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Specificity of Codling Moth (Lepidoptera: Tortricidae) for the Host Plant Kairomone, Ethyl (2*E*,4*Z*)-2,4-Decadienoate: Field Bioassays with Pome Fruit Volatiles, Analogue, and Isomeric Compounds

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Codling moth, *Cydia pomonella* (L.), is a severe pest of apples, pears, and walnuts worldwide, and new approaches for precise monitoring and management would be beneficial. Ninety-two pome fruit volatiles were formulated in 23 distinct blends, of which a single 4-component blend of 10-carbon esters showed the only significant attraction of moths in field bioassays conducted in both walnut and apple orchards. A single constituent of this blend, ethyl (2*E*,4*Z*)-2,4-decadienoate—the "pear ester", was the major contributing attractant. The pear ester attracted both male and female moths in combined numbers that were comparable to the attractiveness of conspecific sex pheromone. Structure−activity tests were conducted in a series of orchard trials to determine the specificity of attraction of codling moths to the pear ester kairomone. No analogue 10-carbon alcohols, aldehydes, acetates, or other esters elicited significant moth capture responses. Tests with various analogue esters with alcohol chain length moiety substitutions of the (2*E*,4*Z*)-2,4-decadienoic acid elicited differential capture responses, with the ethyl exceeding the propyl, methyl, butyl, and hexyl analogues. The (*E*,*Z*) geometric isomers of this series of (2*E*,4*Z*)-2,4-decadienoic acid esters far exceeded the attractiveness of the (*E*,*E*) isomers. The pear ester is a potent attractant of both males and females, and codling moths are highly discriminating and specific in their structure−activity-based attraction to this pear-derived kairomone. These specificity attributes should allow this host plant kairomone to contribute to new abilities for female monitoring and the potential of development of novel and highly selective control practices that should decrease the current dependence on the use of broad-spectrum insecticides.

KEYWORDS: Kairomone; attractant; pear ester; host plant volatiles; codling moth; *Cydia pomonella*; monitoring; isomers; structure−activity

INTRODUCTION

Codling moth (CM), *Cydia pomonella* (L.), is the key pest of pears, *Pyrus communis*, apples, *Malus domestica*, and walnuts, *Juglans regia*, worldwide. Adult populations are typically monitored in orchards by using traps baited with sex pheromone lures, which attract and capture exclusively male moths, to establish both action thresholds and to time insecticide treatments (*1*). The principal goal of pest monitoring is to detect and predict when females are present and ovipositioning and when eggs are hatching. Traditional monitoring of male populations using sex pheromone lures is an indirect, inferred measure of female activity based on male emergence and flight. Confusing the interpretation of pheromone trap captures is the often exhibited "protandry" behavior of male CM, where the overwintering generation of males emerges days to several weeks before females in the late winter−early spring (*1*). Thus, to more precisely observe and predict female emergence and flight behavior, the discovery of a female attractant would be an important tool in improving population monitoring (*2*). A concerted effort has long been put forth to identify volatiles mediating host-finding and oviposition in CM females. CM females are known to be attracted to (*3*−*8*) and stimulated to oviposition (*3*, *8*−*10*) by the odor of apples and by the volatile component (*E*,*E*)-α-farnesene (*4*, *6*, *8*, *9*, *11*). However, attraction of female CM to (*E*,*E*)-α-farnesene has been limited to demonstrations in laboratory, small-arena bioassays. Unfortunately, the use of (*E*,*E*)-α-farnesene as an attractant in orchards is impractical, due to its instability in sunlight, resulting in rapid

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photoautoxidation (*12*). Thus, a need exists for the identification of other potential CM female attractants. Recent studies have demonstrated that the attractancy of (*E*,*E*)-α-farnesene to male CM can be enhanced by several host-plant volatiles (HPVs) of apples (*13*). Moreover, a principal volatile of apple odor, butyl hexanoate, was demonstrated to attract mated female CM in laboratory wind tunnel bioassays (*14*).

Because CM females have a strong preference for apple and pear pome fruits over walnuts (*15*), a recent successful approach took GC-MS-identified HPVs, which are common to both pome fruits and also certain key odor volatiles particular to either apples or pears, and tested synthetic blends of these compounds for their attractiveness to CM in a walnut-orchard context (*16*). Headspace trapping followed by GC-MS analysis has shown that monoterpenoids, sesquiterpenoids, and oxygenated terpenoids are the dominant constituents in the odor profiles of (1) intact leaves of walnut (*17*, *18*), pear (*19*, *20*), and apple (*21*, *22*), (2) walnut hulls (*23*−*25*), and (3) early-season immature apple or pear fruits (*22*). During fruit maturation and ripening, the odor profiles of apple (*22*, *26*−*29*) and pear (*30*−*32*) fruits evolve to be predominantly aliphatic esters, a few short-chain-length aliphatic alcohols, and several sesquiterpenes (*33*), for example, (*E*,*E*)-α-farnesene (*34*−*36*). Furthermore, insect-damaged fruits can be more attractive than uninfested intact fruit, as recently shown for female attraction to CM larval-infested apples (*7*) and mechanically cut or damaged pears (*37*). Insect-damaged or mechanically damaged pome fruits will precociously mature on the tree and emit ripe fruit volatiles (*20*, *21*, *38*).

A key pear volatile indicative of ripened fruit, the "pear ester", ethyl (2*E*,4*Z*)-2,4-decadienoate (*30*, *39*), was recently reported as a potent host plant kairomone attractant for CM adults and larvae and has been demonstrated as a practical and stable lure and successful monitoring tool for CM management (*2*, *16*, *40*−*44*). Ethyl (2*E*,4*Z*)-2,4-decadienoate is attractive to both sexes of codling moth, and traps can be used to assess the timing of female emergence and activity, as well as mating status (*16*). A short paper by Light et al. (*16*) in 2001 identified the pear ester as an effective CM attractant, but did not report the identity or activity of the particular HPVs of apple and pear that were chosen and screened for field attraction of CM. Here we report details of these screening tests and additional structure−activity tests conducted in the field to determine the specificity of CM to selectively detect and behaviorally respond to various analogue 10-carbon saturated and unsaturated alcohols, aldehydes, acetates, and esters related to the pear ester structure. Also, we report the structure−activity attractiveness of the (*E*,*E*) and (*E*,*Z*) geometric isomers of a series of alcohol chain length substitutions of 2,4-decadienoic acid esters.

MATERIALS AND METHODS

Field Test Protocols. Field trials were conducted in 1998 and 1999 to evaluate the attractiveness to wild codling moths of various synthetic compounds (1) previously identified as common host plant volatiles of apple, pear, and/or walnut and (2) analogue esters, alcohols, aldehydes, and geometric isomers of the pear ester, ethyl (2*E*,4*Z*)-2,4-decadienoate. Test compounds were evaluated for their attraction of CM in comparison to standard, commercial "codlemone" [(8*E*,10*E*)-8,10-dodecadien-1-ol] sex pheromone lures (CM-L2, Trécé Inc., Adair, OK). The test compounds were loaded by pipet and impregnated into white (Sigma-Aldrich, Inc., St. Louis, MO) or gray halobutyl elastomer septa (Trécé, Inc.). Both synthetic blends and individual compounds were field tested for their attractiveness to CM and compared to solvent controls and the pheromone-lure standard. Studies were conducted in conventionally and organically managed walnut orchards located near Dixon, Winters, and Esparto, CA, and in conventionally managed apple orchards near Wapato, WA. Either wing-shaped or diamond-shaped sticky traps (Pherocon 1CP and IIB, Trécé Inc.) were used. Traps were hung directly on walnut tree branches at about midcanopy (or 6 m height) and for apple trials traps were attached to PVC poles that were hung in the upper third of the canopy (.∼4 m height). Traps were placed 30−50 m apart within each orchard and arranged in a randomized block design along replicate orchard rows separated by 50−80 m. Traps were checked weekly or more often, moved and rotated in tree position within the orchard row, and replaced when needed or when a catch exceeded 20 moths. Trapped moths were sexed and counted, with females dissected to assess occurrence of mating (i.e., spermatophores).

Pome Fruit Compounds. Experiments to test the field attractiveness to CM of various blends of common apple- and/or pear-derived HPVs were conducted over a 2 year period (1998 and 1999) in organically managed walnut orchards (Chandler and Hartley varieties) in California and conventionally managed apple orchards (Red Delicious variety) in Washington. Test orchards had low to moderate endemic CM populations. Ninety-two HPVs unique to pome fruits were chosen for bioassay screening, on the basis of their identification in odor profiles of apple and/or pears (*33*) and their confirmation by GC-MS analysis of headspace collected fruit volatiles (*16*). Twenty-three distinct blends were formulated, so that each blend was composed of two to nine pome fruit HPV constituents, with a blend's constituents sharing a common carbon-chain length (from 4 to 15 carbons) and/or alcohol, aldehyde, or ester moiety (**Table 1**). All blends were mixtures of equal volumetric proportions of neat constituents, formulated as 10% solutions in hexane (or methylene chloride). One blend of esters, termed "apple maggot lure-derived", was composed of the qualitative constituents defined by Reissig et al. (*45*) but formulated here simply as a blend of equal 1:1 proportions (**Table 1**). All HPV compounds were from sample files at WRRC and had purities of >95% by GC analysis, except for some of the monoterpenes and sesquiterpenes that ranged from 70 to 98% pure. **Table 1** lists the composition of these 23 HPV blends that were tested in direct comparison to (1) the known CM sesquiterpene attractant, (*E*,*E*)-α-farnesene (93.0% pure), (2) solvent controls, and (3) a commercial pheromone lure standard. HPV blends, as 10% solutions in hexane or methylene chloride, were pipetted in 100 μL aliquots into white septa at an approximate blend dose of 10 mg/septum. Septa were replaced every week, due to the volatility (e.g., C_4 and C_5 compound blends) and/or instability [e.g., (*E*,*E*)-α-farnesene] of certain blend constituents. Apple field tests were conducted between July 26 and August 9, 1999, in three replicate apple orchards (Delicious varieties), located near Moxee, WA, with traps checked three times; wheres the walnut tests were conducted in both July 1998 and more extensively repeated in 1999 between June 7 and July 30 with weekly trap checks in two orchards (Esparto, CA).

Constituents of the C_{10} Ester Blend. Experiments to test the field attraction of CM to the four individual and certain binary combinations of the constituents of the synthetic "C_{10} ester blend" were conducted in 1998 in a conventionally managed walnut orchard (Hartley variety) in California. The C_{10} ester blend constituents were the saturated and diene esters: methyl decanoate, ethyl decanoate, methyl (2*E*,4*Z*)-2,4-decadienoate, and ethyl (2*E*,4*Z*)-2,4-decadienoate (Et-*E*,*Z*-DD). Also, certain binary blends (1:1) of constituents were field-tested, including methyl decanoate plus ethyl decanoate, methyl decanoate plus methyl (2*E*,4*Z*)-2,4-decadienoate, ethyl decanoate plus ethyl (2*E*,4*Z*)-2,4-decadienoate, and methyl (2*E*,4*Z*)-2,4-decadienoate plus ethyl (2*E*,4*Z*)-2,4-decadienoate. Purity was greater than 97% for the saturated compounds, 90% for the methyl decadienoate, and 86% for the ethyl decadienoate. The septa were loaded with 10 mg of test compound, and septa were replaced every 2 weeks during the test period of July 6−September 17, 1998.

C_{10} Alcohol, Aldehyde, and Ester Analogues of Et-*E*,*Z*-DD. Field experiments were conducted in 1999 to test the attractiveness of various C_{10} and C_{12} analogues of Et-*E*,*Z*-DD, including (*E*)-2- and (*Z*)-4-monoenes, (2*E*,4*Z*)-2,4-dienes, and saturated alcohols, aldehydes, acetates, and methyl and ethyl esters (**Table 4**). Compounds were attained from Bedoukian Research, Inc. (Danbury, CT), with purities of generally >95% (**Table 4**). Tests were conducted in organically managed walnut orchards (Chandler and Hartley varieties) in California

Table 1. Synthetic Blends of Apple and Pear Volatiles[a] Field-Tested for Codling Moth Attraction

alcohol blends	
C_4 and C_5 alcohols	2-methylpropan-1-ol, butan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol
C_5 alcohols	pentan-1-ol, pentan-2-ol
C_6 alcohols	hexan-1-ol, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, (*E*)-3-hexen-1-ol, (*Z*)-3-hexen-1-ol
C_7, C_8, and C_9 alcohols	heptan-1-ol, octan-1-ol, nonan-2-ol
aldehyde blends	
C_6 aldehydes	hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenal
C_9 and C_{10} aldehydes	nonanal, decanal
ester blends	
C_4 and C_5 acetates	butyl acetate, 2-methylpropyl acetate, pentyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate
C_6 acetates	hexyl acetate, (*E*)-2-hexenyl acetate, (*Z*)-3-hexenyl acetate,
propanoates	propyl propanoate, butyl propanoate, hexyl propanoate
butanoates	methyl butanoate, ethyl butanoate, propyl butanoate, ethyl 2-methylbutyrate, butyl 2-methylpropanoate, butyl butanoate, butyl 2-methylbutanoate, hexyl butanoate, hexyl 2-methylbutanoate
C_7 and C_8 acetates	heptyl acetate, octyl acetate
hexanoates	methyl hexanoate, ethyl hexanoate, butyl hexanoate, hexyl hexanoate
C_4 and C_6 esters, "apple maggot lure"-derived esters	hexyl acetate, butyl hexanoate, hexyl butanoate, propyl hexanoate, butyl 2-methylbutyrate, hexyl propanoate
octanoates	methyl octanoate, ethyl octanoate
decanoates and decadienoates, C_{10} esters	methyl decanoate, ethyl decanoate, methyl (*E*,*Z*)-2,4-decadienoate, ethyl (*E*,*Z*)-2,4-decadienoate
2-methylbutyl esters	2-methylbutyl acetate, 2-methylbutyl propanoate, 2-methylbutyl butanoate, 2-methylbutyl hexanoate
2-methylpropyl esters	2-methylpropyl acetate, 2-methylpropyl propanoate, 2-methylpropyl 2-methylbutyrate
C_4 and C_6 esters	butyl acetate, hexyl acetate, butyl butanoate, hexyl butanoate, butyl hexanoate, hexyl-2-methybutanoate, hexyl hexanoate, 2-methylbutyl acetate, butyl 2-methylbutanoate
$C_{4:6}$ and $C_{6:4}$ esters	butyl hexanoate, hexyl butanoate
hydrocarbon blends	
monoterpene blends	
pear/apple – based	±-α-pinene, ±-limonene, ±-linalool, Δ-3-carene, (*E*)-β-ocimene
walnut -based	γ-terpinene, terpinen-4-ol, *p*-cymene, myrcene, (*E*)-β-ocimene, β-pinene
sesquiterpene blends	
apple/pear/walnut-based	(*E*,*E*)-α-farnesene, β-caryophyllene, germacrene D
pear	α-copaene, δ-cadinene, humulene

[a] Based on GC-MS analysis of headspace trappings of the maturation and development of odors of four apple varieties and Bartlett pears conducted by Bob Flath, USDA-ARS-WRRC.

and conventionally managed apple orchards (Delicious and Fuji varieties) in Washington. The septa were loaded with 1.0 mg of test compound, and septa were replaced every 4 weeks during the test interval of July 6−September 17, 1999, in two replicate walnut orchards (Esparto, CA) and between August 17 and 31, 1999, in three replicate apple orchards (Moxee, WA).

Geometric Isomers and Alcohol Moiety Analogues of Et-*E,Z*-DD. Field experiments were conducted in 1999 to test the attractiveness of (2*E*,4*E*) and (2*E*,4*Z*) isomers of the decadienoic acids possessing methyl, ethyl, propyl, isopropyl, butyl, and hexyl alcohol moieties. Pure ester isomers were synthesized (by Trécé Inc.) using technical grade ethyl (2*E*,4*Z*)-2,4-decadienoate (Sigma-Aldrich, Inc.) by it first being hydrolyzed to the acid, which was purified by repeated recrystallization of the cyclohexylamine salt. The salt was acidified to regenerate the pure acid, which was converted to various esters by forming the acid chloride (via oxalyl chloride) followed by the reaction of the appropriate alcohol and then purified (>98%) through silica gel column chromatography. Synthetic esters were analyzed by GC-MS, and their spectra were found to be authentic in comparison to published data and purity of samples was determined. Field tests were conducted in five organically managed walnut orchards (Chandler, Vina, and Hartley varieties) near Esparto and Winters, CA, and three conventionally managed apple orchards (Delicious varieties) near Moxee, WA. The septa were loaded with 1.0 mg of test ester compound, and septa were replaced every 3 weeks during the test interval of June 30− September 7, 1999, in walnut orchards and between August 17 and 31, 1999, in apple orchards.

Male Attraction to Et-*E,Z*-DD. The possibility that male CM were not being attracted to Et-*E,Z*-DD directly, but might have been attracted by trapped females emitting natural sex pheromone, was tested in walnut orchards in 1998 (August) using a nonsticky, rapid-kill bucket trap. Aqueous antifreeze-filled plastic funnel-bucket-traps baited with Et-*E,Z*-DD septa lures (1.0 mg) were used to drown attracted female moths quickly and, thus, prevent their emission of pheromone. Similar plastic funnel-bucket-traps were baited with standard pheromone lures. Six replicate pairs of traps were placed in three walnut orchards (Hartley var.) in the vicinity of both Dixon and Winters, CA.

Data Analysis. Significant differences in the average moth catch per trap per night for treatment traps baited with each test compound were determined with analysis of variance (ANOVA), $p < 0.05$ (*46*). Average count data were transformed prior to analysis with a square root ($x + 0.01$) formula. Means were separated with least significant difference within all significant ANOVAs. In addition, nonparametric, multiple-range statistical tests were also used to evaluate multiple treatment tests. Nonparametric and paired *t* tests were used to compare gender effects and HPV treatment versus pheromone standard attraction.

RESULTS

Pome Fruit Compounds. In California walnut tests, 22 of the 23 HPV blends and the solvent controls were ineffective, capturing no CM over the 7 week test period. CM captures were elicited only by the C_{10} ester blend (0.88 ± 0.54 mean males/trap/night ± SEM, 1.32 ± 0.82 females/trap/night, and 2.20 ± 1.36 mean CM/trap/night or total of 108 CM/trap/49 days) (**Table 2**). The capture rate of the C_{10} ester blend was significantly greater than that of the only other active test treatment, the single sesquiterpene compound, (*E*,*E*)-α-farnesene (0.05 ± 0.04 CM/trap/night ± SEM, 0.03 ± 0.03 males/trap/

Table 2. Capture Rates (Moths/Trap/Night) of Codling Moth in Traps Baited with Synthetic Blends of Apple- and Pear-Based Host Plant Volatiles

	walnut orchards,[a] mean ± SEM (moths/trap/night)[b]			apple orchards, mean ± SEM (moths/trap/night)		
host plant volatile blends (no. constituents)	males	females	total	males	females	total
C_6 aldehydes (3)	0	0	0	0.02 ± 0.02d	0	0.02 ± 0.02d
C_9 and C_{10} aldehydes (2)	0	0	0	0	0	0
C_4 and C_5 alcohols (4)	0	0	0	0.05 ± 0.02cd	0	0.05 ± 0.02cd
C_5 alcohols (2)	0	0	0	0	0	0
C_6 alcohols (5)	0	0	0	0	0	0
$C_{7,8,9}$ alcohols (3)	0	0	0	0	0	0
C_4 and C_5 acetates (5)	0	0	0	0	0	0
C_6 acetates (3)	0	0	0	0.02 ± 0.02d	0	0.02 ± 0.02d
C_7 and C_8 acetates (2)	0	0	0	0	0	0
propanoates (3)	0	0	0	0	0	0
butanoates (9)	0	0	0	0.02 ± 0.02d	0	0.02 ± 0.02d
hexanoates (4)	0	0	0	0	0	0
C_4 and C_6 esters ("apple maggot lure") (6)	0	0	0	0	0	0
octanoates (2)	0	0	0	0	0	0
C_{10} esters (4)	0.88 ± 0.54b	1.32 ± 0.82a	2.20 ± 1.36a	0.60 ± 0.26b	0.36 ± 0.15a	0.95 ± 0.41b
2-methylbutyl esters (4)	0	0	0	0	0	0
2-methylpropyl esters (3)	0	0	0	0.02 ± 0.02d	0	0.02 ± 0.02d
C_4 and C_6 esters (9)	0	0	0	0	0	0
$C_{4:6}$ and $C_{6:4}$ esters (2)	0	0	0	0.05 ± 0.02cd	0.02 ± 0.02b	0.07 ± 0.03cd
monoterpenes (5)	0	0	0	0.02 ± 0.02d	0	0.02 ± 0.02d
monoterpenes (6)	0	0	0	0.01 ± 0.01d	0.01 ± 0.01d	0.02 ± 0.02d
sesquiterpenes (3)	0	0	0	0.05 ± 0.02cd	0.02 ± 0.02b	0.07 ± 0.03cd
sesquiterpenes (3)	0	0	0	0	0	0
α-farnesene	0.03 ± 0.03c	0.02 ± 0.02b	0.05 ± 0.04b	0.10 ± 0.05c	0.05 ± 0.05b	0.15 ± 0.08c
solvent controls (2 rep)	0	0	0	0	0	
pheromone standards (CM-L2)	3.15 ± 0.98a	0	3.15 ± 0.98a	3.76 ± 0.69a	0	3.76 ± 0.69a

[a] Means (± SEM) are derived from three walnut and three apple orchard replicates with weekly trap check intervals over a 7 week period. Septa were loaded with 10 mg of blend solution, and lures were replaced weekly. [b] Column means followed by the same letter are not significantly different; significant differences are assigned at $p < 0.05$.

night, and 0.02 ± 0.02 females/trap/night). The combined gender average capture with the C_{10} ester blend was lower but not significantly different ($p = 0.35$) from the average number of CM males captured in the standard pheromone-baited traps (3.15 ± 0.98 males/trap/night or 154 males/trap/49 days), although the pheromone standard captured significantly more male CM than did the C_{10} ester blend ($p < 0.001$) (**Table 2**). In the Washington apple orchard context, the C_{10} ester blend elicited combined gender and male capture rates (0.60 + 0.26 males/trap/night, 0.36 ± 0.15 females/trap/night, and 0.96 ± 0.41 CM/trap/night or 47 CM/trap/49 days) that were significantly lower ($p < 0.001$) than male capture rates with the standard pheromone lure (3.76 ± 0.69 males/trap/night or 184 males/trap/49 days) (**Table 2**). In contrast to California walnut context, in Washington apple orchards low CM capture rates, although significantly greater than solvent controls ($p < 0.05$), were evoked by (*E*,*E*)-α-farnesene (0.15 ± 0.08 CM/trap/night, 1:0.5 male/female) an (*E*,*E*)-α-farnesene-containing "sesquiterpene blend" (0.07 ± 0.04 CM/trap/night, 1:0.4 male/female), and a $C_{4:6}$ and $C_{6:4}$ ester blend of butyl hexanoate and hexyl butanoate (0.07 ± 0.03 CM/trap/night, 1:0.4 male/female). Traps baited with six other blends also captured CM in Washington apple orchards (**Table 2**), but their elicited very low capture rates (0.02−0.05 CM/trap/night) were not significantly different from solvent alone (**Table 2**).

Constituents of the C_{10} Ester Blend. Field tests in a walnut orchard to determine the active compound(s) present in the C_{10} ester blend revealed that CM were most strongly attracted to the ethyl (2*E*,4*Z*)-2,4-decadienoate (Et-*E*,*Z*-DD) constituent alone and to three blends containing Et-*E*,*Z*-DD:ethyl decanoate plus Et-*E*,*Z*-DD blend, C_{10} ester blend, and methyl (2*E*,4*Z*)-2,4-decadienoate plus Et-*E*,*Z*-DD blend (**Table 3**). For traps baited with Et-*E*,*Z*-DD and its blends, capture rates of CM (both sexes combined) were lower but not significantly different ($p = 0.07$) from the average male capture in traps baited with the standard sex pheromone lure. Also, comparable capture rates were observed with traps baited with the blend of the two saturated analogues, methyl decanoate plus ethyl decanoate (**Table 3**). However, there were significant differences ($p < 0.05$) observed in other replicated field tests (in orchards with lower CM populations) between these unsaturated analogues and the methyl- and ethyl dienoic acid esters (**Table 4**). The selective attraction of both sexes of CM to these esters followed the order Et-*E*,*Z*-DD > methyl (2*E*,4*Z*)-2,4-decadienoate > ethyl decanoate and/or methyl decanoate (**Tables 3** and **4**).

C_{10} and C_{12} Alcohol, Aldehyde, and Ester Analogues of Et-*E*,*Z*-DD. Field bioassays of numerous oxygenated C_{10} and C_{12} analogues of Et-*E*,*Z*-DD showed Et-*E*,*Z*-DD to be the most attractive to CM (**Table 4**), and this ester attracted no other insects, pest or beneficial. The 19 analogue C_{10} and C_{12} 2,4-diene and 2- or 4-mono-unsaturated alcohols, aldehydes, acetates, and esters elicited a range of low capture activity (0−0.31 CM/trap/night), but all responses were significantly less ($p < 0.007$) than those elicited by Et-*E*,*Z*-DD in both walnut and apple orchards (**Table 4**). Of the analogues tested, only the esters ethyl (*E*)-2-decenoate (0.21 ± 0.10 and 0.17 ± 0.06 CM/trap/night, walnut and apple, respectively), and methyl (2*E*,4*Z*)-2,4-decadienoate (0.17 ± 0.05 and 0.31 ± 0.13 CM/trap/night) elicited minimal rates of attraction, <14% of the Et-*E*,*Z*-DD capture rates in walnut and apple orchards (**Table 4**). However, attraction to Et-*E*,*Z*-DD was comparable to the CM pheromone in California walnut orchards (Et-*E*,*Z*-DD, 1.45 ± 0.33 CM/trap/night or 132 CM/trap/91 days; pheromone, 1.82 ± 0.37 males/trap/night, or 166 males/trap/91 days, $p < 0.67$), but half as potent in this particular second-flight test in Washington apple orchards (Et-*E*,*Z*-DD, 1.38 ± 0.30 CM/trap/night, or 126 CM/trap/91 days; pheromone, 2.83 ± 1.11 males/trap/night, or 258 males/trap/91 days, $p < 0.05$).

Table 3. Capture Rates (Moths/Trap/Night) of Codling Moth in Traps Baited with the C_{10} Ester Blend and Its Saturated and Unsaturated Constituents; Field Tests Conducted in Walnut Orchards

blends and constituents	mean ± SEM (moths/trap/night)[a]		
	males	females	total
C_{10} ester blend	0.80 ± 0.04b	1.20 ± 0.39a	2.02 ± 0.72ab
methyl decanoate + ethyl decanoate	0.87 ± 0.39bcd	0.51 ± 0.22b	1.38 ± 0.56bc
methyl decanoate + methyl (*E*,*Z*)-2,4-decadienoate	0.04 ± 0.04ef	0.35 ± 0.16bc	0.39 ± 0.20de
ethyl decanoate + ethyl (*E*,*Z*)-2,4-decadienoate	0.35 ± 0.10de	1.71 ± 0.92a	2.06 ± 0.98ab
methyl (*E*,*Z*)-2,4-decadienoate + ethyl (*E*,*Z*)-2,4-decadienoate	0.24 ± 0.07ef	1.34 ± 0.56a	1.58 ± 0.60bc
methyl decanoate	0.11 ± 0.06fg	0.12 ± 0.07c	0.23 ± 0.13e
ethyl decanoate	0.23 ± 0.16efg	0.20 ± 0.09bc	0.43 ± 0.17de
methyl (*E*,*Z*)-2,4-decadienoate	0.46 ± 0.16cde	0.35 ± 0.09b	0.81 ± 0.20cd
ethyl (*E*,*Z*)-2,4-decadienoate	0.82 ± 0.24bc	1.41 ± 0.59a	2.23 ± 0.82ab
solvent control	0.04 ± 0.03g	0	0.04 ± 0.03f
pheromone (CM-L2)	3.45 ± 0.96a	0	3.45 ± 0.96a

[a] Means (± SEM) are derived from two replicate walnut orchard tests with 4-day to weekly trap check intervals over a 2 month period. Septa were loaded with 10 mg of test compound or blend solution, and lures were replaced every 2 weeks. Column means followed by the same letter are not significantly different; significant differences are assigned at $p < 0.05$.

Table 4. Capture Rates (Moths/Trap/Night) of Codling Moth in Traps Baited with the C_{10} and C_{12} Saturated and Unsaturated Monoene and Diene Anologue Alcohols, Adehydes, and Esters

C_{10} and C_{12} analogue compound (purity)	walnut orchards,[a] mean ± SEM (moths/trap/night)[b]			apple orchards, mean ± SEM (moths/trap/night)		
	males	females	total	males	females	total
alcohols						
(*E*)-2-decen-1-ol (95.0%)	0	0	0	0	0	0
(*Z*)-4-decen-1-ol (95.0%)	0	0	0	0	0	0
9-decen-1-ol (96.0%)	0	0	0	0	0.02 ± 0.02c	0.02 ± 0.02d
2,4-decadien-1-ol (89.0%)	0	0	0	0	0	0
2,4-dodecadien-1-ol (90.0%)	0.06 ± 0.03c	0	0.06 ± 0.03c	0	0	0
aldehydes						
(*E*)-2-decenal (95.0%)	0	0	0	0	0	0
(*E*)-4-decenal (95.0%)	0	0	0	0	0.02 ± 0.02c	0.02 ± 0.02d
(*Z*)-4-decenal (98.0%)	0	0	0	0	0	0
2,4-decadienal (95.0%)	0.02 ± 0.01c	0	0.02 ± 0.01d	0.05 ± 0.05c	0	0.05 ± 0.05d
2,4-dodecadienal (97.0%)	0.02 ± 0.01c	0	0.02 ± 0.01c	0	0.02 ± 0.01c	0.02 ± 0.01d
(*E*,*Z*)-2,6-dodecadienal (94.0%)	0.03 ± 0.03c	0	0.03 ± 0.03c	0	0	0
esters						
(*E*,*E*)-2,4-hexadienoate (97.0%)	0	0	0	0	0	0
(*E*)-2-decen-1-yl acetate (95.0%)	0.03 ± 0.02c	0.02 ± 0.01c	0.05 ± 0.02c	0	0	0
9-decen-1-yl acetate (95.0%)	0	0	0	0	0	0
methyl decanoate (99.0%)	0.04 ± 0.04c	0.03 ± 0.03c	0.06 ± 0.04c	0	0	0
ethyl decanoate (99.0%)	0	0	0	0	0	0
ethyl (*E*)-2-decenoate (95.0%)	0.04 ± 0.03c	0.17 ± 0.08b	0.21 ± 0.10b	0.07 ± 0.04c	0.10 ± 0.10bc	0.17 ± 0.06c
ethyl (*E*)-4-decenoate (96.0%)	0	0.02 ± 0.02c	0.02 ± 0.02c	0	0	0
methyl (*E*,*Z*)-2,4-decadienoate (98.3%)	0.04 ± 0.02c	0.13 ± 0.03b	0.17 ± 0.05b	0.12 ± 0.09c	0.19 ± 0.06b	0.31 ± 0.13c
ethyl (*E*,*Z*)-2,4-decadienoate (95.3%)	0.44 ± 0.13b	1.02 ± 0.22a	1.45 ± 0.33a	0.55 ± 0.21b	0.83 ± 0.23a	1.38 ± 0.30b
standards						
pheromone (CM-L2)	1.82 ± 0.37a	0	1.82 ± 0.37a	2.83 ± 1.11a	0	2.83 ± 1.11a
solvent controls (2 rep)	0	0	0	0	0	0

[a] Means (± SEM) are derived from three organic walnut orchard and three apple orchard replicated tests, with 4 day to weekly trap check intervals over a 13 week period. Septa were loaded with 1.0 mg of test compound or blend solution, and lures were replaced every four weeks. [b] Column means followed by the same letter are not significantly different; significant differences are assigned at $p < 0.05$.

Geometric Isomers and Alcohol Moiety Analogues of Et-*E*,*Z*-DD. Various 2,4-decadienoic acid ester analogues (with alcohol moieties of C_1-C_6) and (*E*,*E*) and (*E*,*Z*) geometric isomers were found to have significant and specific structure−activity properties in field bioassays (**Table 5**). Both male and female CM had greater affinity and attraction−capture rates in walnut and apple orchard bioassays for (1) ethyl-(*E*,*Z*)- over propyl-(*E*,*Z*)-, methyl-(*E*,*Z*)-, or hexyl-(*E*,*Z*)-2,4-decadienoic acid esters; (2) ethyl-(*E*,*E*)- over propyl-(*E*,*E*)-, isopropyl-(*E*,*E*)-, methyl-(*E*,*E*)-, butyl-(*E*,*E*)-, or hexyl-(*E*,*E*)-2,4-decadienoic acid esters; and (3) (*E*,*Z*)- over (*E*,*E*)-2,4 geometrical isomers (**Table 5**). The differential responsiveness of CM to the (*E*,*Z*)- over the (*E*,*E*)-2,4 geometrical isomers ranged from the (*E*,*E*) isomer being 12% of (*E*,*Z*) isomer response for the ethyl esters, to 22−42% (i.e., walnut−apple) for the propyl esters, to 6−8% for the methyl esters, and to 3−41% for the hexyl esters. The strong attraction of CM males and females (combined gender data) elicited by Et-*E*,*Z*-DD reached the same level as that of male attraction to commercial sex pheromone lures in both walnut orchards (Et-*E*,*Z*-DD, 7.52 ± 0.99 CM/trap/night or 474 CM/trap/63 days; 1:1.3 male/female; pheromone, 7.72 ± 1.70 males/trap/night, or 486 males/trap/63 days, $p < 0.58$) and apple orchards (Et-*E*,*Z*-DD, 1.95 ± 0.31 CM/trap/night, or 123 CM/trap/63 days, 1:1.5 male/female; pheromone, 2.83 ± 1.11 males/trap/night, or 178 males/trap/63 days, $p < 0.15$).

Male Attraction to Et-*E*,*Z*-DD. Use of the funnel-bucket traps to rapidly drown attracted CM showed that males were readily captured in both pheromone- and kairomone-baited

Table 5. Capture Rates (Moths/Trap/Night) of Codling Moth in Traps Baited with the (*E*,*E*) and (*E*,*Z*) Geometric Isomers of C_1–C_6 Alcohol Esters of Decadienoic Acid

esters of (*E*,*Z*) and (*E*,*E*) isomers of 2,4-decadienoic acid (purity)	walnut orchards,[a] mean ± SEM (moths/trap/night)[b]			apple orchards, mean ± SEM (moths/trap/night)		
	males	females	total	males	females	total
synthetic pure esters						
methyl (*E*,*E*)-2,4-decadienoate (99.6%)	0.01 ± 0.01e	0.05 ± 0.03e	0.06 ± 0.03e	0	0.05 ± 0.05ef	0.05 ± 0.05e
methyl (*E*,*Z*)-2,4-decadienoate (98.3%)	0.19 ± 0.06d	0.62 ± 0.15c	0.80 ± 0.19c	0.43 ± 0.11c	0.36 ± 0.07b	0.79 ± 0.15b
ethyl (*E*,*E*)-2,4-decadienoate (99.5%)	0.29 ± 0.12d	0.63 ± 0.19c	0.93 ± 0.30c	0.14 ± 0.08d	0.10 ± 0.05de	0.24 ± 0.13cd
ethyl (*E*,*Z*)-2,4-decadienoate (95.3%)	3.31 ± 0.42b	4.20 ± 0.69a	7.52 ± 0.99a	0.79 ± 0.19b	1.17 ± 0.13a	1.95 ± 0.31a
propyl (*E*,*E*)-2,4-decadienoate (98.5%)	0.22 ± 0.13d	0.26 ± 0.07d	0.48 ± 0.19c	0.12 ± 0.05d	0.21 ± 0.07cd	0.33 ± 0.06c
propyl (*E*,*Z*)-2,4-decadienoate (96.5%)	0.61 ± 0.14c	1.52 ± 0.39b	2.14 ± 0.49b	0.40 ± 0.34cd	0.38 ± 0.20bc	0.79 ± 0.54bc
isopropyl (*E*,*E*)-2,4-decadienoate (99.2%)	0.04 ± 0.03e	0.14 ± 0.04d	0.18 ± 0.07d	0.05 ± 0.02d	0	0.05 ± 0.02e
butyl (*E*,*E*)-2,4-decadienoate (99.2%)	0.04 ± 0.03e	0.05 ± 0.03e	0.09 ± 0.04de	0	0.02 ± 0.02f	0.02 ± 0.02e
hexyl (*E*,*E*)-2,4-decadienoate (99.8%)	0.01 ± 0.01e	0.01 ± 0.01e	0.02 ± 0.01e	0.12 ± 0.12d	0	0.12 ± 0.12de
hexyl (*E*,*Z*)-2,4-decadienoate (91.6%)	0.24 ± 0.11d	0.43 ± 0.13cd	0.67 ± 0.20c	0.26 ± 0.10cd	0.02 ± 0.02f	0.29 ± 0.12cd
standards						
pheromone (CM-L2)	7.71 ± 1.70a	0.01 ± 0.01e	7.72 ± 1.70a	2.83 ± 1.11a		2.83 ± 1.11a
solvent control	0	0.03 ± 0.03e	0.03 ± 0.03e	0	0	0

[a] Means (± SEM) are derived from five organic walnut orchard and three apple orchard replicated tests, with 4 day to weekly trap check intervals over a 9 week period. Septa were loaded with 1.0 mg of test compound or blend solution, and lures were replaced every 3 weeks. [b] Column means followed by the same letter are not significantly different; significant differences are assigned at $p < 0.05$.

bucket traps (pheromone, 3.04 ± 0.83 males/trap/night; Et-*E*,*Z*-DD, 4.10 ± 0.62 CM/trap/night). Females, again, predominated the capture in Et-*E*,*Z*-DD-baited bucket traps, with a sex ratio of 1:3.0 (male/female).

DISCUSSION

Only one blend and category of compounds, the C_{10} methyl and ethyl decanoates and decadienoates, elicited strong and significant attraction and capture responses for CM. The key and dominantly attractive compound was shown to be Et-*E*,*Z*-DD, attracting both CM sexes, with female attraction and capture exceeding male capture over these studies. Moreover, male CM were definitively shown to be attracted to Et-*E*,*Z*-DD and not inadvertently attracted by secondary release of natural sex pheromone, which could possibly be emitted by trapped females stuck on standard sticky traps. Additional evidence of male CM attraction directly to Et-*E*,*Z*-DD is the frequent observation of exclusive capture of male CM in Et-*E*,*Z*-DD-baited sticky traps in late March−early April, when male emergence (protandry) typically precedes females in California walnut orchards (*16*).

Tests with various analogue esters with alcohol chain length moiety substitutions of the (2*E*,4*Z*)-2,4-decadienoic acid elicited significant differential capture responses, with the ethyl exceeding the propyl, methyl, butyl, and hexyl analogues. The (*E*,*Z*) geometric isomers of this series of (2*E*,4*Z*)-2,4-decadienoic acid esters far exceeded the attractiveness of the (*E*,*E*) isomers. These structure−activity tests demonstrate that both sexes of the CM innately possess a specific chemoreceptive affinity for, and a strong behavioral attraction to, the pear ester molecule.

The strong attraction of CM males and females (combined gender data) elicited by Et-*E*,*Z*-DD reached the same level as that of male attraction to commercial sex pheromone lures in both walnut orchards (Et-*E*,*Z*-DD, 7.52 ± 0.99 CM/trap/night, 1:1.3 male/female; pheromone, 7.72 ± 1.70 males/trap/night, $p < 0.58$) and in many trials in apple orchards (Et-*E*,*Z*-DD, 1.95 ± 0.31 CM/trap/night, 1:1.5 male/female; pheromone, 2.83 ± 1.11 males/trap/night, $p < 0.15$). This capture parity was observed in these tests for dose loading rates on septa (e.g., 1 mg) that were low and comparable for these two distinct semiochemicals, further supporting the behavioral potency of this kairomone relative to that established for the CM sex pheromone (*16*). The pear ester also exhibited a degree of species specificity similar to that of pheromone, by being nonattractive to other insect species, both beneficial and pests, including eight key lepidopteran pests of pome fruits and other horticultural fruit and nut crops (*47*).

In the studies reported here, in both walnut and apple orchards, the CM attractant, (*E*,*E*)-α-farnesene, elicited only a low level of field attraction, as also recently reported in apple orchard field trials in Sweden (*13*). Numerous papers defining a stronger attractiveness of (*E*,*E*)-α-farnesene to adult CM have all been limited to only laboratory bioassays (*4*, *6*, *8*, *9*, *11*, *13*). (*E*,*E*)-α-Farnesene is an HPV of all three CM hosts, being a major volatile constituent of apple and pear skins (*33*) and walnut leaves and husks (*18*, *24*, *25*).

Recently, certain other apple-based HPVs have been identified as CM kairomonal attractants (*13*, *14*). Coracini et al. (*13*) found in apple orchard field tests that male CM were attracted to (*E*)-*β*-farnesene and to a lesser extent to (*E*,*E*)-farnesol, whereas (*E*,*E*)-α-farnesene was nonattractive alone but appeared to enhance male capture when combined with (*E*)-*β*-farnesene. However, in flight tunnel studies only (*E*,*E*)-farnesol elicited from male CM the full progression of activation, flight, landing, and wing-fanning behaviors. This series of male orientation behaviors typically is elicited by pheromone, although pheromone was demonstrated to have a 1000-fold lower threshold than these putative kairomones (*13*). Moreover, exclusively male CM were found to be attracted in the field to (*E*)-*β*-farnesene, (*E*,*E*)-farnesol, and/or (*E*,*E*)-α-farnesene, whereas female captures were extremely low and not statistically different from blank septa controls (*13*). Recently, the discovery of a female kairomonal attractant was reported by Hern and Dorn (*14*). They found that a key principal volatile of apple odor, butyl hexanoate, attracted mated females in laboratory wind-tunnel bioassays. Unfortunately, in the field trials reported here, butyl hexanoate was not tested alone, but was a constituent of four ester-based blends (C_4 and/or C_6 chain lengths) of the 23 blends tested. These butyl hexanoate blends elicited no trap captures in the California walnut orchard context, but a particular two-component blend ($C_{4:6}$ and $C_{6:4}$ esters) of butyl hexanoate and hexyl butanoate did elicit a significant, although relatively low level, CM capture in Washington apple orchard trials (**Table 2**). All three other butyl hexanoate-containing blends elicited zero captures, although these blends of C_4 and/or C_6 esters

contained more constituents (**Table 1**), ranging from four to nine compounds, which might possibly have disrupted or masked the butyl hexanoate's activity.

The pear ester is a potent attractant of both male and female codling moths, and the moth is highly discriminating and specific in its structure−activity attraction to this pear-derived kairomone. These attributes of specificity and potency are currently allowing this kairomone to contribute to new abilities for female monitoring in orchards (*2*, *16*, *41*−*44*) and the development of novel and highly selective control practices, targeting either both sexes in mass trapping and attract and kill tactics (*48*) or enhancing−augmenting mating disruption of males (*49*), all of which should decrease the current dependence on use of broad-spectrum insecticides.

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