



Autotoxic effects of old and new wheat straw in conventional-tillage and no-tillage wheat soil

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Abstract. Autotoxic substances found in old and new wheat straw and from soil in no-tillage and conventional-tillage wheat plots near Stillwater, Oklahoma were obtained by aqueous extraction procedures, bioassayed with wheat seeds to determine their effects upon germination and seedling growth, fractionated and analyzed with a capillary gas chromatography/mass spectrometer/data analysis system. The new wheat straw had the strongest inhibitory action against wheat seedling growth, whereas the extracts of the conventional- and no-tillage soil showed similar inhibitory and stimulatory action. Several compounds were identified by CGC/MS/DA analysis, but their individual toxicities toward wheat growth are uncertain.

Key words: Autotoxicity (allelopathy); New wheat straw; Old wheat straw; No-tillage; Conventional-tillage; Soil; Petri dish bioassay; Mass spectrometry; Citric-acid cycle compounds; Nonanedioic acid; Sugars; Methyl-(Z)-9-hexadecanoic acid; Palmitic acid; Stearic acid; 11,14-Eicosadienoic acid; Oleic acid; Plasticizers; Synergistic action; Wheat; *Triticum*.

Introduction

The literature on allelopathy, including substantial amounts of work on crops and their residues, has recently been reviewed by Rice (1984), Putnam and Tang (1986), and Chou and Waller (1989). Allelopathy in wheat (*Triticum aestivum*) and other small grains (McCalla and Duley, 1949; Norstadt and McCalla, 1963; McCalla and Haskins, 1964; Kimber, 1973; Wallace and Elliott, 1979; Wallace and Whitehand, 1980) has been studied. Elliott *et al.* (1984) reviewed the extensive literature on various aspects of rhizosphere

soil; phytotoxic substances (i.e., allelochemicals) can be released into soil as a result of root and microbial exudates and/or metabolism. They can accumulate in the rhizosphere and probably can combine with humus. Samtsevitch (1965) estimated that root mucilage caps of dry matter deposited in the soil of wheat amounted to about the dry weight of grain produced. Though the allelopathic properties of wheat have been studied at several times and places, little research has been done on accumulated allelochemicals in untilled wheat soil, and apart from our own (Waller *et al.*, 1987 and Young *et al.*, 1989), we know of none in the Southern Great Plains region of the United States.

No-tillage (NT) management of wheat farming reduces the growth and yield of successive wheat crops when compared to conventional tillage (CT) (Waller *et al.*, 1987). Autotoxic effects of wheat straw residues are thought to be one factor in this yield reduc-

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tion.

Numerous compounds have been isolated and identified from plant residues, including those of small grains (Rice, 1984; Thompson, 1985; Putnam and Tang, 1986; Waller, 1987). However, we feel that the extraction methods used by some are so severe as to have no counterpart in nature. Also, the presence of an allelochemical in plant residue does not necessarily mean that it can escape into the environment where it can affect recipient plants. Fuerst and Putnam (1983) suggested that, as one of their four guidelines in the proof of allelopathy, suspected compounds must be detected in the environment around the recipient. In the work reported here, only aqueous extractions at 5°C and moderate pH (4.0-8.0) were employed. Using slightly acidic and alkaline aqueous extracts from wheat soil from NT and CT plots, and distilled water for extraction of wheat straw, both old and new (fresh), we used a wheat bioassay to survey the resulting extracts for autotoxic activity, and mass spectrometry to identify several compounds therein.

Materials and Methods

Sampling and Other Characteristics

(a) *Soil*: The soil type from which all soil and straw samples were taken is a Pulaski coarse, loamy, mixed thermic Typic Ustifluent (fine sandy loam with 0-2 percent slope, Oklahoma Department of Agriculture, 1987). Mean annual precipitation is 82 cm (Climatological Data, Agronomy Department, Oklahoma State University, Oklahoma). The samples were collected from plots located in the Agronomy Farm, Stillwater, Oklahoma. Sorghum was planted prior to 1980, and then followed by wheat in continuous plantings. For conventional-tillage soil, residues were turned under as thoroughly as possible with a moldboard plow that inverts and mixes the soil to a depth of about 20-30 cm. In no-tillage soil, all residues were left on the surface of the soil. These plots have been maintained as no-tillage plots since 1981.

Representative soil samples (down to five inches deep) were taken at harvest (June 10, 1985) by subsampling (4-6 times) of both the conventional-tillage and the no-tillage plots. Four plots were represented in the sampling regimens. The subsamples were combined and thoroughly mixed before being placed in quart Mason jars, and immediately frozen with dry ice

in the field and then stored in a freezer at -18°C until further use.

(b) *Wheat*: Pioneer 2157 cultivar of wheat was planted and cultivated throughout the period. The new wheat straw was collected on the day of harvest within one hour after it was dropped by the combine on June 18, 1985. The old wheat straw was collected just prior to combining (June 10, 1985) and represented the straw left on the surface of the soil (accumulated over four years). Samples of NT and CT wheat straw were taken randomly from four plots, air-dried and ground with a micro Wiley Mill to pass a 20-mesh screen (U. S. Standard) and stored at room temperature.

Extraction Procedures

(a) *Straw*: A mixture of 8 g straw and 100 ml distilled water was shaken in a cold room at about 4-6°C for 2 h; after being filtered through glass wool to remove straw residues, the extract was centrifuged (Sorvall Super-speed RC2-B, duPont Co., Irving TX) in 50-ml Teflon tubes at 15×10^3 rpm for 30 min and filtered again through a Millipore filter (type HA, 0.45 μ m porosity, Millipore Corporation, Bedford, MA). Each straw sample was extracted twice and the two crude aqueous extracts were combined after the micro-filtration. The clear crude extract was then lyophilized (Virtis Model 10-MR-T, the Virtis Co., Gardiner, NY), and the residue weighed and stored *in vacuo* at 22-25°C.

(b) *Soil*: Soil (200 g) and 400 ml of distilled water were mixed by stirring. The pH of the mixtures (4.5 ± 0.2 for NT soil and 5.0 ± 0.2 for CT as normal values, respectively) was slowly adjusted to a predetermined pH (acidic 5.4 ± 0.2 and basic 8.0 ± 0.2) by dripping in 1N NaOH (0.1N NaOH for final adjustment). The dispersed slurry was shaken gently for 48 h at 4-6°C. The muddy extract was refrigerated (ca 5°C) while the heavy soil particles settled, and afterward centrifuged and filtered through a Millipore filter. The pH was checked, and a 200-ml portion was lyophilized. The soil extraction procedure is diagrammed in Fig. 1.

Fractionation Procedure

The lyophilized extracts (200-ml portions) of straw and soil were extracted sequentially with 50 ml of each methanol, methylene chloride, and chloroform (J. T. Baker Chemical Co., Phillipsburg, NJ, Baker-Resi Analyzed Grade). We are aware of the recommendation of Young *et al.* (1989) of rotary evapora-

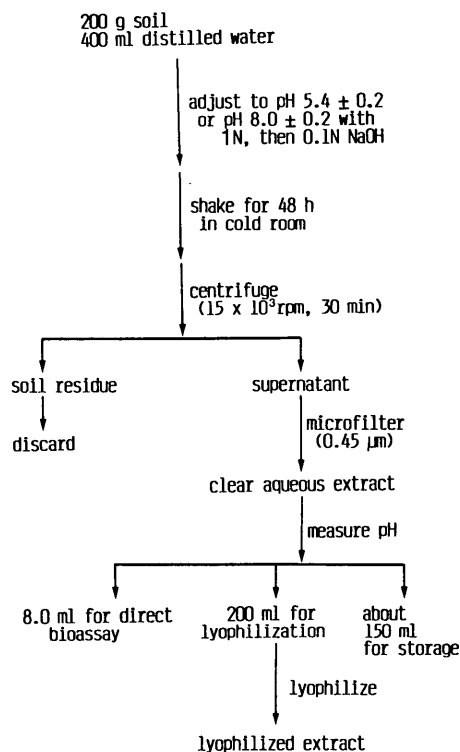


Fig. 1. Soil extraction procedure.

tion being better than the lyophilization method; however, these research programs were being carried out in our laboratories at the same time. Extracted solid residues were air-dried after each extraction. Lyophilized extracts of soil samples were extracted with methanol only. In each extraction, 15 ml solvent was applied and the slurry was gently heated on a hot plate, in a fume hood, with good stirring. The mixture was then filtered through a Millipore filter (type FH, 0.45 μm porosity). A total volume of 45 ml solvent was used (3 15-ml portions), and the three separated sub-extracts were combined. Each fraction was reduced to dryness under N_2 , and prepared as equivalent grams of soil or straw per milliliter of solution for bioassay. This expression can be explained as follows. If the total amount of straw extracted was 8 grams and a total volume of 190 ml crude aqueous extract was obtained, the concentration of this crude extract measured as equivalent amount of straw per milliliter was $8[\text{g straw}]/190[\text{ml}] = 0.04 [\text{g/ml}]$. Thus the concentration of the direct bioassay, in which 8.0 ml of the crude aqueous extract was tested, was also $0.04 [\text{g/ml}]$. The

remaining 182 ml crude aqueous extract was lyophilized and 0.300 grams of dry extract were obtained after lyophilization. If 0.100 grams of lyophilized extract is weighed out and dissolved in 8.0 ml distilled water for bioassay, the concentration of this solution is $0.100 [\text{g lyoph. ext.}]/8.0 [\text{ml}] = 0.0125 [\text{g lyoph. ext./ml}]$. Thus, the concentration expressed as the equivalent amount of straw per milliliter would be: $0.0125 [\text{g lyoph. ext./ml}]/0.300 [\text{g lyoph. ext.}] \times (182/190) [\text{ml/ml}] \times 8 [\text{g straw}] = 0.32 [\text{g straw/ml}]$

The concentrations of organic solvent fractions were calculated in the same manner on the basis of the amount of lyophilized extract that corresponded to grams of straw that was extracted. These concentrations were used for the bioassay.

After organic-solvent extraction, the dried residues were extracted finally with distilled water to test the completeness of removal of bioactive substances by the various organic solvent extractions. The fractionation procedure is illustrated in Fig. 2.

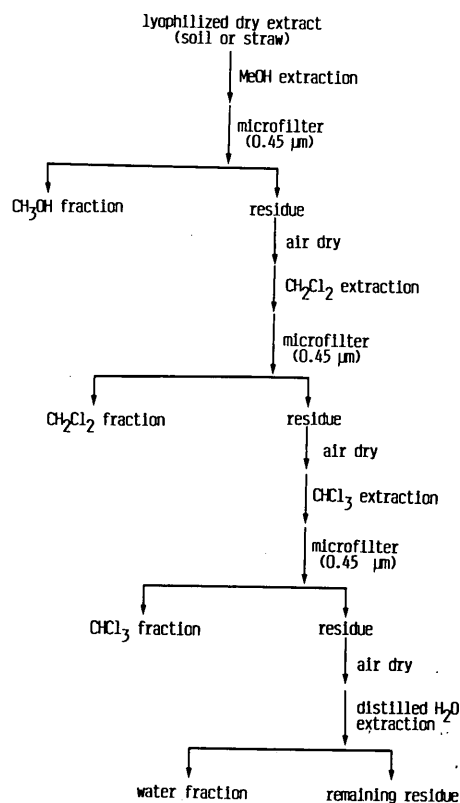


Fig. 2. Scheme of fractionation of lyophilized wheat soil and wheat straw extracts.

Bioassay Procedure

The characterization and identification of allelochemicals require sensitive bioassay methods that are relevant to the critical period of wheat growth. The method used in this study is similar to that described by McPherson and Muller (1969). Glass Petri dishes, 100 x 15 mm, were used as containers with two filter papers (Whatman No. 1, 75 mm) forming the absorptive medium. Ten seeds of Pioneer 2157 wheat were placed between the two sheets of filter paper in a radial pattern with the micropyle end toward the center. Pioneer 2157 seeds were hand-selected for normal size and absence of damage. To reduce moisture evaporation, each Petri dish, after setting the test seeds in the medium, was covered tightly with a square of kitchen-type plastic wrap before pressing the top dish cover over. Distilled water (2.0 ml) was used for the controls. With organic-solvent extracts, 2 ml solution was applied to the filter paper and allowed to evaporate completely before seeds were arranged between them (it usually required from 1-2 h). Distilled water (2 ml) was then applied to permit seed germination and seedling growth. Corresponding pure solvents were applied as controls, the same steps being followed as in the tests. The concentrations of the test samples were expressed as equivalent grams of wheat straw or soil per seed.

Incubation and Replication. Incubation (Precision Model 805, Precision Scientific Corp., Chicago, IL) was at 20°C for 72 h in darkness. Four Petri dishes,

each containing ten seeds, were used for each test, both sample and control. Controls were run with the samples in all treatments.

Statistical Analysis of Data

The lengths of the central root and shoot of each seedling were measured. Means of each set of measurements, including controls, were calculated. The difference between sample and corresponding control was indicated by percent inhibition or stimulation compared to the control as well as by the standard statistical analysis (t-test) at 95% and 99% significance levels (Steel and Torrie, 1980).

Conversion of Fatty Acids to Methyl Esters

Since organic fractions of wheat straw and soil gave unsatisfactory mass spectra on direct analysis, 1 ml of diazomethane in ether was added to each sample and the container was shaken for 5 min or until the yellow color disappeared, indicating completion of the reaction. Any excess diazomethane and its solvent were evaporated under nitrogen. The diazomethane had been synthesized by the method of Ruehle *et al.* (1979).

Low-Resolution Mass Spectrometry

Low-resolution mass spectra of the diazomethane-treated samples were obtained with an LKB-2091 (LKB Produkter, Bromma, Sweden) capillary gas chromatograph-mass spectrometer-data analysis system (CGC/MS/DAS) (McGown and Waller, 1986). These devices were linked so that the chromatography column was directly connected to the ion source of the mass spectrometer and data output went directly to the data-analysis computer. The column was 30 m x 0.25 mm (0.1- μ m film) DW-5 made by J & W Scientific, Folsom, CA. Up to 1.0 μ l of sample in CH₃OH, CH₂Cl₂, or CHCl₃ was injected with a 1:4 split at 60°C and He gas was used at 2-3 ml/min. Temperature was programmed to rise (after 4 min) 10°C/min until 300°C was reached, and then to remain there for 20-30 min.

The mass spectrometer conditions were: injector temperature 280°C, separator temperature 280°C, initial eV 21, scan eV 70, box current 15-20 μ A, accelerating voltage 3.2-3.4 V, filament current 3.3 A, trap current 80-100 A, multiplier voltage 500 mV, and source temperature 265°C.

Table 1. Average measurements of pH and quantities of soil extracts

Sample ^c	Initial pH of slurry	Final pH of extract	Lyophilized extract (g)
Acidic ^a CT extraction	5.43	5.60	0.796
Acidic ^b NT extraction	5.18	5.08	0.541
Basic ^a CT extraction	8.04	7.50	0.507
Basic ^b NT extraction	8.04	7.17	0.672

^aCT-Conventional-tillage soil.

^bNT-No-tillage soil.

^c200 g Soil extracted

For mass spectrometry analysis, 1.0 μl of caffeine dissolved in same solvent was added in each sample as an internal standard. Identification of compounds represented by peaks in spectra was done both visually and by automated means based on comparison of known with unknown spectra and interpretation of the fragmentation patterns. The mass spectrometer was connected with IBM AT microcomputers with Technivent, St. Louis, MO programming systems (McGown and Waller, 1986). Known spectra were also found in the references of the Eight Peak Index of Mass Spectra (1983), EPA/NIH Mass Spectral Data Base (Heller and Miline, 1978), Waller (1972), and Waller and Dermer (1980).

Confirmatory Mass Spectrometry Analysis

Identical samples were analyzed on a 70-ES Mass Spectrometer (CGC/MS/DA) at 70 eV by VG Analytical Limited, Wythenshawe, Manchester, England, the same conditions being used as for the LKB-2091. The spectral data obtained corresponded very well with those obtained from the LKB-2091.

Results and Discussion

Character of Aqueous Extracts

The proportion of water-extractable substances in new wheat straw was about three times (average 0.33 ± 0.002 g) as much as that in old wheat straw (average 0.12 ± 0.002 g) under the same extraction conditions; seven separate extractions were completed. The new-wheat-straw extract was dark and sticky compared with that of old wheat straw, which was lighter and more powdery. These differences suggested that new wheat straw contained more compounds. Most of those in the old straw had been presumably leached into the soil or degraded over a period of three or four years.

The average initial pHs (pH values before shaking), final pHs (pH values after shaking) of both conventional-tillage soil and no-tillage soil extracts, and the weights of the lyophilized extracts are listed in Table 1. The odor and sticky physical appearance of NT soil extracts were similar to that of new wheat straw. This similarity was consistent with the idea that some chemicals escape from straw into the soil.

Bioassay of Old and New Wheat Straw Extracts

The bioassay technique was designed to test the effects of various wheat straw and soil extracts on the early growth and development of wheat seedlings. The concentrations tested were expressed as equivalent grams of raw sample material (straw or soil) per milliliter of bioassay solution (g/ml).

The bioassay was conducted on 40 seeds for each extract. Results of bioassay tests of wheat straw extracts and fractions on growth of wheat seedlings are summarized in Figs. 3a and 3b. The inhibitory biological activity of old wheat straw was present only in the lyophilized aqueous extract and the methanol fraction, whereas for new wheat straw it was in the lyophilized aqueous extract, methanol, methylene chloride, chloroform, and final water fractions. In addition the remaining residue, which was dissolved in distilled water, indicated 26% inhibition on both wheat roots and shoots so not all of the allelopathic material was dissolved. In the bioassays of old wheat straw, no significant amount of toxicity was found in methylene chloride, chloroform, and final water fractions, and there remained too little residue for bioassay tests. The results indicated that some toxic compounds of new straw that are soluble in methylene chloride and/or chloroform but insoluble or less soluble in methanol are missing in old wheat straw. The old wheat straw was a mixture of three previous years that were accumulated on the surface of the field, so many compounds must have disappeared. The sum of the toxicities of the fractions was greater than the toxicity of the lyophilized aqueous extract (Figs. 3a and 3b), indicating they exhibited a synergistic effect.

Bioassays of crude aqueous extracts of both old and new wheat straw showed stimulation of wheat seedling growth and development. This material was about one order of magnitude lower in concentration than the organic solvent-dissolved substances, and thus reflected one of the most important properties of allelochemicals, i.e., that they may act as growth stimulants at very low concentrations, but become inhibitors at higher concentrations (McCalla and Norstadt, 1984).

Bioassay of Conventional-Tillage and No-Tillage Wheat Soil Extracts

The normal pH was about 5.0 for CT soil and 4.5 for NT soil. The extraction of soil was carried out with acidic (pH 5.4 ± 0.2) and basic (pH 8.0 ± 0.2)

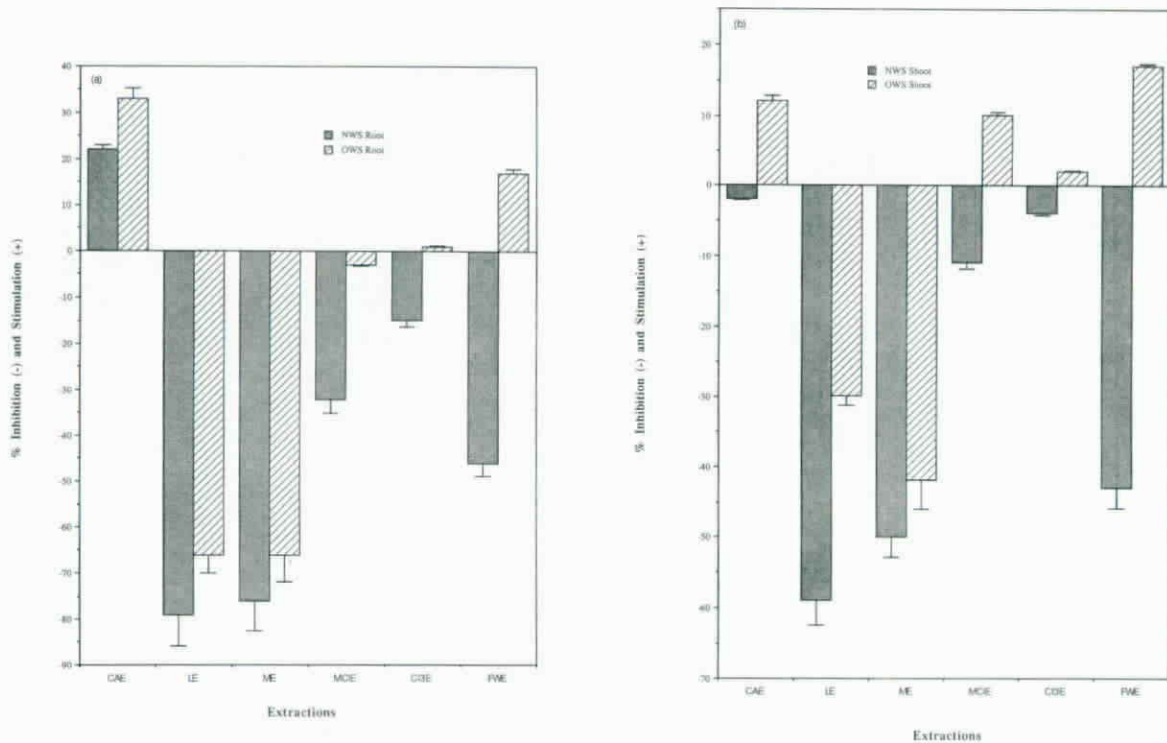


Fig. 3 (a and b). Bioassay results (wheat germination) of lyophilized extracts from new (NWS) and old wheat straw (OWS) collected in June, 1985. Old straw residues from years 1982, 1983 and 1984 were collected as a mixed straw sample. CAE-crude; LE-lyophilized; ME-methanol; MCIE-methylene chloride; Cl₃E-chloroform extract and FWE-final water extracts (see Fig. 2 for isolation procedure). Concentrations 0.05 g/ml CAE, 0.80 g/ml LE, 0.80 g/ml ME, 1.62 g/ml MCIE, 1.62 g/ml Cl₃E, 0.8 g/ml FWE. (See methodology section for computation of concentrations.)

solutions in order to compare the possible differences in biological activities and allelochemical composition of the extracts (all pH values were recorded 2 min after the readings were completely stable because the soil solutions showed fairly strong buffer activity). The reason for extracting the soil under these conditions rather than the neutral ones, as used in straw extractions, is the relatively lower concentrations of water-extractable chemicals in soil than in straw. Extractions at these pH values were not considered to be harsh treatments. In fact, such values were considered to most closely approximate those of the natural environment.

Solutions of aqueous soil extracts were adjusted to pH 7.0 ± 0.2 for bioassays; the results are shown in Fig. 4. Crude aqueous extracts were mostly stimulatory, like those of wheat straw. The acidic extracts of CT and NT soil showed slightly inhibitory action on shoot growth.

Figs. 5a and 5b are graphs of root and shoot

growth versus concentration of the lyophilized soil extracts. These results confirm that allelochemicals may exhibit stimulatory effects on plant growth rather than inhibition at low concentrations, and the stimulation may increase with increasing concentrations until a certain point is reached. As shown in Figs. 5a and 5b, the products of acidic extraction (June soils of both conventional-tillage and no-tillage plots) exhibited a maximum of 17%–26% stimulatory effect on root seedling growth at low concentrations (about 0.5–3.0 g soil/ml) and an inhibitory action began to appear when the concentration was raised to about 6.0–8.0 g soil/ml, while for the extracts made under basic conditions inhibition started at much lower concentrations (about 1.75 g soil/ml) and reached about 22% inhibition at a concentration of 8.0 g soil/ml. Most soils used for wheat production in Oklahoma are slightly acidic (pH 5.8–6.2). These results are interpreted to mean that more chemical compounds were liberated from the soil matrix under slightly basic conditions. Many allelo-

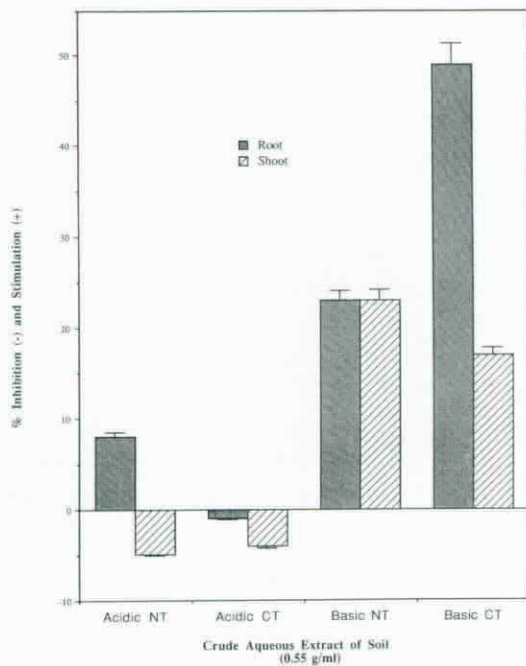
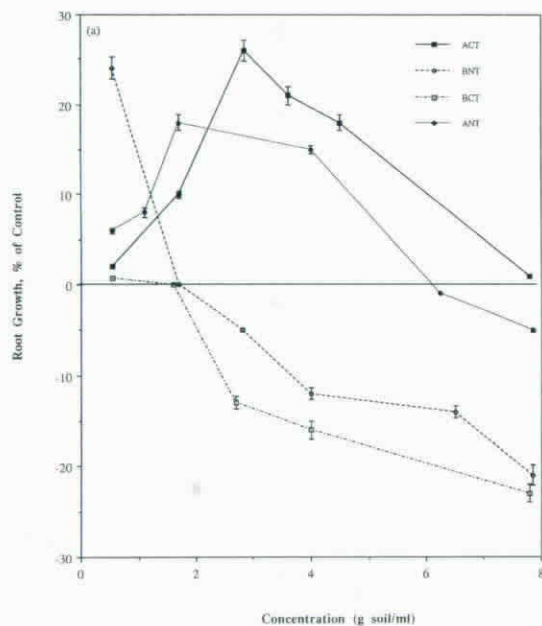


Fig. 4. Bioassay results (wheat germination) of crude aqueous extracts of conventional- and no-tillage wheat soil for June, 1985 at pH 5.4 and pH 8.0.



chemicals may be tightly bonded to clay particles, possibly through noncovalent hydrophilic and hydrophobic bonds, and are not easily released under neutral or acidic conditions. Under slightly basic conditions, however, the hydrophilic bonds, such as hydrogen bonds, or other chemical linkages among biological chemicals and clay particles break down and the chemicals are freed.

The bioassay results on shoot growth generally showed the same trend except that such growth was less sensitive to the difference between CT and NT extracts (Fig. 5 b)

Capillary Gas Chromatography/Mass Spectrometer/Data Analysis (CGC/MS/DA) of the Organic Fractions

Probability-based information on matching of spectra was taken into account only when the given confidence parameters, i.e., the fitness and the quality of the match, were at the same or higher level as those given to the internal standard added (caffeine) to the samples, usually above 60% for fitness and 98% for quality. Such a procedure of identification assured a

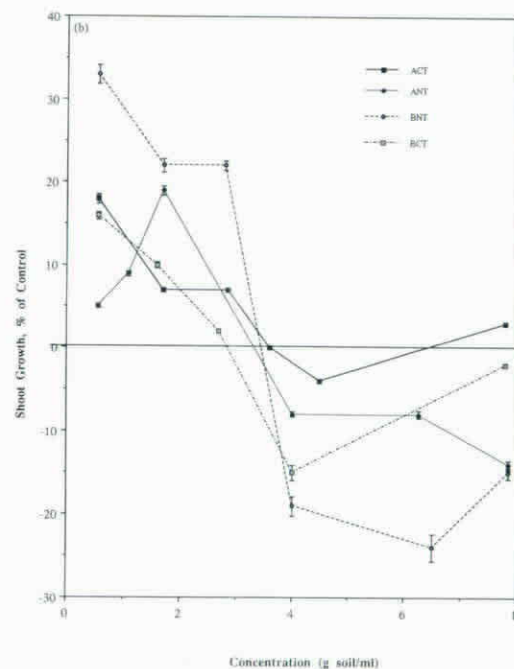


Fig. 5 (a and b). Wheat seedling growth of roots and shoots as a percent of control vs. concentrations of the soil extracts after lyophilization, bioassay results of June, 1985 of conventional-tillage and no-tillage soil extracts. Legend: ACT-acidic conventional-tillage soil extract; ANT-acidic no-tillage soil extract; BCT-basic conventional-tillage soil extract; BNT-basic no-tillage soil extract.

high certainty of correct identification. All mass spectral data are presented as comparisons of fractions of the new wheat extract vs. corresponding fractions of the old wheat straw extracts, and fractions of the aqueous conventional-tillage soil extract vs. corresponding fractions of the no-tillage soil extracts. The differences in chemical composition of each pair of samples are compared and discussed below in relation with their apparent allelopathic effects. The possible allelochemically related linkage between wheat straw and soil is also discussed. As mentioned earlier, all samples were methylated prior to mass spectrometry analysis for better performance. The compounds identified were methyl esters. The natural forms of these compounds, however, were thought to be in their free acid state.

The computer-reconstructed total ion current chromatograms of the methylated methanol fractions of new and old wheat straw are shown in Figs. 6 and 7, respectively. The analysis of the mass spectral data indicated that most of the major components were the same in both straw extracts. Methyl esters of four short-chain dicarboxylic acids were identified at lower retention times. They are dimethyl malonate (peak 5), dimethyl fumarate (peak 8), dimethyl succinate (peak 9) and dimethyl malate (peak 12).

Shilling and his colleagues also isolated malic acid and succinic acid from aqueous extracts of rye (1986). They indicated that malic acid was one of the phytotoxins that inhibited hypocotyl and root growth of *Chenopodium album* and *Amaranthus retroflexus*, while succinic acid had no inhibitory effects. Among these citric-cycle acids, fumaric acid was most often reported as a possible allelopathic substance, inhibiting seedling growth of various weeds and crops (Peters and Luu, 1985; Williams and Hoagland, 1982; Kanchan and Jayachandra, 1980). These citric-cycle acids, however, are not likely to be the major allelochemicals in the inhibitory straw extracts. One study reported that no inhibitions were observed at concentrations up to 10^{-3} - 10^{-2} M of these compounds on oat germination tests (Rudiger and Lohaus, 1987). In nature, they provide substrates for wheat plants and bacteria (Lorber and Muller, 1980; Lovett and Duffield, 1981).

Another methylated dicarboxylic acid identified was dimethyl nonanoate (peak 26). The compound has not previously been reported as an allelopathic substance. This compound was not identified in old wheat

straw though there is a small peak near its retention time in Fig. 7.

Peaks 21 and 22 in the mass spectra of straw were tentatively identified as representing two naturally occurring sugars, 1,2:5, 6-bis-*O*-(1-methylethylidene)- β -D-talofuranose, and 2,3:4, 5-bis-*O*-(1-methylethylidene)- β -D-fructopyranose. The sugars are not changed upon methylation; however, they hang-up in the injection port if the underivatized fraction is used because of the pyrolysis that occurs. Their allelopathic activity is unknown. The identification of the sugars explains the stickiness of the crude aqueous extracts of wheat straw. 2,3:4,5-Bis-*O*-(1-methylethylidene)- β -D-fructopyranose was found in old wheat straw only. It is difficult at the present time to purify and isolate a substantial amount of these sugars, however, we acknowledge that the allelochemical activity of the sugars is an important observation to be made.

The highest peak (peak 28) in the spectra was identified as 1,2:3, 4:5, 6-tris-*O*-(1-methylethylidene)-D-mannitol. No allelopathic effects of this compound have been reported.

Peaks 36 and 39 were identified as being methyl palmitate and methyl stearate. Waller *et al.* (1987) had previously found these fatty acids and more in wheat soil, but palmitate and stearate were always the predominant fatty acids. Although some studies showed that they are allelochemicals (Rice, 1984; Al-sadawi *et al.*, 1983; Spwell, 1984), no inhibition was found by us (K. G. Cast, J. K. McPherson, J. A. Pollard, E. G. Krenzer, Jr., and G. R. Waller, 1989) with either individual standard compounds or their various combinations. Thus they are not responsible for allelopathic effects in wheat straw and soil association.

Two unsaturated long-chain fatty acid esters, methyl 11,14-eicosadienoate and methyl oleate (represented by peaks 37 and 38) were in new wheat straw, whereas only the former was found in old wheat straw; peak 38 was missing. These two unsaturated long-chain fatty acids are more likely to be inhibitory in bioassays than saturated fatty acids. Al-sadawi *et al.* (1983) indicated that the sodium salt of oleic acid was about 18% more inhibitory than that of 11, 14-eicosadienoic acid against seed germination and seedling growth of bermudagrass at 50 ppm level. The oleic-acid-containing methanol fraction (Figs. 3a and 3b) of new wheat straw extract also showed about 10%

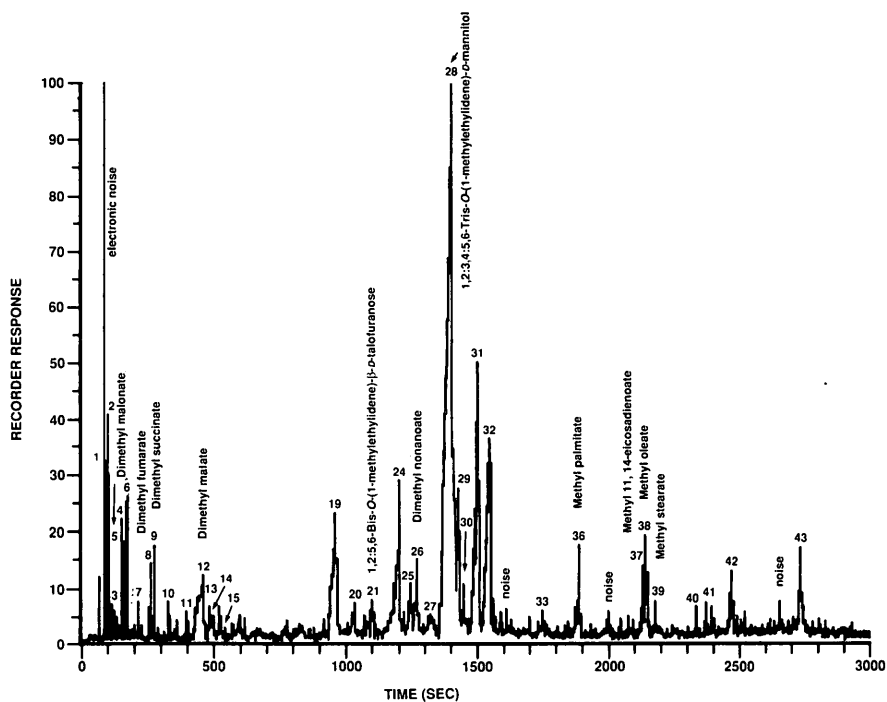


Fig. 6. Reconstructed total ion current chromatogram of methylated methanol-soluble fraction of new wheat straw. Peaks labeled show the mass spectrum number and the identity of the compounds. Instrument type: LKB-2091.

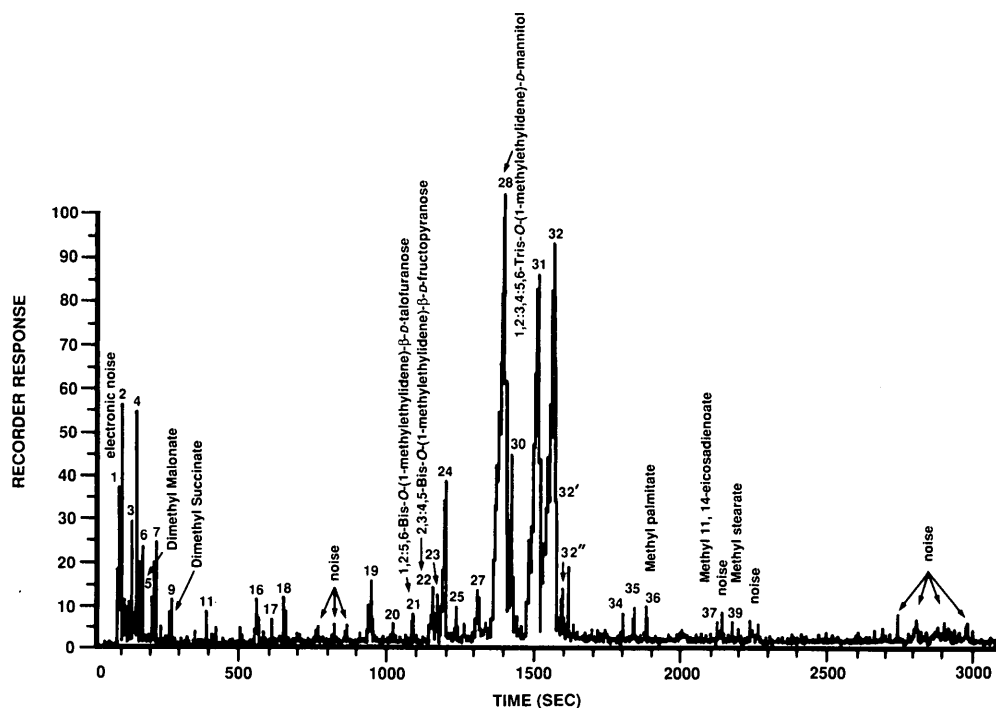


Fig. 7. Reconstructed total ion current chromatogram of methylated methanol-soluble fraction of old wheat straw. Peaks labeled show the mass spectrum number and the identity of the compounds. (Peaks represented as 32' represent the same compound as that labeled by peak 32 according to the mass spectral data.) Instrument type: LKB-2091.

more inhibition than that of old wheat straw in which no oleic acid but 11, 14-eicosadienoic acid was found.

The bioassay results indicated that toxic compounds occurred in both the wheat straw and wheat soil; the new wheat straw extracts had the strongest inhibition on wheat seedling growth, the wheat soil showing less. Bioassays of organic fractions showed that the wheat straw extract showed more inhibition when the more hydrophilic solvent (methanol) was used, rather than hydrophobic solvents (methylene chloride and chloroform). The reconstructed total ion current chromatograms of these two hydrophobic solvents extracts of new wheat straw are shown in Figs. 8 and 9, respectively. The major components isolated in these two fractions were various types of phthalate plasticizers except the peak of 468 in Fig. 8 and 466 in Fig. 9 which represented an unknown compound. This unknown was also found in the June soil (Figs. 10 and 11). The most common types of plasticizers found are: dimethyl terephthalate and diisopropyl phthalate represented by scans 610 and 976 Fig. 8, respectively. Plasticizers were found in almost every extract, but mainly in the CH_2Cl_2 and CHCl_3 fractions. The total quantity of these compounds are relatively high. They

are often considered as laboratory contaminants from various plastic-made wares; however, this interpretation is not believed to be correct in this case. All-glass equipment and Teflon stopcocks and centrifuge tubes were used throughout the experimental procedures. Thus, plasticizers are actual components extracted from wheat straw and soil. They may be naturally occurring compounds or, much more likely, introduced into the natural environment from external sources. Both the CH_2Cl_2 and CHCl_3 fraction of new wheat straw contained methyl palmitate (represented by peak at scan 956 in Fig. 8 and at scan 955 in Fig. 9).

The reconstructed total ion current chromatograms of the CH_2Cl_2 -soluble fraction of old wheat straw and the CHCl_3 -soluble fraction of old wheat straw, respectively (not shown), in contrast with those of new wheat straw, showed no peaks corresponding to chemical compounds except for those for caffeine and a few plasticizers. This result agreed with the lack of activities of these two fractions examined in bioassays (Figs. 3a and 3b).

Figures 10 and 11 show the reconstructed total ion current chromatograms of the methylated CH_3OH fraction made by basic extraction of conventional-till-

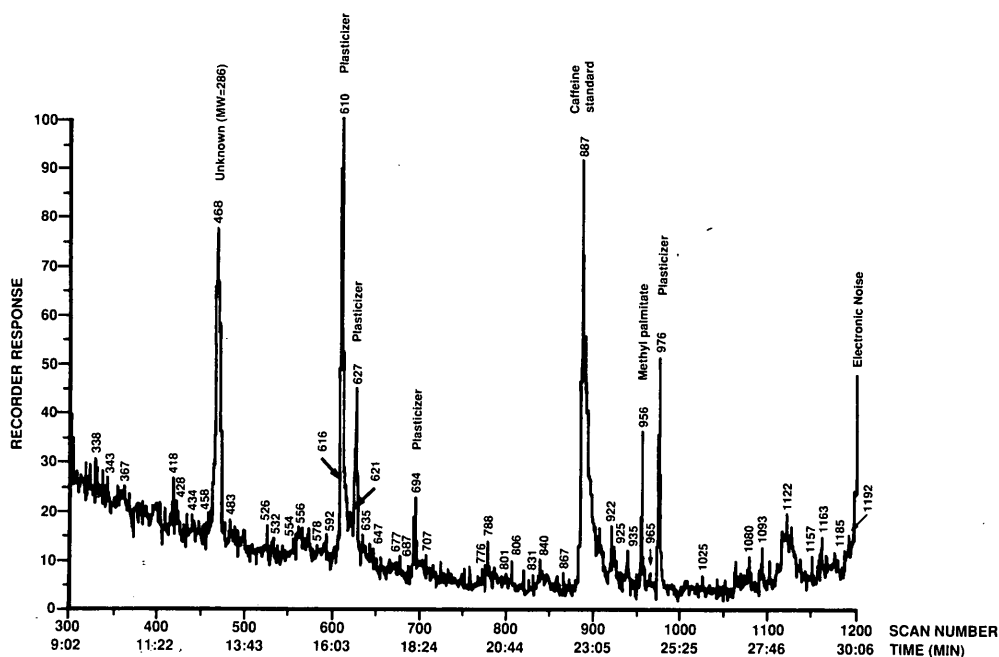


Fig. 8. Reconstructed total ion current chromatogram of the methylene chloride soluble fraction of the lyophilized aqueous leachate of new wheat straw collected on June 10, 1985. (Peaks labeled represent the mass scan number and the identity of the compounds.) Instrument type: VG 70/SE.

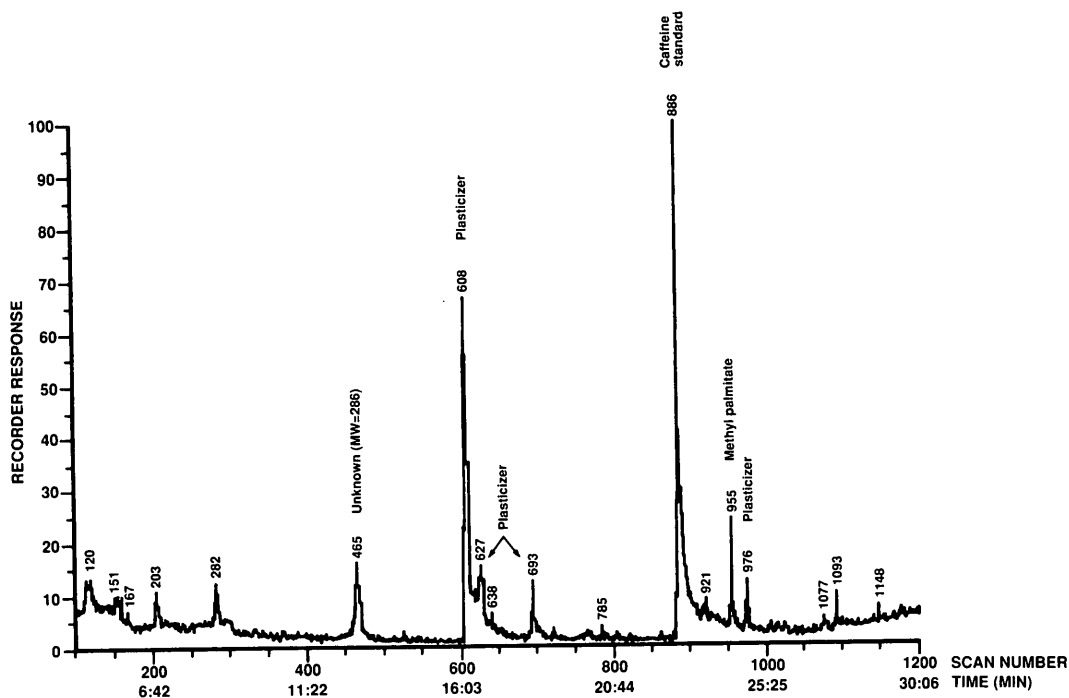


Fig. 9. Reconstructed total ion current chromatogram of the methylated chloroform extract of the lyophilized aqueous leachate of new wheat straw collected on June 10, 1985. (Peaks labeled represent the mass scan number and the identity of the compounds.) Instrument type: VG70/SE.

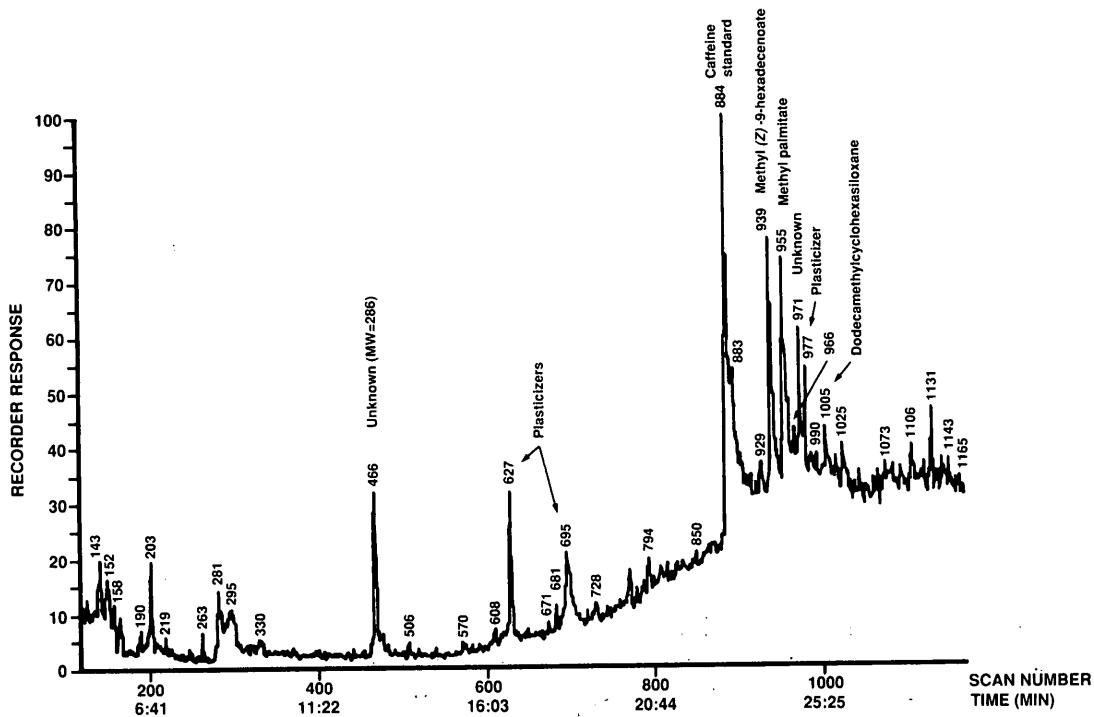


Fig. 10. Reconstructed total ion current chromatogram of methylated methanol fraction of the lyophilized aqueous leachate of CT soil, collected on June 10, 1985. (Peaks labeled represent the mass scan number and the identity of the compounds.) Instrument type: VG 70/SE.

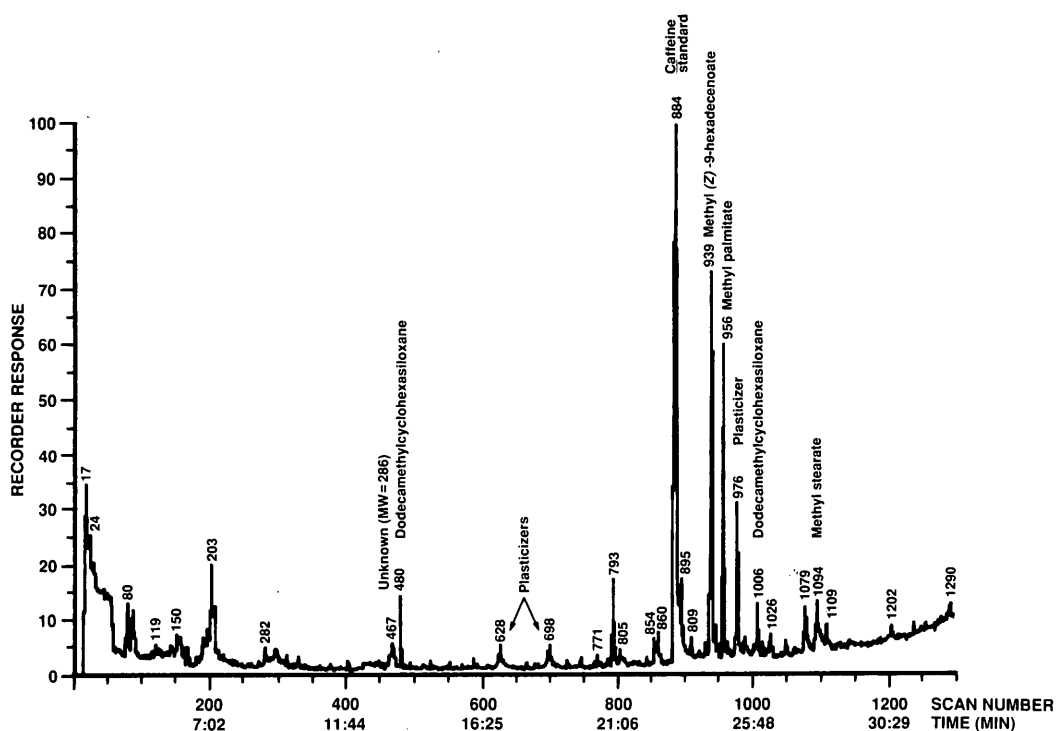


Fig. 11. Reconstructed total ion current chromatogram of methylated methanol fraction of the lyophilized aqueous leachate of NT soil, collected on June 10, 1985. (Peaks labeled represent the mass scan number and the identity of the compounds.) Instrument type: VG 70/SE.

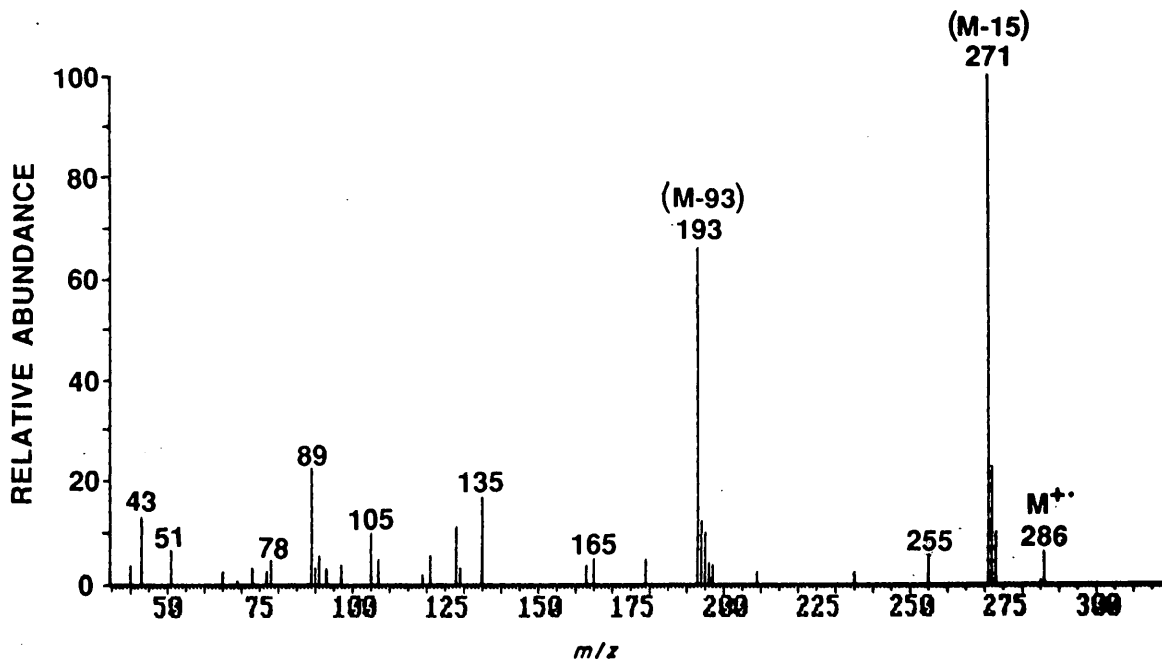


Fig. 12. Mass spectrum of an unknown compound, which gave M^+ 286, corresponding to peak at scan 468, Fig. 7; 465, Fig. 8; 465, Fig. 9; 466, Fig. 10; and 467, Fig. 11.

age soil and of no-tillage soil. Corresponding peaks in these two chromatograms can be seen. Most of the components are the same in both soil extracts except for those represented by peak at scan 466, and scan 971 in Fig. 10 and peak at 467 in Fig. 11. On the basis of the retention times and the mass spectral data obtained, most peaks were identified as labeled in Figs. 10 and 11. Peak at scan 884 in both chromatograms represented the caffeine internal-standard peak. Corresponding plasticizer peaks are also indicated on the chromatograms; the spectra of two such contained peaks were very similar to those shown in Fig. 8 and Fig. 9.

Methyl palmitate (scan 955~956), methyl stearate (scan 1094) were found in both soil extracts (Figs. 10 and 11). A methyl ester of another unsaturated fatty acid was found in both soil extracts. It was represented by peak at scan 939 in Figs. 10 and 11, and was identified as methyl-2-9-hexadecanoate or palmitoleic acidmethyl ester. It has not been determined whether it is phytotoxic.

Peaks at scan 480 and scan 1005-1006 (Figs. 10 and 11) were identified as dodecamethyl cyclohexasiloxane. It is probably the column coating material, which bled during the runs and spread throughout the column, with the concentration increasing as the temperature increased.

One previously unknown compound that was found in wheat straw and soil is thought to be an allelopathic substance (Fig. 12). This compound is represented by peaks 468 in Fig. 7; 465 in Fig. 8; 465 in Fig. 9; 466 in Fig. 10; and 467 in Fig. 11. The mass spectrum of this compound obtained with the LKB mass spectrometer showed it to have a molecular weight of 286 and to correspond to the molecular formula consisting of carbon, hydrogen, and oxygen. It is possible to draw some tentative conclusions: 1) We think that it is a methoxybenzoic ester of C_{10} alcohol or a C_{10} ene-yne corresponding to a molecular formula of $C_{18}H_{22}O_3$; 2) A partial interpretation of the mass spectra indicates that the loss of methyl group ($-CH_3$), probably from the methoxy group, gives rise to the most abundant ion at m/z 271, which is further fragmented to give ion at m/z 193, which corresponds to the loss of a $C_6 H_5 (-78)$ moiety; 3) This interpretation would agree with the allelochemical activity (32% of shoots and 11% inhibition of root growth) of germinating wheat seeds. The unknown compound appears in each of the soil extracts

during June, July, and August, but not before or after during the year. This means that this compound or its precursor was derived from the wheat plant at the time of maturity and has some capability of resisting microorganisms at least for around three months during the summers. Summers in Oklahoma are the time when the soil moisture is at its lowest level; usually in late August or early September, when rain begins, the compound is apparently degraded by soil microorganisms. Attempts to reproduce this finding of 1985 wheat crop have been frustrating; the wheat crops of 1986, 1987, 1988 have all proved not to contain this compound. If it is responsible for the inhibitory activities of the methylene chloride and chloroform extracts of new wheat straw, it must be active at microgram or picogram levels; if it is, it might be used for the preparation of herbicides or nematocides. The fact that it occurs relatively early in the elution pattern of the column indicates that it is moderately volatile. Old wheat straw had none or only traces of the compounds identified in new wheat straw and soil; this must mean that most of these compounds had either been leached out by rainfall or, since the wheat was lying on top of the soil, been decomposed photochemically or by microorganisms in or on the wheat straw. This has been reported in a preliminary manner (Waller, 1989a and b).

Conclusions

The biological activities are the results of the apparent synergistic effects of heterogeneous organic matter in the wheat straw and in the soil. The mode of action cannot be determined because of the complexity of the wheat straw-soil system. We know little about individual concentrations of phytotoxic compounds or their synergistic activity, as well as the total soil microbial activity. In general, the bioassay results indicated that the new wheat straw extracts had stronger inhibition on wheat seedling growth than that of old wheat straw; that the soil extracts were stimulatory at low concentrations, and that the basic extracts were more inhibitory than acidic extracts. The allelopathic activities between soils of CT and NT were comparative. Although the concentrations were increased from 0.55 g/ml to 2.43 g/ml, the equivalent amount of soil present per seed in the bioassays was still less than the soil mass in the natural seedling environment. Wheat seeds

planted in the natural soil environment are surrounded by more soil mass and are therefore exposed to more organic matter. Since significant inhibitions were indicated in these bioassays at moderate concentrations, it can be expected that more serious allelopathic effects on wheat seedling development may occur in the normal field environment. Fewer allelochemicals were identified in extracts of old wheat straw than those of new straw and soil corresponded with the bioassay results. These chemical characterizations may imply an allelochemical linkage between wheat straw residue and soil. Toxins may leach from wheat straw into the surrounding soil, resulting in suppression of the growth of successive plantings of wheat. It remains to be seen whether the allelopathic effects of soil can be demonstrated in the wheat field.

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新舊小麥藁在傳統耕犁及不耕犁 小麥田土中的自毒作用

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在奧克拉荷馬州 Stillwater 附近的不耕犁及傳統耕犁區的新舊小麥藁及土壤中，經由水的萃取及小麥種子的生物分析測定發芽及幼苗生長，並經分段分離後以毛細管氣相層析儀/質譜儀/資料系統分析，發現具有自毒物質的存在。新鮮的小麥藁對小麥幼苗生長有最強的抑制作用，然而在傳統耕犁及不耕犁的土壤中也顯示有相似的抑制及促進作用。有數個化合物已在氣相層析儀/質譜儀/資料系統中鑑定出來，但其各別對小麥生長的毒性作用尚未確定。