JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Competitive Sorption−Desorption Behavior of Triazine Herbicides with Plant Cuticular Fractions

MICHAL SHECHTER,† BAOSHAN XING,‡ FRANK-DIETER KOPINKE,§ AND BENNY CHEFETZ*,†

Department of Soil and Water Sciences, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel, Department of Plant, Soil and Insect Sciences, University of Massachusetts, Amherst, Massachusetts 01003, and Department of Remediation Research, UFZ - Centre for Environmental Research, Leipzig-Halle, Permoserstrasse 15, D-04318 Leipzig, Germany

Sorption interactions of plant cuticular matter with organic compounds are not yet fully understood. The objective of this study was to examine the competitive sorption−desorption interactions of the triazine herbicides (atrazine and ametryn) with cuticular fractions isolated from tomato fruits and leaves of *Agave americana*. The ^{13}C NMR data suggest a rubber-like nature for the cutin. This biopolymer exhibited reversible and noncompetitive sorption. Enhanced desorption of atrazine was recorded in the bi-solute system with bulk and dewaxed *A. americana* cuticles. ^{13}C NMR analyses of these samples suggested that the sorbed competitor ametryn facilitated a physical phase transition of rigid paraffinic sorption domain to mobile and flexible domain during sorption process. We suggest that the different sorption−desorption behavior obtained for the two cuticles is related to the higher content of waxes (14% vs 2.6%) and lower content of cutin (46% vs 75%) in the *A. americana* versus tomato fruit cuticle.

KEYWORDS: Plant cuticle; cutin; cutan; atrazine; NMR; sorption; desorption; glass transition temperature; paraffinic carbon

INTRODUCTION

Sorption−desorption interactions of herbicides and other hydrophobic organic compounds (HOCs) are one of the major processes affecting the fate of these compounds in soils. The predominant sorbent for such compounds is the soil organic matter (SOM). One of the precursors for SOM is plant cuticle (*1*, *2*), a thin layer of predominantly lipids that covers all primary aerial surfaces of vascular plants (*3*). The outer surface of the cuticle is covered with waxes, which consist of a complex mixture of long-chain aliphatic and cyclic components (*4*). In most plant species, the major structural component of the plant cuticle is the cutin biopolymer (between 30 and 70% by weight). This is a high-molecular-weight, insoluble, polyester-like biopolymer composed of various inter-esterified aliphatic hydroxy acids with chain lengths of C_{16} and C_{18} (*5*). In some plant species, such as *Agave americana*, the base and acid hydrolysis-resistant biopolymer, known as cutan, is a major constituent of the cuticle layer together with cutin (*3*, *6*). Cutan is composed of a small aromatic skeleton ether bonded to a long chain of *n*-alkenes and *n*-alkanes (*7*). In addition to these two aliphatic-rich biopolymers, the plant cuticle contains polysaccharides (mainly pectin) that are layered between the epidermal cell wall and the cuticle membrane (*8*).

Several studies have shown the selective preservation of cutin and cutan biopolymers in soils (*1*, *5*, *9*−*11*) and their incorporation into humic macromolecules (*12*). Recent studies have reported that these cuticle-derived biopolymers can sorb significant amounts of highly hydrophobic as well as relatively polar organic compounds (*13*−*17*). The aliphatic-rich structure of the cuticle has been suggested to provide both hydrophobic nonspecific sorption domain and specific adsorption sites, facilitating hydrophobic and H-bonding interactions, respectively (*13*, *15*).

Several studies have suggested that SOM consists of at least two types of sorption domains (*16*, *18*, *19*). These domains can be characterized as expanded and condensed organic structures, analogous to rubbery and glassy synthetic polymers (*18*, *20*, *21*). Sorption to the rubbery gel-like domain is governed by a solid-phase dissolution (partitioning) process, resulting in reversible, noncompetitive sorption and linear sorption isotherms. However, sorption to the glassy (condensed) domain of SOM is generally nonlinear, exhibiting partially irreversible sorption behavior (desorption hysteresis), and is expected to be affected by competition of a similar solute (*8*, *16*, *22*, *23*).

The physicochemical properties of the sorbent (i.e., SOM) in soils and sediments have been suggested to affect the sorption affinity and mechanism of HOCs (*24*−*26*). Thus, to elucidate

* Corresponding author. Tel: +972 948-9384. Fax: +972 947-5181. E-mail: chefetz@agri.huji.ac.il.
† The Hebrew University of Jerusalem.
‡ University of Massachusetts.
§ UFZ - Centre for Environmental Research.

10.1021/jf0614488 CCC: $33.50
Published on Web 09/06/2006

the fate of HOCs in the environment, a better understanding of the role of SOM constituents in the sorption process is essential. Therefore, the objective of this study was to examine the competitive sorption−desorption behavior between *s*-triazine herbicides (atrazine and ametryn) to improve our mechanistic understanding of the sorption capabilities of the plant cuticular fractions based on their unique properties and structures and motivated by their ubiquitous abundance.

MATERIALS AND METHODS

Isolation of Cuticular Fractions. Cuticular fractions were isolated from the fruits of tomato (*Lycopersicon esculentum* Mill) and leaves of the succulent plant *Agave americana*. Tomato cuticle is a cutan-free cuticle, whereas the cuticle of *A. americana* has been reported to be composed of both cutin and cutan biopolymers (*3*, *6*). The cuticular fractions (bulk cuticle, dewaxed cuticle, cutin and cutan biopolymers) were isolated using the method reported previously by Chefetz (*13*). Briefly, cuticle sheets were manually peeled from the fresh fruits or leaves after boiling in water. Then the bulk cuticle sheets were treated with an oxalic acid (4 g/L) and ammonium oxalate (16 g/L) solution at 90 °C for 24 h and washed to remove any residual materials. Waxes were removed by Soxhlet extraction with chloroform/methanol (1:1, v/v) for 6 h. To obtain the cutin biopolymer, the dewaxed tomato cuticles were hydrolyzed with 6 M HCl (6 h under reflux). To obtain the cutan fraction, the dewaxed cuticular material isolated from *A. americana* was saponified (1% w/v KOH in methanol for 3 h at 70 °C), and then hydrolyzed (6 M HCl; 6 h under reflux). All treatments were performed twice to ensure complete removal of the desired fraction. All purified fractions were washed with deionized water, freeze-dried, ground, and sieved (<0.5 mm).

Analyses of the Cuticular Fractions. Elemental (C, H, N) analyses were conducted in duplicate using an automated elemental analyzer (EA 1108, Fisons Instruments, Milan, Italy). The cross-polarization magic angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) spectra were acquired with a Bruker Avance 300 MHz NMR spectrometer (Bruker, Analytic, Billerica, MA). The acquisition parameters were as follows: spectral frequency 75 MHz, spinning rate 5 kHz, contact time 1.5 ms, 1 s recycle delay, and line broadening 40 Hz. The spectra were integrated into the following chemical-shift regions: paraffinic carbon (0−50 ppm); alcohols, amines, carbohydrates, ethers, and methoxyl carbons (50−96 ppm); aromatic and phenolic carbons (96−163 ppm); and carboxyl, carbonyl, and amide carbons (163−220 ppm).

Specific heats of the corresponding cuticular materials were determined in a Shimadzu DSC-50 differential scanning calorimeter (Shimadzu Corp., Kyoto, Japan) with computer-aided data analysis, following the procedure described by Casado and Heredia (*27*). The dried samples (10−15 mg) were heated from 273 to 323 K at 5 K/min. The heat flow into the sample was calculated using the following equation: $dH/dt = mC_p(dT/dt)$ where dH/dt is the measured heat flow (J/min), m is the sample mass (g), C_p is the specific heat (J/g·K) and dT/dt is the scan rate (K/min). To establish an optimal baseline under cooling conditions, the program was carried out on two empty pans. This baseline was used for correction of the baseline with sample runs.

Batch Sorption−Desorption Experiments. Atrazine (2-chloro-4-ethylamino-6-isopropylamino- *s*-triazine) and ametryn (2-ethylamino-4-isopropylamino-6-methylthio-1,3,5-*s*-triazine) were kindly provided by Agan Chemical Manufacturers (Ashdod, Israel). Atrazine exhibits an aqueous solubility of 30 mg/L and log K_{OW} of 2.7. Ametryn's properties are as follows: aqueous solubility of 185 mg/L and log K_{OW} of 3.07. Both compounds are weak bases exhibiting pK_a values of 1.7 and 4.1, respectively (*28*).

Short-term batch sorption experiments were conducted for 3 d (based on kinetic experiments performed for 21 d). Aliquots from a concentrated HPLC-grade methanol stock of atrazine were dissolved in a background solution containing 5 mM $CaCl_2$ (to maintain a constant ionic strength), 100 mg/L NaN_3 (to inhibit microbial activity), and 50 mg/L $NaHCO_3$ (pH 7). Atrazine solutions (10 mL) at various concentrations (0.5−15 mg/L) were added to the cuticle samples previously weighed into 20-mL Teflon centrifuge tubes. The mass of sorbents were selected to achieve 40−60% sorption. The tubes (three replicates and a blank for each concentration) were agitated in the dark at 200 rpm for 3 d (25 °C).

After the sorption experiments, desorption was performed by replacing 60% of the supernatant with fresh (sorbate-free) background solution. Then the tubes were re-agitated under the conditions used in the sorption experiments. Desorption was performed for the periods tested in kinetic experiments to determine the desorption equilibrium time for each sorbent−sorbate pair. Based on these tests, desorption was performed for 3 d for all sorbents except for the cutan residue, which exhibited a longer equilibrium time (7 d). At the end of the sorption and desorption steps, all tubes were centrifuged (4000*g*, 20 min), and 1-mL aliquots were removed and filtered (0.45 μm) for quantitative HPLC analysis.

Competitive sorption between atrazine and ametryn was performed at a constant ametryn concentration (150 mg/L) and varying atrazine concentrations. Competitive desorption was performed by the above-described procedure with the background solution containing the equilibrium concentration of the competitive ametryn (40−60 mg/L). This allowed us to perform the desorption experiment for atrazine at constant ametryn concentration.

Atrazine and ametryn concentrations were detected using an L-7100 LaChrom HPLC with a LiChrospher RP-18 column (25 cm × 4.0 mm, 5 μm), equipped with a photodiode-array detector. Atrazine and ametryn were eluted using acetonitrile/water (70:30, v/v) as the mobile phase and detected by absorbance at 222 nm.

Data Analysis. The Freundlich parameters (K_F and N) were calculated using the logarithmic form of the equation $q = K_F \times C_e^N$, where q is the sorbed amount per unit weight of sorbent (mg/kg), C_e is the equilibrium concentration (mg/L), K_F [(mg/kg)·(mg/L)$^{-N}$] is the Freundlich capacity coefficient, and N (dimensionless) describes the isotherm curvature. Values for the C-normalized capacity coefficient ($K_{F,OC}$) were calculated by normalizing K_F to the C content in each sorbent. Since the $K_{F,OC}$ value depends on the N value, it is not possible to compare the $K_{F,OC}$ values for isotherms with different N values. Thus, the organic C-normalized distribution coefficients (K_{OC}) were calculated for a C_e of 0.1 and 5 mg/L using the equation $K_{OC} = K_{F,OC} \times C_e^{(N-1)}$. Statistical analysis (All Pairs, Tukey−Kramer, $P = 0.05$) was performed by JMPIN software, version 4.0.4. (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Sorption in a Single-Solute System. Sorption and desorption data of atrazine with cuticular fractions isolated from the fruits of tomato and leaves of *A. americana* are presented in **Figures 1** and **2**, respectively. The sorption parameters are summarized in **Table 1**. With the tomato cuticle fractions, the $K_{F,OC}$ values calculated for the bulk cuticle and the cutin biopolymer were 284 and 760 (mg/kg OC)·(mg/L)$^{-N}$, respectively. In addition, the bulk sorption isotherm was highly nonlinear exhibiting a Freundlich N value of 0.74 as compared to 0.94 for atrazine isotherm with the cutin. Due to the highly nonlinear isotherm with the bulk cuticle, at equilibrium concentration C_e of 5 mg/L the calculated K_{OC} value for atrazine with the cutin biopolymer was 3.7 times higher than the value calculated for the bulk cuticle (690 and 186 L/kg OC, respectively). At C_e of 0.1 mg/L, the K_{OC} value for atrazine with the cutin biopolymer was 872 versus 516 (L/kg OC) for the bulk cuticle.

Similar to the atrazine sorption behavior obtained with the tomato cuticular matter, with the *A. americana* cuticles fractions, atrazine exhibited the lowest $K_{F,OC}$ value with the bulk cuticle. Removal of the cuticular waxes (14% by weight) resulted in a 53% increase in the calculated atrazine $K_{F,OC}$ value (**Table 1**). Further removal of the polysaccharides and cutin (19 and 47% by weight, respectively) resulted in a significant increase in the $K_{F,OC}$, from 160 for the bulk cuticle to 347 (mg/kg OC)·(mg/L)$^{-N}$ for the cutan biopolymer. With the *A. americana* cuticles

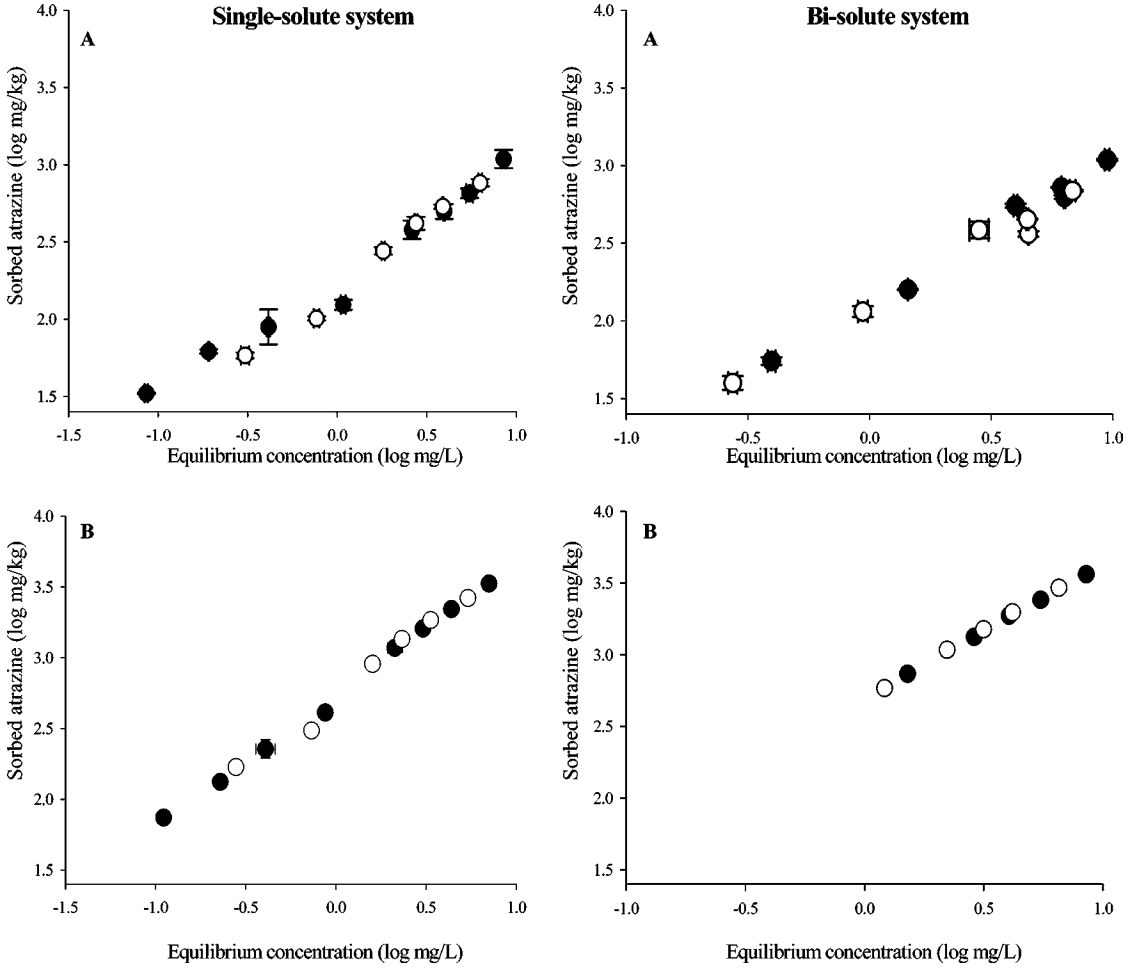

Figure 1. Atrazine sorption (filled) and desorption (empty) data with tomato fruit cuticular fractions: bulk cuticle (**A**) and cutin (**B**) in single-solute (left) and bi-solute (right) systems. Each point represents the mean of triplicate vials; bars represent standard error.

fractions the $K_{F,OC}$ values can be compared since all isotherms were linear and exhibited statistically similar Freundlich N values (**Table 1**). It is important to note that at C_e of 0.1 mg/L, atrazine's K_{OC} values with cutan were significantly lower than that obtained for the cutin biopolymer isolated from the tomato (323 and 872 L/kg OC, respectively). Moreover, the atrazine affinity to the bulk tomato cuticle was higher than to the bulk *A. americana*, probably due to the higher cutin content in the bulk tomato as compared to its level in the bulk *A. Americana* (75% vs 47%, respectively).

Similar to Chefetz (*13*) and Chamel and Vitton (*29*), our data emphasize the importance of the cutin biopolymer as a sorbent for triazine herbicides. The cutin, a major component of the cuticle, exhibited a strongly paraffinic nature. The paraffinic C made up 76% of the total C in the cutin sample as calculated from the ^{13}C NMR spectra (**Figure 3**), and the H/C ratio of this sample was 1.64 (**Table 2**). This ratio was lower for the bulk tomato cuticle, probably due to the presence of unsaturated aliphatic chains and cyclic components in the cuticular waxes (*4*). Removal of the waxy layer (2.6% by weight) and pectin (21% by weight) from the bulk tomato cuticle resulted in a significant increase in the sorption ability of the cutin biopolymer. It was suggested that these two structural fractions of the cuticle (waxes and pectin) are attached to the cutin biopolymer and therefore prevent solute uptake (*13*).

In this study, the physical conformation of the sorbents was studied by ^{13}C NMR spectroscopy (**Figure 3**) and differential scanning calorimeter (DSC) to obtain the glass-transition temperature (T_g) (**Figure 4**). Several studies have reported T_g values for bulk tomato fruit cuticle at −47 and 25 °C and for the cutin biopolymer at 23 °C (*27*, *30*, *31*). The phase transitions were assigned to the melting of waxes and the phase transition inside the cutin biopolymer. In our study, a continuous change of C_p between 10 and 40 °C rather than a distinct glass transition was obtained with the tomato bulk and cutin cuticles (**Figure 4A,B**). The T_g of the tomato cuticle has been reported to vary with fruit maturation. Mature cuticles did not show a clear glass transition (*31*). Our cuticles were isolated from mature tomato fruits; therefore, T_g was not observed in the investigated temperature range. This physical conformation of the bulk tomato cuticle and the cutin biopolymer is supported by the ^{13}C NMR spectra (**Figure 3**). The major peaks in the paraffinic region (0−50 ppm) of the bulk tomato cuticle ^{13}C NMR spectrum were at 25 ppm (CH_3) and 29 ppm (mobile amorphous C domain) (*32*, *33*). The 32-ppm signal (crystalline or rigid C) appeared only as a shoulder in the ^{13}C NMR spectrum of the bulk tomato cuticle (**Figure 3F**), whereas in the cutin spectrum, this shoulder is negligible (**Figure 3E**). This suggests that the level of rigid paraffinic domain which facilitates nonlinear sorption is higher in the bulk cuticle than in the cutin, which appears to be rubbery-like biopolymer. The condensed structure of the bulk cuticle is probably attributed to the pectin which hold together the cutin in a complexes network. These data are supported by the significantly lower linearity of the atrazine sorption isotherm with the bulk tomato cuticle versus the isotherm with the more flexible cutin biopolymer (Freundlich N values of 0.74 and 0.94, respectively). With this biopolymer,

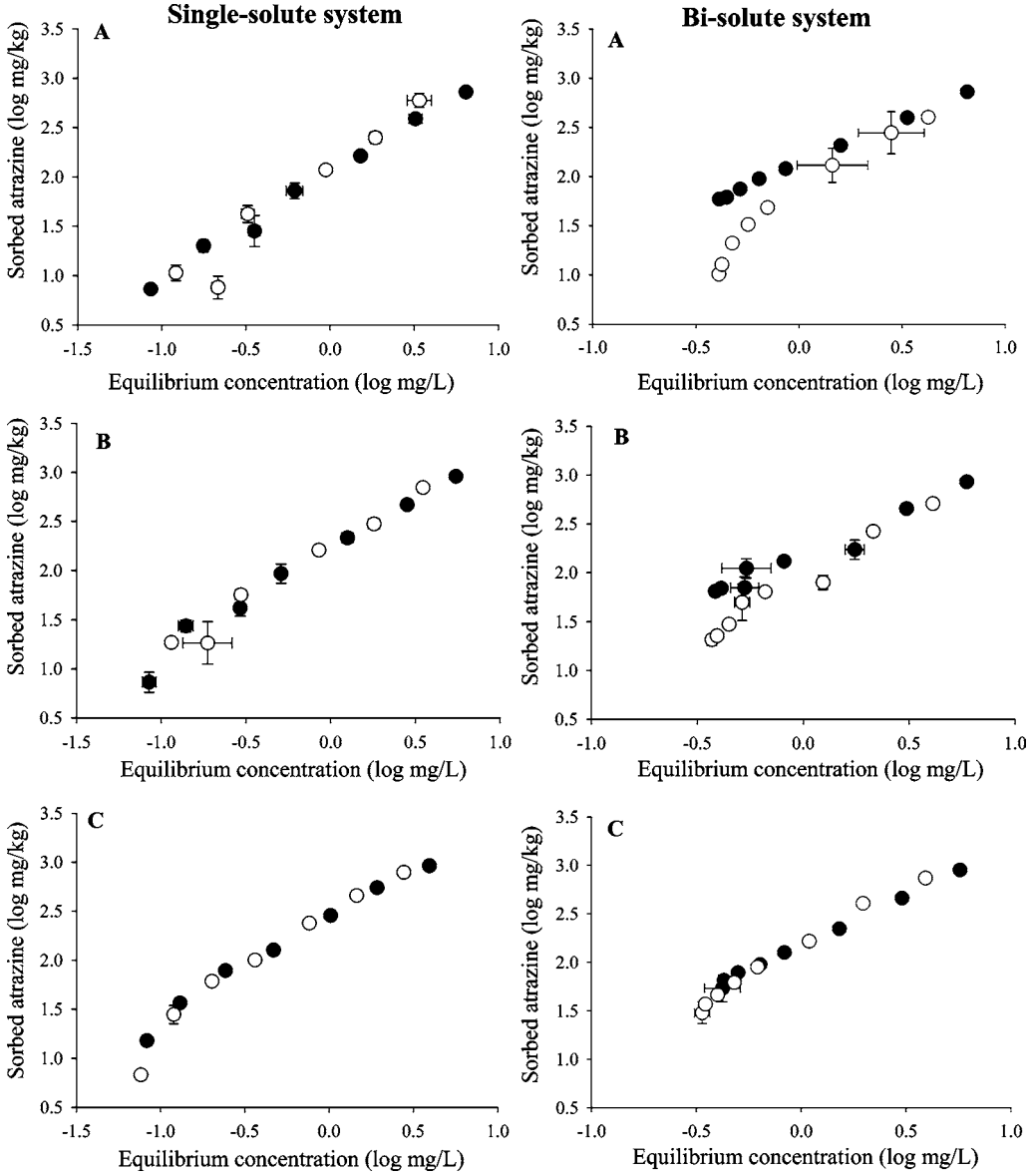

Figure 2. Atrazine sorption (filled) and desorption (empty) data with *Agave americana* cuticular fractions: bulk cuticle (**A**), dewaxed cuticle (**B**), and cutan (**C**) in single-solute (left) and bi-solute (right) systems. Each point represents the mean of triplicate vials; bars represent standard error.

Table 1. Atrazine Sorption and Desorption Parameters

		sorption			desorption		
	sorbent	$K_{F,OC}$[a]	N[b]	R^2	$K_{F,OC}$[a]	N[b]	R^2
		Tomato Fruit-Derived Cuticular Matter					
single-solute system	bulk	284[a]	0.74 ± 0.04	0.95	240[a]	0.90 ± 0.03	0.99
	cutin	760[b]	0.94 ± 0.01	1	750[b]	0.99 ± 0.03	0.98
bi-solute system	bulk	200[a]	0.93 ± 0.02	0.98	193[a]	0.86 ± 0.05	0.87
	cutin	743[b]	0.89 ± 0.01	1	694[b]	0.96 ± 0.01	1
		Agave americana Leaf-Derived Cuticular Matter					
single-solute system	bulk	160[a]	1.06 ± 0.03	0.98	180[a]	1.29 ± 0.09	0.93
	dewaxed	245[d]	1.04 ± 0.03	0.97	265[d]	1.09 ± 0.08	0.93
	cutan	347[f]	1.03 ± 0.03	0.98	384[f]	1.22 ± 0.06	0.95
bi-solute system	bulk	200[b]	0.90 ± 0.01	1	90[c]	1.5 ± 0.07	0.95
	dewaxed	230[d]	0.90 ± 0.06	0.91	130[e]	1.28 ± 0.07	0.94
	cutan	194[h]	1.02 ± 0.04	0.97	192[h]	1.28 ± 0.04	0.98

[a] $K_{F,OC}$ is the C-normalized distribution coefficient [(mg/kg OC)·(mg/L)$^{-N}$]. Means with different superscripts (a–h) are significantly different ($p < 0.05$). Comparisons are made only within the same sorbent type. [b] ± one standard error.

atrazine is likely to perform H bonding interactions with H acceptor groups (*13*).

The significant increase in atrazine sorption affinity to the dewaxed fraction of the *A. americana* cuticle fraction is probably related to the crystalline nature of the epicuticular waxes present in the bulk cuticle (*34*). These waxes have very little binding

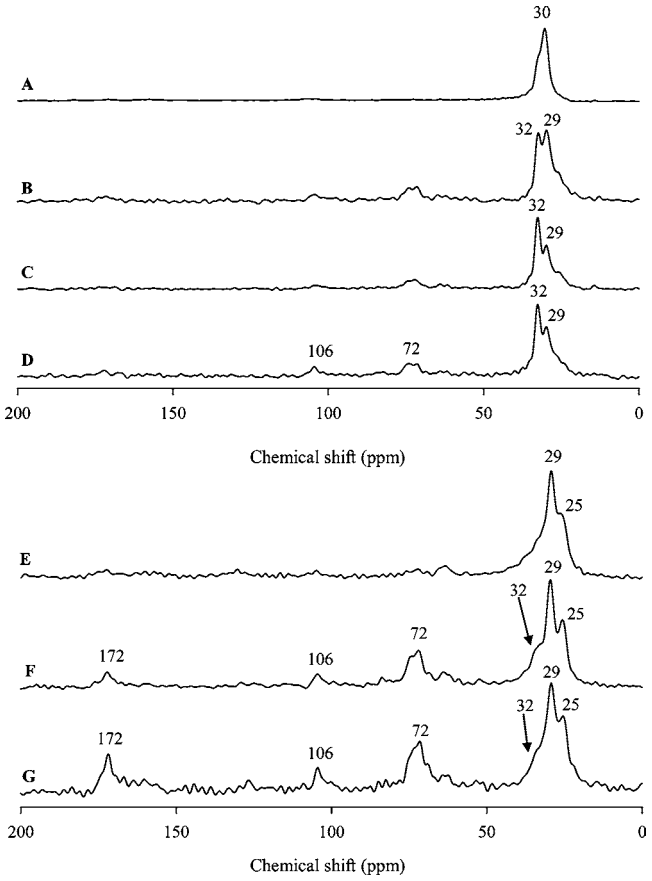

Figure 3. Solid-state ^{13}C NMR spectra of cuticular matter isolated from *A. americana* leaves [cutan (**A**), dewaxed (**B**), and bulk (**C**)] and cuticular fractions isolated from tomato fruits [cutin (**E**) and bulk (**F**)]. Spectra **D** and **G** refer to the bulk cuticles (*A. americana* and tomato, respectively) loaded with 2.6 (% wt) ametryn.

capabilities to polar solutes such as atrazine. A crystalline or condensed structure of the epicuticular waxes is supported by the ^{13}C NMR data presented in **Figure 3**. The spectrum of the bulk *A. americana* cuticle (**Figure 3C**) is characterized by a sharp peak at 32 ppm and a smaller peak at 29 ppm assigned to crystalline and mobile amorphous paraffinic domains, respectively (*32*, *33*). The ratio of the two peak intensities H_{32} ppm: H_{29} ppm (rigid to mobile-amorphous domain) was 1.64 for this sample. When waxes were removed (dewaxed cuticle, **Figure 3B**) this peak-intensity ratio decreased to 0.98. This suggests an increase in the relative level of mobile amorphous state paraffinic domains in the dewaxed cuticle. This change was followed by a 53% increase in the calculated $K_{F,OC}$ for atrazine with the dewaxed cuticle compared to the wax-containing (i.e., bulk) cuticle. The DSC measurements (**Figure 4**) show a distinct T_g in the bulk *A. americana* cuticle in the temperature range between 13 and 16 °C. This T_g was not observed with the dewaxed sample and therefore is assigned to the epicuticular waxes.

The high affinity of atrazine to the cutan biopolymer is related mainly to its hydrophobic nature. The cutan was characterized by a low O content and exhibited a low polarity index (calculated by the atomic (O + N)/C ratio, **Table 2**). Moreover, the ^{13}C NMR spectrum of this sample did not exhibit any peaks assigned to O-containing C functionalities (e.g., carboxyl or carbonyl structures). Therefore, we assume that the atrazine interactions with this sorbent are governed by hydrophobic-type interactions. In contrast, with the cutin biopolymer, both hydrophobic and polar (H-bonding) interactions can occur(*13*, *15*).

Sorption in a Bi-solute System. Additional information on the sorption mechanism can be obtained by studying the competitive behavior of similar sorbates such as atrazine and ametryn. Structurally similar sorbates are expected to compete more strongly with each other than with sorbates exhibiting different physicochemical properties (*19*, *35*, *36*). Xing et al. (*19*) have reported the nonlinear and competitive sorption behavior of *s*-triazines with glassy polymers and linear, noncompetitive behavior with rubbery polymers such as polyethylene, chitin, and cellulose. In our study, for the tomato cuticle fractions (bulk and cutin) the atrazine $K_{F,OC}$ values were statistically similar in the absence and presence of ametryn (**Figure 1**, **Table 1**). However, at C_e of 0.1 mg/L the K_{OC} value for atrazine with the bulk tomato cuticle was decreased by from 516 to 235 L/kg OC in the presence of the competitor ametryn. At C_e of 5 mg/L, the calculated *K*oc values in the presence and absence of ametryn were not significantly different. This trend was resulted from the significant increase in the Freundlich *N* value from 0.74 in the single-solute to 0.93 in the bi-solute system with the bulk tomato cuticle. This suggests that at low atrazine concentrations, the ametryn competes successfully with atrazine for sorption sites in the bulk tomato cuticle, probably due to the relatively high level of rigid paraffinic domain in this fraction. However, with the cutin biopolymer, similar K_{OC} values were obtained for atrazine at both systems (single- and bi-solute). This is probably due to the rubbery nature of this fraction where competition between solutes is not expected. Although the cutin biopolymer made up 75% by weight of the bulk tomato cuticle and it is the main sorbent within the bulk cuticle, its physical nature within the cuticle (attached by pectin and waxes) limits the accessibility of its sorption sites. This results in a successful competition of ametryn over atrazine in low atrazine concentration with the bulk cuticle sorbent.

In competitive sorption experiment with glassy type of sorbent, the sorption affinity of the tested solute is expected to decrease, while with rubbery-like sorbent the sorption affinity is not affected (*19*, *37*). However, in our study with the bulk *A. americana* cuticle, atrazine exhibited an increase in sorption affinity in the bi-solute system. The calculated $K_{F,OC}$ values for the sorption of atrazine with the bulk *A. americana* cuticle were 160 and 200 (mg/kg OC)·(mg/L)$^{-N}$ in the single- and bi-solute systems, respectively (**Table 1**). In addition, atrazine sorption isotherm had more curvature in the presence of ametryn (Freundlich *N* value was decreased from 1.06 to 0.9). At C_e of 0.1 mg/L, the atrazine K_{OC} values with the bulk *A. americana* cuticle exhibited an increase of 78% in the bi-solute system as compared to the single-solute system (251 vs 139 L/kg OC, respectively). A similar trend of decreasing in sorption nonlinearity and increasing of K_{OC} value at low C_e was observed for the dewaxed cuticle. In this case, Freundlich *N* value was decreased from 1.04 to 0.9 and the K_{OC} value at C_e of 0.1 mg/L was increased from 223 to 290 L/kg OC. However, the presence of ametryn significantly reduced the sorption affinity of atrazine to the cutan biopolymer (the $K_{F,OC}$ value decreased by 44%).

Desorption in Single- and Bi-Solute Systems. Desorption data and sorption−desorption hysteresis can provide further insight into the sorption mechanism and structural composition of the sorbent. With the bulk and cutin fractions isolated from the tomato fruit, atrazine exhibited a reversible sorption with no observable desorption hysteresis in the absence and presence of ametryn (**Figure 1**). Similar to the tomato cuticular matter,

Table 2. Relative Yields of Cuticular Fractions, Their Elemental Composition, and Atomic Ratios

	sample	yield[a]	C	H	O[b]	N	H/C	O/C	(O + N)/C
tomato fruit cuticle	bulk	100	64.0	8.2	26.6	1.2	1.55	0.31	0.33
	cutin	75	71.5	9.8	18.6	0.1	1.64	0.19	0.20
Agave americana leaf cuticle	bulk	100	67.0	10.4	21.8	0.5	1.87	0.24	0.25
	dewaxed	86	65.2	10.2	23.6	0.5	1.89	0.27	0.28
	cutan	20	76.8	11.7	11.2	0.4	1.82	0.11	0.11

[a] % weight. [b] Calculated by mass difference.

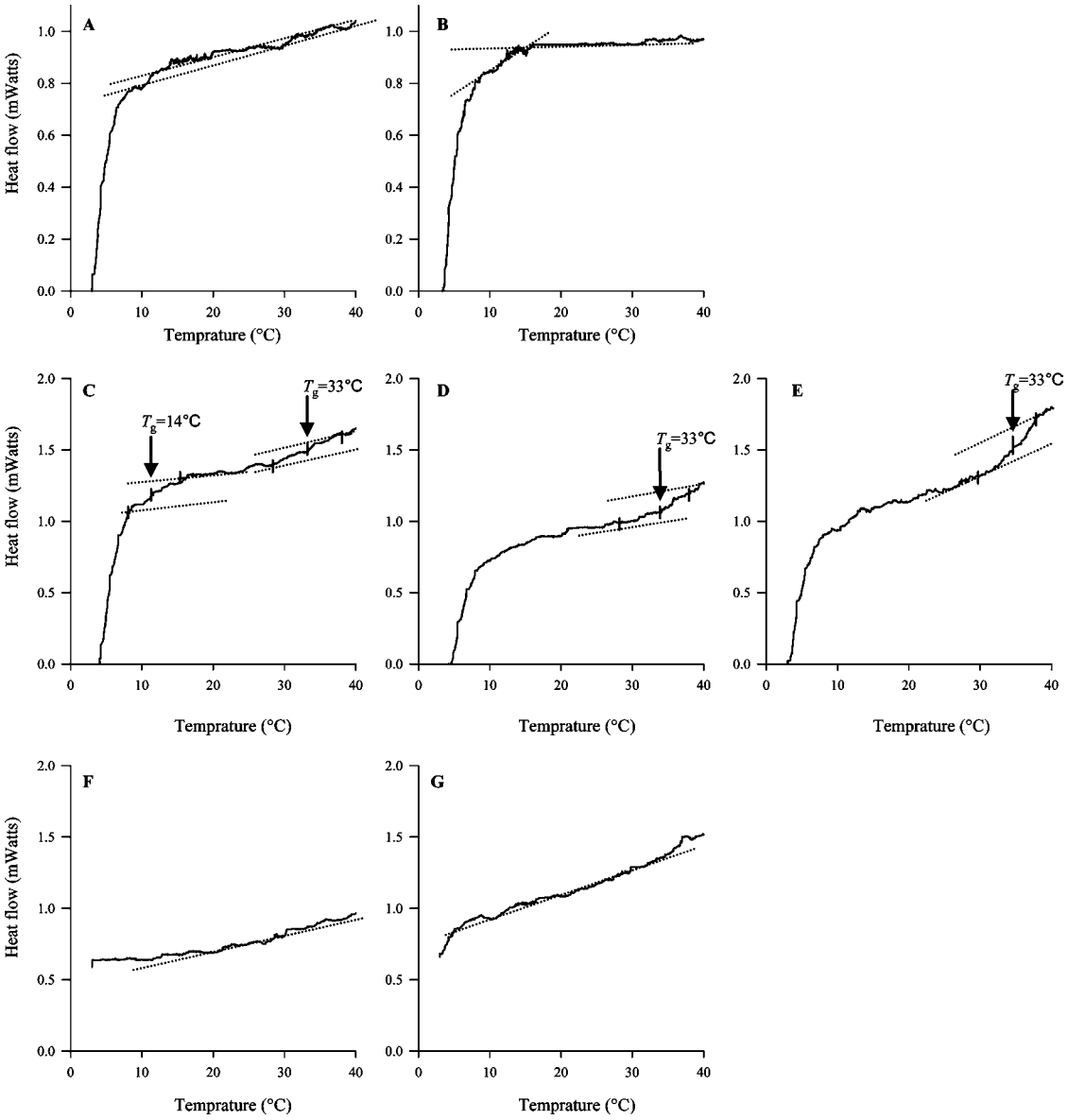

Figure 4. Differential scanning calorimeter heating thermograms of cuticular matter isolated from tomato fruits [bulk (**A**) and cutin (**B**)] and cuticular fractions isolated from *A. americana* leaves [bulk (**C**), dewaxed (**D**), and cutan (**E**)]. Thermograms **F** and **G** refer to the bulk cuticles (tomato and *A. americana*, respectively) loaded with 2.6 (% wt) ametryn.

the atrazine sorption isotherms with all *A. americana* fractions exhibited reversible sorption behavior in single-solute system (**Figure 2**, left side; **Table 1**). With the cutan biopolymer, no desorption hysteresis of atrazine was obtained in the bi-solute system as well. However, in the bi-solute system, the atrazine desorption data obtained with the bulk and dewaxed *A. americana* cuticles exhibited enhanced desorption (**Figure 2**, right side). For these two sorbents, the desorption $K_{F,OC}$ values were significantly lower than the values obtained for the sorption isotherms (**Table 1**). This indicates that it was easier to desorb atrazine from the sorbent matrix (i.e., bulk and dewaxed cuticles) in the presence of a competitor (ametryn). In general, enhanced desorption is observed in systems in which surfactants or dissolved organic matter are present or have been added (*38*). These materials operate as a third medium, assisting to desorb bound molecules. In our study, the concentration of dissolved organic matter in the test tubes was negligible, suggesting that this mechanism was not controlling the enhanced desorption observed for atrazine in the bi-solute system with bulk and dewaxed *A. americana* cuticles.

Another possible mechanism that could assist in enhancing desorption is phase transition of the sorption domain due to a

high load of competitor (*20*, *39*). To check this hypothesis, we analyzed the bulk cuticles (tomato and *A. americana*) by solid-state ^{13}C NMR and by DCS after they had been loaded with ametryn. The samples were interacted with ametryn under the conditions described for the sorption experiments. The ^{13}C NMR spectra of the samples before and after loading with ametryn are presented in **Figure 3C,D** (for *A. americana* bulk cuticle) and **Figure 3F,G** (for tomato bulk cuticle), respectively. The two NMR peaks of 32 ppm (rigid domain: crystalline or solid amorphous domain) and 29 ppm (mobile amorphous domain) in paraffinic carbons (0−50 ppm) have been used to characterize the physical conformation of organic matter (*40*). With the bulk tomato cuticle, the levels of mobile amorphous paraffinic (28−30.5 ppm) and rigid paraffinic (30.5−33 ppm) domains were similar in the presence and absence of the competitor ametryn (27 mg sorbed ametryn/g cuticle). The mobile amorphous domain was 25 and 26%, and the rigid domain was 14.5 and 15% (from the total paraffinic region, 0−50 ppm) in the presence and absence of the competitor ametryn, respectively. Thus, we conclude that ametryn did not influence a matrix conformation of the tomato bulk cuticle.

With the bulk *A. americana* cuticle, the relative level of the solid-amorphous (rigid) paraffinic C domain was decreased from 29.4 to 24.3%, and the level of the mobile-amorphous paraffinic domain was increased from 20.0 to 22.1% in the presence of 26 mg/g ametryn loading. The H_{32} ppm:H_{29} ppm peak intensity ratio (rigid to mobile-amorphous domain) was 1.44, whereas a ratio of 1.64 was calculated for the unloaded cuticle sample (**Figure 3**, panels **D** and **C**, respectively). Thus, with this sorbent (rich in waxes), a matrix transition of rigid to mobile-amorphous paraffinic domains in the presence of sorbed ametryn was occurred. Chen and Xing (*40*) reported that a physical phase transition (melting) of reconstituted waxes was induced by sorbed polycyclic aromatic hydrocarbons during sorption to cuticular waxes isolated from green pepper fruits. This mechanism is similar to the transformation of a glassy polymer to a rubbery stage above the T_g (*20*, *39*, *41*). Our DSC measurements support this phase transition: the bulk *A. americana* cuticle exhibited two T_g, the first between 13 and 16 °C and the second between 30 and 40 °C (**Figure 4C**)); however, no distinct glass transition was obtained with the bulk *A. americana* after loading with ametryn (**Figure 4G**). We assume that these physical phase-transition processes took place mainly with the waxes (which exhibited a glassy nature and made up 14% by weight of the cuticle). This is supported by the lower trend of enhanced desorption observed for the dewaxed cuticle. We assume that the presence of a high concentration of ametryn facilitated the phase transition (melting), which in turn created more low-energy partition-like sorption sites, resulted in higher sorption capacity and easier desorption. This trend was not observed in the tomato cuticle probably due to the low concentration of waxes (3% by weight).

This study demonstrates the markedly high sorption capacity of tomato and *A. americana* cuticular matter for atrazine. The cutin biopolymer seems to facilitate reversible and noncompetitive sorption, probably due to its rubbery nature. On the other hand, the epicuticular waxes facilitate enhance desorption in a bi-solute system. These processes are possibly related to phase-transitions that occur in the presence of high solute loading.

ABBREVIATIONS USED

C_e, equilibrium concentration; DSC, differential scanning calorimeter; HOC, hydrophobic organic compound; $K_{F,OC}$, C-normalized Freundlich capacity coefficient; K_{OC}, C-normalized distribution coefficient; NMR, nuclear magnetic resonance; OC, organic carbon; SOM, soil organic matter; T_g, glass-transition temperature.

LITERATURE CITED

(1) Nierop, K. G. J. Origin of aliphatic compounds in a forest soil. *Org. Geochem.* **1998**, *29*, 1009−1016.

(2) Kogel-Knabner, I. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biol. Biochem.* **2002**, *34*, 139−162.

(3) Jeffree, C. E. Structure and ontogeny of plant cuticle. In *Plant Cuticles: An Integrated Functional Approach*; Kerstiens, G., Ed.; BIOS Scientific Publishers: Oxford, UK, 1996; pp 33−82.

(4) Santier, S.; Chamel, A. Reassessment of the role of cuticular waxes in the transfer of organic molecules through plant cuticles. *Plant Physiol. Biochem.* (*Paris*) **1998**, *36*, 225−231.

(5) Kolattukudy, P. E. Cutin from plants. *Biopolymers* **2001**, *3A*, 1−35.

(6) Villena, J. F.; Dominguez, E.; Stewart, D.; Heredia, A. Characterization and biosynthesis of non-degradable polymers in plant cuticles. *Planta* **1999**, *208*, 181−187.

(7) Boom, A.; Damste, J. S. D.; Leeuw, J. W. Cutan, a common aliphatic biopolymer in cuticles of drought-adapted plants. *Org. Geochem.* **2005**, *36*, 595−601.

(8) Heredia, A. Biophysical and biochemical characteristics of cutin, a plant barrier biopolymer. *Biochim. Biophys. Acta* **2003**, *1620*, 1−7.

(9) Almendros, G.; Guadalix, M. E.; Gonzalez-Vila, F. J.; Martin, F. J. Preservation of aliphatic macromolecules in soil humins. *Org. Geochem.* **1996**, *24*, 651−659.

(10) Derenne, S.; Largeau, C. A review of some important families of refractory macromolecules: composition, origin, and fate in soils and sediments. *Soil Sci.* **2001**, *166*, 833−847.

(11) Lichtfouse, E.; Bardoux, G.; Mariotti, A.; Balesdent, J.; Ballentine, D. C.; Macko, S. A. Molecular, ^{13}C, and ^{14}C evidence for the allochthonous and ancient origin of C_{16}-C_{18} *n*-alkanes in modern soils. *Geochim. Cosmochim. Acta* **1997**, *61*, 1891−1898.

(12) Chefetz, B.; Salloum, M. J.; Deshmukh, A. P.; Hatcher, P. G. Structural components of humic acids as determined by chemical modifications and carbon-13 NMR, pyrolysis-, and thermochemolysis-gas chromatography/mass spectrometry. *Soil Sci. Soc. Am. J.* **2002**, *66*, 1159−1172.

(13) Chefetz, B. Sorption of phenanthrene and atrazine by plant cuticular fractions. *Environ. Toxicol. Chem.* **2003**, *22*, 2492−2498.

(14) Chefetz, B.; Deshmukh, A. P.; Hatcher, P. G.; Guthrie, E. A. Pyrene sorption by natural organic matter. *Environ. Sci. Technol.* **2000**, *34*, 2925−2930.

(15) Chen, B.; Johnson, E. J.; Chefetz, B.; Zhu, L.; Xing, B. Sorption of polar and nonpolar aromatic organic contaminants by plant cuticular materials: role of polarity and accessibility. *Environ. Sci. Technol.* **2005**, *39*, 6138−6146.

(16) Gunasekara, A. S.; Xing, B. Sorption and desorption of naphthalene by soil organic matter: importance of aromatic and aliphatic components. *J. Environ. Qual.* **2003**, *32*, 240−246.

(17) Salloum, M. J.; Chefetz, B.; Hatcher, P. G. Phenanthrene sorption by aliphatic-rich natural organic matter. *Environ. Sci. Technol.* **2002**, *36*, 1953−1958.

(18) Weber, W. J.; McGinley, P. M.; Katz, L. E. A distributed reactivity model for sorption by soils and sediments. 1. Conceptual basis and equilibrium assessments. *Environ. Sci. Technol.* **1992**, *26*, 1955−1962.

(19) Xing, B.; Pignatello, J. J.; Gigliotti, B. Competitive sorption between atrazine and other organic compounds in soils and model sorbents. *Environ. Sci. Technol.* **1996**, *30*, 2432−2440.

(20) Ju, D.; Young, T. M. The influence of natural organic matter rigidity on the sorption, desorption, and competitive displacement rates of 1,2-dichlorobenzene. *Environ. Sci. Technol.* **2005**, *39*, 7956−7963.

(21) Weber, W. J.; Huang, W.; Yu, H. Hysteresis in the sorption and desorption of hydrophobic organic contaminants by soils and sediments. 2. Effects of soil organic matter heterogeneity. *J. Contam. Hydrol.* **1998**, *31*, 149−165.
(22) Hulscher, T. E. M. T.; Cornelissen, G. Effect of temperature on sorption equilibrium and sorption kinetics of organic micropollutants. *Chemosphere* **1996**, *32*, 609−626.
(23) Weber, W.; Leboeuf, E. J.; Young, T. M.; Huang, W. Contaminant interactions with geosorbent organic mater: insight drawn from polymer sciences. *Water Res.* **2001**, *35*, 853−868.
(24) Huang, W.; Weber, W. J. A distributed reactivity model for sorption by soils and sediments. 10. Relationship between desorption, hysteresis, and the chemical characteristics of organic domain. *Environ. Sci. Technol.* **1997**, *31*, 2562−2569.
(25) LeBoeuf, E. J.; Weber, W. J. Macromolecular characteristics of natural organic matter. 2. Sorption and desorption behavior. *Environ. Sci. Technol.* **2000**, *34*, 3632−3640.
(26) Stevenson, F. J. *Humus Chemistry—Genesis, Composition, Reactions*, 2nd ed.; John Wiley & Sons: New York, 1994.
(27) Casado, C. G.; Heredia, A. Specific heat determination of plant barrier lipophilic components: biological implications. *BBA—Biomembranes* **2001**, *1511*, 291−296.
(28) Charles, R. W.; Barrie, W. S. *The Pesticide Manual*, 7th ed.; The British Crop Protection Council: London, 1983.
(29) Chamel, A.; Vitton, N. Sorption and diffusion of ^{14}C-atrazine through isolated plant cuticle. *Chemosphere* **1996**, *33*, 995−1003.
(30) Luque, P.; Heredia, A. The glassy state in isolated cuticles: differential scanning calorimetry of tomato fruit cuticular membranes. *Plant Physoil. Biochem.* **1997**, *35*, 251−256.
(31) Matas, A. J.; Cuartero, J.; Heredia, A. Phase transition in the biopolyester cutin isolated from tomato fruits cuticles. *Thermochim. Acta* **2004**, *409*, 165−168.
(32) Hu, W. G.; Mao, J.; Xing, B.; Schmidt-Rohr, K. Poly(methylene) crystallites in humic substances detected by nuclear magnetic resonance. *Environ. Sci. Technol.* **2000**, *34*, 530−534.
(33) Sachleben, J.; Chefetz, B.; Deshmukh, A. P.; Hatcher, P. G. Solid-state NMR characterization of pyrene−cuticular matter interactions. *Environ. Sci. Technol.* **2004**, *38*, 4369−4376.
(34) Merk, S.; Blume, A.; Riederer, M. Phase behavior and crystallinity of plant cuticular waxes studied by Fourier transform infrared spectroscopy. *Planta* **1998**, *204*, 44−53.
(35) Heuvel, H. V. D.; Noort, V. Competition for adsorption between added phenanthrene and in situ PAHs in two sediments. *Chemosphere* **2003**, *52*, 1097−1103.
(36) Ju, D.; Young, T. M. Effect of competitor and natural organic matter characteristics on the equilibrium sorption of 1,2-dichlorobenzene in soil and shale. *Environ. Sci. Technol.* **2004**, *38*, 5863−5870.
(37) Graber, E. R.; Borisover, M. Competitive sorption of organic contaminants in chalk. *J. Contam. Hydrol.* **2003**, *67*, 159−175.
(38) Sanchez-Camazano, M.; Sanchez-Martin, M. J.; Rodriguez-Cruz, M. S. Sodium dodecyl sulphate-enhanced desorption of atrazine: effect of surfactant concentration and of organic matter content of soils. *Chemosphere* **2000**, *41*, 1301−1305.
(39) Weber, W. J.; Kim, S. H.; Johnson, M. D. A distributed reactivity model for sorption by soils and sediments. 15. High concentration effects on phenanthrene sorption and desorption. *Environ. Sci. Technol.* **2002**, *36*, 3625−3634.
(40) Chen, B.; Xing, B. Sorption and conformational characteristics of reconstituted plant cuticular waxes on montmorillonite. *Environ. Sci. Technol.* **2005**, *39*, 8315−8323.
(41) Tremblay, L.; Kohl, S. D.; Rice, J. A.; Gagne, J. P. Effects of lipids on the sorption of hydrophobic organic compounds on geosorbents: a case study using phenanthrene. *Chemosphere* **2005**, *58*, 1609−1620.

Received for review May 23, 2006. Revised manuscript received August 3, 2006. Accepted August 4, 2006. This research was supported by a research grant from BARD (No. IS-3385-03), the United States−Israeli Binational Agricultural Research and Development Fund.

JF0614488