PHENOLICS ADSORPTION TO SOIL REDUCES THEIR ALLELOCHEMICAL ACTIVITY [1]

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ABSTRACT – Allelopathy could be used to reduce weed infestations, but adsorption of allelochemicals to soil can reduce its effects in agro-ecosystems. The objectives of this work were to study the effect of caffeic, ferulic, and salicilic acids, and catechol on *Setaria faberi* (SETFA) germination in Petri-dish and soil bioassays, and to determine the adsorption of these phenolic compounds to a silt clay loam soil. Petri-dish experiments, using SETFA as the indicator species, showed that the allelopathic potential of the phenolics tested was: ferulic acid > catechol > salicilic acid > caffeic acid. Caffeic acid, up to 27 mM, did not affect SETFA germination. SETFA germinated better in soils than in Petri-dish experiments, suggesting that adsorption plays an important role on the performance of the phenolic acids. Adsorption of the phenolics to soil ranged from 10 to 40 % of the applied concentration. Caffeic acid and catechol had more affinity with the soil than salicilic and ferulic acids. The rate of these phenolics in 12 t/ha of wheat straw is not enough to reduce SETFA germination in the field. These results indicate that allelochemical activity may be limited by their adsorption to the soil.

Key words: no-tillage, wheat, *Setaria faberi*, giant foxtail

ADSORÇÃO DE FENÓLICOS AO SOLO REDUZ SUA ATIVIDADE ALELOPÁTICA

RESUMO – Alelopatia poderia ser utilizada para reduzir infestações de plantas daninhas, mas a adsorção de aleloquímicos ao solo pode reduzir sua eficácia. Os objetivos deste trabalho foram avaliar o efeito de catecol e dos ácidos cafeico, ferúlico e salicílico na inibição da germinação de *Setaria faberi* (SETFA) em experimentos em placa de Petri e em vasos; e quantificar a adsorção destes fenólicos num solo argiloso. Experimentos em placa de Petri, utilizando SETFA como espécie indicadora, demonstraram que o potencial alelopático dos fenólicos testados foi: ácido ferúlico > catecol > ácido salicílico > ácido cafeico. Ácido cafeico, até 27 mM, não afetou a germinação de SETFA. Ocorreu melhor germinação de SETFA no solo do que em placas-de-petri, sugerindo que a adsorção afetou o desempenho dos fenólicos. A adsorção destes fenólicos ao solo variou entre 10 e 40 % do total aplicado. Ácido cafeico e catecol tiveram maior afinidade pelo solo do que os ácidos salicílico e ferúlico. A quantidade destes fenólicos em 12 t/ha de palha de trigo não foi suficiente para reduzir significativamente a germinação de SETFA no campo. Estes resultados indicam que os efeitos inibitórios dos aleloquímicos são limitados pela sua dose e adsorção ao solo.

Palavras-chave: plantio direto, trigo, *Setaria faberi*, capim setária.

INTRODUCTION

Annual weed infestation is reduced after several years of no-till farming. Allelopathy is one mechanism weed scientists think may cause the observed results. However, there are indications that allelochemical activity in the soil depend on processes of removal or dissipation such as adsorption, leaching, volatilization, photolysis and chemical or biological degradation (VIDAL and BAUMAN, 1997).

Adsorption is the most important soil factor controlling the fate of chemicals in the environment because it controls the chemical concentration present in the soil solution (SHEA, 1989). Allelochemicals are also adsorbed to soil particles and may have their concentration drastically reduced (DALTON, 1989; DALTON et al., 1989; GUENZI and McCALLA, 1966 a; HORRIE et al., 1989). DALTON et al. (1989) reported 20 to 60 % adsorption of ferulic acid one day after incubation in several sterilized soils. Likewise, GUENZI and McCALLA (1966 b) observed that the phenolic acids from wheat residues were highly adsorbed to soils, whereas WANG et al. (1971) documented 60 to 98 % adsorption of syringic or ferulic acids only three hours after incubation in soil.

The Freundlich equation is the most common mathematical description of adsorption:

$$x/m = K_f C^{1/n}$$

where x = amount adsorbed, m = amount of adsorbent, C = chemical concentration at equilibrium, K_f and n are empirical constants reflecting the degree of binding and the degree of linearity between the amount adsorbed and the concentration at equilibrium. If $1/n > 1$, the adsorption isotherm has a concave shape (S-type isotherm). If $1/n < 1$, the adsorption isotherm has a convex shape (L-type isotherm). If $1/n = 1$, K_f becomes a partition coefficient (K_D), and the linear equation is called a C-type isotherm (for constant partition) (SHEA, 1989; WEBER et al., 1989).

The objectives of this work were to examine whether germination media affect germination inhibition activity of caffeic, ferulic, salicilic acids, catechol, and to determine the adsorption of these phenolics.

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MATERIAL AND METHODS

Preparation of phenolic solution and controls

The phenolics caffeic, ferulic, and salicilic acids, and catechol, purchased from Sigma (Sigma Chemical Company), were dissolved in methanol, which constituted 10% of the final water solution. In all experiments, methanol 10% in deionized water was used as control.

Effect of media on phenolics performance

The effect of three media on the performance of wheat extracts, caffeic, ferulic, and salicilic acids, and catechol was determined with a bioassay in controllable environment chamber, using *Setaria faberi* (SETFA) as reagent species. The experimental design was a randomized complete block with 15 treatments and 3 replications.

Wheat extract (10% w/w) was obtained with methanol 10% in deionized water. The rates of caffeic, ferulic, and salicilic acids, and catechol were 27, 5, 36, and 45 mM (or 5000, 970, 5000, and 5000 ppm), respectively.

The media tested included filter paper, placed in Petri dishes, and either soil or sterilized soil, placed in pots. The sterilized soil was autoclaved for 1 h at 120 C and 220 kPa. Each petri-dish had 2 filter papers, 7 ml of the phenolic solution, and 250 SETFA seeds. Each pot had 300 cm^3 soil and 250 SETFA seeds placed 1 cm bellow the surface. The pots were placed in containers with the phenolic solution to provide constant sub-irrigation. The chambers were maintained at 27° C with 14/10 h photoperiod. SETFA seedlings were counted 7 days after the installation of the experiment. The data were expressed as a percentage germination in relation to the water control for each media.

Adsorption of selected phenolics

Adsorption of catechol, caffeic, ferulic, and salicilic acids was determined using the batch method adapted from WEBER et al. (1986) using 1.0 g soil in an Erlenmeyer flask with 40 ml solution of a known phenolic concentration. The soil was a Chalmer silty clay loam collected from the upper 5 cm from the Purdue Agronomy Research Center at West Lafayette, IN, thoroughly mixed, air dried at 24° C, and passed through a 2 mm mesh screen. The flask was shaken on a rotating table shaker at 160 rotations per minute (RPM) for 48 h; the solution was centrifuged at 6000 RPM for 10 min, and the supernatant was filtered through 0.45 µm mesh filter papers. Phenolic concentration were determined by high pressure liquid chromatographic (HPLC) analysis.

Each phenolic was tested at four concentrations with three replications. The initial concentrations were: 0.0, 4.5, 7.5, and 10.0 mM for catechol; 0.0, 0.7, 1.5, and 3.0 mM for caffeic acid; and 0.0, 1.2, 2.5, and 5.0 mM for salicilic and ferulic acids.

Final concentrations were determined in 20 µl samples injected in a Hewlett-Packard Series 1050 HPLC equipped with a quaternary pump and multiple wavelength detector. The HPLC had a Spherisorb ODS Hypersil, 5 µm, 20 by 4 mm guard column and a Spherisorb ODS 5 µm, 250 by 4 mm column, which worked at 24° C constant temperature. Elution was performed during 10 min using 30% of solution A and 70% solution B at 1 ml/min flow rate. Solution A was 1% acetic acid in deionized water and B was 100% acetonitrile for catechol and salicilic acid analysis, whereas A was 100% acetonitrile and B was 1% acetic acid in deionized water for caffeic and ferulic acid analysis.

Catechol was detected at 275 nm wavelength, and caffeic, ferulic and salicilic acids were detected at 300 nm. Peaks occurred at 3.3, 3.8, 4.6, and 5.8 min for caffeic acid, catechol, ferulic acid, and salicilic acid, respectively. The relative concentration of each phenolic was determined using external standards as follows: known concentration of the standard was subjected to the same HPLC analysis as the supernatant reported above; the resultant peak area for each standard was used to determine the concentration for the area obtained from the supernatant peaks. The final concentration in the solution was subtracted from the initial concentration and the result was assumed to be the amount adsorbed to the soil.

SETFA response to rates of selected phenolics

Four experiments were conducted in controllable environment chambers to determine SETFA germination in response to rates of caffeic, ferulic, and salicilic acids, and catechol. Each experiment had 4 treatments (rates) and 3 replications. Rates tested were: 0.0, 2.2, 3.0, and 27.0 mM for caffeic acid; 0.0, 2.5, 9.0, and 45.0 mM for catechol; 0.0, 1.2, 2.5, and 5.0 mM for ferulic acid; and 0.0, 2.5, 5.0, and 36.0 mM for salicilic acid.

Each plot consisted of a Petri dish with two filter papers, 7 ml of phenolic solution, and 250 SETFA seeds. The chambers were maintained at 27° C with 14/10 h photoperiod.

SETFA seedlings were counted 7 days after the installation of the experiment. The data were expressed as a percentage germination in relation to the water control.

Data analysis

All data was subjected to analysis of variance (ANOVA). To ensure homocedasticity, ANOVA was also performed in the data transformed to log(x+1), where x was the observed data. In the experiment testing the media effect on phenolic performance, the separation of means was performed with LSD. In the experiment of adsorption, the concentration adsorbed and concentration at equilibrium (final) for each phenolic were log-transformed and used to solve the Freundlich equation (SHEA, 1989; WEBER et al., 1989). In the experiments of rate response, the degrees of freedom of treatments were partitioned into effect linear, quadratic, and deviation from quadratic by orthogonal contrasts.

RESULTS AND DISCUSSION

Effect of media on phenolics performance

Media and treatments interacted affecting the performance of phenolics on SETFA germination (Table 1). The number of SETFA seedlings was similar in all three media in control pots (water + methanol treatments). Caffeic acid did not affect SETFA germination in the three media tested (Table 1). Catechol and salicilic acid were highly inhibitory to SETFA germination in Petri-dish bioassays, but had no effect when soil was the media. Ferulic acid strongly inhibited SETFA germination in Petri-dish bioassays, partially inhibited germination in soil media, and did not affect SETFA germination when sterilized soil was the media. Germination of SETFA in Petri dishes and sterilized soil containing wheat extracts was reduced in relation to the water control, whereas the SETFA germination in non-sterilized soil containing wheat extracts increased in relation to the water control (Table 1).

TABLE 1 – Effect of media and solution treatments on SETFA germination. Purdue University, West Lafayette, IN

Treatment	Petri-dish	Soil	Sterilized soil
		---------%---------	
Caffeic acid	86 Ax [1]	96 CDx	78 Cx
Catechol	0 Dy	114 BCx	137 Ax
Ferulic acid	19 Cdy	70 Dx	102 Bx
Salicilic acid	0 Dy	94 CDx	97 BCx
Wheat extract	49 BCy	155 Ax	67 Cy

1. Uppercase letters compare means within treatment, lowercase letters compare means within media.

Several researchers have observed that binding of allelochemicals to soil would reduce their performance in nature (DALTON, 1989; DALTON et al., 1989; GUENZI and McCALLA, 1966 a; HORRIE et al., 1989). Binding of the phenolics to soil particles could explain, in part, the reduced performance of catechol, ferulic acid, salicilic acid, and wheat extracts on SETFA. Soil binding and microbial decomposition could account for the increased SETFA germination in soil treated with wheat extracts.

Adsorption of selected phenolics

The amount of caffeic acid, catechol, ferulic acid, and salicilic acid adsorbed to soil is presented in Table 2. For all four phenolics, the amount adsorbed increased with increasing initial concentration.

TABLE 2 – Amount of phenolic adsorbed to soil after incubation in three initial concentrations, and Freundlich equation parameters. Purdue University West

Phenolic	Amount adsorbed after Initial Concentrations [1]			Equation parameters [2]	
	1	2	3	Kf	1/n
	---- mg/g soil (%) ----				
Caffeic	41 (30) [3]	85 (31)	114 (21)	2.45	0.6
Catechol	106 (21)	276 (33)	418 (38)	0.09	1.3
Ferulic	48 (19)	91 (19)	290 (29)	0.03	1.4
Salicilic	14 (8)	30 (8)	51 (7)	0.37	0.7

1 Initial concentrations were: 135, 270, 540 mg/l for caffeic acid; 505, 825, 1101 mg/l for catechol; 242, 485, 971 mg/l for ferulic acid; and 172, 345, 690 mg/l for salicilic acid, respectively.

2 Freundlich equation is: $x/m = K_f C^{1/n}$, where x=amount adsorbed, m=amount of soil, C = chemical concentration at equilibrium, K_f and n = empirical constants.

3 Number in parenthesis represent percentage adsorbed in relation to The initial concentration.

The adsorption of caffeic and salicilic acid followed an L-type isotherm (1/n < 1). The L-type isotherm indicates that these phenolics have a preferential sorption initially compared to water or other solutes, with sorption decreasing as more phenolic is sorbed because of the difficulty for a bombarding phenolic molecule to find a vacant site in the adsorbent (WEBER and MILLER, 1989). These authors indicate that L-type isotherms occur when specific bondings are involved, or sorbed molecules are not vertically oriented, or water molecules are not strong competitors for adsorption sites, or when a mixture of several adsorbents (organic matter plus clay for instance) is present (WEBER and MILLER, 1989). Herbicides that follow an L-type adsorption to soils are alachlor, atrazine, bromacil, diquat, diuron, fluridone, glyphosate, paraquat, and prometryn (WEBER and MILLER, 1989).

The adsorption of catechol and ferulic acid followed an S-type isotherm (1/n > 1). The S-type isotherm indicates that these phenolics have a relatively low adsorption initially, and adsorption increases as the number of molecules on the surface increases (also called cooperative sorption mechanism) (WEBER and MILLER, 1989). Three conditions are required: the molecule has one point attachment, the molecule has moderate intermolecular attraction because orient vertically when sorbed, and suffers strong competition for adsorption sites from water and other molecules (WEBER and MILLER, 1989). Herbicides that follow an S-type adsorption to montmorillonite are acetochlor, alachlor, and metolachlor (WEBER and MILLER, 1989; WEBER et al., 1986).

High K_f values indicate a higher affinity between the phenolic and the adsorbents (SHEA, 1989; WEBER and MILLER, 1989). In the L-type isotherm, caffeic acid had more affinity with the soil than salicilic acid (Table 2), and this compound was less adsorbed than the former in all concentrations tested. In the S-type isotherm, catechol had more affinity with the soil than ferulic acid (Table 2). In fact, 20% of catechol or ferulic acid was adsorbed at the lower concentration tested, but 38% of catechol and only 29 % of ferulic acid was adsorbed to soil at the highest concentration tested (Table 2).

SETFA response to rates of selected phenolics

Catechol had the classical quadratic effect on SETFA germination. Increasing catechol rate from 0 to 2.5 mM increased the germination, subsequent rate increase to 45 mM resulted in decreased SETFA germination (Table 3). Ferulic and salicilic acid up to 2.5 mM did not affect SETFA germination, but increasing the rates to 5.0 and 36.0 mM, respectively, severely reduced SETFA germination (Table 3). Caffeic acid, up to 27 mM, did not affect SETFA germination (Table 3).

TABLE 3 – SETFA germination as influenced by selected phenolic compounds at various concentrations. Purdue University, West Lafayette, IN

Rate [1]	Phenolics			
	Caffeic	Cathecol	Ferulic	Salicilic
1	100 a [2]	100 b	100 a	100 a
2	116 a	170 a	114 a	92 a
3	94 a	28 c	91 a	52 b
4	76 a	0 d	25 b	0 c

1 Rates were: 0.0, 2.2, 3.0, 27.0 mM for caffeic acid; 0.0, 2.5, 9.0, 45.0 mM for catechol; 0.0, 1.2, 2.5, 5.0 Mm for ferulic acid; and 0.0, 2.5, 5.0, 36.0 mM for salicilic acid, respectively.

2 Means in the column followed by the same letter are not significantly different (p=95%).

The allelopathic potential of the phenolics tested in Petri-dish experiments was: ferulic acid > catechol > salicilic acid. The germination of SETFA was inhibited by 50% with rates between 2.5 to 5.0 mM of ferulic acid, 2.5 to 9.0 mM of catechol, and with 5 mM of salicilic acid (Table 3). These results coincide with those reported by DUKE (1986) were rates up to 5 mM of several phenolics were necessary to inhibit germination of several test species by 50%.

Ferulic acid is the most abundant in wheat residues of the phenolics studied (GUENZI and MACCALLA, 1966, a; GUENZI and MAcCALLA, 1966, b). Assuming the concentration of ferulic acid as 120 mg/kg of wheat dry mass (GUENZI and MAcCALLA, 1966, a), then 12 t/ha of wheat residues would contribute 1.44 kg/ha of ferulic acid if all the ferulic acid was immediately released to the soil. Assuming that after release ferulic acid accumulated in the top 100 μm (0.1 mm) of the soil, the concentration of this phenolic acid would be 1100 mg/kg soil (assuming soil density of 1.3 g/cm^3). This concentration would dilute linearly to 5 mg/kg soil if ferulic acid diffused uniformly to 2 cm depth in the soil. This reasoning illustrates that in the surroundings of the decomposing wheat residues the concentration of ferulic acid could be high enough to inhibit SETFA germination by 75%, only if all of the ferulic acid in the residue were released and no removal (such as adsorption) or degradation processes occurred.

This reasoning allow us to speculate that, in no-till soils, allelochemicals present in wheat residues have activity limited by time and space. Time limitation occurs because the allelochemicals are not released at once from the decaying wheat residues, and because adsorption and degradation reduce the concentration available in the soil solution. Space limitations occur because, even if all the allelochemicals in wheat residues were released at once, and microbial degradation and adsorption were precluded, the performance of the phenolics would be spatially limited to seeds placed near the decomposing wheat residues, because of reduced concentration.

KIMBER (1973) indicated that , in nature, the concentrations range from inhibitory, for some allelochemicals, to stimulatory, for other allelochemicals, and the resultant net effect may be lower inhibition or stimulation or no effect at all. Some authors have argued that allelochemicals act synergistically, thus magnifying their phytotoxic capabilities (EINHELLIG and

RASMUSSEN, 1978). Few experiments were conducted to test this hypothesis. However, herbicide science indicates that synergism is a rare occurrence and usually antagonistic (GREEN, 1989; ZHANG et al., 1995) or additive (GREEN, 1989) effects are the norm. As expected, DUKE et al. (1984) reported antagonism between p-coumaric and ferulic acids on lettuce seed germination, and BLUM et al. (1984) observed antagonism between ferulic, caffeic, and vanillic acids on cucumber radicle growth.

CONCLUSIONS

This work indicated that SETFA germinated better in soil than in Petri-dish experiments, suggesting that adsorption plays an important role on the performance of the phenolic acids. Adsorption of the phenolics to soil ranged between 10 to 40 % of the applied concentration. Petri-dish experiments using SETFA as the indicator species showed that the allelopathic potential of the phenolics tested was: ferulic acid > catechol > salicilic acid > caffeic acid. Caffeic acid, up to 27 mM, did not affect SETFA germination. These results indicate that allelochemical activity is limited by their adsorption to soil.

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