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# Comparative behavioral and EAG responses of female obliquebanded and redbanded leafroller moths (Lepidoptera: Tortricidae) to their sex pheromone components

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**Key words.** Tortricidae, obliquebanded leafroller, *Choristoneura rosaceana*, redbanded leafroller, *Argyrotaenia velutinana*, electroantennogram recording, sex-attractant pheromone, female response, autodetection, calling behaviour, oviposition, mating disruption

**Abstract.** Studies were conducted investigating the responses of female obliquebanded leafrollers, *Choristoneura rosaceana* (Harris) and redbanded leafrollers, *Argyrotaenia velutinana* (Walker) (Lepidoptera: Tortricidae), to components of their sex pheromone. Electroantennogram (EAG) recordings revealed significant responses from antennae of female moths of both species to the major pheromone component, (*Z*)-11-tetradecenyl acetate, at dosages ranging from 2 µg – 2 mg. However, tested individually, the minor pheromone components of the obliquebanded leafroller, (*E*)-11-tetradecenyl acetate and (*Z*)-11-tetradecenol, elicited little or no antennal response from conspecific females. This result was consistent for redbanded leafroller females, which showed only weak responses to the minor component (*E*)-11-tetradecenyl acetate at a 2 mg dosage. For both species, species-specific blend ratios of the *Z* and *E* isomers of tetradecenyl acetate did not elicit a greater antennal response than the *Z* isomer alone. Virgin females of each species (2–4 d old) were placed into 1-liter plastic assay chambers with constant throughput of carbon-filtered air passed through 1-liter flasks containing rubber septa loaded with (*Z*)- and (*E*)-11-tetradecenyl acetates and (*Z*)-11-tetradecenol for trials with female obliquebanded leafrollers or with (*Z*)- and (*E*)-11-tetradecenyl acetates and dodecyl acetate for trials with female redbanded leafrollers. Exposure to pheromone-permeated air delayed the onset of calling by 1 h and terminated the calling period 1 h earlier for both species compared with solvent-control exposed females. Furthermore, the total proportion of calling females was reduced by half in chambers receiving constant throughput of pheromone-permeated air compared with solvent controls. Exposure to pheromone-permeated air also significantly reduced egg-laying in both species compared with clean-air controls. Furthermore, application of the major pheromone component, (*Z*)-11-tetradecenyl acetate, at dosages ranging from 2 µg–2 mg to wax-paper ovipositional substrates, deterred oviposition by females of both species. Our data suggest that application of synthetic sex-attractant pheromones for mating disruption of leafroller species may have deleterious effects on female moth behavior, which may contribute to pest control. Field investigations will need to be conducted to test this hypothesis.

## INTRODUCTION

The obliquebanded leafroller, *Choristoneura rosaceana* (Harris) and redbanded leafroller, *Argyrotaenia velutinana* (Walker) (Lepidoptera: Tortricidae), are pests of apple throughout the Midwestern and Eastern United States and Eastern and Western Canada (Howitt, 1993; Hull et al., 1995). High population densities of the former species can cause especially severe crop loss resulting in upwards of 20% fruit damage in New York State, USA (Lawson et al., 1996). Both species are characterized by a wide host range and attack many common fruits and vegetables (Chapman, 1973; Hull et al., 1995). Past conventional control strategies have included organophosphorous insecticides (Reissig, 1978); however, increasing incidence of resistance to these toxins (Pree et al., 2001, 2002) and U.S. government-imposed restrictions have mandated pursuit of other control tactics. One alternative insecticide that has been explored for obliquebanded leafroller is the insect growth regulator tebufenozide (Waldstein & Reissig, 2001).

Since the identification of the pheromone components mediating sexual communication in both species, serious effort has been channelled towards development of mating disruption as an alternative or complementary behavioral control tactic to conventional insecticides. These two sympatric species share the major and a minor component of their pheromone blends: (*Z*)-11-tetradecenyl acetate and (*E*)-11-tetradecenyl acetate in approximately a 98 : 2 ratio for the obliquebanded leafroller and 92 : 8 ratio for the redbanded leafroller (Roelofs & Arn, 1968; Roelofs & Tette, 1970; Roelofs et al., 1975; Cardé & Roelofs, 1977; Hill & Roelofs, 1979). However, the pheromone blends of these species are further differentiated by other minor components; these include (*Z*)-11-tetradecenol and (*Z*)-11-tetradecenal in the obliquebanded leafroller blend (El-Sayed et al., 2003) and dodecyl acetate in the redbanded leafroller blend (Roelofs

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et al., 1975). Control of redbanded leafroller by mating disruption using synthetic pheromones has been judged successful (Cardé et al., 1975; Novak et al., 1978; Novak & Roelofs, 1985; Cardé & Minks, 1995); however, results have been inconsistent for the obliquebanded leafroller (Reissig et al., 1978; Agnello et al., 1996; Lawson et al., 1996). Greater success has been recorded with a Western population of obliquebanded leafroller in British Columbia, Canada (Evenden et al., 1999a, b). The females of this Western population contain different amounts and ratios of the four-component pheromone and the males are characterized by different behavioral and antennal responsiveness to blends of these four components as compared with Eastern U.S. populations of this species (Vakenti et al., 1988, Thomson et al., 1991; El-Sayed et al., 2003). Despite numerous studies on mating disruption of these two species, the mechanisms behind its effectiveness are still poorly understood. Specifically, little is known about the effect of synthetic pheromone applications on female behavior and how that may contribute to the effectiveness of mating disruption.

Many studies have been conducted examining male moth antennal and behavioral responses to pheromone for both the redbanded leafroller (Bartell & Roelofs, 1973, 1976; Cardé & Roelofs, 1977; Novak & Roelofs, 1985; El-Sayed et al., 2003; Stelinski et al., 2003a, b, 2004a) and obliquebanded leafroller moths (Evenden et al., 2000; Stelinski et al., 2003a, b, 2004a). Some of these investigations have suggested that high-dosage exposure of male moths to their pheromone blend components decreases subsequent responses of male moths due to adaptation, habituation, or a combination of both. This decreased responsiveness following previous exposure has been implicated as a potential contributing factor to mating disruption (Bartell & Roelofs, 1973; Evenden et al., 2000; Stelinski et al., 2004a). Despite great attention to the effects of pheromone exposure on male moth behavior, similar effects on the responses of conspecific female moths have not been investigated in depth.

It is known that females of many tortricid species are capable of detecting their sex pheromone (Palanaswamy & Seabrook, 1978; Barnes et al., 1992; Stelinski at el., 2003c, 2006; DeLury et al., 2005; Gökçe et al., 2006). In addition to tortricid moths, noctuid (Mitchell et al., 1972; Birch, 1977; Light & Birch, 1979; Ljungberg et al., 1993; Groot et al., 2005), yponomeutid (van der Pers & den Otter, 1978), and arctiid (Schneider et al., 1998) females are reported to be capable of detecting their sex pheromone. Furthermore, exposure to pheromone is known to either advance or delay the onset of calling periodicity and/or increase the total proportion of calling female moths (Palanaswamy & Seabrook, 1978, 1985; Noguchi & Tamaki, 1985; Weissling & Knight, 1996; Stelinski et al., 2006).

Despite extensive evidence among a wide variety of species that female moths are capable of detecting their sex pheromone and that this modifies their behavior as compared with moths in clean air, little is known about how this may contribute to mating disruption. Stelinski et al. (2003c) provided initial evidence that female obliquebanded and redbanded leafroller moths are sensitive to their major sex pheromone component as measured by electroantennograms (EAGs). The objectives of the current study were to (1) describe EAG dose-response relationships of obliquebanded and redbanded leafroller females to their major and selected minor pheromone components and species-specific blends of the major component and its geometric isomer, (2) determine whether exposure to a blend of synthetic pheromone components that is highly attractive to males affects calling behavior and/or oviposition of female moths, and (3) determine whether pheromone applied to ovipositional substrates affects egg laying. Our hypotheses were that females of both species are capable of detecting their sex pheromone components and that exposure to these chemicals affects calling and oviposition behavior.

## MATERIAL AND METHODS

### Insect cultures

Female obliquebanded leafroller moths were drawn from a six-year-old laboratory colony originally collected from unsprayed apple orchards in Southwestern Michigan. The culture was established by collecting moths as 1st and 2nd generation pupae. Redbanded leafroller females originated from a long-established laboratory colony maintained at Geneva, NY, USA by W. Roelofs. Both species were reared year-round without diapause at 24°C and 60% RH on pinto bean-based diet (Shorey & Hale, 1965) under a 16L : 8D photoperiod. Females of each species were sorted out as pupae and adults emerged in 1 l plastic cages containing 5% sucrose solution in plastic cups with cotton dental wicks protruding from their lids.

### Electroantennograms (EAGs)

The EAG system and test protocols have been described in detail elsewhere (Stelinski et al., 2003a, b). EAG cartridges were made by pipetting various dosages (2 μg – 2 mg) of pheromone components in hexane (20 μl total solution) onto 1.4 × 0.5 cm strips of Whatman No. 1 filter paper. Four pheromone treatments were compared for female obliquebanded learollers. These were the major pheromone component: (*Z*)-11-tetradecenyl acetate [(*Z*)11-14:Ac], two minor pheromone components: (*E*)-11-tetradecenyl acetate [(*E*)11-14:Ac] and (*Z*)-11-tetradecenol [(*Z*)11-14:OH], and the major pheromone component and its geometric isomer in a 98 : 2 ratio, which approximates the natural ratio of components produced by females of this species (Hill & Roelofs, 1979). EAG responses of female redbanded leafrollers were compared using three pheromone treatments. These were the same major [(*Z*)-11-tetradecenyl acetate] and minor [(*E*)-11-tetradecenyl acetate] components as for the obliquebanded learoller, and a blend of these two components in a 92 : 8 ratio, which more closely approximates the ratio of components produced by females of this species (Cardé & Roelofs, 1977). Pheromone components were obtained from Shin Etsu (Tokyo, Japan, > 98% isomerically pure). After 5 min in a fume hood for solvent evaporation, pheromone-treated filter paper strips were inserted into disposable glass Pasteur pipettes. EAGs were measured as the maximum amplitude of depolarization to 1-ml puffs of air through EAG-cartridges directed over live-insect preparations.

Virgin female moths of each species were 2–4 d old when used for EAGs. Live insects were restrained on a 3.5 cm diameter Petri dish filled with wax by placing a strip of clay (8 × 3 mm) over their thorax and abdomen. The terminal 2 segments of the antenna used for recording EAGs were excised and the recording electrode was attached at the severed end. The reference electrode was gently inserted into the head capsule near the base of the antenna used for recording. EAGs were measured using ten to thirteen moths per pheromone treatment and cartridge-loading dosage combination. Solvent-only control stimulations (using filter paper impregnated with 20 ml of hexane) were administered before and after each pheromone stimulus presentation spaced 12 s apart; the response to both blank puffs was averaged to calculate the control. Each replicate moth was stimulated with each pheromone component treatment and dosage. Between 12 to 20 s elapsed between different treatment stimulations to minimize potential onset of adaptation. Treatment (component and dosage) order applied per insect was randomized. The experiment was conducted in a two-factor randomized complete block (blocked by antenna) design. The two treatment factors considered were pheromone-blend treatment and loading dosage.

### Female calling behavior

This experiment tested the hypothesis that exposure to pheromone-permeated air alters the calling periodicity of female obliquebanded and redbanded leafroller moths. Virgin females of each species (2–4 d old) were placed into 1-liter plastic assay chambers (140 mm in height and 110 mm in diameter) equipped with two 0.64 cm openings in their lids as described in Stelinski et al. (2006). Glass inlets and outlets were affixed to the lids, which allowed for constant throughput of carbon-filtered air (50 ml/min) through the chambers. Carbon-filtered (Model 100 Safe Glass Hydrocarbon Trap, Chromatography Research Supplies, Louisville, KY) air entering chambers was passed through 1-liter flasks containing rubber septa loaded with 0.485 mg of (*Z*)- and 0.015mg (*E*)-11-tetradecenyl acetates and 0.026 mg of (*Z*)-11-tetradecenol for trials with female obliquebanded leafrollers or with 0.93 mg (*Z*)- and 0.07 mg (*E*)-11-tetradecenyl acetates and 2.0 mg dodecyl acetate for trials with female redbanded leafrollers. Such rubber septa are known to be highly attractive to conspecific males of each species in both laboratory flight-tunnel assays (Stelinski et al., 2004a) and in field traps (Stelinski et al., 2005). Control chambers received input from flasks containing septa loaded with a hexane solvent only. Air emerging from assay chambers was exhausted into a fume hood. Assay chambers were housed in a room at 23°C and 50–70% RH. Light intensity during photophase was 610–650 lux and was generated by 2 fluorescent bulbs (Philips model F96T12, 95 Watt) mounted 40 cm above the chambers. During scotophase, light intensity was ca. 0.5–3 lux.

The experiment was repeated on eight different days for each species and pheromone treatment and replicated with 4 assay chambers per treatment on each day. Five virgin female moths were placed into each treatment and control chamber per replicate ($N = 160$). Females were acclimated in chambers for 30 min prior to initiating the experiment. The experiment commenced at 20:00 h and was terminated at 8:00 h; onset of scotophase was at 18:00 h. The number of females observed calling was recorded hourly. Female calling was quantified by counting the number of females assuming a posture characterized by raised wings and a protruding abdomen as has been observed with other tortricids (Baker & Cardé, 1979; DeLury et al., 2005). During scotophase, observations were carried out using night-vision goggles as described by Stelinski et al. (2004b). Experiments were conducted separately for each species.

### Oviposition frequency

Two experiments were conducted to determine the effect of pheromone on female oviposition behavior. The first experiment tested the hypothesis that exposing female obliquebanded and redbanded leafrollers to pheromone-permeated air would affect oviposition frequency. First, mated, 2–4 d old female moths were placed in groups of 5 in 1-l plastic cups as described above that were lined internally with wax paper and maintained at the temperature, humidity, and light intensity conditions described above. The experiment was conducted 16–24 h after mating. Carbon-filtered air was pushed (50 ml/min) through the assay chambers via 1-l flasks containing the above-described rubber septa loaded with pheromone or hexane only. Air emerging from assay chambers was exhausted into a fume hood. The experiment was replicated on six different days with 4 assay chambers containing 5 mated female moths per treatment replicate for a total of 24 replicated chambers. The experiment ran for 24 h with a 16L : 8D photoperiod after which the total number of eggs deposited on wax paper was recorded. Experiments were conducted separately for each species.

The second experiment tested the hypothesis that application of the major pheromone component, (*Z*)-11-tetradecenyl acetate, to a wax-paper ovipositional substrate would affect oviposition by mated female obliquebanded and redbanded leafrollers. The experimental protocol was a slight modification of Gökçe et al. (2005). In brief, moth oviposition was assayed using 1-liter plastic cups with four windows (30 × 30 mm) cut 90° apart around their circumference and covered with fine mesh for air ventilation. Each cup received four pieces of wax paper (40 × 60 mm) attached around the circumference of the interior wall. For the control treatment, 20 µl of hexane was applied to each side of wax paper and spread with a hexane-rinsed bent glass rod. In each pheromone treatment, 20 µl of a hexane solution of pheromone was evenly applied to each side of the wax paper with a hexane-rinsed glass rod. The three dosages of pheromone compared were 20 µg, 200 µg, and 2 mg. The wax papers were dried in a fume hood for 15 min following application of treatments. In each replicate, the cups contained one hexane-treated wax paper and the three dosages of pheromone-treated wax papers hung in random order around the circumference of the walls of the bioassay cup. Wax papers were attached vertically with cellophane tape to the interior wall of each bioassay cup 10 mm apart. A 5% sucrose solution was provided within bioassay cups. Five female and 3 male leafroller adults (one day post emergence) were transferred into each bioassay cup. Cups were maintained in a fume hood for the duration of the experiment and the number of individual eggs within egg masses was counted and removed every 24 h for 7 days. Freshly treated wax paper was replaced daily. The experiment was replicated eight times and conducted separately for each species. Female moths were dissected following both experiments to inspect their bursa copulatrix for presence of a spermatophore, confirming mating status.

### Statistical analyses

A two-factor randomized complete block analysis of variance (ANOVA) was conducted on the EAG data. Differences in pairs of means between pheromone blend treatments within each dosage tested and among dosages within each pheromone treatment were separated using Fisher's protected least significant difference (LSD) multiple comparison procedure (SAS Institute, 2000). Differences between the proportions of females calling for each hour of observation in pheromone-treated versus control chambers were analyzed using paired *t*-tests (Minitab Release 14, McKenzie & Goldman, 2005). The mean number of fertile eggs oviposited by female leafrollers in pheromone-permeated versus control air was compared with paired *t*-tests. The mean number of eggs laid by female leafrollers on hexane-treated wax paper versus wax paper receiving various dosages

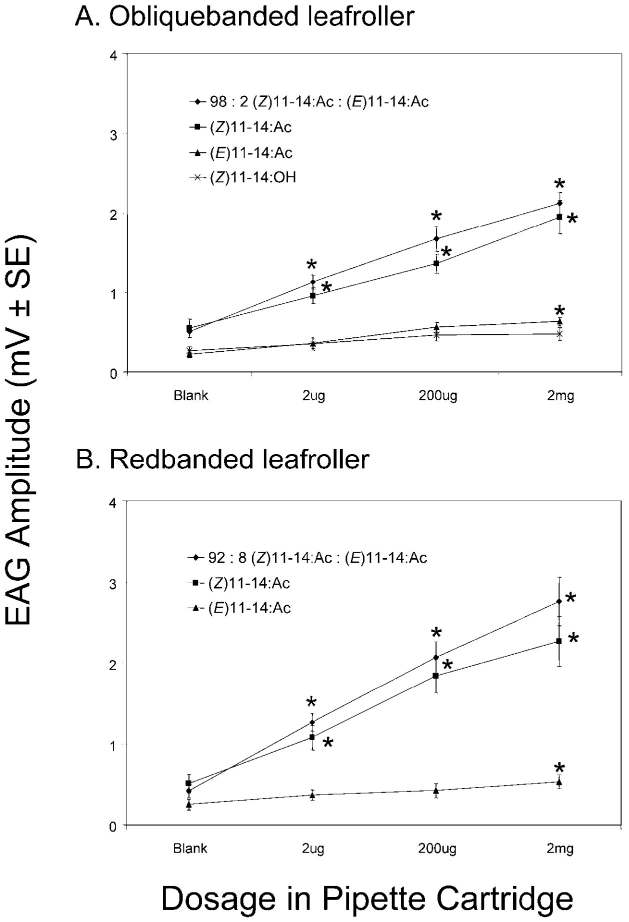

Fig. 1. Dosage-response relationships for female obliquebanded leafroller (A) and redbanded leafroller (B) live-insect antennal preparations. (*Z*)11-14:Ac, (*E*)11-14:Ac, (*Z*)11-14:OH are abbreviations for (*Z*)-11-tetradecenyl acetate, (*E*)-11-tetradecenyl acetate, and (*Z*)-11-tetradecenol, respectively. Significant ($P < 0.05$) differences between mean response to the solvent blank versus a pheromone component at a particular dosage are indicated by *.

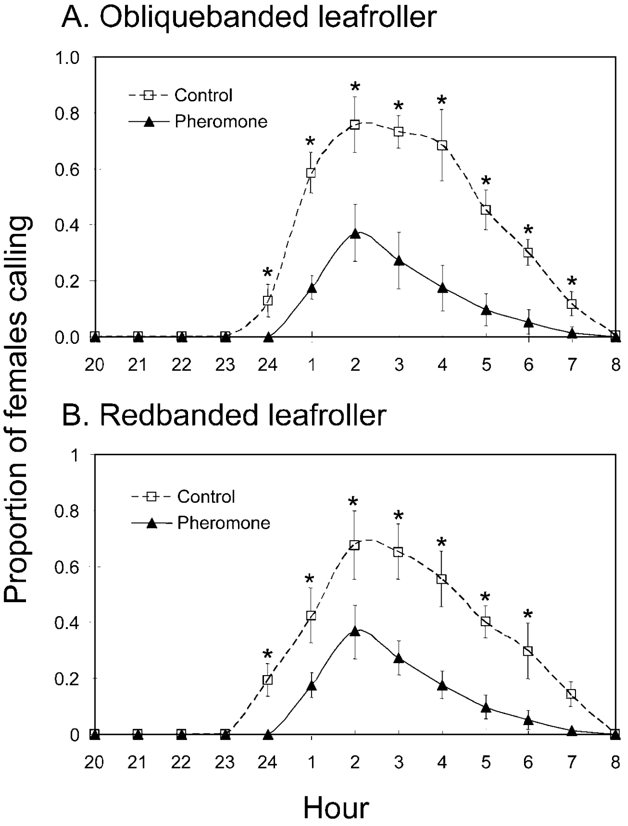

Fig. 2. Proportion of female obliquebanded leafroller (A) and redbanded leafroller (B) moths calling throughout the diel cycle. Significant ($P = 0.05$) differences between the proportions of female moths calling in the pheromone treatment versus the solvent control are indicated by *. Onset of scotophase was at 18:00 h.

of pheromone was analyzed by ANOVA followed by LSD. EAG and egg laying data were transformed to ln (x + 1) (which normalized the distributions and homogenized variance) prior to ANOVAs. Proportional data from the oviposition frequency experiment were normalized using an arcsine transformation.

## RESULTS

### Electroantennograms (EAGs)

There was no significant pheromone component-by-dosage stimulus interaction for either leafroller species; therefore, analyses for the effects of both treatment factors are presented. EAG responses of obliquebanded leafroller females were significantly ($F = 18.7$, $df = 3, 180$, $P < 0.001$) higher to the major pheromone component, (*Z*)-11-tetradecenyl acetate, and 2-component 98 : 2 blend of *Z* and *E* isomers than to the two minor components, (*E*)-11-tetradecenyl acetate and (*Z*)-11-tetradecenol, at each cartridge dosage tested (Fig. 1A). Responses of female obliquebanded leafrollers were significantly ($F = 13.2$, $df = 3, 180$, $P = 0.01$) higher to the major pheromone component, (*Z*)-11-tetradecenyl acetate, and to the 98 : 2 ratio of *Z* and *E* isomers than to the hexane blank at cartridge dosages ranging from 2 µg – 2 mg (Fig. 1A). However, responses to the minor component, (*E*)-11-tetradecenyl acetate, were significantly higher ($F = 4.5$, df $= 3, 180$, $P = 0.03$) than responses to the hexane blank only at the 2 mg dosage while responses to (*Z*)-11-tetradecenol were not significantly ($F = 0.9$, $df = 3, 180$, $P = 0.2$) different from the hexane control at any of the dosages tested (Fig. 1 A).

For female redbanded leafrollers, EAG responses to the major pheromone component and 2-component 92 : 8 blend of *Z* and *E* isomers were significantly ($F = 7.8$, $df = 3, 135$, $P = 0.002$) higher than to the minor pheromone component, (*E*)-11-tetradecenyl acetate, at each dosage tested (Fig 1B). Responses to the major pheromone component and to the 92 : 8 blend were significantly ($F = 9.0$, df $= 3, 135$, $P = 0.006$) higher than to the hexane control at 2 µg – 2 mg cartridge dosages (Fig. 1B). As observed with obliquebanded leafroller, responses of female redbanded leafrollers to the minor component, (*E*)-11-tetradecenyl acetate, were significantly ($F = 4.9$, $df = 3, 135$, $P = 0.02$) higher than responses to the hexane blank only at the 2 mg dosage.

TABLE 1. Effect of (*Z*)-11-tetradecenyl acetate applied to wax paper at dosages ranging from 20 µg – 2 mg on oviposition rates of female obliquebanded and redbanded leafroller moths.

| | Mean number of eggs ± SEM laid per treatment | | | |
|---|---|---|---|---|
| Species | Control | 20 µg | 200 µg | 2 mg |
| Obliquebanded leafroller | 833.3 ± 165.0a[1] | 26.6 ± 13.7b | 34.5 ± 29.0b | 47.5 ± 32.0b |
| Redbanded leafroller | 190.3 ± 52.6a | 41.5 ± 24.9b | 38.1 ± 24.4b | 25.3 ± 13.0b |

[1] Means within rows followed by the same letter are not significantly different ($P < 0.05$, ANOVA followed by LSD).

### Female calling behavior

In the clean-air control, female calling for both the obliquebanded and redbanded leafrollers commenced after 23:00 h and terminated after 7:00 h (Fig. 2A, B). For both species, exposure to pheromone significantly reduced ($t's = 2.4$–$6.1$, $df = 1$, $P's = 0.05$) the proportion of females calling compared with paired control responses for nearly each hour of calling activity (Fig. 2A, B). Furthermore, the onset of calling for both species was delayed by 1 h and terminated 1 h earlier in pheromone-exposed moths compared with the no pheromone control (Fig. 2A, B).

### Oviposition frequency

Female obliquebanded leafrollers laid significantly ($t = 2.3$, $df = 1$, $P = 0.04$) fewer mean (± SE) eggs in chambers with constant throughput of pheromone-treated air (25.2 ± 7.4) than in chambers with throughput of solvent-control air (468.7 ± 163.7) per 24 h. Similarly, the mean (± SE) number of eggs laid by redbanded leafrollers in pheromone-permeated air (50.5 ± 22.3) was significantly ($t = 3.2$, $df = 1$, $P = 0.02$) lower than in control air (91.7 ± 16.2). Ninety-eight percent of the females in this experiment had mated based on dissections.

For both species, significantly ($F's = 11.8$ and $7.1$, $df = 3, 28$, $P's = 0.004$ and $0.03$) fewer eggs were laid on wax paper treated with pheromone, at each dosage tested, compared with the solvent-treated control (Table 1). Overall, the mean number of eggs laid by obliquebanded and redbanded leafroller females on pheromone-treated wax paper was reduced 23 and 5 fold, respectively. All of the females in this experiment had mated.

## DISCUSSION

Both species of leafrollers were capable of detecting their major sex pheromone components and exposure to pheromone had an effect on calling periodicity and oviposition rates. Behavioral changes due to pheromone exposure appeared to be mainly due to detection of the major sex pheromone component, (*Z*)-11-tetradecenyl acetate, given that there was little EAG response to the minor pheromone components and responses to species-specific blends of the *Z* and *E* isomers were not greater than to the major component alone. "Autodetection" (den Otter et al., 1978; Ochieng et al., 1995), known as antennal sensitivity of female moths to their sex pheromone components, is considered to be less common among moth species in general than female anosmia to their sex pheromone. However, among tortricids, in those instances where female moths have been investigated, they are capable of detecting their pheromone (Palanaswamy & Seabrook, 1978, 1985; Noguchi & Tamaki 1985; Barnes et al., 1992; Weissling & Knight, 1996; DeLury et al., 2005; Gökçe et al., 2006; Stelinski et al., 2006). To our knowledge, there are no published reports describing female anosmia to their sex pheromone in a tortricid species. Given broad incidence of autodetection among tortricids, further work is warranted to determine its potential evolutionary significance in this family of Lepidoptera.

Female antennal sensitivity to pheromone quantified here was below that of their conspecific males (Stelinski et al., 2003c), which is typical of autodetection among female moth species (Schneider et al., 1998). It is known that in moth species in which females are anosmic to their pheromone, females lack a male-type macroglomerular complex (Boeckh & Boeckh, 1979; Hildebrand, 1996) and have sexually-dimorphic antennae that are morphologically much simpler than that of males (Schneider et al., 1964, 1998). Further work should be conducted comparing the peripheral and central morphology of olfactory structures in male and female leafroller species.

Constant exposure to pheromone reduced the total duration of calling by a total of two hours by both delaying the onset of calling and prematurely advancing termination of calling (Fig. 2A, B). Furthermore, the total proportion of calling females was reduced by half due to pheromone exposure in both species. Constant exposure to pheromone is also known to delay the onset of calling by up to 40 min, both in the laboratory and field, for two other tortricid species [*Adoxophyes orana* (Fischer von Rösslerstamm) and *Homona magnanima* Diakonoff (Noguchi & Tamaki, 1985)]. However, there are several cases in which exposure to pheromone has been shown to either advance the onset of calling and/or increase the total proportion of calling females in tortricid moths [eg., *Choristoneura fumiferana* (Clemens) (Palanaswamy & Seabrook, 1985; and *Grapholita molesta* (Busck) (Stelinski et al., 2006)]. Also, in the tortricid female, *Cydia pomonella* (L.), exposure to pheromone increases the incidence of calling behavior, but does not advance its onset (Weissling & Knight, 1996). Finally there are examples where pheromone exposure has no effect on time of onset, frequency, and duration of calling, including the tortricids *Pandemis limitata* (Robinson) (DeLury et al., 2005), *Eupoecillia ambiguella* (Hübner), *Lobesia botrana* (Denis & Schiffermüller), and the noctuid *Spodoptera littoralis* (Boisduval) (El-Sayed & Suckling, 2005).

Several investigators have speculated on the significance of autodetection in moths. It has been postulated that female capability for detecting their own pheromone under high population densities may be advantageous if females modify their resultant calling behavior to their advantage. Specifically, exposure to pheromone advances and/or increases the level of female calling in some species, which is thought to increase the probability of attracting a mate (Palanaswamy & Seabrook, 1985; Stelinski et al., 2006). It has also been suggested that autodetection may provide a mechanism by which females maximally distribute themselves among limited host-plant resources (den Otter et al., 1996). Others have speculated that female capability for detecting their pheromone may result in aggregations of females increasing local probability of attracting males (Birch, 1977) or that it may serve as a mechanism for lek formation (Schneider et al., 1998). In the current study, approximately ½ of females of both leafroller species ceased calling under the conditions of constant exposure to their pheromone throughout their normal calling period, which was also truncated by approximately 2 h. Future investigations will need to be conducted to determine the mechanism responsible for this behavioral modification and/or its potential evolutionary or ecological implications.

Interestingly, given that females of the two species investigated here share the major pheromone component, (*Z*)-11-tetradecenyl acetate, and that antennal sensitivity was recorded mainly to this compound with little sensitivity to the selected minor components tested, it is possible that interspecific interactions may also occur. More detailed single sensillum work will need to be conducted before it can be concluded that female leafrollers are not sensitive to their minor pheromone components and that these do not affect their behavior. From an applied perspective, decreasing the proportion of calling females under synthetic pheromone treatment in the field should positively impact the success of mating disruption for both species.

Exposure to pheromone-permeated air reduced egg-laying in females of both species. Furthermore, application of the major pheromone component, (*Z*)-11-tetradecenyl acetate, to an ovipositional substrate deterred oviposition. These results are in contrast to those reported for *C. fumiferana*, where pheromone exposure stimulated egg-laying (Palanaswamy & Seabrook, 1978). There are other examples where exposure to the female-produced pheromone did not affect oviposition rates, eg. *G. molesta* females (Stelinski et al., 2006) and *C. pomonella* (Weissling & Knight, 1996). However, application of the female-produced codling moth sex pheromone, (*E*,*E*)-8,10-dodecadien-1-ol, to wax paper ovipositional substrates also reduced oviposition rates in the tortricid *C. pomonella* (Weissling & Knight, 1996); a result that is consistent with the current study. Poirier & Borden (1991) demonstrated that female obliquebanded leafrollers recognize and avoid previously-laid egg masses of conspecific females. These authors postulated that female obliquebanded leafrollers deposit an oviposition-deterring pheromone along with the egg mass while ovipositing, providing a mechanism for spacing egg masses to avoid potential larval crowding and intraspecific competition. Our data support the hypothesis that the major sex-attractant pheromone component, (*Z*)-11-tetradecenyl acetate, may play a second function as this putative oviposition-deterring pheromone deposited by females in trace amounts while ovipositing. If so, such a chemical marker could act both intraspecifically and interspecifically between obliquebanded and redbanded leafrollers. Chemical analyses of leafroller egg masses are planned to test this hypothesis. Reduced oviposition by both species of leafrollers investigated here suggests that synthetic pheromone applied for mating disruption may affect female egg laying in treated orchards. This may be particularly true with sprayable microencapsulated formulations of synthetic pheromone for leafrollers (Judd et al., 2005), which are applied directly to tree foliage. In a field trial, Evenden (1998) observed a delay in the onset of calling in female obliquebanded leafrollers and a potential reduction in time spent calling compared with counterparts in clean air; a result consistent with the laboratory data presented herein. Further field testing in which female calling behavior and oviposition rates are measured in pheromone-treated versus untreated plots should improve our understanding of how mating disruption contributes to pest control by affecting female leafroller behavior.

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