Volatile Compounds from *Medicago* Spp. as Potential Signals for Alfalfa Weevil Response

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Alfalfa weevils (AW), *Hypera postica* (Gyllenhal), are important pests of alfalfa, *Medicago sativa* L. Although no resistant cultivars exist, certain wild *Medicago* species with erect glandular hairs impart repellency to AW. It is felt that leaf and stem volatile compounds work in conjunction with trichomes to produce antixenosis. Our study isolated and tentatively identified volatile compound differences among four alfalfa cultivars and three wild *Medicago* species using supercritical fluid extraction (SFE) followed by gas chromatography–mass spectrometry. Ninety-six volatile compounds present in the leaves and stems of at least one of the entries were observed. Of these, 17 were unique to the wild species. Three unique compounds, 1-methyl-4-(1-methylethyl)cyclohexanol, (*Z*)-geraniol, and hexahydrofarnesol are particularly interesting because of their similarity to known insect pheromones. Knowledge of *Medicago* species volatile compounds should provide a nascent foundation for the pursuit of AW antixenosis.

Keywords: *Alfalfa; volatiles; glandular hairs; insect repellents*

INTRODUCTION

An apparent overlapping of the Egyptian and western strains of alfalfa weevil (AW) affects alfalfa production in many areas of the western United States (Hsiao, 1993). Yield reduction is caused by losses attributed to both larval and adult feeding (Bjork and Davis, 1984; Manglitz and Ratcliffe, 1988). No AW resistant cultivars exist (Hodgson and Posler, 1986), and the degree of tolerance available in some varieties is unacceptable to alfalfa growers (Kennedy et al., 1987).

Biological control efforts toward weevils in the western United States have been hampered by the aforementioned mixing of strains. Eggs of an AW parasitoid, *Bathyplectes curculionis,* are unaffected by western weevil strains but are encapsulated and destroyed by the Egyptian variety; the effectiveness of this parasitoid is therefore reduced in overlapping zones (Hsiao, 1993).

Successful control measures include early harvest and chemical control (Danielson et al., 1987b; Keith et al., 1982). Low biomass accumulation, stand decline, and decrease in stand productivity are undesirable results of early harvest (Manglitz and Ratcliffe, 1988). Chemical controls are effective but have severe limitations, namely, excessive cost, environmental side effects, threat to beneficial insects, and unavailability due to loss of registry (Moffett et al., 1986).

We seek a genetic solution to overcome the drawbacks associated with the previously discussed approaches. Previous research with AW repellency has uncovered a number of wild *Medicago* species that have shown significant (albeit inconsistent in some cases) AW resistance (Barnes and Ratcliffe, 1969; Johnson et al., 1980a,b; Shade et al., 1975). Nonpreference in these cases was attributed to tactile antixenosis caused by erect glandular hairs present on *Medicago* leaves and stems. Mere trichome density was thought to be the sole basis of repellency (Danielson et al., 1986, 1987a,b; Johnson et al., 1981). Behavioral research has been done with the volatile (*Z*)-oxacyclotridec-10-enone found in *Medicago rugosa* Desr. as a potential AW repellent, but the results have been discouraging (Doss et al., 1989; Doss and Johnson, 1991). Our hypothesis, echoing that of Doss et al. (1989), is that plant volatile compounds, in conjunction with trichomes, impart antixenosis. The objective of this research was to identify differences in volatile compounds among four alfalfa cultivars and three wild *Medicago* species.

MATERIALS AND METHODS

Seven entries representing different *Medicago* accessions were chosen for testing. Four of these were alfalfa cultivars: Caliverde, Caliverde 65, CUF 101, and Moapa 69. The others were wild *Medicago* germplasm sources: KS94GH6, *M. glandulosa* David (Sorensen et al., 1986), KS108GH5, *M. glutinosa* Marshall von Bieberstein (Sorensen et al., 1985), and *M. scutellata* (L.) Mill (PI505433). All plant materials were grown and maintained in a glasshouse (16:8 h, light/dark photoperiod, 30 ± 3 °C, day, 24 ± 3 °C, night). All tested varieties were seeded into prepared beds in the glasshouse except *M. scutellata* and an individual *M. glandulosa* clone 18 (selected for numerous and large trichomes), which were grown in pots with a 1:2 mixture of vermiculite/potting soil (Metromix 360 from Greenhouse and Garden Supplies, Albuquerque, NM). The entries were sampled for plant volatile compounds four weeks after being cut back. Plant tissue samples consisted of stem and leaf tissue from the apical meristem to the fifth internode (as measured from the apical meristem).

Fresh plant tissue (1.5 g) was (1) harvested from plants during peak volatile emanation of volatile compounds (11:00 a.m. to 1:00 p.m.) (Loper and Lapioli, 1972), (2) sealed in plastic resealable bags and stored at −86 °C until prepared for SFE, (3) macerated and placed into a 7 mL extraction

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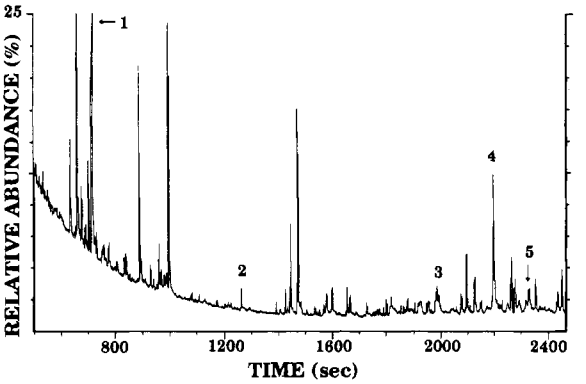

Figure 1. Chromatogram from wild *Medicago* accession KS94GH6 (*M. glandulosa* David) demonstrating the relative abundances of (1) 1-hepten-3-ol, (2) (*Z*)-geraniol, (3) 1-methyl-4-(1-methylethyl)cyclohexanol, (4) 7-tetradecene, and (5) siloxane contaminant.

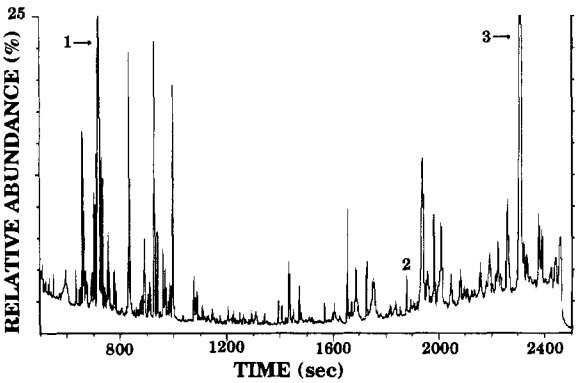

Figure 2. Chromatogram from alfalfa (*M. sativa* L.) variety CUF101 demonstrating the relative abundances of (1) 1-hepten-3-ol, (2) 1-methyl-4-(1-methylethyl)cyclohexanol, and (3) siloxane contaminant.

vessel, and (4) selectively extracted for volatile compounds by SFE (Hewlett-Packard Model 7680A; Palo Alto, CA), using CO_2 as the supercritical fluid. The SFE methodology used for extraction of plant volatiles was that reported by Henning et al. (1994). The resulting extracted compounds, in 0.5 mL of HPLC grade hexane, were transferred into 2 mL automatic injection vials, sealed with caps and septa, and immediately analyzed by GC–MS.

Samples were assayed qualitatively on a Varian Inc. (Sugarland, TX) ion-trap GC–MS, Saturn model. The GC–MS utilized a Restek Corp. (Bellefonte, PA) R_{tx}-5 capillary column (30 m × 0.25 mm i.d.) with 0.25 μm coating of 95% dimethyl/5% diphenyl polysiloxane. The temperature program followed a linear ramp of 5 °C/min from 45 °C to the final temperature of 200 °C. Both the initial and final temperatures were held constant for five minutes. The National Institute of Standards and Technology (NIST) library (1990) of mass spectra was the reference for volatile identification. We report only those compounds exhibiting a greater than 90% match to the spectra from a known compound in the NIST library.

RESULTS AND DISCUSSION

All genetic sources yielded interpretable mass spectra. We were able to identify 96 different volatile compounds from at least one of the seven genetic sources (Table 1). Although intended primarily to augment a previous list of alfalfa leaf and stem volatiles (Buttery and Kamm, 1980), a few comparisons seem appropriate: (1) Buttery and Kamm (1980) found 1-octen-3-ol to be by far their most abundant compound—we found 1-hepten-3-ol to be similarly abundant (Figures 1 and 2); (2) most of their

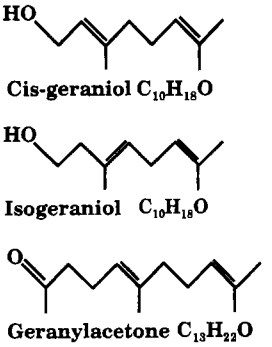

Figure 3. Chemical structures of (*Z*)-geraniol, isogeraniol (Hedin et al., 1988), and geranylacetone (Buttery and Kamm, 1980).

volatiles were unmethylated, whereas our findings showed considerable methylation, i.e., about 10% of their compounds have methyl side chains in contrast to almost 50% methyl group attachment with our volatiles; (3) they reported geranylacetone and we isolated (*Z*)-geraniol. These two volatile compounds differ in mass and structure (Figure 3). Any differences between Buttery and Kamm (1980) and our report could be attributed to species and varietal differences, environmental growing conditions [field grown (Buttery and Kamm) versus greenhouse], or the extraction method [Soxhlet and headspace (Buttery and Kamm) versus SFE].

Interestingly, the four alfalfa cultivars exhibited similar volatile compound makeup (Table 1). Only germacrene B and megastigma-4,6(*Z*),8(*Z*)-triene were isolated from one alfalfa cultivar (CUF 101) but not from the others. On the other hand, wild *Medicago* species showed marked variability in their chemical constitutions, both among and within species, i.e., KS94GH6 seeded into a bed versus KS94GH6 grown in a pot. This result may be due to intraspecific differences or to the method of growth. Contaminants containing siloxane were present and were generally observed as compounds with late retention times greater than 35 min. We believe the majority of these contaminants may have been generated when the autosampler needle poked a hole through the extraction vial septum.

Because our ultimate objective was to identify AW-repelling volatile compounds, we were primarily interested in volatiles that were present in at least one AW-resistant wild species but not (or in small amounts) detectable in *M. sativa*. We observed 17 volatile compounds that met this criterion: 3-octanol; 2,7-dimethyl-2,6-octadien-1-ol (much higher abundances in wild species); (*Z*)-geraniol; 1-methyl-4-(1-methylethyl)cyclohexanol; hexahydrofarnesol; (*Z*,*E*)-5,10-pentadecadien-1-ol; heptanal; 3,7-dimethyl-2,6-octadienal; 4-(1-hydroxyethyl)benzaldehyde; ethyl undecanoate; 1-(2-propenyloxy)heptane; 2,9-dimethyldecane; (*E*,*E*)-1,5-cyclododecadiene; 1,(*E*)8,(*Z*)10-tetradecatriene; 7-tetradecene; 2-methoxy-4-(2-propenyl)phenol; 3,4-diethylphenol (Table 1).

One of these compounds unique to the wild germplasm accessions, (*Z*)-geraniol, resembles isogeraniol, documented as a possible AW pheromone by Hedin et al. (1988) (Figure 3). Hexahydofarnesol is also interesting because a similar compound, (*E*)-β-farnesene, is a known alarm pheromone of pea aphids *Acyrthosiphon pisum* (Harris) (Nault et al., 1973).

We observed seven volatile compounds that were present in *M. sativa* but not in the wild germplasm

Table 1. Tentative Presence or Absence of Volatile Compounds in Four Alfalfa (*Medicago sativa* L.) Cultivars [Caliverde (CAL), CUF101 (CUF), Caliverde 65 (C65), and Moapa 69 (M69)] and Three Wild *Medicago* Accessions [KS94GH6 (K94 and C18), KS108GH5 (K108), and *M. scutellata* (MSC)] with Their Chemical Abstracts Service Registry Numbers[a] (CAS) and Their Retention Times

compound	CAS	*Medicago sativa*				wild species				retention time (s)
		CAL	CUF	C65	M69	K94	C18	K108	MSC	
Alcohols										
1,5-heptadiene-3,4-diol	51945-98-3	X	X	X	X	X	X	X		521
4,5-octanediol	22607-10-9	X	X	X	X					596
2-nonanol	628-99-9	X	X	X	X		X	X	X	634[b]
5-methyl-3-hexanol	623-55-2	X	X	X	X	X		X	X	659
2-hexanol	626-93-7	X	X	X	X	X	X			670
1-methyl-1-cyclopentanol	1462-03-9	X	X	X	X		X	X	X	690
1-hepten-3-ol	4938-52-7	X	X	X	X	X	X	X		718
3-octanol	589-98-0						X			750[b,c]
benzenemethanol	100-51-6	X	X	X	X	X	X	X	X	834
(*Z*)-2-octen-1-ol	26001-58-1	X	X	X	X	X	X	X	X	905[b,c]
(*E*)-2-decen-1-ol	18409-18-2	X	X	X	X	X	X	X	X	978
benzeneethanol	60-12-8	X	X	X	X	X	X	X	X	995
2,7-dimethyl-2,6-octadien-1-ol	22410-74-8	X	X	X	X	X	X	X		1216
3,3,4-trimethyl-1-pentanol	65502-58-1	X	X	X	X			X		1249
(*Z*)-geraniol	106-25-2					X	X	X		1262
2-ethyl-2-hexen-1-ol	50634-00-4	X	X	X	X					1433
4-ethylbenzenemethanol	768-59-2	X	X	X	X	X	X	X		1802
cyclododecanol	1724-39-6	X	X	X	X	X		X	X	1838
(*E*,*E*)-5,10-pentadecadien-2-ol	64275-46-3	X	X	X	X	X	X	X	X	1857
1-methyl-4-(1-methylethyl)cyclohexanol	21129-27-1					X	X		X	1983
2-pentadecyn-1-ol	2834-00-6	X	X	X	X	X	X	X	X	2099
6(*Z*),9(*Z*)-pentadecadien-1-ol	77899-11-7	X	X	X	X	X	X	X		2216
hexahydrofarnesol	6750-34-1					X	X		X	2291
(*Z*,*E*)-5,10-pentadecadien-1-ol	64275-60-1					X				2366
Aldehydes										
heptanal	111-71-7								X	541[b]
(*E*)2,(*E*)4-heptadienal	4313-03-5	X	X	X	X	X	X	X		755
(*Z*)-2-heptanal	57266-86-1	X	X	X	X		X			823
nonadien-2(*E*),6(*Z*)-al	557-48-2	X	X	X	X		X	X		1075
(*E*)-2-nonenal	18829-56-6	X	X	X	X		X	X		1088[c]
β-cyclocitral	432-25-7	X	X	X	X	X	X	X		1205[b]
3,7-dimethyl-2,6-octadienal	5392-40-5					X	X			1283
2,2-dimethylpropanal	630-19-3	X	X	X	X		X	X		1406
4-(1-hydroxyethyl)benzaldehyde	80463-21-4					X	X	X		1424
2-isononenal	53966-58-8	X	X	X	X	X	X	X	X	1599
(*Z*)-3,7-dimethyl-6-oxo-2-octenal	70856-11-0	X	X	X	X	X	X	X		1904
tridecanal	10486-19-8	X	X	X	X			X		1982
Amines										
3-pyrroline	109-96-6	X	X	X	X	X	X	X	X	1479
Aromatic Hydrocarbons										
1,2,3-trimethylbenzene	526-73-8	X	X	X	X	X		X		743
Esters										
(*Z*)-3-hexenyl acetate	3681-71-8	X	X	X	X	X	X	X		778[b,c]
(*Z*)-3-hexen-1-ol benzoate	25152-85-6	X	X	X	X	X	X			1783
10-methyldodecanoic acid methyl ester	5129-65-7	X	X	X	X	X	X	X	X	2129
8-methyldecanoic acid methyl ester	5129-64-6	X	X	X	X	X	X	X	X	2267
ethyl undecanoate	627-90-7					X	X	X		2375
Ethers										
(butoxymethyl)oxirane	2426-08-6	X	X	X	X	X		X		550
2-ethyl-3-propyloxirane	53897-32-8	X	X	X	X	X		X	X	647
Hydrocarbons										
5-(pentyloxy)-1-pentene	56052-85-8	X	X	X	X	X	X	X	X	535
1-methyl-3-ethylcyclopentane	3726-47-4	X	X	X	X	X	X	X		675
4-methyl-3-heptene	4485-16-9	X	X	X	X			X		694
3,5,5-trimethyl-1-hexene	4316-65-8	X	X	X	X	X	X	X	X	703
2,4,4-trimethylhexane	16747-30-1	X	X	X	X	X	X	X		733
2,2,3,3-tetramethylhexane	13475-81-5	X	X	X	X	X	X			758
2-propenylidenecyclobutene	52097-85-5	X	X	X	X		X	X		852
2,6-dimethylheptane	1072-05-5	X	X	X	X					882
1-(2-propenyloxy)heptane	16519-24-7					X	X	X	X	909
1-nonyne	3452-09-3	X	X	X	X					912
2,3-nonadiene	22433-34-7	X	X	X	X	X	X	X	X	927
2,6,7-triethyoldecane	62108-25-2	X	X	X	X	X	X	X	X	969
2,3,6-trimethyl-1,6-heptadiene	74421-35-5	X	X	X	X	X	X	X	X	1175
butenylcyclohexene	74664-14-5	X	X	X	X			X		1293
butenylcyclohexene (isomer)	74664-14-5	X	X	X	X			X		1311
2,9-dimethyldecane	1002-17-1					X	X	X		1439
(*Z*)-4-tridecen-6-yne	74744-42-6	X	X	X	X	X	X	X	X	1473
2-methyl-8-propyldodecane	55045-07-3	X	X	X	X					1511

Table 1 (Continued)

compound	CAS	*Medicago sativa*				wild species				retention time (s)
		CAL	CUF	C65	M69	K94	C18	K108	MSC	
	Hydrocarbons									
(*E*)-β-farnesene	18794-84-8	X	X	X	X			X		1605[c]
3-octadecyne	61886-64-4	X	X	X	X			X	X	1687
(*E*,*E*)-1,5-cyclododecadiene	1684-05-5							X	X	1778
1,(*E*)8,(*Z*)10-tetradecatriene	80625-31-6					X	X		X	1886
2,6,10-trimethyldodecane	3891-98-3	X	X	X	X	X	X		X	1938
megastigma-4,6(*Z*),8(*Z*)-triene	71186-25-9		X			X	X	X	X	2078
7-tetradecene	10374-74-0					X	X	X	X	2197
3-methylene-1,6-heptadiene	16626-48-5	X	X	X	X	X	X	X	X	2224
3-methyltridecane	6418-41-3	X	X	X	X					2227
1,6,9-tetradecatriene	61233-71-4	X	X	X	X	X	X	X	X	2279
heptadecane	629-78-7	X	X	X	X	X	X	X	X	2390
germacrene B	15423-57-1		X				X	X		2447
	Imides									
methylethylmaleimide	20189-42-8	X	X	X	X	X	X	X	X	1225
	Ketones									
3,4-epoxy-4-methyl-2-pentanone	4478-63-1	X	X	X	X	X	X	X		509
5,6-decanedione	5579-73-7	X	X	X	X	X	X	X	X	889
dihydro-3-methylene-2,5-furandione	2170-03-8	X	X	X	X	X	X	X		939
(*E*)-3,7-octadien-2-one	25172-06-9	X	X	X	X	X	X	X		959
2-methyl-6-heptanone	928-68-7	X	X	X	X	X	X	X	X	987
2-nitrothiopene	609-40-5	X	X	X	X	X	X	X	X	1108
3-methyl-4-methylene-2-hexanone	20690-71-5	X	X	X	X			X		1342
2-propyl-1,3-cyclohexanedione	54244-73-4	X	X	X	X					1420
2-*tert*-butyl-4-methylfuran	6141-68-0	X	X	X	X					1449
6-methyl-5-nitro-2-heptanone	66972-02-9	X	X	X	X	X	X			1520
5-ethyl-2-methyl-4-hepten-3-one	49833-96-7	X	X	X	X		X	X	X	1569
2,2,7,7-tetramethyl-4,5-octadien-3-one	19377-97-0	X	X	X	X			X		1629
(*E*)-8-ionone	79-77-6	X	X	X	X	X	X	X	X	1655[b]
5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4*H*)-benzufuranone	15356-74-8	X	X	X	X	X	X	X	X	1729
4-(2,6,6-trimethyl-2-cyclohexen-1-ylidene)-2-butanone	56052-61-0	X	X	X	X	X	X			1818
allethrolone	551-45-1	X	X	X	X	X	X	X	X	1880
6,10-dimethyl-9-undecen-2-one	4433-36-7	X	X	X	X	X	X			2154
	Lactones									
2,3-dimethyl-4-hydroxy-2-butenoic lactone	1575-46-8	X	X	X	X	X	X	X		839
	Phenols									
2-methoxy-4-(2-propenyl)phenol	97-53-0					X	X			1433
3,4-diethylphenol	875-85-4					X	X			1577

[a] CAS Registry Numbers provided by the author. [b] Compounds previously reported by Buttery and Kamm (1980). [c] Compounds previously reported by Buttery et al. (1982).

accessions. These include 2,6-dimethylheptane, 1-nonyne, 2-propyl-1,3-cyclohexanedione, 2-ethyl-2-hexen-1-ol, 2-*tert*-butyl-4-methylfuran, 2-methyl-8-propyldodecane, and 3-methyltridecane. Because alfalfa has demonstrated attractiveness to AW (Dreyer et al., 1987), one or several of these volatiles may act as attractants.

While the identification of unique volatile compounds may point toward potential AW allomones, compounds that act as repellents or attractants can be determined efficiently only by electroantennogram testing followed by behavioral bioassays. This work is currently in progress. Our eventual goal is the introgression of, or selective increase in, volatile compounds invoking antixenosis toward AW in *M. sativa*.

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