Table IV. Soil Analyses

area	field	sampling date, mo-yr	linuron, ppbw
Tuckahoe Creek	1	7-78	41
		9-78	<10
	2	7-78	47
		9-78	14

taken from two soybean fields adjacent to the Tuckahoe Creek on which linuron was known to have been applied at 0.55 kg/ha between mid-May and mid-June. These results show that the persistence of linuron in these field soils is extremely low as the concentration decreased very rapidly in only 2 months. Mud and water samples from adjacent waterways were among those analyzed, and these showed no detectable linuron transfer.

CONCLUSION

The analyses of mud and water samples taken during two successive summers in diverse areas of the Chesapeake Bay, including one river basin where 45 000 kg of linuron is used annually, showed no evidence of linuron accumulation. On this basis, it is concluded that linuron usage on fields which border the Chesapeake Bay and its tributaries is not a contributing factor to the recent declines in the abundance of aquatic plants.

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Volatile Components of Alfalfa: Possible Insect Host Plant Attractants

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Capillary GLC–mass spectrometry analysis of the vacuum steam volatile oil of alfalfa leaves and stems identified 48 components. Major components included 1-octen-3-ol, (*Z*)-3-hexenol, (*E*)-2-hexenal, 2-phenylethanol, linalool, (*Z*)-3-hexenyl acetate and β-ionone. Unusual components include 1-octen-3-one, octan-3-one, α-bergamotene, umbellulone, β-cyclocitral, and 2,2,6-trimethylcyclohexanone.

The alfalfa seed chalcid (*Bruchophagus roddi* Guss.) lays its eggs in the developing alfalfa seed. The chalcid larvae develop inside the seed and can destroy up to 85% of a seedcrop (Kamm and Fronk, 1964). Studies of this insect have indicated that it is probably attracted to the alfalfa plant and stimulated to lay eggs by volatile odor compounds associated with the alfalfa plant (Kamm and Fronk, 1964). Knowledge of the volatile constituents associated with alfalfa provides information necessary in determining which particular volatile chemical compounds attract the alfalfa chalcid and other insect pests of alfalfa. Such knowledge may be useful in an integrated pest control program.

Some studies have been carried out on the volatile components of alfalfa flowers in regard to their attraction for honeybees (Loper et al., 1971), but the flower components seem to be different from those found in the leaves and stems.

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EXPERIMENTAL SECTION

Materials. The alfalfa (*Medicago sativa*) was Germains variety No. 318 grown in the Californian Sacramento valley. For a study of the whole (intact) alfalfa, whole stems (with leaves attached) were cut from the plants.

Authentic chemical compounds were obtained from commercial sources (e.g., Aldrich Chemical Co.) or synthesized by established methods.

Isolation of Volatile Oil from Whole Alfalfa. Whole alfalfa stems, with leaves attached (1 kg), were placed in a 12-L round-bottom flask together with 6 L of odor-free water. A Likens–Nickerson steam distillation continuous extraction head (Likens and Nickerson, 1964) was attached to the flask. Purified hexane (100 mL) was placed in a 250-mL flask attached to the solvent arm of the head. A dry ice reflux condensor was attached to the outlet of the extraction head whose internal condensor was cooled with water–ethanol at 0 °C. The isolation was carried out at reduced pressure (100–110 mm) for 3 h with the alfalfa at a temperature of 45–50 °C. After the isolation the hexane extract was dried by freezing out the water and then concentrated by using low hold up Vigreux distillation columns to give the whole alfalfa volatile oil which was stored at −20 °C with a trace of Ethyl antioxidant 330.

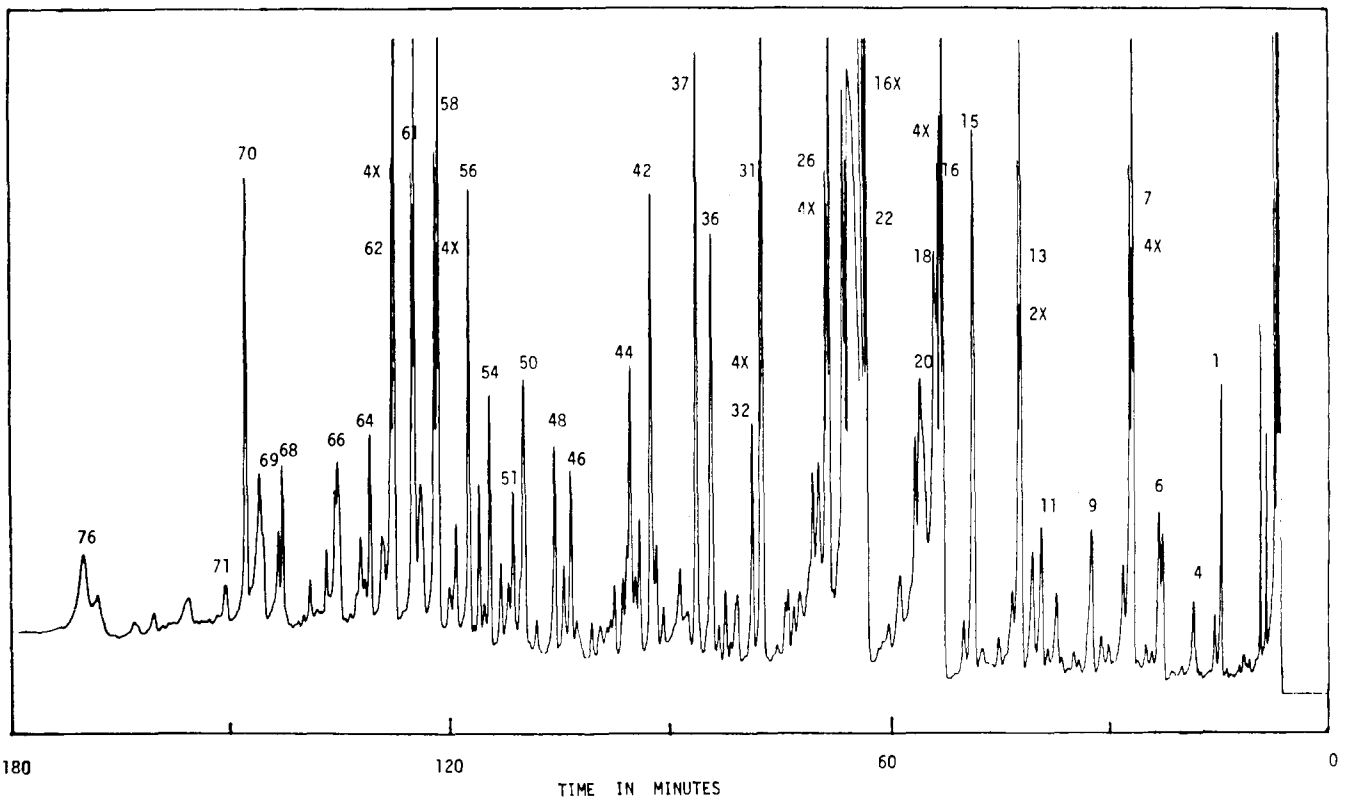

Figure 1. Capillary GLC analysis of the vacuum steam volatile oil from whole alfalfa leaves and stems using the Carbowax 20-M coated Pyrex glass capillary and conditions described in the text.

Macerated Alfalfa. A large quantity of alfalfa was macerated (finely chopped) to a liquid form for other work in progress at our laboratory. A quantity (6 kg) of this macerated alfalfa was treated, using vacuum steam distillation continuous extraction, as for the whole alfalfa except that no additional water was added.

Separation into Hydrocarbon, Aldehyde–Ester and Alcohol Fractions. This was carried out by taking a portion of the alfalfa volatile oil and placing it on a column of activated alumina (Woelm neutral No. 1). Elution with hexane and subsequent removal of the solvent gave the "hydrocarbon fraction". This was followed by elution with diethyl ether (freshly distilled) which gave an intermediate fraction which was called the "aldehyde–ester fraction". Finally elution with ether–methanol (90:10) gave an "alcohol fraction".

Capillary Gas–Liquid Chromatography–Mass Spectrometry (GLC/MS) Analysis. This was carried out on the whole oils and fractions by using a 150 m long by 0.64 mm i.d. Pyrex glass capillary column coated with Carbowax 20-M. The hydrocarbon fraction was also analyzed by using a 150 m × 0.75 mm i.d. stainless steel capillary coated with Silicone SF96(50) containing 5% Igepal CO-880. The usual temperature programming conditions for both columns were to hold the column at 50 °C for the first 30 min after injection and then to program at 1 °C/min from 50 to 170 °C, holding at the upper limit. The column inlet pressure was 16 psi helium. A single-stage Lewellyn–Littlejohn silicone rubber membrane molecular separator was used to couple the end of the capillary column to the mass spectrometer (a modified Consolidated 21-620, cycloidal type). Electron ionization was at 70 eV.

Kovat's GLC indexes were measured relative to other components in the mixture as internal standards. There is some normal relative shifting between the different polarity groups, such as the hydrocarbon group, the aldehyde–ester group, and the alcohol group, depending upon the type of mixture injected, the different programming conditions, and the age of the column. The Kovat's index for each compound was determined relative to members of its own group. This was compared to those of authentic samples determined in the standard way against a series of normal hydrocarbons.

RESULTS AND DISCUSSION

Volatile oils from alfalfa were obtained both from the whole alfalfa (leaves and stems) and macerated (finely chopped) alfalfa by using vacuum steam distillation continuous extraction. The amounts of oils obtained in this way were 5 parts per million (ppm) for the whole alfalfa and 60 ppm for the macerated alfalfa. Figure 1 shows a capillary GLC analysis obtained for the whole alfalfa volatile oil. Table I lists the compounds characterized by GLC–MS. Also listed in Table I is the relative percentage of each component found in the whole alfalfa volatile oil. There is some variation with different samples, and these figures are only meant to give a general idea for a typical sample. By far the major component was 1-octen-3-ol, a compound long considered a mushroom aroma compound (and used for this purpose in synthetic flavors). 1-Octen-3-ol had been found previously as a major volatile component of the edible part of some other legumes, notably beans (Buttery et al., 1975; Stevens et al., 1967). Other major components found included (*Z*)-3-hexenol, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate, which seem to be common to all green leafy plants [cf. Visser et al. (1979)]. Major components which are somewhat less common include linalool, 2-phenylethanol, and β-ionone.

Unusual compounds found include 1-octen-3-one, which is also considered to have a mushroom–metallic aroma, octan-3-one, α-bergamotene, umbellulone, β-cyclocitral, and 2,2,6-trimethylcyclohexanone. Some additional unusual compounds, occurring in reasonable amounts, could

Table I. Compounds Identified in the Vacuum Steam Volatile Oil of Whole Alfalfa

peak no.	compd[a]	characteristic mass spectral ions,[b] m/e	Kovat's GLC index[c]	relative %
		Alkanals		
1	hexanal	29, *44*, 56, 72, 82, 100 (M^+)	1108	0.3
5	heptanal	*44*, 70, 81, 86, 96, 114 (M^+)	1190	0.2
10	octanal	44, 56, 84, 95, 100, 128 (M^+)	1290	0.2
18	nonanal	44, *57*, 82, 96, 98, 142 (M^+)	1390	0.8
27	decanal	44, *57*, 82, 95, 112, 156 (M^+)	1500	0.4
		Alkenals and Alkadienals		
7	(*E*)-2-hexenal	*41*, 55, 69, 83, 97, 98 (M^+)	1230	4
33	(*E*,*E*)-2,4-octadienal	39, 54, 67, *81*, 95, 124 (M^+)	1590	0.1
39	(*E*)-2-decenal	70, 83, 97, 107, 136, 154 (M^+)	1630	0.1
47	(*E*,*Z*)-2,4-decadienal	67, *81*, 95, 109, 123, 152 (M^+)	1740	0.1
50	(*E*,*E*)-2,4-decadienal	67, *81*, 95, 109, 123, 152 (M^+)	1790	0.5
		Alkanones and Alkenones		
9	octan-3-one	*43*, 57, 72, 85, 99, 128 (M^+)	1240	0.3
11	1-octen-3-one	43, *55*, 70, 83, 97,	1290	0.3
28	(*E*,*E*)-3,5-octadien-2-one	*43*, 53, 81, 95, 109, 124 (M^+)	1550	0.1
		Alkanols and Alkenols		
15	hexanol	31, 42, *56*, 69, 84	1330	1
16	(*Z*)-3-hexenol	*41*, 55, 67, 69, 82, 100 (M^+)	1370	5
21	octan-3-ol	41, 55, *59*, 83, 101	1390	0.7
20a	(*E*)-2-hexenol	31, 41, *57*, 67, 82, 100 (M^+)	1380	0.2
22	1-octen-3-ol	*57*, 72, 85, 99, 110	1420	61
32	octanol	31, 42, *56*, 70, 84	1530	0.4
37	(*E*)-2-octenol	41, 44, *57*, 68, 81	1590	1
42	nonanol	31, *56*, 70, 83, 98	1630	0.7
48	decanol	31, 70, 83, 97, 112	1740	0.3
		Terpenoids		
	α-pinene[d]	67, 77, *93*, 105, 121, 136 (M^+)	1020	0.1
2	β-pinene	69, 79, *93*, 107, 121, 136 (M^+)	1120	<0.1
4	myrcene	*41*, 69, 93, 107, 121, 136 (M^+)	1150	0.1
4	Δ^3-carene	67, 79, *93*, 105, 121, 136 (M^+)	1150	
6	limonene	*68*, 79, 93, 107, 121, 136 (M^+)	1180	0.3
	(*E*)-β-ocimene[d]	69, 80, *93*, 105, 121, 136 (M^+)	1250	<0.1
	p-cymene[d]	39, 51, 65, 77, 91, *119*, 134 (M^+)	1280	<0.1
	terpinolene[d]	67, 79, *93*, 105, 121, 136 (M^+)	1290	<0.1
	α-bergamotene[d]	69, *93*, 107, 119, 161, 204 (M^+)	1610	0.1
12	2,2,6-trimethylcyclohexanone	41, 56, 69, *82*, 97, 140 (M^+)	1320	0.2
14a	2-methyl-2-hepten-6-one	*43*, 55, 69, 83, 108, 126 (M^+)	1340	<0.1
31	linalool	55, 71, 80, *93*, 121, 136	1545	2
	camphor[d]	69, 81, *95*, 108, 137, 152 (M^+)	1490	<0.1
36	β-cyclocitral	*41*, 81, 109, 123, 137, 152 (M^+)	1600	0.7
	umbellulone[d]	79, 91, *108*, 122, 135, 150 (M^+)	1610	<0.1
55	geranylacetone	*43*, 69, 93, 125, 151, 194 (M^+)	1850	0.3
61	β-ionone	43, 122, 135, 149, *177*, 192 (M^+)	1920	1
		Benzene, Naphthalene, and Furan Compounds		
8	2-pentylfuran	53, 68, *81*, 95, 109, 138 (M^+)	1220	0.2
38	phenylacetaldehyde	39, 51, 65, *91*, 92, 120 (M^+)	1650	<0.1
45	naphthalene	51, 64, 77, 102, 127, *128* (M^+)	1690	<0.1
46	methyl salicylate	39, 65, 92, *120*, 121, 152 (M^+)	1730	0.3
51	2-methylnaphthalene	39, 51, 57.5, 71, 115, *142* (M^+)	1800	0.2
58	2-phenylethanol	39, 51, 65, *91*, 92, 122 (M^+)	1890	3
70	eugenol	77, 91, 103, 131, 149, *164* (M^+)	2150	0.7
71	*p*-vinylguaiacol	39, 51, 77, 107, 135, 150 (M^+)	2160	0.1
		Others		
13	(*Z*)-3-hexenyl acetate	*43*, 54, 67, 73, 82	1310	2

[a] Mass spectrum (complete spectrum) and Kovat's GLC retention index of all compounds listed are consistent with those of authentic samples. [b] Not necessarily the most intense ions but five of those considered the most unique for that compound. The most abundant ion is shown in italic type and the molecular ion indicated by M^+. [c] Kovat's index for the Carbowax 20-M coated Pyrex glass capillary column described under Experimental Section. [d] Identified in hydrocarbon or aldehyde-ester fractions.

not be identified. These include peak 44 (molecular weight, M_r, 134), peak 56 (M_r 136), peak 68 (M_r 180), and peak 69 (no molecular ion).

In the volatile oil from the macerated alfalfa the common C_6 leaf components (*E*)-2-hexenal, hexanol, (*Z*)-hex-3-enol, and (*E*)-2-hexenol are increased many-fold. A comparison of the concentration of these compounds and some others in the whole and macerated alfalfa is shown in Table II. There is little significant change in the concentrations of 1-octen-3-ol and linalool and only a small change in the concentration of (*Z*)-3-hexenyl acetate. The major change is with (*E*)-2-hexenol. With the alfalfa chalcid the volatiles in the whole undamaged alfalfa are probably the most important although there may be some instances where certain types of other insects are attracted by tissue damage.

Components listed in Table I under Alkanals, Alkenals and Alkadienals, Alkanols and Alkenols, and Alkanones

Table II. Comparison of Relative Concentration of Some Components Found in Whole Alfalfa and Macerated Alfalfa

component	concn, ppm[a] whole alfalfa	macerated alfalfa
whole volatile oil	5	60
hexanal	0.01	1
(*E*)-2-hexenal	0.2	10
octan-3-one	0.01	3
1-octen-3-one	0.01	0.2
(*Z*)-3-hexenyl acetate	0.1	0.6
hexanol	0.05	17
(*Z*)-3-hexenol	0.25	8
(*E*)-2-hexenol	<0.01	19
1-octen-3-ol	3	2
linalool	0.1	0.3

[a] ppm = parts of compound per 10^6 parts of alfalfa.

and Alkenones as well as the compound 2-pentylfuran all seem to be derived from some kind of oxidative breakdown of the unsaturated fatty acids in the lipid of the alfalfa. These types of compounds seem to be found to some extent in all plant and animal tissues. Unsaturated fatty acids, of course, seem to be a necessary component of all cell walls. The exact nature of the fatty acid degradation products and their relative concentration in the different materials seem to vary considerably depending on the type of plant. The direction of the oxidative breakdown apparently is influenced considerably by the different catalysts (including enzymes) and other conditions peculiar to that material.

Some of the compounds listed in Table I under Terpenoids such as geranylacetone, β-ionone, 2,2,6-trimethylcyclohexanone, and β-cyclocitral seem to be derived from the carotenoids. These do not appear to increase significantly in the macerated alfalfa. The enzyme systems involved with the broken cells seem to be fairly specific for the production of only the C_6 fatty acid fragments. The compounds related to β-carotene are shown in Figure 2. From mass spectral fragmentation patterns a number of the unidentified peaks in Figure 1 also seem to be related carotenoid fragmentation products.

Although the main GLC–MS study was done with the whole oil, identification of some of the terpenoid components was facilitated by separation of the whole oil into three main fractions by liquid adsorption chromatography on neutral activated alumina. These fractions were a hydrocarbon fraction, an intermediate polarity fraction called an aldehyde–ester fraction, and a highly polar fraction called the alcohol fraction. Even with the high resolution of the capillary column some minor components are often hidden under major peaks. Separate capillary GLC–MS analyses were carried out on these fractions.

Testing with the Alfalfa Chalcid. Before this work was begun as many as 95 chemical compounds had already been tested for their olfactory attractancy to the alfalfa chalcid (Kamm and Fronk, 1964). These early studies did not include any of the compounds found in the present work and listed in Table I. However, some preliminary studies (Kamm, 1980) have been recently carried out on some of the major components in Table I, using a laboratory olfactometer with female alfalfa seed chalcids. Generally, several concentrations (0.001, 0.01, 0.1, and 1%) in hexane solution were tested (after initial evaporation of hexane). These tests indicated that the whole alfalfa volatile oil, 1-octen-3-ol, β-cyclocitral, (*E*)-β-ocimene, and hexanol showed some attractivity with at least one or more of the concentrations tested. The compounds β-ionone, 2-phenylethanol, linalool, (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, (*E*)-2-hexenal, and nonanal showed essentially no effect at the concentrations tested. Nonanol at the 1% level has a strong repelling effect. Further testing, including field testing with promising compounds, is intended in the near future.

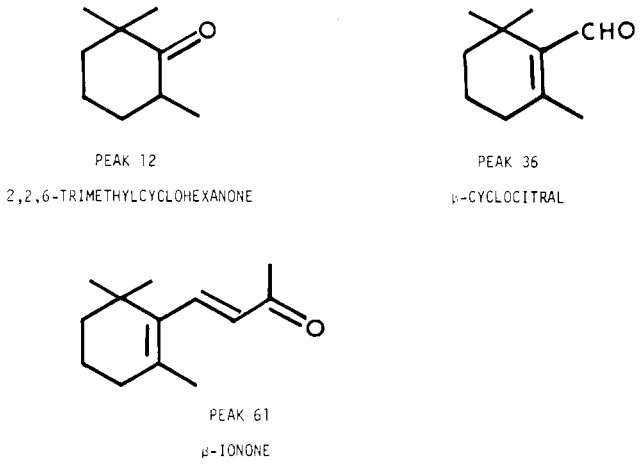

Figure 2. Alfalfa volatile components which are probable β-carotene degradation products.

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