Table V. Characteristics of De Chaunac Grape Juice in Relation to Three Formulations of Bravo Sprayed in Six- and Three-Application Programs

fungicide and program	juice/ berries, mL/kg	sol solids, °B	acidity, pH	tartaric acid equiv, g/100 mL
full season:				
6 applications				
Bravo 75 WP	555 A[a]	15.9 A	3.1 A	1.08 A
Bravo 7.2 F	529 A	15.8 A	3.1 A	1.15 ABC
Bravo 500 F	552 A	15.3 AB	3.0 AB	1.16 ABC
late season:				
3 applications				
Bravo 75 WP	542 A	14.8 AB	3.1 AB	1.17 ABC
Bravo 7.2 F	531 A	14.8 AB	3.0 B	1.19 BC
Bravo 500 F	551 A	15.2 AB	3.0 AB	1.23 C
unsprayed check	542 A	14.1 B	3.1 AB	1.09 AB

[a] Means in the same column followed by different letters are significantly different ($P = 0.05$) by Duncan's Multiple Range Test.

protection against powdery mildew infection for 37 days after the completion of a five-spray program (Northover and Neufeld, 1980).

The decline in chlorothalonil residues from 7.5 to 2.5 μg/g over 36 days represented a reduction of 67%, but the growth of berries from 1.40 g to 1.90 g during this period accounted for a dilution of 26%, leaving only 41% reduction due to loss of residues.

Berry and Juice Residues. Grapes that were refrigerated at 1 °C for 15 days before being frozen showed appreciably lower residues (Table IV) than those associated with grapes frozen immediately after harvest (Table II). This may have been due to the different storage conditions and partially to the dissipative effect of 6 mm of rain between the two harvest dates. The chlorothalonil and 4-OH-Daconil residues from the wettable powder formulation were lower than those from the flowable formulations, confirming the trend in the harvest samples. The chlorothalonil residues in the cold-expressed juice were similar to those in the refrigerated berries, but the very low levels of 4-OH-Daconil appeared lower in the juice than in the berries (Table IV).

Compared with the unsprayed check, the several Bravo programs had no effect on juice yield/kg of grapes (Table V). Soluble solids were higher in the six-spray program of Bravo WP and Bravo 7.2 F than in the check, but the differences in pH and titratable acid were minor. The soluble solids and acidity were lower, and titratable acid was slightly higher than average levels for Ontario-produced De Chaunac (S9549) juice: respectively, 16.6 °B, pH 3.27, and 1.00 g of tartaric acid/100 mL (Zubeckis, 1963).

ACKNOWLEDGMENT

The authors are most appreciative of the assistance given by Ralph Crowther and Pam Gadd (Horticultural Research Institute of Ontario) for juice characterization, to Austin Maitland (Diamond Shamrock Canada Ltd.) for advice and Bravo formulations, to Herman Neufeld and Tracy Lund (Agriculture Canada) for technical and statistical services, and to Robin M. Young (Provincial Pesticide Residue Testing Laboratory) for laboratory assistance.

LITERATURE CITED

Ballee, D. L., Duane, W. C., Stallard, D. E., Wolfe, A. L., in "Analytical Methods for Pesticides and Plant Growth Regulators (VIII)", Zweig, G., Sherma, J., Eds., Academic Press, New York, 1976, p 263.

Braun, A. J., Fungicide–Nematocide Tests, *Am. Phytopathol. Soc.* **19**, 61 (1964).

Chiba, M., Fisher, R. W., Northover, J., Herne, D. C., Neff, A., *Can. J. Plant Sci.* **53**, 189 (1973).

Dzikowski, P. A., Ripley, B. D., 1978 Pesticide Research Report, ECPUA, Ottawa, Canada, 1979, p 557.

Gilbert, M., *J. Agric. Food Chem.* **24**, 1004 (1976).

Maitland, A. E., Diamond Shamrock, Toronto, Canada, Ltd., personal communication, 1978.

Neely, D., *Phytopathology* **60**, 1583 (1970).

Northover, J., Neufeld, H. W., Pesticide Research Report 1979, ECPUA, Agriculture Canada, Ottawa, Canada, 1980, p 375.

Ontario Ministry of Agriculture and Food, Toronto, Canada, Fruit Production Recommendations, Publ. 360, 1976.

Pesticide Analytical Manual, U.S. Department of Health, Education and Welfare, FDA, 1970, Vol. II, Section 120.275.

Ripley, B. D., Cox, D. F., Weibe, J., Frank, R., *J. Agric. Food Chem.* **26**, 134 (1978).

Zubeckis, E., 1962 Report of the Horticultural Experiment Station and Products Laboratory, Ontario Department of Agriculture, Toronto, Canada, 1963, p 97.

Received for review November 29, 1979. Accepted April 22, 1980.

Search for Linuron Residues in Tributaries of the Chesapeake Bay

Edward W. Zahnow* and James D. Riggleman

It has been suggested that herbicides which are used on corn and soybean fields bordering the Chesapeake Bay and its tributaries may be a contributing factor to reported declines in the abundance of grasses in the Bay. As part of the program to determine the contribution, if any, of linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] to the problem, samples of mud and water have been taken from areas likely to show linuron if it is being transferred from fields into adjacent bodies of water. Samples from drainage basins receiving up to 45 000 kg of linuron annually showed no linuron residue, i.e., less than 10 ng/g (10 ppbw) in the mud and less than 0.2 μg/L (0.2 ppb, w/v) in the water. These samples were analyzed by using an extraction procedure followed by measurement with liquid chromatography. From these data, it is concluded that linuron is not accumulating in the Chesapeake Bay.

Declines in the abundance of aquatic grasses in the Chesapeake Bay have been reported recently, and this situation is of concern because of the role which these grasses play in the propagation of waterfowl, shellfish, and finfish. Similar declines have been noted in the past, and the reasons for these cycles are not understood to any significant degree.

E. I. du Pont de Nemours & Co., Biochemicals Department, Wilmington, Delaware 19898.

0021-8561/80/1428-0974$01.00/0

It has been suggested that herbicides (such as linuron and others) which are used on corn and soybean fields bordering the Chesapeake Bay and its tributaries may be a contributing factor to the declines in Bay grasses. Since linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] is not a particularly persistent herbicide, having a soil half-life of 2–5 months, it seems unlikely that it would accumulate in the water or mud of the Chesapeake Bay. This study was undertaken to determine to what extent, if any, linuron is being transferred from farm fields into the Bay. The metabolites of linuron have not been studied in this investigation since they are not known to have herbicidal activity.

A number of methods can be found in the literature for the analysis of linuron by gas chromatography. Some of these (Baunok and Geissbuehler, 1968; Caverly and Denney, 1978; Glad et al., 1978) include reaction of the compound to form a volatile derivative while others (Khan et al., 1975; Lawrence, 1976b; McKone, 1969; McKone and Hance, 1968; Onley and Yip, 1969) measure linuron directly without derivatization.

The technique of liquid chromatography has also been applied to the analysis of linuron (Byast, 1977; Farrington et al., 1977; Glad et al., 1978; Lawrence, 1976a,b; Pribyl, 1977; Pribyl and Hertzel, 1976; Sidwell and Ruzicka, 1976). This approach has two major advantages: first, the conditions of analysis are milder in that the solutions are kept at or near room temperature, thereby minimizing the possibility of thermal decomposition; second, for some types of samples, cleanup procedures can often be minimized or even eliminated entirely since separations of impurities from linuron can be performed directly on the chromatographic column and in this way save much processing time.

The work of Khan et al. (1975) and McKone (1969) indicates that methanol is the solvent of choice for the removal of linuron from soils, and Farrington et al. (1977) have developed methods for the determination of linuron in grain, soil, and river water based on extraction with methanol or methylene chloride and measurement by liquid chromatography.

Our approach is an adaptation of the work of Farrington et al. (1977) with some modifications to improve the sensitivity of the method.

EXPERIMENTAL SECTION

Herbicide Treatment and Sampling. In the sampling area, the principle usage of linuron is on soybeans at 0.55 kg/ha. Treatment is made between mid-May and early July. The first sampling followed the first major rain at the end of the treating season. This occurred in July in 1977 and in August in 1978. The second sampling of each year was 2 months later.

Figure 1 is a map of the Chesapeake Bay, and our three sampling areas are designated as A, B, and C. These were chosen to provide a broad spectrum of sampling locations.

Sampling area A is the Rhode River on the western side of the Bay, somewhat south of Annapolis. It is a small drainage basin with very little agriculture and less than 55 kg of linuron used annually. Sampling area B is Poplar Island, located toward the eastern shore of the Bay. There is no known linuron usage in the immediate area. Sampling area C is the Choptank River and its tributary the Tuckahoe Creek, located east and north of Cambridge. Approximately 45 000 kg of linuron is applied annually in sampling area C.

The Rhode River was sampled in five locations, Poplar Island in four locations and the Choptank River–Tuckahoe Creek region in thirteen locations. In addition, soil samples

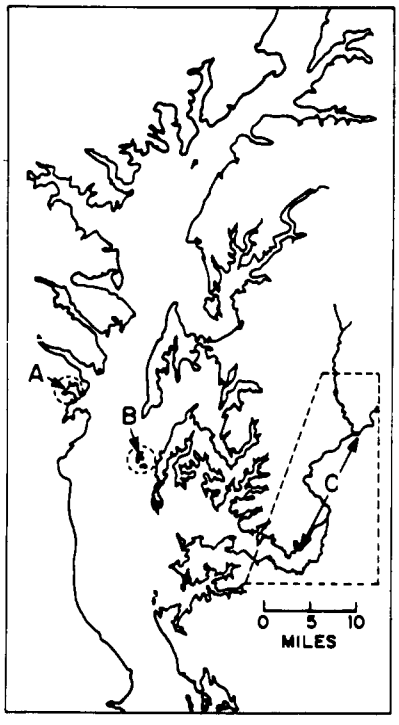

Figure 1. Map of upper Chesapeake Bay.

were taken from two soybean fields bordering the Tuckahoe Creek which had linuron applied at 0.55 kg/ha between mid-May and mid-June in 1977 and 1978.

Water samples were taken from the top 10 cm of the location directly into plastic bottles.

Mud samples were taken by means of a cylindrical steel coring tool. The core taken was 5 cm in diameter and 7–8 cm long.

Soil samples were obtained with a standard soil sampler which took a core of soil down to 15 cm.

All samples were frozen as soon as practicable, generally within 48 h of sampling, and these were kept frozen until they were analyzed.

Mud Extraction. Mud samples were processed by draining off excess water after the sample had settled thoroughly. Then the mud was mixed by stirring with a large spatula, and a 50-g sample was weighed into a 250-mL polypropylene centrifuge bottle. After 100 mL of methanol was added, the mixture was shaken vigorously for 1 h by a mechanical shaker (wrist or platform). Then the mixture was centrifuged at about 1500 rpm for 10–15 min to make a clean separation, and the liquid was decanted through a bed of cotton held in a funnel into a 500-mL round-bottom flask. This extraction was repeated a second time, and the liquid phases were combined.

The liquid was evaporated by using a rotary evaporator at 40 °C until only about 1 mL remained. This remaining liquid was evaporated by a gentle nitrogen stream at room temperature.

The residue in the flask was treated with small amounts (1 mL) of acetonitrile–water (45:55 v/v) which were transferred by means of a Pasteur capillary pipet to a 5-mL volumetric flask. Final dilution to volume was made with the same solvent mixture.

Soil Extraction. Soils were ball-milled as received for 15 min, and a 50-g sample was weighed directly into a 250-mL polypropylene centrifuge bottle. Ten grams of water was added, and the sample was then processed as described for mud.

Water Extraction. Water was filtered through a Whatman No. 40 paper, and 500 mL was measured into

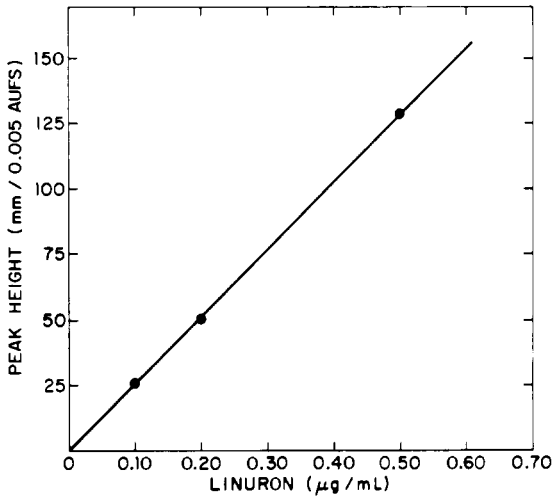

Figure 2. Linuron response (2–10 ng).

a 1-L separatory funnel along with 75 mL of methylene chloride. The mixture was shaken for 1 min after which the methylene chloride layer (lower) was drained through a 50-g bed of anhydrous sodium sulfate held in a funnel into a 500-mL, round-bottom flask. This extraction was repeated twice more, and the lower liquid layers were combined with the first.

The liquid was evaporated by using a rotary evaporator at 40 °C until only about 1 mL remained. The remaining liquid was evaporated by a gentle nitrogen stream at room temperature.

The residue in the flask was treated with two successive portions (<0.5 mL) of acetonitrile–water (45:55 v/v) which were transferred by means of a Pasteur capillary pipet to a 1-mL volumetric flask. Final dilution to volume was made with the same solvent mixture.

Liquid Chromatography. Chromatograms were obtained with a Du Pont Model 850 liquid chromatograph. UV absorbance was measured at 254 nm by using a sensitivity of 0.005 absorbance unit full-scale (aufs).

The column was a reverse-phase type (Du Pont Zorbax ODS) fitted with a reverse-phase guard column filled with the same packing material. The oven temperature was maintained at 35 °C.

The mobile phase was acetonitrile–water (45:55 v/v) pumped at a rate of 1.5 mL/min. Under these conditions, linuron elutes from the column in 12.8 min.

A sample valve (Valco special design) was used with a 20-μL sample loop for manual injection of standards and samples. Before injection, all solutions were filtered by means of a Swinney filter holder (Millipore Corp.) mounted on a 1-mL hypodermic syringe and containing a 0.5-μm filter.

Standardization. A standard stock solution of linuron in acetonitrile was prepared by weighing out 10.0 mg, dissolving it, and diluting to 100 mL in a volumetric flask.

The standards used for liquid chromatography were prepared by diluting 1.0 mL of the stock solution to 100 mL with mobile phase in a volumetric flask. Standards of concentrations 0.50, 0.20, and 0.10 μg/mL were prepared from this solution by appropriate dilution with mobile phase. These three concentrations were injected before each group of samples in order to check the reproducibility of the detector response. A typical calibration curve, comprising the working range of this investigation, is shown in Figure 2, and it can be seen that the response is linear with the line passing through the origin. A chromatogram of the 0.10 μg/mL solution, corresponding to the maximum sensitivity of the method, is shown in

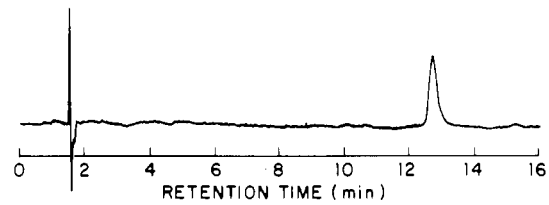

Figure 3. Linuron standard (0.005 aufs).

Figure 3. The peak corresponds to 2 ng of linuron through the column and is 25 mm in height by using a 1-mV recorder with a chart width of 250 mm. This sensitivity is possible because of the following chromatographic factors: use of a pump which has relatively small pressure pulses; use of a highly sensitive and stable detector; use of a high-efficiency column; enhancement of the column efficiency by use of the lower viscosity acetonitrile–water mobile phase.

Sample Analysis. The mud, soil, and water extracts were chromatographed in the same manner as the standards with one exception. For 15 min following sample injection, the mobile phase was maintained at 45% acetonitrile, following which the concentration of acetonitrile in the mobile phase was programmed linearly from 45 to 100% in 5 min, held at 100% acetonitrile for 10 min, programmed linearly from 100 to 45% acetonitrile in 5 min, and then held at the normal mobile phase composition for 5 min. This procedure served to rapidly remove any late-eluting materials present in the samples which would otherwise interfere with subsequent chromatograms.

Samples of mud, soil, and water were spiked with appropriate volumes of standard linuron solutions prepared in acetonitrile by dilution of the standard stock solution. For mud and soil spiking, the concentration used was 1.0 μg/mL, while for water it was 0.10 μg/mL. Recoveries were measured with each set of samples processed. All recovery data were obtained by spiking samples of soil, mud, and water which did not contain linuron with the exception of two field soil samples which were spiked at 10 and 20 ppbw. In these two cases, a correction was made to calculate the recovery values. Figure 5 is the chromatogram of one of these samples.

RESULTS AND DISCUSSION

Figure 4 is a chromatogram of a typical mud sample extract and a chromatogram of the extract of that same mud spiked with 10 ppbw of linuron. The upper chromatogram shows no indication of linuron at all, and the lower chromatogram demonstrates the high rate of recovery in this case, 96%, at these very low levels.

Figure 5 shows chromatograms of the extract of a soil taken from a field bordering the Tuckahoe Creek which was known to have been treated with linuron and of the extract of this same soil spiked with 20 ppbw of linuron. The upper chromatogram shows 14 ppbw of linuron to be present in the treated soil. The lower chromatogram demonstrates a recovery of 89% from the spiked sample after a correction was made for the linuron originally present in the sample.

Figure 6 includes chromatograms of a water sample extract and an extract of this water sample spiked with 0.2 ppb, w/v, of linuron. The upper chromatogram shows no detectable linuron, whereas the lower shows 100% recovery of linuron from a 0.2 ppb, w/v, spiked sample.

Table I shows the recovery data for linuron. There is no evidence of a dependence of degree of recovery on the spike level, showing that there is no significant interference and no serious loss by adsorption, incomplete removal, or processing errors.

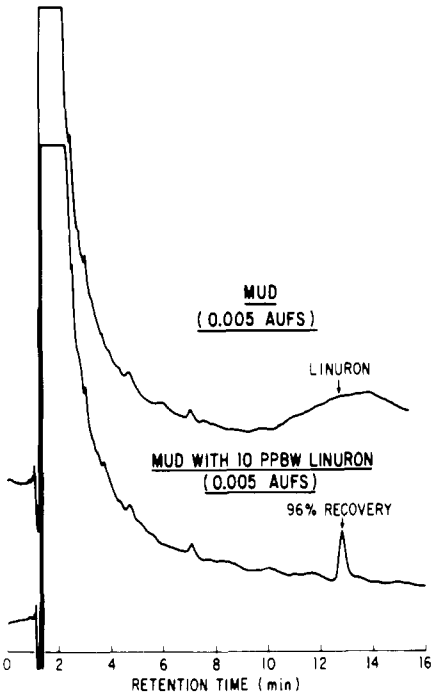

Figure 4. Mud sample extracts.

Table I. Recovery Study

material	spike level, ppbw	no. of samples	av recovery, %	SD, %
mud	10	12	87	±5
	20	9	89	±9
	50	4	81	±2
soil	10	4	85	±19
	20	4	93	±5
	40	7	91	±17
water	0.20[a]	14	96	±13
	0.40[a]	6	90	±5

[a] In units of ppb (w/v).

Table II. Mud Analyses

area	no. of sample locations	sampling date, mo-yr	no. of samples analyzed	linuron, ppbw
Rhode River	5	8-77	5	<10
		7-78	5	<10
		9-78	5	<10
Poplar Island	4	8-77	4	<10
		7-78	4	<10
		9-78	4	<10
Choptank River–Tuckahoe Creek	13	8-77	13	<10
		10-77	13	<10
		7-78	13	<10
		9-78	13	<10

Table II shows the results of the mud analyses. In all, 79 different samples were taken during the growing seasons of 2 consecutive years and were analyzed, and all of them showed less than 10 ppbw of linuron. These include 15 from the Rhode River, 12 from Poplar Island, and 52 from the Choptank River–Tuckahoe Creek area where there is a record of high linuron usage.

Table III summarizes the water analyses corresponding to those of the mud. Samples of mud and water were taken in exactly the same locations. Again, in 79 samples taken over 2 years, none showed linuron above the lower detection limit of 0.2 ppb, w/v.

Table IV illustrates the rapidity with which linuron decomposes when applied to field soil. All samples were

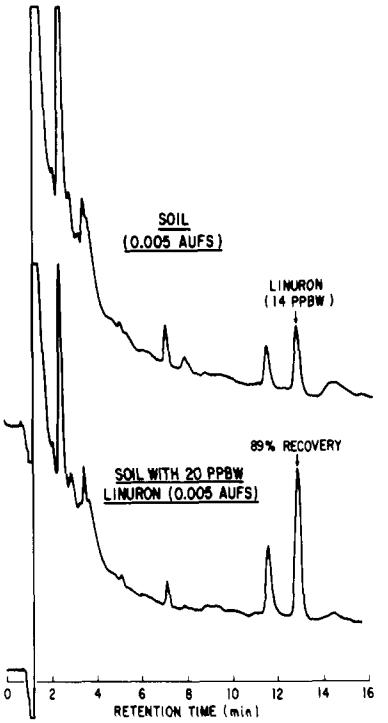

Figure 5. Soil sample extracts.

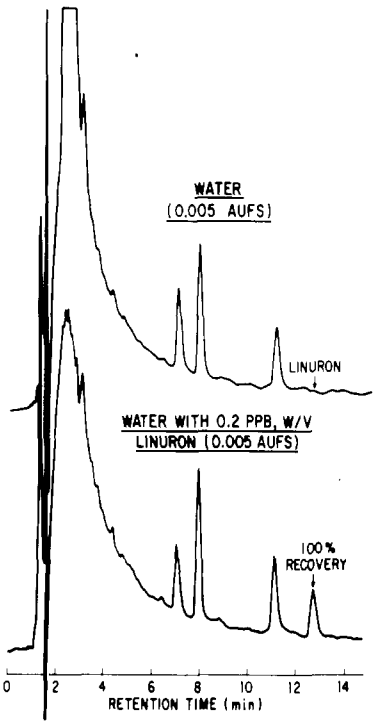

Figure 6. Water sample extracts.

Table III. Water Analyses

area	no. of sample locations	sampling date, mo-yr	no. of samples analyzed	linuron, ppb (w/v)
Rhode River	5	8-77	5	<0.2
		7-78	5	<0.2
		9-78	5	<0.2
Poplar Island	4	8-77	4	<0.2
		7-78	4	<0.2
		9-78	4	<0.2
Choptank River–Tuckahoe Creek	13	8-77	13	<0.2
		10-77	13	<0.2
		7-78	13	<0.2
		9-78	13	<0.2

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.

ACKNOWLEDGMENT

The authors express their appreciation to R. F. Holt, E. E. Puletz, and G. B. Montgomery for their assistance in gathering the samples.

LITERATURE CITED

Baunok, I., Geissbuehler, H., *Bull. Environ. Contam. Toxicol.* **3**, 7 (1968).
Byast, J. H., *J. Chromatogr.* **134**, 216 (1977).
Caverly, D. J., Denney, R. C. *Analyst (London)* **103**, 368 (1978).
Farrington, D. S., Hopkins, R. G., Ruzicka, J. H. A., *Analyst (London)* **102**, 377 (1977).
Glad, G., Popoff, J., Theander, O., *J. Chromatogr. Sci.* **16**, 118 (1978).
Khan, S. U., Greenhalgh, R., Cochrane, W. P., *Bull. Environ. Contam. Toxicol.* **13**, 602 (1975).
Lawrence, J. F., *J. Assoc. Off. Anal. Chem.* **59**, 1066 (1976a).
Lawrence, J. F., *J. Chromatogr. Sci.* **14**, 557 (1976b).
McKone, C. E., *J. Chromatogr.* **44**, 60 (1969).
McKone, C. E., Hance, R. J., *J. Chromatogr.* **36**, 234 (1968).
Onley, J. H., Yip, G., *J. Assoc. Off. Anal. Chem.* **52**, 526 (1969).
Pribyl, J., *Chromatographia* **10**, 753 (1977).
Pribyl, J. Hertzel, F., *J. Chromatogr.* **125**, 487 (1976).
Sidwell, J. A., Ruzicka, J. H. A., *Analyst (London)* **101**, 111 (1976).

Received for review January 21, 1980. Accepted April 23, 1980.

Volatile Components of Alfalfa: Possible Insect Host Plant Attractants

Ron G. Buttery* and James A. Kamm

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.

Western Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, Berkeley, California 94710 (R.G.B.), and the U.S. Department of Agriculture, Department of Entomology, Oregon State University, Corvallis, Oregon 97331 (J.A.K.).

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.