

Sexual biology and mating disruption of orange tortrix, *Argyrotaenia citrana* (Lepidoptera: Tortricidae)

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ABSTRACT

Studies were conducted to characterize the sexual biology of *Argyrotaenia citrana* (Fernald) and to evaluate the potential of sex pheromones to disrupt moth communication. Both males and females are sexually active during their first scotophase. Virgin females start calling 3 hrs into scotophase and continue until sunrise. Calling frequency by virgins is lower during the first than in subsequent nights. Females generally mate once during a scotophase. Calling is reduced after mating for one scotophase and then increases though mated females continue to call less frequently than virgins. Peak calling by mated females is delayed several hours compared with virgins. Females may remate after 1-3 days. Males can mate more than once per scotophase. Oviposition is concentrated during early scotophase. Females laid an average of five egg masses. Communication and mating disruption were evaluated in replicated 0.1 ha plots and 100 m² field cages treated with field-aged polyethylene tube dispensers releasing 0.7-1.2 mg/d of either (Z)-11-tetradecenyl acetate alone or in a 15:1 blend with (Z)-11-tetradecenal. Mating of tethered females in field cages and catches of lure and female-baited traps in small field plots were nearly completely disrupted with the two component blend. Dispensers emitting only the acetate pheromone were less effective in disrupting moth communication in similar tests.

Key words: *Argyrotaenia citrana*, orange tortrix, leafrollers, *Rubus*, mating disruption, sexual behavior

INTRODUCTION

The use of mating disruption as an alternative to broad-spectrum insecticides for control of tortricid pests of horticultural crops is being widely investigated (Pfeiffer *et al.* 1993, Deland *et al.* 1994, Felland *et al.* 1995, Suckling and Shaw 1995, Shorey *et al.* 1995, Agnello *et al.* 1996). Many of the tortricid pests found in North American tree fruits share one or more pheromone components (Arn *et al.* 1992). The most important component for these species in western North America is (Z)-11-tetradecenyl acetate (Z11-14:OAc). A generic, multi-species approach to mating disruption may be possible using this single component or in combination with one or more minor components (Knight 1992, Deland *et al.* 1994, Cardé and Minks 1995). Knowledge of the adult sexual behaviors of each of these species is a prerequisite for development of this behavior-based control tactic (McNeil 1991).

The orange tortrix, *Argyrotaenia citrana* (Fernald), is an economic pest of small fruits, tree fruits, and grapes in areas with relatively moderate winter temperatures in the western United States, i.e., Willamette Valley in Oregon and coastal areas of California (Breakey and Batchelor 1948, Madsen and Falcon 1960, Kido *et al.* 1981). Bassinger (1938) provided the first detailed summary of the bionomics of *A. citrana*. He noted that females generally mated only once, but males were polygamous. The sex pheromone of orange tortrix was identified by Hill *et al.* (1975) as a two component blend of Z11-

14:OAc and (Z)-11-tetradecenal (Z11-14:ALD). Sex pheromone traps are used to recommend and time insecticide sprays (Knight *et al.* 1988). Knight *et al.* (1994) used a pheromone-baited timing trap in the field and a laboratory ultrasound motion detector to determine the circadian periodicity of moth activity. Other important aspects of *A. citrana* behavioral ecology such as temporal patterns of calling, mating, and oviposition have not been reported.

The present paper reports on laboratory studies conducted with *A. citrana* to characterize several aspects of its sexual behavior, and field trials to evaluate the effectiveness of using either Z11-14:OAc alone or in combination with Z11-14:ALD to disrupt communication. Dispensers emitting the complete pheromone blend caused nearly 100% disruption over the entire season.

MATERIALS AND METHODS

Laboratory studies. A laboratory colony was established with larvae collected from caneberry *Rubus spp.* in Linn County, Oregon in 1992. Larvae were reared on a synthetic pinto bean diet (Shorey and Hale 1965) at 24°C and a 16:8 (L:D) photoperiod in 30 ml plastic cups. Adult sexual behaviors were studied at 22±2°C, 50-65% relative humidity, and a 16:8 (L:D) photoperiod. A reversed photoperiod was used to facilitate observations of moth behavior. Light levels were controlled by time clocks which switched on a series of incandescent light sources during the 60 min dusk (0800-0900 h) and sunrise (1700-1800 h) periods (Knight *et al.* 1994). Illumination during scotophase was provided by a light covered with a red acetate filter. Adults were supplied with a cotton wick saturated with a 10% honey solution.

The effect of mating on calling behavior was studied by recording the calling behavior of fifty newly-mated and virgin females for 30 s every 30 min for five and six nights, respectively. Observations of calling behavior were made of females in 250 ml waxed paper cups covered with a clear polyethylene film. Calling behavior was distinguished by wing elevation up to 45° and a downward extension of their abdomen for 20-60 s every 1-3 min. Calling frequency (the number of 30-min intervals during which calling was observed in each scotophase) for mated and virgin females was transformed (square root [$x+0.01$]) and subjected to ANOVA (GLM Procedure, SAS Institute 1985). Means were separated where significant differences occurred with a least significance difference test (LSD). To test whether males and females mate more than once, 100 virgin pairs were placed in cups, and following each successful copulation, males were replaced with a virgin male (< 48 h-old) in half of the cups and females were replaced with virgin females (< 24 h-old) in the other half. Temporal patterns of oviposition on a wax paper substrate were measured for 50 mated females using a clock-driven rotating oviposition apparatus at 20°C and a 16:8 (L:D) with lights-off at 2200 h (Weissling and Knight 1996). Newly emerged females were placed in cups with a male for 24 h and females were transferred to the apparatus between 1000-1100 hours. Oviposition was measured for four nights. Four percent of females laid an egg mass within 30 min of being transferred to the apparatus. These data plus any egg masses laid by unfertilized females were not used in the analysis.

Pheromone dispensers. Two types of polyethylene tube dispensers (Pacific Biocontrol, Vancouver, WA) were evaluated in field studies: Hamaki-con (Lot # TT-52003), a translucent dispenser containing 165 mg 11-14:OAc (94:6 ratio of Z:E isomers); and OT (Lot # OTX53004), a reddish-brown dispenser loaded with 180 mg of pheromone in a 15:1 ratio of 11-14:OAc and 11-14:ALD (94:6 and 93:7 ratio of Z:E

isomers, respectively). Residual pheromone content of new dispensers and those aged in the field and collected every 14 d ($n=4$) from 21 April to 9 Aug 1993 were analyzed with gas chromatography. Dispensers were cut into 2 cm pieces and rinsed continuously with dichloromethane for 3 h. Undecanol was used as the internal standard. Samples were processed with a HP7673 automatic sampler and a Series II 5890 gas chromatograph (Hewlett Packard, Wilmington, DE) using a 60 m x 0.32 mm capillary column coated with dimethylpolysiloxane (DB-1, J&W Scientific, Folsom, CA).

Field trials. Experiment 1 was run in a mixed planting of boysenberry and marionberry (*Rubus* spp.) located near Woodburn, OR for two weeks beginning 10 Aug 1992. Experiment 2 was conducted in a mixed planting of marionberry and raspberry located near Scholls, OR from 14 April to 17 Aug 1993. Both studies were conducted as randomized complete block designs with five replicates of the two pheromone treatments plus an untreated check. Each plot was ca. 30 m x 30 m and plots were separated by > 50 m. Dispensers were evaluated at a density of 1000 per ha. The canopies of the caneberry fields was ca. 1.5 m in height and dispensers were placed at 1.0 - 1.5 m. In both experiments, one rubber septa-baited sex pheromone trap (wing trap, Scentry Inc., Buckeye, AZ) was placed in the center of each plot. In addition, in the 1992 experiment, five virgin-female-baited wing traps (baited with two 1-3 day-old virgin moths placed in a screened pvc cage) were spaced 10 m apart and 5 m inside the edge of each plot. Virgin females were replaced after seven days. Rubber septa were replaced every four weeks in 1993. No insecticides were applied during either study. The number of males captured in pheromone-baited and female-baited traps and the percentage of female-baited traps catching at least one male were transformed (square root $[x + 0.01]$ and arcsin $[x]$, respectively) and subjected to ANOVA (GLM Procedure, SAS Institute 1985). Where significant differences occurred, means were separated with LSD.

Field cage experiments. Six cages (10 x 10 x 2.5 m) were used to measure the level of mating disruption attained under each pheromone treatment versus an untreated check. Each cage contained nine potted apple trees 2.5 m in height (a mix of 'Delicious' and 'Golden Delicious'). Trees were spaced 2.5 m apart in three rows. Nine dispensers (one on each tree at a height of 2.0 m) were placed in each cage in the pheromone treatments. Virgin female moths were tethered at a height of 1.5 - 2.0 m by tying a fine thread (25 cm) around one forewing and taping the end of the thread to a bamboo pole hung vertically from wires within each cage. Tethered females were situated within 0.5 m of foliage. Fifteen females were tethered per cage for each replicate. The same number of males (20-50) was added to each cage per day. Females were left in cages overnight and dissected the next day to determine their mating status. Approximately 20% of the females were either missing or partially eaten by spiders during these tests. On each date each treatment was replicated twice. Dispensers were removed from cages and treatments were rotated among cages after 48-72 h. Experiments were repeated five times between 25 Aug - 15 Sept 1993. The percentage of females mated was transformed (arcsin $[x]$) and subjected to ANOVA (SAS Institute 1985).

RESULTS

Calling behavior. Virgin females started calling approximately 3 h into the first scotophase, with peak levels of calling occurring at 4.5 h (Fig. 1A). Moth calling frequency was lower during the first scotophase, but did not differ for 2- to 6-day-old moths ($F = 8.9$; $df = 5, 282$; $p < 0.0001$). Unmated females called until dawn. Frequency of calling by mated females was significantly lower during the scotophase following

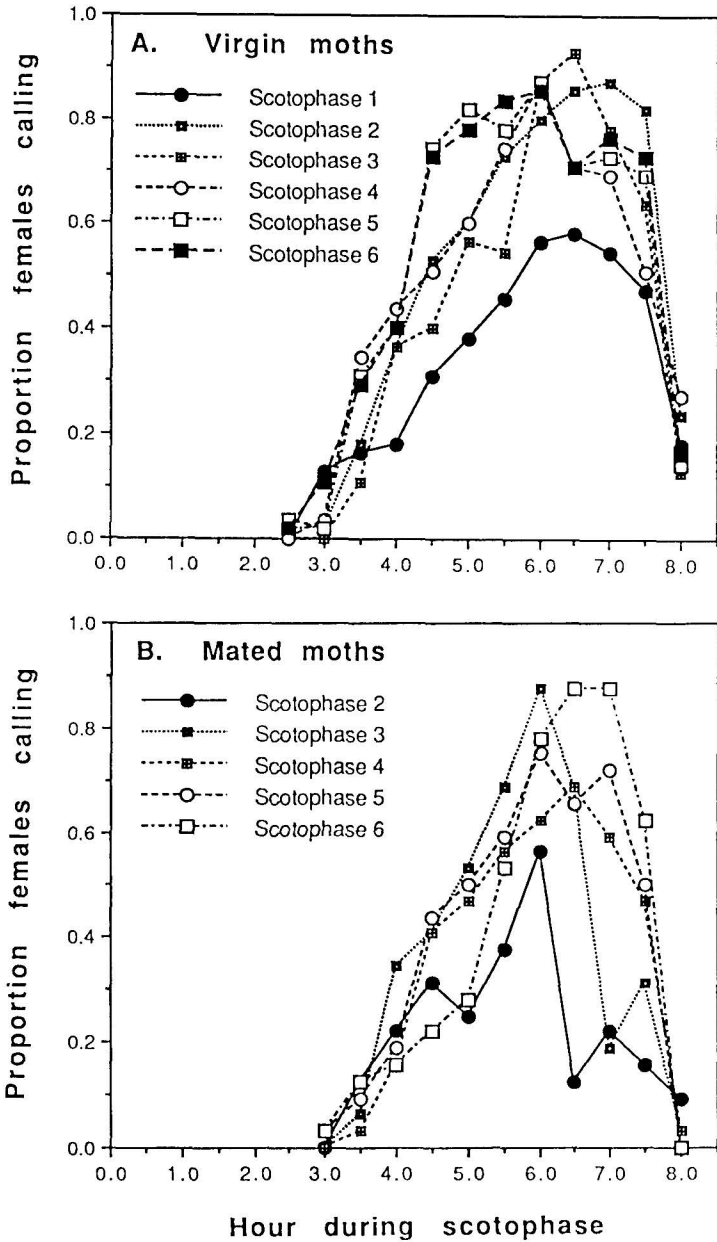


Figure 1. Proportion of female *Argyrotaenia citrana* moths observed calling during an 8-h scotophase. (A) Virgin moths < 12 h-old observed for six nights; and (B) Moths, 36 h-old mated the previous scotophase and then observed for five nights

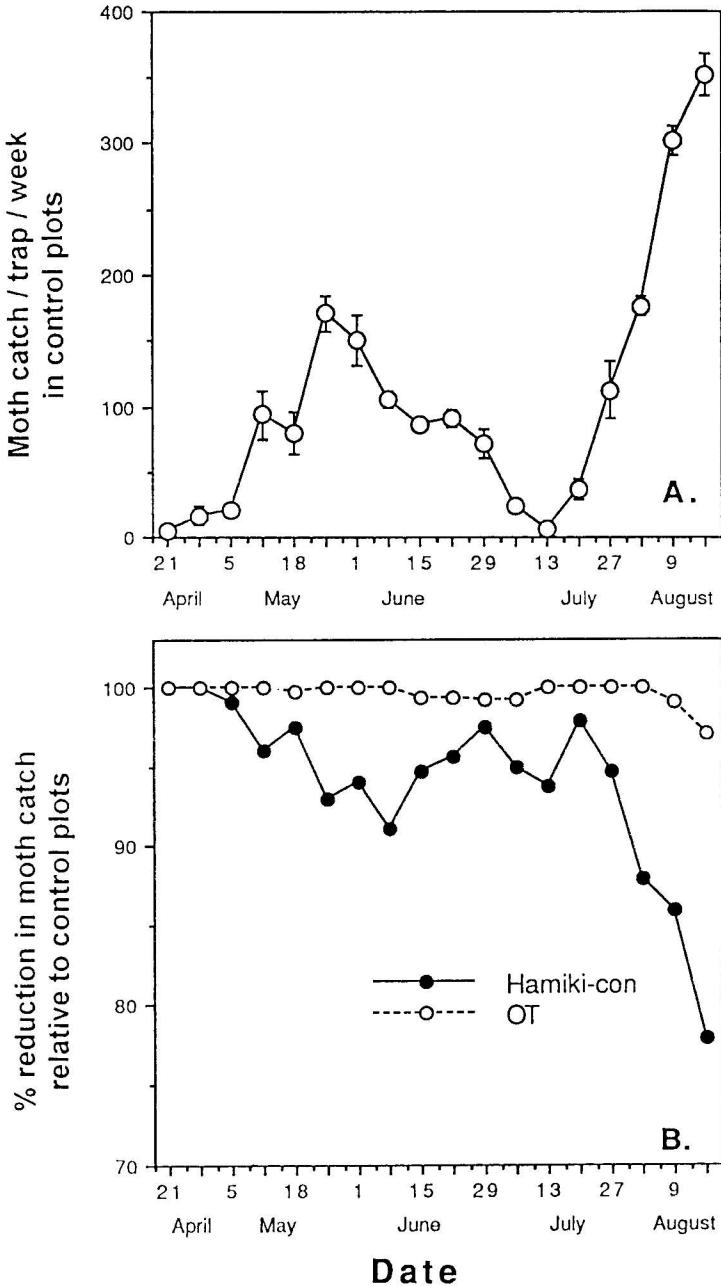


Figure 2. Sex-pheromone-based disruption of *A. citrana* using polyethylene tube dispensers applied at 1,000 / ha releasing either (Z)-11 tetradecenyl acetate (Z11-14:OAc) Hamaki-con dispenser; or a 15:1 blend of Z11-14:OAc and (Z)-11-tetradecenal - OT dispenser, from 14 April to 17 Aug. in a commercial marionberry field, Schols, OR. (A) Weekly catch of males in a lure-baited trap in the control plot; and (B) Percentage reduction of weekly trap catch in the Hamaki-con and OT pheromone treatments compared with control

mating ($F = 4.9$; $df = 4, 140$; $p = 0.001$), and then increased, and remained unchanged during the next four nights (Fig. 1B). Frequency of calling by mated females was significantly lower than for virgin moths ($F = 58.2$; $df = 1,375$; $P < 0.0001$), but a significant interaction with age occurred ($F = 3.2$; $df = 1,375$; $P = 0.01$). All linear contrasts for mating status by age were significant except for 3-day-old moths, $P = 0.23$. Timing of peak calling by mated females was more restricted than for virgin moths and was shifted 1-2 h later into scotophase (Fig. 1A, B).

Mating. Mating on average (\pm SE) began 4.8 ± 0.2 h into scotophase and lasted 63 ± 4 min. Eighty-seven percent of females were mated when paired with a male in cups. Only 4% of these females mated more than once per evening and this occurred only when the first mating event lasted < 30 min. Dissection of these females found only one spermatophore. Thirty-eight percent of the females mated more than once during the six nights and had a mean refractory period of 2.1 ± 0.2 d. Twenty-seven percent of males mated more than once and no male mated more than three times per evening. Males mated an average of 2.9 ± 0.3 times during the six nights of the test.

Oviposition. Eggs were laid 1-3 d after females mated. Females laid an average of 4.8 ± 0.3 egg masses in cups. The first egg mass averaged 60.1 ± 6.9 eggs and was significantly larger than subsequent egg masses ($F = 4.61$; $df = 6, 263$; $p = 0.003$). The mean number of eggs laid per female was 218.2 ± 9.4 . Females laid an average of 5.1 ± 0.8 egg masses on the oviposition apparatus. Eggs were laid between 1700 and 0700 hours, however, oviposition was highly concentrated at the beginning of scotophase (lights off at 2200 h), mean = 2311 ± 0.09 h.

Analysis of dispensers. Hamaki-con dispensers exhibited a linear decline in their residual pheromone content over 110 d, $r^2 = 0.97$, mean release rate = 0.87 mg/d during the experiment (Table 1). A new dispenser contained an average of 5.7% E11-14:OAc and this increased to 9.3% after 110 d in the field. Nearly 70 mg of pheromone remained in the dispenser after 110 d.

Table 1

Analysis of field-aged polyethylene tube dispensers. The Hamaki-con dispensers contained an average of 165.4 mg of 11-tetradecenyl acetate (11-14:OAc) and the OT dispenser contained an average of 180.5 mg of a 15:1 ratio of 11-14:OAc and 11-tetradecenal (11-14:ALD). Dispensers were placed in the field on 21 April, 1992 and collected every two weeks.

Field age (D)	Hamaki-con Dispenser		OT Dispenser		
	Pheromone loss mg/day ^a	Isomeric purity (Z:E) ratio 11-14:OAc	Pheromone loss mg/day ^a	Isomeric purity (Z:E) ratio 11-14:OAc	Isomeric purity (Z:E) ratio 11-14:ALD
New	-	94.3:5.7	-	94.3:5.7	92.8:7.2
0-14	1.40	94.0:6.0	1.01	94.1:5.9	93.3:6.7
15-28	0.55	93.5:6.5	0.98	94.2:5.8	92.4:7.6
29-41	1.19	93.5:6.5	1.44	94.2:5.8	90.0:10.0
42-55	0.88	92.9:7.1	0.89	94.2:5.8	92.0:8.0
56-69	0.58	92.3:7.7	0.68	94.2:5.8	92.6:7.4
70-83	0.85	91.8:7.9	0.43	94.2:5.8	92.4:7.6
84-96	0.51	91.2:8.8	1.39	94.2:5.8	90.2:9.8
97-110	1.00	90.7:9.3	1.32	94.2:5.8	86.7:13.3

^a Change in residual content of 4 dispensers sampled on each date expressed as loss (mg) per d.

Pheromone loss from the OT dispenser declined linearly, $r^2 = 0.99$, mean release rate = 1.01 mg/d (Table 1). This dispenser contained 6.2% 11-14:ALD initially with ca. 92.8% Z-isomer. After 110 d, the pheromone blend left in the dispenser contained 3.2% 11-14:ALD with a 86.7% Z-isomer. The percentage of E11-14:OAc was stable during the season (Table 1). Nearly 70 mg of pheromone remained in the dispenser after 110 d.

Field tests. In the 1992 experiment there were significant differences in moth catches between treatments in both lure-baited ($F = 85.4$; $df=2,12$; $p = 0.0001$) and female-baited traps (average number of males caught: $F = 7.5$; $df = 2,12$; $p = 0.001$; and the proportion of traps catching ≥ 1 moth: $F = 174.4$; $df = 2,12$; $p = 0.001$). No significant differences in cumulative moth catches occurred between the two pheromone treatments (Table 2).

Table 2.

Sex pheromone-based disruption of *Argyrotaenia citrana* using polyethylene tube dispensers at 1,000 per ha releasing either (Z)-11-tetradecenyl acetate (Z11-14:OAc) or a 15:1 blend of Z11-14:OAc and (Z)-11-tetradecenal (Z11-14:ALD). Tests were conducted from 10-21 August, 1992 in mixed marionberry / boysenberry field, Woodburn, OR

Treatment	Mean (\pm SE) catch per trap				Proportion female-baited traps catching	
	Lure-baited	% reduc. ¹	Female-baited	% reduc. ¹	> 1 moth	% reduc. ¹
Z11-14:OAc	2.4 \pm 0.7b	98	0.5 \pm 0.2b	90	0.08 \pm 0.03b	96
Z11-14:OAc + Z11-14:ALD	0.0 \pm 0.0b	100	0.0 \pm 0.0b	100	0.00 \pm 0.00b	100
Control	144.2 \pm 15.4a	-	24.7 \pm 6.5a	-	0.76 \pm 0.05a	-

¹ Percent reduction relative to the control plot. Column means followed by different letters are significantly different, LSD, $p < 0.05$.

In 1993 the first moths were caught in the check plots during the week of 21 April and the first adult flight continued until 13 July (Fig. 2A). In comparison, the first moths were caught in the Hamaki-con and OT-treated plots on 5 May and 18 May, respectively. Moth flight during the second generation began the week of 20 July and moth catch increased sharply each week until the study was terminated on 17 Aug (Fig. 2A). Moth catch varied significantly among treatments during both generations (first generation: $F = 142.5$; $df = 2, 12$; $p < 0.0001$; second generation: $F = 370.0$; $df = 2, 12$; $P < 0.0001$ [Table 3]).

Table 3.

Sex pheromone-based disruption of *Argyrotaenia citrana* using polyethylene tube dispensers at 1,000 per ha releasing either (Z)-11-tetradecenyl acetate (Z11-14:OAc) or a 15:1 blend of Z11-14:OAc and (Z)-11-tetradecenal (Z11-14:ALD) from 14 April to 17 August, 1993, in a marionberry field, Scholls, OR.

Treatment	Mean catch (\pm SE) per trap			
	14 April - 13 July		14 July - 17 August	
	Lure-baited	% reduc. ¹	Lure-baited	% reduc. ¹
Z11-14 acetate	49.6 \pm 7.3b	95	148.4 \pm 18.6b	85
Z11-14 acetate + Z11-14 aldehyde	2.4 \pm 1.1b	>99	14.6 \pm 3.7c	99
Control	21.4 \pm 74.7a	-	980.8 \pm 41.8a	-

¹ Percent reduction relative to the control. Column means followed by different letters are significantly different, LSD, $p < 0.05$.

Weekly moth catch in the OT dispenser treatment was reduced by > 99% compared to traps in the control and then declined to 96% the last week of the study (Fig. 2B). Moth counts in the Hamaki-con treatment compared with the control were reduced 95% during the first flight, but were only reduced 85% during the second flight. Disruption declined sharply during the last three weeks of the study (Fig. 2B). During the second moth flight, the percentage reduction in trap catch was significantly different between the two pheromone treatments, $P < 0.05$ (Table 3)

Cage experiments. Thirty-five percent of the tethered females were mated in the untreated cages. This was significantly higher ($F = 10.0$, $df = 2, 12$; $p = 0.01$) than the level of mating under either pheromone treatment, OT = 0.0% and Hamaki-con = 1.9%. The level of mating in the two pheromone treatments did not differ, $p > 0.05$.

DISCUSSION

Successful incorporation of mating disruption technology into pest management programs first requires a basic knowledge of the insect's biology and behavior and then depends on a reliable dispenser system and reasonable cost. This study provides more information on the temporal aspects of female calling, mating, and oviposition of *A. citrana*. Further insights into the myriad attributes of this species' mating behavior are needed, including a female's pheromone emission rate, moth behavior within a pheromone-flooded field, and the effects of canopy structure on the pheromone plume (Cardé and Minks 1995).

A preliminary test of mating disruption with an acetate:aldehyde blend was done at the same time as mine by R. LaLone (Smuckers Inc., Woodburn, OR) in commercial fields, but the role of population density and immigration on its results was not clear. Capture of males was suppressed, but in some cases, larval populations were high and supplemental sprays were needed. At present, growers control *A. citrana* in caneberry fields with 1-2 applications of conventional insecticides (Knight *et al.* 1988). Adoption of mating disruption for control of *A. citrana* is hampered by its expected higher cost and the need to treat fields with pesticides to avoid the presence of arthropods in processed fruit (Martin and Lawrence 1976). Market forces that demand no pesticide residues may be needed for the registration and grower adoption of this technology (Ott *et al.* 1991).

Both dispensers appeared to provide relatively stable pheromone emissions for 110 d in the field. The percentage of E11-14:OAc in the Hamaki-con increased continuously with field aging similarly to that reported by Deland (1992) but at a low rate, 0.03% per d. This isomerization did not occur in the OT dispenser. The formulation in the OT also appeared to stabilize the isomer ratio of the aldehyde for 80 d. Over the last 30 d of the study, however, the percentage of the E11-14:ALD nearly doubled (Table 1). The ratio of acetate/aldehyde remaining in the dispenser dropped from 6.2% to <4.0%; this may have been due to a higher emission rate of the aldehyde during the season or to degradation. Aldehydes can be oxidized and trimerized (Dunkelblum *et al.* 1984), but I did not measure these byproducts. The stabilizers in these dispensers are proprietary and not known to me.

The effect of these changes in the blend and isomer ratios of pheromones in these dispensers for disruption of *A. citrana* or for other species is unknown. Studies are needed to assess the level of disruption caused by dispensers of various ages. The second flight of *A. citrana* can extend into late Sept and disruption of both flights requires dispensers to last up to 150 d. Further analyses of these dispenser's performance after 110 d are needed.

The Hamaki-con dispenser has been used by several researchers to test a generic blend for mating disruption of tortricid pest species (two species in tea in Asia [Nagata 1989], and several on apple in Japan [Oku 1993], and North America [Deland *et al.* 1994, Agnello *et al.* 1996]). However, the Hamaki-con dispenser used in their trials had less pheromone (80 mg A.I.) and a lower emission rate (ca. 0.48 mg/d [Deland *et al.* 1994, Agnello *et al.* 1996]). A generic dispenser containing only a partial pheromone blend was used in these trials because the pheromone of all these species contains Z11-14:OAc as a major component (Arn *et al.* 1992). Its performance has been inconsistent; Deland *et al.* (1994) obtained > 99% reduction of male catch of *Archips argyrospila* (Walker) in lure-baited traps with plots treated with Hamaki-con, but only 90% for *Archips rosana* (L.) and *Choristoneura rosaceana* (Harris). The poorer performance of the Hamaki-con dispenser for the last two species was probably because its binary blend was not different enough from the female's complete blend to cause a significant imbalance in sensory input nor was it similar enough to cause false trail following (Deland *et al.* 1994). Agnello *et al.* (1996) obtained 94% reduction in catch of *C. rosaceana* in plots treated with Hamaki-con dispensers, however, using the complete three-component pheromone blend that included 5% Z11-14:OH, they improved trap catch shut-down to 99%.

These findings are consistent with my results with *A. citrana*. Hill *et al.* (1975) showed that Z11-14:OAc is not attractive to male moths and that attraction of the natural blend was not affected by up to 10% of the E-isomer. Thus sensory imbalance is not likely to be an important mechanism due to the similarity in the percentage of Z11-14:OAc in the natural blend and the dispenser. The high level of disruption provided by the natural blend suggests that false trail following could be a major mechanism of disruption of *A. citrana* with the OT dispenser.

I suggest that the OT dispenser's blend should be investigated as a generic blend for some species of leafrollers in the western U.S. For instance, the sex pheromone of *C. rosaceana* in western North America contains Z11-14:ALD (Thomson *et al.* 1991). Lures loaded with the natural blend of *A. citrana* are 7-10 \times more attractive than lures loaded with the three-component blend (Hill and Roelofs 1979) of *C. rosaceana* from the eastern U.S. (unpublished data). Yet, small-plot trials conducted in Washington with the OT and Hamaki-con dispensers for *C. rosaceana* have not shown any significant difference in the activity of these two dispensers (unpublished data). The effect of Z11-14:ALD on the other horticultural tortricid pests in the western U.S. is unknown.

The major limitation in the development of mating disruption to control leafrollers may be economics. Leafrollers have been a secondary pest in most crops and have only become a problem after the development of resistance or softer conventional programs. Companies have not been interested in developing many specific pheromone products for the various species of leafrollers. In apple, dual dispensers containing the sex pheromone of both codling moth, *Cydia pomonella* L. and leafrollers have been tested (reviewed by Cardé and Minks 1995). These dispensers have always contained Z11-14:OAc for generic disruption of leafrollers and their performance has been mixed. My results with *A. citrana* support the use of the natural pheromone blend but more research is needed on other leafroller species and to show how this approach can best be developed and used in IPM.

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