact. Chloridized silver wires in drawn-out glass capillaries filled with 0.1% KCl + 1% polyvinylpyrrolidone were used as reference and recording electrodes. The antenna was held in a humidified airstream (90%–100% RH, 23°C±2°C) delivered at 1 m/sec via a water-jacketed glass tube (6-mm i.d.) whose outlet was about 1 cm from the preparation. The EAG signal was fed into an AC/DC amplifier (×100) via a high impedance preamplifier (×10), recorded on the hard disk of a PC via a 16-bit analogue-digital IDAC card (Syntech, The Netherlands), and monitored simultaneously with an oscilloscope (Tektronix 5103, USA).

TABLE 1. SYNTHETIC PLANT VOLATILES TESTED AS CHEMOSTIMULI FOR LBM ANTENNAL RECEPTORS

No.[a]	Compound	Source	Purity (% GC)
1	(−)-Bornyl acetate	Firmenich	Unknown
2	(−)-Camphene	Fluka	85
3	(+)-Camphor	Fluka	97
4	(+)-3-Carene	Fluka	98
5	(+)-Carvon[a,b,c]	Fluka	99
6	(−)-β-Caryophellene[b]	Fluka	99
7	Citral (cis+trans)	Fluka	96
8	Citronellal	Fluka	>98
9	Eugenol[b,c]	Fluka	99
10	Geraniol[b,c]	Fluka	99.3
11	(*E*)-2-Hexenal[b,c]	Aldrich	99
12	(*E*)-2-Hexenol[b,c]	Fluka	95
13	Isoprene	Fluka	99.5
14	(*R*)-(+)-Limonene[b,c]	Fluka	99
15	*p*-(−)-Menthyl acetate[b,c]	Fluka	99
16	β-Myrcene[b,c]	Sigma	90
17	(*E*)-β-Ocimine	Firmenich	Unknown
18	(1*R*)-(+)-α-pinene[b]	Fluka	99
19	(+)-β-Pinene	Fluka	99
20	γ-Terpinene[b,c]	Fluka	99
21	(+)-α-Terpineol[b,c]	Fluka	99
22	α-Terpinolene[b]	Fluka	90
23	Sabinene[b]	Unknown	Unknown
24	(*E*)-β-farnesene[b,c]	Bedoukian	Unknown

Note:Except where indicated, compounds with chiral center(s) were racemic mixtures.
[a] Compounds 1–22 were included in the 22-component mixture tested by GC-EAD and EAG (see text).
[b] Compounds tested by EAG.
[c] Compounds included in the 11-component mixture.

Antennae were stimulated as described in Guerenstein and Guerin (2001) by passing 1 ml of charcoal-filtered air through a 5 ml polypropylene syringe containing the stimulus. The latter consisted of either a synthetic plant volatile at 1 μg source dose (Table 1), the LBM pheromone components *E*11-14: Ac and *E*9-12: Ac (Institute for Pesticide Research, Wageningen, NL) at 100 ng source doses, mixtures of 11 and 22 synthetic plant volatiles (Table 1) with each compound at a source dose of 0.1, 1, and 100 μg, fresh or LBM-defoliated larch, and cembran pine needles (2 g each). An aliquot of a stimulus chemical dissolved in dichloromethane (DCM, Merck, analytical grade) was deposited on a filter paper strip that was placed in the syringe after evaporation of the solvent; DCM alone

was used as a control. The EAG amplitudes presented are the absolute amplitudes in millivolts generated by the stimuli minus the control value (if any).

Gas-Chromatography-Coupled Electroantennogram Detection (GC-EAD). The methodology is described in Steullet and Guerin (1994). LBM antennae were employed as detectors to locate biologically active volatiles in the odors of fresh and LBM-defoliated larch, and cembran pine. These odors were separated on a high-resolution gas chromatography capillary column (30 m DBWAX, 0.25-mm i.d., 0.25-μm film thickness, J&W Scientific, CA, USA) in a gas chromatograph (Carlo Erba Instruments 5160, Mega series, Milan, Italy) with a split–splitless injector at 200°C (for direct headspace vapor injection), an on-column injector (for analysis of extracts), and a flame ionization detector (FID, at 260°C). The carrier gas was H_2 (30 cm/sec at 40°C). On-column injections were made at 40°C, and the column temperature was programmed at 5°C/min to 230°C (held for 10 min). The column effluent was split (50:50) between the FID and the antennographic detector (EAD) and simultaneously monitored by the FID and a LBM antenna, and both signals were recorded simultaneously on a PC using a GC-EAD software program (Syntech, The Netherlands). Kovats retention indices (KIs) for the biologically active plant volatiles detected were calculated with reference to *n*-alkanes (C_{10}–C_{30}) injected under the same GC conditions as the analyte.

Direct Analysis of Plant Volatiles. Some 20 g of fresh foliage of *P. cembra* (Pinaceae), fresh and LBM-defoliated *L. decidua* (Pinaceae), or fennel *Foeniculum vulgare* (Apiaceae) were placed in airtight glass bottles (200 ml) with a rubber septum in the lid for 1 hr at room temperature to allow headspace vapor sampling with a 5 ml gas sampling syringe. A 2 ml sample was injected splitless (method described in McMahon et al., 2001) onto the column for GC-EAD analysis.

Porous Polymer Extracts of Plant Volatiles. Some 800 g of fresh foliage of cembran pine and fresh and LBM-defoliated larch foliage (800 g each) were put in airtight dessicators (2 l) fitted with inlet and outlet tubes. Charcoal-filtered air entered through one tube, and a glass cartridge containing 5 g preconditioned (Byrne et al., 1975) Porapak Q® (60–80 mesh, Millipore Corporation, USA) was attached to the other. A water pump sucked air (50 ml/min) over the plant material to the adsorbent. The porous polymer was extracted with 500 μl DCM (Merck, analytical grade) and 2 μl of the extract were injected on-column.

Gas-Chromatography-Coupled Mass Spectrometry (GC-MS). Plant odor extracts analyzed by GC-EAD were subsequently analyzed by GC-MS in as HP 5890 series II chromatograph linked to a HP 5971A mass selective detector (MSD; Hewlett Packard, USA), with the column and conditions as in the GC-EAD analysis (above). Blank controls were analyzed as for the respective headspace extracts. Two microliters of extract were injected on-column; the column was connected via a 1-m deactivated fused-silica capillary (0.25-mm i.d.) to the MSD ion source (temperature 160°C, ionization energy 70 eV) with helium as carrier gas at constant

flow (linear velocity ~30 m/sec at 40°C). Headspace vapor analysis by GC-MS with splitless injection was performed as described in McMahon et al. (2001) under the same conditions as for GC-EAD, but in a Varian 3400-Saturn 3 (CA, U.S.A.), with the MSD, column, and carrier gas flow as above.

Biologically active components of headspace vapors and volatile extracts located by GC-EAD analysis were relocated by GC-MS using KIs, and by comparison of chromatogram profiles. Identification of an electrophysiologically active peak in an extract was first based on the match of its mass spectrum with that of a known product stored in a computer-based library using the HP-Chemstation software. The KI of an unknown chemostimulus from larch and pine was then compared with that of the library-proposed synthetic analogue injected under the same conditions. Biological activity with synthetic analogues of four chemostimuli was established by GC-EAD with LBM antennae (Table 2). Specific enantiomers of compounds identified in extracts with chiral center(s) were not determined, but enantiomers of synthetic chiral products used are indicated in Table 1.

Statistical Analysis. Because of the variation in EAG responses between LBM antennae, responses of a given antenna to different chemostimuli in either EAG or GC-EAD experiments were normalized by summing the responses in millivolts to all the chemostimuli. The percent contribution of each compound to this sum was then square root transformed for statistical analysis. Where responses of the sexes did not differ significantly ($P > 0.05$, ANOVA), male and female antennal responses were pooled within a host race. EAG and GC-EAD response amplitudes of the host races were then compared by ANOVA. All the chemostimuli identified in the GC-EAD analyses of larch and cembran pine volatiles were included in paired comparisons, except the cubebol/epicubebol peak in the cembran pine bouquet that elicited varied responses from the two host race antennae, and an unidentified peak (unidentified 1, M^+ 150) in the larch foliage bouquet that selectively elicited responses from the cembran pine race only. The EAG responses to fresh and LBM-defoliated larch foliage, and to fresh cembran pine foliage, were compared by *t* test (unpaired). The vapors emanating from LBM-defoliated larch were analyzed by GC-EAD with both larch and cembran pine host race antennae, but the porous polymer collected volatiles from LBM-defoliated larch were analyzed only with cembran pine race antennae (the only ones available).

RESULTS

EAG Responses to Larch and Cembran Pine Foliage. Both sexes of the larch and cembran pine LBM host races responded similarly to larch and cembran pine foliage vapors collected in the stimulus cartridge: cembran pine foliage elicited EAG responses of 2.25 ± 0.86 (mean EAG response $\pm$ standard deviation) and 1.71 ± 0.97 mV from the cembran pine and larch host races, respectively, whereas

TABLE 2. ANTENNAL CHEMOSTIMULI IDENTIFIED FOR THE LARCH BUD MOTH AND FREQUENCY OF THEIR DETECTION BY LBM HOST RACE ANTENNAE IN THE ODOR OF HOST PLANTS (LARCH AND CEMBRAN PINE), AND IN THAT OF A NONHOST PLANT (FENNEL)

	Direct headspace vapor injection[a]								Porous polymer trapped volatile extract[b]					
	P. cembra		*L. decidua*				*F. vulgare*[d]		*P. cembra*		*L. decidua*			
	Fresh		Fresh		Defoliated		Fresh		Fresh		Fresh		Defoliated	
Compound	LR ($N = 7$)	PR ($N = 12$)	LR ($N = 7$)	PR ($N = 8$)	LR ($N = 3$)	PR ($N = 6$)	LR ($N = 3$)	PR ($N = 2$)	LR ($N = 4$)	PR ($N = 4$)	LR ($N = 4$)	PR ($N = 4$)	PR ($N = 3$)	Identification criteria[c]
Camphene	7	8	1		1									MRE
β-Myrcene	7	12	1	1	1		3	2	4	4	4	4	3	MRE
Limonene	7	11	1	1	1				4	4	2	4	3	MRE
β-Phellandrene											3	3	3	MR
β-Ocimene							3	2						MR
γ-Terpinene											2	4	3	MRE
p-Cymene	7	12	7	8	3	6	3	2			3	4	3	MR
Unidentified 1 (M^+ 150)											0	4	3	
Unidentified 2									4	4				
Unidentified 3									4	2				
Isopinocamphone											4	4	3	M
Thymol methyl ether									4	4				M
Trans-pinocarveol											4	4	3	M
β-Farnesene											4	4	3	MR
Methyl chavicol							3	2						MR
α-Terpinyl acetate									4	4				MR
Isogermacrene-D											4	4	2	M
α-Farnesene											4	4	3	M
Cubebol/epi-cubebol									3	4				M

Note: Pooled data from male and female antennae; LR = larch race and PR = pine race. Absence of a compound down a column denotes no response from antennae to the dose present in the headspace or extract in GC-EAD analysis (see text).

[a] Foliage headspace injected directly.

[b] Foliage headspace components trapped over a porous polymer.

[c] Criteria for identification of an EAD active peak: M: matching mass spectra with products in the HP-Chemstation database, R: matching retention time of the synthetic analogue, and E: matching electrophysiological activity with that of the synthetic analogue.

[d] Analysis only by females of two host races.

the larch foliage elicited clearly smaller responses of 0.91 ± 0.58 and 1.06 ± 0.35 mV, respectively, ($N = 12$; 6 males and females for each host race). These responses were not different either between sexes of a given host race or between the host races ($P > 0.05$). The higher responses recorded from the antennae of both host races to cembran pine foliage can be explained by the higher amount of volatiles emanating from this host plant (Figures 1 and 2). Using the direct headspace sampling method described here, we estimated camphene at 3 times more, β-myrcene at 10 times more, limonene at 75 times more, and *p*-cymene at 2 times more in the headspace of cembran pine over larch. LBM-defoliated larch elicited EAG responses of 0.69 ± 0.31 mV, no different from the 1.05 ± 0.16 mV responses generated in pine host race antennae to fresh larch foliage ($N = 6$, $P > 0.05$).

GC-EAD Analysis of Larch, Cembran Pine, and Fennel Headspace Vapors. In fresh larch foliage headspace vapor, only the aromatic *p*-cymene elicited a conspicuous and consistent EAG response from male and female antennae of both host races; the response was recorded from all 7 larch and 8 cembran pine host race antennae employed (Figure 1 and Table 2). The EAD responses of 3 larch and 6 cembran pine host race antennae to LBM-defoliated larch vapors were similar to those to fresh larch, where *p*-cymene was again the most active chemostimulus in LBM-defoliated vapors. As with fresh larch, responses were recorded occasionally to camphene, β-myrcene, and limonene in LBM-defoliated larch vapor whenever the injected sample contained the compounds in sufficient quantity.

In cembran pine headspace vapor, in addition to *p*-cymene, the monoterpenes camphene, β-myrcene, and limonene elicited antennal responses from both sexes of the two host races (Figure 2, Table 2). Despite the similarity of the responses of the two host races to chemostimuli from both host plants, cembran pine released a greater number of chemostimuli at a sufficiently high dose to stimulate antennae of both the host races. The vapors released from the two host plants therefore induce a specific array of olfactory responses. GC-EAD analysis of odors of a nonhost plant, fennel, demonstrated the presence of another array of chemostimuli for the LBM composed of monoterpenes β-myrcene and β-ocimene, and the aromatics *p*−cymene and methyl chavicol; these four compounds elicited responses in all the female antennae of the two host races that were used (Table 2).

GC-EAD Analysis of Volatiles Trapped from Larch and Cembran pine. Ten constituents of the porous polymer trapped volatiles from larch elicited responses from antennae of both sexes of the two host races and were identified as the monoterpenes β-myrcene, limonene, β-phellandrene, γ-terpinene, the sesquiterpene β-farnesene, and the aromatic hydrocarbon *p*-cymene (Figure 3 and Table 2). In addition, isopinocamphone, trans-pinocarveol, isogermacrene-Δ, and α-farnesene were tentatively identified by mass spectral matches with computerized database spectra. An unidentified peak (M^+ 150, KI 1316, unidentified 1; Table 2) elicited responses from antennae of both sexes of the cembran pine host

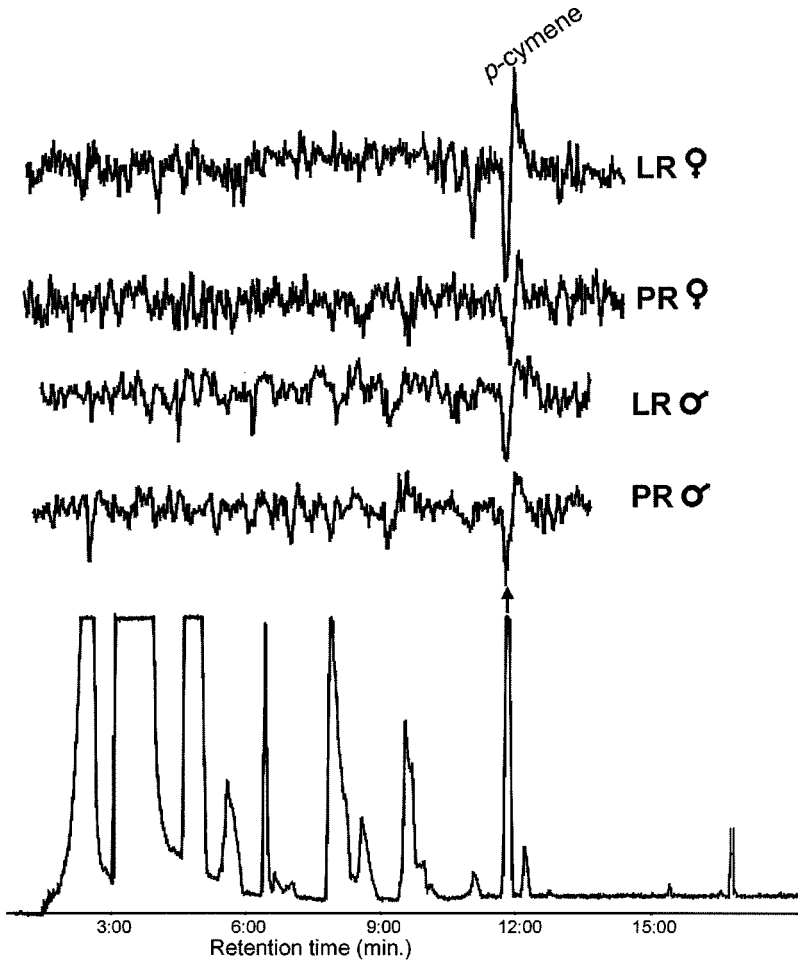

FIG. 1. Analysis of directly injected larch foliage headspace volatiles by GC-EAD with LBM larch (LR) and cembran pine host race (PR) antennae. The *lower trace* is the flame ionization detector (FID) response and the *upper four traces* are EAD responses generated during elution of the biologically active constituents of the headspace odor from the gas chromatographic column.

race only. This product was not included in the paired comparisons of response amplitudes (Figure 5). The porous polymer extract of LBM-defoliated larch volatiles induced similar EAD response profiles as induced by fresh larch foliage in the three cembran pine race female antennae employed. Moreover, the FID detector response of the GC revealed that the amounts of 10 chemostimuli in the porous

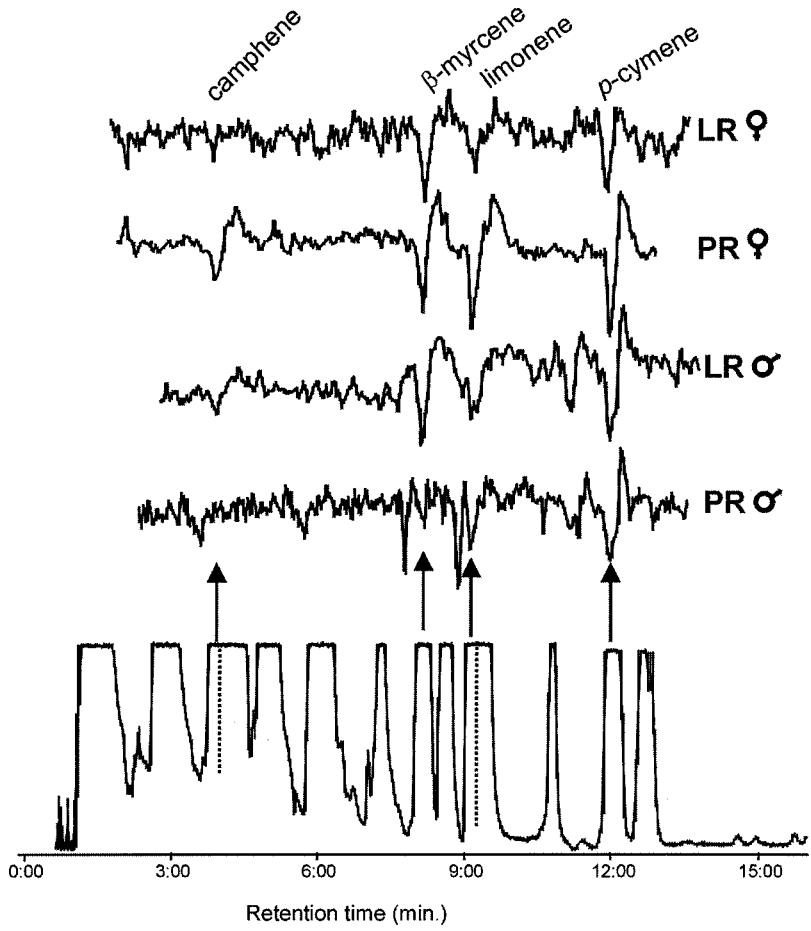

FIG. 2. Analysis of directly injected cembran pine foliage headspace volatiles by GC-EAD with LBM larch (LR) and cembran pine (PR) host race antennae. For further details see text and legend to Figure 1.

polymer extract of fresh larch volatiles were approximately the same in the vapors of LBM-defoliated larch, but the latter did contain additional components.

Constituents of the volatiles trapped from cembran pine elicited EAD responses and were identified as β-myrcene and limonene, and the aliphatic ester α-terpinyl acetate. Thymol methyl ether and a sesquiterpene, which was either cubebol or epicubebol (Figure 4 and Table 2), were also tentatively identified by matches with database spectra. The cubebol/epicubebol peak (KI 1932) induced the strongest antennal response from both host races. However, the response was

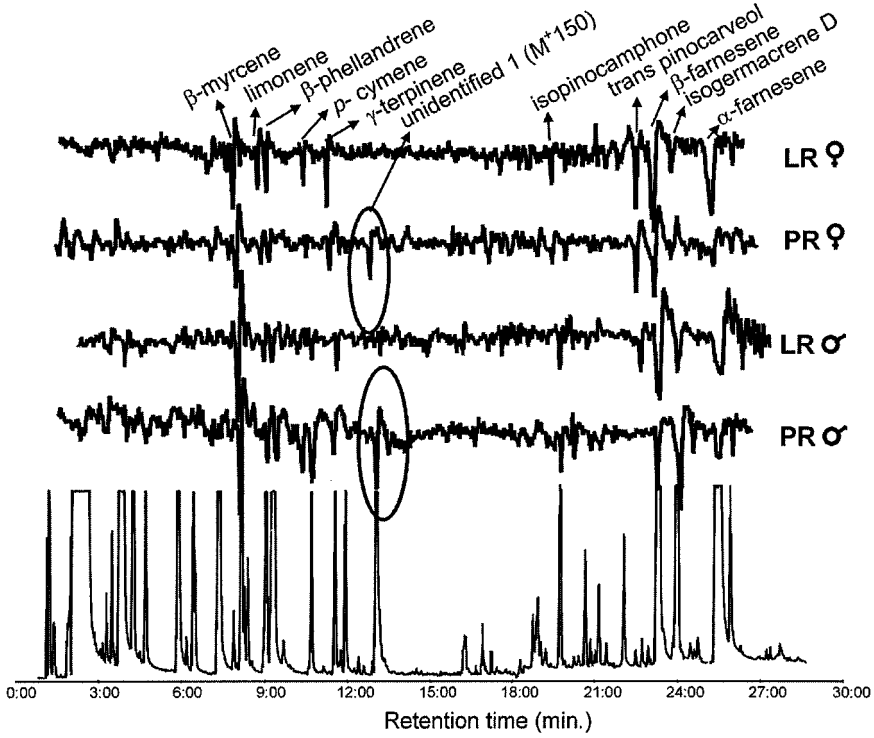

FIG. 3. Analysis of larch foliage odor as collected on a porous polymer by GC-EAD with LBM larch (LR) and cembran pine host race (PR) antennae. For further details see text and legend to Figure 1.

not consistent (observed in 4 of 4 analyses with the cembran pine host race but in only 3 of 4 analyses with larch host race antennae), and so the cubebol/epicubebol peak was not included in the paired comparisons (below). Exact chemical identity of the two chemostimuli with KIs of 1361 (unidentified 2) and 1512 (unidentified 3) could not be established (Table 2, Figure 4). Comparisons of the EAD response amplitudes of male and female antennae of each host race to the 10 larch- and 6 cembran-pine-identified chemostimuli indicated no significant differences either between sexes of the same host race or between the two host races ($P > 0.05$; Figure 5). It is clear, however, that the number and quantity of volatile chemostimuli collected from each host plant is unique, and correspondingly the olfactory response profiles of the LBM host races.

GC-EAD Analysis and EAG Screening of Synthetic Plant Volatiles and Pheromone Components. In the 22-component mixture of generally occurring plant volatiles analyzed by the GC-EAD, 13 compounds elicited electrophysiological

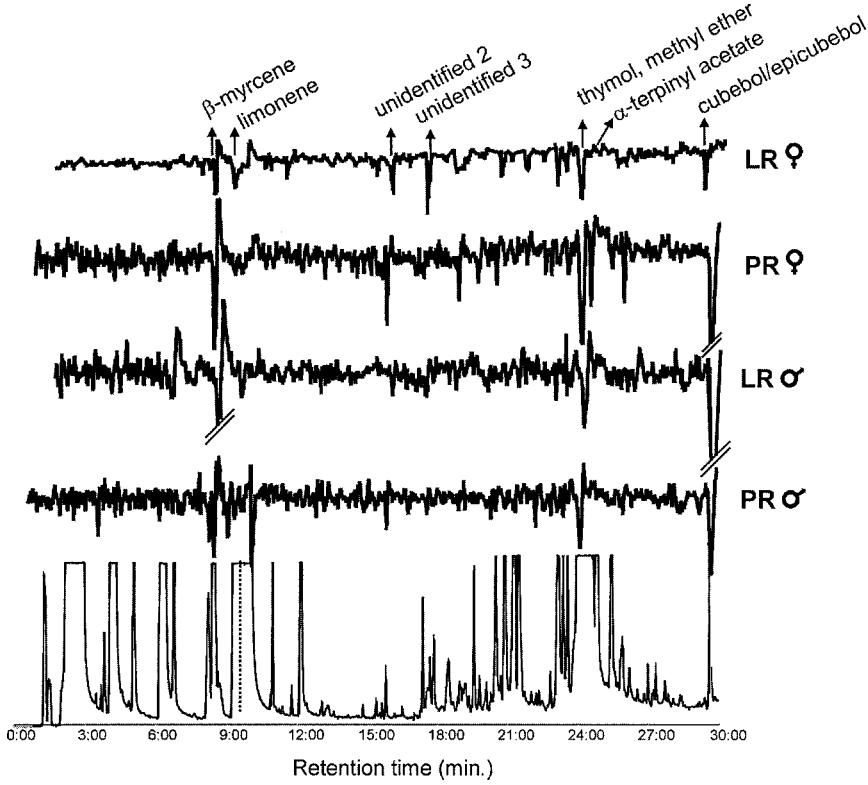

FIG. 4. Analysis of cembran pine foliage odor as collected on a porous polymer by GC-EAD with LBM larch (LR) and cembran pine host race (PR) antennae. For further details see text and legend to Figure 1.

responses from male and female antennae of both host races. In addition to three of the chemostimuli identified above from both larch and cembran pine, i.e., β-myrcene, limonene, and γ- terpinene, 10 other compounds elicited antennal responses, i.e., citronellal, (+)-camphor, (+)-α-terpineol, (+)-carvone, geraniol, (*E*)-2-hexenal, (*E*)-2-hexenol, *p*-(−)-menthyl acetate, (−)-bornyl acetate, and eugenol. In follow-up EAG tests, 15 compounds identified as chemostimuli for the LBM (above) were tested at a 1 μg source dose in the stimulus cartridge. Each of these compounds elicited similar EAG responses from antennae of either host race (Figure 6; $P > 0.05$ between sexes and between the host races). Two mixtures of synthetic plant volatiles containing 11 and 22 components (Table 1) elicited linear dose-dependent EAG responses, covering 3 orders of magnitude (0.1, 1, and 10 μg) from both male and female antennae of the two host races; the 22-component mixture elicited higher responses at all doses tested.

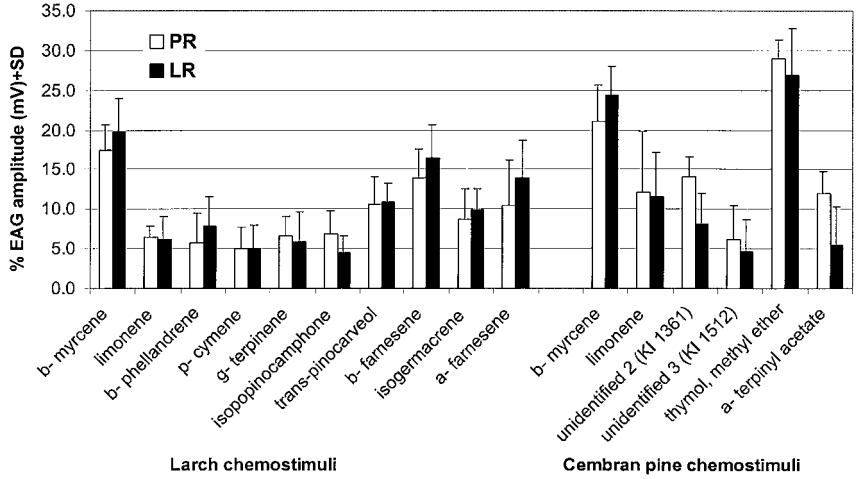

FIG. 5. Electroantennograhic detector responses (mean EAD responses + SD) of LBM larch (LR, $N = 4$) and cembran pine host race (PR, $N = 4$) antennae to chemostimuli eluting from the chromatographic column in the GC-EAD analysis of larch and cembran pine headspace volatiles as collected on a porous polymer (from Figures 3 and 4). Values are percent contribution of each stimulus to the pooled amplitudes generated by all the chemostimuli from a given antenna, and the standard deviation. Male and female responses were pooled for each host race as the effect of sex was not significant ($P > 0.05$).

In order to establish that we were dealing with LBM host races similar to those previously described (Priesner, 1979), the responses of the male antennae were recorded to the LBM host race pheromone components. Male antennae of the two host races showed higher responses to the respective host race principal pheromone components, i.e., antennae of the larch host race responded best to *E*11-14: Ac, whereas those of the cembran pine host race responded best to *E*9-12: Ac ($N = 6$ in each case).

DISCUSSION

The two LBM host races were equally sensitive to the particular suites of volatile chemostimuli released from larch and cembran pine. Mono- and sesquiterpenes accounted for over 80% of the chemostimuli identified for the LBM from larch and cembran pine volatiles. Because both host races feed exclusively on conifers (Baltensweiler et al., 1977), the predominance of terpenes as chemostimuli is not surprising. Antennae of both host races also responded to green leaf volatiles such as (*E*)-2-hexenol and (*E*)-2-hexenal and to the aromatics *p*-cymene,

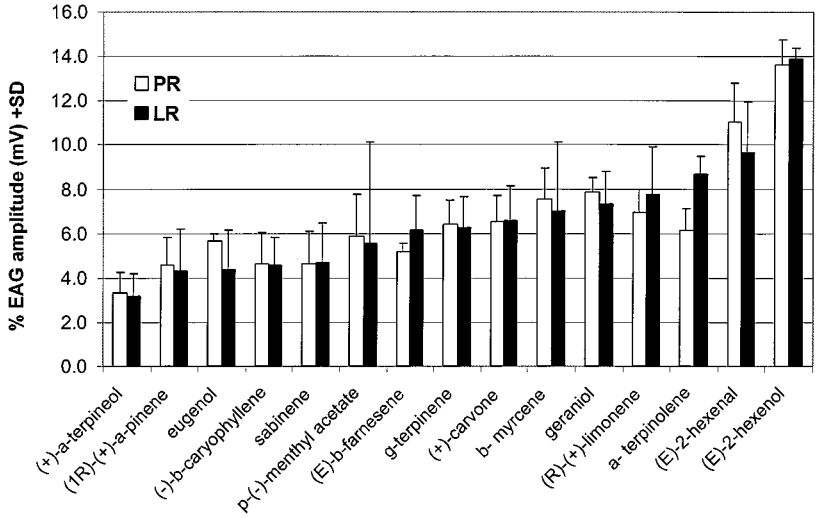

FIG. 6. EAG responses of LBM larch (LR) and cembran pine host race (PR) antennae to a range of plant volatiles. Values are percent contribution of each stimulus to the pooled amplitudes generated by all the compounds tested on a given antenna, and the standard deviation ($N = 7$ and 8 for cembran pine and larch host races, respectively). Male and female responses were pooled for each host race, as the effect of sex was not significant ($P > 0.05$).

eugenol, and methyl chavicol. The responses induced by the green leaf volatiles were the highest for both host races.

However, there was one unidentified compound in the larch porous polymer volatile extract that elicited EAD responses only from male and female cembran pine host race antennae. Otherwise there was no difference in EAG responses of the sexes of either host race to any of the chemostimuli. Furthermore, females of both host races responded equally to a suite of chemostimuli from fennel, a nonhost plant.

Among the 10 compounds chemically identified as stimuli in the porous polymer extracts of larch, 8 of these could be located in cembran pine by single ion monitoring (SIM) (isopinocamphone and α-farnesene were not detected), whereas of the 5 identified cembran pine chemostimuli, 4 were detected by SIM in larch (thymol methyl ether was not detected). High yields of chemostimuli sometimes interfered with the resolution of products: two distinct FID and corresponding EAD responses were recorded from both host races to limonene and β-phellandrene from the porous polymer extract of larch, but these chemostimuli were not resolved in the cembran pine extract because of overloaded nonresolved peaks. The presence of both products in cembran pine was, however, confirmed by SIM in GC-MS.

Antennae of both the LBM host races showed dose-dependent responses to mixtures of plant volatiles, underlining the graded responses of the antennal receptors to the chemostimuli. Clearly, behavioral tests are required to investigate how host plant odors affect LBM host race behaviors.

The EAD response profiles of the LBM larch race antennae to vapors of freshly cut larch foliage and LBM-defoliated larch were the same. However, the EAD profiles induced by direct injection of LBM-defoliated larch vapors or by the porous polymer trapped extract of these volatiles were similar to those induced by fresh larch, but not to those induced by cembran pine. Furthermore, the quantities of the chemostimuli were approximately the same in the LBM-defoliated porous polymer extract as in the fresh larch extract. This would suggest that the LBM-defoliated larch is probably perceived as "larch" by the LBM. Despite this, the LBM-defoliated larch appears not to provide the LBM with an adequate substrate, especially contact chemostimuli, to induce oviposition (Baltensweiler and Rubli, 1999). This may explain the mass migration previously noted from LBM-defoliated larch stands in the Engadine (Baltensweiler and von Salis, 1975).

Cross-attraction between the LBM host races in the field has been documented (Emelianov et al., 2001): larch race females calling from pine attracted significantly more alien males (37% of the total number of males attracted) than did larch race females calling from larch (2.4% alien males). Pine females calling from either pine or larch invariably attracted only 3.6% alien males. This indicates two important points. First, females of both host races could be mated on either host plant. The pheromone of either host race is perceived by at least some males, independent of host odors, i.e. the specificity of chemostimuli from larch or cembran pine does not interfere with pheromone perception. Second, and more important, assortative mating for the larch host race is enhanced when females call from their own host plant, but this almost breaks down when these females call from cembran pine. However, the attraction of pine race males to larch race females is probably more related to the mixed pheromone signals of some larch host race females, which may contain small amounts of *E*9-12: Ac (Guerin et al., 1984; Baltensweiler and Priesner, 1988), than to the context of host plant volatiles in which the pheromone is perceived. Nevertheless, in mixed forests with equal numbers of larch and cembran pines, the two host races showed 80%–90% alighting preferences for their own hosts, and in laboratory choice experiments, larch and pine races preferred their own host of 63%–69% (Emelianov et al., 2003). Even though the olfactory systems of two host races share a common array of receptors responding to a variety of rather nonspecific host plant odors, it is the response of an array of olfactory receptors to the bouquet unique to each host plant that may allow each race to discriminate among different host plants by the across-fiber pattern of activated peripheral receptors (Visser, 1986).

The best-studied insect host races show remarkable similarity in their transition patterns between host plants. The apple maggot fly, *Rhagoletis pomonella*,

has host races on hawthorn and apples, and volatiles identified from apples that attract the apple maggot fly (Fein et al., 1982) were also identified from hawthorn (Carle et al., 1987). The close similarity in volatile profile between apple and hawthorn led Carle et al. (1987) to suggest that the chemical similarity in volatile composition facilitated the shift of *R. pomonella* from hawthorn to apple. Despite the differences in the suites of chemostimuli we collected from larch and cembran pine, a close similarity in the volatile profile has been reported by Rappaport et al. (1996) for alpine larch and cembran pine. We did not, however, investigate the details of the volatile profile of larch and cembran pine, but compared the olfactory responses across both sexes and host races of the LBM to chemostimuli from either host plant.

There is evidence in phytophagous insects for the evolution of monophagy from polyphagy (Ehrlich and Raven, 1964; Bernays and Graham, 1988), with the possible benefit of reduced predation (Jeffries and Lawton, 1984). Among sympatrically evolving host races, the more nutritive resource is usually preferred. This is equivalent to the exploitation of larch with its high nutritive value by the larch host race [nitrogen in larch reaches 25 mg/g needles but only 15 mg/g in evergreen Norway spruce (Baltensweiler, 1992)]. By contrast, LBM larvae exploiting cembran pine must cope with higher amounts of toxic oleoresins (Norin, 1972). It has been suggested that the more generalist cembran pine race surviving on evergreens represents the original form because of its more adaptive characteristics like slower rate of post-diapause development, significantly smaller adult size, and greater survival under nutritional stress (Baltensweiler, 1993; Khomentovsky et al., 1997). Seasonal and annual variation in relative host abundance and suitability, a common occurrence in nature (Boughton, 2000), tends to oppose the increase in host choice. Given a choice between two constant plant resources, an insect should evolve increased fidelity for higher quality or the more abundant host (Fry, 1996). However, predictable seasonal variation in host abundance makes it difficult to eliminate completely the vestiges of choice of an alternative plant (Berlocher and Feder, 2002). In the LBM system, the disadvantage for the larch host race is that larch, despite being more nutritive, is susceptible to defoliation at high moth densities. This results in a less suitable resource for the larch race at regular 8-year intervals. At this point in the cycle, however, the proportion of light morphs capable of exploiting cembran pine (Baltensweiler, 1993) is highest on larch (Baltensweiler, unpublished). Since our data suggest that the larch host race does perceive chemostimuli from cembran pine, we have to assume the host plants may still function as rendezvous sites for both host races, but whether olfactory perception serves to permit utilization of the alternate resource is an open question. Nevertheless, olfactory perception does not act as a definite selective barrier for either host race to restrict genetic diversity within the species *Z. diniana*.

Acknowledgments—We are grateful to Dr C. McMahon for initial help in preparing plant volatile extracts and reading the manuscript, to Dr S. Mohottalage, Institute of Chemistry, University of Neuchâtel, for his help with identification of products, and to Mrs J. Moret of the Institute of Mathematics, University of Neuchâtel, for statistical advise. This paper is part of the PhD thesis submitted by Z. Syed at the University of Neuchâtel.

REFERENCES

ARN, H., STÄDLER, E., and RAUSCHER, S. 1975. The electroantennographic detector—A selective and sensitive tool in gas chromatographic analysis of insect pheromones. *Z. Naturforsch.* 30:722–725.

BALTENSWEILER, W. 1992. The role of stress in population dynamics of forest pests. The case of the larch bud moth (*Zeiraphera diniana* Gn. Lep., Tortricidae), pp. 2–13, *in* IUFRO Conference S.2.07-06 "Population Dynamics," Zakopane, September 1991. Instytut badawczy Lesnictwa, Warszawa.

BALTENSWEILER, W. 1993. A contribution to the explanation of the larch bud moth cycle, the polymorphic fitness hypothesis. *Oecologia* 93:251–255.

BALTENSWEILER, W., BENZ, G., BOVEY, P., and DELUCCI, V. 1977. Dynamics of larch bud moth populations. *Annu. Rev. Entomol.* 22:79–100.

BALTENSWEILER, W. and PRIESNER, E. 1988. A study of pheromone polymorphism in *Zeiraphera diniana* Gn. (Lep., Tortricidae). 3. Specificity of attraction to synthetic pheromone sources by different male-response types from 2 host races. *J. Appl. Entomol.* 106:217–231.

BALTENSWEILER, W. and RUBLI, D. 1999. Dispersal: An important driving force of the cyclic population dynamics of the larch bud moth, *Zeiraphera diniana* Gn. *For. Snow Landsc. Res.* 74:3–153.

BALTENSWEILER, W. and VON SALIS, G. 1975. Long range dispersal of the larch bud moth (*Zeiraphera diniana* Gn., Tortricidae). *J. Appl. Entomol.* 3:251–257.

BERLOCHER, S. H. and FEDER, J. L. 2002. Sympatric speciation in phytophagous insects: Moving beyond controversy? *Annu. Rev. Entomol.* 47:773–815.

BERNAYS, E. A. and GRAHAM, M. 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* 69:886–892.

BOUGHTON, D. A. 2000. The dispersal system of a butterfly: A test of source–sink theory suggests the intermediate-scale hypothesis. *Am. Naturalist* 156:131–144.

BOVEY, P. and MAKSYMOV, J. K. 1959. Le problème des races biologiques chez la tordeuse grise du mélèze *Zeiraphera griseana* (HB). *Vierteljahrsschr. Naturforsch. Ges. Zürich* 104:264–274.

BYRNE, K. J., GORE, W. E., PEARCE, G. T., and SILVERSTEIN, R. M. 1975. Porapak-Q collection of airborne organic compounds serving as models for insect pheromones. *J. Chem. Ecol.* 1:1–7.

CARLE, S. A., AVERILL, A. L., RULE, G. S., REISSIG, W. H., and ROELOFS, W. L. 1987. Variation in host fruit volatiles attractive to apple maggot fly, *Rhagoletis pomonella*. *J. Chem. Ecol.* 13:795–805.

DRÈS, M. 2000. Gene flow between host races of the larch bud moth, *Zeiraphera diniana* (Lepidoptera: Tortricidae), PhD Dissertation. University College, London.

EHRLICH, P. R. and RAVEN, P. H. 1964. Butterflies and plants: A study in coevolution. *Evolution* 18: 586–608.

EMELIANOV, I., DRÈS, M., BALTENSWEILER, W., and MALLET, J. 2001. Host induced assortative mating in host races of the larch bud moth. *Evolution* 55:2002–2010.

EMELIANOV, I., SIMPSON, F., NORANG, P., and MALLET, J. 2003. Host choice promotes reproductive isolation between host races of the larch birdmoth *Zeiraphera diniana*. *J. Evol. Biol.* 16:208–218.

EMELIANOV, I., MALLET, J., and BALTENSWEILER, W. 1995. Genetic differentiation in the larch bud moth *Zeiraphera diniana* (Lepidoptera: Tortricidae): Polymorphism, host races or sibling species? *Heredity* 75:416–424.

FEIN, B. L., REISSIG, W. H., and ROELOFS, W. L. 1982. Identification of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella* (Diptera, Tephritidae). *J. Chem. Ecol.* 8:1473–1487.

FRY, J. D. 1996. The evolution of host specialization: Are trade-offs overrated? *Am. Naturalist* 148:S84–S107.

GUERENSTEIN, P. G. and GUERIN, P. M. 2001. Olfactory and behavioural responses of the blood-sucking bug *Triatoma infestans* to odors of vertebrate hosts. *J. Exp. Biol.* 204:585–597.

GUERIN, P., BALTENSWEILER, E., ARN, H., and BUSER, H. 1984. Host race pheromone polymorphism in the larch bud moth. *Experientia* 40:892–894.

JEFFRIES, M. J. and LAWTON, J. H. 1984. Enemy free space and the structure of ecological communities. *Biol. J. Linn. Soc.* 23:269–286.

KHOMENTOVSKY, P. A., BALTENSWEILER, W., and MARYCHEVA, E. 1997. The first record of an outbreak of the larch bud moth, *Zeiraphera diniana* Gn. (Lep., Tortricidae) on an evergreen conifer host (*Pinus pumila* [Pall.] Regel) in North Eastern Asia. *J. Appl. Entomol.* 121:1–7.

MCMAHON, C., GUERIN, P. M., and SYED, Z. 2001. 1-Octen-3-ol isolated from bont ticks attracts *Amblyomma variegatum. J. Chem. Ecol.* 27:471–486.

NORIN, T. 1972. Some aspects of the chemistry of the order Pinales. *Phytochemistry* 11:1231–1242.

PRIESNER, E. 1979. Specificity studies on pheromone receptors of noctuid and tortricid Lepidoptera, pp. 57–71, *in* F. J. Ritter (ed.). Chemical Ecology: Odor Communication in Animals. Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands.

PRIESNER, E. and BALTENSWEILER, W. 1987. A study of pheromone polymorphism in *Zeiraphera diniana* Gn. (Lep., Tortricidae). 2. Pheromonal response types in F1 hybrids between three host races. *J. Appl. Entomol*. 104:433–448.

RAPPAPORT, N., JENKINS, M. J., and ROQUES, A. 1996. Cone and foliage volatiles from Douglas-fir and European Larch: Relationship to attack by cone and seed insects, pp. 57–79, *in* G. L. DeBarr, A. Roques, J. H. Sun, and J. J. Turgeon (eds.). Proc. 4th Int. Conf. on Cone and Seed Insects Working Party (IUFRO S2.07-01), Beijing and Harbin. USDA For. Serv. Southeast. For. Exp. Stn., Athens, Georgia.

SCHNEIDER, D. 1957. Elektrophysiologische Untersuchungen von Chemo- und Mechanorezeptoren der Antenne des Seidenspinners *Bombyx mori* L. *Z. Vergl. Physiol.* 40:8–41.

STEULLET, P. and GUERIN, P. M. 1994. Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae). I. Receptors within the Haller's organ capsule. *J. Comp. Physiol. A* 174:27–38.