Biological Activity of Dhurrin and Other Compounds from Johnson Grass (*Sorghum halepense*)

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Johnson grass (*Sorghum halepense* L. Pers.), a severe weed pest in the southeastern United States, has been shown by several workers to release compounds toxic to higher plants. Here, several previously isolated phytotoxins and taxiphyllin, not previously found in Johnson grass, have been isolated from methanolic extracts of Johnson grass rhizomes. These compounds were active against tomato and radish seedling root growth and also inhibited growth of several of nine bacteria tested. Exudates from living rhizomes did not affect radish and tomato root growth but did affect bacterial growth and slowed germination of three of five crop species tested. A ^{13}C NMR method is presented for distinguishing the epimers dhurrin and taxiphyllin which were found in Johnson grass rhizomes.

Johnson grass (*Sorghum halepense* L. Pers.) is a highly competitive species that is a severe pest in many row crops, particularly in the southeastern United States. Johnson grass allowed to grow unchecked may grow in almost pure stands for several years, which has led many workers to suggest that Johnson grass may release phytotoxic compounds into the environment and thereby reduce the growth of competing species. Abdul-Wahab (1964) has shown that extracts of the rhizomes and the rhizosphere soil were inhibitory to primary root growth of rice seedlings. Abdul-Wahab and Rice (1967) tested water extracts of Johnson grass leaves and rhizomes against germination and seedling growth of seven associated species and found significant inhibitory activity. Later experiments showed that soil incorporated decaying leaves and rhizomes and exudates from living rhizomes also were active against germination and seedling growth. They identified chlorogenic acid, *p*-coumaric acid, and *p*-hydroxybenzaldehyde as the major inhibitors in the leaf and rhizome extracts and postulated the presence of dhurrin, a cyanogenic glycoside which breaks down to *p*-hydroxybenzaldehyde, HCN, and glucose. Dhurrin had been identified in other *Sorghum* species by Dunstan and Henry (1902). Kovacs (1972) confirmed the presence of dhurrin in Johnson grass rhizomes and rhizome exudates. He tested the effect of rhizome exudates containing dhurrin, HCN released by enzymatic action on rhizome exudates, synthetic *p*-hydroxybenzaldehyde, and synthetic *p*-hydroxybenzoic acid on barley seedling growth. Although he did not test pure dhurrin, he found all the constituents active and that HCN contributed the major part of the inhibitory activity.

Parks and Rice (1969) found that the growth of certain soil algae was inhibited by rhizome extracts. They suggested that plant released toxins might directly affect other higher plants by influencing the soil microflora.

In order to further clarify the nature and biological activity of some of the compounds contained in and emitted from Johnson grass rhizomes, the following study was conducted. Dhurrin, its epimer taxiphyllin, and *p*-hydroxybenzaldehyde were isolated and identified from rhizome extracts. These compounds, some of their biological precursors, and structurally similar compounds were bioassayed on radish and tomato root growth and the growth of several common soil bacteria. Concurrently, exudates from living rhizomes were bioassayed on radish and tomato root growth, bacterial growth, and germination of five crop species. The cyanide (CN^-) content of the rhizome exudates was measured to see if the cyanide content could explain any biological activity. The ^{13}C NMR of dhurrin and taxiphyllin were studied by using gated decoupling spectra to give completely coupled spectra, providing the ^{13}C–H coupling constants which differ from one epimer to the other.

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MATERIALS AND METHODS

Extraction of Johnson Grass. Johnson grass rhizomes were obtained from a field at Stoneville, MS. Excess soil and debris were removed from the rhizomes (1 kg) by rinsing under cold running water. They were chopped and extracted (1 kg) with methanol under reflux for 2 h. The methanol was removed under vacuum to give 40 g (4%) dry extractables.

Fractionation of the Methanol Extract (See Figure 1). The methanol extract of rhizomes was chromatographed on a silica gel column (l = 150 cm, i.d. = 5 cm) by using the solvents benzene (1 L), benzene–ethyl acetate, 5:5 (v/v, 1 L), and chloroform–methanol–water, 65:35:20 (v/v/v, 1 L). The eluates from the second and third solvents were reduced to dryness and taken up with methanol, the second was separated on a cellulose column (l = 150 cm, i.d. = 5 cm) by using 10% AcOH and the third by DCC (Droplet counter current apparatus, Tokyo Rikakikai, Toyama-cho, Kanda Chiyoda-ku, Tokyo, Japan) by using the upper phase of a solution of chloroform–methanol–propanol–water (5:6:1:4 v/v) as the stationary phase and the lower phase of the solvent mixture as the moving phase. The eluates were collected in 10-mL fractions and monitored by TLC on silica gel plates. Fraction C was separated by HPLC on a Partisil 10 C-8 column (Whatmann, 50 cm, i.d. = 4.6 mm) using dioxane as the solvent, thus improving the separation reported by Nahrstedt (1978).

Extraction and Separation of Taxiphyllin. The procedure of Schwarzmaier (1976) was used to obtain taxiphyllin from *Bambusa vulgaris*, gathered in Puerto Rico.

Root Length Bioassay. The experimental unit in the root length bioassay consisted of twenty-five seeds arranged on three layers of Anchor germination paper in a 100 mm square by 15 mm deep plastic Petri dish. The paper was moistened with 5 mL of the appropriate test solution. The bioassays were incubated at 20 °C in a temperature-controlled chamber. Two species were used in these bioassays: *Lycopersicon esculentum* Miller "Homestead 24" (tomato) and *Raphanus sativus* L. "Champion" (radish). Results given are mean lengths of germinated seeds; germination varied from 96 to 100%.

Radish root length was measured after 96 h; tomato root length was measured after 168 h of incubation. The experiment was a completely randomized design with three replications per treatment per species per week.

Greenhouse Experiment. The units used to grow the Johnson grass plants consisted of eight 10-cm PVC T joints connected in series by 16.5-cm sections of 10-cm PVC drain pipe. A PVC elbow joint was connected at one end, and a plastic mesh screen was placed over the other end to allow drainage while keeping the soil mixture in place. The units were supported on a wooden framework with the elbow end elevated to facilitate drainage. An artificial soil consisting of perlite–coarse sand–vermiculite, 3:2:1 v/v/v, was used to fill the units. Johnson grass rhizomes were planted in the upright arms of the T joints. Water and nutrient solution were poured into the elbow joint and allowed to move through the unit past the plant roots until it flowed out the screened end. Peter's Hydro-sol (0.97 g/L) with $Ca(NO_3)_2$ (0.45 g/L) added was used as a nutrient solution. The solution was pH 6.5. Four liters of solution was added to each unit on each Tuesday and Friday. Tap water was used to water as needed at other times. On Mondays, 8 L of tap water was added to each unit, and the first liter of water emitted was collected for the bioassay and chemical identification work. The control consisted of a growing unit treated identically but without plants.

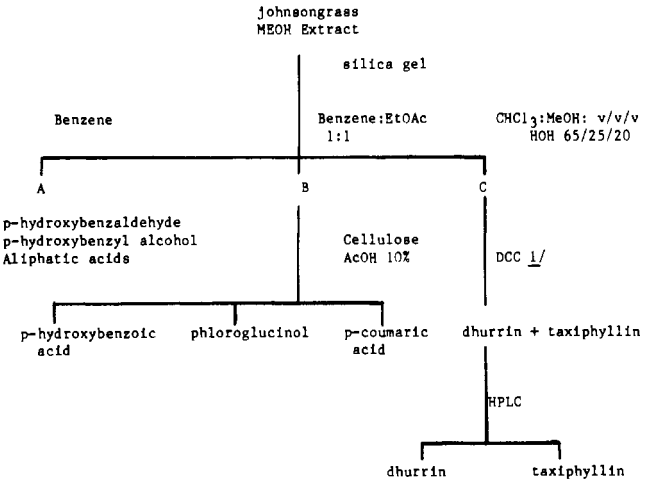
Germination Bioassay. The experimental unit in the germination bioassay was identical with the root length bioassay except germination counts were taken at 24, 48, 72, 96, and 168 h. A seed was considered germinated when the radicle extended 2 mm. Five species were used in those bioassays: *Abelmoschus esculentus* (L.) Moench "Clemson Spineless" (okra); *Beta vulgaris* L. "Asgrow Wonder" (beet); *Daucus carota* L. "Red Core Chantenay 503" (carrot); *L. esculentum* Miller "Homestead 24" (tomato); *R. sativus* L. "Champion" (radish). Two replications of each treatment–seed combination were used each week for the 23-week experiment.

Bacterial Test. The filter paper disk diffusion method (Rohde, 1971) was used to test the antibacterial properties of compounds isolated from Johnson grass. *Pseudomonas maltophilia*, *Pseudomones mendocina*, *Pseudomones delafidlii*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter airogenes*, and *Enterobacter cloacae* were obtained from American Type Culture Collection (Rockville, MD) and *B. subtilis* was obtained from insectary-reared boll weevils. Disks impregnated with 100 μg of the test compound were placed near the periphery of an inoculated plate. The plates were incubated for 24 h at 37 °C and the inhibition zones measured in millimeters.

Cyanide Quantification. A 100-mg sample of freeze-dried root washings was added to a boiling flask containing 40 mL of 62.5% H_2SO_4 and 7.5 g of $MgCl_2$ dissolved in 20 mL of H_2O. The gas washer contained 10 mL of 0.25 N NaOH. The train consisting of a boiling flask, an air inlet, a condenser, a gas washer, a suction flask trap, and an aspirator was connected and the suction adjusted so that one bubble of air per second entered the boiling flask through the air inlet. The boiling flask was heated to rapid boiling and refluxed for 1 h, the air flow was discontinued, the flask was cooled for 15 min, and the condenser was rinsed into the gas washer with distilled water. Cyanide content was determined by the colorimetric method of Asmus and Garschagen (1953).

RESULTS AND DISCUSSION

Figure 1 diagrams the chromatographic methods used for the isolation and purification of the methanolic extract from Johnson grass rhizomes. Fraction A was found to contain a mixture of *p*-hydroxybenzaldehyde, *p*-hydroxybenzyl alcohol, and aliphatic acids. Fraction B contained *p*-hydroxybenzoic acid, phloroglucinol, and *p*-coumaric acid. Fraction C contained dhurrin and its epimer taxiphillin in a ratio of 9 to 1. The presence of taxiphyllin [not reported by Abdul-Wahab and Rice (1967) or Kovacs (1972)] could be the result of epimerization of dhurrin during extraction and separation.



1/ $CHCl_3:CH_3OH:C_2H_5OH.\ H_2O$ 5:6:1:4.

Figure 1. Scheme for the chromatographic separation of Johnson grass extract.

Table I. ^{13}C NMR Chemical Shift Values (ppm) of Dhurrin, Taxiphyllin, and Prunasin

carbons	dhurrin (*S*)	taxiphyllin (*R*)	prunasin
1	124.90	124.89	132.20
2	130.27	130.28	128.91
3	115.80	116.57	130.98
4	158.87	159.00	130.08
5	115.80	116.57	130.09
6	130.27	130.28	128.91
7	68.45	68.45	68.76
CN	119.39	119.10	119.47
1′	100.87	101.37	102.34
2′	73.91	73.91	74.85
3′	77.31[a]	77.31[a]	78.00[a]
4′	70.60	70.60	71.61
5′	76.92[a]	76.92[a]	78.34[a]
6′	61.85	61.85	62.91

[a] Can be reversed.

Identification. All compounds of fractions A, B, and C were identified by comparing their R_f values on silica gel TLC (benzene–EtOAc, 1:1 v/v) with those of standard compounds. Proton NMR spectra of dhurrin and taxiphyllin have been reported by Towers et al. (1964) and our shifts were similar. Carbon resonance spectra are given in Table I for dhurrin (*S*), taxiphyllin (*R*), and the related glucoside prunasin. The shifts between the two epimers *R* and *S* are quite similar with the exception of the resonance of the anomeric carbons $C_{1'}$ (0.6-pm difference) and C_3 and C_5 (0.7-ppm difference).

To assign the configuration, coupling constant values were measured for dhurrin and taxiphyllin by using gated decoupling spectra (Table II). The methine carbons of the aromatic ring C_2, C_3, C_5, C_6, and C_7 show proton–carbon coupling constants $^1J_{CH}$ and long-range coupling constants $^3J_{CCCH}$. C_3, C_5, and C_7 are doublet of doublets and C_2 and C_6 are d × d × d). The major difference in those coupling constant values between dhurrin and taxiphyllin is sig-

Table II. Coupling Constant Values (Hertz) of Dhurrin and Taxiphyllin

$^1J_{CH}$	$^2J_{CCH}$	$^3J_{CCCH}$
	Dhurrin	
C_2-H_2 = 159.91	C_1-H_7 = 6.1	C_1-H_3 = 8.0
C_3-H_3 = 160.45	C_4-H_3 = 2.6[a]	C_1-H_5 = 8.2
C_5-H_5 = 160.45	C_4-H_5 = 2.2[a]	C_2-H_6 = 7.5[b]
C_6-H_6 = 159.91	CN-H_7 = 5.3	C_2-H_7 = 4.2
C_7-H_7 = 151.95		C_3-H_6 = 4.8[c]
		C_4-H_2 = 8.2[a]
C_1'-H_1' = 157.90		C_4-H_6 = 8.4[a]
C_2'-H_2' = 144.85		C_5-H_3 = 4.9[c]
C_3'-H_3' = 144.55[a]		C_6-H_2 = 7.15[b]
C_4'-H_4' = 144.66		C_6-H_7 = 4.3
C_5'-H_5' = 143.05[a]		C_7-H_2 = 4.7
C_6'-H_6' = 144.00		
	Taxiphyllin	
C_2-H_2 = 160.31	C_1-H_7 = 5.6	C_1-H_3 = 7.70[c]
C_3-H_3 = 161.20	C_4-H_3 = 2.5[a]	C_1-H_5 = 7.60[c]
C_5-H_5 = 161.20	C_4-H_5 = 2.3[a]	C_2-H_6 = 6.95
C_6-H_6 = 160.31	CN-H_7 = 7.7	C_2-H_7 = 4.30
C_7-H_7 = 154.00		C_3-H_5 = 4.70
C_1'-H_1' = 159.51		C_4-H_2 = 9.20[a]
C_2'-H_2' = 144.50		C_4-H_6 = 8.83[a]
C_3'-H_3' = 144.05[a]		C_5-H_3 = 4.70
C_4'-H_4' = 142.95		C_6-H_2 = 6.95
C_5'-H_5' = 144.65[a]		C_6-H_7 = 4.3
C_6'-H_6' = 144.50		C_7-H_2 = 4.6

[a-c] Can be reversed inside the column.

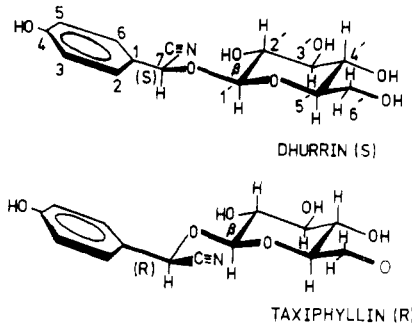


Figure 2. Structures of dhurrin (*S*) and taxiphyllin (*R*).

nificant only for $^1J_{CH}$ C_7-H_7 (2.05-ppm difference). $^2J_{CCH}$ for those methine carbons is too small (1–2 Hz) to be measured in contrast to the vicinal $^3J_{CCCH}$ (7–10 Hz). The quaternary carbons C_1 and C_4 have different patterns, depending on whether they have two (C_4) or three (C_1) vicinal protons. The C_4 has a nine-line pattern from which coupling constants $^3J_{CCCH}$ and $^2J_{CCH}$ have been extracted. The $^3J_{CCCH}$ for C_4 is larger in the case of taxiphyllin and results in an anisotropic rotational diffusion (Levy et al., 1973) depending on the mass and polarity of the substituent in C_1 and in conjunction with the rotation around the C_2 axis. The C_1, a six-line pattern, also presents differences in the coupling constants between the two epimers, particularly $^2J_{CCH}$ for C_1-H_7. The molecular model of dhurrin (Figure 2) suggests that the most stable conformation for this compound having the aglycon with the L configuration would result in the –CN group and the anomeric hydrogen of the glucose being almost eclipsed. On the other hand, the compound having the aglycon with the D configuration (taxiphyllin) has the –CN group lying in the same plane as the glucose. Since Schwarcz and Perlin (1972) found that $^2J_{CCH}$ is largest when the substituent is in the plane of the C–C–H coupling, it is easy to understand the difference of 2.4 Hz for CN-H_7 between the epimers. The $^2J_{CCH}$ for CN-H_7 in prunasin is the same as that in taxiphyllin, confirming the Towers et al. (1964) observation.

Bioassay Results. Figures 3 and 4 show the root growth inhibition of tomato and radish seedlings with

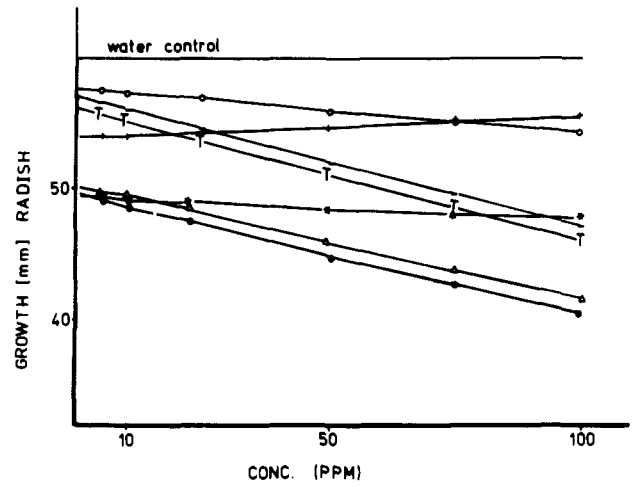


Figure 3. Regression analysis of radish root growth inhibition with concentation (ppm). (T) *p*-Hydroxybenzaldehyde, $y = 56.34e^{-0.10x}$; (–) prunasin, $y = 56.61e^{-0.10x}$; (△) *p*-hydroxymandelonitrile, $y = 50.41e^{-0.08x}$; (○) dhurrin, $y = 54.91e^{-0.04x}$; (●) mandelonitrile, $y = 49.62e^{-0.02x}$; (*) NaCN (pH 5.5), $y = 49.65e^{-0.02x}$; (+) taxiphyllin, $y = 53.81e^{-0.02x}$.

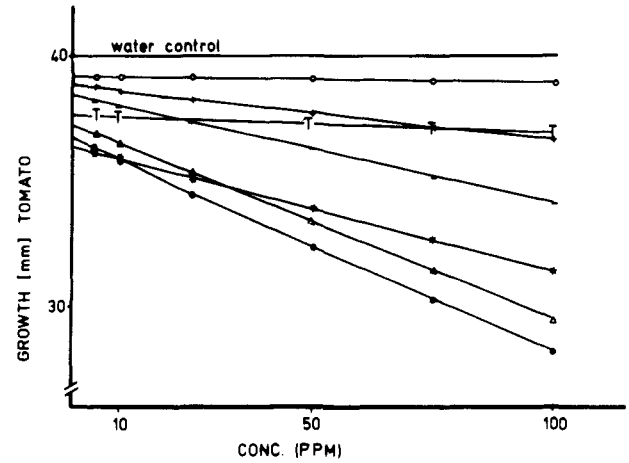
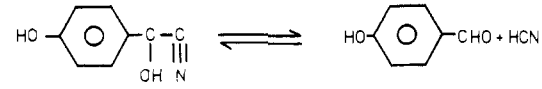


Figure 4. Regression analysis of tomato root growth inhibition with concentration (ppm). (T) *p*-Hydroxybenzaldehyde, $y = 41.39e^{-0.01x}$; (–) prunasin, $y = 38.51e^{-0.04x}$; (△) *p*-hydroxymandelonitrile, $y = 37.27e^{-0.08}$; (○) = dhurrin, $y = 39.21e^{-0.002x}$; (●) madelonitrile, $y = 36.77e^{-0.08x}$; (*) NaCN (pH 5.5), $y = 36.38e^{-0.05x}$; (+) taxiphyllin, $y = 38.87e^{-0.02x}$.

respect to concentration (ppm) of chemical isolates from Johnson grass and related cyanogenic compounds. All the compounds tested showed an increase in inhibition in the tomato and radish root growth assay (Figure 4) with increased concentration, except taxiphyllin, which showed a slight increase in growth of radish with increasing concentration. However, the slope of the taxiphyllin inhibition curve was not statistically significantly different from zero. The difference in absolute reduction of seedling growth for dhurrin treated plants and the water control is significant. The radish assay (Figure 3) was more sensitive than the tomato assay to all compounds except NaCN. The reduction in seedling growth by *p*-hydroxymandelonitrile parallels that of *p*-hydroxybenzaldehyde due to the equilibrium



The presence of a sugar moiety (prunasin, dhurrin, and taxiphyllin, Figures 3 and 4) decreases the inhibition of tomato and radish root growth. Although the presence of

Table III. Germination Test of Selected Seed Treated with Johnson Grass Root Washings

bioassay, seed[b]	mean % germination[a] for time of germination count of				
	24 h	48 h	72 h	96 h	168 h
okra					
H_2O	0.2	6.17	19.0	42.4	82.7
SH	0.0	2.87[d]	11.2[d]	34.0[d]	83.6
tomato					
H_2O	0.0	1.6	82.1	97.4	98.8
SH	0.0	2.5	77.7[c]	96.5	98.7
radish					
H_2O	87.0	99.4	99.4	99.5	99.6
SH	84.4[d]	99.1	99.5	99.5	99.7
beet					
H_2O	0.0	15.1	61.2	79.6	88.1
SH	0.3	14.9	58.1	78.4	87.1
carrot					
H_2O	0.0	4.9	38.2	68.3	87.4
SH	0.1	4.4	36.4	66.3	87.7

[a] Calculated for the 23-week experiment. [b] SH = *Sorghum halepense*. H_2O = water control. [c] Significant at the 5% level by Duncan's multiple range test. [d] Significant at the 1% level.

a *p*-hydroxyl group has been shown to increase the phytotoxic properties in aromatic substituted acids (Nicollier and Thompson, 1982); mandelonitrile was consistently (though not significantly) more inhibitory than *p*-hydroxymandelonitrile in both radish and tomato bioassays.

Figure 5 shows the effect of the Johnson grass root exudates on radish and tomato root growth plotted as the percent of the water control during the weeks of the experiment. The data do not show significant inhibition and imply that the root exudate is not very phytotoxic to radish and tomato.

The germination test of selected seeds treated with Johnson grass root exudates shows that the effect of the toxin(s) varies considerably with the seed species used for bioassay (Table III). Okra seed germination was strongly delayed, that of radish moderately delayed, that of tomato slightly delayed, and beet and carrot germination was not affected. In no case was total germination at 168 h affected; rather, germination was slowed but not stopped. Both the slowing of germination and the differential activity against different species could be important factors in weed–crop interactions in the field.

Figure 6 shows the amount of cyanide present in the root exudates from Johnson grass during the 23-week test. There was an increase of CN– in the middle of the experiment but the highest level measured (approximately 0.02 ppm) is far below the lowest concentration tested (5 ppm) in the bioassay of NaCN. The 0.2-ppm concentration apparently exerted no measurable effect on tomato or radish root growth.

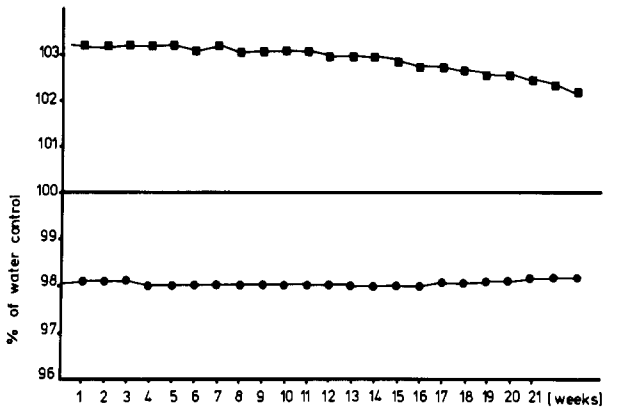


Figure 5. Regression analysis of radish and tomato root growth with Johnson grass leachate as percentage of water control with time. (●) Radish; (■) tomato.

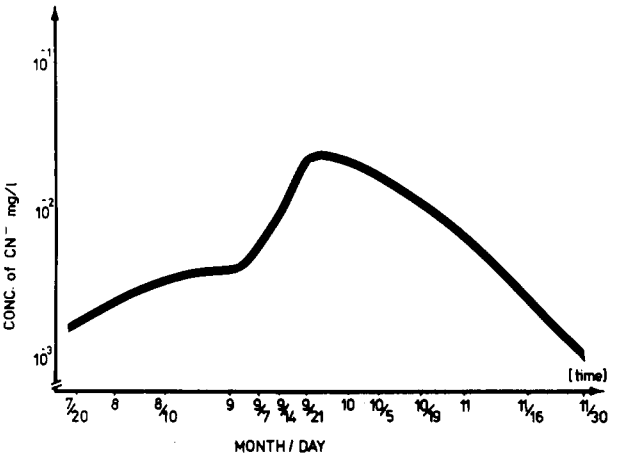


Figure 6. Cyanide released by Johnson grass with time.

Johnson grass constituents were tested against four genera (nine species) of bacteria (Table IV) in order to determine if these compounds might influence the soil bacteria populations and thereby possibly indirectly influence other higher plants. Dhurrin was active against all bacteria except *P. mendocina*. Prunasin was active against *Bacillus subtilis*. Johnson grass root washings were active at the concentration tested against *P. maltophilia* and *B. subtilis* and *Bacillus thuringiensis*. All other compounds were inactive against all bacteria tested.

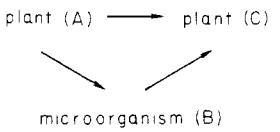
It appears that dhurrin, taxiphyllin and possibly other allelopathic compounds can act on bacteria as well as higher plants and therefore the allelopathic mechanism in this case may not be simply a plant–plant interaction

Table IV. Bacterial Inhibition by Dhurrin, Taxiphyllin, Prunasin, *p*-Hydroxybenzaldehyde, and Johnson Grass Root Washings

bacteria	inhibition zone, mm, ± SD				
	dhurrin	taxiphyllin	*p*-hydroxy-benzaldehyde	prunasin	root washings[a]
Pseudomonas mendocina	0	0	0	0	
Pseudomonas maltophilia	8.0 ± 0.4	8.0 ± 0.1	0	0	8.0 ± 0.5
Pseudomonas maltophilia	10.0 ± 0.5	10.0 ± 2.0	12.0 ± 1.0	0	
Bacillus sphaericus	8.0 ± 1.6	8.0 ± 0.5	0	0	
Bacillus subtilis	12.0 ± 1.0	14.0 ± 1.0	8.0 ± 0.5	8.0 ± 0.5	8.0 ± 0.5
Bacillus thuringiensis	12.0 ± 0.5	10.0 ± 1.0	0	0	8.0 ± 0.5
Enterobacter aerogenes	8.0 ± 0.1	0	0	0	
Enterobacter cloacae	10.0 ± 0.5	9.0 ± 0.5	0	0	
Staphylococcus aureus	9.0 ± 0.4	9.0 ± 0.5	0	0	

[a] 1000 μg was applied to a bacteriological test disk paper in this case.

but may involve the mechanism



Interactions A–C and A–B were studied but a modification of the microorganism (B) can modulate the growth of other plants (C); thus, the edaphic environment must be considered in studying allelopathy in the real world.

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Registry No. Dhurrin, 499-20-7; taxiphyllin, 21401-21-8; *p*-hydroxybenzaldehyde, 123-08-0.

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Capillary Gas Chromatography–Electron Impact and Chemical Ionization Mass Spectrometry of Toxaphene

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Analysis of toxaphene by fused silica capillary column gas chromatography coupled with electron impact, positive and negative chemical ionization mass spectrometry reveals a complex mixture of at least 202 compounds. Among the toxaphene components, 76% are polychlorobornane isomers, 18% are polychlorobornenes, 2% are polychlorobornadienes, 1% are other chlorinated hydrocarbons, and 3% are nonchlorinated compounds. Single ion monitoring in the electron impact mode at m/e 159 provides a highly selective and sensitive technique for analysis of toxaphene and toxaphene-like materials in the presence of other chlorinated hydrocarbon insecticides.

Toxaphene is an extremely complex mixture of polychlorinated compounds with an average elemental composition of $C_{10}H_{10}Cl_8$ (Holmstead et al., 1974; Casida and Saleh, 1978). It is produced by intensive chlorination of technical-grade camphene to an overall chlorine content of 67–69% (Buntin, 1951). Only 10 toxaphene components have been isolated and identified, including the most toxic ingredients. However, these account for less than 25% of the mixture (Saleh and Casida, 1979).

Toxaphene is carcinogenic in rodents (National Institutes of Health, 1979) and is mutagenic in the Ames *Salmonella* test (Hopper et al., 1979). It is highly persistent in soils and lake sediments with a half-life estimated at 11–20 years (Nash and Woolson, 1967; Hermanson et al., 1971; Nash et al., 1973) and also accumulates in fish (Sanborn et al., 1976; Ribick et al., 1982).

More than 5×10^5 tons of toxaphene have been used in the United States since 1947. Toxaphene and toxaphene-like pesticides (Saleh and Casida, 1977) are still used extensively in many other countries (U.S. Trade Commission, 1977). Despite the wide use of toxaphene and its apparent health hazard, little is known about its distribution and residue in mammalian species or in the environment. This stems largely because of the difficulties in analyzing such a complex mixture and its degradation products (Pollock and Kilgore, 1978) and because of interference by other chlorinated hydrocarbons. This report presents the results of an investigation of capillary column gas chromatography–mass spectrometry (capillary GC–MS) analysis of toxaphene using electron impact (EI), positive chemical ionization (PICI), and negative chemical ionization (NICI) techniques. The use of the single ion monitoring (SIM) technique in the EI mode for residue analysis of toxaphene is also discussed.

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MATERIALS AND METHODS

Chemicals. The same sample of toxaphene (Lot X-18825-6 from Hercules, Inc., Wilmington, DE) was used throughout this investigation. The chlorinated hydrocarbon insecticide mixture containing hexachlorocyclohexane isomers, heptachlor, heptachlor epoxide, aldrin, dieldrin, chlordane, DDT isomers, DDE, DDD, endrin, mirex, kepone, and polychlorinated biphenyl isomers was obtained from Supelco-Chromatography Supplies (Bellefonte, PA).

Sample Preparation. A standard solution of toxaphene in hexane (1 mg/mL) was used for the GC/MS analysis. Samples of rat body fat (2g) were fortified either

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