

Attraction of *Choristoneura Rosaceana* (Lepidoptera: Tortricidae) to Pheromone Blends in Ratios Produced in Females' Pheromone Gland or Emitted by the Females in Michigan Apple Orchards

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Research Article

Keywords: Pheromone blend, Pheromone emission, Pheromone gland, Attraction, Lure.

Posted Date: May 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-548468/v1>

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Abstract

Trap captures of obliquebanded leafroller, *Choristoneura rosaceana* (Harris) to pheromone blends in ratios approximating those reported in pheromone glands and a novel blend based on a volatile headspace collection from live virgin females were evaluated in field experiments in Michigan apple orchards. In an initial field trapping study, pheromone lures composed of either a three- or four-component blend approximation of the blend present in female pheromone glands at doses ranging from 0.1 to 20 mg/lure were compared. The four-component blend was a combination of (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac), (*E*)-11-tetradecenyl acetate (*E*11-14:Ac), (*Z*)-11-tetradecen-1-ol (*Z*11-14:OH), and (*Z*)-11-tetradecenal (*Z*11-14:Al) in a ratio of 96.5:1.8:1.4:0.2, respectively, while the three-component blend lacked *Z*11-14:Al. Pheromone emissions by groups of virgin females and commercial lures were collected in the laboratory and analyzed by gas chromatography. These data were used to formulate a new pheromone lure that was compared to a commercial lure in a second trapping study. In the first field study, traps baited with 10 mg pheromones or above captured significantly more moths than traps baited with 1 mg or less, regardless of the blend. Surprisingly, groups of virgin females only emitted two detectable pheromone components, *Z*11-14:Ac and *Z*11-14:OH in a ratio of 37:63 which was substantially different from the blends detected in pheromone glands in the literature. The newly formulated pheromone lure based on females' emission was more than twice as attractive as the commercial lure which emitted a 74:5:21 three-component blend of *Z*11-14:Ac, *E*11-14:Ac, and *Z*11-14:OH, indicating that the response of *C. rosaceana* to its pheromone was more strongly mediated by the pheromone quantity relative to the blend ratio.

Introduction

The obliquebanded leafroller, *Choristoneura rosaceana* (Harris), is native to North America and has become an important pest of apples, *Malus domestica* (L.) (Agnello et al. 1996); pears, *Pyrus communis* (L.) (Barnett et al. 1991); sweet cherry, *Prunus avium*; and tart cherries, *Prunus cerasus* (Long et al. 1997). In Michigan, the pest has two generations per year. Damage occurs when larvae feed on buds, leaves, and flowers in the spring and leaves and the surface of fruit in the summer. Larvae roll leaves and remain concealed unless feeding. Apples damaged by *C. rosaceana* during the first generation often fall to the ground, however feeding by the summer generation results in feeding scars on harvested fruit. In tart cherries, larvae that feed in webbed fruit clusters are often able to survive insecticide applications and can contaminate harvested fruit. This can lead to the rejection of whole truckloads of fruit valued at many thousands of dollars per load.

Monitoring *C. rosaceana* is accomplished using traps baited with synthetic sex pheromone. The sex pheromone of *C. rosaceana* females consists of a blend of a major sex pheromone component, (*Z*)-11-tetradecenyl acetate (*Z*11-14:Ac) (Roelofs and Tette 1970), and minor components: (*E*)-11-tetradecenyl acetate (*E*11-14:Ac), (*Z*)-11-tetradecenol (*Z*11-14:OH), or (*Z*)-11-tetradecenal (*Z*11-14:Al) (Hill and Roelofs 1979; Vakenti et al. 1988; El-Sayed et al. 2003). Multiple monitoring lures have been developed based on sex pheromone blends that differ both in the number of components present and their ratios (Solymar

1999). 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 was recommended to monitor *C. rosaceana* population from Ontario (Vakenti et al. 1988; El-Sayed et al. 2001). The same four-component blend but at a 100:4:5:2 ratio was used to capture males of *C. rosaceana* in Michigan, but the addition of Z11-14:Al had no effect on male catches (Stelinski et al. 2007). In Oregon, a 100:1:1:2 four-component blend captured more males than 100:4:1:2 and 100:10:1:2 blends (Stelinski et al. 2007). A three-component blend of Z11-14:Ac, E11-14:Ac, and Z11-14:OH in a ratio of 97:2:1 with an addition of either 1 or 2% Z11:14Al was proposed by El-Sayed et al. (2003) for monitoring *C. rosaceana* in North America.

Nevertheless, current pheromone blends are less than 50% as attractive compared to pheromone extracted from virgin females or calling females both in the laboratory (Trimble and Marshal 2008) and in the field (El-Sayed and Trimble 2002; Stelinski et al. 2004). Furthermore, as mentioned above, performance of the various lures differs regionally. Suboptimal lures greatly reduce the utility of monitoring programs as they may lead to underestimates of *C. rosaceana* populations, leading to management failures.

Mating disruption with synthetic pheromones presents an alternative to traditional, lethal insecticide-based *C. rosaceana* management. Mating disruption has been attempted for *C. rosaceana* for both western and eastern populations in North America with mixed results and little improvement achieved by increasing dispenser density or the use of different blends (Trimble and Appleby 2004; Stelinski et al. 2003, 2004). Lack of understanding regarding optimal pheromone blends is one potential explanation for inconsistent mating disruption.

Nearly all previous studies have developed *C. rosaceana* sex pheromone blend ratios based on the amounts of each component added to lure substrates. However, the blend ratio in the substrate may not reflect the actual ratio released into the atmosphere by the substrate. This is a relevant and crucial knowledge gap because male obliquebanded leafroller responds to “plumes” of pheromone in the atmosphere to orient to potential mates, traps, or attract and kill devices. Therefore, the aims of this study were (i) to re-evaluate whether the addition of Z11-14Al to a 3-component blend in a ratio produced by females’ pheromone gland as well as the blend dosages would affect *C. rosaceana* male captures in Michigan orchards; (ii) to quantify sex pheromone emissions by *C. rosaceana* females using a volatile capture system and use that information to develop a new pheromone lure; and (iii) to determine the effectiveness of this new lure on male captures in the field.

Methods And Materials

An initial field trapping experiment (Field Experiment 1: Effects of Blend Ratios and their Amounts on Male Captures) was conducted to determine whether pheromone blend ratio and amount affected trap captures in the field. This experiment was followed by laboratory evaluation (Laboratory Bioassay: Evaluation of Sex Pheromone Volatile Emissions) of the pheromone volatiles released by groups of laboratory reared females. Two subsequent field trapping experiments were conducted comparing the

efficacy of newly formulated lures (Field Experiment 2: Evaluation of New Pheromone Blends with and without *E11-14:Ac* and Field Experiment 3: Comparison of Male Captures between New Lures and Commercial lures).

Insect Colonies. *C. rosaceana* adults were obtained from a laboratory colony originated from unsprayed orchards in Fennville, Michigan and maintained at the Michigan State University (East Lansing, MI, USA). Larvae were reared on a pinto bean-based diet (Shorey and Hale 1965) at 24 °C under a 16:8 light:dark photoperiod and 50% RH in an environmental chamber. To prevent mating by emerging adults, pupae were sorted by sex and kept separately in Bugdorm-1 cages (30 × 30 × 30 cm, Megaview Science Education Services Co., Taiwan) with 5% sucrose solution for adult emergence.

Pheromones. Pheromone compounds, *Z11-14:Ac* (98% purity), *E11-14:Ac* (95% purity), *Z11-14:OH* (95% purity), *Z11-14:Al* (95% purity) were purchased from Bedoukian Research, Inc. (Danbury, CT). Pheromone solutions were prepared using HPLC grade hexane (Aldrich, Milwaukee, WI).

Field Study Plots. Field studies were conducted in 2012 and 2014 in unmanaged apple plantings at the Michigan State University Clarksville Research Center (42.8423°N, 85.2425°W). Plots consisted of eight 0.21-ha apple orchards with 12 rows spaced at 4.5 m intervals and 26 apple trees (ca. 3 m height) within each row spaced at 1.5 m apart. Each plot was at least 100 m apart and planted with three apple cultivars (Idared, Empire, and Liberty) with four rows per cultivar (Gut et al. 2005). Among these eight plots, four were surrounded by a hedgerow barrier along its perimeter, consisting of three rows of hybrid poplar, one row of Italian alder, and one row of white pine (Gut et al. 2005), the other four plots had no such hedgerow-barrier.

Field Experiment 1: Effects of Blend Ratios and their Dosages on Male Captures. The relative attractiveness of lures containing three- or four-component blends at different dosages was evaluated in the field between May 26 to July 5, 2012. The three-component blend at five doses (0.01, 0.1, 1, 10, and 20 mg) was randomized deployed in a plot surrounded by a hedgerow barrier, whereas the four-component blend at five doses was conducted in a plot without the barrier.

The four-component blend of *Z11-14:Ac*, *E11-14:Ac*, *Z11-14:OH*, and *Z11-14Al* was prepared in a ratio of 96.5:1.8:1.4:0.2 based on the ratio reported from female pheromone gland effluvium from a colony established from the same origin maintained the same way at Michigan State University as described above (El-Sayed et al. 2003). The 3-component blend of *Z11-14:Ac*, *E11-14:Ac*, and *Z11-14:OH* in a ratio of 96.5:1.8:1.4 was prepared similarly but without *Z11-14Al*. Either three- or four-component blends (200 µl) containing 0.01, 0.1, 1, 10, and 20 mg were applied to the sleeves of gray rubber septa (6 mm ID, 19 mm height, ISCA Technology, CA) in a fume hood. Pheromone impregnated septa were stored in a freezer at -20°C after hexane was evaporated.

Lures were deployed in Pherocon® VI orange delta traps (Trécé Incorporated, Adair, OK) with a sticky liner (18.5 × 18.5 cm) at the bottom by pinned centrally to the inside ceiling of the trap. Traps baited with one of 5 three-component lures were randomly placed at least 10 m apart in the 2nd and 11th rows in a plot

with the hedgerow barrier and repeated three more times in the rest of the plots with the barrier. Traps were hung in the upper third of the tree canopy. Four-component lures were deployed in the same way but in four plots with no barrier. All the traps in each plot were checked weekly and sticky liners replaced as needed. Treatments within a plot were rotated weekly in a clockwise pattern to eliminate trap position bias.

Both weekly and total male captures over a 6-week trapping period were analyzed and compared among varying dosages within each blend. Since two blends were deployed in separate plots with or without hedgerow barriers and each plot had different levels of moth populations, the effect of blend type on male captures was not able to be directly compared. Instead, the proportion of male capture by each trap was generated for both blends by dividing its total capture over the 6-week trapping period by the grand total male capture from all the traps within each plot (i.e., sum of male captures by traps baited with 0.01, 0.1, 1, 10, and 20 mg dose). A linear regression analysis was performed on the newly generated data set for each blend; and their constants and slope coefficients were compared between the blends.

Laboratory Bioassay: Evaluation of Sex Pheromone Volatile Emissions from Live Females. Volatile collections were performed on groups of 42 1-2d old virgin females, males, a commercial lure, or an empty vessel (blank control) under dark conditions over a 16h period corresponding to the scotophase experienced by the source colony. Multiple moths were used to ensure adequate volatile collection, because each female pheromone gland contains a tiny fraction of pheromone ($<10\text{ng.gland}^{-1}$) and not every female will call on a given night. The volatile collection was repeated 3 more times with additional groups of 42, 45, and 65 virgin females and males along with commercial lures.

Volatile collection chambers were constructed from 1-liter Teflon containers equipped with two 0.64-cm ports in their lids (Jensen, Coral Springs, Florida). The lid was completely sealed using Teflon tape to ensure that 100% of the air was directed through the trap. Air was passed through the collection chambers at a rate of 958 ml.min^{-1} after it passed through a moisture trap and a charcoal column. Volatiles were trapped using a trap containing 25 mg of Super Q adsorbent (Alltech Assoc., Deerfield, IL). Following collections, the chambers were cleaned with acetone three times and baked in an oven $120\text{ }^{\circ}\text{C}$ for at least 2 h.

Volatile chemicals were eluted from individual traps with $150\text{ }\mu\text{l}$ hexane three times. The elute was concentrated under a nitrogen stream to approximately $20\text{ }\mu\text{l}$ of each sample, of which $1\text{ }\mu\text{l}$ was analyzed by capillary gas chromatography (Hewlett-Packard HP6890 equipped with a Hewlett Packard 7863 autosampler) and an HP-Innowax polyethylene glycol column ($30\text{ m} \times 250\text{ }\mu\text{m}$ i.d., $0.25\text{ }\mu\text{m}$ film thickness) with a splitless injector at $250\text{ }^{\circ}\text{C}$ and flame ionization detector at $300\text{ }^{\circ}\text{C}$. Following injection, column temperature was held at $50\text{ }^{\circ}\text{C}$ for 5min, increased at $25\text{ }^{\circ}\text{C.min}^{-1}$ to $155\text{ }^{\circ}\text{C}$ and held for 5 min; then increased at $0.5\text{ }^{\circ}\text{C.min}^{-1}$ to $165\text{ }^{\circ}\text{C}$ and held for 3 min; finally increased at $30\text{ }^{\circ}\text{C.min}^{-1}$ to 225 and held for 2 min. Helium was used as a carrier gas at a flow rate of 1.1 ml.min^{-1} . Pheromone peaks released by the females were identified based on their retention times compared to those of synthetic compounds and further confirmed by their mass spectrums with those of synthetic ones by GC-MS using HP-5 5%

phenyl methyl siloxane column. Data were collected with Hewlett- Packard ChemStation® software. Due to not knowing exactly how many females released pheromones during the collection period, the actual amount of each pheromone compound released was not calculated, instead, the percentage of each compound released was reported and calculated by $100 \times$ its peak area divided by the total peak areas from all the pheromones combined.

Field Experiment 2: Evaluation of New Pheromone Blends with and without E11-14:Ac. This experiment was conducted to test how the addition of E11-14:Ac to a newly formulated 2-component pheromone lure (NL-2) would affect male capture. The 2-component lure contained 10 mg of Z11-14Ac and Z11-14OH in a ratio of 37:64 and the three-component lure contained 10 mg of Z11-14Ac, Z11-14OH, and E11-14Ac in a ratio of 37:64:0.68 (NL-3). Otherwise, lures were manufactured using the methods described above. Traps baited with either NL-2 or NL-3 were deployed on two adjacent trees in the middle 2nd row of one of four plots with the hedgerow barrier from June 23 to September 26, 2014. The numbers of males in each trap were counted and removed weekly. Moth captures from the week of 7/2 to the week of 7/23 were summed together to represent the total capture during the first flight, and data from 8/6 to 9/24 was added together to represent the total capture during the second flight. Both weekly and total male captures in the 1st and 2nd generations between the lures were compared.

Field Experiment 3: Comparison of Male Captures between New Lures and Commercial lures. This experiment was conducted to compare the relative attractiveness of the 3-component new blend lure (NL-3) with a commercial lure (3 mg, Trécé Incorporated, Adair, OK). Based on the results from Exp. 1 indicating that blend dosage significantly affected moth captures, 3 mg NL-3 was used to match the quantity of pheromones that was loaded in the commercial lure. Two traps, each baited with one of the two lures, were deployed on two adjacent trees in the center row of one of four plots without the hedgerow barrier and serviced the same way as described above during the second flight from August 15 to September 26, 2014. Both weekly and total male captures over the 6-week trapping period between the new and commercial lures were compared.

Statistical Analysis. In Field Experiment 1, weekly moth captures over time among traps baited with varying dosages of three-component or four-component blends were analyzed by repeated measures ANOVA with the dosage as the subject factor and time (week) as the within subject factor after data were $\log(X+1)$ transformed to meet their normality and homoscedasticity assumption (SAS Institute, 2020). Total numbers of moths captured over a 6-week period by traps baited with three- or four-component blends at various dosages were both analyzed by a RCBD design with study plots as blocks since original data met normality and homoscedasticity assumption tests (SAS Institute, 2020). Mean separations were performed via a post hoc Tukey's HSD test ($\alpha=0.05$). The proportion of male captured for both blends at varying dosages was analyzed using a linear regression model using Proc Reg after data was arcsine of square root transformed to meet normality and homoscedasticity assumption test (SAS Institute, 2020).

In Field Experiment 2, weekly moth captures over time between traps baited with NL-2 or NL-3, and moth captures between traps baited by NL-3 or the commercial lure in Field Experiment 3 were analyzed by

repeated measures ANOVA with the lure type as the subject factor and time (week) as the within subject factor after data were $\log(X+1)$ transformed to meet normality and homoscedasticity assumptions (SAS Institute, 2020). Total numbers of moths captured during the first and second flight in Field Experiment 2 and total moth captures after $\log(X)$ transformed in Field Experiment 3 were analyzed by ANOVA with a randomized complete block design where the study plots served as blocks.

Results

Field Trial 1: Effects of Blend Ratios and their Amounts on Male Captures. Dosage (between subject effects; $F_{4,12} = 16.29$, $P < 0.001$) and week (within subject effects; $F_{5,60} = 12.78$, $P < 0.001$) significantly affected moth captures for the three-component blend (Fig. 1A). There was no significant block effect ($F_{3,12} = 0.61$, $P = 0.62$) or interaction between the week and the block ($F_{15,60} = 0.54$, $P = 0.9$), and between the week and the dosage ($F_{20,60} = 0.48$, $P = 0.97$). Likewise, both the dosage ($F_{4,12} = 49.67$, $P < 0.001$) and the week ($F_{5,60} = 17.64$, $P < 0.001$) significantly affected moth captures for the four-component blend (Fig. 1B). There was no significant interaction between the week and the block ($F_{15,60} = 1.76$, $P = 0.06$), but a significant block effect ($F_{3,12} = 5.81$, $P = 0.011$) and interaction ($F_{20,60} = 2.06$, $P = 0.02$) were found between the week and the dosage. Overall, doses of pheromone lures at 10 mg and above captured more males weekly regardless of the blend type (Fig. 1A and 1B). However, significant differences were only found for three-component lures in the week of 5/31 ($F_{4,12} = 6.44$, $P = 0.005$) and 6/14 ($F_{4,12} = 7.59$, $P = 0.003$) (Fig. 1A). In contrast, significant differences in moth captures among dosages were found every week for four-component blend lures (5/31: $F_{4,12} = 12.79$, $P < 0.001$; 6/7: $F_{4,12} = 10.58$, $P < 0.001$; 6/14: $F_{4,12} = 9.52$, $P = 0.001$; 6/21: $F_{4,12} = 17.01$, $P < 0.001$, 6/28: $F_{4,12} = 11.04$, $P < 0.001$, 7/5: $F_{4,12} = 13.57$, $P < 0.001$) (Fig. 1B).

For total males captured during the 6-week trapping period, both three- and four-component blends had a significant dosage effect on moth captures (three-blend: $F_{4,12} = 12.88$, $P < 0.001$; four-blend: $F_{4,12} = 12.31$, $P < 0.001$) (Fig. 1C). There was no block effect on moth captures for the three-blend ($F_{3,12} = 1.47$, $P = 0.7$), but the block effect for the four-component blend was significant ($F_{3,1} = 4.68$, $P = 0.02$). Traps baited with 20 mg either three- or four-component blends captured the highest numbers of male *C. rosaceana*, similar to traps baited with 10 mg blend, but significantly different from those baited with lower doses at 1, 0.1, or 0.01 mg. Traps baited with 10 mg three- or four-component blends captured significantly more moths than those baited with 0.1, 0.01, or 1 mg pheromones except for the three-component blend at 1 mg. There was no significant difference in moth captures among traps baited with 1 mg or lower doses of pheromones.

A strong linear regression was detected between the proportion of male captures and the dose of three- ($F_{1,18} = 55.60$, $P < 0.001$) or four-component blend ($F_{1,18} = 93.35$, $P < 0.001$) (Table. 1). Both regression lines had a slope and y-intercepts that overlapped within 95% confidence intervals, thus the proportion of moths responding to three-component blend was similar to the four-component blend.

Laboratory Bioassay: Evaluation of Sex Pheromone Volatile Emissions. The commercial pheromone lure (Trécé) emitted a pheromone blend consisting of a major compound Z11-14:Ac and two minors of E11-14:Ac and Z11-14:OH in a ratio of 74:5:21. Z11-14:Al was under the detection limit (Fig. 2 and 3). In contrast, groups of virgin females emitted a pheromone blend containing only Z11-14:Ac and Z11-14:OH in a ratio of 37:63. Both E11-14:Ac and Z11-14:Al were under the detection level. None of the pheromone components were detected from volatile collection chambers containing nothing or groups of males.

Field Trial 2: Evaluation of New Pheromone Blends with and without E11-14:Ac. There was no significant effect of lure type on male captures ($F_{1,3} = 3.07$, $P = 0.18$), but week significantly affected moth captures ($F_{9,27} = 13.64$, $P < 0.001$) (Fig. 4A). The interaction between the lure type and week/time was not significant ($F_{9,27} = 1.21$, $P = 0.35$). There was a numerical increase in moth captures when E11-14:Ac was added to the 2-component blend of Z11-14:Ac and Z11-14:OH, but this increase was not statistically significant during either the first or second flights.

Numerically, 3-component lures captured more males than 2-component lures during the first and second flight (Fig. 4B), but the difference was not significant (first generation: $F_{1,3} = 7.58$, $P = 0.07$; second generation: $F_{1,3} = 3.50$, $P = 0.16$). There was no block effect on moth capture during the first flight ($F_{3,3} = 2.08$, $P = 0.28$), but block effect was significant during the second flight ($F_{3,3} = 16.98$, $P = 0.02$).

Field Trial 3: Comparison of Male Captures between New Lures and Commercial lures. There was a significant effect of lure type ($F_{1,3} = 13.56$, $P = 0.03$) and week ($F_{5,15} = 28.08$, $P < 0.001$) on moth captures in the second flight (Fig. 5); however, block effect was not significant ($F_{3,3} = 2.9$, $P = 0.20$). Within the week of 8/29 and 9/5, NL-3 lure captured significant higher numbers of male moths compared to the commercial lure (8/29: $F_{1,3} = 9.43$, $P = 0.05$; 9/5: $F_{1,3} = 17.16$, $P = 0.03$); moth catches were not significant for the other weeks (8/22: $F_{1,3} = 3.95$, $P = 0.14$; 9/12: $F_{1,3} = 0.49$, $P = 0.53$; 9/19: $F_{1,3} = 3.00$, $P = 0.18$; 9/26: $F_{1,3} = 1.0$, $P = 0.39$).

Traps baited with NL-3 captured 22.0 ± 6.0 (mean \pm SEM) males in total over the 6-week trapping period, significantly higher than those baited with the commercial lure (8.8 ± 2.9) ($F_{1,3} = 21.76$, $P = 0.02$). There was no block effect ($F_{3,3} = 4.95$, $P = 0.11$).

Discussion

The sex pheromone of *C. rosaceana* was discovered and identified nearly a half century prior to this study (Roelofs and Tetter 1970; Cardé et al. 1977; Hill and Roelofs 1979; Vakenti et al. 1988), yet at present there is not an optimized, universal sex pheromone blend suitable for monitoring populations across its North American geographic range. Both western and eastern *C. rosaceana* females have been confirmed to produce the same pheromone components of Z11-14:Ac, E11-14:Ac, Z11-14:OH, and Z11-14:Al but in different ratios (El-Sayed et al. 2003). So far, numerous pheromone blends have been proposed in the literature to use to monitor this species (Hill and Roelofs 1979; Thomson et al. 1991; Vakenti et al. 1988;

El-Sayed et al. 2001, 2003; Stelinski et al. 2007); all of which are composed of Z11-14:Ac at least 90 percent and Z11-14:OH at less than 2 percent. However in this study, groups of Michigan females released less Z11-14:Ac than Z11-14:OH in a ratio of 37:63 (Fig. 2 and 3), a markedly different profile than previously reported from laboratory reared Michigan female pheromone glands: Z11-14:Ac, E11-14:Ac, Z11-14:OH in a ratio of 96.5:1.8:1.3:0.2 (El-Sayed et al. 2003). This suggests that the actual volatiles released by females may not translate directly to the content of pheromone glands. This is important because, pheromone ratios and concentrations in the air, not in pheromone glands, are what *C. rosaneana* males respond to when mate foraging. On the other hand, it is possible that the blend ratio reported by El-Sayed et al. (2003) may be a little different from what females produced in this study. However, while there were 10 years between El-Sayed et al. (2003) and the present study, both groups of females were from the same colony continuously maintained using the same procedures. Sex pheromones are highly species specific; they should be under strong stabilizing selection against small changes (Symonds and Elgar 2008). Thus, it is unlikely that there would have been major changes in pheromone blends between the two groups.

The addition of Z11-14:OH to a blend of Z11-14:Ac and E11-14:Ac has been found to increase male captures significantly (Hill and Roelofs 1979). In another study, adding this compound at 1, 4, or 10% relative to Z11-14:Ac to a blend of Z11-14:Ac, E11-14:Ac, and Z11-14:Al didn't significantly affect moth captures in Michigan, but significantly decreased moth captures in Oregon as its dose increased (Stelinski et al. 2007). In addition, virgin females from this study released nearly twice as much Z11-14:OH as Z11-14:Ac, a major component of pheromone blend reported in the literature. Therefore, the significance of Z11-14:OH in *C. rosaneana* response to the pheromones might be underestimated and deserves further study.

In Field Experiment 1, traps baited with high doses of either three- or four-component pheromone blends captured significantly more *C. rosaceana* than traps baited with low dosages (Fig. 1). This agrees with the findings by Stelinski et al. (2005) showing that an increase in lure loading by varying numbers of equally loaded lures resulted in more moth captures. However, the attraction of *C. rosaceana* to lures can reach an upper limit (Klun and Robinson 1972; Stelinski et al. 2005), as also seen in this study that rate of increase in moth capture plateaued at 10 mg/lure. Moreover, the addition of Z11-14:Al to the three-component blend had a comparable effect on the number of moths captured as the blend without. This result was similar to previous findings reporting that captures of male *C. rosaceana* in Michigan were not affected by the presence or absence of the aldehyde (El-Sayed et al. 2003; Stelinski et al. 2007).

In the Laboratory Bioassay, minor compound E11-14:Ac was not detected in pheromone emission by *C. rosaneana* females, but the importance of this minor compound was previously documented in the literature. For example, Trimble et al. (2008) reported that the addition of E11-14:Ac to Z11-14:Ac increased the incidence of *C. rosaneana* male upwind flight but did not increase source contact in wind tunnel assays. This agrees with the present study as the addition of E11-14:Ac to a newly developed two-component blend of Z11-14:Ac and Z11-14:OH in a ratio of 37:63 didn't have a significant effect on moth captures (Fig. 4).

However, the NL-3 lure captured more than double the moths compared to a commercial lure releasing Z11-14:Ac, E11-14:Ac, and Z11-14:OH in a ratio of 74:5:20 (Fig. 5). This finding further supports the hypothesis supported in Field Experiment 1 that *C. rosaceana* males' response is more strongly mediated by pheromone blend quantity relative to blend ratio (Fig. 1). This hypothesis was also proposed by Stelinski et al. (2007) who found females originating from western populations were more attractive to males in Michigan than local females, which was correlated to greater pheromone production by the western compared to Michigan females. Additional studies should be undertaken to fine tune the release rate by the pheromone lure to match the one by the female of *C. rosaceana* to improve monitoring and detection of this species for timely application of management tactics. These studies might also shed some light on why authentic females are more attractive than the pheromone lure in the field and why mating disruption has not been successful for this species.

Declarations

Acknowledgements The authors thank Dana Blanchard and Thomas Gut for rearing the moth colony and Katelin Aho for assisting the field trapping study. Thanks also go to ISCA Technology, CA for donating gray septa.

Funding by the Michigan Apple Research Committee.

Conflict of Interest /Competing interests The authors declare no conflict of interest.

Availability of data and material excel files and available upon request

Code Availability Not applicable.

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Tables

Table 1. Statistical parameters of linear regressions of the proportion of male captures by the dosage of three- or four-component blend. Values were generated from the arcsine of square root transformed data.

Blend Type	Parameter name	Parameter value	t-Value	Pr > t	95% Confidence limits		R-Square
Three-component	slope	0.01854	7.46	<0.001	0.01332	0.02377	0.7554
	intercept	0.32619	13.10	<0.001	0.27389	0.37849	
Four-component	slope	0.02677	9.66	<0.001	0.02095	0.03260	0.8383
	intercept	0.25477	9.18	<0.001	0.19649	0.31305	

Figures

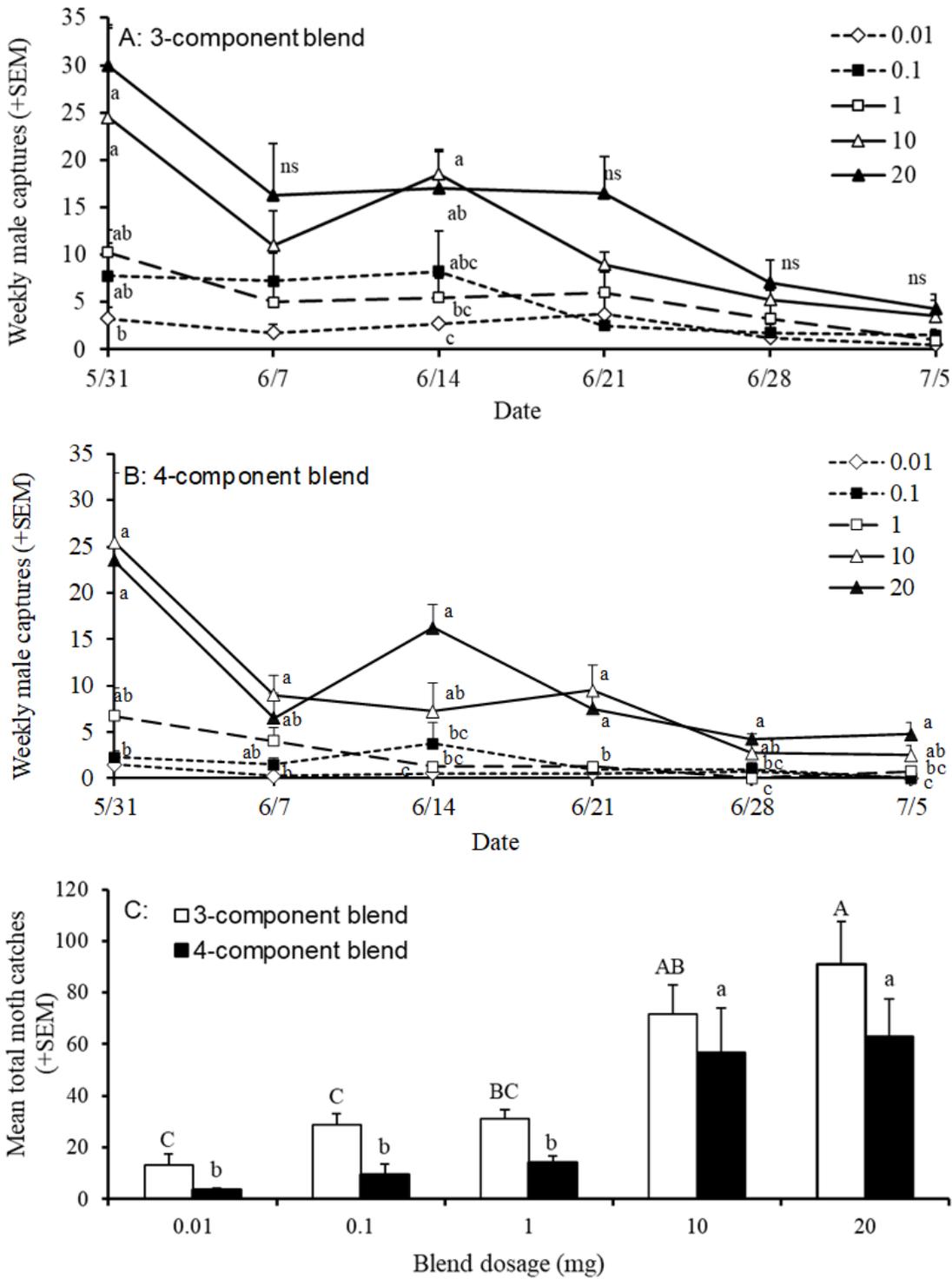


Figure 1

Mean (+SEM) weekly (A, B) and total (C) *Choristoneura rosaceana* males captured over a 6-week period in traps baited with a gray septum lure loaded with various doses of either a three-component blend of Z11-14:Ac, E11-14:Ac, Z11-14:OH in a ratio of 96.5:1.8:1.4 produced in females' pheromone glands (A) or a four-component blend by adding Z11-14:Al to the three-component blend (B). Means followed by the same letter within weeks (A, B) are not significantly different by Tukey's HSD test at $P=0.05$. Bars topped

with different letters (lowercase letters for four-component blend and capitals for three-component blend) were significant different among their respective dosages (C).

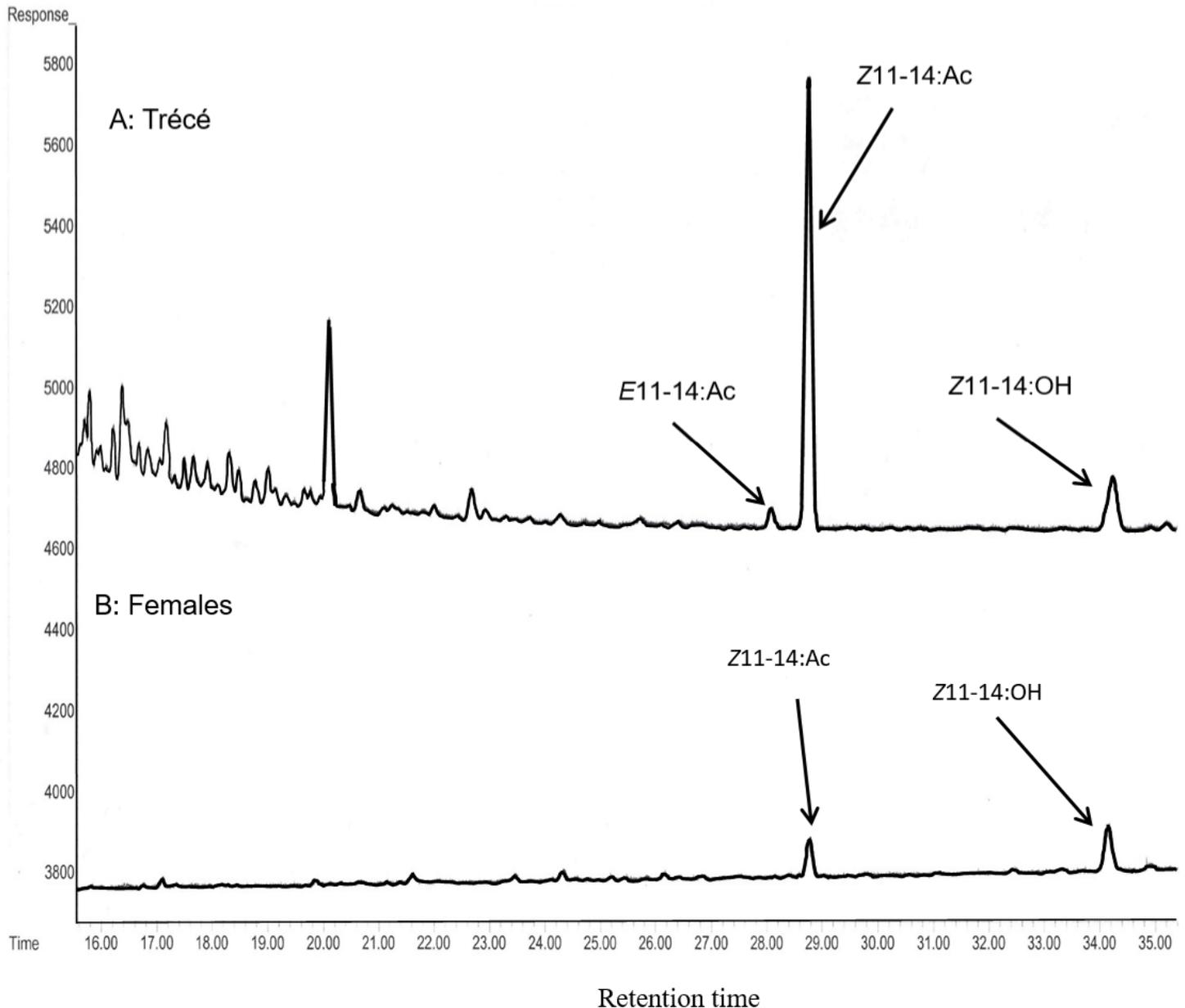


Figure 2

Gas chromatographic profiles of sex pheromone compounds released by a commercial Trécé lure (A) and a group of *C. rosaceana* females (B). The compounds represented by peaks in the chromatograms were identified as E11-14:Ac, Z11-14:Ac and Z11-14:OH.

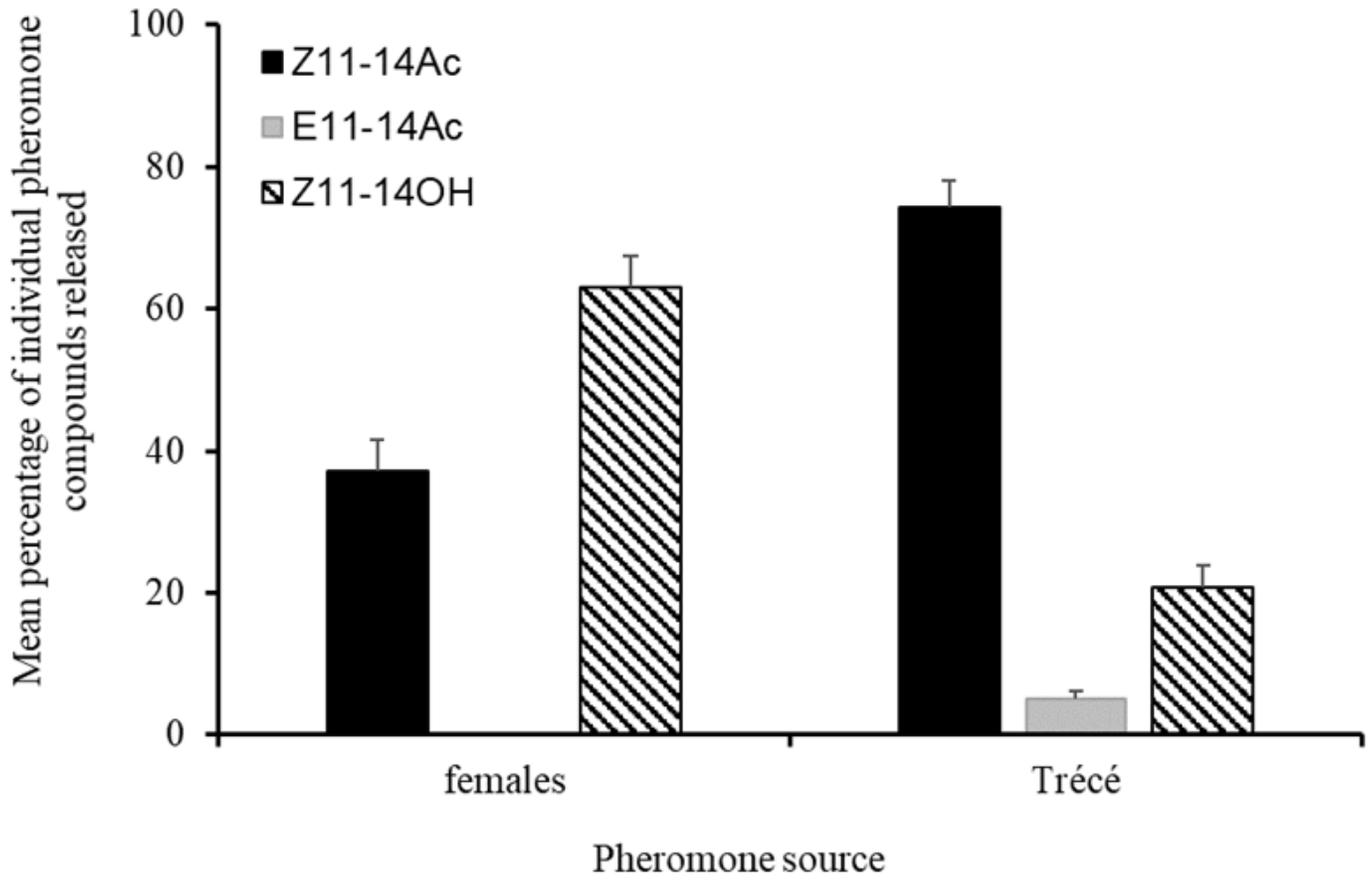


Figure 3

Mean (+SEM) relative percentage of individual pheromone compounds released by a commercial lure (Trécé) and a group of *C. rosaceana* females.

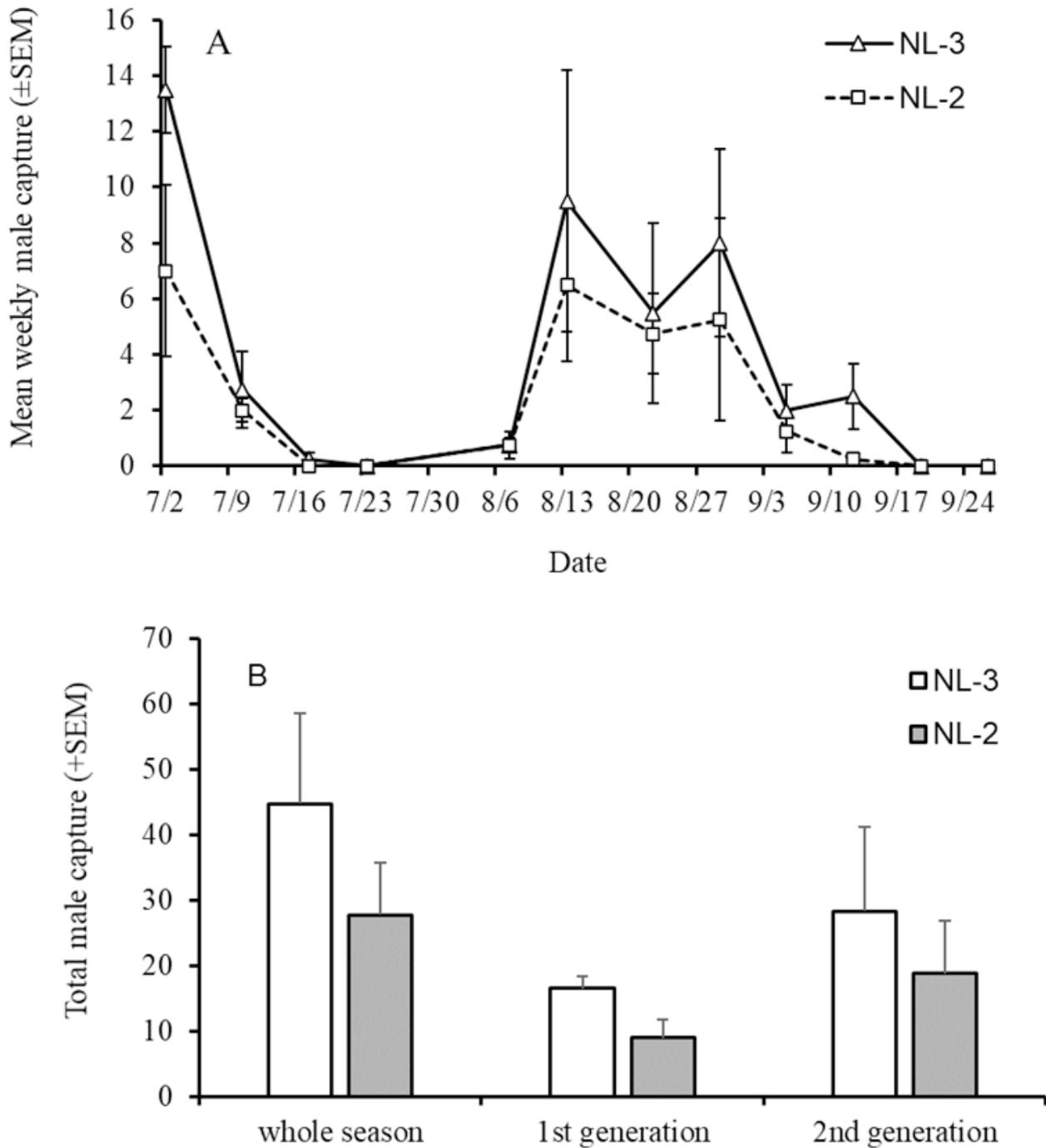


Figure 4

Mean (\pm SEM) weekly captures of *C. rosaceana* males in traps baited with a newly formulated gray septum lure loaded with 10 mg three-component blend of Z11-14:Ac, E11:14:Ac, and Z11-14OH (NL-3) or a two-component blend without E11-14:Ac (NL-2) in a ratio based on pheromone emissions by the conspecific females.

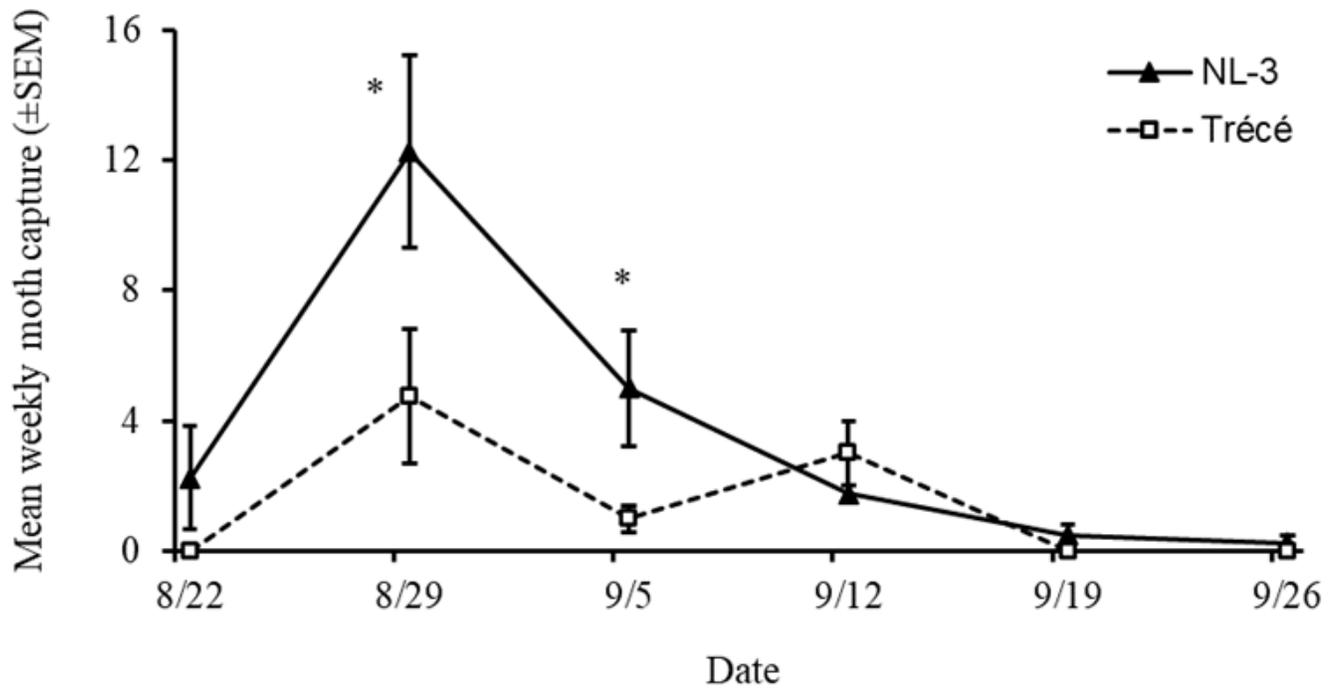


Figure 5

Mean (\pm SEM) weekly captures of *C. rosaceana* males in traps baited with a newly formulated gray septum lure loaded with 3 mg three-component blend of Z11-14:Ac, E11:14:Ac, and Z11-14:OH (NL-3) in a ratio based on pheromone emissions by the conspecific females or a commercial lure (Trécé). Within the weeks, pairs of means marked with an asterisk were significantly different between the lures.