Compounds from Host Fruit Odor Attractive to Adult Plum Curculios (Coleoptera: Curculionidae)[1]

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Abstract Three release rates of each of 30 compounds identified as components of the odor of unripe host plum or apple fruit were evaluated in field tests in 1999 for attractiveness to adult plum curculios, *Conotrachelus nenuphar* (Herbst). Compounds were introduced into polyethylene vials and assayed in association with boll weevil traps placed beneath infested apple trees in Ohio and Massachusetts. Results confirmed previously reported attractiveness of limonene and ethyl isovalerate to this insect. In addition, at least six other compounds showed good evidence of attractiveness (benzaldehyde, benzyl alcohol, decanal, E-2-hexenal, geranyl propionate and hexyl acetate), and five other compounds appeared worthy of further evaluation for attractiveness (2-hexanol, 1-pentanol, 2-pentanol, phenylacetaldehyde and 2-propanol). Degree of attractiveness of compounds varied according to release rate.

Key Words *Conotrachelus nenuphar,* plum curculio, odor attractants, fruit volatiles

Several weevil species are known to respond positively to odor from host plants (Roseland et al. 1992, Jaffe et al. 1993, Bundenburg et al. 1993, Giblin-Davis et al. 1994, Hardee and Mitchell 1997, Smart and Blight 1997, Collins et al. 1997, Landon et al. 1997, Gunawardena et al. 1998). For some species, attractive host plant volatiles have been identified and employed alone or in combination with pheromone in traps for monitoring weevil abundance or directly controlling weevils (Jaffe et al. 1993, Giblin-Davis et al. 1994, Perez et al. 1997).

Recently, it was reported that two volatile components (limonene and ethyl isovalerate) of unripe host plum fruit were significantly attractive to plum curculio adults, *Conotrachelus nenuphar* (Herbst), in both laboratory and field assays (Leskey et al. 1998, 2001). This insect is a major pest of stone and pome fruit in eastern and central North America (Racette et al. 1992). Developing effective traps employing attractive odor would markedly improve the capability of monitoring abundance of plum curculios in commercial orchards, which currently relies upon examining host fruit for extent of feeding or ovipositional injury (Vincent et al. 1999).

Here, we report on field responses of plum curculio adults to three release rates of each of 30 compounds identified as components of the odor of unripe host plum or apple fruit, known to be attractive to this insect (Leskey and Prokopy 2000).

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Materials and Methods

Uninfested plums (var. 'Fellenburg'), *Prunus domestica* L., and uninfested green apples (var. 'McIntosh'), *Malus domestica* Borkhausen, were picked 1 to 3 wks after bloom from an unmanaged orchard at the Ohio Agricultural Research and Development Center in Wooster. Odor of plum and apple fruit is most attractive to plum curculios when fruit is at this stage of development (Leskey and Prokopy 2000). Ten to 40 fruit of the same species were placed in an 8-dram vial, held on ice, and transported to the laboratory. Methods used in collection and identification of volatiles are described in detail by Leskey et al. (2001).

From the collections made in Ohio, 16 compounds were identified from plum odor and 23 compounds from apple odor (Table 1). Of these, two compounds were found present in odor of both plums and apples, 14 compounds were unique to odor of plums, and 21 compounds were unique to odor of apples. Of the compounds from apples and plums, eight were the same as those identified by Boeve et al. (1996) from odor of apples (mixed cultivars, picked 1 to 4 wks after bloom) from trees in Switzerland (Table 1). In addition, Boeve et al. (1996) identified 10 volatile compounds from apples (Table 1) that were not identified from collections made in Ohio. Also, nine unique volatiles were detected in Ohio emanating from apples during a time when plum curculio females were feeding on collected apples during volatile collection (Table 1).

Of the 56 compounds listed in Table 1, 30 were chosen here for evaluation for attractiveness to plum curculios. Sixteen of these were evaluated in laboratory and field studies in 1998 (Leskey et al. 1998, 2001), and part of the intent here was to confirm their findings. The other 14 compounds were selected for evaluation here on the basis of availability from a commercial supplier (Aldrich Chemical Co. Inc., Milwaukee, WI) and cost (those costing more than $5.00/g were excluded). In Leskey et al. (1998, 2001), each compound evaluated in field tests was diluted with mineral oil and applied to cotton dental wick wrapped in aluminum foil (one end open to permit release). Here, each compound was introduced into a 2-dram polyethylene vial (Israel Andler and Sons, Everett, MA) and assessed at three different release rates (Table 2). Release rates were varied either by adding mineral oil to the contents of a vial to reduce release rate or drilling 2-mm-diam holes in a vial just beneath the cap to increase release rate. Our initial intent was that the high, middle and low release rate of each compound would be about 48, 12, and 3 mg/day, respectively, or 4-fold levels of difference between adjacent release rates. Owing to constraints associated with our approach to adjusting release rates, it was not possible in every case to achieve our original intent. Each release rate was established by assessing the amount of weight lost per vial over a period of 30 d at 25°C and 50% RH.

Compounds were assayed in association with green boll weevil traps obtained from Gemplers Inc. (Belleville, WI). Each trap was baited with one vial containing a compound or one empty vial. Vials were suspended vertically by wire attached to the base of the screen funnel top of the trap and positioned midway between the top and base of the trap. We reasoned that positioning vials in this fashion could elicit attraction of adults to traps and was not as likely to cause repulsion at close range as might the placing of vials within the screen funnel top.

All 30 compounds were evaluated over a 56-d period in two apple orchards at the Ohio Agricultural Research and Development Center in Wooster. The first orchard consisted of unmanaged 40-yr-old 'Cortland' trees, each about 10 m in canopy diam.

Table 1. Compounds identified from odor of unripe domestic plums alone, unripe domestic apples alone and unripe domestic apples during feeding of plum curculio (PC) females

Compound	Plum**	Apple+	Apple++	PC on Apple+++
*benzaldehyde	X			
*benzonitrile	X			
*benzothiazole		X		
*benzyl alcohol				X
bicyclo-oct-5-ene-2-one		X		
beta-bourbonene		X	X	
butyl isopentyl ether		X		
1,2-butanediol			X	
2,3-butanediol			X	
E-beta-caryophyllene		X	X	
alpha cubebene		X		
1,3,5-cycloheptatriene				X
E,Z-2,4-decadienal			X	
*decanal		X		
E-4,8-dimethyl-1,3,7-nonatriene			X	
E-2(3)-epoxy-2,6-dimethyl-6,8-nonadiene			X	
*ethyl acetate	X			
ethyl benzene				X
*ethyl butyrate	X			
*ethyl isovalerate	X			
E,E-alpha-farnesene		X	X	
*geranyl propionate				X
**E*-2-hexenal	X	X	X	
*1-hexanol		X		
*2-hexanol	X			
*3-hexanol	X			
*2-hexanone	X			
*3-hexanone	X			
3-hexen-1-ol		X		
Z-3-hexenyl acetate		X	X	
*hexyl acetate		X		

Table 1. Continued

Compound	Plum**	Apple+	Apple++	PC on Apple+++
*3-hydroxy-2-butanone	X		X	
E-3-hexenyl-butyrate		X		
*isopropyl acetate	X			
*limonene	X			
*linalool	X	X	X	
*3-methyl-1-butanol			X	
*2-methyl-3-buten-2-ol				X
3-(4-methyl-3-pentenyl) furan		X		
methyl salicylate			X	
nonanal		X		
**E*-2-nonenal				X
E-beta-ocimene		X	X	
*1-pentanol		X		
*2-pentanol	X			
*3-pentanol		X		
*1-penten-3-ol		X		
3-penten-2-ol				X
4-penten-2-one		X		
pentyl formate		X		
*phenylacetaldehyde			X	
phenylacetonitrile			X	
*2-phenylethanol			X	
*2-propanol	X			
1,3,3-trimethylnonal benzene				X
2,6,6-trimethyl octane				X

* Compound assayed here for attractiveness.
** Identification from uninfested plums by PLP in Ohio.
+ Identification from uninfested apples by PLP in Ohio.
++ Identification from uninfested apples by Boeve et al. (1996) in Switzerland.
+++ Identification from apples during feeding of plum curculio females by PLP in Ohio.

Prior to assays, brush was removed and ground vegetation was mowed to a height of 10 cm. There were ten traps per tree, each positioned about 3 m from the tree trunk and about 2 m apart. The second orchard consisted of 9-yr-old 'Empire', 'Red Delicious', 'Golden Delicious', and 'Rome' trees, each about 2 m in canopy diam. This

Table 2. Attractiveness of odor components of unripe host fruit to plum curculio adults in field tests in Ohio and Massachusetts in 1999. Compounds were evaluated in association with boll weevil traps placed beneath infested apple trees

	Ohio*				Massachusetts*			
Compound release rate (mg/day)	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++
benzaldehyde								
0	.06				0.5			
1.3	.12		32	.06	1.4		46	.011
11.7	.12		32	.06	0.3			
46.7	.08				1.1			
benzonitrile								
0	.06							
2.1	.01							
12.4	.05		−7					
49.5	.05		−7					
benzothiazole								
0	.06				1.1			
4.1	.08				0.7			
17.6	.05				0.8			
70.3	.10		27		1.5		17	
benzyl alcohol								
0	.06				0.5			
2.2	.15	.06	44	.04	0.6			
14.1	.01				0.7			
56.2	.05				1.0		35	.056
decanal								
0	.06				0.2			
3.6	.05				1.0		64	.0001
21.9	.19	.03	53	.002	0.7			
87.5	.06				0.7			
ethyl acetate								
0	.06				1.3			
2.0	.05				0.4			
9.7	.06				0.6		−36	
50.2	.08		13		0.6		−36	

Table 2. Continued

	Ohio*				Massachusetts*			
Compound release rate (mg/day)	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++
ethyl butyrate								
0	.06							
4.7	.05							
15.1	.06		4					
58.1	.04							
ethyl isovalerate								
0	.06				0.4			
3.9	.10				0.7			
13.0	.06		27		1.0		40	.029
72.9	.06				0.5			
geranyl propionate								
0	.06				0.2			
6.3	.06				0.3			
21.0	.12		32	.06	0.8		59	.007
109.4	.06				0.8		59	.007
1-hexanol								
0	.06							
2.0	.04							
13.3	.08		13					
53.1	.04							
2-hexanol								
0	.06							
3.1	.10							
13.3	.01							
53.1	.12		32	.06				
3-hexanol								
0	.06							
3.1	.06		4					
13.3	.06		4					
53.1	.06		4					

Table 2. Continued

	Ohio*				Massachusetts*			
Compound release rate (mg/day)	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++
2-hexanone								
0	.06							
3.0	.06		4					
12.5	.06		4					
50.1	.06		4					
3-hexanone								
0	.06							
2.0	.03							
13.0	.03							
38.1	.08		13					
E-2-hexenal								
0	.06				0.1			
2.1	.06				0.4			
12.8	.15	.07	44	.04	1.1	0.009	90	<.0001
51.0	.05				0.4			
hexyl acetate								
0	.06				0.4			
3.6	.01				1.4	0.03		
21.6	.04				1.2	0.10		
65.0	.10		27		2.0	0.006	67	.0001
3-hydroxy-2-butanone								
0	.06							
1.8	.06							
10.6	.03							
59.9	.10		27					
isopropyl acetate								
0	.06							
3.1	.09		20					
9.2	.08							
55.2	.09		20					

Table 2. Continued

	Ohio*				Massachusetts*			
Compound release rate (mg/day)	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++
limonene								
0	.06				0.4			
3.4	.08				0.5			
20.4	.09				1.7	0.007	64	.0001
63.7	.19	.02	53	.002	0.5			
linalool								
0	.06							
3.1	.05							
9.3	.01							
18.5	.08		13					
3-methyl-1-butanol								
0	.06							
2.6	.05		−7					
11.5	.05		−7					
45.8	.03							
2-methyl-3-buten-2-ol								
0	.06				0.7			
2.6	.05				0.1			
11.2	.08		13		0.6		−5	
44.8	.06				0.6		−5	
1-pentanol								
0	.06				0.2			
2.6	.05				0.5			
11.5	.09		20		0.9		59	.0007
45.8	.09		20		0.6			
2-pentanol								
0	.06				0.6			
2.6	.08		13		0.4			
11.5	.04				0.8			
45.8	.05				1.2		35	.056

Table 2. Continued

	Ohio*				Massachusetts*			
Compound release rate (mg/day)	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++	Mean no. PC/ replicate	*P* catch> control**	RI+	*P* RI> control++
3-pentanol								
0	.06							
2.6	.03							
11.5	.06		4					
45.8	0.0							
1-penten-3-ol								
0	.06							
2.6	.05							
11.2	.03							
44.8	.06		4					
phenylacetaldehyde								
0	.06							
6.0	.09							
12.0	.05							
24.0	.12		32	.06				
2-phenylethanol								
0	.06							
3.7	.09		20					
15.9	.03							
63.5	.01							
2-propanol								
0	.06							
1.3	.04							
5.4	.12		32	.06				
32.5	.01							
E-2-nonenal								
0	.06				0.7			
2.8	.04				0.0			
16.8	.05		−7		0.7		0	
67.3	.04				0.5			

* Ohio, n = 26; Massachusetts n = 6 with 14 compounds tested.

** Probability that treatment catch is greater than control catch as measured by Dunnett's test. Only probabilities <0.10 are displayed.

\+ RI = ((treatment − control)/(treatment + control)) × 100.

++ Probabilities based on Monte Carlo simulation of 1000 random values generated from control trap values. Only probabilities <0.10 are displayed.

orchard was sprayed with fungicides but no insecticides, and the ground beneath tree canopies was sprayed with herbicide to eliminate understory vegetation. There were three traps per tree, each positioned about 60 cm from the tree trunk and about 90 cm apart.

Using a randomized complete block design, we assigned one block of treatments to the Cortland orchard and two blocks of treatments to the mixed-cultivar orchard. Each block of treatments consisted of 120 traps deployed in randomized fashion: 90 traps with odor (30 compounds each at three release rates) and 30 traps without odor (controls). Assays began 3 May (4 d after petal fall) and continued until 28 June. Traps were checked every 1 to 2 d, for a total of 31 sampling dates. Each sampling date was considered to be a replicate, except that no plum curculios were found on five dates (these were excluded from analysis). Captured adults were released between tree rows. Traps were re-randomized within a block every 3 to 4 d.

Fourteen of the 30 compounds were evaluated over a 30-d period in Conway, MA, beginning 9 June. Choice of compounds for evaluation was based on those 14 compounds that appeared to be most attractive as of 1 June in the Ohio tests. Assays were conducted beneath 14 unmanaged backyard apple trees of mixed cultivars, each about 6 m in canopy diam. Grass beneath tree canopies was maintained at a height of 5 to 10 cm throughout testing. There were four traps per tree: one for each release rate of a single compound and one control trap. The four traps were positioned in a circle about 1.8 m from the tree trunk and 2.5 m apart. Traps were examined and rotated clockwise daily for 4 d. Captured adults were released at bases of tree trunks. A replicate consisted of total adults captured per treatment across the 4 d when traps were beneath the same tree. Compounds were re-randomized among trees after each replicate.

Data from Ohio were analyzed after log (x + 1) transformation by balanced ANOVA for effects of block (3), date (26), and level (4) for each chemical. The control values were the mean of 30 traps for each date and block. These values were shared for testing effects of level of each chemical. Data from Massachusetts were analyzed after log (x + 1) transformation by 2-way ANOVA, separating effects of blocks (6) and levels (4) for each chemical. For both locations, a Dunnett's test (Steel and Torrie 1960) was conducted when significant effect of level was measured to determine which levels were significantly greater than control. In addition, to facilitate across-experiment ranking of compounds, we used a Response Index (RI) developed by Phillips et al. (1993): the total number of adults responding to unbaited control traps (C) was subtracted from the total number responding to that release rate of a compound that "attracted" the greatest numbers of adults (T), divided by the value (T + C) and multiplied by 100. The greater the RI value, the more attractive that release rate of a compound. The statistical significance of RI values at each location was determined by generating 1000 random values using mean and standard deviation of the population of control trap values at that location. Next, a Monte Carlo simulation of RI values was conducted using these random trap numbers to quantify the probability of getting a RI value by chance (Sokal and Rohlf 1981).

Results and Discussion

Analysis of variance of the trapping data from Ohio indicated significant attraction ($P \leq 0.10$) of plum curculios to four of the 30 compounds tested: benzyl alcohol, decanal, E-2-hexenal and limonene (Table 2). In all cases, only one release rate elicited trap captures significantly greater than control: low rate of benzyl alcohol, medium rate of decanal, medium rate of E-2-hexenal, and high rate of limonene. Analysis of variance of Massachusetts trapping data showed a significant treatment effect ($P \leq 0.10$) for three of the 14 compounds tested: E-2-hexenal, hexyl acetate, and limonene (Table 2). E-2-hexenal and limonene were significantly attractive at the medium release rate, while hexyl acetate was attractive at all levels of release.

In addition to the four compounds identified by ANOVA as attractive in Ohio, RI values from Ohio trapping suggested significant attraction to at least one release rate for five other compounds (RI = 32, $P = 0.06$ for each): benzaldehyde, geranyl propionate, 2-hexanol, phenylacetaldehyde, and 2-propanol. In Massachusetts, RI values indicated significant activity ($P \leq 0.06$) for at least one level of seven compounds in addition to the three listed above for Massachusetts: benzaldehyde (RI = 46), benzyl alcohol (35), decanal (64), ethyl isovalerate (40), geranyl propionate (59), 1-pentanol (59), and 2-pentanol (35).

In this study, based on the criterion of ANOVA, two compounds (E-2-hexenal and limonene) were significantly attractive to plum curculios both in Ohio and Massachusetts, and three compounds (benzyl alcohol, decanal, and hexyl acetate) were significantly attractive either in Ohio or Massachusetts at one or more release rates. Based on the criterion of RI value, two additional compounds (benzaldehyde and geranyl propionate) were significantly attractive both in Ohio and Massachusetts, and six additional compounds (ethyl isovalerate, 2-hexanol, 1-pentanol, 2-pentanol, phenylacetaldehyde and 2-propanol) were significantly attractive either in Ohio or Massachusetts at one or more release rates.

In field tests conducted in Massachusetts in 1998 (Leskey et al. 1998, 2001) using the same approach (but a different release vehicle) for evaluating compounds for attractiveness to plum curculios as used in Massachusetts in 1999 in this study, two of the 16 compounds tested (ethyl isovalerate and limonene) were statistically significantly attractive. Findings in this study confirm the attractiveness of these two compounds. In addition, two compounds (benzaldehyde and E-2-hexenal) were significantly attractive in this study in both Ohio and Massachusetts and three compounds (2-hexanol, 2-pentanol, and 2-propanol) were significantly attractive in this study either in Ohio or Massachusetts but were not significantly attractive in Massachusetts in 1998 field studies. Six compounds showing significant attractiveness in this study either in Ohio or Massachusetts were not tested in Massachusetts in 1998: benzyl alcohol, decanal, geranyl propionate, hexyl acetate, 1-pentanol and phenylacetaldehyde.

The degree and nature of response (attraction or repulsion) of an insect to a stimulus can be strongly affected by the strength of the stimulus. Findings here indicate that for the six compounds having a RI value of 32 or greater in both Ohio and Massachusetts, attractiveness was greatest in Ohio and Massachusetts, respectively, at the following release rates: benzaldehyde (low, medium; low); benzyl alcohol (low; high); decanal (medium; low); geranyl propionate (medium; medium, high); E-2-hexenal (medium; medium); and limonene (high; medium). For the 16 compounds evaluated in the field in Massachusetts in 1998 as well as in this study, the single release rates used in 1998 were bracketed by the range of release rates used in this study. Three of the above six compounds were evaluated in Massachusetts in 1998 and at the following release rates: limonene (low); E-2-hexenal (low); and benzaldehyde (medium). Attraction to limonene and benzyl alcohol appears to have occurred over the entire range of release rates used, whereas attraction to the other four compounds seems to have been confined to a narrower range of release rates.

In summary, findings here support findings of Leskey et al. (1998, 2001) that the host fruit volatiles limonene and ethyl isovalerate are attractive to plum curculio adults. In addition, findings here suggest that at least six other host fruit volatiles also are attractive to this insect: benzaldehyde, benzyl alcohol, decanal, E-2-hexenal, geranyl propionate and hexyl acetate. Also, our findings suggest that five other compounds are worthy of additional evaluation for potential attractiveness to plum curculios: 2-hexanol, 1-pentanol, 2-pentanol, phenylacetaldehyde and 2-propanol. Future studies should consider optimizing release vehicles and release rates of each of these compounds as components of an attractive blend, using such a blend in conjunction with synthetic pheromone of plum curculio (Eller and Bartelt 1996), and optimizing deployment of an attractive blend of fruit volatiles and pheromone in association with attractive visual traps (Prokopy et al. 2000).

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