ALLELOCHEMICAL INTERACTIONS IN THE SOIL FROM NO-TILLAGE VERSUS CONVENTIONAL-TILLAGE WHEAT (*TRITICUM AESTIVUM*) SYSTEMS

By

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PREFACE

Although today's farmers have some of the most modern techniques at their disposal, one thing has not changed since man's first agricultural enterprise--lost topsoil can never be reclaimed. Therefore, the choice of an efficient tillage system is paramount in a farmer's mind. In general terms, farmers have only two choices: conventional tillage or conservation tillage. In a conventional till system, the residue from harvest is turned under the soil to facilitate decomposition. This has been the "conventional" system used ever since the necessary machinery was invented. Included in this strategy are implements such as the moldboard, V-blade, disk, and others. In conservation tillage, the residue is left exposed on the soil to "conserve" the natural resources, including topsoil. No-till, stubble mulch, and others are included in this strategy. Therefore, when CT is used in this paper, it implies conventional till, while NT means no-till, a type of conservation-tillage system.

I wish to express my sincere gratitude to all of the individuals who helped me in my course work and research, and who had confidence in me during my years at Oklahoma State University. Specifically, I thank Dr. James K. McPherson, my major adviser, for his understanding and critical comments. I also thank Drs. A. J. Pollard and G. R. Waller, who also comprised my thesis committee. Dr. Pollard's assistance with the statistical analysis was invaluable. Dr. Waller, my "companion in zealous research," had the greatest effect on the results of this thesis, and I

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Special thanks are offered to Dad and Pat for their critical reviews and constant support. Gratitude also goes to Cindy Wyman, Gene Wilde, and Lori Thorne for their many "bull sessions" over my rough drafts, statistics, and CGC/MS data. Also, a very special thanks to Danielle for her long-distance support, the most valuable of all.

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LIST OF ABBREVIATIONS

A	amperes
A.D.	Anno Domini
ANOVA	Analysis of Variance
bu/acre	bushels per acre
C	Celsius
CGC/MS/DA	Capillary Gas Chromatograph/Mass Spectrometer/ Data Analysis System
cm	centimeter
CT	conventional-tillage
DW	distilled water control
EDTA	ethylenedinitrilotetraacetic acid
eV	electron volts
HCl	hydrochloric acid
hr	hour
g	gram
m^2	meter squared
M	Molar
mg	milligram
min	minute
ml	milliliter
mM	millimolar
mV	millivolts
NaOH	sodium hydroxide
ns	not significant

NT	no-tillage
P	probability
rpm	rotations per minute
VFA	volatile fatty acids
μA	microamperes
μG	micrograms
μL	microliters

CHAPTER I

INTRODUCTION

Biochemical interactions between crop plants, weeds, and crop residues have become of great interest to investigators of allelopathy in the last decade. While many studies have suggested the incorporation of allelopathy into agricultural management systems, few have attempted to understand the complex interactions of any one specific monoculture crop and its residue management for a complete year.

Conservation tillage practices have more than doubled in the past 10 years. In 1986, it was estimated that over 43 million acres of wheat were involved in some type of conservation tillage system (Krenzer, 1987). Farmers are becoming more aware of the benefits associated with conservation tillage systems such as decreased fuel consumption, moisture conservation, and reduced soil erosion. However, with the increasing conservation tillage practices, some deleterious aspects have arisen. Weeds, microbial pathogens, and other pests seem to be more prevalent in these systems. Additionally, reduced and erratic yields are often recorded, especially in cool, moist areas.

Nevertheless, farmers must consider both options. Conventional management systems emphasize high production and high technology. This frequently translates into costly herbicide, pesticide, and fertilizer applications, as well as expensive machinery and increased fuel consumption. Conversely, conservation management systems attempt low-cost, resource-conserving practices. It has been estimated that the United

States loses 5.5 billion tons of topsoil each year (Becker, 1981)--topsoil that we cannot afford to lose. Only 12.1% of our land is free from physiochemical problems (Boyer, 1982). At this rate of loss, lower yields due to poor soil conditions are imminent. As little as three tons per acre of surface residue can reduce the soil erosion from an experimental plot by 85% (Becker, 1981). Additionally, it has been recorded that after 25 years of stubble mulch wheat farming, 14.5% more carbon and 12.2% more nitrogen are found in the top 18" of conservation-tilled soil than are found in adjacent conventionally tilled soils (Bauer, 1983).

Many researchers have isolated and identified numerous compounds from the residue of small grains (Rice, 1984; Thompson, 1985). However, some criticism has arisen concerning the severity of extraction methods. Furthermore, there is no guarantee that compounds extracted from crop residues in the laboratory are biologically active in the soil. Fuerst and Putnam (1983) suggested that, as one of the four guidelines in the proof of allelopathy, suspected compounds must be detected in the environment (soil, air, etc.) around the recipient. Buttery, Cheng-ji, and Ling (1985) isolated and identified numerous compounds from whole wheat leaves and stems by using a Tenax absorbent trap system and capillary GC/MS. These compounds included aliphatic aldehydes and ketones, aliphatic alcohols and esters, and terpenoids. The concentration of total volatile compounds isolated from the leaves and stems was estimated at 10-50 ppb. These volatiles have never been considered allelochemicals. Consequently, the primary location which allelochemicals could be harmful to wheat plants is in the soil.

The purpose of this study was to determine if surface residue on no-tillage wheat plots renders the soil allelopathic. Allelochemicals were extracted from the soil of both conventional-tillage (CT) and no-tillage

(NT) plots under mild alkaline-aqueous conditions. In an attempt to fully understand the biochemical interactions in these plots, extraction data for a full year were analyzed. Bioassays were completed on aqueous extracts (both direct aqueous extracts and the methanol-soluble portion of lyophilyzed extracts) to determine the biological activity of the allelochemicals in the soil. In addition, low resolution mass spectrometry was carried out on methanol extracts of the lyophilized extracts in an attempt to identify the putative allelochemicals in the soil.

CHAPTER II

LITERATURE REVIEW

Early History

For centuries, man has observed and recorded biochemical interactions among plants. This has been especially important in crop plants, for it is beneficial for man to understand fully that which he depends upon for survival. In 300 BC, Theophrastus described the deleterious effect that chick pea (Cicer arietinum) had on surrounding plants. He stated that chick pea "exhausted" the soil rather than reinvigorating it, as other legumes did. In about 1 AD, Plinius reported that chick pea (Cicer arietinum), barley (Hordeum vulgare), fenugreek (Trigonella foenum-graecum), and bitter vetch (Vicia ervilia), all "scorched up" the land. Plinius also gave a lengthy description on the "shade" of walnut (Juglans regia), and how it could cause headache to man and injury to plants. He reasoned that the nature of some plants (though not actually deadly), was

> . . . injurious, to its blend of scents or of juice--for instance, the radish and the laurel are harmful to the vine: for the vine can be inferred to possess a sense of smell and to be affected by odors to a marvelous degree. . . . (Plinius Secundus, 1 A.D., p. 76).

In 1822, Tull was one of the first researchers to explore tillage practices. He noted that tillage systems were spared the "soil sickness" commonly associated with conservation-tillage practices. However, it was not until DeCandolle's (cited in Rice, 1984) work in 1832 that the monoculture crops themselves were suspected as being the causative agents in

reduced yields. DeCandolle reasoned that the "soil sickness" problem in no-tillage systems could be alleviated by simple crop rotation. DeCandolle was also the first to expand the idea of plants actively exuding chemicals from their roots.

Although these early investigators had observed allelopathic interactions in detail, a formal concept of these processes did not arise until 1840, and publication of von Liebig's Organic Chemistry in its Application to Agriculture and Physiology. In this publication, von Liebig coined the term "agricultural chemistry," and described many interactions between crop plants, fertilizers, residue management systems, and the environment. However, with increasing research and interest in plant biochemical interactions, Molisch (cited in Rice, 1984) saw the need for a new and unique field of study, and thus the term "allelopathy" was coined. Molisch meant this term to refer to biochemical interactions, positive and negative, between all plants, including microorganisms.

Wheat (Triticum aestivum) and Other Cereal Grains

The majority of research dealing with allelopathy in agroecosystems, past and present, has focused on crop residues. Most researchers have reasoned that either the allelochemicals are being leached directly from the residues (especially in conservation tillage systems), or that the microflora associated with the residues are the responsible agents of release.

The first recorded work investigating crop residue toxicity was by Schreiner and Reed (1907). They demonstrated that certain crop plant residues, including wheat and oats (Avena sativa), exude materials into

the growth medium, resulting in chemotrophic responses by the roots of the same plants.

Benedict (1941) demonstrated that toxic compounds were produced by the roots of smooth brome (Bromus inermis). Oven-dried roots, either incorporated into a soil medium or extracted with water, produced autotoxic effects on seedlings.

The most complete work on allelopathy in wheat agroecosystems was initiated by McCalla and Duley in 1949 at the University of Nebraska at Lincoln. McCalla and Duley completed preliminary greenhouse experiments with wheat straw (at a rate of two to four tons/acre) and soil collected from experimental plots. Both mulched and ummulched plots were considered, and the effect on corn seedlings was noted for both. The mulched plots resulted in 44% corn germination, as opposed to 92% in the unmulched plots. Subsequent experiments were run by soaking corn seeds in aqueous extracts of wheat straw. This had little or no effect on the corn growth until ammonium nitrate was added. Then, the combination of the toxins from the straw and the increased microbial growth retarded germination and growth drastically. Guenzi and McCalla (1962) extracted numerous crop residues, including wheat and oats, with water and ethanol. In all cases, they found water and ethanol soluble substances to be toxic to test wheat seedlings.

Nordstat and McCalla (1963) further investigated the association between toxins from crop residues and microorganisms. Experimental plots involved in a crop rotation of corn, oats, and wheat were studied in an attempt to understand the reduced yields observed in all three when involved in stubble-mulch farming. The fungus Penicillium urticae was demonstrated to produce a substance toxic to the three crop seedlings. Nordstat and McCalla identified the toxin as the antibiotic patulin, an

unsaturated lactone. In the range of 20 to 75 ppm in solution, sand, and soil, it can inhibit wheat germination and root and shoot growth by 50%. It has also been shown to decrease weed emergence, and affect dicotyledons more than monocotyledons. McCalla and Haskins (1964) reported on 318 fungi isolated from the soil of stubble-mulch plots. Of these, approximately 219 produced compounds with some degree of toxicity to wheat seedlings. Ellis and McCalla (1973) reported that, of all these fungi, P. urticae comprised 90% of the total fungal population. Thus, great emphasis has been placed on P. urticae as a causative agent of reduced yields of crops involved in conservation tillage systems in Nebraska.

Further attempts by McCalla and colleagues to understand the complex interactions in these plots focused on phenolic acids. Guenzi and McCalla (1966a, 1966b) isolated and tentatively identified numerous phytotoxins from wheat residue and the soil of stubble-mulch plots. Of these, they focused upon five phenolic acids: p-coumaric, syringic, vanillic, ferulic, and p-hydroxybenzoic acids. They reported p-coumaric to be the most prevalent and the most toxic to wheat seedlings. They emphasized that both localized and synergistic effects of these compounds in the field must be considered.

Kimber (1973), in an attempt to understand how allelopathy affects the nitrogen cycle, performed a set of experiments near Adelaide, Australia, to determine why wheat residues depress the yield of subsequent wheat crops. He demonstrated that unidentified toxins are released from wheat residue, and these could have an effect on nitrogen mobilization. One interesting aspect of Kimber's study was that the addition of nitrogen fertilizer did not overcome the toxic effects of the straw. This appeared to be accentuated when the straw was allowed to remain on the surface of the plots, as in conservation-tillage systems.

A few researchers of allelopathy in cereal crops have chosen to focus on short-chain aliphatic or volatile fatty acids (VFA), especially acetic acid (Fujii, Kobayashi, and Takahashi, 1972, Lynch, 1977; Tang and Waiss, 1978; Wallace and Elliot, 1979; Cochran et al., 1983). They have all suggested that VFA are the only compounds produced in sufficient quantities from decaying residues to be toxic in the field. Cochrane (1963) was the first to have suspected VFA toxicity. However, he implied that most of the VFA, especially acetic acid, resulted from bacterial metabolism.

Barnes and Putnam (1983) investigated the use of rye as a cover crop in reduced-tillage systems. In a series of experiments, they demonstrated that rye root leachates reduced tomato dry weight by 25-30%. Additionally, spring-planted rye reduced weed biomass by 93% over plots without a cover crop of rye. It was suggested that toxins could be released from rye roots and taken up by other plants. However, Barnes and Putnam also indicated the need for radioactive tracer studies to demonstrate this conclusively.

Grodzinsky et al. (cited in Waller, 1987) reported on a method for isolation of allelochemicals by using ion-exchange resins. This mild technique allows the desired allelochemicals to be collected without destruction to the humic material. They found that cinnamic, p-coumaric, p-hydroxybenzoic, and other phenolic acids accumulate under monocultures of wheat, rye, and other cereal crops. The concentrations of these compounds seemed to increase drastically under permanent wheat culture. To alleviate this problem, they suggested and briefly discussed the addition of certain chemicals to the soil for accelerating the detoxification of these allelochemicals.

Elliott and Lynch (1984) reported on pseudomonads that inhibit winter wheat growth by the colonization of root surfaces and the production of toxins. More pseudomonads colonized the roots of plants grown in plots where wheat residue remained than where residue was removed by burning. In further support of this, Fredrickson and Elliott (1985) found that the pseudomonads produced compounds inhibitory to Escherichia coli C-1a. These inhibitory effects on the bacteria, as well as wheat seedling root inhibition, were reversed by the addition of methionine (a bacteriocide to certain pseudomonads), providing more evidence that a toxin was being produced by the pseudomonads.

Most recently, Waller (1987) investigated allelopathy in no-tillage versus conventional-tillage wheat production. He reported that both organic solvent extracts and neutral acidic and basic steam distillates of wheat soil from both conventional and no-tillage plots contain inhibitory substances. Numerous compounds, including fatty acids, alcohols, ketones, and hydrocarbons were identified by low-resolution mass spectrometry (Table I). However, no specific compound nor group of compounds were identified as the primary toxic allelochemicals in the soil.

Decomposing Residue--Soil Interactions

Guenzi and McCalla (1966b) found that aqueous acidic extractions of stubble-mulched soil contained compounds inhibitory to germinating wheat seedlings. They realized that the concentrations of the compounds may be minimal; however, they hypothesized that localized and synergistic effects of the compounds could account for the inhibition. Guenzi, McCalla, and Nordstat (1967) followed this research with a subsequent report on water extracts of decomposing residues of wheat, oats, and other crops. Nine varieties of wheat were tested in bioassays against

wheat seedlings. These included: Omaha, Nebred, Warrior, Cheyenne, Bison, Pawnee, Ponca, and Wichita. Ponca residue extracts depressed wheat germination more than the other varieties, while Nebred extracts were the least toxic. For all varieties, the unidentified toxins disappeared after eight weeks of decomposition.

TABLE I

COMPOUND GROUPS OBTAINED FROM SOIL BY STEAM DISTILLATION AS IDENTIFIED BY CGC/MS/DA SYSTEM*

	Initial	Acidic	Basic
Fatty Acids	8	20	0
Fatty Acid Esters	0	3**	0
Alcohols	1	2	3
Aldehydes	3	6	8
Ketones	1	4	2
C-H and Other N-Containing Compounds	4	5	17
S-Containing Compounds	1	1	2
Cl-Containing Compounds	2	0	0
Aromatics Not Otherwise Included	7	1	23
Aliphatics Not Otherwise Included	11	33	16
Totals	39	75	75

**All ethyl esters.

Source: G. R. Waller, Allelochemicals: Role in Agriculture and Forestry (1987).

Patrick, Toussoun, and Snyder (1963, 1964) investigated phytotoxins in the soil that originate from decomposing crop residues. They determined that toxic compounds from decomposing residues exist in the field. However, it appeared that the toxic compounds did not move very far from their origin. Thus, effects on the crop plant depend upon the frequency of encounters between the root system and the decomposing residues. Patrick, Toussoun, and Snyder also gave evidence of the rapid breakdown of these phytotoxins.

Tang and Waiss (1978) reported that the allelochemicals released from decomposing wheat residues were primarily organic acids. These included: acetic, propionic, butyric, and pentanoic acids. These, as well as numerous other compounds, have been found in decomposing rye and corn residues (Chou and Patrick, 1976).

Current Reviews of Allelopathy

The increasing amount of research investigating allelopathic interactions has created a need for reviews of current publications. Rice (1984) published Allelopathy, the most complete review of research concerning all aspects of allelopathy to date. Thompson (1985) edited the American Chemical Society Symposium (Series 168), "The Chemistry of Allelopathy: Biochemical Interactions Amont Plants," which was primarily concerned with the chemistry of plant biochemical interactions. Putnam and Tang (1986) co-edited The Science of Allelopathy, which presented the latest scientific developments regarding research in the field of allelopathy. Waller (1987) edited the American Chemical Society Symposium (Series 330), "Allelochemicals: Role in Agriculture and Forestry," which focused on allelopathy in these two respective fields.

CHAPTER III

MATERIALS AND METHODS

Sample Characteristics and Methods

Location of the Fields

Soil samples were collected from plots located at the Oklahoma State University Agronomy Farm, Efaw plots, Payne County (Range 2E, Township 19N, Section 9), Stillwater, Oklahoma. The Efaw plots were established in the fall of 1981, and have been part of a ongoing, multidisciplinary research project measuring the effects of residue management systems and planting dates on wheat production.

Characteristics of the Soil

The following were soil sample characteristics for this study:

1. Soil. All soil sampled was Easpur, occasionally flooded, fine sandy loam (Oklahoma Department of Agriculture, 1987).

2. Residue. At any one time, up to three years' accumulation of wheat residue could be recognized on the NT plots. Immediately after harvest time in June, it was possible to discern up to four years of layered residue in various stages of decomposition. Since the residue was turned under in the CT plots, minimum residue was observed.

3. Rainfall. Rainfall for the period of February, 1986, through January, 1987, was 82 cm.

Treatment of the Plots

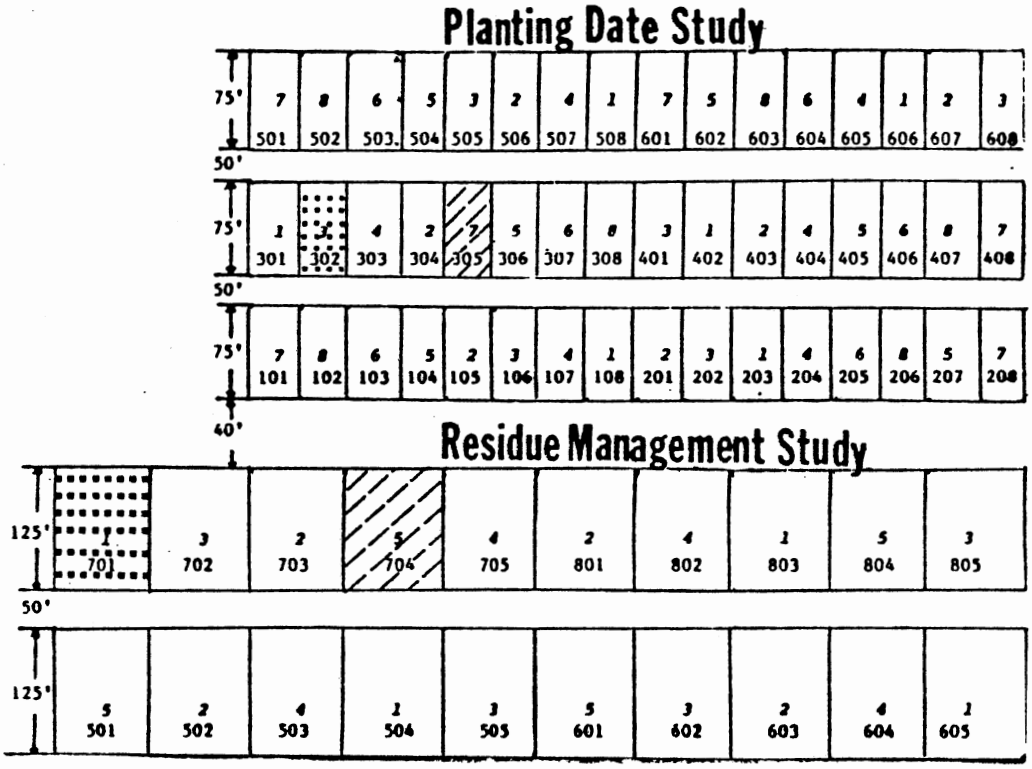
For the plots sampled, all treatments (application of herbicides, pesticides, etc.) were identical, except for the tillage systems. After harvest, the residue on the CT plots was turned under the surface to an approximate depth of 8-12" with a moldboard plow. In the NT plots, the residue was left exposed on the surface as it fell from the combine.

Sampling of the Soil

Representative soil samples were collected monthly (8-10 randomly located subsamples per plot) from the surface to a depth of 3 cm from both the CT and NT plots. Sampling began in February, 1986, and continued through January, 1987. Excess surface residue on the NT plots was brushed away so primarily soil would be collected. After thoroughly mixing the subsamples, the soil was immediately placed into two Mason quart jars, frozen with dry ice in the field, transported back to the laboratory, and stored at -18^{o}C until analysis.

Specific Plots Sampled

Throughout the study, plots 701 (CT) and 704 (NT) were sampled, excluding the months of May through September (Figure 1). An overspray of simazine (2-chloro-4, 6-bis (ethylamino)-s-triazine) in late May resulted in the killing of the NT plants, which forced the sampling of analogous plots (plots 302-CT and 305-NT of the planting data study) that had not been sprayed. However, after September, additional wheat straw was added at a rate of two to three tons per acre to the original plots. This was determined to be a sufficient amount to reinstate the plots to



Planting Date Key

1 = August CT
2 = September CT
3 = October CT
4 = November CT
5 = August NT
6 = September NT
7 = October NT
8 = November NT

Residue Management Key

1 = Moldboard Plow
2 = Disc
3 = V-Blade
4 = No-Till
5 = No-Till

(Note: •• = CT Plots Sampled; // = NT Plots Sampled)

Figure 1. Location of Conventional Till and No-Till Plots Sampled (Agronomy Farm--Efaw Plots--Stillwater, Oklahoma)

their original condition, and sampling of these plots was resumed for October.

Percentage of Soil Moisture Determination

Soil samples were removed from cold storage, allowed to thaw for 30 minutes, and approximately 100g of both the NT and CT soil was placed in a preweighed tin container. The samples were weighed to determine the wet weight, dried for 24 hours at 105^{o}C, and weighed again to determine the amount of water lost. Percentage of soil moisture was calculated as a percentage of the dry weight.

Chemical Reagents and Apparatus

Chemical Reagents

The methanol and methylene chloride used were Baker Resi-Analyzed obtained from the J. T. Baker Chemical Company, Phillipsburg, New Jersey. Ethylenedinitrilotetraacetic acid (EDTA) was also obtained from this company. Fatty acids were obtained from Nu-Chek-Prep Incorporated, Elysian, Minnesota.

Apparatus

Following is a list of apparatus used in this study:

1. pH Meter: Orion Research Model 231 S/N, pH/mV/temperature meter, Taiwan, R.O.C.

2. Triple Beam Balance: DIAL-O-GRAM, OHAUS Scale Corporation, Florham, Park, New Jersey.

3. Shaker: Rotary shaker, Model 29071, Type SA, General Electric Company, Schenectady, New York.

4. Centrifuge: Sorvall Superspeed RC2-B, automatic refrigerated centrifuge, Irving, Texas.

5. Lyophilizer: Virtis Equipment Model 10-MR-ST, Virtis Company, Gardineer, New York. Hand-built and assembled lyophilizer, utilizing Pyrex 4-port suction device with built-in cold finger; cooled with a CryoCool CC-60 from NesLab Instruments, Incorporated, Portsmouth, New Hampshire, and utilizing a SPEEDIVAC High Vacuum Pump, Model ED 35, Edwards High Vacuum Limited, Manor Royal, Crawley, Sussex, England.

6. Balance: Semimicro analytical balance, Ainsworth Type 24W, WM, Ainsworth and Sons, Incorporated, Denver, Colorado.

7. Incubator: Precision Model 805, Range $5^{o}C$ to $50^{o}C$, Precision Scientific Corporation, Chicago, Illinois.

8. CGC/MS/DA System: CGS--United Technologies Packard Model 438A capillary gas chromatograph, Model 642 Recorder, Downers Grove, Illinois; MS--LKB 2091 Gas Chromatograph/Mass Spectrometer, LKB Producter AB, Stockholm, Sweden, modified as a capillary gas chromatograph mass spectrometer (McGowan and Waller, 1986); DA--IBM Personal Computer AT, Tecknivent Model 1050 mass spectrometer data system, Tecknivent Corporation, St. Louis, Missouri.

9. Millipore MULTIFIT 2.0 and 5.0 cc Glass Syringe: Millipore Corporation, Bedford, Massachusetts, using MSI MAGNA 0.45 um, 13 mm, nylon 66 membrane filter, Fisher Scientific, Honeoye Falls, New York.

10. POWER-O-MATIC 60 Oven Dryer: Model POM-136C, Blue M Electric Company, Blue Island, Illinois.

Extraction and Purification Procedure

Attempted Extraction Techniques

Various procedures were used in an attempt to extract allelochemicals from the soil. In the first procedure, 100 g soil was combined with 100 ml methanol and shaken at 10°C for 24 hours. After being centrifuged and filtered, the extract was flash-evaporated, the residue reextracted sequentially with methanol and water, and the extracts bioassayed. These data indicated that although some of the compounds were insoluble in pure methanol or distilled water, they might be soluble in a mixture of the two solvents. Consequently, 100 ml of a 1:1 mixture of methanol and water was combined with 100 g soil, and by following similar procedures mentioned previously, bioassays were run on the resulting extracts. However, any bioassay results were considered to be a result of residual methanol, and not to extracted compounds.

In an attempt to rule out any possible inorganic influence, extracts were made with EDTA to chelate any ions and possibly free the organic acids and alcohols for extraction. For this procedure, 100 g soil was combined with 100 ml 1.0 M EDTA and mixed with a magnetic stirrer for 20 minutes. The pH of one extraction was adjusted to pH 8.0 $\pm$ 0.2 with 1 M NaOH, while the pH of another remained at pH 4.0 $\pm$ 0.2. Both mixtures were shaken for eight hours at 10°C, centrifuged, filtered, and extracted with methylene chloride in a separatory funnel. Bioassays were run on both the methylene chloride fraction and the aqueous EDTA fraction. These data indicated that no compounds with biological activity were present in the methylene chloride fraction, and while the EDTA fraction had activity, the EDTA itself was extremely toxic to the seedlings.

Next, a milder aqueous extraction was employed. In this procedure, both acidic (pH 4.0) and basic (pH 8.0) extracts were prepared. After a general procedure of combining 200 g soil with 400 ml water, adjusting the pH (to either pH 4.0 or pH 8.0), filtering, centrifuging, and lyophilizing, bioassays were completed on both extracts.

Introduction to Extraction Techniques

Mild procedures were finally adopted in an attempt to focus on those chemicals actually available to plants. Extracts were obtained with slightly basic aqueous soil mixtures (Figure 2). After the basic soil slurry had been shaken for two hours at 5-10^{o}C, the mixtures were filtered and centrifuged to obtain clear solutions. After lyophilization, the dry residues were weighed and then extracted with methanol to produce two different concentrations. Additionally, a quantity of the dry residue was extracted with methanol for CGC/MS data analysis. All extracts, excluding those solely for the purpose of CGC/MS data analysis, were bioassayed by testing the response of wheat seedling radicle and shoot growth. This procedure was repeated at least twice for every month of sampling.

Soil Extraction Procedure

To obtain data for each month, both the CT and the NT samples were taken from storage at -18^{o}C and allowed to thaw at room temperature for approximately 30 minutes, or until the soil could be removed from the Mason jar. A 400 g sample of this soil was combined with 800 ml of triply distilled water in two Mason quart jars. After the initial pH of the soil-water slurry had been measured, it was adjusted to pH 8.0 $\pm$ 0.2 by using 1M NaOH, and the slurry was shaken for two hours at 5-10^{o}C. The

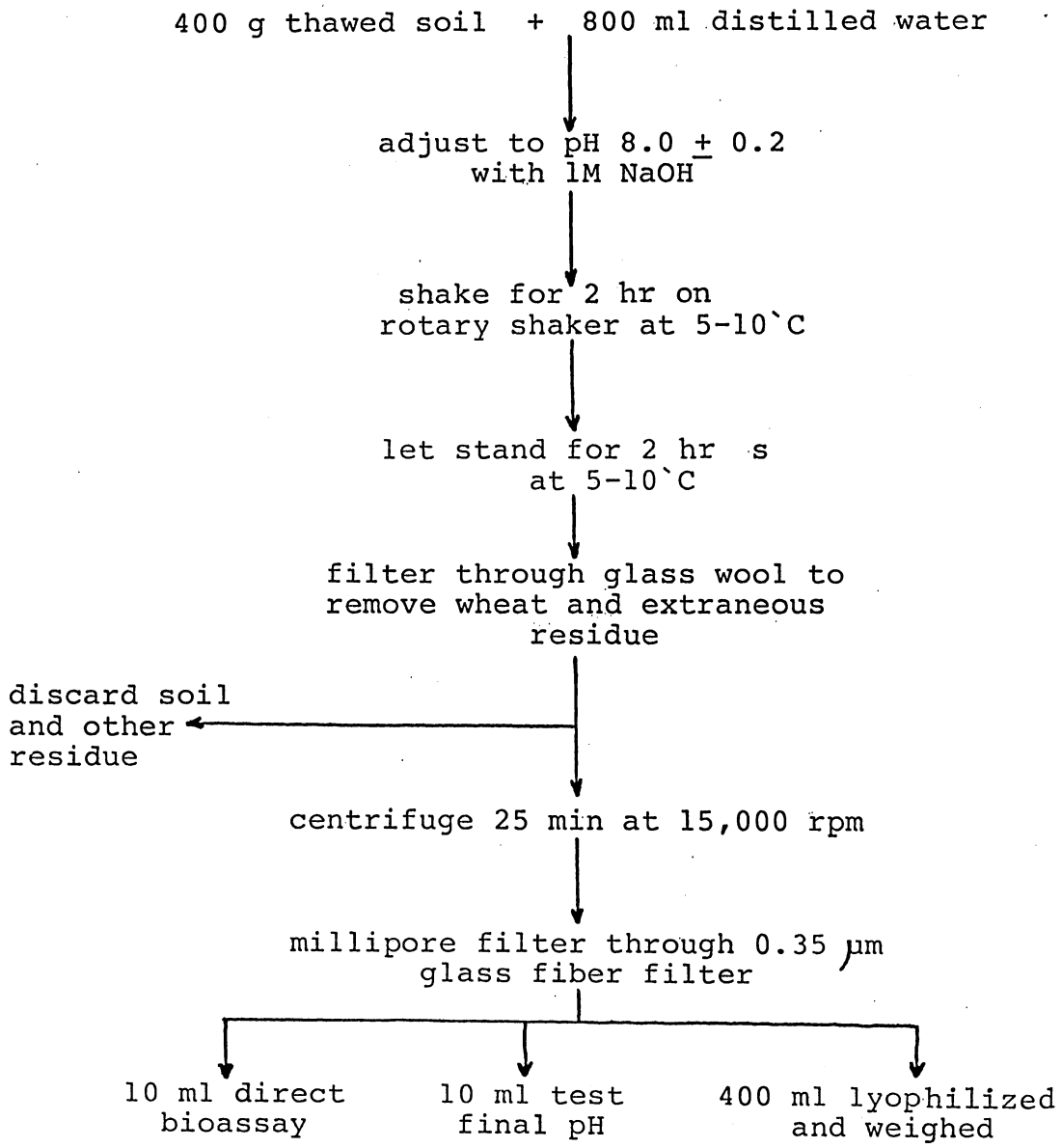


Figure 2. Extraction Technique Used to Isolate Organics From Soil

heavy soil particles were then allowed to settle before the samples were filtered through glass wool to remove any floating wheat residue. This extract was subjected to refrigerated centrifuging in teflon centrifuge tubes for 25 minutes at 15,000 rpm, and was filtered through Gelman type A/E 0.35 um glass fiber filter. Ten milliliters of the crude extract was set aside for a direct bioassay, 10 ml was used to check the final pH, and 400 ml was lyophilized. The remaining soil-water slurry was discarded, and the lyophilized residue was weighed and stored at room temperature in amber bottles for further analysis. This procedure was repeated at least twice for each month of sampling.

Preparation of Extracts for Bioassay

The following extracts were utilized in this study:

1. Aqueous Bioassay: A bioassay was performed on 10 ml of the crude aqueous extract.

2. Bioassay of Methanol Soluble Material: Two concentrations of the lyophilized residue were tested for biological activity by using simple calculations based on the amount of residue obtained (Appendix A). In each case, the predetermined amount of residue was extracted three times with 10 ml portions of hot methanol, and any insoluble residue was removed by water suction filtration through 4.25 cm Whatman #1 qualitative filter paper. The methanol was removed by evaporation overnight under a hood, and the residue was dissolved in 10 ml of methanol for bioassay.

Bioassay Procedure and Analysis

Bioassay Procedure of Unknowns

For each bioassay, four glass Petri dishes (100 x 15 cm) were used, with two sheets of 7.5 cm Whatman #1 qualitative filter paper. Ten seeds of Pioneer 2157 wheat were placed in a radial pattern with the micropyle end toward the center between the two sheets. Two milliliters of the appropriate solvent or extract was applied to each dish, and a square of plastic wrap was placed over the bottom half of the dish before the top was pressed on, to reduce moisture loss.

Three different bioassays were conducted for each soil sample extracted (CT and NT): a direct aqueous bioassay, amethanol bioassay of the lyophilized extract at the same concentration as the direct aqueous bioassay, and a methanol bioassay of the lyophilized residue at three times the direct aqueous bioassay concentration. After 72 hours of incubation in the dark, the roots and shoots of each seedling were measured in millimeters. For the methanol bioassays, the solvent control and the methanol extracts were applied to the filter paper and allowed to evaporate completely (approximately two hours) before exposure to seeds. Two milliliters of distilled water was then used to moisten the paper.

Statistical Analysis of Bioassay Data

Analyses of Variance (ANOVA) were completed for each months and the entire year of data. The purpose of these tests was to determine whether root or shoot lengths showed statistically significant differences associated with the tillage treatment of the soils, associated with the date on which soils were collected, or associated with a date x treatment interaction. The latter would imply that the effects of tillage

practices vary at different times during the year. For each specific date, a one-way ANOVA examining differences due to treatment (NT, CT, or Control) was carried out. More effects and interactions for the entire year included treatment, date, and the date x treatment interactions. Analyses were carried out using the SAS mainframe computer package, general linear models procedure for unbalanced ANOVA (Torrie and Steel, 1917).

In order to tell which treatments differed from which others, Tukey's Studentized Range Test for root and shoot means was completed for each specific date and for the entire year. Additionally, a correlation analysis between the growth of the aqueous bioassay seedlings, as expressed as a percentage of control, and the amount of lyophilized residue obtained was completed for both root and shoot variables to determine whether quantitative amounts of extractable material explained the variation in growth.

Bioassay Procedure for Pure Fatty Acids

To determine any biological activity, bioassays were carried out on five pure fatty acids. Solutions of palmitic, stearic, myristic, eicosanoic, and heptadecanoic acids were made at 1.0 mM concentrations. Then, following procedures like those used for the bioassay of the unknowns, each fatty acid was studied separately, in every possible combination of two, three, four, and all five collectively. In all cases, the total concentration was held at 1.0 mM. Additionally, a bioassay of all five fatty acids at a total concentration of 5.0 mM was carried out.

Analysis of Organic Compounds From the Soil

Extraction of Lyophilized Residue

To obtain data for each month, 150 mg of the lyophilized residue was extracted three times with 25 ml portions of hot methanol. The solvent-residue mixture was placed on a hot plate under a hood and, with frequent stirring, allowed to begin to boil. To separate the methanol soluble organic fraction from the insoluble particulate fraction, the mixture was suction-filtered through 4.25 cm Whatman #1 qualitative filter paper, the methanol was removed by evaporation under a hood overnight, and the remaining organic compounds were reextracted three times with 1.0 ml portions of hot methanol. The extract was further purified by filtering with a 2.0-5.0 cc Multifit type B-D syringe and 0.45 μm MSI nylon membrane filter. The sample was then placed into a 2 ml conical Reacti-Vial, and evaporated to dryness under nitrogen.

Conversion of Compounds to Methyl Esters

Approximately 0.5-1.0 ml of diazomethane was added to the dry, crude residue for the conversion. The diazomethane was obtained from Dr. E. J. Eisenbraun of the Chemistry Department at Oklahoma State University, and had been synthesized as described by Ruehle, Browne, and Eisenbraun (1979). Upon addition, the sample was shaken until the characteristic yellow color disappeared (approximately two hours), indicating completion of the reaction. The excess diazomethane and solvent (ethyl ether) were removed under nitrogen. As an internal standard, 1.0 ul of caffeine (1 mg/μl) was added to each sample.

Low-Resolution Mass Spectrometry

1. Low-resolution mass spectra of the samples treated with diazomethane were obtained using a LKB-2091 capillary gas chromatograph/mass spectrometer/data analysis system (GCG/MA/DA). The capillary column was a J&W DW-5, 60 m x 0.32 mm, connected directly to the ion source of the mass spectrometer (McGowan and Waller, 1986). Up to 2.0 μl of the sample in methanol was injected with a 1:4 split at 65°C. After four minutes, the temperature was programmed to rise 10°C per minute until it reached 300°C, and was held there for 10 minutes. After the sample passed through the mass spectrometer, the incoming data were transferred through an electron multiplier and analog-to-digital converter, to an IBM AT computer equipped with the Tecknivent programming system. The data system employed in both the acquisition and analysis of the data was the Tecknivent Model 1050 MS data analysis system.

2. Mass spectrometer conditions: separator temperature (171°C, initial eV (21), scan eV (70), box current (15-20 μA), accelerating voltage (3.2-3.4V), filament current (3.3A), trap current (80-100 A), multiplier voltage (600 mV), and source temperature (265°C).

3. Identification of spectra: the reconstructed spectra of the unknown compounds were identified by comparison with known spectra of standard compounds given in the Eight Peak Index of Mass Spectra (1983), Waller (1972), and Waller and Dermer (1980).

CHAPTER IV

EXPERIMENTAL RESULTS AND DISCUSSION

Percentage of Soil Moisture

The percentage of soil moisture values appeared to have no significant correlation with the bioassay results (Table II). This could be due to several factors. The soil moisture values were obtained on July 29, 1987, from stored soil samples. Thus, some samples had been in cold storage for over a year. It is not known what effect this had on the moisture in the soil, but a loss of moisture could be expected. Additionally, the specific date of collection and corresponding environmental conditions must be considered. Thus, these percentages were not representative of an entire month.

Previous studies by McCalla and Duley (1949), McCalla and Haskins (1964), and Guenzi and McCalla (1966a, 1966b) indicated that the reduced and erratic yields often associated with conservation-tillage systems in Nebraska are influenced by rainfall. Therefore, yield reduction may be a reflection of the amount of annual precipitation in any given geographic area. There is reason to believe that this connection between moisture availability and crop residue toxicity is occurring in Oklahoma and other states. However, the harmful effects may be "masked" by the beneficial retention of moisture. These results do support moisture retention as a definite advantage in conservation-tillage systems.

TABLE II

PERCENTAGE OF SOIL MOISTURE PER MONTH OF SAMPLED SOIL

Month	NT	CT
February	19.2	17.0
March	18.9	18.8
April	23.9	20.8
May	20.4	22.3
June	19.0	16.0
July	9.2	3.5
August	10.1	9.7
September	8.0	6.0
October	17.0	17.2
November	17.5	14.0
December	17.3	17.4
January	22.5	13.4

Statistical Analysis of Bioassay Data

Analysis of Direct Aqueous Bioassay Data

As indicated by the ANOVA results of the yearly aqueous bioassay data for both dependent variables, all main effects and interactions were highly significant ($p < 0.0001$) (Table III). Thus, there was significant biological activity between dates and treatments. Closer examination of the root and shoot means by Tukey's Test (Table IV) illustrated that, for an entire year, bioassays of conventional-till soil extracts had the highest mean, followed by the distilled water control and then the no-till extracts. ANOVA results month-by-month (Table V) showed considerable significant differences among the main effects. Closer analysis by Tukey's Test (Table VI) illustrated that, most often for both root and

shoot, bioassays of the conventional-till soil extracts had the highest mean.

TABLE III

ANOVA OF DIRECT AQUEOUS BIOASSAY DATA
FOR COMPLETE YEAR

Dependent Variable: Root				
Source	DF	Type III SS	F Value	PR>F
Date	11	28142.04	42.92	0.0001
Treatment	2	3408.77	28.60	0.0001
Date * Treatment	22	8532.54	6.51	0.0001
Dependent Variable: Shoot				
Source	DF	Type III SS	F Value	PR>F
Date	11	1050.84	16.04	0.0001
Treatment	2	148.39	12.46	0.0001
Date * Treatment	22	438.59	3.35	0.0001

Graphic illustration of these data expressing growth as a percentage of control showed the statistical significance (Figure 3). Also illustrated are three months of severe inhibition. The months of June, July, and August showed significant inhibition for both NT and CT treatments. This was expected, as harvest was in early July, and fresh residue was lying on the surface of both the NT and the CT plots. This was

supportive of the observations of Guenzi, McCalla, and Nordstat (1967). They found that residue toxicity changed during initial decomposition in the field. The toxicity of wheat residue extract was approximately the same for the first four weeks, but was virtually absent by eight weeks. Thus, with harvest of the experimental plots in early July, it was expected that numerous compounds were being released by the initial decomposition of the residue. By September, most of the compounds had already been released, and the residue on the CT plots had been turned under. Consequently, a drastic decrease in toxicity was recorded in the CT extracts, while a slight decrease was recorded in the NT plots.

TABLE IV

TUKEY'S STUDENTIZED RANGE TEST OF DIRECT AQUEOUS BIOASSAY DATA FOR COMPLETE YEAR

	Grouping	Mean (mm)	Treatment
Root			
	a	28.83	CT
	b	27.56	DW
	c	26.04	NT
Shoot			
	a	7.47	CT
	a	7.42	DW
	a	6.97	NT

Note: Means with the same letter are not significantly different. (CT = Conventional-tillage, NT = No-tillage, and DW = Distilled water control.)

TABLE V

SUMMARY FOR MONTHLY ANOVA OF DIRECT AQUEOUS BIOASSAY DATA TESTING SIGNIFICANCE OF TREATMENTS

Month	Root	Shoot
January	ns	ns
February	***	**
March	***	**
April	***	ns
May	***	*
June	***	ns
July	***	*
August	***	**
September	*	*
October	**	ns
November	ns	ns
December	***	***

Note: ***$p < 0.0001$, **$p < 0.001$, *$p < 0.05$, and ns $p > 0.05$.

The next time of highly significant inhibition was recorded in February and March. Here, environmental conditions must be considered. The CT extracts showed very little inhibition, or even significant stimulation. By now, the turned-under residue in the CT plots was almost entirely decomposed, and thus there were very few components being released in a diffuse manner. Conversely, the NT extracts showed highly significant exhibition. The warm, moist conditions are thought to facilitate the decomposition of residue from the last year's harvest and all previous harvests.

TABLE VI

RESULT OF TUKEY'S STUDENTIZED TEST, MONTH-BY-MONTH,
FOR DIRECT AQUEOUS BIOASSAY DATA

ROOT

JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
CT-a	CT-a	CT-a	CT-a	CT-a	CT-a	DW-a	DW-a	CT-a	CT-a	DW-a	CT-a
DW-a	DW-a	DW-a	NT-b	NT-a	DW-b	NT-b	CT-b	DW-a	DW-ab	CT-a	DW-ab
NT-a	NT-b	NT-b	DW-b	DW-b	NT-c	CT-b	NT-b	NT-a	NT- b	NT-a	NT- b

SHOOT

CT-a	DW-a	CT-a	CT-a	CT-a	CT-a	DW-a	DW-a	CT-a	CT-a	CT-a	CT-a
NT-a	CT-ab	DW-ab	NT-a	NT-ab	DW-a	NT-ab	NT-b	NT-ab	DW-a	DW-a	DW-a
DW-a	NT- b	NT- b	DW-a	DW- b	NT-a	CT- b	CT-b	DW- b	NT-a	NT-a	NT- b

Note: Means with the same letter are not significantly different; means arranged in descending order in columns. (CT = Conventional-tillage, NT = No-tillage, and DW = Distilled water control.)

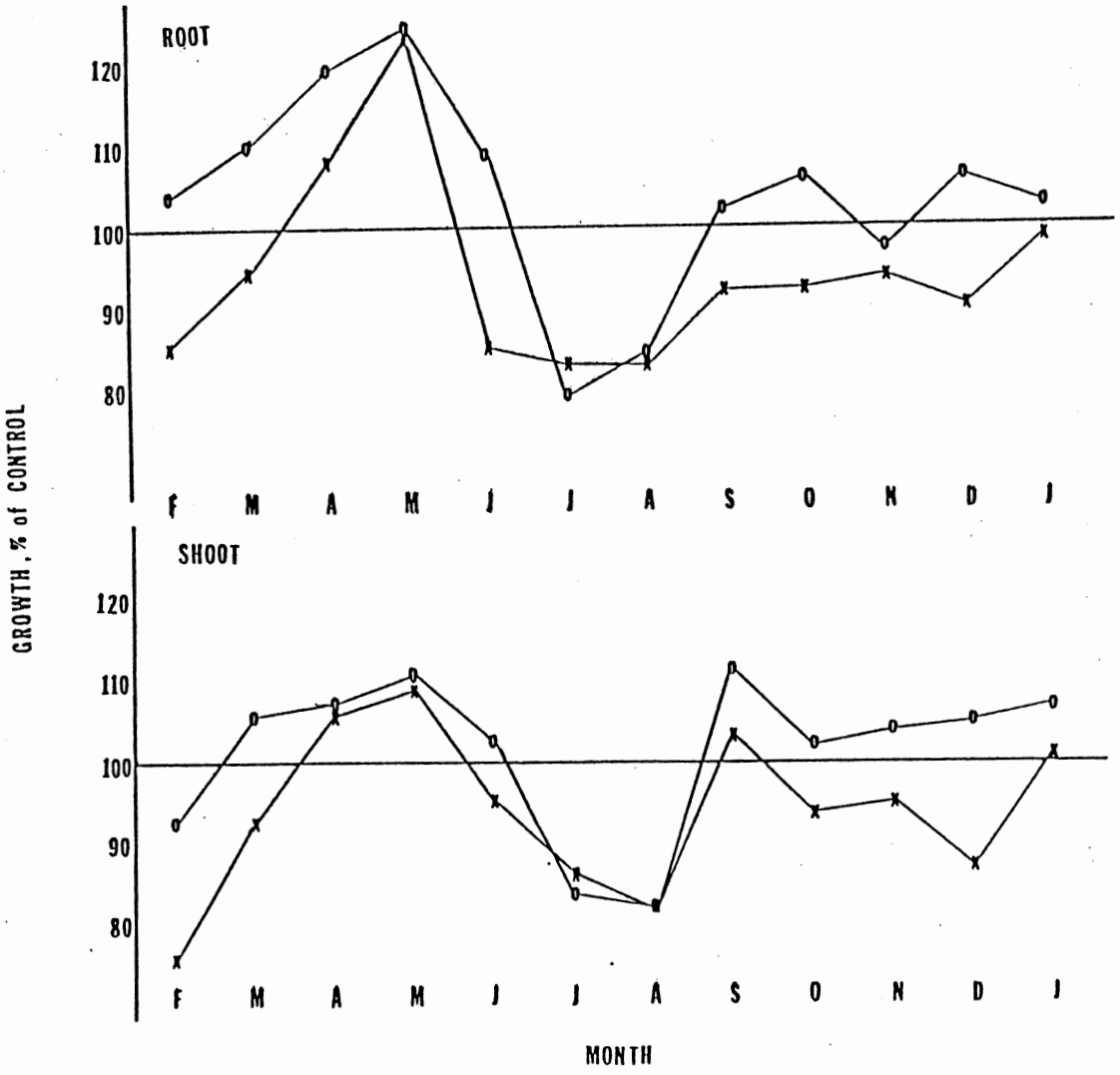


Figure 3. Growth as a Percentage of Control From Bioassay of Direct Aqueous Extract of NT (X) and CT (O) Soils, From February, 1986, to January, 1987

Analysis of Bioassay Results of Methanol Soluble Compounds in the Lyophilized Aqueous Extract at Approximately the Same Concentration as Aqueous Bioassay (Dilute Methanol Bioassay)

Theoretically, if the putative allelochemicals in the extracts are soluble in water, it is probable that they are soluble in methanol as well. Therefore, these data should be a reflection of the aqueous bioassay data. Analysis of these data indicated that this was the case. ANOVA results of the yearly methanol bioassay data showed that all interactions and effects were highly significant (Table VII). To better understand these data, Tukey's Test was completed (Table VIII). As with the aqueous extracts, the NT extracts showed significant inhibition; however, for the root means, the CT extract was significantly less than the distilled water means. Similarly, the shoot means of the two were not significantly different. This indicated that there were possible toxic allelochemicals in both the CT and NT extracts. Again, ANOVA of the monthly data followed a pattern similar to that of the aqueous bioassay data, but less significance was present (Table IX). Closer analysis by Tukey's Test indicated that the NT extract was almost always inhibited (Table X). However, the extract from CT and the control are interchangeable.

Graphic illustration of these data followed the same trends as for the aqueous bioassay (Figure 4). The two main periods of significant inhibition (June, July, and August; February and March) were recorded. It was clear that less deviation from the control line (100%) was present in these data points. This lack of deviation was a reflection of the purification of the extracts. Primarily organic compounds were present

in the methanol bioassay that could affect seedling germination and growth, while inorganics could be present in the aqueous extract. This was further supported by the lack of substantial stimulation caused by fertilizer application (as in the aqueous bioassay). The high amount of shoot stimulation in December was unexplained, as was the root inhibition in July.

TABLE VII

ANOVA OF BIOASSAY DATA FOR DILUTE METHANOL SOLUTIONS OF LYOPHILIZED MATERIAL FOR COMPLETE YEAR

	Dependent Variable: Root			
Source	DF	Type III SS	F Value	PR>F
Date	11	21045.91	44.64	0.0001
Treatment	2	1761.73	20.55	0.0001
Date * Treatment	22	3507.78	3.72	0.0001
	Dependent Variable: Shoot			
Source	DF	Type III SS	F Value	PR>F
Date	11	1342.61	23.69	0.0001
Treatment	2	123.25	11.96	0.0001
Date * Treatment	22	689.72	6.08	0.0001

TABLE VIII

TUKEY'S STUDENTIZED RANGE TEST FOR BIOASSAY DATA FOR DILUTE METHANOL SOLUTIONS OF LYOPHILIZED MATERIAL FOR COMPLETE YEAR

	Grouping	Mean (mm)	Treatment
Root			
	a	26.29	DW
	b	25.19	CT
	c	24.46	NT
Shoot			
	a	7.36	CT
	a	7.29	DW
	b	6.85	NT

Note: Means with the same letter are not significantly different. (CT = Conventional-tillage, NT = No-tillage, and DW = Distilled water control.)

Analysis of Bioassay Data for Concentrated Methanol Solutions of Lyophilized Material

ANOVA of root bioassay data showed highly significant differences associated with date, treatment, and date * treatment. However, only differences due to the date in the shoot data were significant (Table XI). This indicated that biological activity was present in both the NT and CT extracts. Closer analysis of the significant variation in root length by Tukey's Test indicated that the NT and CT extracts were significantly inhibitory compared to the control (Table XII). Also, the NT extracts were inhibited significantly in comparison to the CT extracts. Thus, biological activity was present in both CT and NT extracts, but was

more noticeable in the NT extracts. ANOVA for the monthly data showed a great deal of significance, especially in the more sensitive root means (Table XIII). Analysis by Tukey's Test showed that only a few of the sample dates had significant differences between treatments (Table XIV). Therefore, at high concentrations, both CT and NT extracts contained compounds that were inhibitory to test seedlings.

TABLE IX

SUMMARY FOR MONTHLY ANOVA OF BIOASSAY DATA FOR DILUTE METHANOL SOLUTIONS OF LYOPHILIZED MATERIAL FOR COMPLETE YEAR

Month	Root	Shoot
January	ns	***
February	ns	*
March	ns	ns
April	ns	ns
May	ns	ns
June	*	*
July	***	*
August	***	***
September	***	ns
October	***	ns
November	**	***
December	**	***

Note: ***$p < 0.0001$, **$p < 0.001$, *$p < 0.05$, and ns $p > 0.05$

TABLE X

RESULT OF TUKEY'S STUDENTIZED TEST, MONTH-BY-MONTH, FOR BIOASSAY DATA FOR DILUTE METHANOL SOLUTIONS OF LYOPHILIZED MATERIAL FOR COMPLETE YEAR

ROOT											
JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
DW-a	DW-a	CT-a	DW-a	NT-a	DW-a	DW-a	DW-a	DW-a	DW-a	DW-a	CT-a
NT-a	CT-a	NT-a	CT-a	CT-a	CT-ab	NT-b	NT-b	CT-b	CT-a	CT-ab	DW-ab
CT-a	NT-a	DW-a	NT-a	DW-a	NT- b	CT-c	CT-b	NT-b	NT-b	NT- b	NT- b
SHOOT											
DW-a	CT-a	CT-a	CT-a	NT-a	DW-a	DW-a	DW-a	CT-a	DW-a	DW-a	CT-a
CT-b	DW-ab	DW-a	NT-a	DW-a	CT-ab	CT-ab	NT-b	DW-a	CT-a	NT-a	DW-b
NT-b	NT- b	DW-a	CT-a	NT-b	NT- b	CT- b	NT-a	NT-a	CT-b	NT-b	NT-b

Note: Means with the same letter are not significantly different; means arranged in descending order in columns. (CT = Conventional-tillage, NT = No-tillage, and DW = Distilled water control.)

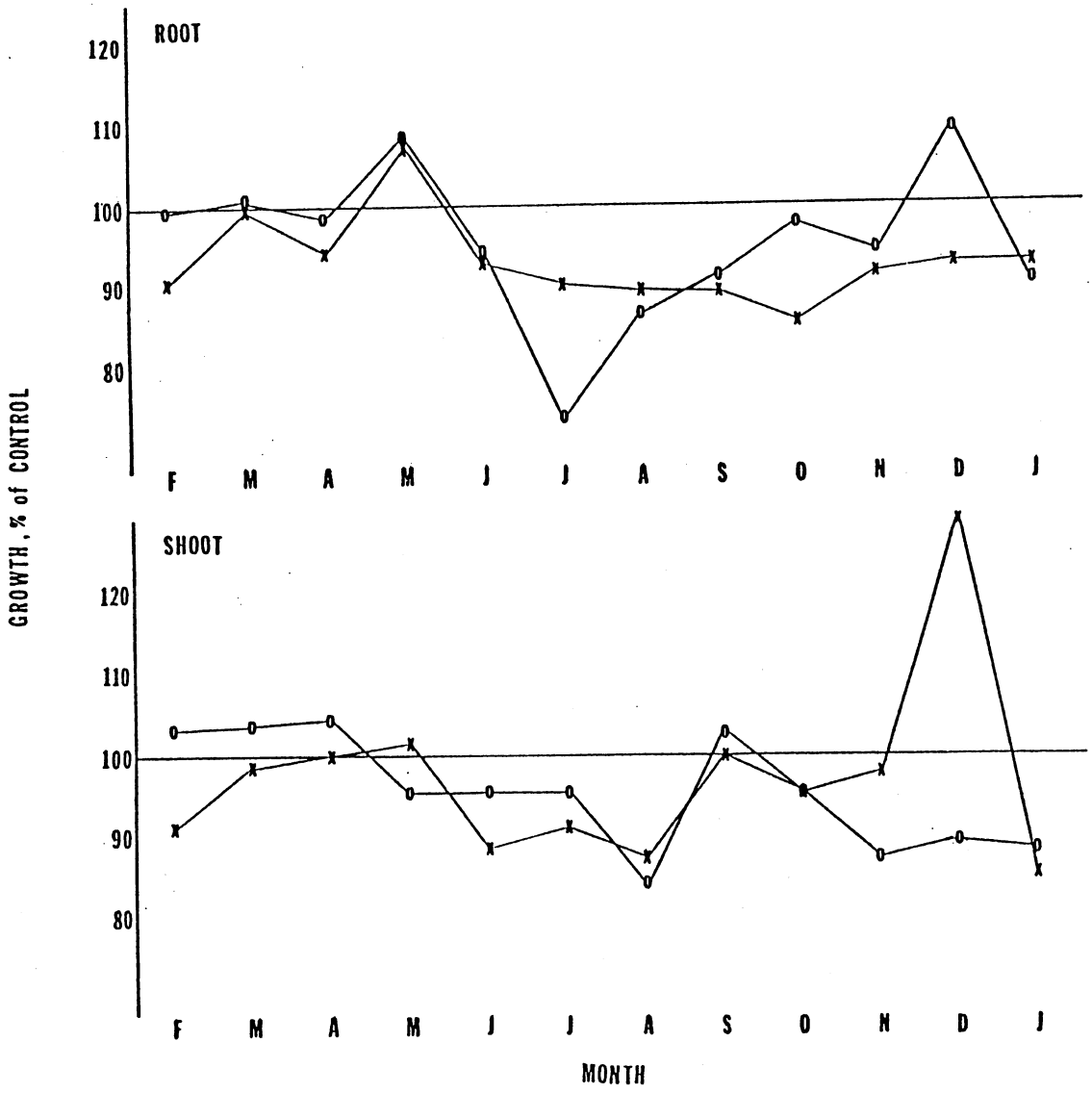


Figure 4. Growth as a Percentage of Control From Bioassay of Methanol Extract of Lyophilized Residue at Approximately the Same Concentration as the Aqueous Bioassay of NT (X) and CT (O) Soils, From February, 1986, to January, 1987

TABLE XI

ANOVA OF BIOASSAY DATA FOR CONCENTRATED SOLUTIONS OF LYOPHILIZED MATERIAL IN METHANOL

Dependent Variable: Root				
Source	DF	Type III SS	F Value	PR>F
Date	11	19199.52	40.42	0.0001
Treatment	2	5118.47	59.27	0.0001
Date * Treatment	22	4363.25	4.59	0.0001
Dependent Variable: Shoot				
Source	DF	Type III SS	F Value	PR>F
Date	11	1649.53	2.73	0.0016
Treatment	2	175.87	1.60	0.2019
Date * Treatment	22	1112.95	0.92	0.5667

Graphic illustration of these data show that inhibition was the rule at high concentrations (Figure 5). Again, the greatest inhibition occurred in June, July, and August, Nevertheless, the general trend still indicated that NT extracts resulted in more inhibition.

Correlation Analysis Between Growth, Percentage of Control, and Amount of Lyophilized Residue

Scatter diagrams were computed for root and shoot, NT, and CT to test if the total amount of lyophilized residue had any effect on the

aqueous bioassay results (Figures 6 and 7). The lack of significance indicated that no correlation existed. Therefore, the chance of toxic allelochemicals being present in the extracts was strengthened.

TABLE XII

TUKEY'S STUDENTIZED RANGE TEST OF BIOASSAY DATA FOR CONCENTRATED SOLUTIONS OF LYOPHILIZED MATERIAL IN METHANOL

	Grouping	Mean	Treatment
Root			
	a	26.29	DW
	b	24.49	CT
	c	23.09	NT
Shoot			
	a	7.54	CT
	a	7.27	DW
	a	6.84	NT

Note: Means with the same letter are not significantly different. (CT = Conventional-tillage, NT = No-tillage, and DW = Distilled water control.)

Low-Resolution Mass Spectrometry Analysis Identification of Methanol-Soluble Compounds in the Lyophilized Extract

Gas chromatograms and mass spectra of such compounds from both treatments for February, March, June, and May samples were analyzed by

the researcher and Dr. G. R. Waller of Oklahoma State University, using a LKB-2091 CGC/MS/DA system (Figures 8-14).

TABLE XIII

SUMMARY FOR MONTHLY ANOVA OF BIOASSAY DATA FOR CONCENTRATED SOLUTIONS OF LYOPHILIZED MATERIAL IN METHANOL

Month	Root	Shoot
January	ns	*
February	***	ns
March	ns	ns
April	*	ns
May	ns	ns
June	***	***
July	***	*
August	***	***
September	***	**
October	***	**
November	***	***
December	***	ns

Note: ***$p < 0.0001$, **$p < 0.001$, *$p < 0.05$, and ns $p > 0.05$

In each sample, all possible compounds were identified, with particular emphasis on the fatty acids. It was evident that there were more peaks in the NT chromatograms than in the CT chromatograms. In all cases, caffeine peaks represented the internal standard, and plasticizers constituted artifactual products in the soil (Waller, 1987). In all months and treatments, the most prevalent fatty acids were the methyl esters of palmitic and stearic acids. Shorter-chain fatty acids included

TABLE XIV

RESULT OF TUKEY'S STUDENTIZED TEST, MONTH-BY-MONTH, BIOASSAY DATA FOR CONCENTRATED SOLUTIONS OF LYOPHILIZED MATERIAL IN METHANOL

ROOT											
JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
DW-a	DW-a	DW-a	DW-a	DW-a	DW-a	DW-a	DW-a	DW-a	DW-a	DW-a	CT-a
NT-a	CT-ab	CT-a	CT-ab	CT-a	CT-a	NT-a	CT-b	NT-b	CT-b	CT-a	DW-a
NT-b	NT- b	NT-a	NT- b	NT-a	NT-b	CT-b	NT-b	CT-b	NT-b	NT-b	NT- b
SHOOT											
CT-a	CT-a	NT-a	CT-a	DW-a	CT-a	DW-a	DW-a	CT-a	DW-a	DW-a	DW-a
DW-ab	DW-a	CT-a	NT-a	CT-a	DW-a	NT-ab	NT-b	DW-a	CT-b	NT-a	NT-a
NT- b	NT-a	DW-a	DW-a	NT-a	NT-b	CT- b	CT-b	NT-b	NT-b	CT-b	CT-a

Note: Means witn the same letter are not significantly different; means arranged in descending order in columns. (CT = Conventional-tillage, NT = No-tillage, and DW = Distilled water control.)

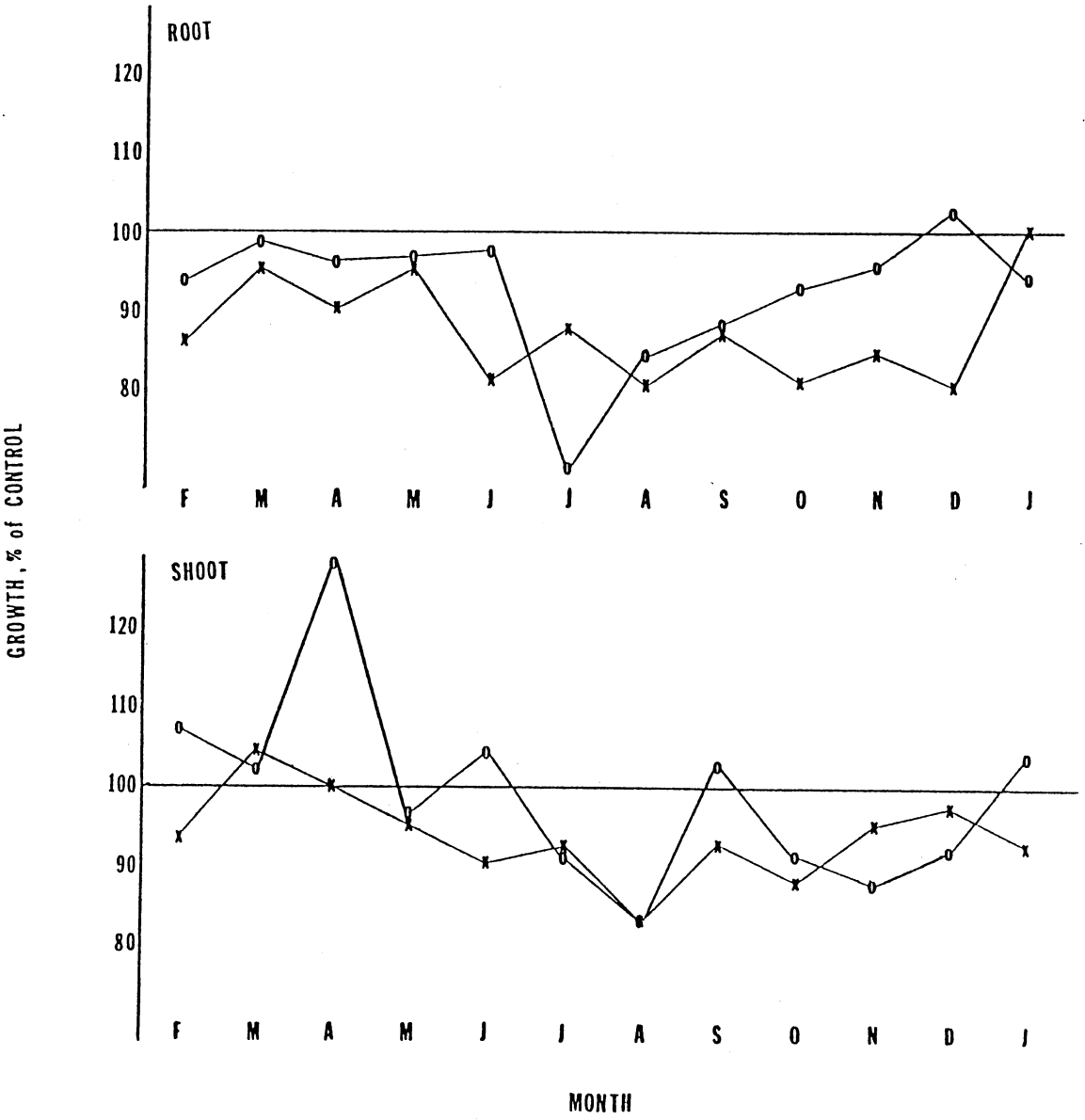


Figure 5. Growth as a Percentage of Control From Bioassay of Methanol Extract of Lyophilized Residue at Approximately Three Times the Concentration as the Aqueous Bioassay of NT (X) and CT (O) Soils, From February, 1985, to January, 1986

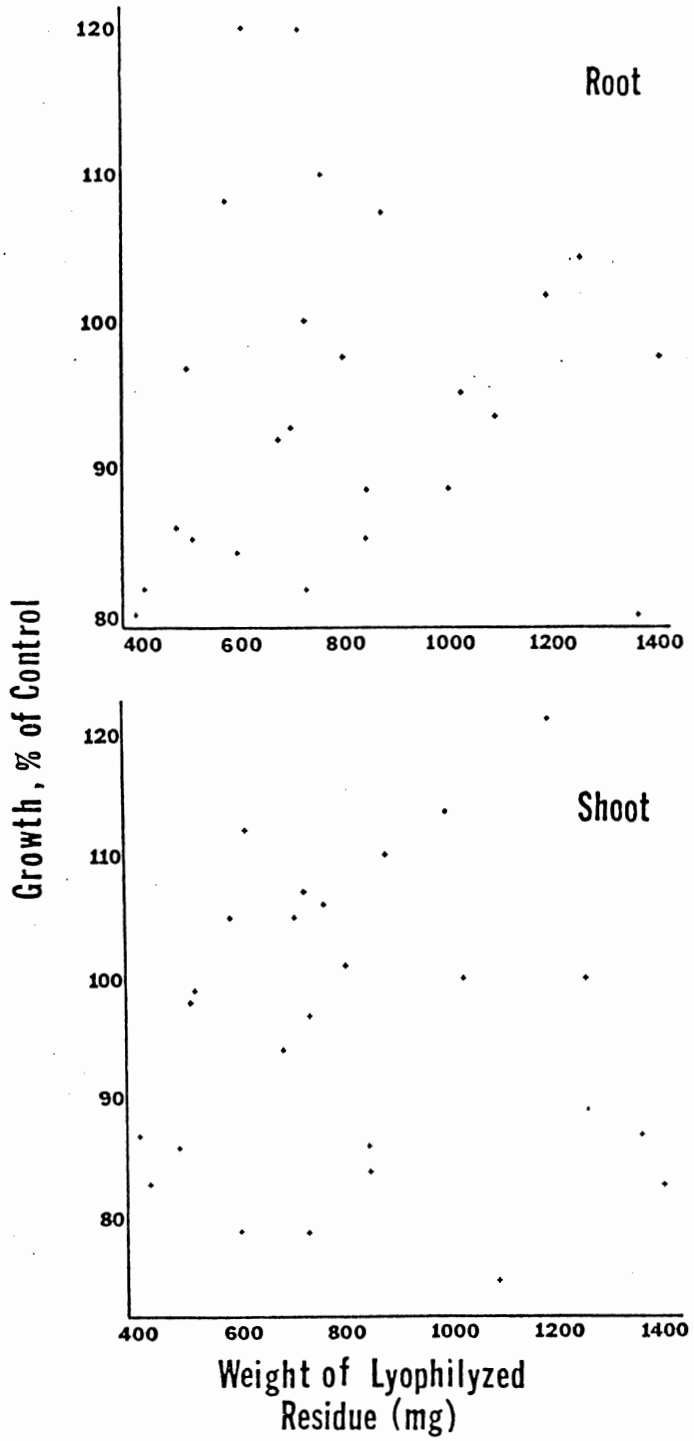


Figure 6. Scatter Diagram Showing No Significant Correlation Between Growth, Percentage of Control, and Amount of Lyophilized Residue Obtained From NT Extracts

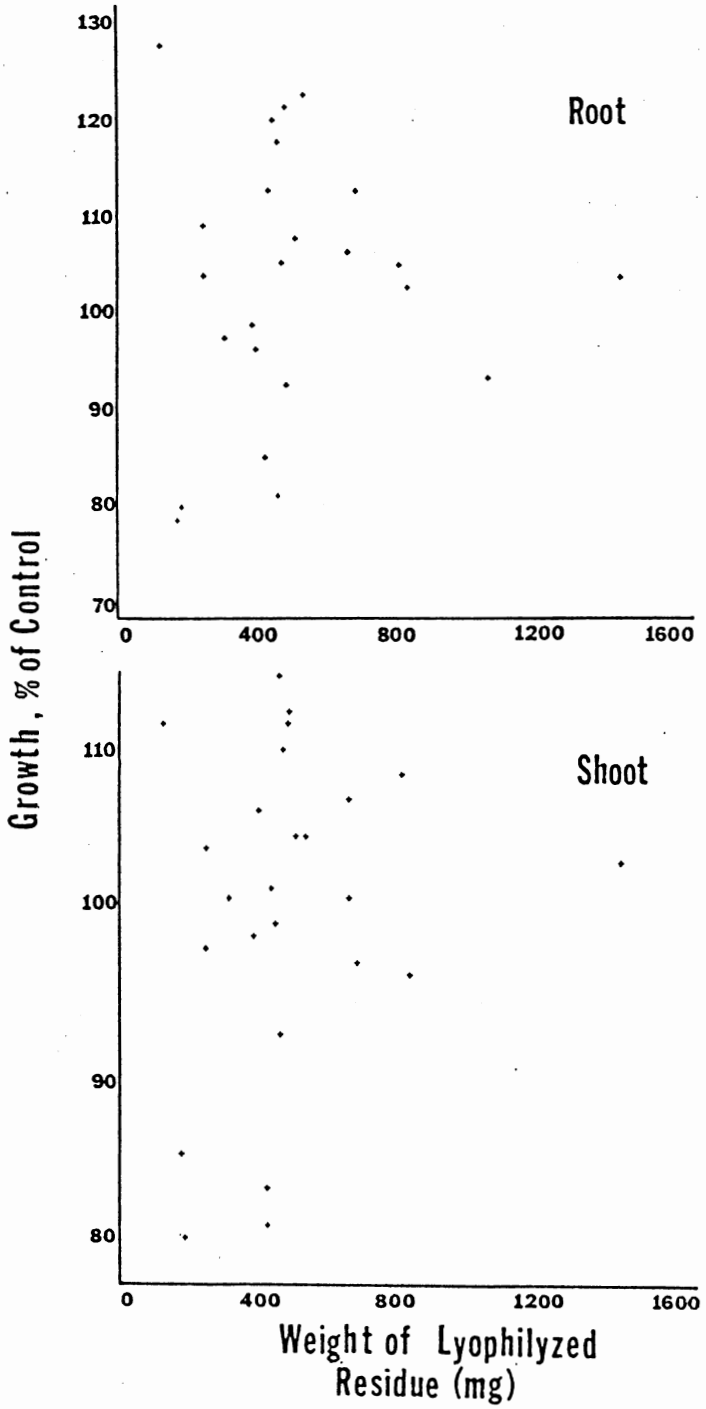


Figure 7. Scatter Diagram Showing No Significant Correlation Between Growth, Percentage of Control, and Amount of Lyophilized Residue Obtained From CT Extracts

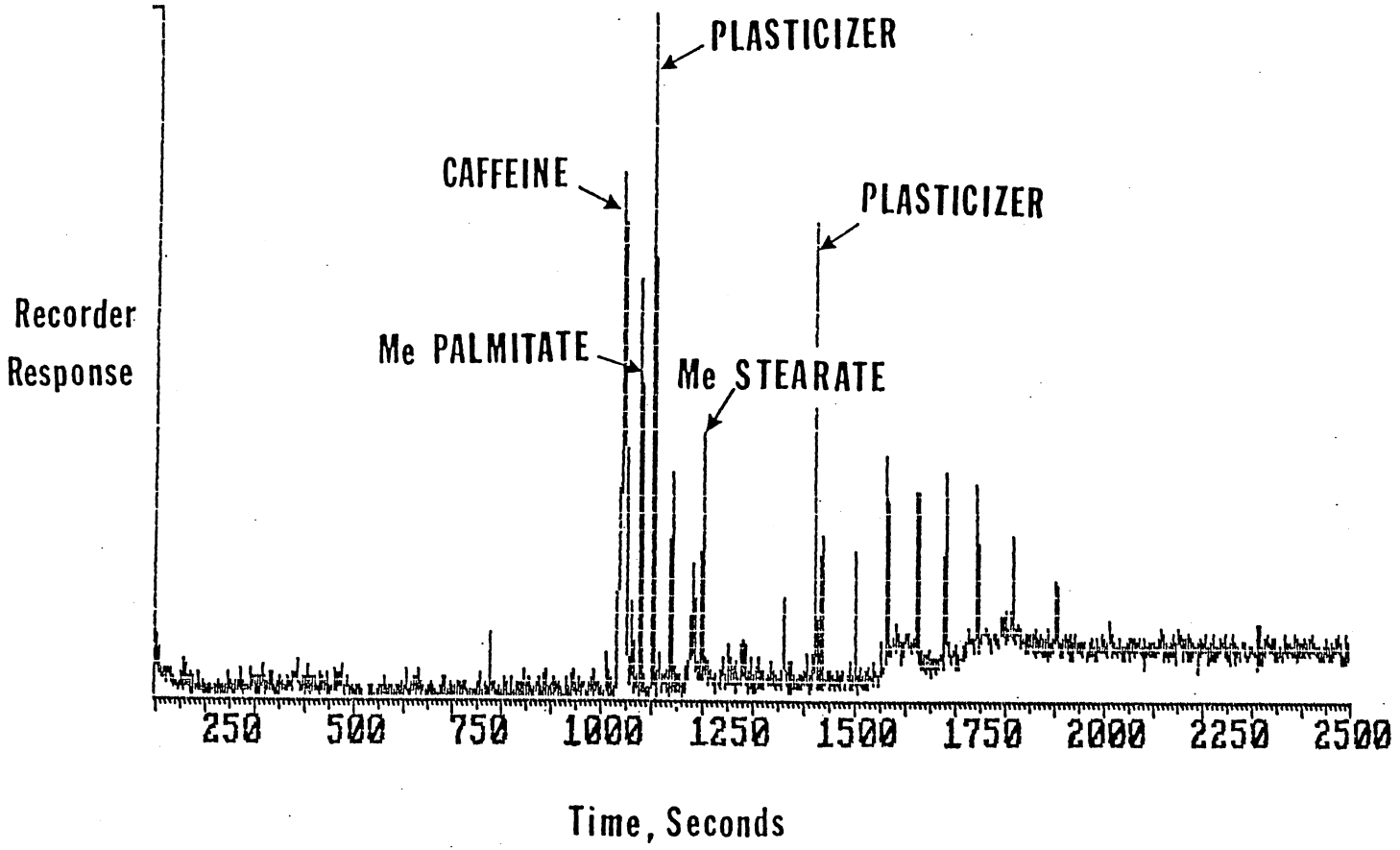


Figure 8. Reconstructed Total Ion Current Chromatogram of a NT Soil Extract (LKB-2091 CGC/MS/DA): Peaks Represent Compounds, Identified Peaks Labeled. Soil Sample: February, 1986

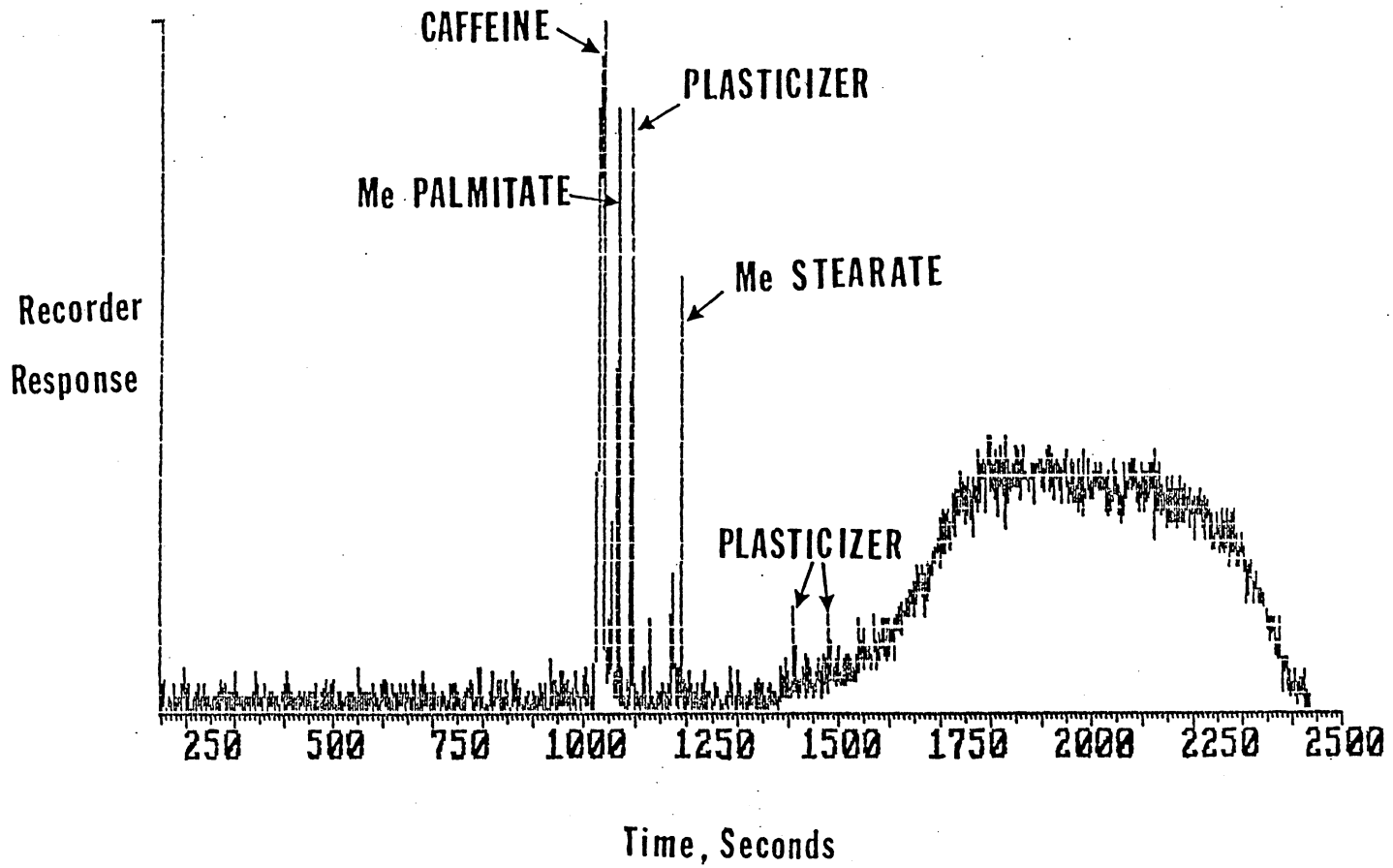


Figure 9. Reconstructed Total Ion Current Chromatogram of a CT Soil Extract (LKB-2091 CGC/MS/DA): Peaks Represent Compounds, Identified Peaks Labeled. Soil Sample: February, 1986

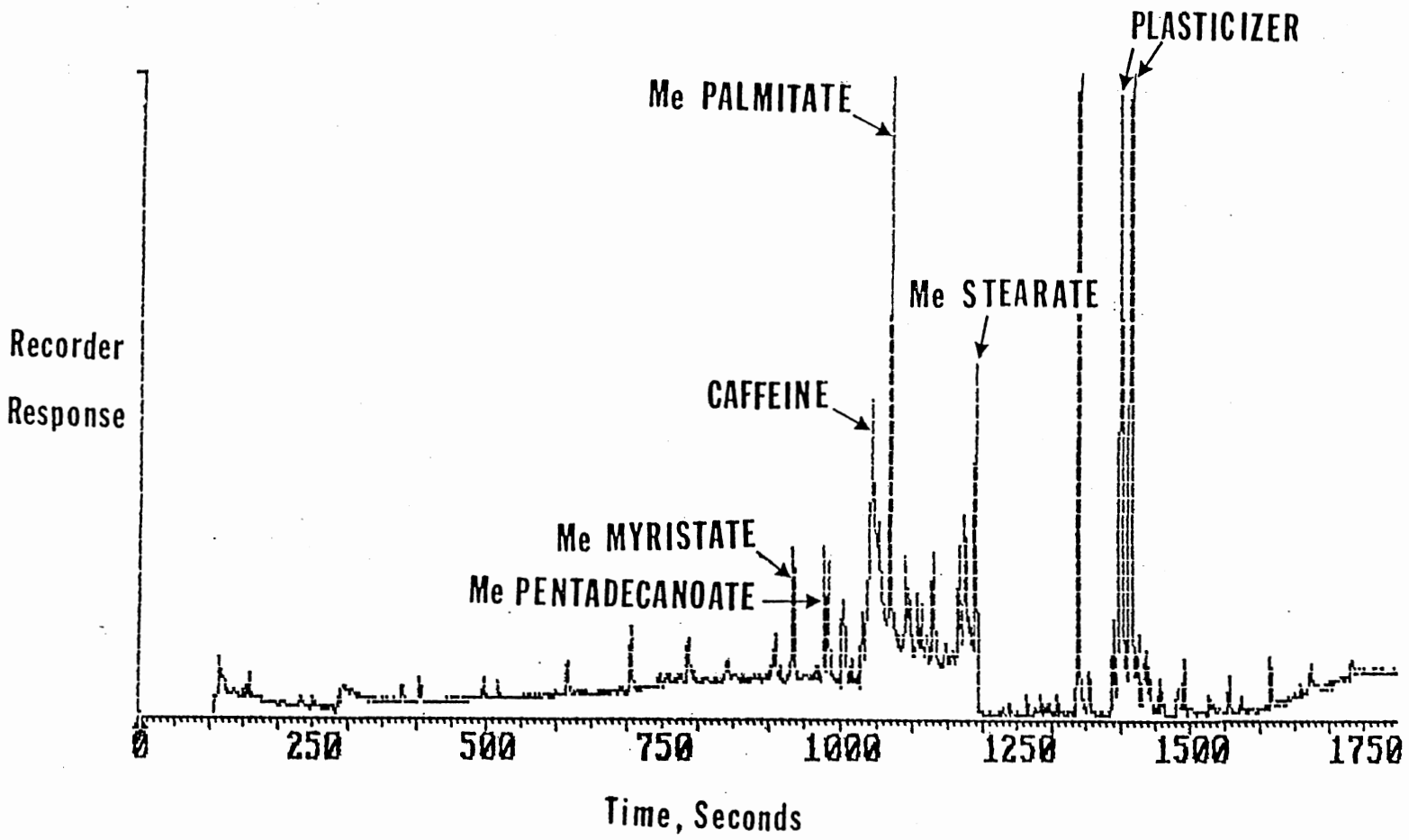


Figure 10. Reconstructed Total Ion Current Chromatogram of a NT Soil Extract (LKB-2091 CGC/MS/DA): Peaks Represent Compounds, Identified Peaks Labeled. Soil Sample: April, 1986

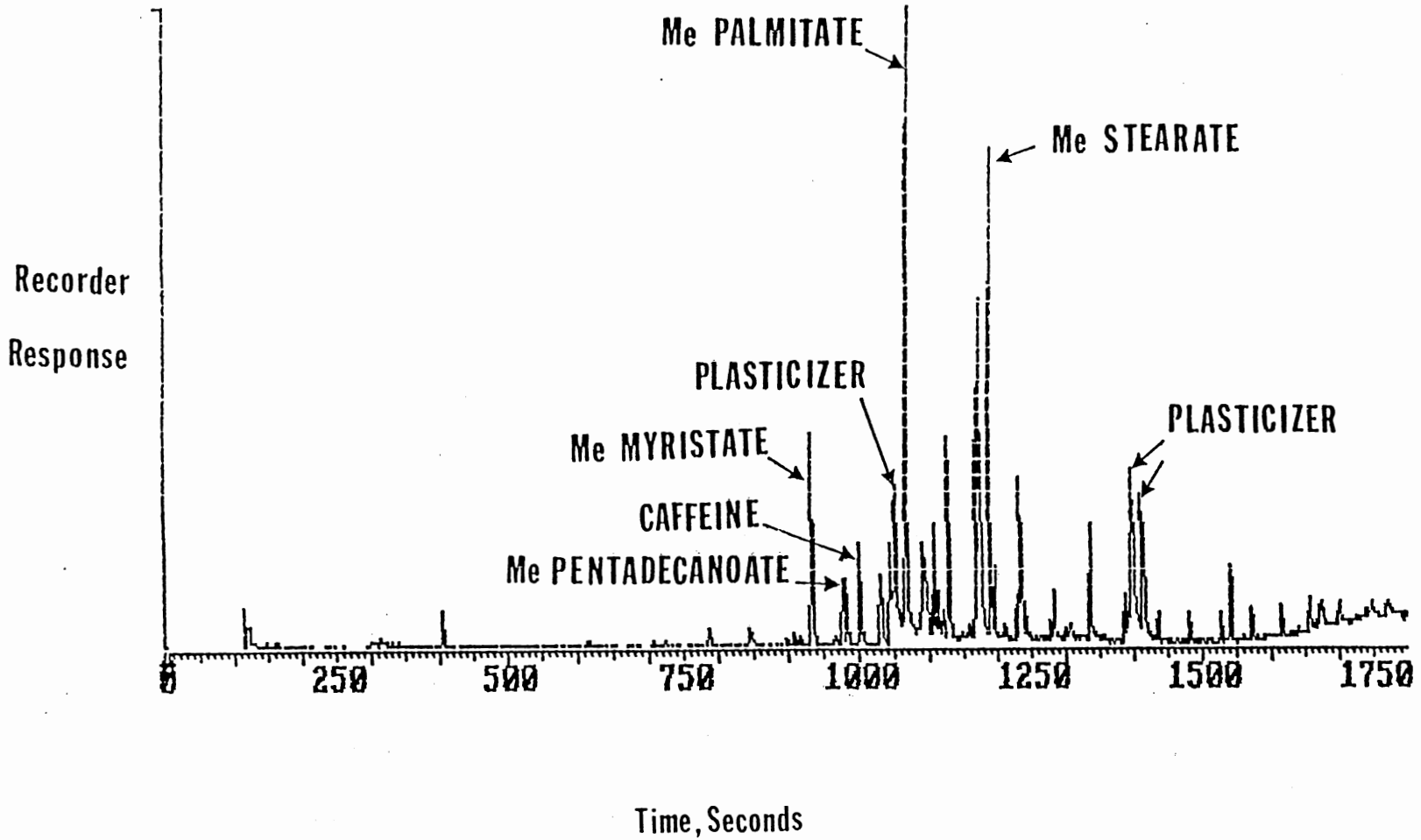


Figure 11. Reconstructed Total Ion Current Chromatogram of a CT Soil Extract (LKB-2091 CGC/MS/DA): Peaks Represent Compounds, Identified Peaks Labeled. Soil Sample: April, 1986

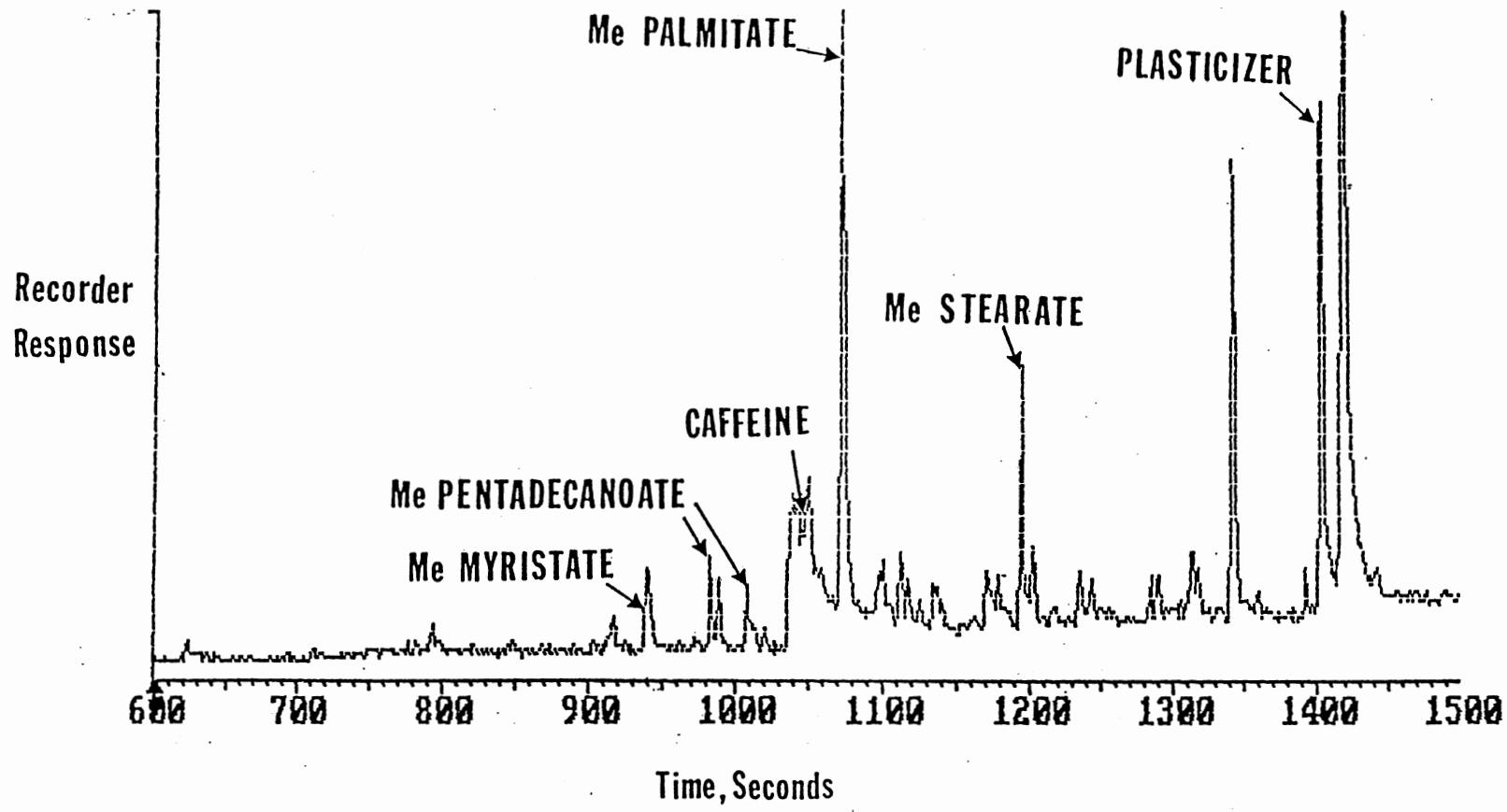


Figure 12. Reconstructed Total Ion Current Chromatogram of a NT Soil Extract (LKB-2091 CGC/MS/DA): Peaks Represent Compounds, Identified Peaks Labeled. Soil Sample: May, 1986

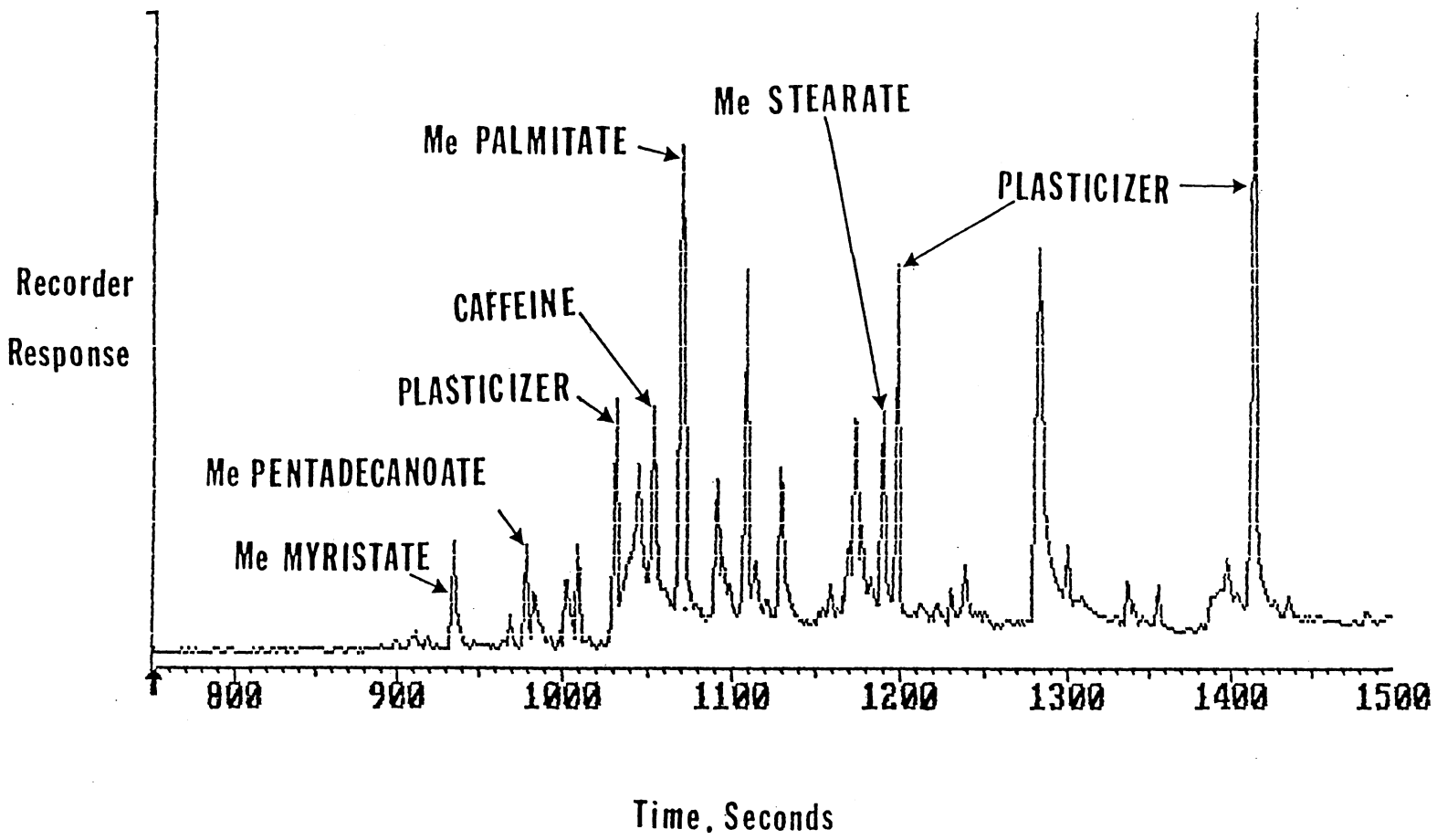


Figure 13. Reconstructed Total Ion Current Chromatogram of a NT Soil Extract (LKB-2091 CGC/MS/DA): Peaks Represent Compounds, Identified Peaks Labeled. Soil Sample: June, 1986

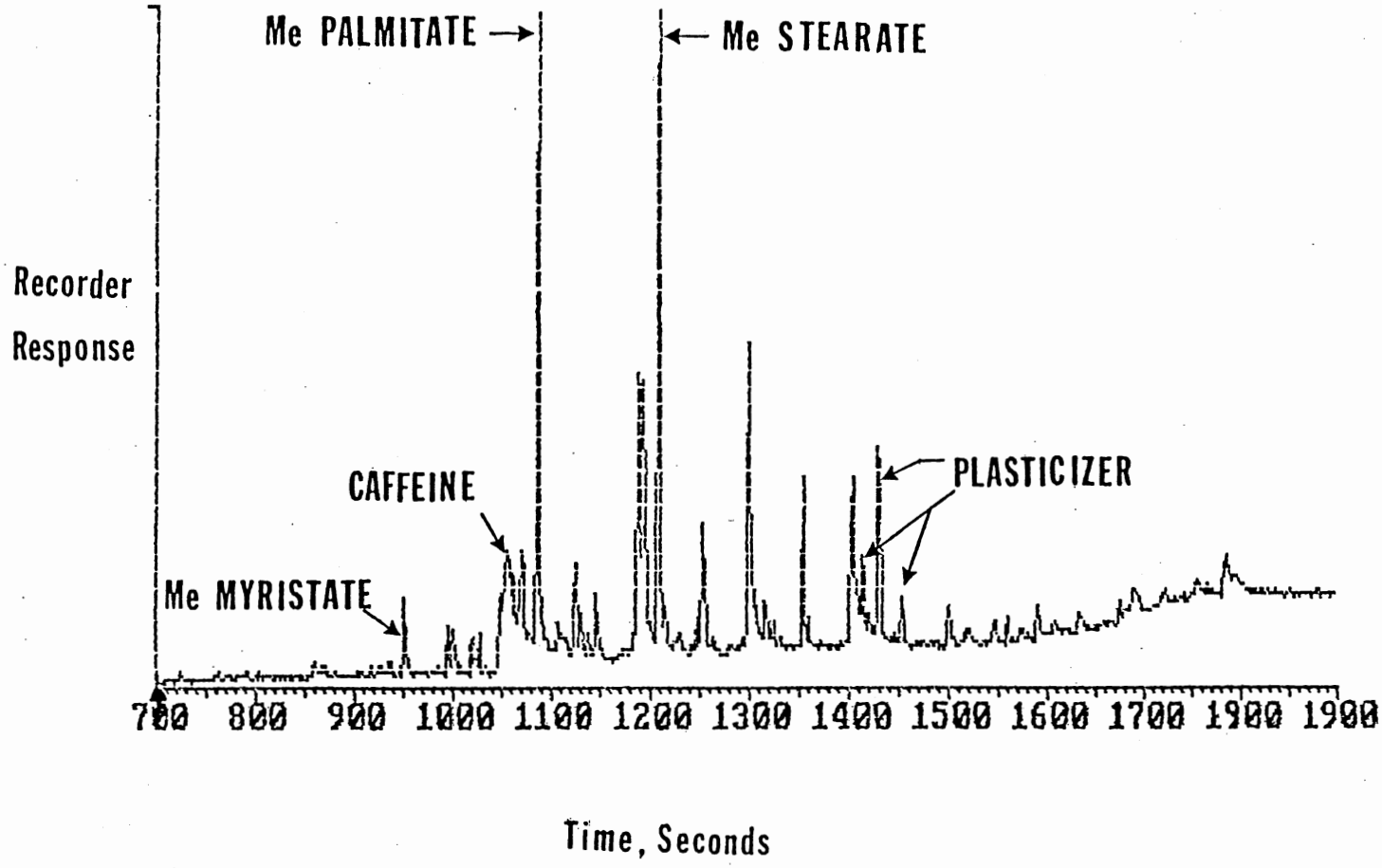


Figure 14. Reconstructed Total Ion Current Chromatogram of a CT Soil Extract (LKB-2091 CGC/MS/DA): Peaks Represent Compounds, Identified Peaks Labeled. Soil Sample: June, 1986

methyl myristate and methyl pentadecanoate. These compounds, caffeine, and the phalate plasticizer gave separate spectra and chromatograms shown in Figures 15 through 20.

In relation to the bioassays, the low-resolution analysis was invaluable. The high degree of inhibition recorded in February and June was reflected here. In each case, NT extracts were the most inhibitory, and NT chromatograms contained the most peaks. Therefore, the NT extracts have some compounds not found in the CT extracts. The February NT chromatogram contained 17 definite peaks, while the February CT contained only 8. Similarly, the NT June chromatogram contained 26 definite peaks, while the CT June had only 20. This concept was further supported by the April chromatograms. April NT and CT bioassay data were not significantly different, and this was reflected by a similar number of peaks.

As previously mentioned, fatty acids were regarded in this investigation as possible allelochemicals in the soil of conservation-tillage wheat systems. Not only were these the main organic compounds in the extracts, but other researchers have investigated the role of fatty acids as possible allelochemicals. Most of this research has focused upon volatile, short-chain fatty acids (Tang and Waiss, 1978; Lynch, 1977), but some long-chain ones have been considered (Spoehr et al., 1949). Alsaadawi, Rice, and Karns (1983) investigated allelopathic interactions between Polygonum aviculare and Cynodon dactylon. They reported that the sodium salts of long-chain fatty acids found in the littler and soil surrounding P. aviculare were toxic to the grass, as well as some nitrogen-fixing bacteria. More recently, fatty acids have been suspected of toxicity in aquatic ecosystems (Rice, 1984; Waller, 1987).

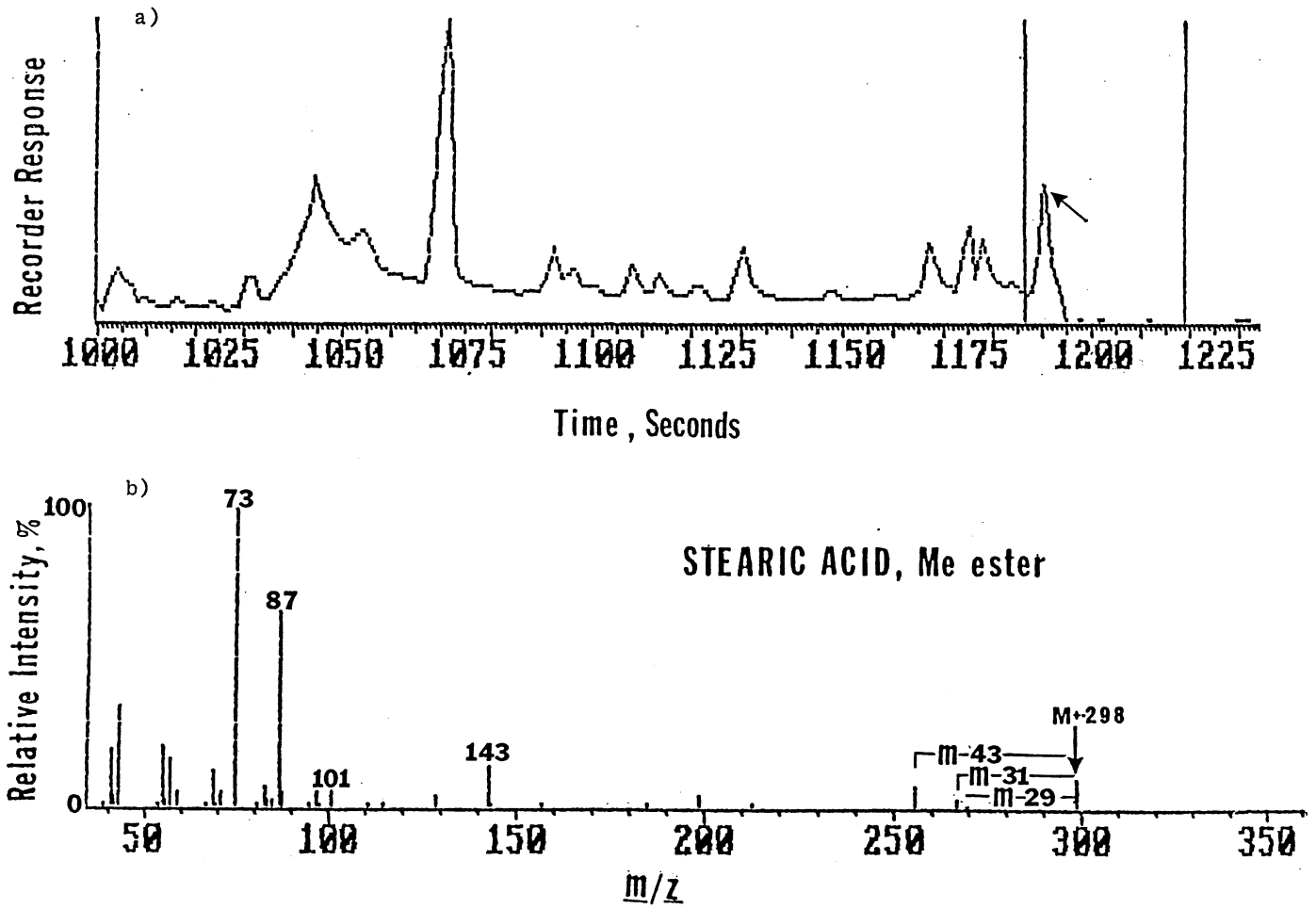


Figure 15. (a) Reconstructed Part of Total Ion Current Chromatogram of a Soil Extract--(LCB-2091 CGC/MS/DA): Peaks Represent Compounds

(b) Mass Spectrum of Palmitic Acid, Methyl Ester, That Corresponds to Peak 10885 Marked With Cursor

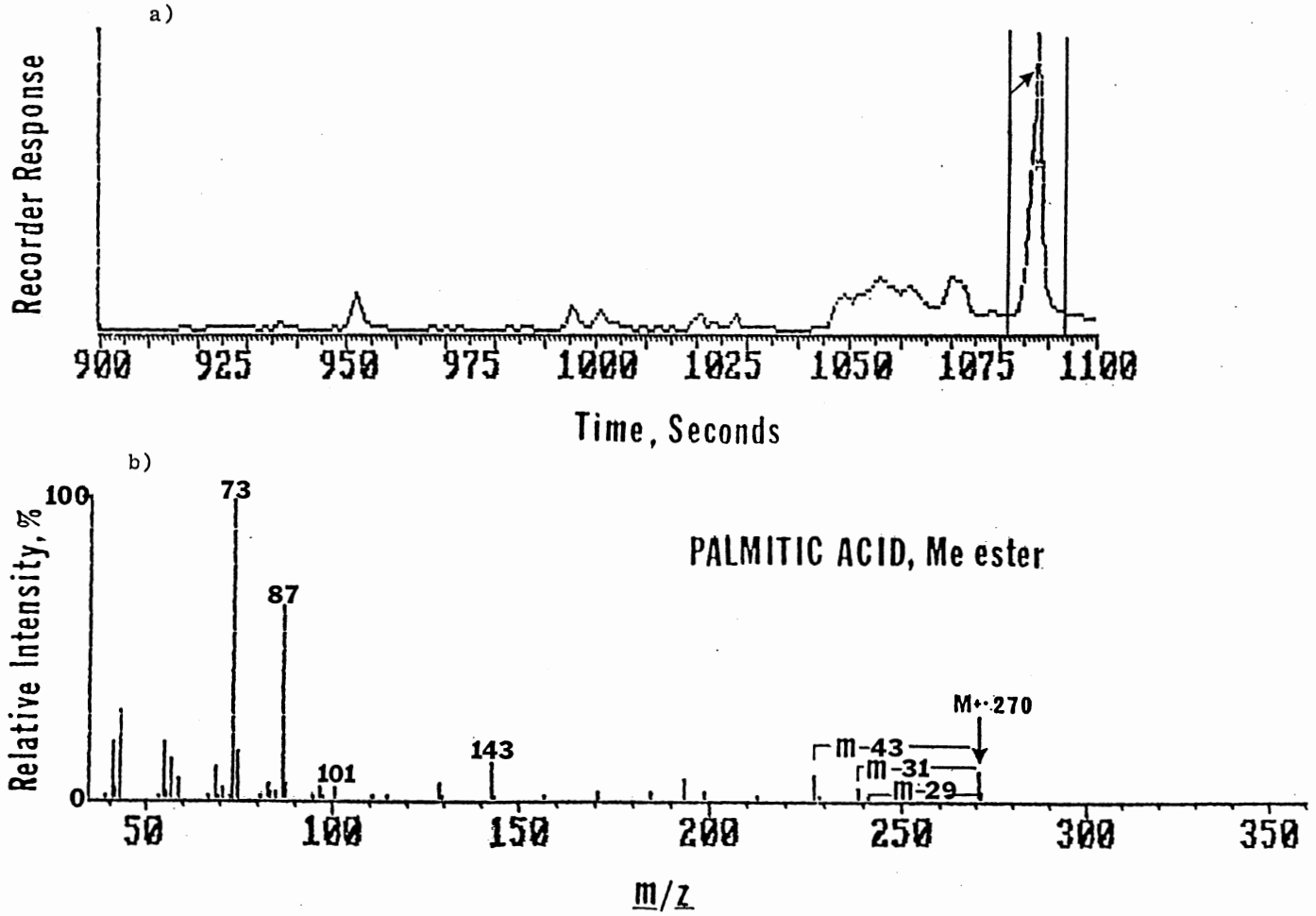


Figure 16. (a) Reconstructed Part of Total Ion Current Chromatogram of a Soil Extract--(LKB-2091 CGC/MS/DA): Peaks Represent Compounds

(b) Mass Spectrum of Pentadecanoic Acid, Methyl Ester, That Corresponds to Peak 670 Marked With Cursor

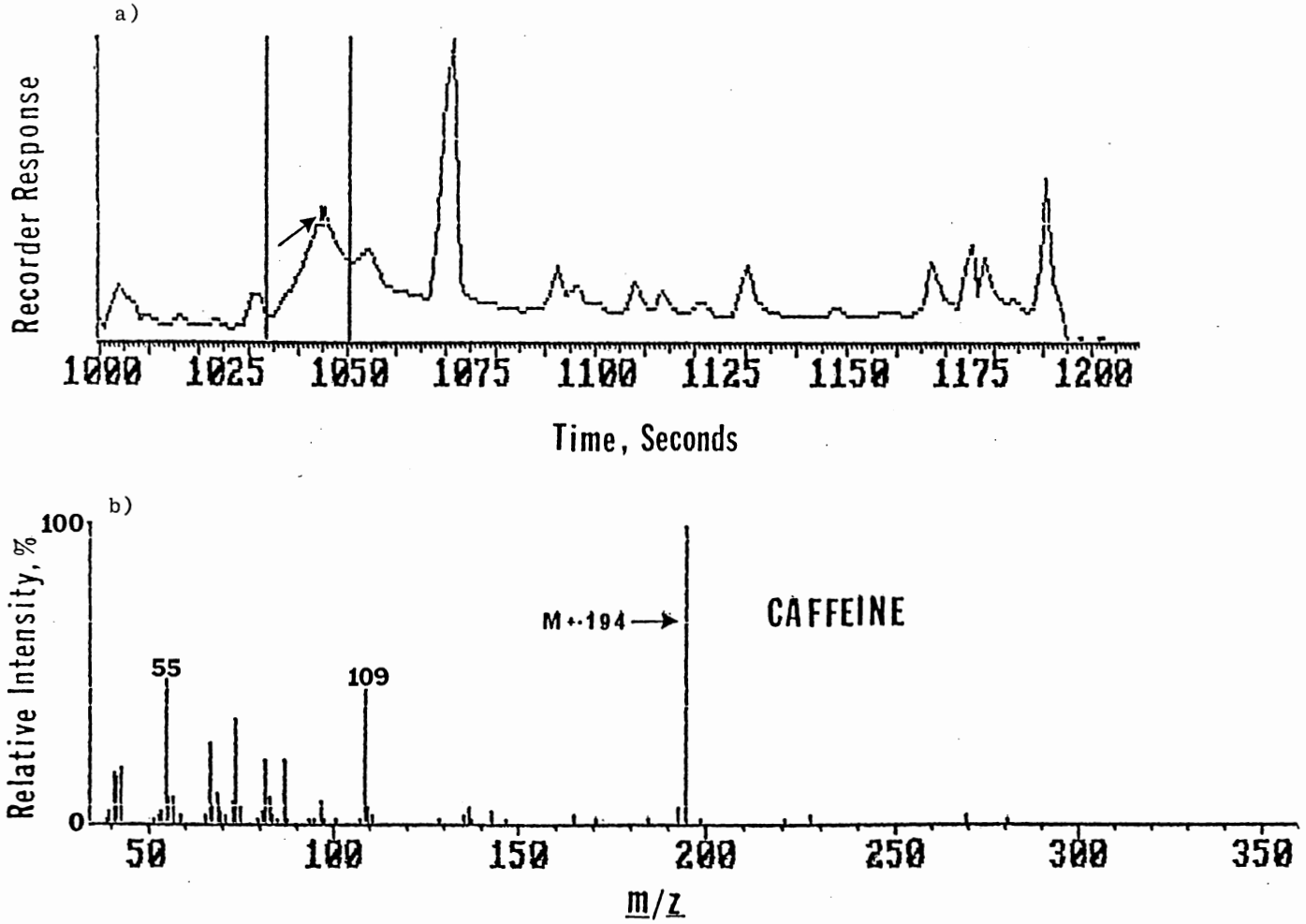


Figure 17. (a) Reconstructed Part of Total Ion Current Chromatogram of a Soil Extract--(LKB-2091 CGC/MS/DA): Peaks Represent Compounds

(b) Mass Spectrum of Stearic Acid, Methyl Ester, That Corresponds to Peak 1186 Marked With Cursor

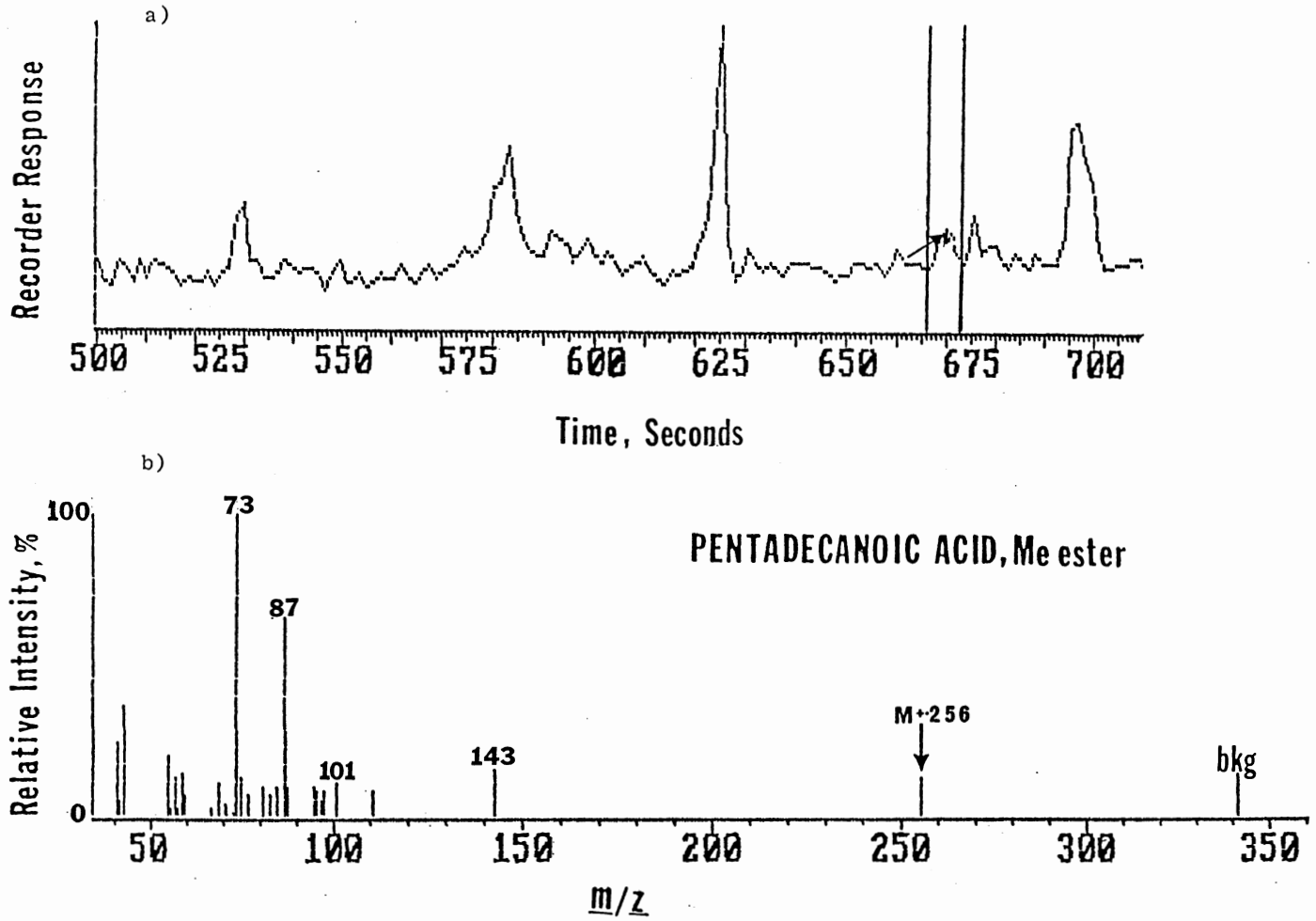


Figure 18. (a) Reconstructed Part of Total Ion Current Chromatogram of a Soil Extract--(LKB-2091 CGC/MS/DA): Peaks Represent Compounds

(b) Mass Spectrum of Myristic Acid, Methyl Ester, That Corresponds to Peak 935 Marked With Cursor

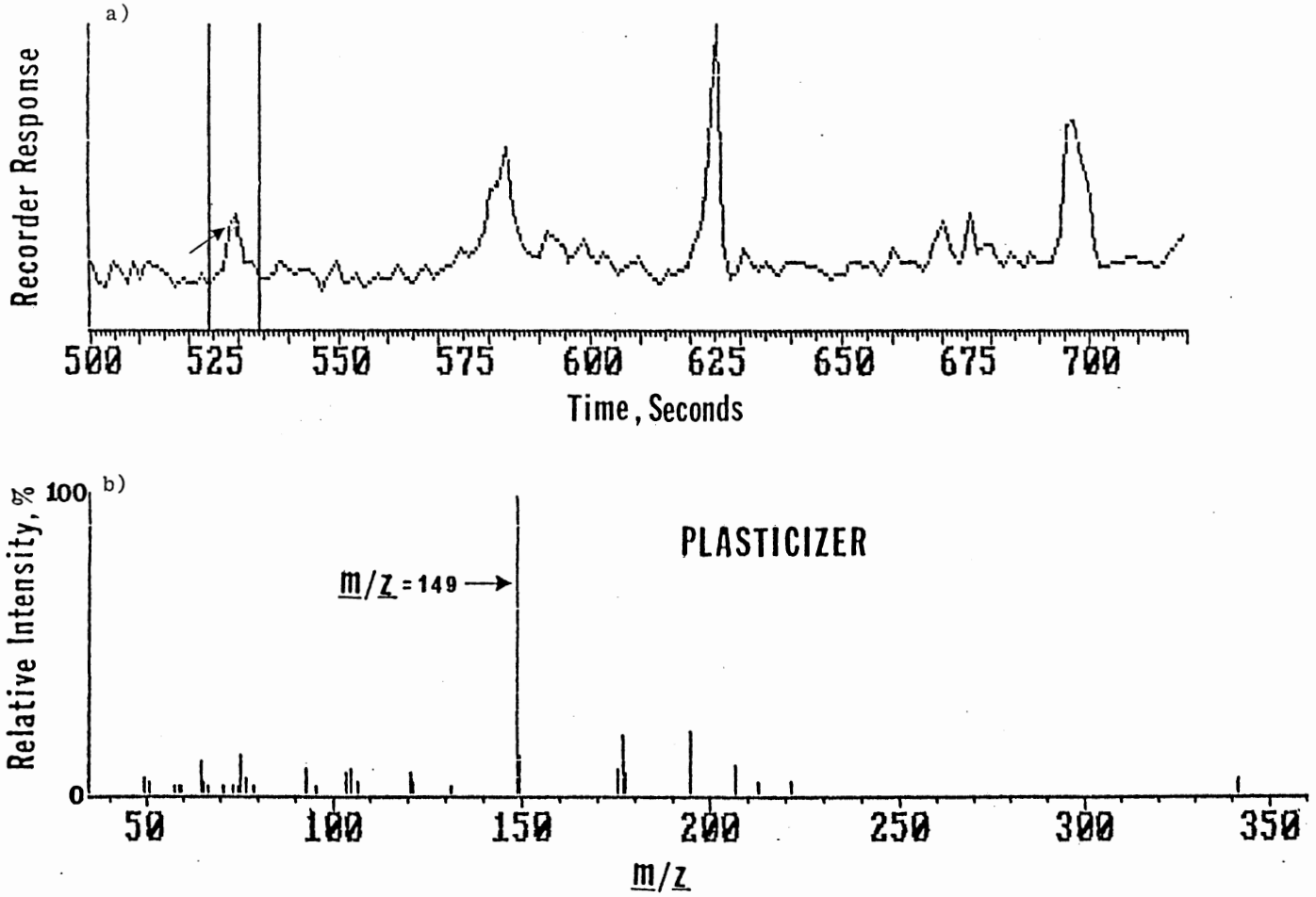


Figure 19. (a) Reconstructed Part of Total Ion Current Chromatogram of a Soil Extract--(LKB-2091 CGC/MS/DA): Peaks Represent Compounds

(b) Mass Spectrum of Plasticer, That Corresponds to Peak 529 Marked With Cursor

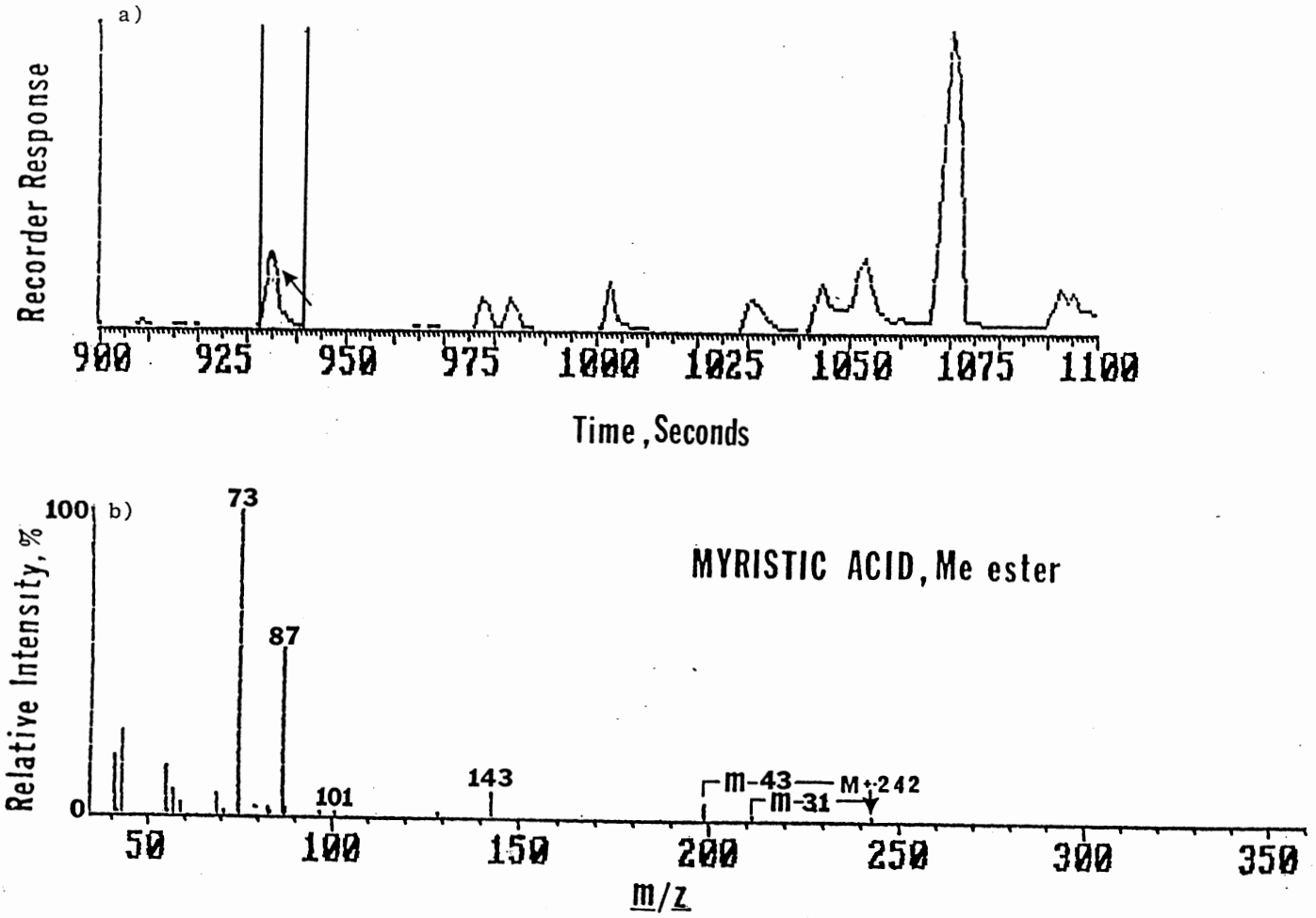


Figure 20. (a) Reconstructed Part of Total Ion Current Chromatogram of a Soil Extract--(LKB-2091 CGC/MS/DA): Peaks Represent Compounds

(b) Mass Spectrum of Caffeine, That Corresponds to Peak 1045 Marked With Cursor

Analysis of Fatty Acid Bioassay

The test to determine if fatty acids could act as allelochemicals resulted in nonsignificant results (Tables XV and XVI). For all fatty acids assayed at 1.0 mM, no statistical inhibition nor stimulation was noted. Additionally, synergistic effects were sought with five fatty acids, and again, negative results were recorded. Therefore, at least these fatty acids showed no biological activity. Other tests that have demonstrated fatty acid toxicity used sodium salts, or other slight variations (Alsaadawi, Rice, and Karns, 1983). This study indicated that free fatty acids were not damaging to wheat seedling growth.

TABLE XV

RESULT OF BIOASSAY OF PURE FATTY ACIDS AT 1.0 mM, EXPRESSED AS A PERCENTAGE OF CONTROL

	Growth, % of Control	
Fatty Acid	Root	Shoot
Palmitic	89.9	81.3
Stearic	97.6	85.0
Myristic	107.1	94.4
Eicosanoic	100.9	96.7
Heptadecanoic	112.9	98.1

TABLE XVI

RESULT OF BIOASSAY OF THE SYNERGISTIC EFFECTS OF FIVE FATTY ACIDS AT TWO CONCENTRATIONS

	Growth, % of Control			
	1.0 mM		5.0 mM	
	Root	Shoot	Root	Shoot
All Five Fatty Acids	107.1	88.8	115.0	97.0

CHAPTER V

CONCLUSIONS AND SUMMARY

These data indicated that there were some "additional factors" in the no-till extracts that were not present in the conventional-till extracts. When this idea was extrapolated, it could be argued that these "additional factors" must be contended with in all wheat no-tillage monoculture cropping systems, for a number of reasons. First, it is true that environmental conditions, specifically moisture and temperature, have a great effect on the chemical interactions in these no-till systems. Cool, moist conditions will permit the residue decomposing bacteria and fungi to flourish, and thus to release a vast quantity and variety of chemicals into the soil as compared to dry, hot conditions. Since geographic location is a primary determinant of environmental conditions, it must also be considered. Therefore, it could be postulated that, in the Panhandle of Oklahoma, where summer conditions are generally dryer and warmer, the results could differ from those related here. Consequently, wheat in no-till plots may not be inhibited significantly, compared to that in conventional-till systems. Similar arguments could be made throughout areas of the United States.

These "additional factors" may be organic or inorganic. This research was directed only at the organic chemicals in the soil extracts, specifically the fatty acids. This latter class of chemicals was chosen because not only were they the most prevalent in the extracts, but they had also been previously considered as allelochemicals. Although these

data did not confirm the phytotoxicity of fatty acids, they cannot be completely discounted. It is the opinion of the researcher that free fatty acids are not inhibitory to wheat seedling growth, but the salts of fatty acids may have toxic properties. It was the design of the CGC/MS/DA investigations simply to narrow the possibilities for the "additional factors" present in the no-till extracts. As indicated in the Appendixes, something is present in no-till systems that is not present in conventional-till systems. These "additional factors" are probably not fatty acids. Therefore, future research should focus on the other compounds present in the extracts.

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APPENDIXES

APPENDIX A

EQUATIONS USED TO DETERMINE AMOUNT OF LYOPHILIZED RESIDUE TO BE USED IN BIOASSAYS OF METHANOL SOLUBLE MATERIALS

400 g soil + 800 ml water

$$\frac{\text{400 g soil}}{\text{800 ml water}} \times \frac{\text{? g}}{\text{10 ml to bioassay}}$$

400 ml of the aqueous extract is lyophilized--this represents 56% of the total aqueous extract

0.56 x 400 ml = 224 g soil represented in the 400 ml lyophilized aqueous extract

Therefore:

X mg lyophilized residue/224 g soil

(X mg residue/1 g soil) x 5 = bioassay of dilute solution

And:

Amount for the bioassay of dilute solution x 5 = bioassay of centrated solution.

APPENDIX B

GRAIN YIELD (BU/ACRE) FROM EFAW PLOTS, STILLWATER, OKLAHOMA, FOR FIVE CONSECUTIVE YEARS

TABLE XVII

GRAIN YIELD (BU/ACRE) FROM EFAW PLOTS, STILLWATER OKLAHOMA, FOR FIVE CONSECUTIVE YEARS

	Grain Yield (bu/acre)			
	Moldboard	Disc	V-Blade	No-Till
1982-83	36	46	39	41
1983-84	55	61	64	60
1984-85	35	39	23	43
1985-86	18	16	15	3*
1986-87	25	22	23	16

*Wheat killed by simazine herbicide which was applied on March 3, 1986.

Note: Five-year average courtesy of Dr. Gene Krenzer, Department of Agronomy, Oklahoma State University (Krenzer, 1987).

APPENDIX C

DRY WEIGHT (G/M^2) OF STANDING WHEAT FROM EFAW PLOTS, STILLWATER, OKLAHOMA, FOR 1985-86

TABLE XVIII

DRY WEIGHT (G/M^2) OF STANDING WHEAT FROM EFAW PLOTS, STILLWATER, OKLAHOMA, FOR 1985-86

	Dry Weight (g/m^2)			
	Moldboard	Disc	V-Blade	No-Till
January 7	47	37	22	40
March 3	380	181	197	90
March 19	866	489	291	125

Note: Table information courtesy of Dr. Gene Krenzer, Department of Agronomy, Oklahoma State University (Krenzer, 1987).

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