Role of Ground Trash Volatiles in the Selection of Hibernation Sites by Boll Weevils

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Volatiles play a large role in governing the behavior of boll weevils (*Anthonomus grandis* Boheman). They are attracted to cotton plants, and the female is sexually attracted to the male. The attracting compounds in both instances are terpenoids. Primarily in the fall of the year, boll weevils seek hibernation sites in leaf trash, where they remain until the following spring or summer. In the present study, essential oils were prepared by steam distillation from several leaf samples known to be prevalent at hibernation sites, and the oils were analyzed by GLC-MS. On the basis of the resulting presumptive identifications by comparison with those of standards, a number of mixtures were formulated and were field tested, as were the essential oils. The field tests failed to support unambiguously the premise that boll weevils select hibernation sites on the basis of leaf odor alone. However, in the presence of the sex pheromone, β-caryophyllene ($P > T = 0.08$), or a mixture of three sesquiterpene hydrocarbons ($P > T = 0.10$), or a mixture of alkyl alcohols ($P > T = 0.15$) increased captures. The response to formulations of the sex pheromone with β-caryophyllene may be primarily sexual, based on its presence in female boll weevils.

Keywords: *Boll weevil; hibernation; ground trash; essential oils; terpenoids*

INTRODUCTION

Volatiles play a large role in governing the behavior of boll weevils (*Anthonomus grandis* Boheman). They are attracted to cotton plants (*Gossypium* spp.) (Hedin et al., 1973), and the female is sexually attracted to the male (Tumlinson et al., 1969). The attracting compounds in both instances are terpenoids. Primarily in the fall of the year, boll weevils seek hibernation sites in which they remain until the following spring or summer, and this has been the subject of a number of studies since the early 1900s (Hinds and Yothers, 1909). In later studies, boll weevils entering the sites relatively early in the fall were found to emerge relatively late during the next season (Mitchell et al., 1973).

Recently, McKibben et al. (1998) reported that field trapping studies showed that late summer and fall diapausing boll weevils responded better to traps containing Grandlure plus synthetic plant components (mostly terpenoids) than to traps baited with Grandlure alone. They were also weakly attracted to essential oils (steam distillates) of sweet gum (*Liquidambar styraciflua* L.), honeysuckle (*Lonicera* sp.), willow oak (*Quercus phellos*), and red oak (*Quercus rubra* L.) and to extracts of leaf litter.

In the present investigation, the essential oils of these leaves and of ground trash have been evaluated by gas chromatography−mass spectrometry (GLC-MS) and were deployed in field traps during the fall seasons of 1997 and 1998 to evaluate their attractancies. Also, on the basis of GLC-MS analyses of the leaves and ground trash, categories of commercial compounds were formulated and also were evaluated for their attractancies.

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MATERIALS AND METHODS

Collection and Processing of Leaves. Fresh leaves of cotton (*Gossypium* spp.), willow oak (*Quercus phellos* L.), honeysuckle (*Lonicera* sp.), sweet gum (*Liquidambar styraciflua* L.), and mixed ground trash, ∼2 kg of each, were steam distilled into methylene chloride from water employing a variable reflux distilling head distillation−extraction apparatus (Ace Glass, Vineland, NJ). Extracts were partially concentrated with a rotary evaporator at 50 °C and then carefully reduced to their essential oils with a stream of nitrogen. The essential oils were then applied to 10 × 38 mm cotton dental rolls (Patterson Dental Canada Inc., Montreal, Canada) in 50−100 mg (μL) quantities for field testing. The dental rolls were attached to standard boll weevil traps. Aliquots were reserved for analysis by GLC-MS.

Chemical Analyses Procedures. GLC-MS analyses were performed with a DB-1 column (J&W Scientific, Folsom, CA; 30 m × 0.32 mm i.d. × 0.25 μm layer thickness): injection temperature, 280 °C; transfer line, 300 °C; program, 45 °C, 2 min hold, 15 °C/min to 300 °C, 5 min hold; split ratio, 15:1. The column was interfaced to a Hewlett-Packard HP-5989 quadrupole mass spectrometer operated in the EI mode. Spectral interpretations were supported by the NIST/EPA/MSDC Mass Spectral Database 1A PC version 3.0 (Lias and Stein, 1990) and the HP 59944C MS Chem System version 8.05 (Hewlett-Packard, 1992).

Field Tests. Concentrates and specified commercially available compounds and their mixtures were placed in boll weevil field traps for testing in the late summer and early fall seasons of 1997 and 1998 in Coahoma and Quitman Counties of Mississippi. The 36 commercially available compounds used in the field tests were procured from either Fluka Chemika-Biochemika, Ronkonkoma, NY, or Aldrich Chemical Co., Milwaukee, WI (see Results and Discussion for identities). For comparison, standard 10 mg dispensers of Grandlure, the male boll weevil sex pheromone (Tumlinson et al., 1969), were also deployed. Nine replications were conducted over a 5 day period. Statistical analyses were performed using the least squares means separations from the GLM procedure (SAS Institute, 1990).

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Table 1. Terpenoid and Non-Terpenoid Compositions of Several Leaf Essential Oils, Percent of Total

		terpenoids					non-terpenoids[a]				
sample	response, % of blank[b]	C_{10} hydro-carbons	C_{10} oxy-genated	C_{15} hydro-carbons	C_{10} oxy-genated	total	aliphatic	aromatic	nitrog-enous	total	unidentified GLC maxima
cotton	106	35.7	0	19.7	19.2	74.6	9.2	0	0	10.9	14.5
sweet gum	65	29.1	11.7	28.8	9.3	83.5	1.1	0	0	1.1	15.4
willow oak	94	1.8	0.9	11.1	7.0	20.8	20.0	19.1	0	39.1	40.1
honeysuckle	118	1.2	2.6	1.0	10.2	15.0	20.8	11.3	8.6	40.7	44.3
mixed "fresh"		0	1.4	19.2	10.1	30.7	31.2	8.2	0	37.8	31.5
ground trash	124	6.3	2.9	6.3	8.6	24.1	12.6	3.3	0	15.9	60.0

[a] Includes C_5-C_{20} alcohols, aldehydes, ketones, acids, and esters, C_5-C_{12} aromatic aldehydes, alcohols, furans, benzoates, and pyridines, and quinolines. [b] Also pine = 100, cedar = 100.

Studies to determine whether boll weevils were attracted to field hibernation sites on the basis of their responses to leaf essential oils, to commercially available terpenoids, to other compounds found present in the essential oils, and to the synthetic boll weevil pheromone were conducted during the late summer and early fall seasons of 1997 and 1998.

Three related field tests were conducted in 1997. In the first test, "A", 50 mg quantities of the various leaf essential oils were injected into dental rolls for placement in the standard yellow boll weevil traps. However, sex pheromone (Grandlure) was not included in this test. The traps were placed in two locations at intervals 100−200 ft (30−60 m) from cotton fields. Trapped boll weevils were removed and counted daily during a 10 day period in September. In a second test, "B", dental rolls containing 50 mg of β-caryophyllene were tested in adjacent fields over a 3 day period.

In a third test, "C", also conducted during the same period, the responses of weevils to the standard 10 mg boll weevil sex pheromone formulation were compared to a formulation of the sex pheromone plus 50 mg of β-caryophyllene.

In 1998, two tests were conducted. For the first test, "D", 5 kg of ground trash was steam distilled into methylene chloride, giving, on concentration, 5.6 gm of essential oil from which 56 dental rolls, each containing 100 mg, were prepared. The sex pheromone was not incorporated in this study. This test was conducted over a 9 day period and included 46 replicates.

In a second test conducted in 1998, "E", 10 different synthetic mixtures (100 mg; equal weight) were evaluated as additives to the standard 10 mg Grandlure formulation and compared to Grandlure alone. The categories of synthetic mixtures were based on compounds identified by GLC-MS as present in the various leaf essential oils (Tables 1 and 2). Nine replications were conducted over a 5 day period.

RESULTS AND DISCUSSION

GLC-MS Analyses. Initially, structural assignments were suggested on the basis of the MS fragmentation pattern data. Because the literature and preliminary field tests suggested that boll weevils were somewhat attracted under some conditions to terpenoids, they became the major emphasis of the continuing field tests. Nevertheless, for the purpose of completeness, identifications for both terpenoids and non-terpenoids were tentatively assigned, and the totals of categories are listed in Table 1. Because this was a preliminary survey to determine whether there was a dominant leaf preparation, comparisons of spectra were limited to those of major peaks with those in the literature. Forthcoming results did not seem to warrant further GLC-MS analyses of standards.

These data show that sweet gum and cotton leaves were high in terpenoids, although their distributions were somewhat different, whereas honeysuckle and willow oak leaves were much lower in terpenoids. Evidently, the mixed fresh collection and the ground trash were derived mostly from the willow oak and honeysuckle on the basis of their relatively low terpenoid contents. The honeysuckle and willow oak leaves have (by difference) a relatively high content of non-terpenoids, noteably of aromatic character. Because the ground trash is a diverse mixture of leaves, a large number of small GLC peaks were present that provided only weak spectral fragmentation patterns. Thus, only 40% of the peaks had fragmentation patterns adequate to attempt structural assignments.

One of the objectives of these analyses was to investigate whether the selection of hibernation sites by boll weevils was modified by the leaf terpenoids, either as a specific response to one or a few or as a general response based on a higher concentration. A listing of the terpenoids and their percent content in the essential oils of the six is given in Table 2. Tentative structures were assigned to 46 peaks, and fragmentation patterns suggested the presence of at least an additional 8 terpenoids. Cotton and sweet gum essential oils were high in both C_{10} and C_{15} hydrocarbons (Tables 1 and 2). Willow oak and honeysuckle were low in C_{10} terpenoids but relatively high in C_{15} terpenoids (Tables 1 and 2). α-Pinene, β-pinene, limonene, α-fenchene, α- and β-terpineol, β-caryophyllene, and humulene were present in relatively high concentrations in several essential oils.

Field Tests. Studies to determine whether boll weevils were attracted to field hibernation sites on the basis of their responses to leaf essential oils, to commercially available terpenoids, to other compounds found present in the essential oils, and to the synthetic boll weevil pheromone were conducted during the late summer and early fall seasons of 1997 and 1998, as described under Materials and Methods.

Three related field tests were conducted in 1997. In the first test, "A", in which 50 mg quantities of the various leaf essential oils were tested, sex pheromone was not included. A preliminary listing of responses to the oils is given in Table 1. Although the tests were not large enough to allow standard statistical evaluations, the greatest numbers at both locations were attracted to ground trash followed by honeysuckle.

In a second test, "B", dental rolls containing 50 mg of β-caryophyllene were tested in the same fields over a 3 day period. β-Caryophyllene has been shown to be attractive to boll weevils under defined conditions (Minyard et al., 1969). It was also isolated from female boll weevils and found to be specifically attractive to males when present with two of the male boll weevil sex pheromone components (Hedin et al., 1979). However, in the absence of the pheromone, the attractancy to β-caryophyllene was not greater than that to blanks in these tests.

In a third test, "C", conducted in the same fields during the same period, the responses of weevils to the

Table 2. Terpenoids in Six Leaf Essential Oils, Percent[a]

I_K	compound	cotton	sweet gum	willow oak	honeysuckle	mixed "fresh"	ground trash
1005	α-phellandrene						0.7
1010	α-pinene	7.8	8.1		0.5		4.0
1020	camphene		1.4				0.8
1035	cineole						1.3
1040	3-carene						0.8
1050	β-pinene	7.5	6.1				
1070	α-terpinene		0.5				
1080	myrcene	7.8					
1090	limonene	4.8	9.2	1.8	0.7		
1120	α-fenchene	7.8	3.8				
1130	linalool		1.0				
1150	M^+ 150		1.5				
1160	fenchyl alcohol		0.8				
1165	M^+ 154		0.7				
1185	camphene hydrate		1.5				
1195	M^+ 150		0.6				
1205	terpinen-4-ol		4.2				1.6
1210	β-terpeneol				1.4	0.6	
1215	α-terpineol		4.6	0.9	0.6	0.8	0.8
1240	M^+ 150		1.0				
1260	nerol				0.6		
1270	(+)-carvone		0.4				
1360	δ -elemene		1.7				
1380	β-elemene		0.6				
1410	α-farnesene					0.7	
1425	α-cubebene						1.6
1440	α-bergamotene			4.3		1.2	
1460	β-caryophyllene	7.8	4.6	2.6		1.4	3.8
1465	clovene					1.9	
1470	α-guaiene	0.8				3.1	
1480	humulene	6.0	4.4				
1490	δ-guaiene	3.1				4.1	
1495	alloaromodendrene		1.7				
1500	(+)-bergamotene			2.4			
1505	δ-cadinene		3.2		1.0	3.6	0.9
1515	γ-bisabolene	1.0					
1520	γ-cadinene		4.0			1.5	
1530	—	1.0					
1550	α-muurolene		0.9			1.7	
1575	—		2.5	1.8			
1580	γ-muurolene		3.0				
1590	bisabolene oxide	4.5					
1595	palustrol						1.8
1600	β-farnesene		2.2				
1610	—	2.3					
1630	(+)-elemol	1.4					
1650	guaiol				2.2	1.7	
1660	torreyol		2.2				
1670	cedrol				4.7	1.8	
1680	—		3.2		3.3		2.8
1690	hinesol	3.1		1.5			
1695	ledol						4.0
1700	—	2.6	3.9				
1720	β-eudesmol			5.5		6.6	
1740	nerolidol	5.3					
total	—	74.6	83.5	20.8	15.0	30.7	24.1

[a] Cotton, *Gossypium* spp.; sweet gum, *Liquidambar styraciflua* L.; honeysuckle, *Lonicera* spp.; willow oak, *Quercus phallos*; red oak, *Quercus rubra* L.; mixed "fresh", mixtures of honeysuckle, willow oak, red oak, and ground trash, diverse mixture of the above.

standard 10 mg boll weevil sex pheromone formulation were compared to responses to a formulation of the sex pheromone plus 50 mg of β-caryophyllene.

During this period, 1920 boll weevils responded to the sex pheromone, whereas 2351 responded to the sex pheromone plus β-caryophyllene, a 22% increase ($P > T = 0.08$). This increase may be at least partly attributed to a higher initial response by males, thus being actually a sexual response as previously reported (Hedin et al., 1979). However, in this study, insects were not counted or sexed daily, so higher response initially by males would not be noted.

Table 3. Responses of Weevils at Two Locations to 10 Formulations in the Presence of Grandlure[a]

no.	mixture[a]	captures[b]	$P > T$[c]
1	α-pinene, β-pinene, α-terpinene, (*R*)-(+)-limonene	108	0.64
2	α-terpineol, geraniol, carvone, linalool	74	0.61
3	β-caryophyllene, longifoline, humulene	150	0.10
4	farnesol, caryophyllene oxide, α-bisabolol, *cis*-nerolidol	99	0.84
5	1-hexanol, 1-hepten-3-ol, *cis*-3-hexenol, (*R*)-(2)-octenol	143	0.15
6	*trans*-2-decenal, *trans*-2-hexenal, 2-octanone, 1-heptaldehyde	70	0.54
7	oleic acid, maleic acid diethyl ester, valeric acid, caprylic acid	63	0.41
8	eugenol, α-ionone, phenethyl alcohol, methyl salicylate	75	0.63
9	indole, 1,4-diaminobutane	40	0.14
10	geosmin	98	0.86
11	Grandlure	92	

[a] Compounds were procured commercially; each formulation of 100 mg was applied to a dental roll and each test also contained a 10 mg formulation of Grandlure. The test was for 2 days. [b] Average captures/trap/day. [c] Probability that the mean is not significantly different from Grandlure (no. 11) alone according to the SAS GLM procedure.

In 1998, two tests were conducted. In the first test, "D", the essential oil of ground trash was injected into dental rolls so that each contained 100 mg of the oil. In this test, the ground trash was found to be repellent, at variance with the results in the 1997 test (Table 1). The average captures by the controls was 1.17 versus 0.48 by the test.

In a second test conducted in 1998, "E", 10 different synthetic mixtures (100 mg; equal weight) were evaluated as additives to the standard 10 mg Grandlure formulation and compared to Grandlure alone. The categories of synthetic mixtures were based on compounds identified by GLC-MS as present in the various leaf essential oils (Table 1). The mixtures in the absence of Grandlure were not attractive.

No formulation in the presence of Grandlure elicited statistically significant increases in captures at the 5% level. However, two formulations, the sesquiterpene hydrocarbons (Table 3, test 3, $P > T = 0.10$) and the alkyl alcohols (Table 3, test 5, $P > T = 0.15$), appeared to show some promise.

In summary, these field tests failed to support the premise that diapausing boll weevils select hibernation sites on the basis of leaf odors which may arise from the ground trash in which the weevils are found. Although β-caryophyllene when tested with the sex pheromone increased captures in the 1997 test and appeared to increase captures in the 1998 test as a component of the sesquiterpene hydrocarbon formulation (Table 3, test 3), these responses should not necessarily be attributed to its presence in leaves. Its previous identification as a component of the female boll weevil pheromone (Hedin et al., 1979) suggests that the additional captures may have been those of males, and therefore its source was not necessarily from ground trash but rather from female weevils.

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