

Effects of phenolic acids on seed germination and seedling growth in soil

M.J. Krogmeier and J.M. Bremner

Department of Agronomy, Iowa State University, Ames, IA 50011, USA

Summary. It is commonly assumed that the adverse effect of plant residues on crop yields is largely or partly due to phytotoxic compounds leached from these residues or produced by their decomposition. There has been substantial support for the hypothesis that the phytotoxic compounds responsible for reduced crop yields are phenolic acids such as p--coumaric acid, *p*-hydroxybenzoic acid, and ferulic acid. To test the validity of this hypothesis, we studied the effects of nine phenolic acids (caffeic acid, chlorogenic acid, p-coumaric acid, ellagic acid, ferulic acid, gallic acid, p-hydroxybenzoic acid, syringic acid, and vanillic acid) on (1) seed germination of corn (Zea mays L.), barley (Hordeum vulgare L.), oats (Avena sativa L.), rye (Secale cereale L.), sorghum [Sorghum bicolor (L.) Moenchl, wheat (Triticum aestivum L.). and alfalfa (Medicago sativa L.) on germination paper and soil, (2) seedling growth of alfalfa, oats, sorghum, and wheat on germination paper and soil, and (3) early plant growth of corn, barley, oats, rye, sorghum, and wheat in soil. The results showed that although the phenolic acids tested affected germination and seedling growth on germination paper, they had no effect on seed germination, seedling growth, or early plant growth in soil even when the amounts applied were much greater than the amounts detected in soil. We conclude that the adverse effect of plant residues on crop yields is not due to phenolic acids derived from these residues.

Key words: Phenolic acids – Seed germination – Seedling growth – Plant residues

Recent concern about soil erosion and other problems associated with conventional tillage systems has stimu-

lated interest in conservation tillage systems in which crop residues are managed on the soil surface with minimum or no tillage. These systems have the advantage that they reduce soil erosion and conserve energy, but it is well established that conversion to these systems can lead to a reduction in crop yield (Guenzi and McCalla 1962; McCalla and Norstadt 1974). This reduction in yield has been attributed to phytotoxic compounds leached from plant residues or produced by their decomposition (Guenzi and McCalla 1962; McCalla and Haskins 1964; Patrick 1971; Cochran et al. 1977; Elliot et al. 1978), and many workers (Guenzi and McCalla 1966a; Wang et al. 1967; Chou and Patrick 1976; Patterson 1981; Lodhi et al. 1987) have suggested that the phytotoxic compounds responsible for reduced crop yields are phenolic acids such as pcoumaric acid, ferulic acid, and *p*-hydroxybenzoic acid. This hypothesis has stimulated research to identify and estimate phenolic acids in soils (Whitehead 1964; Guenzi and McCalla 1966b; Wang et al. 1967; Whitehead et al. 1981; Dalton et al. 1987), and it has been supported by studies showing that phenolic acids known to occur in soils delay or inhibit germination of seeds and reduce seedling growth on germination paper or agar (Mayer and Evenari 1952, 1953; Varga and Köves 1959; Guenzi and McCalla 1966a; Hennequin and Juste 1967; Rasmussen and Einhellig 1977; Einhellig and Rasmussen 1978; Williams and Hoagland 1982), or reduce growth of plants in nutrient solutions (Hennequin and Juste 1967; Wang et al. 1967; Rasmussen and Einhellig 1977; Einhellig and Rasmussen 1978, 1979; Patterson 1981; Sparling and Vaughan 1981). However, no studies of the effects of phenolic acids on seed germination or growth of agronomic plants in soils have been reported.

We report here the results of studies of the effects of nine phenolic acids (caffeic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, ferulic acid, gallic acid,

Offprint requests to: J.M. Bremner

p-hydroxybenzoic acid, syringic acid, and vanillic acid) on (1) germination of seeds of seven plants (alfalfa, barley, corn, oats, rye, sorghum, and wheat) on germination paper and soil, (2) seedling growth of four plants (alfalfa, oats, sorghum, and wheat) on germination paper and soil, and (3) early growth of six plants (barley, corn, oats, rye, sorghum, and wheat) in soil.

Materials and methods

The seed-germination studies reported were performed with seeds of alfalfa (*Medicago sativa* L.), barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), oats (*Avena sativa* L.), rye (*Secale cereale* L.), sorghum [*Sorghum bicolor* (L.) Moench], and wheat (*Triticum aestivum* L.). The plant-growth studies were performed with plants grown from six of these seeds (barley, corn, oats, rye, sorghum, and wheat). The alfalfa and wheat seeds were obtained from Merschman Seed and Fertilizer Inc., West Point, Iowa and the corn seeds were obtained from Mike Brayton Seeds, Ames, Iowa. The other seeds used were obtained from May Seed and Nursery Co., Shenandoah, Iowa.

The soils used (Table 1) were surface (0-15 cm) samples of soils representative of the Webster, Harps, and Storden series which are used extensively for corn and soybean production in north-central Iowa. Before use, each sample was air-dried and crushed to pass through a 2-mm screen. The soil analyses reported in Table 1 were performed as described by Zantua and Bremner (1975).

The phenolic acids tested were caffeic acid, chlorogenic acid, *p*coumaric acid, ellagic acid, ferulic acid, gallic acid, *p*-hydroxybenzoic acid, syringic acid, and vanillic acid. They were obtained from Sigma Chemical Co., St. Louis, Missouri.

The following procedure, based on the rules for seed testing published by the Association of Official Seed Analysts (1981), was used to study the effects of phenolic acids on germination of seeds on germination paper and soil. Two 85-mm disks of germination paper (Steel Blue Anchor Seed Germination Blotter, Anchor Paper Co., St. Paul, Minn) or 40 g air-dried soil were placed in a 15-×100-mm Petri dish (Fisher Scientific, Pittsburgh, Pennsylvania) and moistened with 10 ml water (control) or with 10 ml water containing the phenolic acid under study. Seeds (25 with corn, 100 with others) were placed between the disks of germination paper or on the soil. and the Petri dish was covered with a lid and kept in the dark for 7 days in an incubator maintained at 20 °C. The number of seeds germinated was then counted and calculated as a percentage of the number of seeds sown. The criterion for germination of the alfalfa seeds was radicle emergence. The criterion for germination of the other seeds studied was the emergence of a radicle and a coleoptile that were longer than the seed. All germination tests reported were performed in quadruplicate.

To determine the effect of the phenolic acids on the rate of germination of the seeds on germination paper and soil, 25 seeds (five rows of five) were placed on a 50-mm disk of germination paper or on 20 g air-dried soil in a $15-\times60$ -mm Petri dish (Fisher Scientific, Pittsburgh, Pa), and the germination paper or soil was moistened with 5 ml water (control) or with 5 ml water containing the phenolic acid under study. The Petri dish was then covered with a lid and placed in an incubator maintained at 20 °C, and the number of germinated seeds was counted every 24 h for 7 days and calculated as a percentage of the number of seeds sown. All germination tests reported were performed in quadruplicate.

To determine the effect of the phenolic acids on seedling growth, 20 seeds were placed between two 50-mm disks of germination paper or on 20 g air-dried soil in a 15×60 -mm Petri dish, and the germination paper or soil was moistened with 5 ml water (control) or

Table 1. Properties of soils used

Soil		pH (H ₂ O)	Organic	Sand (%)		Clay (%)
Series	Subgroup	(1120)	0(70)	(70)	(70)	(,,,,,
Webster	Typic Haplaquoll	6.6	3.3	28	40	32
Harps	Typic Calciaquoll	7.9	4.2	40	49	11
Storden	Typic Udorthent	8.1	1.2	19	24	57

with 5 ml water containing the phenolic acid under study. The Petri dish was then covered with a lid and placed in an incubator maintained at 20 °C. After 7 days, the lengths of the roots and shoots on the germinated seeds were measured. All tests reported were performed in triplicate.

To determine the effect of the phenolic acids on early plant growth in soil, eight seeds of the plant under study were placed 2 cm below the surface of 500 g air-dried soil in an $80 - \times 110$ -mm plastic pot, and the soil was moistened with 125 ml water (control) or with 125 ml water containing the phenolic acid under investigation. After plant emergence, the plants in the pot were thinned back to five plants. The pot was watered daily to replace water lost by evapotranspiration, which was determined by weighing the pot. After 10 and 20 days, the pot was treated with 10 ml water (control) or with 10 ml water containing the phenolic acid under study. After 30 days, the plants were removed from the pot, rinsed with water to remove soil particles, dried for 3 days at 65 °C, and weighed. All plant-growth tests reported were performed in quintuplicate.

Results and discussion

Experiments with germination paper

Although it has been widely reported that phenolic acids inhibit germination of seeds on germination paper (Mayer and Evenari 1952, 1953; Varga and Köves 1959), Williams and Hoagland (1982) studied the effects of several phenolic acids, including caffeic, pcoumaric, ferulic, and gallic acids, on seed germination of nine crop and weed species on germination paper and concluded that, in most cases, inhibition of germination by phenolic acids is not the primary cause of the phytotoxicity of these acids. They also concluded that phenolic acids delay but do not substantially inhibit germination. These conclusions are supported by the data reported in Tables 2 and 3. Table 2 shows that the nine phenolic acids tested did not inhibit germination of alfalfa, barley, corn, rye, oats, sorghum, or wheat seeds over 7 days at a concentration of 10^{-3} mol 1^{-1} H₂O, and that only three of these acids (*p*coumaric, ferulic, and vanillic acids) had any effect on seed germination at a concentration of 10^{-2} mol 1^{-1} H₂O (data for alfalfa seeds in Table 2). Table 3, however, shows that the rate of germination of the alfalfa and wheat seeds over 7 days was reduced by ferulic, pcoumaric, caffeic, p-hydroxybenzoic, and vanillic acids at a concentration of 10^{-2} mol 1^{-1} H₂O, and that the rate of germination of the sorghum seeds was reduced 118

Phenolic acid	Concentration (mol l^{-1} H ₂ O)	Seeds (%)	germination i	n 7 days)		Seeds (% germination in 7 days)											
	$(\text{morr} + \text{H}_2\text{O})$	Alfalfa	Barley	Corn	Rye	Oats	Sorghum	Wheat									
None (control)	_	92	95	84	94	96	85	93									
Caffeic acid	10^{-3}	90	94	85	93	98	85	92									
	10^{-2}	92	93	85	94	94	84	93									
Chlorogenic acid	10^{-3}	90	94	84	94	95	85	93									
	10^{-2}	9 1	94	83	93	95	84	93									
p-Coumaric acid	10 ⁻³	89	93	84	95	96	84	93									
	10^{-2}	67	96	86	94	95	83	92									
Ellagic acid	10^{-3}	90	95	84	96	98	84	93									
	10^{-2}	93	93	86	94	97	88	92									
Ferulic acid	10^{-3}	91	94	86	95	96	84	90									
	10^{-2}	86	95	85	96	95	83	92									
Gallic acid	10^{-3}	90	94	82	96	95	83	91									
	10^{-2}	92	94	85	94	97	86	93									
<i>p</i> -Hydroxybenzoic acid	10^{-3}	93	94	86	96	96	86	93									
	10^{-2}	91	93	87	95	96	84	94									
Syringic acid	10^{-3}	93	95	84	94	95	86	93									
	10^{-2}	91	94	86	93	97	83	93									
Vanillic acid	10 ⁻³	91	96	84	94	97	86	92									
	10^{-2}	88	95	85	95	96	83	94									
LSD ^a (0.05)		2.9	2.7	3.2	2.1	3.0	3.2	2.8									

Table 2. Effects of phenolic acids on germination of seeds on germination paper

^a LSD, least significant difference

Table 3. Effects of phenolic acids on rate of germination over 7 days (d) of seeds on germination paper

Phenolic acid	Concentration	%	Germi	natio	1													
	$(\text{mol } l^{-1} H_2 O)$	Alfalfa seeds				Sorghum seeds						Wheat seeds						
		1d	2d	3d	4d	7d	1d	2d	3d	4d	5d	7d	1d	2d	3d	4d	5d	7d
None (control)		14	74	87	92	92	0	53	83	85	85	85	0	4	41	91	92	92
Caffeic acid	10^{-3}	15	72	89	92	92	0	51	80	85	85	85	0	5	43	93	93	93
	10 ⁻²	4	70	89	93	93	0	55	82	86	86	86	0	4	21	67	89	91
p-Coumaric acid	10^{-3}	12	69	86	93	93	0	54	83	86	86	86	0	3	44	91	93	93
-	10^{-2}	1	8	51	59	72	0	17	34	61	84	84	0	0	8	32	66	91
Ferulic acid	10^{-3}	16	76	86	94	94	0	49	82	84	84	84	0	4	42	92	92	92
	10^{-2}	2	50	73	84	84	0	33	55	76	86	86	0	0	5	36	75	93
<i>p</i> -Hydroxybenzoic acid	10^{-3}	14	72	84	90	90	0	52	84	85	85	85	0	4	37	94	94	94
	10^{-2}	3	63	79	86	93	0	54	83	85	85	85	0	0	27	63	87	92
Vanillic acid	10^{-3}	13	75	85	92	92	0	44	84	86	86	86	0	4	43	66	93	93
	10^{-2}	1	51	70	83	86	0	41	83	84	84	84	0	2	18	48	81	90

by ferulic, *p*-coumaric, and vanillic acids at this concentration.

Many workers have noted that phenolic acids affect seedling growth on germination paper more than germination. For example, Guenzi and McCalla (1966a) reported that although *p*-coumaric, ferulic, *p*hydroxybenzoic, syringic, and vanillic acids had little effect on the germination of wheat seeds on germination paper when applied at concentrations up to 3.6×10^{-2} mol 1^{-1} H₂O, they severely reduced the growth of wheat seedlings on germination paper. Also, Rasmussen and Einhellig (1977) found that whereas sorghum seedling growth on germination paper was reduced when *p*-coumaric or ferulic acid was applied at a concentration of 5×10^{-4} mol 1^{-1} H₂O, germination of sorghum seeds on germination paper was inhibited only when the concentration of these acids was 10 times higher than that required for a reduction in sorghum seedling growth. Table 4 shows the effects of *p*-coumaric, ferulic, *p*-hydroxybenzoic, and vanillic acids at concentrations of 10^{-3} or 10^{-2} mol 1^{-1} H₂O on shoot growth in 7 days from barley, sorghum, and wheat seeds on germination paper. The data show that *p*-coumaric and vanillic acids reduced the shoot growth of sorghum and wheat seedlings at a concentration of 10^{-3} mol 1^{-1} H₂O, and that all four of the phenolic acids tested reduced shoot growth from barley, sorghum, and wheat seeds at a concentration of 10^{-2} mol 1^{-1} H₂O. These observations support the conclusion that seedling growth on germination paper is more sensitive to phenolic acids than is germination.

Experiments with soils

A study of the effects of nine phenolic acids on the germination of seven seeds in samples of Webster, Harps, and Storden soil incubated at 20 °C for 7 days showed that none of the phenolic acids tested affected the germination of seeds in these soils at concentrations of 10^{-3} or 10^{-2} mol 1^{-1} H₂O (0.25 or 2.5 μM g⁻¹ soil) (Table 5).

Studies to determine the effects of *p*-coumaric, ferulic, and vanillic acids on the rate of germination of seeds in samples in samples of Webster and Storden soil incubated at $20 \,^{\circ}$ C for 7 days showed that none of

 Table 4. Effects of phenolic acids on shoot growth of seedlings on germination paper

Phenolic acid	Concentration (mol 1^{-1} H ₂ O)		Seedling shoot growth in 7 days (mm)							
		Barley	Sorghum	Wheat						
None (control)		48	38	45						
p-Coumaric acid	10^{-3}	45	23	34						
	10^{-2}	35	10	29						
Ferulic acid	10^{-3}	49	31	41						
	10^{-2}	44	24	32						
<i>p</i> -Hydroxybenzoic acid	10^{-3}	48	40	40						
	10^{-2}	46	31	34						
Vanillic acid	10^{-3}	47	33	39						
	10^{-2}	40	29	31						
LSD (0.05)		5.4	4.8	5.6						

Table 5. Effects of phenolic acids on germination of seeds in soil

Phenolic acid	Concent	ration	See	ds ^a (º	70 ge	rmin	ation	in 7	day	s)													
	μ mol mol		Alfalfa			Barley		Corn I		Rye		Oats			Sorghum			Wheat					
	g ⁻¹ soil 1 ⁻	1 ⁻¹ water	w	Н	s	w	Н	s	w	Н	s	w	Н	s	w	Н	S	w	Н	S	w	Н	S
None (control)		_	91	9 1	93	95	94	96	84	86	86	94	95	96	96	95	96	86	85	83	92	93	93
p-Coumaric acid	0.25	10^{-3}	93	92	90	95	96	96	83	86	86	96	94	96	95	96	97	84	85	86	93	92	91
-	2.50	10^{-2}	91	90	92	96	95	95	84	83	84	95	96	94	96	94	96	83	85	83	91	93	93
Ferulic acid	0.25	10^{-3}	90	93	92	96	96	96	85	83	85	95	94	96	95	96	95	86	84	82	93	94	92
	2.50	10^{-2}	93	9 1	92	94	95	95	84	85	85	96	93	94	95	96	93	85	85	81	93	93	93
Vanillic acid	0.25	10^{-3}	92	9 1	90	96	95	96	83	84	84	94	96	95	96	94	96	84	85	85	92	92	94
	2.50	10^{-2}	91	93	90	95	94	95	86	86	86	95	96	94	95	95	96	84	86	83	92	91	92
Others ^{b, c}	0.25	10^{-3}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	2.50	10^{-2}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LSD (0.05)				3.2			2.6			2.9			3.1			2.1			3.0			3.3	

^a W, Webster soil; H, Harps soil; S, Storden soil

^b Caffeic acid, chlorogenic acid, ellagic acid, gallic acid, p-hydroxybenzoic acid, and syringic acid

^c NS, not significantly different from control

these acids affected the rate of germination of alfalfa, sorghum, or wheat seeds when they were applied at concentrations of 10^{-3} or 10^{-2} mol 1^{-1} H₂O (0.25 or 2.5 μ M g⁻¹ soil) (Table 6).

A study of the effects of nine phenolic acids on seedling growth in Webster and Storden soil over 7 days showed that the acids tested had no effect on seedling growth from seeds of alfalfa, sorghum, oats, or wheat when they were applied at concentrations of 10^{-3} or 10^{-2} mol 1^{-1} H₂O (0.25 or 2.5 μ M g⁻¹ soil) (Table 7).

Experiments to determine the effects of nine phenolic acids on early plant growth in Webster soil showed that these acids did not affect plant growth over 30 days when applied after 1, 10, and 20 days at a concentration of 3×10^{-3} mol 1^{-1} H₂O (0.75 μM g⁻¹ soil) (Table 8).

It is well established that phenolic acids occur in many plant materials and are released into soil from decaying plant residues (Winter 1961; del Moral and Muller 1970; Patrick 1971; Chou and Patrick 1976; del Moral et al. 1978; Rice 1984). Many of these compounds are rapidly decomposed by soil microorganisms (Henderson and Farmer 1955; Sparling and Vaughan 1981; Sparling et al. 1981; Vaughan et al. 1983), but some appear to be stabilized against microbiological degradation through sorption by organic matter, clay minerals, and hydroxy-aluminum or Fe compounds in soil (Huang et al. 1977; Sparling et al. 1981; Whitehead et al. 1981; Rice 1984), and several phenolic acids have been detected in soils (Whitehead 1964; Wang et al. 1967). However, the amounts of phenolic acids that have been detected in soils are much smaller than the amounts we added to soils in the work reported in Tables 4-8 (up to 2.5 μ mol g⁻¹

Phenolic acid	Concent	ration	%	Gern	ninati	ion																	
	μmol	mol	Alfalfa seeds					Sorghum seeds						Wheat seeds									
	g ⁻¹ soil	soil 1 ⁻¹ water	1d	2d	3d	4d	5d	6d	7d	1d	2d	3d	4d	5d	6d	7d	1d	2d	3d	4d	5d	6d	7d
Webster soil													_										
None (control	_	-	4	73	90	91	91	91	91	0	10	72	86	86	86	86	0	0	22	86	91	92	92
<i>p</i> -Coumaric acid	0.25	10^{-3}	4	70	92	93	93	93	93	0	11	69	85	85	85	85	0	0	20	84	91	91	91
-	2.50	10^{-2}	3	70	9 1	92	92	92	92	0	7	68	82	85	85	85	0	0	19	83	92	92	92
Ferulic acid	0.25	10^{-3}	6	71	90	92	92	92	92	0	8	72	84	86	86	86	0	0	21	84	93	93	93
	2.50	10^{-2}	3	68	88	91	91	91	91	0	10	67	82	85	85	85	0	0	19	82	83	83 -	83
Vanillic acid	0.25	10^{-3}	4	72	92	92	92	92	92	0	10	74	86	87	87	87	0	0	23	87	92	93	93
	2.50	10 ⁻²	7	75	93	93	93	93	93	0	8	70	84	84	85	85	0	0	23	83	90	90	90
Storden soil																							
None (control)			3	75	90	92	92	92	92	0	8	73	85	85	85	85	0	0	19	84	90	92	92
<i>p</i> -Coumaric acid	0.25	10^{-3}	2	67	90	92	92	92	92	0	9	68	83	84	84	84	0	0	17	83	92	92	92
•	2.50	10^{-2}	2	69	92	93	93	93	93	0	6	70	83	83	83	83	0	0	20	86	91	91	91
Ferulic acid	0.25	10^{-3}	4	70	89	90	90	90	90	0	9	67	84	85	85	85	0	0	23	85	93	93	93
	2.50	10^{-2}	4	71	86	90	92	92	92	0	11	72	83	84	84	84	0	0	18	82	90	90	90
Vanillic acid	0.25	10^{-3}	3	70	90	90	90	90	90	0	12	70	86	86	86	86	0	0	20	85	91	92	92
	2.50	10^{-2}	3	72	92	93	93	93	93	0	11	73	84	84	84	84	0	0	19	85	93	93	93

Table 6. Effects of phenolic acids on rate of germination over 7 days (d) of seeds in soils

Table 7. Effects of phenolic acids on seedling growth in soils

Phenolic acid	Concentrat	ion	Seedling g	rowth in 7 d	lays (mm)				
	μmol	mol 1 ⁻¹ water	Alfalfa shoot	Sorghun	1	Oat		Wheat	
	g ⁻¹ soil	g son i water		Root	Shoot	Root	Shoot	Root	Shoot
Webster soil									
None (control)	_	-	32	30	18	83	44	73	42
p-Coumaric acid	0.25	10^{-3}	36	33	19	79	45	75	44
•	2.50	10^{-2}	30	32	17	77	42	70	38
Ferulic acid	0.25	10^{-3}	35	32	17	78	45	72	41
	2.50	10-2	29	33	19	78	44	70	40
Vanillic acid	0.25	10 ⁻³	35	28	20	80	46	74	40
	2.50	10^{-2}	33	30	19	78	43	72	43
Others ^{a, b}	0.25	10^{-3}	NS	NS	NS	NS	NS	NS	NS
	2.50	10^{-2}	NS	NS	NS	NS	NS	NS	NS
Storden soil									
None (control)	_	_	33	32	20	83	46	72	45
p-Coumaric acid	0.25	10^{-3}	36	32	20	81	49	77	45
•	2.50	10^{-2}	31	29	19	7 9	47	73	42
Ferulic acid	0.25	10^{-3}	31	36	23	86	50	70	45
	2.50	10^{-2}	29	33	19	85	44	68	43
Vanillic acid	0.25	10^{-3}	33	34	19	84	45	76	42
	2.50	10^{-2}	32	31	22	82	44	77	43
Others ^{a, b}	0.25	10^{-3}	NS	NS	NS	NS	NS	NS	NS
	2.50	10^{-2}	NS	NS	NS	NS	NS	NS	NS
LSD (0.05)			4.6	6.7	4.7	7.2	6.3	5.3	5.6

^a Caffeic acid, chlorogenic acid, ellagic acid, gallic acid, p-hydroxybenzoic acid, and syringic acid

^b NS, not significantly different from control

soil). For example, Whitehead (1964) found that the amounts of *p*-coumaric, ferulic, *p*-hydroxybenzoic, and vanillic acids in grassland soils ranged from $4.0 \times 10^{-3} \,\mu\text{mol g}^{-1}$ soil to $3.6 \times 10^{-2} \,\mu\text{mol g}^{-1}$ soil,

and Wang et al. (1967) found that surface (0-25 cm)samples of eight soils contained only 0 to $2.9 \times 10^{-3} \mu \text{mol g}^{-1}$ soil of these phenolic acids. It is evident from the data in Tables 4–8, therefore, that

Phenolic acid ^a	Amount applied	Plant growth in 30 days (g dry matter)											
	(µmol g ⁻¹ soil)	Barley	Corn	Rye	Oats	Sorghum	Wheat						
None (control		1.67	2.32	1.39	1.35	1.95	1.50						
<i>p</i> -Coumaric acid	0.75	1.72	2.39	1.42	1.39	1.93	1.46						
Ferulic acid	0.75	1.73	2.38	1.43	1.41	1.96	1.55						
Vanillic acid	0.75	1.64	2.29	1.44	1.38	1.91	1.48						
Others ^{b, c}	0.75	NS	NS	NS	NS	NS	NS						
LSD (0.05)		0.08	0.09	0.06	0.07	0.07	0.06						

Table 8. Effects of phenolic acids on early plant growth over 30 days in Webster soil

^a The phenolic acids specified were applied at a concentration of 10^{-3} mol 1^{-1} water (0.25 µmol g⁻¹ soil) after 1, 10, and 20 days

^b Caffeic acid, chlorogenic acid, ellagic acid, gallic acid, *p*-hydroxybenzoic acid, and syringic acid

^c NS, not significantly different from control

the amounts of phenolic acids detected in soils are too small to have an adverse effect on seed germination, seedling growth, or early plant growth.

It has been suggested (Rasmussen and Einhellig 1977; Einhellig and Rasmussen 1978) that phenolic acids may act synergistically in soils, i.e., that the effects of a combination of phenolic acids on seed germination and seedling growth may be significantly greater than the sum of the individual effects of these acids. We have been unable to confirm this hypothesis by comparing the individual and combined effects of *p*coumaric, vanillic, and ferulic acids on seed germination, seedling growth, and early plant growth in soil (Krogmeier 1988).

To summarize, the work reported here showed that the phenolic acids tested had no effect on seed germination, seedling growth, or early plant growth in soil even when the amounts applied were much greater than the amounts detected in soil. We conclude that the adverse effect of plant residues on crop yields is not due to phenolic acids derived from these residues. Alternative explanations of the adverse effects of plant residues on crop yields are that these residues immobilize nitrogen (Elliot et al. 1981) or reduce soil temperature (Unger and McCalla 1981).

Acknowledgment. Journal Paper No. J-13462 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, USA. Project 2655.

References

- Association of Official Seed Analysts (1981) Rules for testing seeds. J Seed Technol 6:1-126
- Chou CH, Patrick ZA (1976) Identification and phytotoxic activity of compounds produced during decomposition of corn and rye residues in soil. J Chem Ecol 2:369-387
- Cochran VL, Elliot LF, Papendick RI (1977) The production of phytotoxins from surface crop residues. Soil Sci Soc Am J 41:903-907

- Dalton BR, Weed S, Blum U (1987) Plant phenolic acids in soils: A comparison of extraction procedures. Soil Sci Am J 51:1514-1521
- del Moral R, Muller CH (1970) The allelopathic effects of *Eucalyp*tus camaldulensis. Am Midl Nat 83:254-282
- del Moral R, Willis RJ, Ashton DH (1978) Suppression of coastal heath vegetation by *Eucalyptus baxteri*. Aust J Bot 26:203-219
- Einhellig FA, Rasmussen JA (1978) Synergistic inhibitory effects of vanillic and *p*-hydroxybenzoic acids on radish and grain sorghum. J Chem Ecol 4:425-436
- Einhellig FA, Rasmussen JA (1979) Effects of three phenolic acids on chlorophyll content and growth of soybean and grain sorghum seedlings. J Chem Ecol 5:815-824
- Elliot LF, McCalla TM, Wais A (1978) Phytotoxicity associated with residue management. In: Oschwald WR (ed) Crop residue management systems. Am Soc Agron Spec Publ 31, Am Soc Agron, Madison, Wisc, pp 131-146
- Elliott LF, Cochran VL, Papendick RI (1981) Wheat residues and nitrogen effects on wheat growth in the greenhouse. Soil Sci 131:48-52
- Guenzi WD, McCalla TM (1962) Inhibition of germination and seedling development by crop residues. Soil Sci Soc Am Proc 26:456-458
- Guenzi WD, McCalla TM (1966a) Phenolic acids in oats, wheat, sorghum, and corn residues and their phytotoxicity. Agron J 58:303-304
- Guenzi WD, McCalla TM (1966b) Phytotoxic substances extracted from soil. Soil Sci Soc Am Proc 30:214-216
- Henderson MEK, Farmer VC (1955) Utilization by soil fungi of *p*hydroxybenzaldehyde, ferulic acid, syringealdehyde and vanillin. J Gen Microbiol 12:37-46
- Hennequin JR, Juste C (1967) Presence d'acide phénols libres dans le sol: Étude de leur influence sur la germination et al croissance des végétaux. Ann Agron 18:545-569
- Huang PM, Wang TSC, Wang MK, Wu MH, Hsu NW (1977) Retention of phenolic acids by noncrystalline hydroxy-aluminum and iron compounds and clay minerals of soils. Soil Sci 123:213-219
- Krogmeier MJ (1988) Effects of organic acids on seed germination and seedling growth in soil. MS thesis, Iowa State University, Ames, Iowa
- Lodhi MAK, Bilal R; Malik KA (1987) Allelopathy in agroecosystems: Wheat phytotoxicity and its possible roles in crop rotation. J Chem Ecol 13:1881-1891
- Mayer AM, Evenari M (1952) The relation between the structure of coumarin and its derivatives and their activity as germination inhibitors. J Exp Bot 3:246–252
- Mayer AM, Evenari M (1953) The activity of organic acids as germination inhibitors and its relation to pH. J Exp Bot 4:256-263

- McCalla TM, Haskins FA (1964) Phytotoxic substances from soil microorganisms and crop residues. Bacteriol Rev 28:181-207
- McCalla TM, Norstadt FA (1974) Toxicity problems in mulch tillage. Agric Environ 1:153-174
- Patrick ZA (1971) Phytotoxic substances associated with the decomposition in soil of plant residues. Soil Sci 111:13-18
- Patterson DT (1981) Effects of allelopathic chemicals on growth and physiological responses of soybean (*Glycine max*). Weed Sci 29:53-59
- Rasmussen JA, Einhellig FA (1977) Synergistic inhibitory effects of p-coumaric and ferulic acids on germination and growth of grain sorghum. J Chem Ecol 3:197-205
- Rice EL (1984) Allelopathy, 2nd edn. Academic Press, New York
- Sparling GP, Vaughan D (1981) Soil phenolic acids and microbes in relation to plant growth. J Sci Food Agric 32:625-626
- Sparling GP, Ord BG, Vaughan D (1981) Changes in microbial biomass and activity in soils amended with phenolic acids. Soil Biol Biochem 13:455-460
- Unger PW, McCalla TM (1981) Conservation tillage systems. Adv Agron 33:1-58
- Varga M, Köves E (1959) Phenolic acids as growth and germination inhibitors in dry fruits. Nature (Lond) 183:401

- Vaughan D, Sparling GP, Ord BG (1983) Amelioration of the phytotoxicity of phenolic acids by some soil microbes. Soil Biol Biochem 15:613-614
- Wang TSC, Yang TK, Chuang T (1967) Soil phenolic acids as plant growth inhibitors. Soil Sci 103:239-246
- Whitehead DC (1964) Identification of *p*-hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acids in soils. Nature (Lond) 202:417-418
- Whitehead DC, Dibb H, Hartley RD (1981) Extractant pH and the release of phenolic compounds from soils, plant roots and leaf litter. Soil Biol Biochem 13:343-348
- Williams RD, Hoagland RE (1982) The effects of naturally occurring phenolic compounds on seed germination. Weed Sci 30:206-212
- Winter AG (1961) New physiological and biological aspects in the interrelationships between higher plants. Symp Soc Exp Biol 15:229-244
- Zantua MI, Bremner JM (1975) Comparison of methods of assaying urease activity in soils. Soil Biol Biochem 7:291-295

Received February 12, 1989