Journal of Chemical Ecology, Vol. 17, No. 6, 1991

PHENOLIC ACID CONTENT OF SOILS FROM WHEAT–NO TILL, WHEAT–CONVENTIONAL TILL, AND FALLOW–CONVENTIONAL TILL SOYBEAN CROPPING SYSTEMS[1]

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(Received October 31, 1990; accepted February 1, 1991)

Abstract—Soil core (0–2.5 and/or 0–10 cm) samples were taken from wheat–no till, wheat–conventional till, and fallow–conventional till soybean cropping systems from July to October of 1989 and extracted with water in an autoclave. The soil extracts were analyzed for seven common phenolic acids (*p*-coumaric, vanillic, *p*-hydroxybenzoic, syringic, caffeic, ferulic, and sinapic; in order of importance) by high-performance liquid chromatography. The highest concentration observed was 4 μg/g soil for *p*-coumaric acid. Folin & Ciocalteu's phenol reagent was used to determine total phenolic acid content. Total phenolic acid content of 0- to 2.5-cm core samples was approximately 34% higher than that of the 0- to 10-cm core samples. Phenolic acid content of 0- to 2.5-cm core samples from wheat–no till systems was significantly higher than those from all other cropping systems. Individual phenolic acids and total phenolic acid content of soils were highly correlated. The last two observations were confirmed by principal component analysis. The concentrations were confirmed by principal component analysis data of individual phenolic acids extracted from soil samples were related to soil pH, water content of soil samples, total soil carbon, and total soil nitrogen. Indirect evidence suggested that phenolic acids recovered by the water-autoclave procedure used came primarily from bound forms in the soil samples.

Key Words—Wheat, *Triticum aestivum*, soybean, *Glycine max*, no till, con-

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[1]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of products named, nor criticism of similar ones not mentioned.

ventional till, soil extracts, allelopathy, phenolic acids, Folin & Ciocalteu's phenol reagent, HPLC.

INTRODUCTION

Straw residues of wheat, barley, oats, rye, grain sorghum, and sudangrass have effectively suppressed weeds, primarily annual broadleaf weeds (Barnes and Putnam, 1983; Putnam and De Frank, 1983; Putnam et al., 1983; Shilling et al., 1985, 1986b; Liebl and Worsham, 1983). In North Carolina, for example, Worsham (1989) noted that straw management and tilling in no-till-planted crops affected the level of early-season weeds. He found that: (1) removing wheat or rye straw plus tilling resulted in a 9–30% suppression; (2) removing straw without tilling resulted in a 43–50% suppression; (3) removing straw, tilling, and then replacing the straw resulted in a 60% suppression; and (4) leaving straw without tilling resulted in a 76–81% suppression of broadleaf weeds such as redroot pigweed, common lambsquarters, common ragweed, morning-glory, prickly sida, and sicklepod, when compared to no-cover-crop–tilled plots.

Studies have suggested that inhibition of germination and seedling growth by small grain mulches may be due, in part, to allelopathic interactions (Chou and Patrick, 1976; Liebl and Worsham, 1983; Shilling et al., 1985, 1986a,b; Barnes et al., 1986). To actually establish the role of allelopathic interactions in such systems will require: (1) identification of inhibitors involved; (2) determination of the rates of uptake of inhibitors by seeds or roots; and (3) establishing that uptake was of a sufficient magnitude to bring about the observed levels of inhibition.

Concentrations of inhibitors available to interact with seeds and roots of seedlings in soils are determined by the rate of release of potential allelopathic compounds from plant debris, the action of microorganisms, the fixation by the soil, and the rates of soil leaching (Skujins, 1967; Haider and Martin, 1975; Huang et al., 1977; Hartley and Whitehead, 1985, Blum et al., 1987; Dalton et al., 1987; Blum and Shafer, 1988). The level of inhibition observed for a given species will depend on the sensitivity of the species, on an adequate "source" and rate of release of inhibitors, and on the activity and strengths of the various "sinks" (i.e., clays, organic matter, microbes, seeds, and roots) within the soil. Seeds and roots are essentially in "competition" for inhibitors with other sinks in the soil. The "available" fraction in the soil (i.e., that which has not been irreversibly bound, leached, taken up by seeds and roots, or metabolized by microorganisms) at any time represents a residual but dynamic pool through which inhibitors move.

A variety of potentially allelopathic compounds have been identified from small grain mulches, including phenolic acids (Chou and Patrick, 1976; Liebl and Worsham, 1983; Shilling et al., 1985; Barnes et al., 1986). The presence

of simple phenolic acids, usually in bound form, such as caffeic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, protocatechuic, sinapic, syringic, and vanillic, is almost universal in plant tissue (Bates-Smith, 1956; Harborne, 1980). All of the above listed phenolic acids have been isolated from soils (Whitehead et al., 1982; Hartley and Whitehead, 1985; Kuiters and Denneman, 1987) and have been identified as potential allelopathic agents (Rice, 1984).

The objectives of this research were to characterize the phenolic acid pools in soils of no-till and conventional-till wheat/soybean cropping systems and to establish correlations between easily obtained soil characteristics and phenolic acid pools. Good correlations between soil characteristics and phenolic acid pools could help in the rapid identification of systems with potential allelopathic interactions.

METHODS AND MATERIALS

General Background Information. A long-term rotation study was initiated in the fall of 1985 to study various low-input cropping systems on a 6-hectare site located 5 km south of the North Carolina State University campus. Forty treatments (only five of which were used for this study) were arranged in a randomized complete block design with four replicates (only three replicates were used for this study). Blocking was based on landscape position and soil texture. Plots of 30 × 8 m with eight rows were used throughout the study. Tillage, planting, spraying, cultivating, and harvesting were done with two-row farm equipment. A Case International no-till planter was used to plant corn (*Zea mays* L. Dekalb 798 or 689), and soybeans (*Glycine max* L. Merill Deltapine 417), and a KMC no-till drill was used to plant wheat (*Triticum aestivum* L. Coker 916) and clover (*Trifolium incarnatum* L. Tibbee or *Trifolium pratense* L. Kenland). Beginning in 1988, a Hiniker cultivator was used to cultivate for weed control. A partial history of the treatments sampled and their codes is outlined in Table 1. Wheat was harvested on June 14, and soybeans were planted on June 19 or 20, 1989.

Sampling. Treatment plots were divided into four sections (15 × 4 m) for sampling. On July 3 and 31, August 31, and October 12, 1989, two soil cores (5.5 cm in diameter; 0–10 cm) per section were taken and combined. For some treatments, additional cores were taken to a depth of 2.5 cm adjacent to the previous sampling locations. The 0- to 2.5-cm core samples were taken for wheat–no till (WNT) and wheat–conventional till in four-year rotation (WT4R) for all sampling dates and for all treatments at the final sampling date. Soil samples were sieved (3 mm sieve), placed in plastic bags, and frozen (−20°C). Maximum storage in the freezer before soils were extracted was three months. Most soil samples were extracted within a month.

TABLE 1. HISTORY OF TREATMENTS SAMPLED AND IDENTIFICATION CODES[a]

1985–86	1986–87	1987–88	1988–89	Weed control summer of 1989	Code for 1988–1989 season
fallow/corn	wheat/soybean	fallow/corn	wheat/soybean	herbicides	WNT
C clv/corn	wheat/soybean	C clv/corn	wheat/soybean	cultivated	WT2R
C clv/corn	R clv/R clv	R clv/corn	wheat/soybean	cultivated	WT4R
fallow/soybean	fallow/corn	fallow/corn	fallow/soybean	herbicides	FTH
fallow/soybean	fallow/corn	fallow/corn	fallow/soybean	cultivated	FTC

[a] WNT = wheat–no till; WT2R and WT4R = wheat–conventional till in two and four-year rotation, respectively; FTH and FTC = fallow–conventional till with weed control by herbicides and cultivation, respectively; C clv = crimson clover; R clv = red clover; herbicides on soybeans, 1989: 3.4 kg glyphosate, 3.4 kg alachlor + 0.14 kg imazaquin per hectare were applied at planting on June 19; 0.3 kg sethoxydim was applied per hectare on August 7; cultivated: tilled with field cultivator (maximum depth 15 cm) on June 14, disked (maximum depth 8 cm) on June 15 or 16, cultivated for weed control (maximum depth 5 cm) on July 3 and 27.

Aboveground soybean and weed biomass were determined on June 13, July 3 and 31, and August 31 by clipping 0.5-m^2 quadrats centered around the soil cores in each section. Standing and surface debris were collected from the quadrats in the no-till plots on the same dates plus October 12. The soybean, weed, and debris samples in each section were combined by type, oven dried (70°C), and weighed.

Aboveground wheat biomass for litter bags was collected on June 13. Litter bags (N = 54; 19 × 19 cm; constructed of plastic screen with 1-mm mesh) containing 10 g of dried (40°C for five days) material were half buried between soybean rows on June 26 in the winter wheat plots. Two litter bags per plot were recovered on July 31, August 31, and October 12. Material in the litter bags was freeze-dried. Organic remains were separated from the soil material and weighed.

Site Description. Treatment plots were chosen among available plots of a low-input sustainable agriculture (LISA) cropping system rotation study. Soybean plots selected (see Table 1) included plots with wheat stubble (WNT), plots in which the wheat stubble had been tilled under (WT2R, WT4R), and plots without wheat (fallow plots; FTH, FTC). The soils were Cecil and Appling gravelly sandy loams (Typic Kanhaludults) with 2–6% slope and moderate erosion. Soils were composed of 67 ± 2% (mean ± SE, N = 15) sand, 18 ± 0.5% silt, and 16 ± 2% clay with 22 ± 2% gravel. Due to timing of herbicide applications, cultivation, and sampling, weed biomass and surface debris were highly variable (Table 2).

June to November mean maximum and minimum temperatures were 27.8

TABLE 2. SOYBEAN AND WEED BIOMASS, SOIL SURFACE DEBRIS, AND LITTER BAG CONTENT FOR 1989[a]

Sampling date	Code	Soybean (g/m of row)	Weeds (g/m^2)		Surface debris (g/m^2)	Wheat litter in bags (g)
6/13	WNT		32.88 (8.35)	12	110.07 (6.91)	Starting weight 6/26
	WT2R		40.54 (6.93)	12	53.25 (6.85)	
	WT4R		64.92 (13.37)	12	105.16 (10.73)	
	FTH		6.63 (2.62)	12		10.05 (0.05)[b]
	FTC		7.49 (2.57)	12		
7/3	WNT	2.88 (0.23)	6.96 (1.82)	9	200.16 (19.77)	
	WT2R	3.04 (0.13)	1.38 (0.41)	11		
	WT4R	3.23 (0.13)	5.29 (0.99)	12		
	FTH	3.42 (0.19)	0.16 (0.05)	3		
	FTC	4.12 (0.25)	1.09 (0.29)	9		
7/31	WNT	51.59 (3.84)	13.69 (3.95)	9	188.45 (12.09)	7.49 (0.24)[c]
	WT2R	60.71 (3.80)	25.27 (6.81)	12		7.78 (0.14)
	WT4R	78.31 (6.14)	44.33 (8.28)	12		7.46 (0.13)
	FTH	77.38 (6.62)				
	FTC	101.22 (10.91)	21.60 (4.41)	11		
8/31	WNT	349.70 (13.08)			268.04 (25.50)	6.42 (0.18)
	WT2R	397.34 (30.58)	108.00	1		7.16 (0.20)
	WT4R	355.70 (31.34)	94.43 (19.25)	7		6.52 (0.15)
	FTH	348.97 (30.42)				
	FTC	433.16 (26.27)	43.25 (32.25)	2		
10/12	WNT				197.83 (28.69)	6.02 (0.13)
	WT2R					6.01 (0.26)
	WT4R					4.88 (0.31)
	FTH					
	FTC					
		N = 12	N value after ()		N = 12	N = 6

[a] WNT = wheat–no till; WT2R and WT4R = wheat–conventional till in two- and four-year rotation, respectively; FTH and FTC = fallow–conventional till with weed control by herbicides and cultivation, respectively; () = standard errors.
[b] Oven dried at 40°C for five days.
[c] Freeze-dried.

and 17.2°C, respectively. There were 54 rain events, which provided a total of 76 cm (maximum event = 9.52 cm). Weather data were collected 1.6 km from the research site. The max/min temperatures and precipitation were 0.65°C lower/0.94°C higher, and 21 cm higher than normal for Raleigh, North Carolina, respectively.

Soybean plant biomass was not significantly affected by any of the treatments selected during July and August of 1989 (Table 2). Soybean biomass/m row increased 100-fold from July 3 to August 31. This rapid increase was partially due to excellent weather conditions. There were four rain events over 2.54 cm during this period.

The decline of wheat straw in the half-buried litter bags was not significantly different for the WNT, WT2R, and WT4R treatments. A 35% reduction (after adjusting for water content) in biomass was observed in the litter bags after 109 days. No significant differences in surface debris, however, were observed for the WNT plots over the same interval. This is consistent with the observations of Summerell and Burgess (1989) that the rate of decomposition of partially incorporated and buried wheat residue is much faster than that of surface debris.

Soil and Litter Analyses. Soil samples were defrosted overnight in a refrigerator (10°C). Three soil subsamples were taken to determine soil pH, water content, and phenolic acid content. The pH was determined by mixing 25 g soil with 100 ml deionized water and reading the pH 60 min later. Water content was determined gravimetrically on 10–15 g of soil before and after oven drying (100°C). Nitrogen and carbon content of soil samples were determined with a Perkin-Elmer 2400 C, H, N analyzer (Perkin-Elmer, Norwalk, Connecticut).

To determine phenolic acid content, 50 g of soil was placed in a 500-ml Erlenmeyer flask with 100 ml deionized water. Loosely capped flasks were autoclaved for 45 min at 1.2 kg/cm^2 and 121°C, removed from the autoclave, and then allowed to come to room temperature (20–30 min). The slurry in the flask was centrifuged for 10 min at 27,200 *g*. The resulting supernatant was filtered (Whatman No. 42). The filtered supernatant (extract 1) was adjusted to pH 2 with HCl, centrifuged for 10 min at 27,200 *g*, and the resulting supernatant was adjusted to pH 7 with NaOH (extract 2).

To determine the phenolic acid content of wheat litter samples, freeze-dried litter samples were ground in a Wiley mill (20 mesh), and 400-mg samples were extracted by the same procedures used for the soil samples.

HPLC Analysis. Protocatechuic acid (200 μl; 0.25 mM; pH 7) was mixed with 1.8 ml of extract 2 from soil and litter samples. The resulting solution was filtered through a 0.2 -μm Supor-200 filter (Gelman Sciences Inc., Ann Arbor, Michigan). Phenolic acids were determined with a Waters (Milford, Massachusetts) HPLC at 245 nm, using a model 440 absorption detector and a Baseline 810 Chromatographic Workstation (Dynamic Solutions, Ventura, California). A Waters reverse-phase 5-μm Nova-Pak C_{18} column was used to isolate and quantify eight different phenolic acids [i.e., caffeic (CAF), ferulic (FER), *p*-coumaric (PCO), *p*-hydroxybenzoic (POH), protocatechuic (PRO), sinapic (SIN), syringic (SYR), and vanillic (VAN)]. Standard phenolic acids were obtained from Sigma Chemical Company (St. Louis, Missouri). Extracts were analyzed using two mobile phases: (A) 2% methanol, 0.25% ethyl acetate, and 0.5% acetic acid, and (B) 80% methanol, 1% ethyl acetate, and 2% acetic acid. Linear gradients starting with 92% A and ending with 66% A were used over the first 40 min of a 60-min run. The flow rate of the mobile phase was 0.5 ml/min. Protocatechuic acid was used as a marker in each sample. Identification and quantification were confirmed by comparing retention times and areas with those of the appropriate standards (prepared in deionized water) and by spiking unknown samples with standards. Additional confirmations of the identity of the extracted phenolic acids were obtained with paper chromatography and by UV spectral comparisons in ethanol and ethanol plus sodium hydroxide (Harborne, 1984).

FC Method. Extracts 1 or 2 (0.5 ml) of soils plus 4.5 ml deionized water or 2.5 ml of standard phenolic acid solution plus 2.5 ml deionized water (pH 7) were mixed with 0.75 ml of 1.9 M Na_2CO_3, and 0.25 ml Folin & Ciocalteu's phenol reagent (Sigma Chemical Company, St. Louis, Missouri). This mixture was allowed to stand in darkness at room temperature (20–25°C) for at least 1 hr before its absorption was read at 750 nm (Box, 1983). Ferulic acid was chosen as the standard because it has been identified as an allelopathic agent in wheat mulch (Liebl and Worsham, 1983), and it was somewhat intermediate in color development when compared to the other phenolic acids (Figure 1). Units of total phenolic acid in soils determined by the FC method are thus in micrograms ferulic acid equivalents per gram of soil.

Data Analyses. Data were analyzed, using statistical analysis system programs for analysis of variance, regressions, correlations, and principal component analysis (SAS Institute Inc., 1988). Principal component analysis was run on mean plot values. Phenolic acids in soils were analyzed either as a split-plot design with whole plot subsampling (0- to 10-cm core samples for all sampling dates), a bivariate split-plot design with whole plot subsampling (0- to 2.5- and 0- to 10-cm soil core samples for treatments WNT and WT4R at all sampling dates), or a bivariate randomized block design with whole plot subsampling (0- to 2.5- and 0- to 10-cm soil core samples for all treatments at final sampling date). The bivariate was core type, the whole plot was treatment, and the subplot was sampling date. Means comparisons were based on Bonferroni *t* tests.

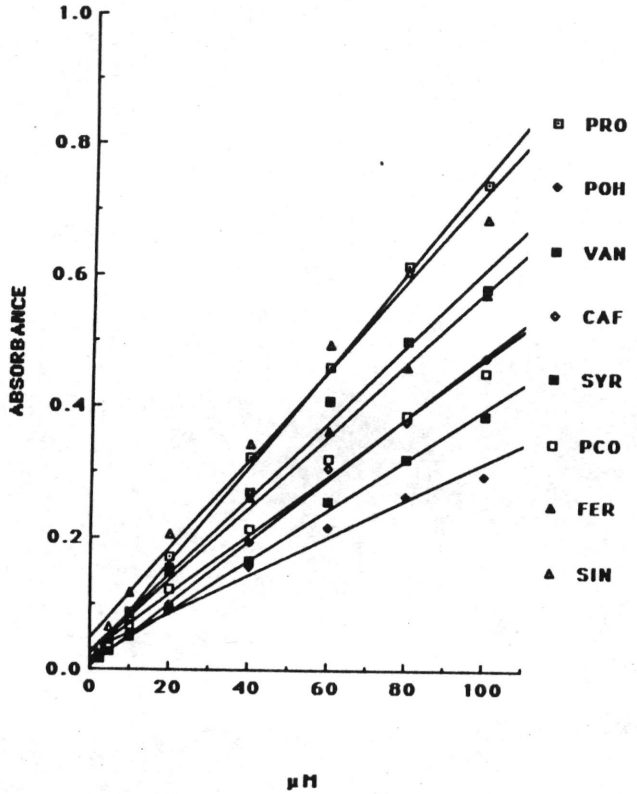


FIG. 1. Standard curves for protocatechuic (PRO), *p*-hydroxybenzoic (POH), vanillic (VAN), caffeic (CAF), syringic (SYR), *p*-coumaric (PCO), ferulic (FER), and sinapic (SIN) acids determined by the FC method. Order based on HPLC retention times.

RESULTS

Soil Extraction Procedures. To determine how the extraction procedures might affect the stability of phenolic acids, we autoclaved, filtered, etc. (see Methods and Materials), a mixture of caffeic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, protocatechuic, sinapic, syringic, and vanillic acids (0.25 mM each, 100 ml, pH 5.5). Caffeic acid and sinapic acid were reduced 20 ± 0.004% (mean ± SE) and 61 ± 0.03% in extract 1 and 28 ± 0.002% and 69 ± 0.02% in extract 2, respectively. No other major losses or gains were observed. Values subsequently presented have not been adjusted for these losses, because changes noted in these solutions may not be representative of changes in soil extracts. This information is presented only to alert the reader to limitations of this extraction procedure.

To estimate the proportion of "available" to "bound" phenolic acids in the extract, soil samples (75 g) from all the treatments ($N = 60$) were placed into Petri dishes with 25 ml water or 25 ml Hoagland's nutrient solution and incubated in the dark at 30°C. Soils were extracted and analyzed by HPLC at the start and after one and two weeks. Total phenolic acid content for the 0- to 2.5-cm soil samples decreased by 8% and 13% in the water treatment and increased by 3% and 18% in the nutrient solution treatment at the ends of week 1 and 2, respectively. When water was added to 0- to 10-cm soil core samples, total phenolic acid content of the samples was not changed. When nutrient solution was added, the total phenolic acid content was reduced by 10% by the end of week 1, but no additional reduction occurred during week 2. This suggested that phenolic acids in these soils were primarily in a bound form and that decomposition in these soil samples was nutrient-limited. Since, however, we do not have data on actual flows in and out of the phenolic acid pools, these conclusions are lacking in certainty. It is also not certain what proportion of the "bound" phenolic acids extracted by the autoclave procedure may be released into the soil solution by the action of microorganisms.

Soil Phenolic Acids. Treatment effects ($P \leq 0.05$) were noted only for *p*-coumaric acid (PCO). PCO of WNT (2.24 μg/g) and FTH (0.97 μg/g) were significantly different (Table 3). Significant effects of sampling date were noted for all but total phenolic acid (sum of seven phenolic acids determined by HPLC). Highest concentrations (μg/g) were observed for *p*-hydroxybenzoic (POH, 0.95), caffeic (CAF, 0.68), syringic (SYR, 0.80), and PCO (1.80) on August 31 and for vanillic (VAN, 1.43), sinapic (SIN, 0.18), and ferulic (FER, 0.53) on October 12. Lowest concentrations (μg/g) were observed for POH (0.82), CAF (0.48), VAN (0.94), and SYR (0.57) on July 31; for SIN (0.05) and FER (0.42) on August 31; and for PCO (1.30) on October 12. The coefficients of variation for all but PCO, FER, and SIN were less than 45% over all sampling dates for each treatment (Table 3).

Sampling dates (for all but PCO and SIN), core depth, and core depth by sampling date interactions (for all but PCO and SIN) were significantly different ($P \leq 0.05$) when individual and total phenolic acid values were compared for WNT and WT4R. For these treatments both 0- to 2.5- and 0- to 10-cm cores were taken at all sampling dates. The 0- to 2.5-cm soil core samples had on average 34% higher concentrations than the 0- to 10-cm soil core samples (Table 3). For example, the micrograms per gram of total phenolic acids for the 0- to 2.5-cm cores were 10.22, 8.14, 9.26, and 11.15 for sampling dates 1, 2, 3, and 4, respectively (July 3 and 31, August 31 and October 12, 1989, respectively). The values for the 0- to 10-cm cores were 6.14, 5.85, 7.27, and 6.41 μg/g, respectively. The differences between cores were 4.08, 2.29, 1.99, and 4.74 μg/g for sampling dates (SD) 1, 2, 3, and 4, respectively. Thus, values of 1.79, 2.09, 0.66, 0.30, 2.45, and 2.75 were observed for SD1–SD2, SD1–

TABLE 3. PHENOLIC ACID CONTENT, pH, AND PERCENTAGE WATER OF SOIL SAMPLES AVERAGED OVER SAMPLE DATES[a]

Variable	Treatment	0– to 2.5-cm cores Mean ± SE (μg/g)	CV	0– to 10-cm cores Mean ± SE (μg/g)	CV
POH	WNT	1.51 ± 0.06	28	0.94 ± 0.04	31
	WT2R	1.33 ± 0.15	38	1.00 ± 0.05	35
	WT4R	1.18 ± 0.05	32	1.03 ± 0.05	35
	FTH	0.96 ± 0.07	24	0.68 ± 0.03	29
	FTC	1.00 ± 0.05	19	0.78 ± 0.04	30
VAN	WNT	2.06 ± 0.11	38	1.34 ± 0.07	35
	WT2R	1.52 ± 0.06	15	1.12 ± 0.04	24
	WT4R	1.35 ± 0.05	25	1.25 ± 0.05	28
	FTH	1.13 ± 0.07	22	0.91 ± 0.03	24
	FTC	1.34 ± 0.07	17	1.10 ± 0.04	26
CAF	WNT	1.30 ± 0.06	34	0.74 ± 0.04	39
	WT2R	0.76 ± 0.03	15	0.57 ± 0.02	21
	WT4R	0.76 ± 0.02	23	0.59 ± 0.02	28
	FTH	0.50 ± 0.04	25	0.42 ± 0.02	29
	FTC	0.57 ± 0.03	19	0.51 ± 0.02	38
SYR	WNT	1.53 ± 0.07	33	0.88 ± 0.05	39
	WT2R	0.90 ± 0.04	15	0.67 ± 0.02	21
	WT4R	0.90 ± 0.03	23	0.70 ± 0.03	28
	FTH	0.60 ± 0.04	25	0.49 ± 0.02	29
	FTC	0.67 ± 0.04	19	0.60 ± 0.03	30
PCO	WNT	4.08 ± 0.22	37	2.24 ± 0.17	53
	WT2R	1.59 ± 0.11	23	1.41 ± 0.08	38
	WT4R	2.10 ± 0.08	41	1.74 ± 0.10	40
	FTH	0.93 ± 0.12	43	0.97 ± 0.06	42
	FTC	1.52 ± 0.11	25	1.55 ± 0.10	42
FER	WNT	1.21 ± 0.08	44	0.59 ± 0.03	40
	WT2R	0.76 ± 0.05	22	0.50 ± 0.03	43
	WT4R	0.65 ± 0.04	41	0.50 ± 0.03	44
	FTH	0.41 ± 0.06	46	0.32 ± 0.03	65
	FTC	0.55 ± 0.03	22	0.44 ± 0.03	51
SIN	WNT	0.60 ± 0.07	86	0.16 ± 0.02	90
	WT2R	0.32 ± 0.02	27	0.13 ± 0.02	132
	WT4R	0.18 ± 0.03	103	0.14 ± 0.02	104
	FTH	0.12 ± 0.02	69	0.05 ± 0.01	159
	FTC	0.23 ± 0.02	36	0.12 ± 0.03	154
Total	WNT	12.30 ± 0.58	32	6.89 ± 0.38	38
	WT2R	7.18 ± 0.32	15	5.83 ± 0.14	18
	WT4R	7.11 ± 0.24	23	5.94 ± 0.23	27
	FTH	4.64 ± 0.34	26	3.83 ± 0.13	23
	FTC	5.88 ± 0.27	16	5.10 ± 0.23	32

TABLE 3. Continued

Variable	Treatment	0 to 2.5-cm cores Mean ± SE	CV	0 to 10-cm cores Mean ± SE	CV
pH	WNT	5.5 ± 0.05	6	5.4 ± 0.05	6
	WT2R	5.3 ± 0.08	5	5.2 ± 0.05	7
	WT4R	5.5 ± 0.06	8	5.3 ± 0.06	8
	FTH	5.3 ± 0.09	6	5.2 ± 0.06	7
	FTC	5.7 ± 0.18	11	5.5 ± 0.06	8
% Water	WNT	16 ± 0.49	21	15 ± 0.50	23
	WT2R	13 ± 0.64	17	13 ± 0.37	19
	WT4R	13 ± 0.38	20	14 ± 0.31	15
	FTH	8 ± 0.36	15	9 ± 0.29	22
	FTC	11 ± 0.58	18	13 ± 0.28	8

[a] POH = *p*-hydroxybenzoic acid CAF = caffeic acid; VAN = vanillic acid; SYR = syringic acid; PCO = *p*-coumaric acid; FER = ferulic acid; SIN = sinapic acid; WNT = wheat–no till; WT2R and WT4R = wheat–conventional till in two- and four-year rotation, respectively; FTH and FTC = fallow–conventional till with weed control by herbicides and cultivation, respectively. Number for 0 to 2.5-cm cores: WNT $N = 47$; WT4R $N = 48$; WT2R, FTH, and FTC $N = 12$ (last sample date only). Number for 0 to 10-cm cores: $N = 48$.

SD3, SD2–SD3, SD4–SD3, SD4–SD2, and SD4–SD3 comparisons, respectively (LSD = 1.26). In each case the first sampling date cited had the greater difference between the cores. Thus, the differences in concentration between the core depths for the sampling dates were significantly different for all but SD2–SD3 and SD4–SD3. For a discussion of a bivariate split-plot design with whole plot subsampling, see chapter 15 for factorial experiments and chapter 16 for split-plot designs in Steel and Torrie (1980).

For the final sampling date, a sampling date where both core depths were taken for all treatments, treatment, core depth, and the treatment by core depth interactions were significant ($P \leq 0.05$) for all phenolic acids and for the total phenolic acid content of soil sampled. Only concentrations of WNT and FTH were significantly different. For example, total phenolic acid concentrations for WNT and FTH were 10.18 and 4.42 μg/g, respectively. The 0- to 2.5-cm soil core samples were 32% higher in phenolic acid concentration than the 0- to 10-cm core soil samples. In all instances, the difference in phenolic acid concentrations between the core depths for WNT was greater than that of all other treatments. All other comparisons were not significantly different. For example, the micrograms per gram of total phenolic acids for the 0- to 2.5-cm core samples were 14.09, 7.18, 8.13, 4.94, and 5.88 for WNT, WT2R, WT4R, FTH, and FTC, respectively. The values for the 0- to 10-cm cores were 6.28, 5.66,

6.54, 3.90, and 4.88, respectively. The differences between cores were 7.81, 1.52, 1.59, 1.04, and 1.00 μg/g for WNT, WT2R, WT4R, FTH, and FTC, respectively (LSD = 5.18). For a discussion of a bivariate randomized block design with whole plot subsampling, see chapters 15 and 16 in Steel and Torrie (1980).

Principal component analysis (PCA) has been successfully used in interpreting patterns of objects with several attributed variables. Objects in this case were treatments and the attributed variables were the seven different phenolic acids. PCA takes data that initially have a high dimensionality and creates new variables, called components, which are linear combinations of the original variables. Typically, the first two or three components (referred to as axes in this paper) account for much of the variation in the data. This results in an efficient summarization, which lends itself well to visual presentation and interpretation (Gauch, 1982).

PCA on the 0- to 10-cm core data did not result in clear groupings of treatments or in significant correlations between phenolic acids and any axes; thus only PCA on 0- to 2.5-cm core data are presented. The results of the PCA on phenolic acid content for WNT and WT4R are given by sampling date in Figure 2. Treatments are grouped with the exception of replicate plot 3 (block 3) of WNT, which appears to be more closely related to WT4R than WNT. The soil in this WNT plot was very sandy (78% sand, 17% silt, and 6% clay) compared to the other WNT plots (mean = 60% sand, 19% silt, and 21% clay). The first PCA axis accounted for 88% of the variation (Table 4). All phenolic acids, as well as total phenolic acid, were positively correlated with the first axis. Individual phenolic acids were also highly correlated with total phenolic acid.

The results of the PCA on phenolic acid content of soils sampled (0–2.5 cm) for all treatments at the final sampling date are given in Figure 3. Treatments are generally grouped with some overlapping of treatments. WNT is the most distinct in its distribution. The first PCA axis accounted for 95% of the variation (Table 4). All phenolic acids, as well as total phenolic acid, were positively correlated with the first axis. Individual phenolic acids were also highly correlated with total phenolic acid. Not only were individual phenolic acids correlated with total phenolic acid, but individual phenolic acids were also correlated with each other (Table 5). This latter correlation was based on an independent phenolic acid analysis of randomly chosen soil samples.

Phenolic Acid Concentrations in Litter Bag Samples. Concentrations of individual phenolic acids in extract 2 from litter bag samples for the WNT, WT2R, and WT4R treatments and the three litter sampling dates (July 31, August 31, and October 12) were not significantly different. The mean values and standard errors (N = 18) for POH, VAN, CAF, SYR, PCO, FER, SIN, and total were 12.49 ± 1.17, 33.35 ± 2.90, 61.52 ± 3.77, 40.03 ± 6.63,

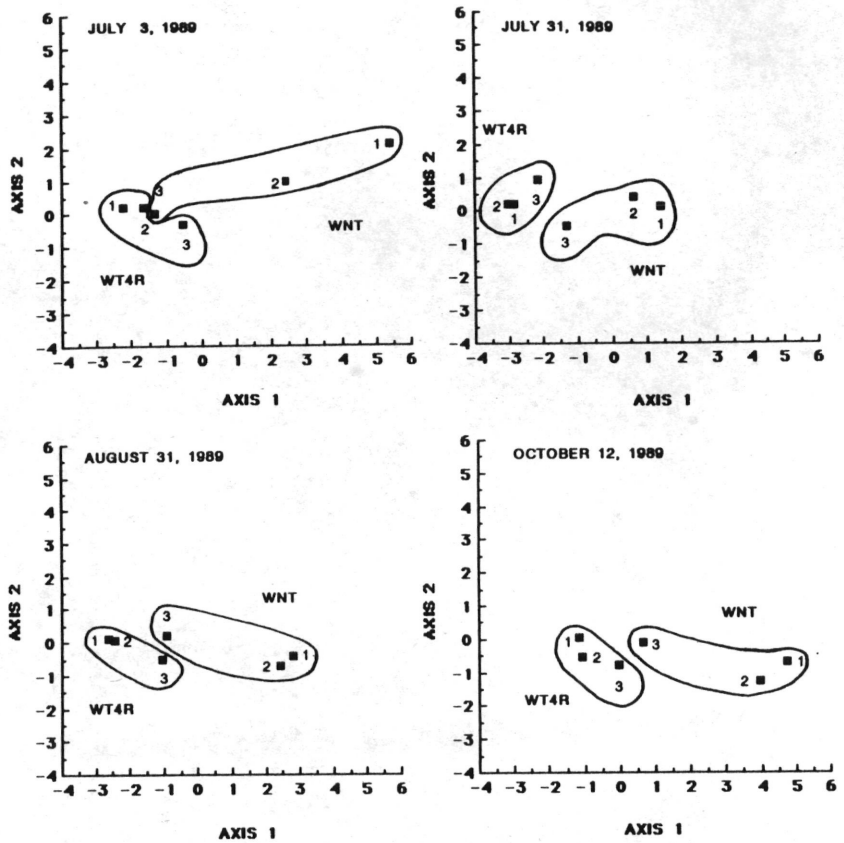


FIG. 2. Principal component analysis for phenolic acid content of soils (0- to 2.5-cm cores) from wheat–conventional till (WT4R) and wheat–no till (WNT) plots. Numbers associated with points are blocks. PCA analysis was based on all four sample dates. Sample dates presented individually only for ease of interpretation.

260.96 ± 26.19, 43.58 ± 3.45, 97.22 ± 22.96, and 549.15 ± 32.41 μg/g, respectively. Concentrations of the individual phenolic acids extracted from wheat straw placed into the litter bags (starting material) were similar to those extracted from the partially decomposed wheat straw, with the exception of FER, SIN, and SYR. FER content was 23% higher and SIN and SYR contents were 49% and 60% lower, respectively.

A 35% reduction in biomass was observed in the partially buried litter bags after 109 days (Table 2). No significant differences in surface debris, however, were observed for the WNT plots over the same interval.

Estimates of Total Phenolic Acids in Soils by FC Method. Phenolic acid

TABLE 4. CORRELATIONS (R) OF INDIVIDUAL PHENOLIC ACIDS FROM 0– TO 2.5-cm SOIL SAMPLES WITH PCA AXIS 1, AND PERCENTAGE OF TOTAL VARIATION ACCOUNTED FOR BY AXIS 1[a]

	Figure 2		Figure 3	
	Axis 1	Total phenolic acid	Axis 1	Total phenolic acid
POH[b]	0.92	0.90	0.92	0.91
VAN	0.95	0.93	0.98	0.98
CAF	0.97	0.97	0.99	0.99
SYR	0.97	0.97	0.99	0.99
PCO	0.94	0.96	0.98	0.98
FER	0.95	0.94	0.99	0.99
SIN	0.84	0.84	0.96	0.96
Total Phenolic Acid	1.00	—	1.00	—
Variation (%)	0.88		0.95	

[a] Correlations of individual phenolic acids with total phenolic acid (HPLC analysis) are also presented.

[b] POH = *p*-hydroxybenzoic; VAN = vanillic; CAF = caffeic; SYR = syringic; PCO = *p*-coumaric; FER = ferulic; SIN = synapic.

content of soil was determined on both extract 1 and extract 2 (injected into the HPLC). Extract 1 (crude extract) was adjusted to pH 2, centrifuged, and then adjusted to pH 7 to produce extract 2. Centrifugation of the acidified extract produced a reddish brown pellet, probably humic acid. The supernatant was straw-yellow in color, probably due to fulvic acid (Stevenson, 1982). Total phenolic acid of extract 2 (μg ferulic acid equivalents/g soil) estimated with the Folin & Ciocalteu's reagent was highly correlated, except for SIN, with the individual phenolic acids and the total phenolic acid (sum of seven phenolic acids) estimated by HPLC (Table 5; Figure 4). Thus, total phenolic acid determined by the FC method could be used for these soil samples to estimate total phenolic acid based on HPLC, as well as individual phenolic acid content of extract 2 (Table 6).

Finally, total phenolic acid of extract 1 based on the FC method was highly correlated with total phenolic acid of extract 2 based on the FC method (Figure 4). This suggested that the amount of reddish brown precipitate removed by acidification and centrifugation of extract 1 was proportional to total concentration, since the relationship of total phenolic acid estimated for extract 1 and extract 2 was linear.

Soil Characteristics and Total Phenolic Acid Content of Extract 1. Total phenolic acid of soil samples estimated by the FC method for extract 1 was

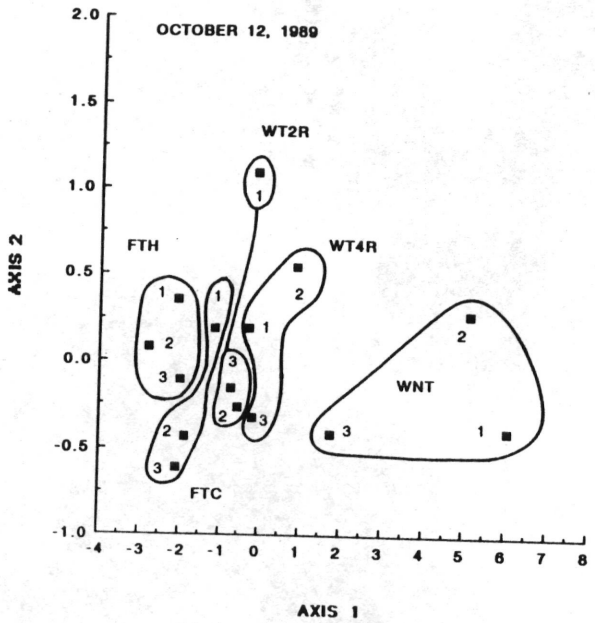


FIG. 3. Principal component analysis of phenolic acids in soils (0- to 2.5-cm cores) from fallow–conventional till (FTH, FTC), wheat–conventional till (WT2R, WT4R) and wheat–no till (WNT) plots for the last sampling date. Numbers associated with points are blocks.

correlated with carbon (C), nitrogen (N), soil pH, and soil water content. R values ranged from 0.51 to 0.83. Stepwise regression with maximum r^2 improvement was used, subsequently, to generate the best one, two, etc., variable models for total phenolic acid. Only nontransformed and squared values (i.e., no interaction terms) were included in the stepwise regressions. The best single-variable model was obtained with N ($r^2 = 0.69$) and the best two-variable model was obtained with N and pH ($r^2 = 0.76$). No improvement in r^2 values occurred with the addition of other variables. Regressions for total phenolic acid content in extract 1 with C, N, soil water content, and pH are presented in Figure 5.

Since C and N were linearly correlated ($r = 0.92$; $\%N = -0.0076 + 0.0527\ \%C$, $r^2 = 0.843$, $P \leq 0.0001$, $N = 48$), N was removed from the data set and the stepwise procedure was repeated. The best single-variable model was now obtained with C ($r^2 = 0.54$), the best two-variable model was obtained with C and the squared soil water content ($r^2 = 0.66$), and the best three-variable model was obtained with C, pH, and the squared soil water content (r^2

TABLE 5. CORRELATION COEFFICIENTS FOR INDIVIDUAL PHENOLIC ACIDS AND TOTAL PHENOLIC ACID DETERMINED BY HPLC (SUM OF INDIVIDUAL PHENOLIC ACID) OR FC METHOD[a]

	POH	VAN	CAF	SYR	PCO	FER	SIN	Total HPLC	Total FC
POH		0.79	0.66	0.79	0.63	0.67	NS	0.79	0.78
VAN			0.81	0.93	0.70	0.89	NS	0.89	0.91
CAF				0.89	0.77	0.83	NS	0.87	0.85
SYR					0.78	0.94	0.28	0.94	0.95
PCO						0.80	0.30	0.91	0.82
FER							0.26	0.92	0.95
SIN								0.45	NS
Total HPLC									0.93

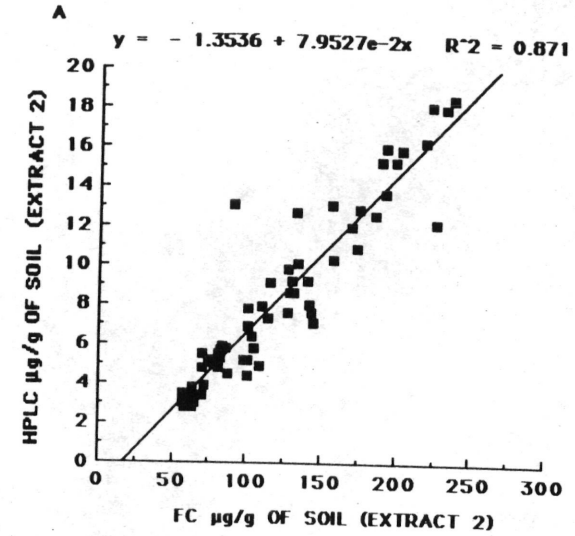
[a]Soil samples were chosen at random from all samples collected during 1989 ($N = 64$). POH = *p*-hydroxybenzoic acid; VAN = vanillic acid; CAF = caffeic acid; SYR = syringic acid; PCO = *p*-coumaric acid; FER = ferulic acid; SIN = sinapic acid; NS = not significantly different at 0.05. Extract 2 data.

= 0.66). No improvement in r^2 values occurred with the addition of other variables.

DISCUSSION

Soil Extraction Procedures. An unequivocal quantification of the "available" fraction of phenolic acids in soil pools has not been made for any soil. The amounts of phenolic acids recovered from a given soil depend on a variety of factors, including the extractant and the extraction procedures used (Hartley and Whitehead, 1985; Dalton et al., 1987). In general, however, most researchers would agree that extractants such as water, low concentrations of calcium hydroxide, sodium acetate, or mild chelating agents provide the most biologically meaningful estimates of the "available" phenolic acid pools. The "available" fraction in the soil is essentially the "free" and/or "readily freed" phenolic acids present at any time.

Phenolic acids, such as ferulic, *p*-coumaric, vanillic, etc., generally are slow to dissolve in water at ambient temperatures. The speed with which phenolic acids solubilize and the amount that can stay in solution can be increased by raising the pH and/or the temperature of the solution. Increasing the temperature and/or the pH of an extraction solution may at times be more desirable than using long-term extractions, because of the presence of microbes. Blum and Shafer (1988), for example, noted that <1% of exogenously applied phe-



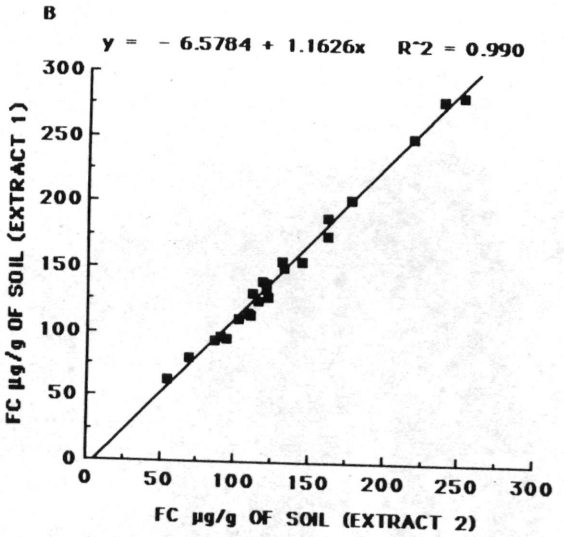


FIG. 4. Relationship of total phenolic acid content of soil determined by HPLC and by the FC method for extract 2 (A). Relationship of total phenolic acid content of soil determined by the FC method for extracts 1 and 2 (B). FC values are in ferulic acid equivalents.

TABLE 6. PARTIAL REGRESSION COEFFICIENTS AND r^2 VALUES FOR INDIVIDUAL PHENOLIC ACIDS AND SUM OF PHENOLIC ACIDS (TOTAL HPLC) REGRESSED AGAINST TOTAL PHENOLIC ACID DETERMINED BY FC METHOD[a]

Phenolic acid	Line intercept	Linear	r^2
POH	0.3444	0.0074	0.61
VAN	−0.0682	0.0158	0.82
CAF	−0.1220	0.0057	0.72
SYR	−0.2254	0.0095	0.90
PCO	−0.8463	0.0278	0.67
FER	−0.3357	0.0095	0.90
SIN	NS		
Total HPLC	−1.3395	0.0794	0.87
Total HPLC-SIN	−1.2532	0.0758	0.91

[a] Soil samples were chosen at random from all soil samples collected during 1989 ($N = 64$). Soil samples in Tables 5 and 6 are identical. POH = *p*-hydroxybenzoic acid; VAN = vanillic acid; CAF = caffeic acid; SYR = syringic acid; PCO = *p*-coumaric acid; FER = ferulic acid; SIN = synaptic acid; $P \leq 0.0001$ in all cases; NS = not significant at 0.05; dependent and independent variables in μg/g of soil. Extract 2 data.

nolic acids could be extracted with water 24 hr after addition to nonsterilized Portsmouth soil, but >90% could be recovered from sterilized Portsmouth soil. Thus, shaking field soil samples for long periods to extract "available" phenolic acids could lead to substantial underestimations of these pools. On the other hand, extractants with artificially high pH or extractions at high temperatures will lead to the extraction of both "available" and some "bound" phenolic acids.

We chose water as the extractant and an extraction procedure that was rapid [i.e., high temperature (121°C) and pressure (1.2 kg/cm^2)], that had only a minimal impact on soil pH, and that eliminated the concern about microbial activity. With the exception of caffeic and sinapic acid, which were reduced in concentration, this procedure appeared to be satisfactory for extracting some of the common phenolic acids found in soil and litter samples. The proportion of "available" and "bound" phenolic acids extracted cannot be stated with certainty, but indirect evidence would suggest that the phenolic acids extracted came primarily from "bound" forms.

Individual Phenolic Acids. With the exception of sinapic acid, the individual phenolic acids were not only correlated with total phenolic acid determined by the FC and HPLC methods, but also to each other. Soil samples analyzed to establish these relationships included samples from all treatments, i.e., WNT, WT2R, WT4R, FTH, and FTC. This indicated that differences in soil phenolic acid content between the crop management systems were primarily quantitative

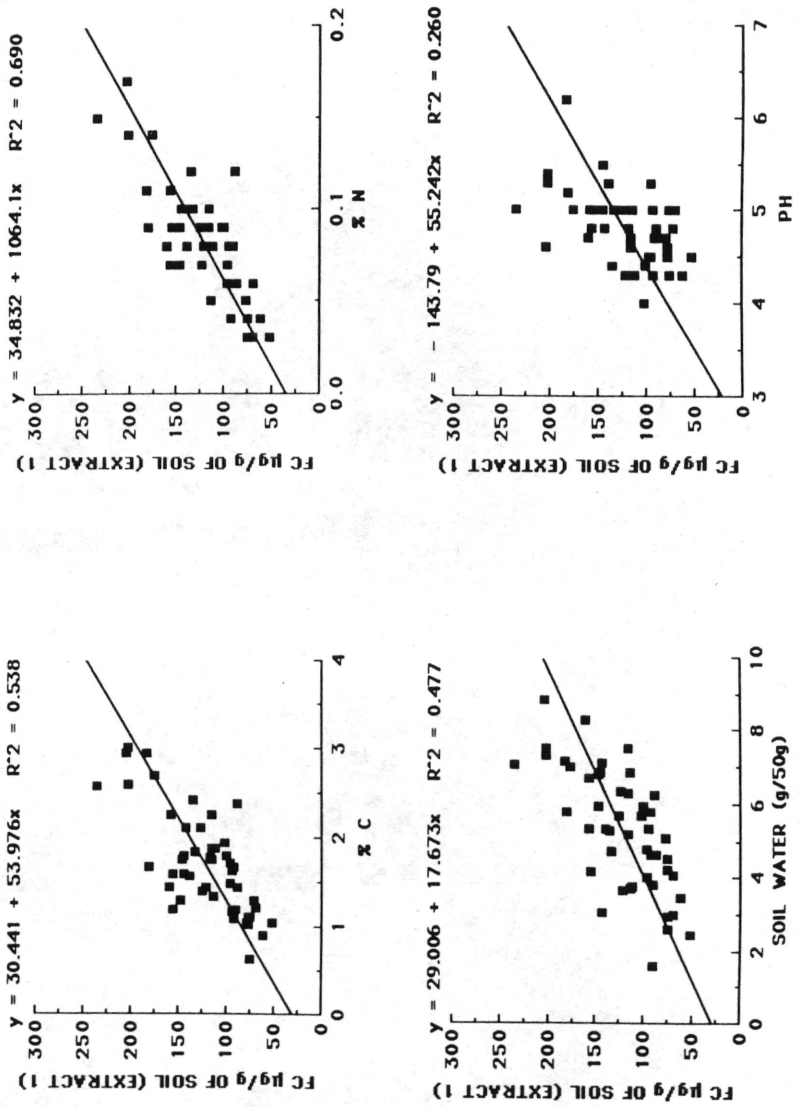


FIG. 5. Relationship of total phenolic acid content of soil and %C, %N, soil water content, and pH. Total phenolic acid values were based on extract 1 and are in ferulic acid equivalents.

and not qualitative. One arrives at the same conclusion by the use of principal component analysis (PCA). For both PCAs (Figures 2 and 3), the strong positive correlations of all individual phenolic acids with the first axis, and the large amount of variance accounted for by the first axis, were indicative of the high level of multicollinearity among the phenolic acids. There is evidently little residual variation that may be attributed to independent behavior of any of the phenolic acids. The first axis, then, is a surrogate for joint variation of all the phenolic acids, as evidenced by its strong positive correlation with total phenolic acid. Positioning of the treatments along the first axis is a reflection of the substantially higher concentrations of all phenolic acids in the WNT treatment and of somewhat greater concentrations in the WT2R and WT4R treatments, relative to the FTH and FTC treatments. This is not to say that concentrations of the individual phenolic acids recovered from soil did not differ from each other. With minor exceptions, PCO > VAN > POH > SYR > CAF > FER > SIN. The benzoic acid derivatives (i.e., POH, SYR, and VAN) were higher in concentration than the cinnamic acid derivatives (i.e., CAF, FER, and SIN), with the exception of PCO.

The types and amounts of plant residue present in the soil and the action of various soil processes (i.e., microbes, soil fixation, leaching, etc.) would obviously be important in determining the absolute and relative amounts of individual phenolic acids present. Such factors may account for the higher concentrations of phenolic acids in the 0- to 2.5-cm than in the 0- to 10-cm core samples (the former were approximately 34% higher than the latter), and the quantitative differences between individual phenolic acids extracted from soil and litter bag samples (i.e., soil samples: PCO > VAN > POH > SYR > CAF > FER > SIN; litter bag samples: PCO > SIN < CAF > FER > SYR > VAN > POH). That the importance of individual phenolic acids in soils is influenced by plant species growing in the soil has been demonstrated by Whitehead et al. (1981, 1982) and Kuiters and Denneman (1987), among others. Whitehead et al. (1981) suggested that substantial proportions of the phenolic acids extracted from soil were either derived from organic residues more than 4 years old or were the result of microbial synthesis. This last observation may suggest one possible reason for the lack of clear differences in the importance of individual phenolic acids in the wheat–no-till (WNT), wheat–conventional-till (WT2R, WT4R), or the fallow–conventional-till (FTH, FTC) system soils.

Phenolic Acids and Sampling Dates. No dramatic changes or clear trends for soil phenolic acid concentrations were observed over the first 109 days of the growing season. Maximum changes for the total phenolic acid content based on HPLC analysis were on the order of 20–30%. Slow changes of phenolic acid concentrations in these nutrient limited soils would be expected if the major sources of these phenolic acids were a result of microbial action on plant litter and/or organic residues (Turner and Rice, 1975; Lodhi, 1978; Whitehead et al., 1979). We suspect that the fluctuations observed were, in part, a result of the proximity of rain events to the soil sampling dates. This observation is based on the correlations between water content of the soil samples and total phenolic acid content. Additional fluctuations resulted from the variation in litter distribution. As stated before, we do not have data on flows in and out of the phenolic acids pools and, thus, cannot state these conclusions with certainty.

Total Phenolic Acid Content of Soil Extracts. The sum of the individual phenolic acids in extract 2 determined by HPLC analysis (μg/g of soil) was highly correlated with total phenolic acid estimated by the FC method (μg ferulic acid equivalents/g soil) for extract 2. Values for extracts 1 and 2 based on the FC method were also highly correlated with each other. Total phenolic acid content of soil extracts estimated by the FC method was 1.11 times greater for extract 1 than for extract 2. The difference between extracts 1 and 2 was most likely the result of the removal of humic acid by acid precipitation. The small difference observed between extracts 1 and 2 suggested that little humic acid was extracted by the procedure. This was not unexpected, since humic acid is only sparingly soluble in water (Hartley and Whitehead, 1985). The total phenolic acid estimated for extracts 1 and 2 by the FC method were 16.75 and 15 times higher, respectively, than that determined by the HPLC method. Extracts 1 and 2 would include phenolic acids not identified, humic and/or fulvic acid, and other compounds that reduce the Folin & Ciocalteu's phenol reagent. Box (1983) provides a list of organic substances with or without a phenolic hydroxyl group and inorganic substances that reduce the FC reagent. Others have used the FC method to estimate total phenolic acid content of soils (for example, Kuiters and Denneman, 1987), but this is the first time that correlations between the FC and HPLC methods have been made. Whether such correlations will occur for data of other soil systems is not certain. Similar correlations between the FC and HPLC methods, however, have been found for Durham sandy loam soils underneath crimson clover, hairy vetch, subterranean clover, and rye cover crops (unpublished data). Once such correlations are defined for a given soil and management system, the FC method would appear to be useful in surveys to identify potential systems in which allelopathic interactions due to phenolic acids may be important. We would, however, not recommend the use of the FC method to estimate absolute total phenolic acid concentrations in soil extracts, since the Folin & Ciocalteu's phenol reagent reacts differently with individual phenolic acids (Figure 1) and also reacts with other organic and inorganic substances (Box, 1983).

Total phenolic acid content of soil as determined by the FC method was also correlated with soil carbon (C), nitrogen (N), pH, and water content. Since the total amount of phenolic acids dissolved is determined by the volume of water and the pH of the solution, this was not entirely unexpected. The percent C for the soil samples was 2% $\pm$ 0.17 for WNT, 1.7% $\pm$ 0.12 for WT2R,

1.9% ± 0.11 for WT4R, 1.3% ± 0.09 for FTH, and 1.5% ± 0.16 for FTC. The C/N ratios of the soils were 20 ± 0.77 for WNT, 21 ± 1.98 for WT2R, 20 ± 0.60 for WT4R, 26 ± 2.17 for FTH, and 24 ± 1.79 for FTC. C/N ratios above about 14 are a strong indication that the soil contains much partially decomposed plant material (Wild, 1988). Both Iritani and Arnold (1960) and Harmsen and Van Schreven (1955) suggested that a C/N ratio of 20 was the approximate dividing line between positive and negative release of N in soils. The relationships between C, N, and total phenolic acid content suggest that the phenolic acid concentrations in this soil were closely related to organic matter content of the soil. This latter relationship may also help to explain the observed soil phenolic acid content of the various treatments and the patterns observed in the principal component analysis, particularly the small distinction between fallow plots (FTH, FTC) and wheat-conventional till plots (WT2R, WT4R).

Concluding Remarks. Pools of "available" phenolic acids in these soils were small. The majority of phenolic acids recovered, by the procedure used, were released from "bound" forms, which were not necessarily recent in origin. The primary source of "available" phenolic acids would be from the action of microorganisms on plant litter and/or organic residues. Thus, the most likely place for allelopathic interactions in these soils would be in the soil surface of the no-till plots.

Since it appears that a substantial portion of the phenolic acids extracted by the autoclave procedure came from plant litter and/or soil organic residues, the procedure used here in conjunction with the FC method could be used to identify potential soil systems for more detailed study. Total C and/or N content of soil also may be helpful in this regard. However, data on phenolic acid pools ("available" and/or "bound") in soils are, by themselves, unlikely to provide much insight concerning allelopathic interactions unless flows in and out of these pools are determined and source–sink relationships within a given soil are understood.

Acknowledgments—The authors wish to thank Lynn D. Holappa for her technical assistance in the field and laboratory. This research was partially supported by the Southern Region Low-Input Sustainable Agricultural Research and Extension Program of USDA.

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