# Geographic Variation in Pheromone Chemistry, Antennal Electrophysiology, and Pheromone-Mediated Trap Catch of North American Populations of the Obliquebanded Leafroller

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**ABSTRACT** The total and relative amounts of (Z)-11-tetradecenyl acetate (Z11-14:Ac), (E)-11-tetradecenyl acetate (E11-14:Ac), (Z)-11-tetradecen-1-ol (Z11-14:OH) and (Z)-11-tetradecenal (Z11-14:Al), and the EAG response of male antennae to these pheromone gland compounds were compared in laboratory reared *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae) from British Columbia, Michigan, Ontario, New York, and Quebec. A field trapping experiment was conducted in each of these locations to determine the effect of Z11-14:Al on the numbers of moths captured. The amount of each of the four pheromone-gland compounds declined successively in moths from British Columbia, Quebec, Ontario, Michigan, and New York. The relative amount of Z11-14:Ac was greatest in moths from New York and smallest in moths from Ontario, whereas the relative amount of E11-14:Ac was greatest in moths from Ontario and smallest in moths from British Columbia. Moths from Ontario, Quebec, British Columbia, Michigan, and New York contained decreasing relative amounts of Z11-14:OH and Z11-14:Al. There was a trend of increasing antennal sensitivity to each of the four pheromone-gland compounds in moths from New York, Michigan, Ontario, Quebec, and British Columbia. The addition of 1% Z11:Al to a three compound blend of Z11-14:Ac, E11-14:Ac and Z11-14:OH (97:2:1) resulted in a >twofold increase in average trap catch in British Columbia, Ontario, and Quebec; this compound had no effect on trap catch in Michigan or New York.

**KEY WORDS** obliquebanded leafroller, *Choristoneura rosaceana*, geographic variation, pheromone chemistry, trap catch

THE OBLIQUEBANDED LEAFROLLER, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), is native to North America where it is distributed throughout southern Canada and most areas of the continental United States, except the arid southwest (Chapman and Lienk 1971). The larvae of *C. rosaceana* are polyphagous, feeding on the foliage and fruit of many deciduous plants, although its primary hosts typically belong to the Rosaceae (Weires and Riedl 1991). *C. rosaceana* has one or two generations per year, depending on climate, and overwinters as a second- or third-instar larva in a hibernaculum (Chapman and Lienk 1971). Organophosphorus insecticides have been used to prevent economic injury by *C. rosaceana* in commercial apple orchards (Reissig 1978), but their effectiveness has been reduced because populations of this pest have developed resistance to these compounds (Reissig et al. 1986, Smirle et al. 1998, Pree et al. 2001).

The sex pheromone of *C. rosaceana* was identified as (Z)-11-tetradecenyl acetate (Z11-14:Ac) (Roelofs and Tette 1970). Subsequent studies revealed the presence of three additional compounds: (*E*)-11-tetradecenyl acetate (E11-14:Ac), (Z)-11-tetradecen-1-ol (Z11-14:OH) and (Z)-11-tetradecenal (Z11-14:Al) (Cardé et al. 1977, Hill and Roelofs 1979, Vakenti et al. 1988). Z11-14:Al was found only in females from the Okanagan Valley of British Columbia (Vakenti et al. 1988). Thomson et al. (1991) found that the addition of Z11-14:Al to lures containing a three-component blend of Z11-14:Ac, E11-14:Ac and Z11-14:OH significantly increased the capture of moths in traps in British Columbia but not in Quebec, and they proposed that the pheromone of western populations of *C. rosaceana* contained four components whereas the

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pheromone of eastern populations contained three components.

The first evidence for the occurrence of Z11-14:Al in an eastern population of *C. rosaceana* was provided by El-Sayed et al. (2001a) who found that trap catch in an apple orchard increased by >50% with the addition of 0.02 mg of Z11-14:Al to lures containing 1.0 mg of a 100:2:1.5 blend of Z11-14:Ac, E11-14:Ac and Z11-14:OH. They recommended using a lure containing a four-component blend of Z11-14:Ac, E11-14:Ac, Z11-14:OH, and Z11-14:Al in a ratio of 100:2:1.5:1 (Vakenti et al. 1988) when monitoring *C. rosaceana* in apple orchards in the Niagara peninsula of Ontario.

In this article, we provide chemical, electrophysiological, and field trapping evidence for the occurrence of Z11-14:Al in several other eastern North American populations of *C. rosaceana.* The total and relative amounts of the four pheromone gland compounds and the response of male antennae to these compounds was compared in laboratory colonies of moths that were established from larvae collected in Michigan, New York, Ontario, Quebec, and British Columbia. A field trapping experiment was conducted in each of these locations to determine the effect of Z11-14:Al on the numbers of moths captured.

## Materials and Methods

**Insects.** Insecticide-susceptible female moths were obtained from colonies that were established from larvae collected in Lyons, NY (Lawson et al. 1997, Waldstein and Reissig 2000), Queenston, Ontario (El-Sayed et al. 2001b), Fennville, MI (Ahmad et al. 2002), Ste. Foy, Quebec (Delisle and Vincent 2002), and Summerland, British Columbia (Pree et al. 2001, 2002). The colonies had been maintained in laboratories for 2 or more years, i.e., >20 generations. Larvae were reared on artificial diet (Shorey and Hale 1965, Smirle et al. 1998) and the colonies were maintained at 23°C and 60% RH using a 16L:8D photoperiod. Pupae were sexed (Guennelon et al. 1972) and held separately in 33 × 33 × 33 cm Plexiglas cages for adult emergence.

**Gas Chromatography-Mass Spectrometry Analysis (GC-MS).** Analysis of pheromone gland extracts and synthetic chemicals was performed with a Fisons MD 800 GC-MS (Fisons Instruments SpA, Milan) with a mass range of 30–260 m/z using a 30 m × 0.25 mm ID, DB-5MS fused silica capillary column (J & W Scientific, Folsom, CA). The temperature of the transfer line from the GC column to the MS ion source was 280°C. The ionization voltage was 70 eV, and the ion source temperature was 180°C. Samples were injected in split/splitless mode with split opened after 1 min. Helium was used as a carrier gas. The oven temperature was maintained at 80°C for 2 min, and then raised to 250°C at a rate of 10°C/min.

**Pheromone-Gland Extraction.** Pheromone glands were removed from 1-d-old calling females during the second hour of the scotophase (Delisle and Royer 1994, El-Sayed and Trimble 2002) and placed individually in the bottom of a 0.25 ml-capacity, glass insert vial (Kimble Glass Inc., Vineland, NJ) and extracted in 5 μl of high performance liquid chromatography (HPLC) grade hexane (Aldrich, Milwaukee, WI) for 3–5 min. The solvent was allowed to evaporate in a fume hood until 2–3 μl remained, and it was then immediately subjected to GC-MS analysis. The glands of 50 females from each location were extracted and analyzed.

The external standard method (Scott 1995) was used to calibrate the GC-MS. Known amounts (0.1, 1.0, 10.0, and 100.0 ng) of each compound were injected into the GC-MS using a Fisons A2005 (Fisons Instruments SpA, Milan) autosampler. The results were subjected to linear regression analysis (SAS Institute Inc. 1998) to determine the relationship between amount of compound injected and the peak area of the chromatogram.

The amount of each compound lost during the extraction and injection process was estimated using an HPLC grade hexane solution containing 10 ng/μl of each of the four pheromone compounds. Five microliters of this solution was placed in the extraction vial and then immediately removed and analyzed. Another 5 μl of solution was allowed to remain in the vial for 5 min before analysis. This process was repeated on ten occasions. The mean (±SD) percentage loss of each compound was: Z11-14:Ac, 4.0 ± 2.4; E11-14:Ac, 5.4 ± 2.9; Z11-14:OH, 4.7 ± 2.8; Z11-14:Al, 5.8 ± 2.5. The amounts of pheromone in glands were computed without correcting for the small loss that may have occurred during extraction and injection.

**Purification and Synthesis of Pheromone Gland Compounds.** Chromatography in silica gel (Merck 60) impregnated with silver nitrate ($AgNO_3$) was used to purify Z11-14:OH (Bedoukian Research Inc., Danbury, CT). Isomeric purity was >99.9%. NMR in $CDCl_3$ at 400 MHz: $\delta_H$ 5.35 (2H, m), 3.60 (2H, t), 2.02 (4H, m), 1.60 (2H, q), 1.30 (14H, m), 0.95 (3H, t). $^{13}C$ NMR in $CDCl_3$ at 100 Mhz: $\delta_C$ 14.80, 20.92, 26.15, 27.50, 29.69, 29.84, 29.93, 29.97, 30.01, 30.19, 33.24, 63.52, 129.75, 131.94. GC-MS (m/z): 252 (M+), 233, 207, 194, 149, 121, 109, 95, 81, 67, 55 (100%).

Pyridinium dichromate (7.08 g, 18.82 mmol) in $CH_2Cl_2$ was used to oxidize Z11-14:OH (2.00 g, 9.41 mmol) (Bedoukian) (Santangelo et al. 2002). The yield after medium pressure liquid chromatography on silica gel (MPLC) (Baeckström et al. 1987) was 1.38 g (70%) of Z11-14:Al. Isomeric purity was 94.9%. NMR: $\delta_H$ 9.80 (1H, s), 5.35 (2H, m), 2.40 (2H, t), 2.00 (4H, m), 1.60 (2H, q), 1.50–1.90 (12H, m), 0.95 (3H, t). GC-MS (m/z): 210 (M+), 192, 163, 149, 135, 121, 111, 98, 83, 81, 69, 55, 41 (100%), 33.

Z11-14:OH (6.5 g, 30 mmol) (Bedoukian) was acetylated by acetic anhydride (8 ml) in pyridine (16 ml) (Unelius et al. 1998). MPLC provided 6.1 g (80%) of Z11-14:Ac in an isomeric purity of 96.2%. NMR: $\delta_H$ 5.35 (2H, m), 4.05 (2H, t), 2.00 (3H, s), 2.00 (4H, m), 1.60 (2H, q), 1.30 (14H, m), 0.95 (3H, t). GC-MS (m/z): 254 (M+), 225, 194, 166, 152, 138, 124, 110, 96, 82 (100%), 68, 55, 43.

Argentation chromatography yielded E11-14:Ac (Bedoukian) with an isomeric purity of >99.9%. NMR:

$\delta_H$ 5.35 (2H, m), 4.10 (2H, t), 2.10 (3H, s), 2.00 (4H, m), 1.65 (2H, q), 1.30 (14H, m), 0.95 (3H, t). GC-MS (m/z): 254 (M+), 194, 166, 152, 138, 123, 109, 96, 82 (100%), 68, 61, 55.

**Electroantennography.** The depolarization of antennae was measured using a Syntech High-Resistance EAG Probe, Type ID-02 Signal Interface Box and Type IDAC-02 Intelligent Data Acquisition Controller (Syntech, Hilversum, Netherlands). Syntech electroantennogram software was used to filter, amplify, visualize, and store antennal depolarization data on a personal computer. The antennae of 2- to 3-d-old males were excised at their base and attached to the electrodes of the probe using Spectra 360 Electrode Gel (Parker Laboratories, Inc., Orange, NJ). Several segments were removed from the distal end of the antenna to facilitate conductivity. Each antennal preparation was continuously exposed to a humidified and charcoal-filtered airstream (0.5 liters/min) supplied through a glass tube (6 mm ID, 9 mm OD). Four amounts, 0.1, 1.0, 10.0, or 100.0 ng of Z11-14:Ac, E11-14:Ac, Z11-14:OH, and Z11-14:Al were tested. Test compounds were applied to a 1-$cm^2$ piece of Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, England) in 10 $\mu$l of HPLC-grade hexane. Ten microliters of hexane was applied to a filter paper square for use as a control stimulus. The filter paper squares were placed in Pasteur pipettes after the solvent had evaporated. Each antenna was first stimulated with the control, and then with increasing concentrations of one compound. The stimuli were delivered in 0.5 s pulses at an airflow rate of 0.5 ml/min using a Syntech Type CS-01 Stimulus Controller. A 60-s recovery period followed application of a stimulus. Pasteur pipettes and filter paper squares were renewed after 4 h of use. The four concentrations of one compound were administered to each of 10 antennae.

**Field Trapping Experiment.** The relative attractiveness of lures containing a 97:2:1 blend of Z11-14:Ac, E11-14:Ac and Z11-14:OH and increasing amounts of Z11-14:Al was tested during the first *C. rosaceana* flight of 2001 in a mixed deciduous woodlot in Ste. Foy, Quebec (15 June–15 July), experimental and commercial apple orchards near Geneva, NY (1–15 June), experimental apple orchards in Jordan Station, Ontario (9 June–6 July) and Fennville, MI (11 June–9 July), and experimental and commercial apple orchards near Summerland, British Columbia (20 June–18 July). The relative attractiveness of five blends was compared: (1) 0.97 mg Z11-14:Ac, 0.02 mg E11-14:Ac, 0.01 mg Z11-14:OH; and (2) blend one plus 0.01 (Vakenti et al. 1988); (3) 0.02; (4) 0.04; and (5) 0.08 mg of Z11-14:Al. The blends of synthetic compounds were applied to the large "well" of natural rubber sleeve stoppers (Chromatographic Specialties, Brockville, Ontario) in 200 $\mu$l of analytical grade hexane (Fisher, Ottawa, Ontario). The solvent was allowed to evaporate in a fume hood and the stoppers were stored until use at −20°C.

A split-plot design (Snedecor and Corchran 1967) was used to compare the relative attractiveness of the five blends. Three plots were located either within one orchard (Ontario and Michigan), or woodlot (Quebec), and separated by at least 100 m, or in separate orchards (New York and British Columbia), again separated by at least 100 m. Five trap lines were established at ≈20-m intervals in each plot and five "trapping stations" were established at ≈20-m intervals in each trap line. Each of the five treatments was randomly assigned to a trapping station within a trap line. White plastic delta traps (Cooper Mill Ltd., Madoc, Ontario) each containing a pheromone-impregnated stopper were placed ≈1.5–2.0 m from the ground within a tree at each trapping station. Trapped moths were removed and counted every 2–7 d and stoppers were renewed after 2 wk of exposure.

**Statistical Analyses.** The significance of between-location differences in the mean total and mean relative amounts of each pheromone gland compound in gland extracts was tested using the Kruskal–Wallis test (SAS Institute Inc. 1998). Significantly different means were identified using a nonparametric Student–Newman–Keuls test (Zar 1974). Linear regression analysis was used to describe the relationship between antennal depolarization and stimulus concentration (SAS Institute Inc. 1998). The significance of location on the slope and intercept of the antennal response-stimulus concentration relationship was tested using analysis of covariance (ANCOVA) (SAS Institute Inc. 1998). Significantly different slopes and intercepts were identified using Fisher's Protected Least Significant Difference test (SAS Institute Inc. 1998). The variance of the mean total number of moths captured using each pheromone blend was stabilized using the $\sqrt{x}$ transformation and the significance of treatment effect was tested using a split-plot analysis of variance (ANOVA). Significantly different treatment means were identified using Fisher's Protected Least Significant Difference test (SAS Institute Inc. 1998).

## Results

The pheromone glands of British Columbia moths contained the greatest, and the glands of New York moths the smallest amount of each of the four pheromone gland compounds (Table 1). The pheromone glands of moths from Quebec, Ontario and Michigan contained decreasing amounts of the four compounds (Table 1). The relative amount of the main pheromone gland compound, Z11-14:Ac, was greatest in moths from New York and smallest in moths from Ontario (Table 2). The relative amounts of this compound were similar in moths from British Columbia, Michigan and Quebec (Table 2). The relative amount of E11-14:Ac was similar in moths from Ontario and New York, and in moths from Michigan and Quebec, and greater than in moths from British Columbia (Table 2). Moths from Ontario, Quebec, British Columbia, Michigan, and New York contained decreasing relative amounts of Z11-14:OH and Z11-14:Al (Table 2).

There was a positive linear relationship between antennal depolarization and the $\log_{10}$ concentration of

**Table 1. Amount (mean ± SD) of four pheromone gland compounds in *C. rosaceana* from five locations in North America**

| Location[a] | Amount, ng[b] | | | |
|---|---|---|---|---|
| | Z11-14:Ac | E11-14:Ac | Z11-14:OH | Z11-14:Al |
| BC | 57.10 ± 21.84a | 0.94 ± 0.72a | 0.77 ± 0.46a | 0.18 ± 0.10a |
| PQ | 42.21 ± 18.31b | 0.69 ± 0.29b | 0.55 ± 0.46b | 0.13 ± 0.09b |
| ON | 35.74 ± 23.88c | 0.65 ± 0.55c | 0.54 ± 0.30c | 0.12 ± 0.06c |
| MI | 27.16 ± 11.45d | 0.50 ± 0.40d | 0.36 ± 0.25d | 0.05 ± 0.06d |
| NY | 14.06 ± 5.60e | 0.26 ± 0.18e | 0.12 ± 0.04e | 0.01 ± 0.01e |

[a] BC = British Columbia; PQ = Quebec; ON = Ontario; MI = Michigan; NY = New York.
[b] $n = 50$.
Means in a column followed by the same letter are not significantly different (nonparametric Student-Newman-Keuls test, $P > 0.05$).

each pheromone gland compound in moths from British Columbia (Z11-14:Ac, $F = 60.5$, $r^2 = 0.51$; E11-14:Ac, $F = 102.8$, $r^2 = 0.64$; Z11-14:OH, $F = 41.8$, $r^2 = 0.42$; Z11-14:Al, $F = 86.7$, $r^2 = 0.60$), Michigan (Z11-14:Ac, $F = 69.8$, $r^2 = 0.55$; E11-14:Ac, $F = 79.8$, $r^2 = 0.58$; Z11-14:OH, $F = 23.4$, $r^2 = 0.29$; Z11-14:Al, $F = 71.4$, $r^2 = 0.55$), Ontario (Z11-14:Ac, $F = 47.9$, $r^2 = 0.45$; E11-14:Ac, $F = 68.7$, $r^2 = 0.54$; Z11-14:OH, $F = 56.0$, $r^2 = 0.49$; Z11-14:Al, $F = 55.5$, $r^2 = 0.49$), New York (Z11-14:Ac, $F = 50.8$, $r^2 = 0.47$; E11-14:Ac, $F = 54.2$, $r^2 = 0.48$; Z11-14:OH, $F = 87.4$, $r^2 = 0.60$; Z11-14:Al, $F = 154.4$, $r^2 = 0.73$); and Quebec (Z11-14:Ac, $F = 29.9$, $r^2 = 0.34$; E11-14:Ac, $F = 35.1$, $r^2 = 0.38$; Z11-14:OH, $F = 63.5$, $r^2 = 0.52$; Z11-14:Al, $F = 79.9$, $r^2 = 0.58$) ($P < 0.0001$, df = 1, 58 for each compound at all five locations). The slope of the concentration-response relationship was similar in moths from each of the five locations when Z11-14:Ac ($F = 1.1$, df = 4, 290, $P = 0.37$), Z11-14:OH ($F = 1.5$, df = 4, 290, $P = 0.19$) and Z11-14:Al ($F = 0.4$, df = 4, 290, $P = 0.78$) were used as stimuli; there was a trend of decreasing slope in moths from New York, Michigan, Ontario, Quebec, and British Columbia when E11-14:Ac ($F = 3.0$, df = 4, 290, $P = 0.02$) was used as a stimulus (Table 3). There was a trend of progressively smaller intercepts in the concentration-response relationship in moths from New York, Michigan, Ontario, Quebec, and British Columbia when Z11-14:Ac ($F = 4.8$, df = 4, 294, $P = 0.001$), E11-14:Ac ($F = 11.6$, df = 4, 294, $P = < 0.0001$), Z11-14:OH ($F = 28.9$, df = 4, 294, $P = < 0.0001$) and Z11-14:Al ($F = 10.9$, df = 4, 294, $P < 0.0001$) were used as stimuli (Table 3).

The addition of Z11-14:Al to lures containing Z11-14:Ac, E11-14:Ac and Z11-14:OH affected the average total number of moths captured in British Columbia (Treatment, $F = 4.5$, df = 4, 60, $P = 0.003$; Block, $F = 72.6$, df = 2, 60, $P < 0.0001$; Treatment × Block, $F = 0.8$, df = 8, 60, $P = 0.63$); Ontario (Treatment, $F = 3.9$, df = 4, 60, $P = 0.007$; Block, $F = 1.1$, df = 2, 60, $P = 0.35$; Treatment × Block, $F = 0.3$, df = 8, 60, $P = 0.98$); and Quebec (Treatment, $F = 6.5$, df = 4, 60, $P = 0.0002$; Block, $F = 2.7$, df = 2, 60, $P = 0.08$; Treatment × Block, $F = 0.3$, df = 8, 60, $P = 0.96$), but not in Michigan (Treatment, $F = 1.3$, df = 4, 60, $P = 0.27$; Block, $F = 25.7$, df = 2, 60, $P < 0.0001$; Treatment × Block, $F = 0.5$, df = 8, 60, $P = 0.86$) or New York (Treatment, $F = 0.7$, df = 4, 60, $P = 0.61$; Block, $F = 170.5$, df = 2, 60, $P < 0.0001$; Treatment × Block, $F = 1.1$, df = 8, 60, $P = 0.37$). In British Columbia and Quebec, increasing the amount of Z11-14:Al from 1 to 8% relative to the main pheromone compound, Z11-14:Ac, did not affect the average number of moths captured, whereas in Ontario, the average number of moths captured in traps baited with lures containing one and 2% Z11-14:Al was greater than the number captured in traps baited with 8% Z11-14:Al (Table 4).

## Discussion

Two types of intra-specific geographic variation in the pheromone chemistry of moths have been observed (Klun and Cooperators 1975, Kochansky et al. 1975, Guerin et al. 1984, Hansson et al. 1990, Toth et al. 1993). "Monomorphic" variation occurs when geographically isolated populations of the same species use the same compounds but in different ratios, whereas "polymorphic" variation occurs when different populations use different compounds in their pheromone (Löfstedt 1990). Coevolution of male pheromone reception is often associated with geo-

**Table 2. Relative amount (mean ± SD) of four pheromone gland compounds in *C. rosaceana* from five locations in North America**

| Z11-14:Ac | | E11-14:Ac | | Z11-14:OH | | Z11-14:Al | |
|---|---|---|---|---|---|---|---|
| Location[a] | Relative amount, %[b] | Location | Relative amount, % | Location | Relative amount, % | Location | Relative amount, % |
| NY | 97.21 ± 1.18a | ON | 2.33 ± 2.29a | ON | 2.20 ± 2.33a | ON | 0.53 ± 0.68a |
| BC | 96.58 ± 1.38b | NY | 1.85 ± 0.96a | PQ | 1.80 ± 1.99b | BC | 0.32 ± 0.17b |
| MI | 96.54 ± 2.08b | MI | 1.83 ± 1.19b | BC | 1.53 ± 1.11c | PQ | 0.30 ± 0.18c |
| PQ | 96.11 ± 2.57b | PQ | 1.78 ± 0.96b | MI | 1.44 ± 1.13d | MI | 0.19 ± 0.27d |
| ON | 94.94 ± 3.71c | BC | 1.58 ± 0.64c | NY | 0.92 ± 0.46e | NY | 0.03 ± 0.05e |

[a] BC = British Columbia; PQ = Quebec; ON = Ontario; MI = Michigan; NY = New York.
[b] $n = 50$.
Means in a column followed by the same letter are not significantly different (nonparametric Student-Newman-Keuls test, $P > 0.05$).

**Table 3. Slopes and intercepts of linear relationships between the amount of pheromone in stimulus and electroantennogram response in *C. rosaceana* from five locations in North America**

| Location[a] | Z11-14:Ac | | E11-14:Ac | | Z11-14:OH | | Z11-14:Al | |
|---|---|---|---|---|---|---|---|---|
| | Slope | Intercept | Slope | Intercept | Slope | Intercept | Slope | Intercept |
| NY | 0.120a | 0.200a | 0.110a | 0.183a | 0.067a | 0.175a | 0.106a | 0.188a |
| MI | 0.104a | 0.176ab | 0.092b | 0.136b | 0.053a | 0.113b | 0.103a | 0.156b |
| ON | 0.095a | 0.163bc | 0.077bc | 0.120bc | 0.050a | 0.084c | 0.092a | 0.122c |
| PQ | 0.087a | 0.148bc | 0.069bc | 0.112c | 0.044a | 0.080c | 0.092a | 0.108c |
| BC | 0.084a | 0.129c | 0.063c | 0.096c | 0.044a | 0.075c | 0.091a | 0.103c |

[a] BC = British Columbia; PQ = Quebec; ON = Ontario; MI = Michigan; NY = New York.
Means in a column followed by the same letter are not significantly different (Fisher's Protected Least Significant Difference test, $P > 0.05$).

graphic variation in pheromone chemistry (Löfstedt 1990). One well-documented example of monomorphic variation occurs in the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Klun and Cooperators 1975). The pheromone consists of Z11-14:Ac and E11-14:Ac and throughout eastern North America and Europe there are stains that respond preferentially to a 97:3 or a 3:97 blend of the Z:E isomers. Heterogeneity of male response is greatest in regions where the two pheromone strains occur sympatrically (Klun and Cooperators 1975, Kochansky et al. 1975). The male antennae of the Z-strain contain two types of receptor neurons, the first type responds to the Z isomer with a large electroantennograph (EAG) spike amplitude, whereas the second type responds to the E isomer with a low EAG spike amplitude. In contrast, antennae of E-strain males have a receptor that responds to the E isomer with a large EAG spike amplitude, and another that responds to the Z isomer with low EAG spike amplitude (Hansson et al. 1987). Additional examples of monomorphic pheromone variation are documented for the turnip moth, *Agrotis segetum* (Schiff) (Lepidoptera: Noctuidae) (Arn et al. 1983, Subchev et al. 1986, Hansson et al. 1990), the common sheep moth, *Hemileuca eglanterina* Boisduval (Lepidoptera: Saturniidae) (McElfresh and Millar 1999, 2001) and the black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) (Gemeno et al. 2000).

Pheromone polymorphism is rare in moths. One example occurs in the larch budmoth, *Zeiraphera diniana* Guenée (Leptidoptera: Tortricidae) where one race feeding on larch, *Larix decidua* Miller uses E11-14:Ac for male attraction, and another race feeding on cembrian pine, *Pinus cembra* L. uses (E)-9-dodecenyl acetate (E9–12:Ac) for male attraction (Guerin et al. 1984). This pheromone polymorphism is associated with monomorphic variation in the sensitivity of male antennae. Males of the larch race are 100-fold more sensitive to E11-14:Ac than to E9–12:Ac, whereas males of the cembrian pine race are 10-fold more sensitive to E9–12:Ac than E11-14:Ac (Priesner and Baltensweiler 1987).

The geographically diverse populations of *C. rosaceana* that we studied exhibited monomorphic variation in their pheromone because moths from all five locations contained the same four compounds but in different amounts and ratios. This variation could also have been due to differences in adult size, temporal variation in pheromone production, insecticide resistance, and variable selection associated with the process of laboratory colonization. The weight of females was not recorded and therefore we could not test for a relationship between mass and pheromone content; however, Delisle and Vincent (2002) found no relationship between pupal mass and the amount of Z11-14:Ac in the pheromone glands of *C. rosaceana.* In addition, Miller and Roelofs (1980) found no relationship between body weight and pheromone content in the redbanded leafroller, *Argyotaenia velutinana* (Walker) (Lepidoptera: Tortricidae). The amount of pheromone in the glands of *C. rosaceana* varies with time of day and age (Delisle and Royer 1994, Delisle and Vincent 2002, El-Sayed and Trimble 2002). Interpopulation variation in the relationship between pheromone content and time of day and/or age could

**Table 4. Total number (mean ± SD) *C. rosaceana* captured in traps baited with lures containing different amounts of Z11-14:Al at five locations in North America**

| Amount Z11-14:Al (%)[a] | Location | | | | |
|---|---|---|---|---|---|
| | BC[b] | MI | ON | NY | PQ |
| 0 | 3.9 ± 7.0 (59)[c]b | 15.8 ± 15.0 (238)a | 16.9 ± 9.0 (253)b | 23.3 ± 28.3 (349)a | 18.3 ± 11.3 (275)b |
| 1 | 19.6 ± 37.9 (294)a | 28.9 ± 24.4 (434)a | 35.7 ± 19.8 (536)a | 30.5 ± 38.9 (458)a | 68.7 ± 57.3 (1,031)a |
| 2 | 22.8 ± 50.7 (342)a | 18.5 ± 16.0 (278)a | 41.6 ± 41.8 (624)a | 32.2 ± 40.1 (483)a | 86.9 ± 44.1 (1,304)a |
| 4 | 21.6 ± 38.4 (324)a | 21.2 ± 18.6 (318)a | 24.3 ± 19.1 (365)ab | 33.4 ± 38.7 (501)a | 54.8 ± 30.4 (822)a |
| 8 | 18.5 ± 31.2 (278)a | 26.8 ± 31.9 (402)a | 18.8 ± 15.9 (282)b | 25.5 ± 37.0 (383)a | 57.6 ± 32.2 (864)a |
| Total moths captured | 1,297 | 1,670 | 2,060 | 2,174 | 4,296 |

[a] Percentage relative to 1.0 mg of a 97:2:1 blend of Z11-14:Ac, E11-14:Ac and Z11-14:OH.
[b] BC = British Columbia; PQ = Quebec; ON = Ontario; MI = Michigan; NY = New York.
[c] Total number moths captured.
Means in a column followed by the same letter are not significantly different (Fisher's Protected Least Significant Difference test, $P > 0.05$).

contribute to variation in the pheromone content of moths from different locations. Azinphosmethyl-resistant *C. rosaceana* contain less pheromone than azinphosmethyl-susceptible *C. rosaceana* (Delisle and Vincent 2002, El-Sayed and Trimble 2002, El-Sayed et al. 2001b), but resistance to insecticides is unlikely to have contributed to the geographic variation in pheromone content that we observed because all of the populations studied were insecticide susceptible (Lawson et al. 1997, Waldstein and Reissig 2000, El-Sayed et al. 2001b, Pree et al. 2001, Ahmad et al. 2002, Delisle and Vincent 2002, Pree et al. 2002). Field collected *A. segetum* were found to contain more pheromone than laboratory reared moths (Svensson et al. 1997). In addition, field collected redbanded leafrollers, *A. velutinana*, contained more pheromone than laboratory reared leafrollers, and the average ratio of two pheromone components, E11-14:Ac and Z11-14:Ac, differed significantly in females from the two sources (Miller and Roelofs 1980). Therefore, it may be possible that the process of laboratory colonization could have contributed to the monomorphic variation that we observed in different populations of *C. rosaceana.*

The slope of an EAG concentration-response relationship describes the rate of increase in response when an antenna is stimulated with increasing amounts of a pheromone compound. There was no difference in the slopes of the concentration-response relationships of the five populations when using Z11-14:Ac, Z11-14:OH, and Z11-14:Al as stimuli, suggesting a similarity in responsiveness to these compounds. By contrast, the trend of decreasing slope in the concentration-response relationship in moths from New York, Michigan, Ontario, Quebec, and British Columbia when using E11-14:Ac as a stimulus suggests a decreasing responsiveness to this compound. The intercept of an EAG concentration-response relationship is the theoretical minimum amount of compound required to ellicit a response from an antenna, and provides a measure of the "sensitivity" of the antenna to a compound. The trend of progressively smaller intercepts in the concentration-response relationships in moths from New York, Michigan, Ontario, Quebec, and British Columbia when Z11-14:Ac, E11-14:Ac, Z11-14:OH, and Z11-14:Al were used as stimuli suggests that the antennae of moths from British Columbia had the greatest sensitivity to these compounds, and the antennae of moths from Michigan and New York had the lowest sensitivity to these compounds. Interestingly, the pheromone glands of females from Michigan and New York contained the smallest relative amounts of Z11-14:Al, and the addition of this compound to lures containing a three compound blend of Z11-14:Ac, E11-14:Ac and Z11-14:OH (97:2:1) had no effect on trap catch in these locations. These results suggest that the ability of male *C. rosaceana* to respond to Z11-14:Al has coevolved with the ability of females to produce this compound.

We recommend the use of a three compound blend of Z11-14:Ac, E11-14:Ac, and Z11-14:OH (97:2:1) with the addition of either one or 2% Z11-14:Al for monitoring *C. rosaceana* in North America. The addition of only 1% Z11-14:Al to the three compound blend resulted in a >two-fold increase in trap catch in British Columbia, Ontario, and Quebec. Pheromone lures prepared using Z11-14:Al would also be suitable for use in Michigan and New York because this compound did not reduce trap catch of *C. rosaceana* in these locations.

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