

ORIGINAL CONTRIBUTION

Variability in the efficacy of sex pheromone lures for monitoring oriental fruit moth (Lepidoptera: Tortricidae)A. L. Knight¹, E. Basoalto² & L. L. Stelinski³¹ Yakima Agricultural Research Laboratory, Agricultural Research Service, USDA, Wapato, WA, USA² Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias, Universidad Austral de Chile, Valdivia, Chile³ Citrus Research and Education Center, Entomology and Nematology Department, University Florida, Lake Alfred, FL, USA**Keywords***Cydia pomonella*, *Grapholita molesta*, monitoring, peach**Correspondence**Alan L. Knight (corresponding author), USDA, Agricultural Research Service, 5230 Konnowac Pass Road, Wapato, WA 98951, USA.
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Abstract

Studies were conducted in Chile and the United States to compare the attractiveness of various commercial sex pheromone lures and two experimental lures for oriental fruit moth, *Grapholita molesta* (Busck), in peach orchards treated with or without sex pheromone dispensers. The experimental lures contained the three-component sex pheromone blend of *G. molesta*: Z-8-dodecenyl acetate, E-8-dodecenyl acetate and Z-8-dodecenol (Z8-12:OH), and the sex pheromone of codling moth, *Cydia pomonella* (L.), (E,E)-8,10-dodecadien-1-ol, (codlemone). Commercial lures varied in their substrate, initial loading and blend ratio of components. Significant differences in male catches were found among commercial lures in orchards treated with or without sex pheromone dispensers. Experimental lures with the addition of codlemone significantly increased the catches of *G. molesta* using lures loaded with 0%, 1% or 5% Z8-12:OH in the *G. molesta* blend compared with the same ratio of components in just the *G. molesta* blend. The experimental lures were significantly more attractive than all commercial lures in the untreated orchard. However, moth catch with the experimental lures in the sex pheromone-treated orchard was only intermediate among all of the lures tested. These findings highlight the need to develop more effective and standardized lures that can be used in trap-based monitoring programme for this important pest.

Introduction

The oriental fruit moth, *Grapholita molesta* (Busck), is a widely distributed tortricid fruit pest attacking stone (*Prunus* spp.) and pome (*Malus* and *Pyrus* spp.) tree fruits. *G. molesta* females release a chemical blend from their sex pheromone gland mainly composed of a 100 : 6 : 3 blend of Z-8-dodecenyl acetate (Z8-12:Ac), E-8-dodecenyl acetate (E8-12:Ac) and Z-8-dodecenol (Z8-12:OH) (Cardé et al. 1979), among other minor components (Linn and Roelofs 1983). The proportion of E8-12:Ac found in the female's natural blend has been fairly consistent across investigations: 5–6% (Cardé et al. 1979; Baker et al. 1980; Lacey and Sanders 1992; El-Sayed and Trimble 2002; Yang et al. 2002; Knight et al. 2015). In comparison, the

percentage of Z8-12:OH compared with Z8-12:Ac in females' effluvia has ranged from trace amounts (Lacey and Sanders 1992) to $\geq 30\%$ (Cardé et al. 1979; Baker et al. 1980). A number of studies have characterized the influence of blend ratios of the two acetates with and without the addition of Z8-12:OH on male flight behaviours (Baker and Cardé 1979; Baker et al. 1981; Linn and Roelofs 1983; Knight et al. 2015). A blend with a 100 : 6 : 10 ratio of these components was reported to be the most attractive (Linn and Roelofs 1983). This blend was evaluated as a tool to monitor worldwide populations of *G. molesta* (Knight et al. 2015). However, publication of the proprietary loading of monitoring lures or pre-registration efficacy testing is not required in the United States, and no previous study has reported the

loading or compared the effectiveness of the various lures available to monitor *G. molesta* in either sex pheromone-treated or untreated orchards. Therefore, variation in lure efficacy among various commercial formulations may be a problem and warranted investigation.

Seasonal monitoring of male *G. molesta* flight and population dynamics within orchards treated with sex pheromone for mating disruption has been difficult with sex pheromone-baited traps (Knight et al. 2013). Furthermore, disruption of moth catch by pheromone-baited traps has been used as a seasonal indicator of the success of mating disruption (Rice and Kirsch 1990); therefore, effective lures are needed within both pheromone-treated and untreated orchards.

Recent testing of a combinational lure loaded with the three-component *G. molesta* blend (93 : 6 : 1) and also containing (*E,E*)-8,10-dodecadien-1-ol, (*E,E*8-12:OH), the main sex pheromone component of the codling moth, *Cydia pomonella* (L.), increased the catch of *G. molesta* 2- to 8-fold in sex pheromone-treated orchards in Argentina, Chile and the United States (Knight et al. 2014). The low moth catch in traps baited with a commercial sex pheromone lure loaded with a purported 93 : 6 : 1 blend in these trials was surprising. We hypothesized that variation among existing lures may contribute to possible significant differences in lure effectiveness for monitoring *G. molesta*. Therefore, here, we compare several commercial sex pheromone lures, as well as lures that also contained the main sex pheromone of *C. pomonella* (*E,E*8-12:OH). Experiments were conducted in both sex pheromone-treated and untreated orchards in the United States and Chile, respectively. Experiments were also conducted to evaluate the importance of Z8-12:OH within the known *G. molesta* sex pheromone lure or within the lure that also contained the *C. pomonella* pheromone, *E,E*8-12:OH.

Materials and Methods

Traps, lures and field study protocol

Studies were conducted with orange delta-shaped traps ((28 × 20 × 20 cm, Pherocon VI, Trécé Inc., Adair, OK) baited with one of several commercially available or experimental lures during 2013-2014. Paper liners coated with a polybutane adhesive (Trécé Inc.) were used in all studies in Chile. Liners coated with a proprietary dry adhesive (Alpha Scents, Portland, OR) were used in the USA. Lures were

attached to the inside roof of the trap. Traps were placed in the upper third of the canopy, 20–30 m apart, and >10 m from the borders of orchards. Treatments were randomized within each orchard with five replicates. Studies were conducted for 7 days; traps were re-randomized following inspection; and experiments were repeated on 2–4 separate dates among experiments.

Proprietary sex pheromone lures were purchased from several companies, including red rubber and grey elastomer septa, as well as a plastic membrane dispenser (table 1). Experimental grey septum lures were prepared by Trécé Inc. chemists in 2013 and loaded with three different ratios of the three-component sex pheromone: *E*8-12:Ac (98.6% purity), Z8-12:Ac (98.1% purity) and Z8-12:OH (98.3% purity). The combined loading of Z8-12:Ac and *E*8-12:Ac was 0.41 mg within each lure type, but the percentage of Z8-12:OH in the total blend was adjusted to be either 0%, 1% or 5% of the total loading. Additional lures were prepared with these three blends of *G. molesta* sex pheromone plus 3.0 mg of *E,E*8-12:OH. The seventh lure type was loaded with only 3.0 mg of *E,E*8-12:OH. Similar experimental lures were prepared in 2014 with either 1% or 5% Z8-12:OH plus *E,E*8-12:OH (table 1).

Lure analysis

Lures were extracted by placing each septum into a 20-ml glass scintillation vial (Fisher Scientific, Pittsburgh, PA) and then adding 10 ml of dichloromethane. Thereafter, the vials were kept at room temperature for 24 h. A 900- μ l aliquot from each extract was pipetted into a 4-ml glass screw-top vial after which 100 μ l of an internal standard was added. The internal standard used was hexadecyl acetate (Sigma-Aldrich, St. Louis, MO) at a concentration of 1.123 μ g/ μ l. A 1 μ l sample of each extract containing the added internal standard was analysed by gas chromatography. The amount of each compound within release devices was calculated by comparing peak areas of extracted compounds to that of the internal standard. The retention times of all compounds in the extract were verified with synthetic standards. The gas chromatograph (GC) was a Varian CP-3800 equipped with a 30-m, 0.25-mm ID, 0.5- μ m-df film Restek RTX5 column (Restek Corp., Bellefonte, PA) and a flame-ionization detector. The GC temperature programme was initially 40°C and increased at 10°C/min to 250°C with a 2.5-min final hold using helium as the carrier gas.

Table 1 Chemical analysis of commercial and experimental lures tested in 2014

Lures manufacturer, substrate, product id	Mean (SE) lure loading (μg) [% of <i>Grapholita molesta</i> 3-component blend]			
	Z8-12:Ac	E8-12:Ac	Z8-12:OH	E,E8,12:OH
Suterra, red septa, #14994	118.3 (20.0) [76]	36.5 (9.4) [24]	0.0 (0.0) [0]	–
Scentry, grey septa, #L111	39.8 (3.9) [93]	2.5 (1.3) [6]	0.5 (0.5) [1]	–
Alpha Scents, red septa, #12J19	47.9 (6.4) [86]	7.1 (3.8) [13]	0.6 (0.1) [1]	–
Suterra, Biolure, #14995	430.7 (79.2) [76]	113.0 (18.9) [20]	25.2 (5.2) [4]	–
Trécé, red septa, #3102	131.4 (14.3) [85]	4.7 (0.7) [3]	17.9 (1.0) [12]	–
Trécé, grey septa, #4102	514.2 (39.9) [91]	31.3 (2.5) [5.5]	20.1 (5.2) [3.5]	–
Experimental, grey septa #643	439.7 (30.7) [92.5]	33.1 (3.4) [7]	3.2 (1.9) [0.5]	3,720 (200)
Experimental, grey septa, #864	471.6 (91.2) [88]	36.2 (6.7) [7]	27.4 (3.7) [5]	4,567 (745)

Comparison of lures with variable rates of Z8-12OH with or without E,E8-12:OH

This experiment was conducted in an unmanaged peach orchard near Parker, Washington (46°30'N, 120°28'W), during 2013. The orchard was adjacent to an abandoned apple orchard. Neither orchard was treated with sex pheromone dispensers for mating disruption. The experiment was initiated on 12 August and repeated on 19 and 26 of August, and 2 and 9 of September. Seven treatments were included in this trial, including three grey septa loaded with a total content of 0.41 mg of a 100 : 6 ratio of Z8-12:Ac and E8-12:Ac with the addition of either 0%, 1% or 5% of Z8-12:OH. Three additional grey septum treatments were tested with the same three blends of *G. molesta* sex pheromone with the addition of 3.0 mg of E,E8-12:OH. The negative control treatment consisted of lures loaded with only 3.0 mg of E,E8-12:OH.

Comparisons of commercial and experimental lures

Identical trials were conducted in a mixed-cultivar canning peach orchard (20 ha) situated near Talca (35°33'S, 71°33'W) in the Maule Region of Chile and in the Parker orchard (USA) during 2014. The orchard in Chile was not treated with sex pheromone dispensers for mating disruption. Traps were placed in this orchard on 8 February 2014, and the study was repeated on 16 February and again on 23 February. In Parker, both the peach and surrounding apple orchards were treated with Cidetrak OFM-L dispensers (Trécé Inc.) loaded with 250 mg of a 93 : 6 : 1 blend of Z8-12:Ac, E8-12:Ac and Z8-12:OH and applied at a rate of 250 ha⁻¹ on 30 June 2014 for the purpose of mating disruption. Traps were placed in the orchard on 7 July, and the experiment was repeated weekly on four additional dates.

Statistical analysis

A square root transformation was used to normalize count data prior to analysis of variance (ANOVA). Data were successfully normalized (Shapiro–Wilk test) and were subjected to a randomized complete block ANOVA with date as the blocking variable. A *P*-value of 0.05 was used to establish significance among means following ANOVAS (Statistix 9, Analytical Software Inc., Tallahassee, FL).

Results

Lure analysis

Loading and blend ratios varied widely among the eight lures analysed in 2014 (table 1). Total sex pheromone loading among the five commercial septum lures varied by >10-fold. Lure loading varied 3-fold among the three red septum treatments, and all were loaded with less than a third of one of the two grey septum treatments analysed. The loading of the plastic membrane lure was similar to the high-load grey septum treatments. Blend ratios among commercial lures ranged from 76% to 93%, 3% to 24% and 0% to 12% for Z8-12:Ac, E8-12:Ac and Z8-12:OH, respectively. Z8-12:OH was not found in one of the commercial red septum treatments (table 1). Both experimental lures tested contained ca. 0.5 mg of the three-component sex pheromone blend plus >3.5 mg of E,E8-12:OH.

Comparison of lures with variable rates of Z8-12OH with or without E,E8-12:OH

All lures loaded with either the two- or three-component *G. molesta* sex pheromone blend caught significantly more male *G. molesta* and fewer male

C. pomonella than lures loaded with only *E,E*8-12:OH (table 2). The percentage of Z8-12:OH in the three-component *G. molesta* blend alone or in combination with *E,E*8-12:OH did not affect catch of either species. The addition of *E,E*8-12:OH significantly increased the catch of *G. molesta* and decreased catch of *C. pomonella* at each of the 3% Z8-12:OH blend ratios tested (table 2).

Comparisons of commercial and experimental lures

Significant differences were found among lures in the peach orchard not treated with sex pheromones in Chile (table 3). The two experimental lures loaded with *E,E*8-12:OH caught significantly more moths (>2-fold) than all of the commercially available sex pheromone lures tested (table 3). In addition, two of

the three red rubber septum, as well as all except one of the grey elastomer septum treatments, resulted in significantly more *G. molesta* males captured than the plastic membrane lure (table 3).

Significant differences were found among lures in the peach orchard treated with sex pheromone dispensers in the USA (table 3). However, significant mean separations were not as clear in this experiment as compared with the one conducted in Chile. The two experimental monitoring lures tested caught significantly more *G. molesta* males than several, but not all, of the commercial lures (table 3). The lowest mean catch was with the low-load grey septum treatment, and this was significantly lower than catch observed with three of the commercial and both experimental lures, but not different from the high-load grey septum lure treatment (table 3).

Table 2 Comparison of male *Grapholita molesta* catches in traps baited with the three-component sex pheromone blend with three percentages of Z8-12:OH alone and in combination with *E,E*8-12:OH added to grey septa, 2013, n = 25

% Z8-12:OH in <i>G. molesta</i> blend ¹	<i>E,E</i> 8-12:OH added to lure	Mean (SE) male moth catch	
		<i>G. molesta</i>	<i>Cydia pomonella</i>
0.0	No	4.2 (1.0)c	0.08 (0.06)c
1.0	No	3.1 (1.3)c	0.04 (0.04)c
5.0	No	7.9 (2.0)bc	0.08 (0.06)c
0.0	Yes	11.4 (2.0)a	7.4 (1.7)b
1.0	Yes	10.5 (2.1)ab	6.2 (1.5)b
5.0	Yes	10.9 (1.7)a	8.8 (1.7)b
–	Yes	0.20 (0.13)d	22.5 (3.0)a
	ANOVA	$F_{6, 164} = 15.77, P < 0.0001$	$F_{6, 164} = 52.36, P < 0.0001$

Data were analysed with a randomized complete block design with date of each assay as the blocking variable. Column means followed by a different letter were significantly different, $P < 0.05$, Tukey's test.

¹All lures with *G. molesta* sex pheromone had a total content of 0.41 mg of the two acetates plus a variable amount of Z8-12:OH. *E,E*8-12:OH was added (3.0 mg) to some lures.

Table 3 Comparison of *Grapholita molesta* male catch in traps baited with commercial and experimental lures in two studies, a peach orchard not treated with mating disruption (MD) in Chile, n = 15, and in an orchard treated with MD in the USA, n = 20, 2014

Lure ^a	<i>E,E</i> 8-12:OH added to lure	Mean (SE) male moth catch	
		Non-MD, Chile	MD, USA
Suterra, Biolure	No	2.5 (0.5)d	8.6 (1.9)abc
Trécé, grey septa	No	5.3 (1.0)cd	7.0 (1.3)bcd
Suterra, red septa	No	6.2 (1.4)bcd	5.7 (1.0)cd
Alpha Scents, red septa	No	12.0 (2.5)bc	8.6 (1.8)abc
Scentry, grey septa	No	15.1 (3.6)bc	2.4 (0.6)d
Trécé, red septa	No	15.7 (4.0)b	8.5 (1.9)abc
Exp. #643, grey septa	Yes	37.6 (5.3)a	12.8 (1.8)ab
Exp., #864, grey septa	Yes	34.8 (4.2)a	14.1 (2.0)a
	ANOVA	$F_{7, 110} = 22.98, P < 0.0001$	$F_{7, 150} = 7.64, P < 0.0001$

Data were analysed with a randomized complete block design with date of each assay as the blocking variable. Column means followed by a different letter were significantly different, $P < 0.05$, Tukey's test.

^aSee Table 1 for lure loadings.

Discussion

The use of sex pheromones to disrupt mating of *G. molesta* has been a major success story in the use of semiochemicals in applied agriculture (Cardé and Minks 1995; Witzgall et al. 2010). Commercial products available in the United States for mating disruption of *G. molesta* include nine solid dispensers, two aerosol sprays, one liquid flowable and one paraffin wax (Agrian Labels, 2015). All of these products use a 93 : 6 : 1 blend of Z8-12:Ac, E8-12:Ac and Z8-12:OH. The low percentage of Z8-12:OH in this blend used for mating disruption of *G. molesta* compared with a more optimal blend used in lures used with traps for monitoring purposes (6–10%), or present in female effluvium (15–30%) may be because Z8-12:OH affects short-range behaviours of male *G. molesta* near the source (moth catch), but does not necessarily disrupt their searching behaviours (Linn and Roelofs 1983). Thus, an attracticide formulation, which required moth contact to be effective, was formulated with a 87 : 4 : 9 blend of Z8-12:Ac, E8-12:Ac and Z8-12:OH (Evenden and McLaughlin 2004).

Our investigation found that Z8-12:OH was not required for a Z:E acetate binary blend to be attractive in the field to *G. molesta* males as shown for one of the commercial lures (table 3) and one of our experimental grey septum treatments (table 2). This result is consistent with data from several previous studies (Roelofs and Cardé 1974; Yang et al. 2002), but has not been found in all investigations of male *G. molesta* response to pheromone blends (Baker and Cardé 1979; Linn and Roelofs 1983). A broad range in the proportion of Z8-12:OH in the female effluvium has been found among geographic populations of *G. molesta*, but the possible effect of this plasticity on host race formation or sympatric speciation has not yet been addressed (Knight et al. 2015). For example, Z8-12:OH inhibits conspecific moth species in North America [*G. prunivora* (Walsh)] and Europe [*G. funebrana* (Tretschke)] (Baker and Cardé 1979; Guerin et al. 1986; Tòth et al. 1991). Whether the trace amounts of Z8-12:OH found in a *G. molesta* population in Australia (Lacey and Sanders 1992) or the relatively low percentage found in a population in Ontario (El-Sayed and Trimble 2002) are associated with causing a lower degree of interspecies competition in stone and pome fruits in these regions is an open question and requires investigation.

The addition of E,E8,12:OH to the binary acetate or to the three-component *G. molesta* blend in the same lure substrate increased the catch of male *G. molesta*. Similar results were reported earlier with a 93 : 6 : 1

blend of *G. molesta* sex pheromone (Knight et al. 2014). But, here, the combinational lures (grey septa) did not outperform all commercial lures (red septa and plastic membrane) in the orchard treated with sex pheromone. The further addition of (*E*)- β -ocimene to grey septa combinational lures has been shown to enhance the catch of male *G. molesta* in orchards treated with pheromone (Knight et al. 2014). Here, we found that the percentage of Z8-12:OH in the three-component *G. molesta* blend did not affect the synergistic contribution of E,E8,12:OH. Although the combinational lures that included the main pheromone component of *C. pomonella* and the main components of the *G. molesta* blend increased the catch of *G. molesta*, the catch of *C. pomonella* was reduced as compared with using E,E8,12:OH alone for this species (Knight et al. 2014). This may slow the adoption of the combinational lure by pest managers to monitor both species with one trap.

Some of the lure comparisons and their effect on catch of male *G. molesta* have additional implications for practical management of this pest. One lure in each of the two field trials performed significantly worse than one or more of the other commercial lures, that is the plastic membrane lure in Chile and the low-load grey septum treatment in the USA. We hypothesize that the former lure performed poorly in the untreated orchard due to a higher than optimal emission rate based on its relatively greater attractiveness to male *G. molesta* in the sex pheromone-treated orchard. In addition, the relatively high percentage of E8-12:OH present in this lure could have further reduced moth catch (Roelofs and Cardé 1974; Baker and Cardé 1979; Baker et al. 1981; Linn and Roelofs 1983). In contrast, we hypothesize that the lower catch of male *G. molesta* by the low-load grey septum lure treatment in the sex pheromone-treated orchard, as compared with what was observed in the untreated orchard, is due to lower loading of active ingredient in these lures and a possible associated lower emission rate over time from grey vs. red elastomeric septa (Knight 2002).

Our comparisons of commercial lures were meant to test current use of these attractants and did not specifically evaluate all possible factors impacting lure performance for monitoring *G. molesta*. For example, three substrates were evaluated among the lures tested, and we discovered that both the initial loading and blend ratios of lure treatments varied and were therefore inadequate for factorial analysis. Also, we were unable to adequately compare the emission rates of the various commercial lures evaluated. Finally, from a practical perspective, it would also be

important to conduct longer duration field trials in the future to further determine the effect of lure age on catch of *G. molesta* with the lures evaluated here. Yet, the significant variability in performance found among the commercial *G. molesta* lures initially tested over a short duration does suggest that current monitoring programmes for *G. molesta* can be improved. This is important not only for monitoring populations of *G. molesta* for possible damage thresholds to time insecticide applications, but also for adequately evaluating the effect of mating disruption for this pest. One final point is that this variability among commercial lures could also impact applied research projects that often rely on donated materials, which can vary among different participating laboratories or in different years during an investigation (Knight et al. 2011).

In summary, despite several decades of research on the pheromone blend of *G. molesta* (examples: Baker and Cardé 1979; Linn and Roelofs 1983 Yang et al. 2002), it is surprising that a standardized monitoring lure is not yet available for *G. molesta* that could provide some consistency for both pest management practitioners and researchers. Our results suggest that developing such a standardized lure based on both well-established chemical and behavioural data and new research findings evaluating kairomone attractants data may improve management of this important pest of stone and pome fruit.

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