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SHORT COMMUNICATION

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Attractants from Bartlett pear for codling moth, *Cydia pomonella* (L.), larvae

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Abstract The alkyl ethyl and methyl esters of (2*E*,4*Z*)-2,4-decadienoic acid found in head-space samples of ripe Bartlett pear (*Pyrus communis* L.) stimulated a response from neonate larvae of the codling moth (CM), *Cydia pomonella* (L.), in both static-air Petri-plate and in upwind Y-tube and straight-tube olfactometer bioassays. In comparison with the known CM neonate attractant, (*E*,*E*)-α-farnesene, ethyl (2*E*,4*Z*)-2,4-decadienoate was attractive at 10-fold and 1,000-fold lower threshold dosages in the Petri-plate and in the Y-tube bioassays, respectively. Methyl (2*E*,4*Z*)-2,4-decadienoate was attractive to CM neonates in these bioassays at much higher doses than ethyl (2*E*,4*Z*)-2,4-decadienoate. Other principal head-space volatiles from ripe pear fruit and pear leaves, including butyl acetate, hexyl acetate, (*Z*)-3-hexenyl acetate, and (*E*)-β-ocimene, were not attractive to CM neonates. The potential uses of these pear kairomones for monitoring and control of CM in walnuts and apple are discussed.

Introduction

Codling moth (CM), *Cydia pomonella* (L.), has a close, world-wide ecological association with the cultivation of apple, pear, and walnut (Shel'deshova 1967). Females rarely lay eggs on non-bearing trees, and individual eggs are generally laid within 20 cm of fruit (Geier 1963). Fruits are typically infested by one or a few larvae. The sesquiterpene found in apple fruits (*Malus domestica* Borkh), (*E*,*E*)-α-farnesene (*E*,*E*-αF) (Murray et al. 1964), was identified as both a key larval attractant and an ovipositional stimulant for CM (Sutherland and Hutchins 1972; Wearing and Hutchins 1973). No other unsaturated or saturated hydrocarbon fractions obtained from crude chloroform extracts of apples were attractive to CM larvae (Sutherland and Hutchins 1972), yet *E*,*E*-αF alone was not as attractive as the complete chemical blend extracted from apple skins (Sutherland et al. 1974). *E*,*E*-αF was subsequently found in fruit (Huelin and Murray 1966) and leaves (Miller et al. 1989) of pears (*Pyrus communis* L.), but the attraction of other pear volatiles for CM have not been reported. Variability in the concentration of *E*,*E*-αF among crops, cultivars, and seasonal effects has been used to explain observed differences in fruit susceptibility to CM (Russ 1976; Sutherland et al.1977). A number of studies published in the past 5 years have strengthened the hypothesis that *E*,*E*-αF is a principal host attractant for CM larvae and adults (Bradley and Suckling 1995; Landolt et al. 1998, 2000; Yan et al. 1999; Hern and Dorn 1999).

Light et al. (2001) evaluated the attractiveness of 92 headspace volatiles, unique to pome fruits, to CM adults in field trials conducted in both walnut and pome fruit orchards. Ethyl (2*E*,4*Z*)-2,4-decadienoate (Et-*E*,*Z*-DD) was found to be the only potent CM adult kairomonal attractant. Methyl (2*E*,4*Z*)-2,4-decadienoate (M-*E*,*Z*-DD) was also attractive but at a significantly lower level for CM adults (7-fold less attractive than Et-*E*,*Z*-DD). In addition, *E*,*E*-αF was a weak attractant and only for the first few days in the field (D.M. Light, unpublished data). Concurrent with this study of adult attractants, we evaluated responses of CM neonate larvae to the principal volatiles released from ripe Bartlett pear and pear leaves. Here we show that both Et-*E*,*Z*-DD and M-*E*,*Z*-DD are potent attractants for CM neonate larvae. In particular, Et-*E*,*Z*-DD has a minimum threshold dose for attraction much lower than that of *E*,*E*-αF.

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Materials and methods

We analyzed the headspace trapped odor composition of ripe Bartlett pears using standard analytical procedures (Light et al. 2001). The five major components, comprising 88.66% of the pear odor, were hexyl acetate (40.75% of concentrated odor), butyl acetate (35.11%), Et-*E*,*Z*-DD (7.89%), M-*E*,*Z*-DD (3.97%), and *E*,*E*-αF (0.94%). Samples of these five compounds were purified (>98.0%) and dissolved in hexane to create decade concentration–dilution series. Samples of two pear leaf volatiles, (*E*)-β-ocimene and (*Z*)-3-hexenyl acetate were also purified (>98.0%) and dissolved and diluted in hexane. α-Farnesene consisted of an isomeric mixture of 99.0% *E*,*E*-αF and 1.0% *E*,*Z*-α-farnesene. All chemicals were stored in septum-capped vials under gaseous nitrogen and kept in the dark at –10°C.

We evaluated the kairomonal activity of each of these seven pear volatiles using three distinct laboratory bioassays. Dual choice bioassays were conducted in closed 50 mm-diameter polystyrene Petri plates (bioassay adapted from Bradley and Suckling 1995). Significant differences ($P<0.05$) between behavior (number of larvae first touching a treated paper strip) towards a host volatile versus hexane alone were analyzed using the Wilcoxin matched pairs test, $n=15$ (Snedecor and Cochran 1967). Host volatiles were initially tested at a dose of 10.0 µg per paper strip. Volatiles that elicited a significant larval response at that dose were tested subsequently with a progressively declining dose series from 1.0 µg to 0.1 ng, until no significant response to the host volatile relative to the control was observed. A second series of tests measured the response of CM neonates in dual choice tests using a modified Y-tube olfactometer (bioassay adapted from Landolt et al. 1998). Host volatiles eliciting a significant choice response at the initial dose (100 µg) were subsequently tested at decreasing order of magnitude dosages (10 µg to 0.1 ng per septum) until no significant response to the host volatile relative to the control was observed (χ^2 test, Snedecor and Cochran 1967). Fifty larvae were tested at each concentration of host volatile. A third series of tests were conducted using a no-choice bioassay to measure the distance larvae walked upwind towards a septum loaded with 100 µg of either host volatile or hexane control. Twenty-five replicates were conducted and distance traveled by neonates in 2 min toward the hexane control versus the host volatile was analyzed with a paired sample *t*-test (Snedecor and Cochran 1967).

Results

CM neonates exhibited a significant chemotactic response to Et-*E*,*Z*-DD, M-*E*,*Z*-DD, and *E*,*E*-αF in both the Petri-plate and Y-tube olfactometer bioassays (Table 1). Each of these compounds also significantly increased the neonate's walking distance in the 2-min straight-tube bioassay by approximately 40% more than hexane alone (Fig. 1). No significant responses by CM neonates to the four other pear volatiles; hexyl acetate, butyl acetate, (*E*)-β-ocimene, or (*Z*)-3-hexenyl acetate, were detected in any of these three bioassays.

The lowest loading of each of these three attractive host volatiles that elicited a significant chemotactic response by CM neonates varied across three orders of magnitude both on filter paper in the Petri-plate bioassay and on septa in the olfactometer bioassay (Table 1). Et-*E*,*Z*-DD was attractive at 1/10th and 1/1,000th the con-

Table 1 The responses of codling moth neonates to selected pear volatiles versus a hexane control

Pear volatile tested	Closed Petri plate[a]					Y-tube olfactometer[b]				
	Mean±SE proportion attracted to					Mean proportion attracted to				
	Pear Volatile	Hexane			Lowest significant response[c]	Pear Volatile	Hexane			Lowest significant response[d]
		Control	*Z*	*P*			Control	χ2	*P*	
Ethyl(2*E*,4*Z*)-2,4-decadienoate	0.37 (0.05)	0.16 (0.04)	–2.67	0.008	1.0 ng	0.42	0.14	7.00	0.008	1.0 ng
Methyl(2*E*,4*Z*)-2,4-decadienoate	0.36 (0.06)	0.09 (0.04)	–2.54	0.01	10.0 µg	0.38	0.16	4.48	0.03	1.0 µg
(*E*,*E*)-α-farnesene	0.36 (0.06)	0.17 (0.03)	–1.98	0.05	10.0 ng	0.26	0.06	3.77	0.05	1.0 µg
Hexyl acetate	0.17 (0.05)	0.21 (0.04)	0.53	0.59	–	0.12	0.15	0.09	0.76	–
Butyl acetate	0.08 (0.04)	0.20 (0.04)	2.19	0.03	–	0.14	0.14	0.00	1.00	–
(*E*)-β-ocimene	0.20 (0.05)	0.24 (0.07)	0.27	0.79	–	0.12	0.12	0.00	1.00	–
(*Z*)-3-hexenyl acetate	0.20 (0.04)	0.33 (0.06)	1.61	0.11	–	0.16	0.18	0.06	0.81	–

[a] A 3-min choice test conducted in a closed 50 mm diameter Petri plate. Five larvae were placed equidistantly (<1.0 cm) from two 20×3 mm strips of white copy paper impregnated with a 10 µl drop of either a pear volatile (10 µg) or the hexane solvent control. The number of larvae to first touch a particular strip was recorded for a 3 min period in a dark room kept at 24°C, 30% RH, and illuminated by red light. Fifteen replicates were run for each host volatile–hexane combination. The relative positions of the two treatments were switched after each replicate.

[b] A 2 min choice test where neonates had to walk >6 cm upwind on a 16-gauge wire suspended in a Y-tube (i.d.=25 mm) towards gray halobutyl septa (previously cleaned with methylene chloride and air-dried for 15–45 min) loaded with either a 100 µg dose of a pear volatile or 100 µl hexane. Charcoal-filtered purified air was pumped through the olfactometer at 100 ml/min. Septa were positioned 5 cm from the Y-junction in the arms of the olfactometer. Individual larvae were placed 3.0 cm downwind from the Y-junction in the stem on a 16-gauge stainless steel wire suspended up the middle of the tube from the stem through both arms of the olfactometer. The number of larvae crawling >3 cm past the Y-junction (6 cm total distance from release point) toward either the solvent or host volatile-impregnated-septum were recorded after 2 min duration. Five consecutive larvae were run for each septa pair and then the Y-tube was turned over, switching the arm positions, and five more larvae were assayed. New septa and clean (washed and baked) glassware were used after ten larvae had been tested.

[c] Mean proportion ± SE of larvae responding at lowest significant pear volatile load: Et-*E*,*Z*-DD at 1.0 ng – 0.47±0.15 (Z=–2.73, P=0.006); M-*E*,*Z*-DD at 10 µg – 0.36±0.06 (Z=–2.554, P=0.01); and *E*,*E*-αF at 10.0 ng – 0.40±0.06 (Z=–2.00, P=0.05).

[d] Mean proportion of larvae responding at lowest significant pear volatile load: Et-*E*,*Z*-DD at 1.0 ng – 0.48 ($\chi2$=12.40, P <0.001); M-*E*,*Z*-DD at 1 µg – 0.30 ($\chi2$=3.86, P=0.04); and *E*,*E*-αF at 1 µg – 0.28 ($\chi2$=4.17, P=0.04).

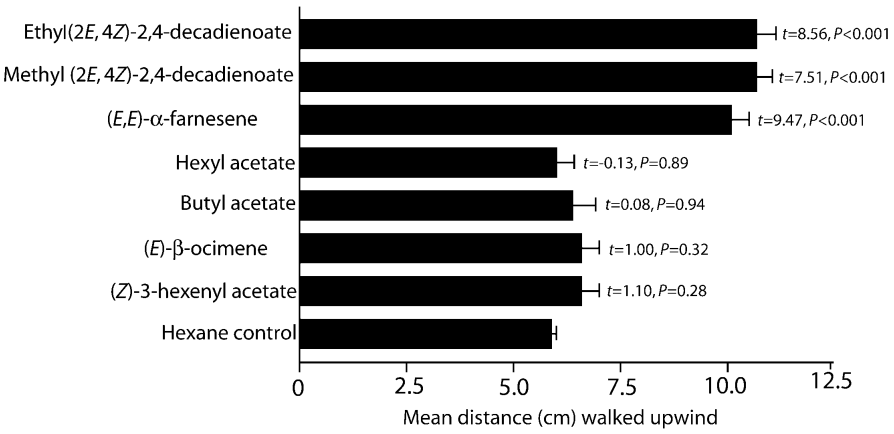

Fig. 1 The mean (SE) distance (cm) that neonate codling moth larvae walked upwind in 2 min towards individual pear volatiles and a hexane control in paired tests. Larvae were placed on a 16-gauge stainless steel wire suspended up the middle of a straight glass tube (i.d.=1.4 cm). Purified air was pumped through each tube at 100 ml/min and larvae were placed 20 cm downwind from the odor septa. Twenty replicates were conducted with each pear volatile and data were analyzed with a paired *t*-test, $P<0.05$

centrations effective for *E*,*E*-αF in these two bioassays, respectively (Table 1). The lowest attractive loading of *E*,*E*-αF on filter paper in the Petri-plate bioassays, 10 ng, was similar to that previously reported by Bradley and Suckling (1995). The lower threshold doses of M-*E*,*Z*-DD to elicit a CM neonate response were 10,000- and 1,000-fold higher than required for Et-*E*,*Z*-DD in the Petri-plate and Y-tube bioassays, respectively (Table 1).

Discussion

Alkyl (2*E*,4*Z*)-2,4-decadienoates are known to be important volatiles of ripening and fully-ripe pears and are responsible for the characteristic aroma of Bartlett pear (Jennings et al. 1964). In contrast, Et-*E*,*Z*-DD and M-*E*,*Z*-DD have been found in ripe apple fruits in only one post-harvest study (Berger and Drawert 1984). Numerous reports have identified a broad range of volatiles, primarily esters, released during the season from apple headspace but have not detected alkyl (2*E*,4*Z*)-2,4-decadienoates (Kakiuchi and Moriguchi 1986; Carle et al. 1987; Mattheis et al. 1991). In addition, alkyl (2*E*,4*Z*)-2,4-decadienoates have not been isolated from the odor of immature pome fruit (R.G. Buttery, unpublished data) or from pear leaves (Miller et al. 1989; Scutareanu et al. 1997). Alkyl (2*E*,4*Z*)-2,4-decadienoates, however, are released from immature pear fruit infested by codling moth (R.G. Buttery, unpublished data); and may contribute to the chemotactic preference of CM neonates for the odor of larval-damaged apple fruits over the odor of intact fruit (Landolt et al. 2000).

The apparent absence of the alkyl (2*E*,4*Z*)-2,4-decadienoates in early-season pome fruit and season-long in walnut orchards creates several practical applications to use this potent kairomone attractant of CM larvae and adults in pest management. Et-*E*,*Z*-DD has already been shown to be an effective, long-lasting lure for monitoring emergence and mating status of female CM in both sex pheromone-treated and conventional walnut and apple orchards (Light et al. 2001). Et-*E*,*Z*-DD can also be formulated with insecticides in "attract and kill" paste drops, bait stations, or sprayable formulations for adults. The chemical stability and potent attraction of alkyl (2*E*,4*Z*)-2,4-decadienoates for larvae may also be used to improve CM management. A sprayable formulation applied to an orchard canopy could increase the time neonates spend walking on foliage prior to entry into fruit or nuts. Such an extended period of larval wandering could increase natural mortality and exposure to biological control agents or to standard or microbial insecticide residues or baits. In addition, mixing alkyl (2*E*,4*Z*)-2,4-decadienoates with insecticides with oral activity against CM, such as *Bacillus thuringiensis* Berliner, codling moth granulosis virus, or ecdysone agonists could improve the efficacy of these selective CM control agents.

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