Use of Ethyl (*E,Z*)-2,4-decadienoate in Codling Moth Management: Stimulation of Oviposition

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ABSTRACT

The effect of the pear volatile, ethyl (*E*,*Z*)-2,4-decadienoate (Et-*E*,*Z*-DD), on oviposition by codling moth, *Cydia pomonella* (L.), was evaluated in a series of choice and no-choice laboratory experiments and in subsequent field tests conducted in apple and walnut. Gray halobutyl elastomer septa loaded with 1.0 and 100.0 µg Et-*E*,*Z*-DD significantly increased the numbers of eggs laid by a laboratory population in 96 h no-choice assays by 2-fold. In addition, the number of eggs laid near the Et-*E*,*Z*-DD versus a solvent blank dispenser was significantly higher in choice bioassays across a similar range of septa loadings. Oviposition rates by a field-collected post-diapause strain of codling moth were significantly increased by the addition of a 1.0-µg septa versus a solvent blank dispenser in a no-choice bioassay. Field trials were conducted in apple and walnut to develop an artificial egg trap baited with Et-*E*,*Z*-DD to monitor codling moth oviposition. Septa loaded with 0.1 to 10.0 mg did not significantly increase oviposition versus solvent blank dispensers on a Mylar plastic collar trap or on the adjacent leaves and fruit in apple. Significantly more eggs were laid on the fruit and foliage than on the plastic collar. No eggs were deposited on non-bearing apple shoots baited with 0.1 – 40.0 mg Et-*E*,*Z*-DD septa. Similarly, no eggs were deposited on cylindrical wax paper-covered plastic traps baited with 10.0 µg to 1.0 mg Et-*E*,*Z*-DD septa in walnut orchards. The potential of Et-*E*,*Z*-DD to monitor codling moth's oviposition in the field, stimulate oviposition by field-collected strains under laboratory conditions, and to improve pest control by disrupting host location are discussed.

Key Words: *Cydia pomonella*, oviposition, kairomone, phenology, egg trap

INTRODUCTION

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) is typically monitored in fruit and nut orchards with sex pheromone-baited traps. The numbers and timing of male moths captured in these traps is used to infer the density of female moths and timing of oviposition (Riedl and Croft 1974, Riedl *et al.* 1976). The development of an effective, inexpensive tool to directly monitor the density of codling moth females and/or oviposition could improve its management. The sesquiterpene, (*E,E*)-α-farnesene (*E,E*-αF), a major constituent of apple fruit and leaf odors, was identified as a key adult and larval attractant for codling moth (Sutherland *et al.* 1974) and was shown to stimulate oviposition in both choice and no-choice laboratory bioassays (Wearing and Hutchins 1973). Unfortunately, *E,E*-αF is unstable in the presence of oxygen and has a very short residual activity (Anet 1969). Field trials evaluating the stimulatory effect of E,E-αF on codling moth oviposition have not been reported.

Direct monitoring of codling moth egg density in orchards through foliage and fruit sampling is labor intensive and often ineffective due to the relatively low population density of this pest in commercial orchards (Elkins 2002). A novel approach to monitor codling moth egg density in

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California pear orchards has recently been reported that uses the artificially cutting or damaging of fruit (Zoller and Zoller 2001). Pear clusters containing one cut-fruit (2.0-3.0 cm wedge cut 0.5-1.0 cm deep) were 95-times more likely to contain a codling moth egg than normal fruit clusters. Yet, a number of factors increase the variability of this approach including cultivar and days before harvest, *i.e.*, Bosc pear clusters were more attractive than Bartlett; and the attractiveness of cut- versus uncut-fruit was only significantly different during the month before harvest (Zoller 2001). Unfortunately, the incidence of eggs among the cut-fruit cluster samples in commercial pear orchards was low (< 1.0%), and the utility of this labor-intensive approach for improving the management of codling moth is unclear (Zoller and Zoller 2001).

Light *et al.* (2001) and Knight and Light (2001) found that ethyl (*E*,*Z)*-2,4-decadienoate (Et-*E*,*Z*-DD), a principal odorant from ripe pear (Jennings *et al.* 1964), was a potent adult and larval kairomonal attractant for codling moth. Et-*E*,*Z*-DD appears to be chemically more stable than *E,E*-αF and can be loaded in septa to provide long-lasting, effective monitoring of adult populations (Light *et al.* 2001; Knight *et al.* 2005). The potential effects of Et-*E*,*Z*-DD on oviposition of codling moth have not been reported. Herein, we report on the effects of Et-*E*,*Z*-DD on codling moth oviposition under laboratory conditions and evaluate its use as a lure to monitor egg laying in apple and walnut orchards.

MATERIALS AND METHODS

Choice and no-choice laboratory experiments. Moths 24-48 hr old were obtained from a laboratory colony reared on artificial diet (Toba and Howell 1991) at the U.S.D.A. Yakima Agricultural Research Laboratory in Wapato, WA. Individual virgin female moths were paired with two male moths for 24 h at 25 °C, > 40% R.H., and a 16:8 L:D photoperiod. Females were then placed in screened (3 x 3 mm) cylindrical cages (11 cm long and 9 cm diameter). Both ends of each cage were capped with a plastic cover lined with wax paper. Gray halobutyl elastomer septa (No. 1888, size No. 1,West Co., Phoenixville. PA) were pinned to the wax paper at each end of the cage. A 10.0% honey water wick was inserted into the middle of each cage.

Choice and no-choice tests were conducted with septa loaded with 1.0 and 100.0 µg Et-*E*,*Z*-DD (93.7% A.I., Trécé Inc., Adair, OK) versus septa treated only with the solvent, dichloromethane as a blank. In choice tests an Et-*E,Z*-DD-loaded septa and a solvent blank septa were pinned to the wax paper at opposite ends of the cage. Identical lures were pinned at each end of the cage in the no-choice tests. Tests were run for 96 h inside greenhouses maintained between 20 – 25 °C. Cages were spaced > 5 m apart and no more than four replicates were run on each of six dates. An additional no-choice test was conducted with 1.0 µg Et-*E*,*Z*-DD septa versus a solvent control septa using moths reared from an overwintering population collected as larvae the previous season in corrugated bands attached to apple trees. Larvae had been maintained under diapause conditions (2 °C and total darkness) for five months and were allowed to emerge in rooms maintained at 25 °C, 16:8 L:D and > 45% R.H. The numbers of eggs laid on each wax paper cap were counted in all experiments and females were dissected to determine their mating status. Only data from mated females were included in the statistical analyses (15 – 20 replicates per comparison).

Development of an egg trap. Studies were conducted in a greenhouse to develop a suitable substrate for an artificial egg trap. The suitability of waxed cardboard and Mylar plastic as sites of codling moth oviposition were compared with natural apple leaves. Three mated females (< 72 h old) were placed inside of screened cages with a 1.2 m potted apple seedling. Artificial substrates were cut to mimic a typical leaf shape (19.8 cm^2) and were clipped to the main stem of the plant. In the first test we compared oviposition on the plastic versus the waxed cardboard substrate. The height of each type of artificial leaf was alternated on the seedling at 0.7 and 1.0 m, and six replicate cages were evaluated from 14 – 19 March 2001. In a second test, we compared oviposition on a selected apple leaf on the seedling versus a similar-sized plastic leaf. Both the natural and artificial leaves were positioned on the seedling at a height of 1.0 m and on opposite sides of the shoot. Twenty-four cage replicates of this test were conducted between 23 March and 26 April 2001. The number of eggs on each artificial and natural apple leaf was counted after 5 d.

Field evaluation of egg traps in apple. Studies were conducted in an unsprayed 'Red Delicious' apple orchard situated near Parker Heights, WA from 18 – 29 May 2001. Square plastic sheets (0.1 m^2) were slit and folded as collars around fruit and foliage clusters and baited with either 0.1, 1.0, or 10.0 mg Et-*E*,*Z*-DD lures or an unbaited solvent-only lure (nine replicates per treatment). Baited trees were spaced > 20 m apart and traps were placed in the upper third of the canopy. Fruit monitoring indicated that no fruit injury was present in the orchard when the experiment was started. Fruit and leaf clusters were removed after 7 d and plastic collars, fruits and leaves were then examined in the laboratory for codling moth eggs and injury. A second test was conducted from 5 – 11 June 2001 in another unsprayed 'Red Delicious' apple orchard situated near Moxee, WA using the plastic collar trap baited with either 3.0 mg Et-*E*,*Z*-DD or solvent-only lures. Twenty replicates of each lure type were placed on shoots with and without fruit. Only uninjured fruit clusters were used in this study. Collars, foliage, and fruit were examined for eggs after 6 d. Eggs in both studies were categorized into developmental stages based on their morphology (Richardson *et al.* 1982). Only 'white' eggs and 'red ring' eggs characterized as having only one visible stemmata (< stage 11) were scored as having been laid during each test.

A third apple trial was conducted during 2002 in the Moxee apple orchard to examine if oviposition could be stimulated to occur on non-bearing shoots baited with Et-*E*,*Z*-DD lures. Septa loaded with either 1.0, 3.0, 10.0, 20.0, or 40.0 mg Et-*E*,*Z*-DD and solvent-only septa (10 replicates per treatment) were pinned to non-bearing shoots on 15 June. One septum was attached per tree and baited-trees were spaced 5 – 6 m apart. Five replicates of each lure loading were collected after 10 d and the rest were collected after 20 d. All shoots were examined for hatched and unhatched eggs. Two Et-*E*,*Z*-DD-baited sticky delta-shaped traps (Trécé Inc., Adair, OK) were used to monitor the activity of female codling moths in this orchard during this test. In addition, 30 randomly selected fruit from 20 trees situated within 100 m of the center of the study site were inspected for codling moth injury at the end of this test to assess pest pressure.

Field evaluation of egg traps in walnut. Studies were conducted from mid-June through September 2002 in a 'Chandler' walnut orchard (Dixon, CA) characterized as having a moderate codling moth population density based on early-season moth catches in sex pheromone-baited traps. An oviposition trap was constructed using a plastic sheet (21.0 x 28.0 cm) covered with wax paper on both sides. The plastic sheet was rolled into a 9 cm diameter tube, then secured and hung horizontally by a wire trap hanger. Septa impregnated with either 10.0, 100.0, or 1,000.0 µg Et-*E*,*Z*-DD or solvent-only were attached by a plastic septa holder from the center of the inner tube of the trap. Traps were hung in the upper third of the 8.0 m canopy and baited trees were separated by 80 m. Three replicate blocks were established within three orchards (9 replicates of each lure rate). Traps were checked weekly for eggs and septa were replaced every two weeks. In July, a 500-

nut sample was collected from the canopy in each replicate block and examined for codling moth injury.

Statistical analysis. The total number of eggs laid in cages baited either with Et-*E*,*Z*-DD or solvent-only septa in choice and non-choice tests were compared with paired and unpaired t tests, respectively, at a $P < 0.05$ level of significance (Analytical Software 2000). A paired t-test was used to compare the suitability of substrates for oviposition in the greenhouse studies. Analysis of variance (ANOVA) was used to evaluate the influence of lure loading rate on oviposition on Mylar traps and associated fruit and foliage in apple and to compare oviposition on the Mylar collar versus fruit and foliage. Mean separation following a significant ANOVA was determined with a LSD test, $P < 0.05$.

RESULTS

Choice and no-choice laboratory experiments. Septa loaded with Et-*E*,*Z*-DD significantly increased oviposition by a laboratory strain of codling moth in greenhouse tests in both choice and no-choice tests with lures baited with 1.0 and 100.0 µg Et-*E*,*Z*-DD versus solvent-only lures (Table 1). The mean number of eggs laid on wax paper next to the kairomone lure was 2-5 fold higher than next to a blank lure in choice-tests. In addition, approximately 2-fold more eggs were laid in cages baited with Et-*E*,*Z*-DD lures versus with solvent-only lures in no-choice tests. Field-collected codling moth females laid twice the number of eggs in cages baited with Et-*E*,*Z*-DD than in cages baited with the solvent control (Table 1).

Development of an egg trap. No difference was found in the number of eggs laid on the waxed cardboard leaf-model (mean $\pm$ SE = 9.7 $\pm$ 2.5) versus the plastic leaf-model (mean $\pm$ SE = 8.3 $\pm$ 2.3) (t = 0.39, df = 10, P = 0.71). The Mylar plastic leaf-model was chosen for subsequent field tests due to its greater stability when exposed to adverse field conditions, i.e. precipitation. However, significantly more eggs were deposited on the apple leaf (mean $\pm$ SE = 9.6 $\pm$ 2.4) versus the plastic leaf-models (mean $\pm$ SE = 4.3 $\pm$ 1.2) (t = -3.73, df = 46, $P < 0.001$).

Field evaluation of egg traps in apple. The mean $\pm$ SE number of fruit within the Mylar collar trap in the May 2001 test was 2.9 $\pm$ 0.3 and there was no significant difference in fruit density among lure treatments, F = 0.35; df = 3, 32; P = 0.79. The mean $\pm$ SE number of eggs that had already been deposited (> stage 11 plus hatched eggs) on the fruit and foliage surrounded by the Mylar collar was 1.3 $\pm$ 0.3 per cluster. There was no significant difference in the mean density of already deposited eggs among lure treatments in this test, F = 0.93; df = 3, 32; P = 0.44. Few new eggs (< stage 11) were laid on either the Mylar trap or the associated foliage and fruit during the May 2001 field experiment (Table 2). Total egg density on the Mylar, foliage, and fruit did not differ among lure types, F = 0.87; df = 3, 32, P = 0.47. Significantly more eggs were laid on the foliage and fruit than on the Mylar plastic collar across all lure loadings and the solvent blank, F = 5.69; df = 1, 70; $P < 0.05$. An average of 23% of the fruit within the Mylar collars were injured at the end of the experiment.

No eggs were deposited on Mylar collars or foliage in non-bearing shoots baited with either the 3.0 mg Et-*E*,*Z*-DD or a blank lure in the June 2001 test. Similarly, no eggs were deposited on the Mylar collars surrounding fruit. The mean $\pm$ SE number of fruit within the Mylar collar trap was 2.4 $\pm$ 0.1 and there was no significant difference in fruit density between lure treatments on fruit-bearing shoots, F = 0.29; df = 1, 38; P = 0.59. The mean $\pm$ SE number of eggs that had already been deposited (> stage 11 plus hatched eggs) on the fruit and foliage surrounded by the Mylar collar was 1.2 $\pm$ 0.3 per cluster. There was no significant difference in the mean density of already deposited eggs

Table 1.
The effects of ethyl (*E*,*Z*)-2,4-decadienoate loaded in gray halobutyl elastomer septa on egg laying of mated codling moth in choice and non-choice laboratory tests conducted for 96 h.

Test Type	Treatment	No. replicates	Mean ± SE no. eggs laid per cage	Statistical test [1] *P*-value
Choice	100.0 µg	18	21.0 ± 3.7	< 0.05
	Blank		11.6 ± 3.4	
Choice	1.0 µg	20	25.0 ± 5.2	< 0.001
	Blank		4.4 ± 1.4	
No-choice	100.0 µg	20	31.0 ± 3.4	< 0.001
	Blank		14.2 ± 2.8	
No-choice	1.0 µg	20	27.9 ± 5.2	< 0.05
	Blank		12.3 ± 3.0	
No-choice[2]	1.0 µg	15	13.1 ± 3.8	< 0.05
	Blank		5.7 ± 3.2	

[1] A paired and unpaired t-test were used to analyze data from the choice and no-choice tests, respectively.
[2] Field collected adults reared from pupae collected in corrugated bands placed in an unsprayed apple orchard.

Table 2.
Density of codling moth eggs deposited on Mylar traps and associated fruit and leaf clusters baited with ethyl (*E*,*Z*)-2,4-decadienoate or a solvent-only lure in an apple orchard during May (nine replicates per lure) and June (20 replicates per lure) 2001.

	Mean + SE no. eggs laid on		
Lure load (mg)	Mylar collar	Foliage and fruit	Both
May 2001			
Solvent only	0.11 ± 0.11	0.22 ± 0.15	0.33 ± 0.17
0.1	0.00 ± 0.00	0.33 ± 0.17	0.33 ± 0.17
1.0	0.00 ± 0.00	1.11 ± 0.59	1.11 ± 0.59
10.0	0.22 ± 0.15	0.44 ± 0.34	0.67 ± 0.47
June 2001			
Solvent only	0.00 ± 0.00	0.10 ± 0.10	0.10 ± 0.10
3.0	0.00 ± 0.00	0.35 ± 0.17	0.35 ± 0.17

between lure treatments on fruit-bearing shoots in this test, $F = 1.18$; df = 1, 38; $P = 0.28$. The number of eggs laid on foliage and fruit in collars baited with a 3.0 mg versus the solvent-only lure was not significant ($F = 1.66$; df = 1, 38; $P = 0.21$). An average of 11% of the fruit within Mylar collars were injured at the end of the experiment.

No eggs were deposited on non-bearing apple shoots baited with Et-*E*,*Z*-DD or the solvent control lures during the 2002 study. The mean ± SE capture of female codling moth in sticky traps baited with a Et-*E*,*Z*-DD lure was 0.3 ± 0.1 moths per night. Fruit injury by codling moth on a sample of trees within 100 m of the study site averaged 18.7% in mid July.

Field evaluation of egg traps in walnut. No eggs were laid on the wax paper-covered plastic tube traps placed in walnut orchards during the 14 wk study. However, the population of codling moth in this orchard was moderate to high. Nut damage in July averaged 3.0% in canopy samples. In addition, traps baited with both sex pheromone and Et-*E*,*Z*-DD lures caught a large number of moths, 3 – 6 moths per trap per night during the test. Codling moth adults were observed on several occasions resting on the plastic egg traps.

DISCUSSION

Our studies to develop an effective monitoring trap for codling moth oviposition with Et-*E*,*Z*-DD in apple and walnut were largely unsuccessful. Mylar and waxed paper were poor artificial substrates for oviposition by codling moth compared with fruit and foliage. Despite working in orchards with relatively very high population densities of codling moth (levels of fruit and nut injury $\geq$ 3.0%), rates of oviposition on fruit and leaves were low, and the addition of a Et-*E*,*Z*-DD lure did not significantly increase the number of eggs deposited. Baiting non-bearing shoots with Et-*E*,*Z*-DD lures did not stimulate oviposition.

Several factors likely contribute to the difficulty in developing an effective egg trap for codling moth. The density of codling moth eggs in most commercial orchards is very low and a large number of traps may be necessary to detect oviposition (Zoller and Zoller 2001). Females typically deposit eggs near or on fruit clusters that emit E,E-αF and other volatiles that stimulate oviposition (Sutherland *et al.* 1974). It is unclear whether adding a Et-*E*,*Z*-DD lure could further increase this stimulation. For example, the addition of Et-*E*,*Z*-DD to an attractive blend of E,E-αF and (*E*)-*β*-farnesene did enhance male upwind flight but not 'landing at source' in flight tunnel studies (Coracini *et al.* 2004). Studies addressing the influence of these volatile blends on short-range female orientation and oviposition have not been conducted. The cut-pear method is the only study that has stimulated codling moth oviposition in the field with the addition of kairomones (Zoller 2001), but the blend of volatiles and their emission rates from artificially injured pears has not been characterized. While a wide range of Et-*E*,*Z*-DD lure loadings was evaluated in our studies we have not compared the emission rate of Et-*E*,*Z*-DD from cut pears versus these synthetic lures. Very low Et-*E*,*Z*-DD lure loadings (1.0 – 100.0 μg) stimulated oviposition under laboratory conditions. However, higher rates of Et-*E*,*Z*-DD ($\geq$ 1.0 mg) are needed to attract adequate numbers of female moths to traps under field conditions (Light *et al.* 2001). The successful development of an egg trap for codling moth must consider the trade-off between increasing the number of eggs laid on or near the trap (short-range stimulation) versus increasing the likelihood of detecting a single egg near or on the trap (long-range attraction). The complexity of visual and olfactory cues that may stimulate short-range host location behaviors in codling moth is not well understood.

Additional factors associated with developing a practical egg-monitoring program for codling moth should be considered. Lombarkia and Derridj (2002) found that several primary water-soluble metabolites (sugar alcohols and sugars) isolated from apple leaves and fruits stimulated oviposition by codling moth. Whether the suitability of a plastic surface might be improved or the leaf and fruit surfaces enhanced by the addition of these or other chemicals should be investigated. Microencapsulated formulations of Et-*E*,*Z*-DD alone and in blends with other attractive volatiles could be applied to fruit clusters to create attractive point sources. Other physical aspects of the trap could be improved to increase oviposition, such as providing grooves or folds in the trap's surface and the influence of trap color (Knight and Miliczky 2003).

The attraction of codling moth adults and neonates plus the stimulation of oviposition by Et-*E*,*Z*-DD could be utilized to improve management of this pest (Light *et al.* 2001; Knight and Light 2001). Laboratory studies with Et-*E*,*Z*-DD have examined the use of a paste formulation laced with insecticides applied as coarse droplets to control larvae (A. L. K., unpublished data). The use of attractive kill stations for females has been evaluated in field trials and offers promise (Knight *et al.* 2002). Hughes *et al.* (2003) evaluated the use of apple odor and *E,E*-αF to disrupt host location of neonates and mated females in laboratory trials. They suggest that natural mortality of neonates could be increased with a competitive kairomone-based approach. A similar design using Et-*E*,*Z*-DD could be effective and should be further evaluated.

Another potential use of Et-*E*,*Z*-DD can be to increase oviposition by post-diapause field-collected strains of codling moth. The overwintering generation of field-collected populations of codling moth typically has a much lower fecundity and mating success under laboratory conditions than either laboratory-adapted or summer generation field-collected strains (Howell 1991). Increasing egg production of codling moth under laboratory conditions has been facilitated by the addition of ripe apple fruits, water, or molasses baits (Van Leeuwen 1947; Wearing *et al.* 1973). The use of a dry, long-lasting Et-*E*,*Z*-DD lure to increase egg laying would facilitate insecticide-susceptibility testing of field populations and in establishing laboratory colonies (Knight *et al.* 2001).

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