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Bioavailability of diuron in soil containing wheat-straw-derived char

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Abstract

This study evaluated the bioavailability of diuron in soil as influenced by char arising from the burning of wheat straw. The wheat char was a highly effective sorbent for diuron. The presence of 1% wheat char in soil resulted in a 7–80 times higher diuron sorption. A 10-week incubation resulted in <40% of 0.5 mg/kg diuron in 0.5% char-amended soil microbially degraded, as compared to 50% in char-free soil under the same conditions. Over the experimental range of diuron application rates from 0 to 12 mg/kg and of char contents from 0% to 1.0%, a 4-week bioassay indicated that both the barnyardgrass survival rating and the fresh weight of aboveground biomass decreased with increasing diuron application at given char contents but increased with increasing char content at potentially damaging diuron application rates. Residual analyses of bioassayed soils showed that the soils with char contents of 0.5% and higher and diuron application rates of 3.0 mg/kg and higher, as compared to those with no or low (0.05%) char and a diuron application rate of 1.5 mg/kg, had higher residual diuron levels but higher barnyardgrass survival ratings and fresh weights. These results suggest that enhanced sorption of diuron in soil in the presence of wheat char reduced the bioavailability of diuron, as manifested by reduced microbial degradation of diuron and its herbicidal efficacy to barnyardgrass. This study may have greater implication than for burning of wheat straw that field burning of vegetations may reduce bioavailability of pesticides.

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1. Introduction

Many studies have suggested that soil-bound organic contaminants are unavailable for microbial

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degradation (Ogram et al., 1985; Shimp and Young, 1988; Steen et al., 1980). Although recent research suggested that limited biodegradation of soil-sorbed pesticides may occur (Feng et al., 2000; Park et al., 2001, 2002, 2003), liquid-phase contaminants are much more bioaccessible to soil microorganisms (Ogram et al., 1985; Guerin and Boyd, 1992; Lahlou and Ortega-Calvo, 1999). Other studies have estab-

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lished that phytotoxicity of herbicides is directly related to their concentrations in the soil solution (e.g., Lambert, 1966; Pillay and Tchan, 1971). It is generally true that only dissolved nutrients and organic chemicals in the soil interstitial bulk-like water (i.e., plant-available water above the wilting point) are available for plant-root uptake. Sorption largely controls concentrations of organic contaminants in the soil solution and thus is the major determinant of the bioavailability of pesticides to both microorganisms and plants. Enhancing sorption by increasing soil organic matter content leading to a reduction in the solution-phase concentrations results in a decreased microbial degradation of organic contaminants (e.g., Guerin and Boyd, 1993). Charcoal dipping or banding effectively protects many plants from herbicidal injury (Arle et al., 1948; Burr et al., 1972; Chandler et al., 1978; Jordan and Smith, 1971; William and Romanowski, 1972).

Sorptive properties of agricultural soils, and thus bioavailability of pesticides, are often influenced by agricultural practices. Field burning of crop residues worldwide incorporates the resulting chars into soils. Hilton and Yuen (1963) postulated that the retained sorptivity of many Hawaiian soils for ureas and striazines after oxidative removal of soil organic matter by hydrogen peroxide was a result of the peroxideresistant soil chars arising from burning of sugarcane trash. Toth et al. (1999) observed a reduction in the phytotoxicity of diuron applied over the ash of recently burned kangaroo grass, due primarily to the diuron sorption by the ash. We confirmed their postulation by measuring the sorption of pesticides on soil-free chars from burning of crop residues. Sorption of diuron by the chars from burning of wheat straw and rice residue was 400-2500 times higher than that by a soil with 2.1% organic matter (Yang and Sheng, 2003a). Amendment of the soil with the wheat char up to 1% (by weight) enhanced the sorptivity of the soil for diuron in proportion to the char content. The char aged in the soil for one month remained highly effective for diuron sorption and dominated the sorption, although a small reduction (<30%) in sorptivity was observed (Yang and Sheng, 2003b). Further aging of the char for up to 12 months did not result in a further sorptivity reduction, indicating that the char was resistant to degradation. One direct consequence of the high sorptivity of crop-residuederived chars may be the reduced bioavailability of pesticides in soils to both microbes and plants. The reduction in microbial degradation of benzonitrile in a soil in the presence of wheat char has been reported (Zhang et al., 2004). Reduced microbial degradation increases the persistence of pesticides and thus the environmental risk associated with pesticide use. The high sorptivity of crop-residue-derived chars may also reduce herbicidal efficacy to weeds. Poor herbicidal efficacy is a concern both economically and environmentally due to additional application of herbicides for weed control.

In this study, we determined the bioavailability of diuron to soil microorganisms and to barnyardgrass (Echinochloa crus-galli (L.) Beauv.) in soil in the presence of wheat-straw-derived char. Use of barnyardgrass, a common rice weed, has both agronomic and environmental significance. Our objectives were to determine the microbial degradation of diuron and its efficacy to barnyardgrass in relation to soil sorption in the presence of wheat char and to evaluate the impact of crop-residue-derived chars on the bioavailability of pesticides in soil. Results from laboratory measurements and greenhouse bioassays indicated that wheat char was a highly effective sorbent for diuron and its presence in soil resulted in enhanced diuron sorption and subsequently diminished bioavailability.

2. Materials and methods

2.1. Materials

Wheat char used in this study was from our previous studies (Yang and Sheng, 2003a,b). Airdried wheat (*Triticum aestivum L.*) straw (10 kg) was collected from the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas. Wheat char was obtained by burning the straw on a stainless steel plate (1 m \times 1 m) in an open field under natural conditions in a July afternoon. The BET surface area of the char was determined to be 10.1 m²/g in a commercial service laboratory. Chemical analysis showed that the char contained 13% elemental carbon and 42% silica.

Soil, classified as Stuttgart silt loam, was collected at the Rice Research and Extension Center, Stuttgart, Arkansas. The soil had a mechanical composition of 17.1% sand, 60.4% silt, and 22.5% clay. The soil contained 2.1% organic matter and had a cation-exchange capacity of 8.5 cmol_c/kg. The soil, without records of crop residue burns, was presumed to contain minimal levels of chars. The soil was air-dried, ground, and passed through a 1-mm sieve. Char-amended soils were prepared by thoroughly mixing soil with accurately weighed char in exact char contents of 0.05%, 0.1%, 0.5%, and 1.0% (by weight).

Diuron with a purity of 99% was purchased from ChemService (West Chester, PA) and used as received. Diuron is an electroneutral molecule. The pesticide has a water solubility of ~42 mg/l and a log $K_{\rm ow}$ of 2.68 at room temperature (Howard and Meylan, 1997). The Henry's Law constant of 2.7×10^{-6} atm m³/mol indicates that the pesticide is non-volatile and therefore suitable for prolonged laboratory and greenhouse studies. Diuron is a urea compound primarily used as a preemergence herbicide in soils to control germinating grasses and broad-leaved weeds. As a photosynthesis inhibitor, diuron injures weeds with symptoms of foliar chlorosis concentrated around veins (sometimes interveinal) followed by necrosis.

A mixed diuron-degrading culture was isolated from soil collected in a cotton field where diuron has been applied annually for over 15 years. One gram of the soil was inoculated into 100 ml of medium containing mineral salts (Stanier et al., 1966), 0.1 ml of vitamin solution (Wolin et al., 1963), and saturated diuron. The mixture was incubated for 1 week at 28 °C on a platform shaker at 150 rpm. After two serial transfers to fresh medium, the culture enrichment was obtained. Preliminary tests showed that the isolated culture readily degraded diuron in solution.

Barnyardgrass seeds, collected from the plant grown in a rice field in Stuttgart, AR in 1983, were acid-scarified in 1 N nitric acid for 1 h at room temperature. The seeds were washed with deionized water, spread evenly on filter paper in petri dishes, covered with another filter paper, watered with 3 ml of deionized water, and incubated in the dark at room temperature for 72 h for germination.

2.2. Sorption isotherms for diuron

Sorption of diuron by soil, wheat char, and 1% char-amended soil was measured by the batch

equilibration technique. Various quantities of diuron in 0.005 M CaCl₂ solution were added into 25-ml Corex glass centrifuge tubes containing sorbents with a constant mass between 0.01 and 3.5 g. The mass of sorbents was adjusted to allow for >40% of added diuron to be adsorbed. Additional 0.005 M CaCl₂ solution was added to bring the total liquid volume to 10 ml. Initial concentrations of diuron ranged from 1.25 to 12.5 mg/l. The tubes were closed with Teflonlined screw caps and rotated (40 rpm) at room temperature (~25 °C) for 48 h. Other measurements have shown that sorption of diuron by both the soil and the char reached apparent equilibria within 24 h; the sorption by char-amended soil was thus assumed to also reach equilibrium within the same duration.

After the establishment of sorption equilibria, sorbents and aqueous phases were separated by centrifugation at 6000 rpm (RCF=5210 g) for 30 min. The diuron concentrations in supernatants were analyzed by high-performance liquid chromatography (HPLC). The amount of diuron sorbed was calculated from the difference between the amount initially added and that remaining in equilibrium solution. All measurements were in duplicate with a variation generally <5%, and the calculated average data were reported. The measurements with blanks not containing sorbents found that glass tubes did not adsorb diuron and no processes other than sorption contributed to the loss of solution-phase diuron.

2.3. Microbial degradation of diuron

Sterilized soil and 0.5% char-amended soil (200 g each) were placed in 1000-ml glass beakers, spiked with 2 ml of 50 mg/l diuron stock solution in acetone, thoroughly mixed, and allowed for acetone to evaporate for 2 days. The soils were then watered to near the field capacity and aged for another 2 days to allow the diuron sorption to complete. Three 5-g samples from each of the soils were extracted with 5 ml of H₂O and 5 ml of water-saturated ethyl acetate for 72 h. Following the phase separation, 2 ml of ethyl acetate were dried under N2 gas and redissolved in 1 ml of methanol. The diuron concentrations in methanol were analyzed by HPLC. The recoveries were 95.2% and 85.2% for soil and char-amended soil, respectively. Each soil was inoculated with 5 ml of the isolated culture enrichment. Preliminary tests found that 5 ml of the enrichment in 200 g of soil gave an appreciable degree of diuron degradation in one week. The soils were thoroughly mixed again. The beakers were then covered with aluminum foil and placed in the dark at room temperature. Three 5-g soil samples from each beaker were taken weekly for 10 weeks for diuron analysis. The soils were extracted following the same extraction procedures as those for the recoveries, except that extracting water contained 0.5% Ag₂SO₄ to immediately terminate biodegradation. The diuron concentrations were adjusted for the recoveries. The soils, watered when necessary, were maintained moist throughout the degradation experiment.

2.4. Barnyardgrass bioassay for diuron

A series of 4 diuron solutions with concentrations of 60, 120, 240, and 480 mg/l in acetone were prepared. Five milliliters of each of the solutions, along with acetone only, were added to 200 g of charfree soil or char-amended soils in plastic bags, resulting in the diuron levels of 0, 1.5, 3.0, 6.0, and 12 mg/kg in the soils, respectively. The five diuron levels were referred to as 0X, 1X, 2X, 4X, and 8X, where X=1.5 mg/kg and is within the range of recommended field application rates. The five char contents were 0%, 0.05%, 0.1%, 0.5%, and 1%. The combination of the 5 diuron rates and the 5 char contents resulted in a total of 25 treatments. Each treatment was in triplicate. The soils were thoroughly mixed in the bags, and transferred to plastic cups following the evaporation of acetone from the opened bags.

A small quantity of soil (~20 g) was first removed from each cup. Ten pregerminated barnyardgrass seeds with extended radicles and hypocotyls were placed evenly on the soil surface in each cup and then covered with the previously removed soil. Following planting, the cups were placed in a completely randomized block design in a greenhouse, watered with 40 ml of deionized water, and maintained moist throughout the experiment. The greenhouse was maintained at 34/20 °C day/night temperatures with a 14-h lighting cycle. Barnyardgrass seedling survival was visually rated two weeks after planting as percent of survival between 0 and 100, with 0% representing no survival and 100% complete survival (no injury).

The survival rating was performed independently by three individuals. Average survival ratings were calculated using the data from the replicated samples from all the individuals. Four weeks after planting, plants were cut at the soil level and immediately weighed to obtain the fresh weights of the aboveground biomass. All the visual survival rating and fresh weight data were statistically analyzed using the SAS program.

Following the bioassay, the soil samples of selected treatments were analyzed for residual diuron by HPLC using the extraction procedures described earlier. The average diuron concentration of the replicate soils of the same treatment was reported. The soils from the following 6 treatments were selected based on the char contents, diuron rates, and barnyardgrass aboveground fresh weights: S0-0, S0-1.5, S0.05-1.5, S0.5-3.0, S1-3.0, and S1-6.0, where the suffix C-D to S represents the char content (C)-applied soil concentration of diuron (D).

2.5. Analysis of diuron

Diuron in the supernatants from sorption experiments and in the extracts from microbial degradation and greenhouse bioassay tests was analyzed on a Hitachi reversed-phase high-performance liquid chromatograph (Hitachi High-Technologies Tokyo, Japan) fitted with a UV-visible detector set at the maximum absorption wavelength for diuron (252 nm). A Phenomenex Prodigy C18 column was used (Alltech Assoc., Deerfield, IL). The mobile phase was a mixture of acetonitrile and water (50:50, v/v) at a flow rate of 1.0 ml/min. The injection volume was 20 μ l.

3. Results and discussion

Isotherms for the sorption of diuron by soil, wheat char and 1% char-amended soil are presented in Fig. 1, in which the amount of diuron sorbed (mg/kg) is plotted against the equilibrium concentration (mg/l) in water. No single mathematical models provided adequate fits for the sorption data. The curves were drawn to assist in visualization and comparison of the data. Soil effectively sorbed diuron, rather consistent with the prediction from the log $K_{\rm ow}$ value of diuron

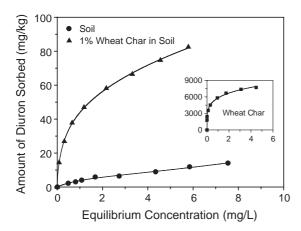


Fig. 1. Isotherms for sorption of diuron from water by soil, wheat char, and char-amended soil.

and the soil organic matter content. Wheat char had a much higher sorptivity than soil for diuron (see the inset in Fig. 1). From the curves, we estimated that the char was 700–37,000 times more effective than the soil in sorbing diuron over the experimental concentration range (0–6 mg/l). These results are similar to those obtained in our previous study (Yang and Sheng, 2003a). It has also been reported in the literature that chars (the term ash was used) derived from burning of vegetation are effective adsorbents for pesticides (Yang and Sheng, 2003b; Toth and Milham, 1975).

Much higher sorptivity of wheat char than of soil resulted in enhanced sorption of diuron by the soil in the presence of the char, as indicated in Fig. 1. Calculations show that 1% char-amended soil sorbed about 7-80 times more diuron than char-free soil over the experimental concentration range. Assuming that the amendment of soil with 1% char did not change the sorptivity of the soil for diuron, 1% char contributed >86% to the total sorption of diuron, indicating the predominance of the char for diuron sorption. Normalization of the sorption to char content resulted in a diuron isotherm slightly lower than that for soil-free char, which may have resulted from the competitive sorption of dissolved soil organic matter on the char. While the diuron sorption by charamended soil was evaluated with only one char content, enhanced sorption at other char contents is expected. At recommended field application rates, enhanced diuron sorption in the presence of wheat char may decrease the concentration of diuron in the soil solution, leading to reduced biodegradation and loss of herbicidal efficacy.

An evaluation of the bioavailability of diuron to soil microorganisms in the presence and absence of wheat char in soil was made by comparing the degradation of diuron in char-free soil and 0.5% char-amended soil, both inoculated with the same size of an isolated diuron-degrading culture. The initial diuron concentration in both soils was 0.5 mg/kg. We simply measured the dissipation of diuron over incubation time and did not identify the degradation products, thus offering no mechanistical information on the diuron transformation reaction. In Fig. 2, the degradation is expressed as the percent of total diuron degraded at given time. Although diuron is a rather persistent pesticide in field soils, it slowly degraded in field soils (Hill et al., 1955). It has been reported that mixed cultures from pond water and sediment aerobically degraded diuron to several identified products and carbon dioxide (Ellis and Camper, 1982). While we did not know the species and the population of the isolated cultures, diuron in both soils was degraded with time. The degradation was slower in charamended soil than in char-free soil. Over a 10-week incubation, <40% of diuron in 0.5% char-amended soil was degraded, in comparison to about 55% in char-free soil. While direct degradation of soil-sorbed organic compounds by bacteria may occur, we did not know whether such a process was involved in diuron degradation. However, our results clearly show that

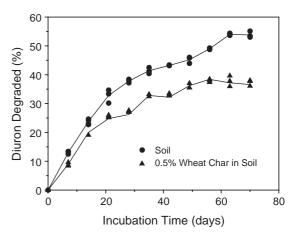


Fig. 2. Microbial degradation of diuron over time in sterilized soil and char-amended soil inoculated with a mixed enrichment culture.

enhanced sorption in the presence of wheat char reduced the bioavailability of diuron to soil microorganisms. Reduced biodegradation of benzonitrile by a bacterium in soil in the presence of wheat char was also due to enhanced sorption (Zhang et al., 2004). In addition to elemental carbon and silica, wheat char contained 21% potassium, 1.5% phosphorous, 0.64% nitrogen, and other microelements (Yang and Sheng, 2003b). When in their available forms in soil, some of these nutrients may stimulate microbial activity. We have found that when benzonitrile was not limiting, 1% wheat char provided nutritional stimulation on benzonitrile degradation (data not shown). Such a stimulation on diuron degradation in 0.5% charamended soil was not obvious.

Fig. 3 is the photograph comparing the barnyard-grass growth among the soil samples subjected to diuron applications in the absence and presence of wheat char just prior to cutting the plants for their fresh weights. The samples consisted of 25 cups, each representing one of the three replicates for each of all the treatments, and were placed de-randomized to aid visualization. In the absence of diuron application, barnyardgrass showed a normal growth without observable growth stimulation by wheat char nutrients. One week after planting, the barnyardgrass injury was obvious (photograph not shown). The injury increased with increasing diuron application

rate at a given char content and decreased with increasing char content at a given diuron application rate. The observation is consistent with the earlier sorption measurements that the presence of wheat char enhanced the diuron sorption by soil. By four weeks after planting (prior to cutting), uninjured barnyardgrass showed continued growth, whereas injured ones did not recover (Fig. 3).

More quantitative barnyardgrass bioassay to evaluate the impact of wheat char on the bioavailability of diuron was obtained by visually rating barnyardgrass survival two weeks after planting and by weighing aboveground fresh biomass four weeks after planting (Fig. 4). Without application of diuron, barnyardgrass was somehow injured in char-free soil. In the presence of wheat char ($\geq 0.05\%$), the injury was eliminated. This suggests that unknown herbicides likely phytotoxic to barnyardgrass may be present in char-free soil but deactivated by the char. A full rate of diuron (1.5 mg/kg) in char-free soil showed almost complete injury to barnyardgrass. However, both the barnyardgrass survival rating and fresh weight increased with increasing char content. A char content of 0.5% or higher was sufficiently high that diuron completely lost its efficacy to barnyardgrass. The application rates of 3 mg/kg and higher resulted in complete-to-partial losses of diuron efficacy to barnyardgrass. Similar to the observations with the full rate of diuron, the

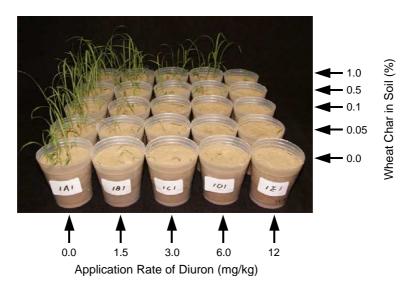


Fig. 3. Photograph showing barnyardgrass growth in soils as a function of diuron application rate and wheat char content four weeks after planting.

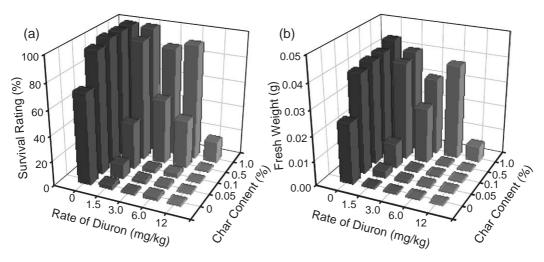


Fig. 4. Growth of barnyardgrass in soils as a function of diuron application rate and wheat char content showing (a) survival rating of barnyardgrass two weeks after planting, and (b) fresh weight of barnyardgrass four weeks after planting.

survival rating and fresh weight at higher application rates increased with increasing char content over the tested range of char contents in soil. The Student's t test at the 95% level of confidence (α =0.05) was used to compare the individual survival ratings and fresh biomass weights at various char contents and diuron application rates. Survival ratings and fresh weights were statistically different (α =0.05) for \leq 0.05% char with no diuron application, for ≤0.5% char with 1.5 mg diuron/kg, for $\geq 0.1\%$ char with 3.0 mg or 6.0 mg diuron/kg (fresh weights were significantly different for≥0.5% char with 6.0 mg diuron/kg), and for $\geq 0.5\%$ char with 12 mg diuron/kg. These results suggest the decreased bioavailability of diuron to barnyardgrass with increasing char content in soil, due presumably to enhanced sorption of diuron in the presence of the char.

The sorptive role of wheat char in reducing diuron bioavailability (efficacy) to barnyardgrass is confirmed by measuring residual concentrations of diuron in soils after cutting barnyardgrass. Soils from 6 selected treatments, where barnyardgrass fresh weights differed significantly, were analyzed for residual diuron. The concentrations in the three replicate soils of each treatment were highly invariant, with the difference <4.3%. The average concentrations were calculated and presented in Table 1. Charfree soil (S0-0) did not contain a measurable level of diuron. All other soils that had received diuron

contained residual diuron with levels of about 40–49% of their respective application rates. The soils S0-1.5 and S0.05-1.5 containing no or low char (0% and 0.05%, respectively) had residual diuron concentrations of ~0.7 mg/kg and produced much lower barnyardgrass fresh weights than the soil S0-0, indicating the availability of diuron to barnyardgrass in these soils. Although the soils S0.5-3.0 and S1-3.0 containing 0.5% and 1% char, respectively, had residual diuron concentrations almost twice those in the soils S0-1.5 and S0.05-1.5, the barnyardgrass fresh weights associated with the former two soils were much higher than those with the latter two. In fact, the barnyardgrass fresh weights with the soils

Table 1
Relationship between measured residual concentrations of diuron and barnyardgrass fresh weights in selected soil and char-amended soil samples subjected to various treatments four weeks after planting as influenced by percent char content and application rate of diuron

Soil	Char content (%)	Rate of diuron fresh weight (g)	Barnyardgrass fresh weight (g)	Residual concentration (mg/kg)
S0-0	0	0	0.0242	0.00
S0-1.5	0	1.5	0.0007	0.73
S0.05-1.5	0.05	1.5	0.0030	0.69
S0.5-3.0	0.5	3.0	0.0224	1.28
S1-3.0	1	3.0	0.0308	1.20
S1-6.0	1	6.0	0.0378	2.42

S0.5-3.0 and S1-3.0 were similar to that with char-free soil without receiving diuron application (i.e. the soil S0-0). The barnyardgrass fresh weight with the soil S1-6.0 containing an even higher level of residual diuron remained high. These results indicate that diuron in soils with high contents of wheat char was largely unavailable to barnyardgrass.

Our measurements indicate that at typical application rates of diuron, a char content of as low as 0.1% may appreciably reduce the bioavailability of diuron in soil. We reported that burning of wheat straw produced wheat char at ~6% of the straw weight (Yang and Sheng, 2003a). Using the average production of wheat straw of ca. 6000 kg/ha, its burning would generate ~360 kg/ha wheat char. If this wheat char were mixed in soil with a density of 1.4 g/cm³ to the depth of furrow slice (~0.15 m), a single burning would result in a wheat char content of~0.02%. Cropresidue-derived chars are expected to accumulate in soils, as crop residues are repeatedly burned, and the resulting chars are expected to remain to be highly effective sorbents for pesticides (Yang and Sheng, 2003b). As such, field burning of crop residues may effectively reduce the bioavailability of pesticides.

4. Conclusions

Wheat char is a highly effective sorbent for diuron. Field burning of wheat straw incorporates the resulting wheat char into soil and enhances the sorption of diuron by the soil. A direct consequence of this agricultural practice is the reduced bioavailability of diuron in soil. We found that diuron was less biodegradable in soil in the presence of wheat char. Its herbicidal efficacy to barnyardgrass decreased with increasing char content in soil and, at recommended field application rates, could be completely lost when the soil char content was 0.5% or higher. Reduced bioavailability of diuron appeared to result from the enhanced sorption in soil in the presence of wheat char. Although only wheat char and diuron were tested in this study, it is expected that chars arising from field burning of other crop residues and vegetations also effectively sorb other pesticides and reduce their bioavailability. The presence of crop-residue-derived chars in soil may increase the environmental risk of pesticides and reduce their efficacy to pests.

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