

## Preferential Sorption of Phenolic Phytotoxins To Soil: Implications for Altering the Availability of Allelochemicals

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Allelopathy, secondary metabolite-mediated plant-to-plant interaction, is gaining application in current agricultural science as well as in invasion ecology. However, the role of sorption to soil in modifying the bioavailability of components in complex allelochemical mixtures is still obscure. Hence, the role of preferential sorption to soil in altering the chemical composition of plant exudates was studied in a silt loam soil using representative mixtures of plant phenolic acids, namely, hydroxybenzoic acid, vanillic acid, coumaric acid, and ferulic acid. The experiment was conducted using a batch equilibration technique, and data were fitted to a Freundlich isotherm. The concentration-dependent sorption coefficient ( $K_d$ ) at  $10 \mu\text{g mL}^{-1}$  was used to assess the sorption affinity of phenolic acids across different systems. Along with solid phase dissolution, all of the phenolic acids exhibited strong site-specific sorption, as evident from their nonlinear isotherms. Removal of organic matter substantially decreased the sorption affinity of all phenolic acids. Direct competition for sorption sites was observed even at low concentrations of phenolic acids. The  $K_d$  of hydroxybenzoic acid was decreased more than 90% in the presence of coumaric acid. About 95% of sorbed vanillic acid was displaced into the soil solution in the presence of ferulic acid. Hydroxybenzoic acid did not affect the sorption affinity of other phenolic acids significantly, whereas ferulic acid showed low displacement by other phenolic acids. The displacement pattern indicated directional sorption of phenolic acids with  $-\text{OH}$  and  $-\text{COOH}$  groups. Soil organic matter was associated with preferential sorption. This is the first study to elucidate competitive sorption characteristics of plant secondary metabolites in soil matrix. The results demonstrate that preferential sorption to soil can alter the availability of plant exudates in mixtures and thus may mediate their phytotoxic effects.

**KEYWORDS:** Preferential sorption; competitive sorption; allelopathy; phenolic acids; plant exudates; water solubility

### INTRODUCTION

Allelopathy, as coined by Molisch (1), encompasses positive and negative, direct and indirect biochemical interactions between all types of plants including microorganisms. These plant-to-plant interactions are mediated by a wide variety of plant secondary metabolites including phenolic compounds, benzoxazinoids, glucosinolates, and sesquiterpenoids (2, 3). This plant secondary metabolite-mediated “chemical warfare” among plants has been gaining application in present-day agricultural science, especially in the areas of exotic plant invasions (4–6) and identification of allelopathic crops and cover crops for integrated weed management (7–9). Because of increased environmental concerns, herbicide costs, and development of herbicide-resistant weed species biotypes, alternative nonchemical weed management practices are receiving attention (8). Allelopathy offers the potential for selective biological weed management and could be incorporated into organic farming.

Unlike herbicides, allelochemicals are environmentally benign, less persistent, and exhibit bioactivity at low concentration (10, 11). Because of the multiple modes of activity, the development of weed species resistant to allelochemicals is rare. Significant success has been achieved in screening cultivars of rice, wheat, sorghum, and rye that have allelopathic potential (7–9, 12, 13). Chemical screening of genotypes with higher allelochemical content is currently employed in identifying allelopathic cultivars (7, 13, 14). Although quite successful in identifying potential varieties, chemical screening has some drawbacks in field applicability. The phytotoxicity of allelopathic cultivars under field conditions could not be attributed solely to the presence of a single compound (15), and phytotoxicity assessments in laboratory bioassays are difficult to duplicate under field conditions as the soil aspect of allelopathy is usually ignored (16).

The effect of soil in modifying the phytotoxicity of allelochemicals is not well-understood (16). Soils are capable of altering the phytotoxicity of plant secondary metabolites by changing their bioavailability, persistence, and fate under field conditions. Sorption is one of the prominent factors affecting

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the phytoavailability of allelochemicals in soil. Soil sorption can also protect compounds from microbial degradation and thus indirectly alter their phytotoxic effects (17, 18). The soil sorption properties of some individual allelochemicals have previously been studied. Various soil components such as organic matter, sesquioxides, and clay minerals have been found to affect the phytoavailable concentration of allelochemicals (19–21). Because allelochemicals are generally exuded in mixtures of metabolites that often include other allelochemicals (14, 22), preferential sorption of compounds onto the soil matrix could further alter availability. Even if an observed phytotoxicity is entirely due to a single compound in plant exudates (4–6), the rate at which the compound becomes available will be the primary factor governing its phytotoxicity. Therefore, apart from the physiological joint toxic activity of compounds in a mixture (23), preferential sorption of compounds onto the soil matrix, by altering the chemical composition of exudates, could be another mechanism that mediates the phytotoxicity of allelopathic mixtures in field situations.

Studies investigating the sorption of individual compounds fail to assess the final composition of the multicomponent exudates that become bioavailable. This is because sorption of compounds in a multisolute system differs from that in a single-solute system and is affected by the presence of companion solutes. Xing and Pignatello (24) reported that naturally occurring plant phenolic acids competed with anthropogenic contaminants for sorption onto the soil matrix, which in turn resulted in increased bioavailability of the contaminants. Similar results were also reported by Yu and Huang (25) while studying the sorption behavior of hormones onto soil. In addition, an increase in soil phosphorus availability was reported in the presence of dissolved organic matter (26). However, a companion solute may also increase the sorption affinity of a primary solute because of swelling of the organic matter matrix (27). Competitive sorption is due to the varied affinity of nonidentical molecules for similar sorption sites. To date, the phenomenon of preferential sorption of multicomponent plant exudates onto a soil matrix has not been investigated in detail. Considering the fact that compounds in the soil solution are more phytoavailable than those sorbed to the soil matrix (28, 29), the varied sorption affinity of compounds in a mixture will change the composition of plant exudates that become bioavailable. Different compounds in plant exudates differ widely in their phytotoxicity (15, 22); hence, preferential sorption to soil could in turn alter the phytotoxicity of these exudates.

In this study, mixtures of phenolic acids were chosen as representative plant exudates. Phenolic compounds are the most widely distributed class of secondary metabolites in plants. Considering the total phenolic content in plant roots, litter, and organic matter, it is estimated that more than 100 kg ha<sup>-1</sup> of phenolic compounds is added to grassland soils annually (30). Phenolic acids and their derivatives are the major phenolic compounds in most plant ecosystems (2). Phenolic acids in the plant–soil environment represent a dynamic system undergoing continuous cycles of deposition, decomposition, plant uptake, leaching, chemical immobilization, and microbial synthesis (30). Because of their ability to modify plant growth, most phenolic acids are implicated in plant–plant interactions (3). Four phenolic acids, namely, *p*-hydroxybenzoic acid (HYB), vanillic acid (VAN), *p*-coumaric acid (COU), and ferulic acid (FER), were used for the experiment because they are ubiquitous in plant exudates (22, 30) and have been implicated in allelopathy (3). HYB, VAN, COU, and FER have been detected as primary phytotoxic compounds in the rhizosphere of many plant species,

for example, *Sasa cernua* (31), *Quercus mongolica* (32), *Echinochloa crus-galli* (33), and *Allium ursinum* (34). Also, the allelopathic activity of rice (35), sorghum (36, 37), and wheat (38, 39) has been attributed partly to the presence of these phenolic acids.

The objectives of this study were to (i) identify the presence of site-specific sorption to soil of compounds typical of plant exudates in soil, (ii) identify the soil fraction leading to site-specific sorption, and (iii) investigate the occurrence of preferential sorption of compounds in plant exudates onto the soil matrix.

## MATERIALS AND METHODS

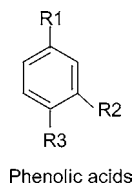
**Soil and Chemicals.** The sorption studies were carried out using a Scitico silt loam soil (Typic Haplaquepts) with a pH of 5.15 (measured in CaCl<sub>2</sub> solution) and an organic matter content of 6%. The soil was air-dried, sieved (0.5 mm), and stored at 10 °C. The sieved soil composition was 32% sand, 40% silt, and 28% clay. The dithionate–citrate extractable Fe, Al, and Mn of the soil were 0.98, 0.22, and 0.047%, respectively.

To assess the soil fraction involved with site-specific sorption, soil organic matter was removed by oxidation (40). This involved adding increments of H<sub>2</sub>O<sub>2</sub> (30%) to a 1:2 soil–water mixture kept on a hot plate at 60 °C until the effervescence disappeared. The samples were then centrifuged at 3000g for 15 min to settle all soil particles, washed, and air-dried. The organic C content was determined by dry combustion.

Four phenolic acids, namely, *p*-hydroxybenzoic acid (HYB), vanillic acid (VAN), *p*-coumaric acid (COU), and ferulic acid (FER), of analytical grade (>99% purity) were purchased from Sigma (St. Louis, MO). The characteristics of the phenolic acids used are given in **Table 1**. Because of the lack of published solubility data for these phenolic acids, the water solubility of the compounds was determined using the saturation shake-flask method (41) at a temperature of 24 °C and a pH of 5.1.

**Sorption Experiments.** The sorption studies were conducted using a batch equilibration technique at 24 ± 1 °C. The experiments were conducted in 10 mL Teflon-lined screw-capped vials containing 200 µg mL<sup>-1</sup> HgCl<sub>2</sub> as a bioinhibitor. Previous work has demonstrated that HgCl<sub>2</sub> is as effective as autoclaving three times in restricting microbial growth and has little impact on other soil properties (42). The efficiency of HgCl<sub>2</sub> in inhibiting microbial degradation of phenolic acids was confirmed in this study by complete recovery of individual phenolic acids from the soil solution after 16 days of incubation, during which HgCl<sub>2</sub> was added to maintain 200 ppm, and by the absence of breakdown products in individual sorption experiments. The pH of the phenolic acid stock solution was adjusted to that of the soil using 1 M NaOH. The soil–solution ratio was adjusted to achieve 20–80% sorption of the principle solute, that is, 4 g of soil and 8 mL of solution for HYB, VAN, and COU and 1 g of soil and 7 mL of solution for FER. The concentration ranges of the phenolic acids used for the experiment varied according to the water solubility of the acids, and the concentration used to obtain isotherms ranged between 0.01 and 12 mM. In all cases, the highest concentration chosen was below 70% of the maximum water solubility of the acids (**Table 1**). The suspension was continuously mixed on a hematology mixer for 4 days to reach sorption equilibrium. Preliminary studies indicated that there was no increase in sorption of any of the phenolic acids beyond 3 days in a 16 day sorption experiment. In selected cases, the pH of the suspension was measured at the end of the equilibration period and was found to be unchanged. At the end of the equilibration period, vials were centrifuged at 3000g for 15 min and the phenolic acid concentration of the supernatant was analyzed. Because of the absence of sorption by glass vials and lack of biodegradation, the amount of phenolic acids sorbed to soil was calculated by mass difference. The amounts of naturally occurring phenolic acids in the experiment soil were below 0.01 µg g<sup>-1</sup> soil.

Competitive sorption experiments for each phenolic acid were carried out under the same experimental conditions as described above

**Table 1.** Characteristics of Phenolic Acids Used in the Study

phenolic acid	R1	R2	R3	$pK_a^a$	ionized fraction (pH 5.15)	water solubility ( $\mu\text{g L}^{-1}$ ) <sup>b</sup>
HYB	COOH	H	OH	4.5	0.82	$23.6 \pm 0.1$
VAN	COOH	OCH <sub>3</sub>	OH	4.5	0.82	$9.2 \pm 0.1$
COU	CH=CHCOOH	H	OH	4.7	0.73	$3.4 \pm 0.1$
FER <sup>c</sup>	CH=CHCOOH	OCH <sub>3</sub>	OH	4.7	0.73	$3.1 \pm 0.1$

<sup>a</sup> For -COOH; 24. <sup>b</sup> At pH 5.1 and  $24 \pm 1$  °C. <sup>c</sup> >99% trans form.

at a fixed initial concentration of competing solute (3 mM). In all cases, the soil–solution ratio was maintained as that of the primary solute. The effects of varying concentrations of competing solutes on primary solutes were also studied by keeping the concentrations of primary solute constant and varying the competitor concentration. All sorption studies were done in duplicate. To prevent trans–cis conversion of the compounds, the experiments were conducted in partial light.

The supernatant was analyzed with high-performance liquid chromatography (HPLC) (PerkinElmer Instruments, Shelton, CT) on a C-18 column (25 cm  $\times$  4.6 mm, 5  $\mu\text{m}$ , Supelco). For measurements on individual phenolics, the solvent system consisted of 20% acetonitrile with an isocratic flow rate of 1 mL  $\text{min}^{-1}$ . To help differentiate the peaks for the different acids in the competitive sorption studies, acetonitrile concentrations varied between 15 and 25% depending on the compounds in the sample. The solvents were buffered with 1% acetic acid to a pH of 2.8 to convert the dissociated acids to their protonated form. Phenolic acids were quantified with a UV–vis diode array detector with dual channel absorption at 254 and 280 nm. The HPLC detection limit for the phenolic acids was 0.01  $\mu\text{g mL}^{-1}$ . The sorption and competitive sorption data were fitted using a logarithmic form of the Freundlich equation:

$$\log S = \log K_F + N \log C$$

where  $S$  is the sorbed concentration of phenolic acid to soil ( $\mu\text{g g}^{-1}$ ),  $C$  is the solution phase equilibrium concentration ( $\mu\text{g mL}^{-1}$ ), and  $K_F$  [ $(\mu\text{g g}^{-1})(\mu\text{g mL}^{-1})^{-N}$ ] and  $N$  (dimensionless) are constants. The Freundlich exponent ( $N$ ) and affinity constant ( $K_F$ ) were determined by linear regression of log-transformed data.

Data were analyzed using one-way analysis of variance. Duncan's new multiple range test (DNMRT) was used to compare various parameters across the systems. Significance is reported at the 99% confidence interval. All statistical tests were performed with SAS 9.1 (SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

**Single-Solute Sorption Isotherms.** Because of the proximity of  $pK_a$  values of the phenolic acids (**Table 1**), they had similar amounts of ionized species in the soil system. Studies have shown that at a soil pH near 5 the sorption affinities of phenolic acids are less influenced by the ratio of dissociated to undissociated forms of the acids (24). As the concentration-dependent error in measurement was minimal and since the concentrations were intentionally spread over the log scale, the isotherms were fitted to a straight line using least squares regression of log–log transformed data. Freundlich parameters  $K_F$  and  $N$  of single-solute isotherms of phenolic acids are shown in **Table 2**. High values of  $K_F$  indicate that a solute is more strongly

sorbed onto a soil, which in turn could result in the compound being immobile, evading microbial degradation, and possibly accumulating in ecosystems. The Freundlich exponent ( $N$ ) denotes the curvature of the isotherm. Soils that are homogeneous at the macroscopic level can be implicitly heterogeneous at a finer scale as different matrices and interfaces may exhibit varied affinities for organic solutes. As this reactivity is unevenly distributed in different soil fractions,  $N$  can be used to infer the energy distribution of the sorption sites (43).

The data fitted well to the Freundlich model, and the sorption isotherms did not plateau at higher concentrations, which indicates sorption site abundance (**Table 2**). The sorption affinity coefficient ( $K_F$ ) ranged from 1.54 for HYB to 480 for FER. However, because the sorption isotherms were nonlinear, precise comparison of the sorption affinities of different phenolic acids could not be made using the Freundlich affinity coefficient (44). Therefore, the concentration-dependent sorption coefficient ( $K_d$ ) at two selected concentrations ( $C = 10$  and  $500 \mu\text{g mL}^{-1}$ ) was employed to compare the sorption affinities of different phenolic acids. At  $10 \mu\text{g mL}^{-1}$ ,  $K_d$  follows the order FER > VAN > COU > HYB ( $P < 0.01$ ; DNMRT). This is in accordance with other studies (20). Sorption of FER and COU is in accordance with their hydrophobicity (**Table 1**). Sorption affinity of VAN could be attributed to their methoxy substitution. Compounds with methoxy group (VAN and FER) could be sorbed more readily as the methoxy group repels electrons and increases the electron density of the ring, thereby increasing its binding affinity toward organic material and Lewis acids (polyvalent metal cations). Compounds with a higher sorption affinity could evade microbial degradation due to their close association with the soil matrix (18). All of the sorption isotherms were nonlinear, and the  $N$  value decreased in the order COU > HYB > VAN > FER ( $P < 0.01$ ; DNMRT). The nonlinearity of the isotherms indicates site-specific sorption of phenolic acids to the soil matrix. Adsorption at a solid–water interface can be linear, where sorption occurs to an infinite surface of uniform sorption free energies ( $N = 1$ ), or it could be nonlinear, which indicates heterogeneous adsorption potentials of sorption sites (43, 45). Nonlinearity arises from a progressive decrease in affinity of the phenolic acids to the sorption sites as the solute concentration increases. As the equilibration concentration ( $C$ ) increased, the sorption affinity of the solute decreased (**Table 2**), and  $K_d$  values at higher concentrations ( $C = 500 \mu\text{g mL}^{-1}$ ) were in the order FER > COU > VAN > HYB ( $P < 0.01$ ; DNMRT). Such an order of affinity for phenolic acids was reported earlier (19). The decrease in  $K_d$  at higher concentration further implies the nonlinearity of the sorption isotherms: As the solution concentration increases, the added sorbates are bound to weaker and weaker sorption sites. Again, the comparatively higher sorption of COU over VAN at the higher equilibration concentration ( $C = 500 \mu\text{g mL}^{-1}$ ) could be explained by the higher nonlinearity of VAN isotherm ( $N = 0.52$ , **Table 2**). The  $K_d$  values decreased over the concentration range ( $C = 10$  and  $500 \mu\text{g mL}^{-1}$ ) by factors of 2.7, 6.5, 2.1, and 16 for HYB, VAN, COU, and FER, respectively. The sharp decrease in sorption affinity of VAN and FER is due to their higher site-specific sorption (lower  $N$ ) as compared to HYB and COU.

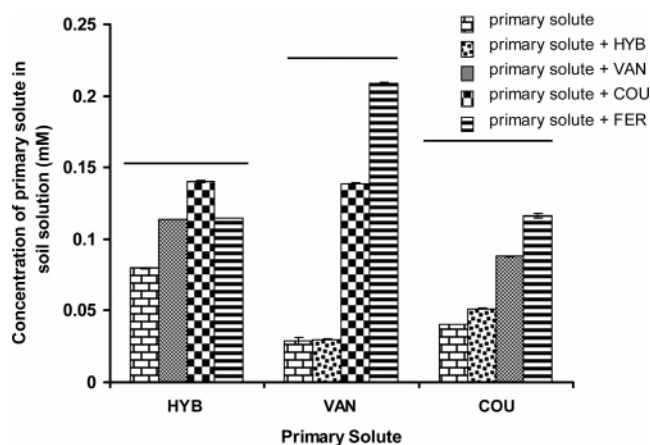
In a soil matrix, phenolic acids can sorb onto organic matter, they can sorb onto clay minerals through cation bridging, polymerization onto mineral surfaces (19, 21), or inner and outer sphere complexation with hydroxylated iron and aluminum compounds, or they can be oxidized by iron and manganese (20, 46). Sorption studies with phenolic acids were done on

**Table 2.** Freundlich Isotherm Parameters and Concentration-Dependent Sorption Coefficients ( $K_d$ ) for Single-Solute Isotherms of Phenolic Acids<sup>a</sup>

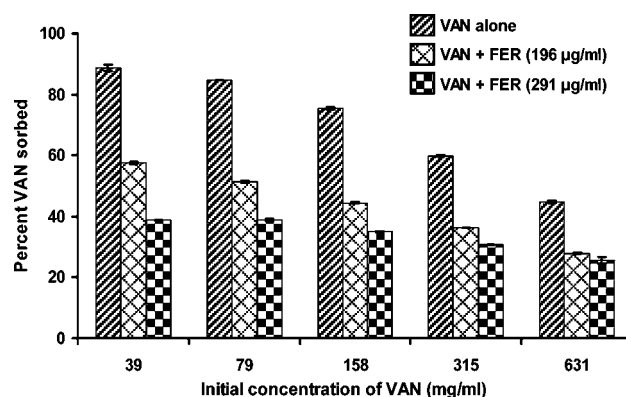
phenolic acid	soil with organic matter			soil pretreated with H <sub>2</sub> O <sub>2</sub>			
	$K_F$	N	$r^2$	concentration-dependent $K_d$			N
				C = 10 $\mu\text{g mL}^{-1}$	C = 500 $\mu\text{g mL}^{-1}$	C = 10 $\mu\text{g mL}^{-1}$	
HYB	1.54 d	0.78 ± 0.01	0.998	0.95 d	0.41 d	0.17 b	1.04 ± 0.01
VAN	32.7 b	0.52 ± 0.01	0.995	10.8 b	1.67 c	0.19 b	1.05 ± 0.01
COU	13.5 c	0.79 ± 0.01	0.995	8.51 c	3.88 b	0.15 b	0.94 ± 0.01
FER	480 a	0.30 ± 0.01	0.991	92.6 a	5.66 a	23.5 a	0.79 ± 0.01

<sup>a</sup> Means with the same letter are not significantly different at  $P < 0.01$  (DNMRT).

soils treated to remove organic matter to further specify the site for the sorption. H<sub>2</sub>O<sub>2</sub> treatment of soil reduced the organic matter content from 6.0 to 3.8%. Oxidation of organic matter with H<sub>2</sub>O<sub>2</sub> was employed in similar studies (47). The low removal of organic matter could be attributed to the high clay content of the soil. Organic matter in close association with mineral surfaces could escape chemical destruction (48). H<sub>2</sub>O<sub>2</sub> treatment had little effect on the sesquioxide composition of the soil (dithionate–citrate extractable Fe, Al, and Mn in treated soil was 1.01, 0.22, and 0.038%, respectively). The concentration-dependent sorption affinity ( $K_d$ ) of the phenolic acids decreased upon removal of organic matter from the soil (Table 2). The decrease was drastic in the cases of VAN and COU (i.e.,  $K_d$  dropped by factors of 57 and 56, respectively). The nonlinearity of the isotherms also decreased; that is,  $N$  values increased for all compounds. Linear isotherms are often observed during the sorption of compounds onto mineral surfaces (47). However, the more linear isotherms coupled with the reduction in sorption affinity indicate that soil organic matter contributes most to the sorption of these phenolic acids in the soil matrix. Also, if clay minerals were the primary sites of sorption, removal of organic matter, by exposing more mineral surfaces, would have increased the sorption of phenolic acids. Hence, organic matter seems to act as the main sorption sites. Because the hydrophobic organic substances are not covered with water, the undissociated forms of the acids can also associate with organic matter with little expenditure of energy (49). In treated soil, FER still had a considerable sorption affinity and nonlinear isotherms as compared to the other three phenolic acids. This could be due to an affinity of FER for the Fe and Al oxides and clay minerals present in the soil matrix. A high affinity of FER to soil sesquioxides has been reported (20). Also, the process of organic matter removal could expose some of the sorption sites, thus further increasing the sorption of FER (50). Additionally, the protonated form of FER, due to its comparatively high hydrophobicity, could displace water molecules and associate itself with mineral surfaces by London dispersive interactions (47). The residual organic matter left after the H<sub>2</sub>O<sub>2</sub> treatment apparently did not have much effect on the sorption of phenolic acids. Clay could physically protect organic matter in micropores. Given the fact that H<sub>2</sub>O<sub>2</sub> treatment itself did not remove all organic matter, we argue that the reduced sorption might have been more influenced by the inaccessibility of phenolic acids to the protected organic matter. Intercalation, which includes the replacement of interlayer cations of clay minerals by organic molecules, reduces the accessibility to the protected organic matter (48, 51). Also, reduced sorption could partly be attributed to the possible alteration of the nature of the remaining organic matter from the H<sub>2</sub>O<sub>2</sub> treatment. The results of single-solute sorption establish that the sorption of phenolic acids to soil matrix is site-specific (Table 2), and compounds exhibiting site-specific sorption are sorbed more



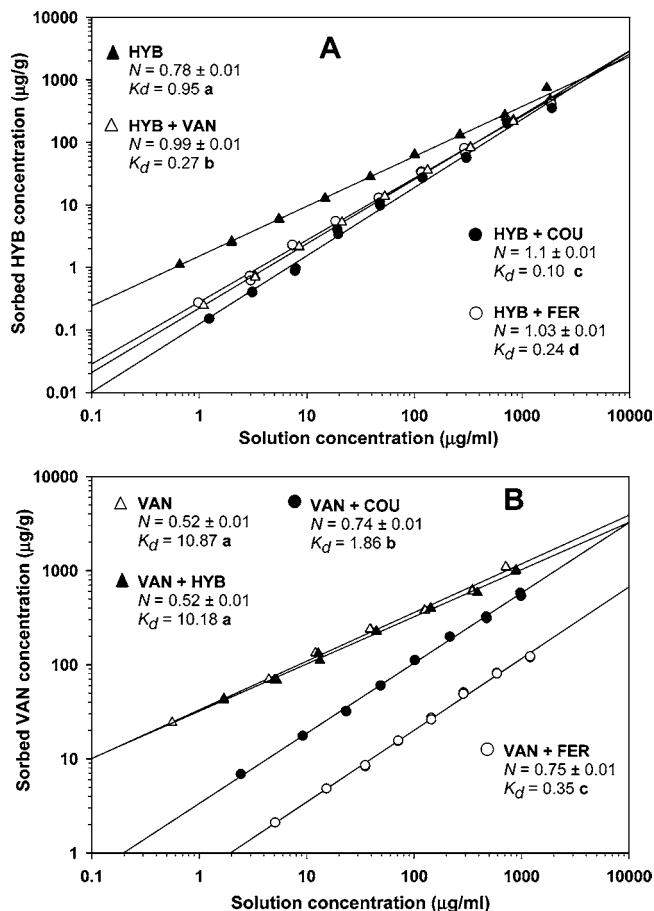
**Figure 1.** Amount of primary solute in soil solution during single-solute and binary-competitive sorption experiments (means ± range bars;  $n = 2$ ). The solid line above each group bar represents the initial amount of primary solute added to each system. The competitor concentration was 3 mM in all cases.



**Figure 2.** Sorption of vanillic acid (VAN) as influenced by the presence of varying concentrations of a competing solute (ferulic acid, FER). Values are means ± range bars ( $n = 2$ ).

strongly at lower concentrations than at higher concentrations. This concentration-dependent sorption could partly explain the difference in sorption affinity of phenolic acids observed by other authors (19, 20).

**Competitive Sorption.** The amount of three primary solutes (i.e., HYB, VAN, and COU) in the soil solution increased in the presence of a competing solute (Figure 1). There was no decrease in sorption of FER in the presence of other solutes (data not shown). The sorption of VAN in the presence of FER was nonlinear (Figure 2). When the concentration of FER was increased by 50%, more VAN was displaced into solution. The amounts of FER in the supernatant at both 196 and 291  $\mu\text{g mL}^{-1}$  were below our detection limit (Figure 2). Hence, at both concentrations, the sorption sites were in short supply of FER,



**Figure 3.** Competitive sorption isotherms of hydroxybenzoic acid (A) and vanillic acid (B) at a fixed initial concentration of other phenolic acids. The concentration-dependent sorption coefficient ( $K_d$ ) value is at a solution concentration of  $10 \mu\text{g mL}^{-1}$ . Abbreviations are as follows: hydroxybenzoic acid (HYB), vanillic acid (VAN), coumaric acid (COU), and ferulic acid (FER). Means of  $K_d$  with the same letter are not significantly different at  $P < 0.01$  (DNMRT).

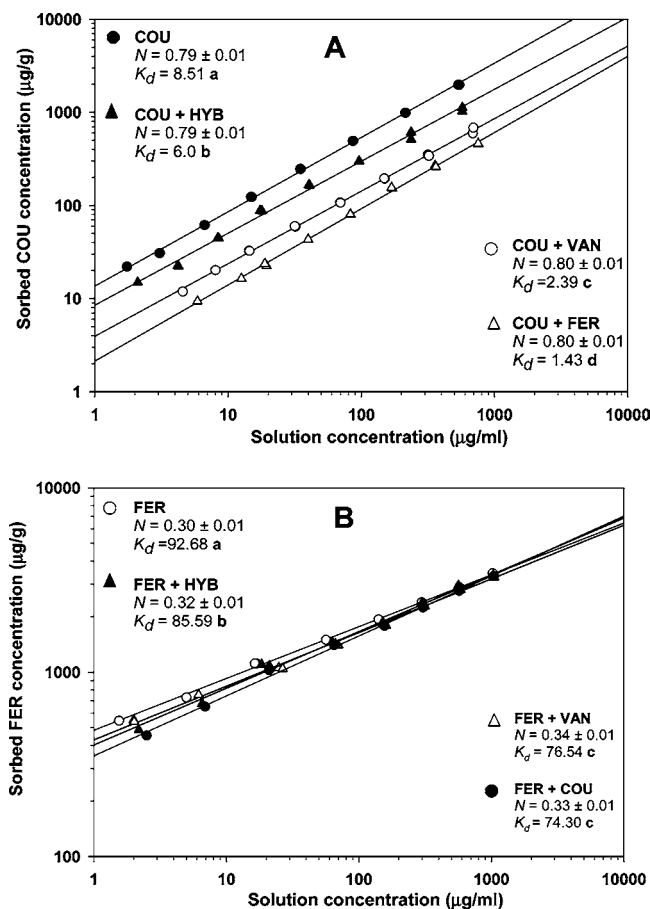
which caused the higher displacement (reduced sorption) of VAN as the FER concentration increased. This further clarifies the sorption site preference for FER over VAN. At high VAN concentrations, however, the concentration effect of FER was nullified (Figure 2). This is because at a low concentration of VAN, a higher proportion of VAN will be sorbed to specific sites, and as the VAN concentration increased, the proportion sorbed on specific sites decreased. Hence, the concentration-dependent ability of FER to displace VAN also decreased with an increase in VAN concentration.

Because of differences in slopes of sorption isotherms and because the concentration of phenolic acids in soil systems is always low (31), the concentration-dependent sorption coefficient ( $K_d$ ) at  $C = 10 \mu\text{g mL}^{-1}$  was used to compare the competitive sorption data between different systems. Competitive sorption data clearly indicate that other phenolic acids competed with HYB for sorption sites in binary solute systems (Figure 3A). The  $K_d$  decreased by factors of 4, 10, and 4 in the presence of VAN, COU, and FER, respectively. Competition for sorption sites arises if the same sites can be occupied by more than one nonidentical molecule (45). Because of the presence of hydroxyl and carboxyl groups, phenolic acids can adsorb in a directional manner onto the soil matrix (24). Considering structural differences among the phenolic acids (Table 1), the presence of a methoxy functional group in FER and VAN may act as a hindrance for their occupying the sites

that are normally sorbed by HYB. Thus, directional sorption explains the fact that, although FER had the highest affinity to sorption sites in single-solute systems, COU was more efficient than FER in competing with HYB for sorption sites. Also, the linearity of the HYB isotherm increased in the presence of other phenolic acids. The decrease in isotherm nonlinearity with a competing solute has been previously described (27). Because the specific sorption sites for HYB are limited in the soil matrix, at low concentrations, a higher proportion of HYB will be occupying these sites than at high concentrations. Hence, in the presence of a competing solute, a larger proportion of HYB will be displaced from the specific sites at a low concentration than at a high concentration. This causes the lower portion of the HYB isotherm (low concentrations) to shift downward more drastically than the upper portion (high concentrations), which in turn makes the isotherm more linear. Also, VAN was able to outcompete HYB for sorption sites, which indicated that  $pK_a$  was not a main factor affecting sorption ability (VAN and HYB had similar  $pK_a$  values, Table 1).

Sorption of VAN was affected in binary systems (Figure 3B). COU and FER reduced the  $K_d$  of VAN by 6 and 31 times, respectively. HYB had little influence of sorption of VAN. This again shows that directional bonding might be involved in the sorption of VAN, as it is competitively displaced with another phenolic acid having a methoxy substitution. The nonlinearity of the VAN sorption isotherms decreased due to the presence of another phenolic acid in the system. The decrease in sorption affinity of COU was less drastic in the presence of other phenolic acids (two, four, and six times for HYB, VAN, and FER, respectively; Figure 4A). Although VAN and COU had similar sorption affinities (Table 1), FER was five times more efficient in displacing VAN than COU (Figures 3B and 4A). This could be partly due to methoxy group hindrance of FER, which prevents it from occupying the COU sorption sites, and partly due to the comparatively high hydrophobicity of COU (Table 1). None of the phenolic acids were able to compete efficiently with FER for the sorption sites, as evident by the low change in sorption affinity of the FER isotherm (Figure 4B). Also, there was low deviation in the linearity of the FER isotherm in the presence of other phenolic acids. The low influence of a competitor on FER sorption could be explained by the higher sorption affinity of FER. The higher sorption of FER could be partly attributed to the presence of a methoxy group (ring activator) and lack of a carboxy group directly attached to the ring (ring deactivator) and partly to its higher hydrophobicity (19, Table 1). Also, the  $\alpha,\beta$ -unsaturated carbonyl arrangement of the acrylic side chain is susceptible to electrophilic addition; hence, it could increase the binding affinity of FER to organic matter and to polyvalent cations (19). The high sorption affinity of FER was further evident from the 16 day equilibration studies where 50% of added FER ( $0.25 \text{ mM}$ ) disappeared from the soil solution within 10 min of equilibration time (data not shown). Such high reactivity of FER was reported earlier (17).

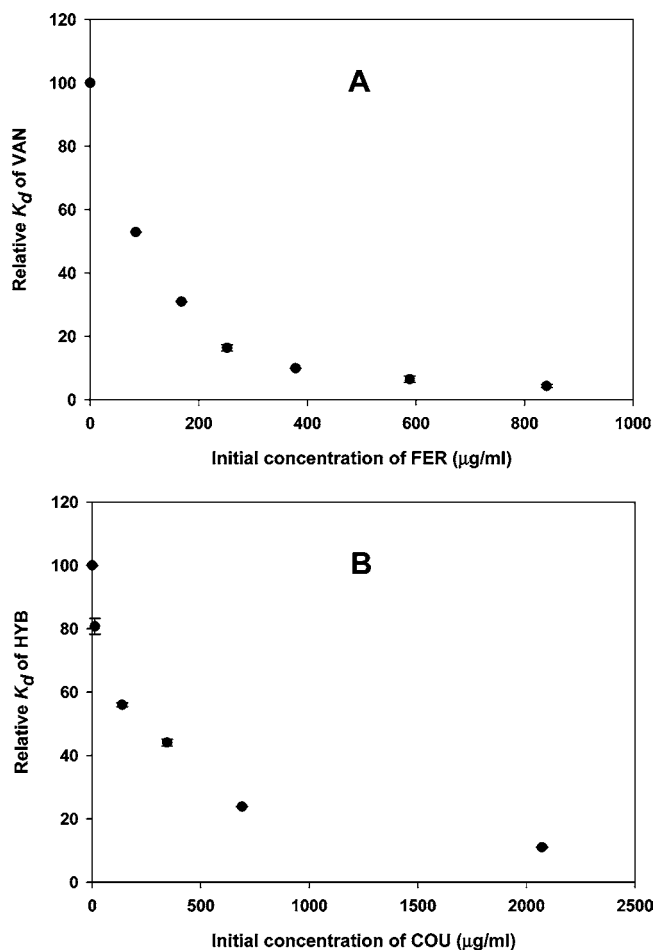
We further investigated the effect of varying concentrations of a competitor (FER and COU) on the sorption affinity of selected primary solutes (VAN and HYB, respectively). A drastic decrease in the sorption affinity of the primary solutes was noticed in both cases. The exponential decrease of  $K_d$  was sharper at a low vs high concentration of the competitor. As the competitor concentration increased above  $500 \mu\text{g mL}^{-1}$ , the decrease leveled off in both cases (Figure 5). A similar exponential decrease in sorption affinity of the primary solute was observed during competitive sorption of phenolic acids and



**Figure 4.** Competitive sorption isotherms of coumaric acid (A) and ferulic acid (B) at a fixed initial concentration of other phenolic acids. The concentration-dependent sorption coefficient ( $K_d$ ) value is at a solution concentration of  $10 \mu\text{g mL}^{-1}$ . Abbreviations are as follows: hydroxybenzoic acid (HYB), vanillic acid (VAN), coumaric acid (COU), and ferulic acid (FER). Means of  $K_d$  with the same letter are not significantly different at  $P < 0.01$  (DNMRT).

anthropogenic soil contaminants (24) and *S*-triazines and their analogues (45). The exponential decrease of  $K_d$  at low competitor concentrations further confirms that the change in sorption affinity is not because of the competitor modifying the soil properties or because of the primary solute altering the solution properties. In the presence of either of those effects,  $K_d$  will drop marginally at a lower cosolute concentration and will then drop exponentially as the concentration of cosolute increases. We were unable to conduct competitive sorption studies with treated soils (organic matter removed), as the phenolic acid sorption to the soil was too low to give reliable results. However, on the basis of the single-solute sorption on treated soils, we infer that the majority of the sorption was occurring in organic matter (Table 1). Hence, the competition for sorption sites may also occur in soil organic matter. In their work with anthropogenic pollutants and aromatic acids, Xing and Pignatello (24) demonstrated the same. Competitive sorption is shown to take place in soil organic matter in hole-filling domains where solute specificity is exhibited (52).

Although the sorption studies were carried out by exogenously applying the phenolic acids, to our knowledge, this is the first extensive study to incorporate both the complexities of soil sorption as well as plant exudates. Our study clearly indicates that plant secondary metabolites do exhibit preferential sorption toward specific sorption sites in the soil matrix. This in turn can drastically change the composition of the exudates that



**Figure 5.** Competitive sorptions of vanillic acid (VAN) at variable concentrations of ferulic acid (FER, A) and of hydroxybenzoic acid (HYB) at variable concentrations of coumaric acid (COU, B). Values are means  $\pm$  SE. Each  $K_d$  value is based on an eight point isotherm at VAN and HYB solution equilibrium concentrations of  $10 \mu\text{g mL}^{-1}$ .

becomes available for plant absorption (Figures 1 and 2). Also, this preferential sorption could result in increased mobility of compounds in multicomponent systems. Hence, caution should be exercised while selecting allelopathic cultivars based on chemical screening of the primary allelopathic compound alone. In cases of invasions facilitated by allelopathy, soil sorption properties could be another important factor in the invasibility of a habitat. Because sorption isotherms are macroscopic measurements, a detailed sorption mechanism could not be identified in this study; however, organic matter appears to be the major factor affecting site-specific sorption. Organic matter serving as sites for competitive sorption is of further relevance in agricultural scenarios, where the decomposing plant residues on topsoils can serve as potential sorption sites. Although sorption could offer some protection against microbial degradation, how such degradation can further modify the phytoavailable concentration of exudates is beyond the scope of our experiment. Finally, our study provides some explanation why laboratory bioassays with a single compound are difficult to duplicate under field conditions.

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