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Soil characteristics and water potential effects on plant-available clomazone in rice

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Clomazone has been successfully used for weed control in rice, but crop injury is a potential problem on light-textured soils. Experiments were conducted to determine the effect of soil characteristics and water potential on plant-available clomazone and rice injury. A centrifugal double-tube technique was used to determine plant-available concentration in soil solution (ACSS), total amount available in soil solution (TASS), and K_d values for clomazone on four soils at four water potentials. A rice bioassay was conducted parallel to the plant-available study to correlate biological availability to ACSS, TASS, and K_d. TASS was significantly different in all soils. The order of increasing TASS for the soils studied was Morey < Edna < Nada < Crowley, which correlated well with soil characteristics. The order of increasing TASS after equilibrium was $-90 < -75 < -33 < 0$ kPa. TASS values at 0 kPa were greater than two times the TASS values at -90 kPa. It appears that severe rice injury from clomazone on these soils could occur if TASS > 110 ng g^{-1} and $K_d < 1.1$ ml g^{-1}. We propose that the double-tube technique provides a more accurate estimate of available herbicide because the solution–soil ratios are < 0.33:1 and would be more representative of a plant root–herbicide relationship. This technique or some variation possibly could be further developed such that clomazone rates could be more clearly defined particularly on lighter-textured soils. TASS may be a better predictor of plant-available herbicide than ACSS when evaluating moderately to highly water-soluble herbicides in a nonsaturated soil environment.

Nomenclature: Clomazone.

Key words: Adsorption, chlorophyll content, K_d, water potential.

Clomazone is taken up by plant roots and shoots and moves primarily in the xylem to plant leaves (Duke and Paul 1986). Clomazone indirectly inhibits 1-deoxy-D-xyulose-5-phosphate synthase (Vencill 2002). Ultimately, biosynthesis of chlorophyll and carotenoid pigments is inhibited, causing a bleached appearance in susceptible plant species, producing white, yellow, or light-green plants (Duke and Paul 1986; Scott et al. 1994). Clomazone is used in row crops including soybean [*Glycine max* (L.) Merr.], tobacco (*Nicotiana tabacum* L.), pepper (*Piperaceae* spp.), pumpkin (*Cucurbita pepo* L.), and sugarcane (*Saccharum officinarum* L.) (Vencill 2002). Clomazone has recently been introduced as an herbicide for rice weed control for control of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] (Jordan et al. 1998; Webster et al. 1999) and other grasses (Vencill 2002). However, rice injury by clomazone has been an important issue on light-textured soils (J. M. Chandler, personal communication). This injury could be due to the unique chemical characteristics of clomazone including a relatively high water solubility (1,100 mg L^{-1}), high vapor pressure (19.2 mPa at 25 C) (Vencill 2002), and distinctive symptomology.

Several studies have documented clomazone adsorption to soil. However, a batch equilibrium technique using a relatively large volume of water per unit of soil was used in each case, which would represent more of a flooded field condition and not a representative soil–water environment for most agricultural situations. These studies used sorbent to solution ratios 1:10 (Loux et al. 1989), 1:5 (Cumming et al. 2002; Mervosh et al. 1995), and 1:2 (Kirksey et al. 1996). As a relative adsorption technique, these methods are acceptable; however, they do not accurately estimate the amount of herbicide available for plant uptake.

The concentration of herbicide in soil water is primarily dependent on dissolution into the liquid phase, adsorption on the soil components, leaching, and degradation (Gaillardon et al. 1991). Determination of the herbicide concentration in soil solution is important for improving our understanding of herbicide availability to weeds, crops, and soil microorganisms and herbicide movement in soil. This has practical consequences for efficacy, selectivity, persistence, and distribution of soil-applied compounds (Gaillardon et al. 1991).

Several techniques have been developed for the extraction of soil solution for dissolved herbicide determinations (Gaillardon et al. 1991). Centrifugation (Moyer et al. 1972), suction (Green and Obien 1969), pressure (Goetz et al. 1986; Hance and Embling 1979; Walker 1973), and displacement (Wolt et al. 1989) have been used as techniques for more accurately determining available herbicide in soil solution. Unfortunately, most of these techniques require a relatively large amount of soil, high soil moisture, and large time periods for completion (Gaillardon et al. 1991).

Another technique has been effectively used to estimate plant-available water by equating water potential to centrifugal gravity (Kobayashi et al. 1994, 1996, 1999; Wolt 1994; Lee et al. 1996, 1998). This technique uses a double–cen-

trifuge tube apparatus where the soil is placed in an inner tube with a perforated end, which is then placed in an outer centrifuge tube. When the tube is placed in a centrifuge and rotated at 13,000 × *g,* plant-available soil water from the soil sample is dispensed in the outer tube (Kobayashi et al. 1994). Centrifuging at this force equates to a soil water potential of − 1,500 kPa. This soil water potential represents the permanent wilting point for plant material (Brady and Weil 1996; Kobayashi et al. 1994). Therefore, any soil water above − 1,500-kPa water potential is assumed to be available for plant uptake.

Soil moisture variations can affect herbicide availability (Dao and Lavy 1978; Green and Obien 1969; Moyer 1987). In an upland soil (nonflooded), thiobencarb concentrations in soil solution at soil moistures of 35, 45, 55, 65, and 75% were not statistically different (Lee et al. 1996). However, in lowland soils (flooded), concentration of thenylchlor, clomeprop, and mefenacet in soil water was the most important parameter for determining phytotoxic activity (Kobayashi et al. 1994, 1996, 1999).

Several researchers have examined the relationship between rice injury caused by clomazone and soil properties and soil moisture levels. Cumming et al. (2002), using field dissipation studies with clomazone on several soils, projected that estimation of phytotoxicity should not be based purely on soil concentrations. Lee et al. (1998) suggested that total available amount of herbicide in soil solution could vary as a result of varying water volumes, potentially enhancing availability and phytotoxicity as soil moisture increases. Therefore, the objective of this study was to determine the effect of water potential on plant-available concentration in soil solution (ACSS), total amount available in soil solution (TASS), and K_d values for clomazone in four soils.

Materials and Methods

Soil Collection and Preparation

Surface soil from a 8-cm depth was collected in September 2002 from rice fields located near Beaumont, Eagle Lake, Ganado, and Provident City, TX. Approximately, 6 kg of soil was collected at each location that had not received herbicide applications for at least 2 yr. The soil was air-dried for 30 d at 25 C and passed through a 2-mm sieve. Soil moisture for the air-dried soil was determined by oven drying subsamples at 105 C for 48 h. Soil moistures ranged from 0.5 to 3.7% depending on the soil. Soils were characterized by the Texas Agricultural Experiment Station Soil Characterization Laboratory, and results are presented in Table 1.

A water retention curve was constructed for each soil to accurately determine the various moisture levels needed for each moisture treatment (Romano et al. 2002). Water potentials used for constructing the water retention curves were − 10, − 33, − 100, − 250, − 500, and − 1,500 kPa (Figure 1). Mass water content was calculated for each soil and each pressure from the following equation:

$$\text{mass water content } (\theta_m) = \left[\frac{(\text{weight of wet soil} - \text{weight of dry soil})}{(\text{weight of dry soil})}\right] \quad [1]$$

Mass water content was determined for each soil in this

TABLE 1. Soil characterization of Edna (fine, smectitic, hyperthermic Aquertic Chromic Hapludalfs), Morey (fine-silty, siliceous, superactive, hyperthermic Oxyaquic Argiudolls), Nada (fine-loamy, siliceous, active, hyperthermic Albaquic Hapludalfs), and Crowley (fine, smectitic, hyperthermic Typic Albaqualfs) rice soils.[a]

Soil series name	Location	Sand content[b]						Silt content[c]		Clay content[d]		Textural classification	Organic carbon content	pH (1:1)[e]
		VC	C	M	F	VF	Total	F	Total	F	Total			
		%											%	
Edna	Ganado	0.5	0.4	2.5	38.0	25.1	66.5	9.0	18.9	10.2	14.6	Fine sandy loam	0.84	6.1
Morey	Beaumont	0.2	0.2	0.2	2.4	16.4	19.4	28.4	45.3	20.8	35.5	Silty clay loam	1.32	7.3
Nada	Eagle Lake	0.5	3.4	11.4	28.2	17.9	61.4	14.8	31.2	4.1	7.4	Fine sandy loam	0.75	6.1
Crowley	Provident City	0.4	2.5	10.6	35.8	17.0	66.3	13.1	25.0	4.7	8.7	Fine sandy loam	0.50	5.3

[a] Soil Characterization Laboratory, Texas Agricultural Experiment Station, TAMU, College Station, TX.
[b] VC, very coarse sand (2.0 to 1.0 mm); C, coarse sand (1.0 to 0.5 mm); M, medium sand (0.5 to 0.25 mm); F, fine sand (0.25 to 0.1 mm); VF, very fine sand (0.1 to 0.05 mm); Total sand (2.0 to 0.05 mm).
[c] F, fine silt (0.02 to 0.002 mm); total silt (0.05 to 0.002 mm).
[d] F, fine clay (< 0.0002 mm); total clay (< 0.002 mm).
[e] Soil:H_2O.

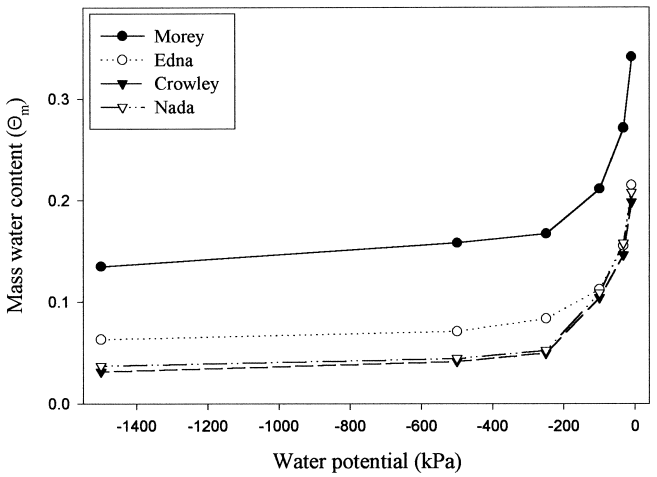

FIGURE 1. Relationship between mass water content (θ_m) and water potential (kPa) of four soils. The soil moisture on a weight basis of each air-dried soil was Morey, 3.5%; Edna, 1.2%; Nada, 0.7%; and Crowley, 0.5%.

manner and plotted vs. pressure (Figure 1). The four water potentials used in the plant-available clomazone study from soil included − 90, − 75, − 33, and 0 kPa as shown as in Figure 1. The bioassay included only − 75, − 33, and 0 kPa because of poor rice growth at − 90 kPa. These water potentials were chosen based on plant-available water estimates (Brady and Weil 1996) that would represent (1) a relatively wet soil environment that approaches a flooded condition (0 kPa), (2) field capacity and optimal conditions for plant growth (− 33 kPa), (3) a relatively moderately dry soil environment capable of sustaining seed germination and plant growth (− 75 kPa), and (4) a more severe dry soil environment (− 90 kPa).

Determination of Plant-Available Clomazone From Soil

Soil Treatment of Clomazone

Technical-grade clomazone (98% pure) was obtained from Chemservice.[1] Ring-labeled ^{14}C-clomazone (98% pure, 2.76 kBq μg^{-1} specific activity) was obtained from the FMC Corporation.[2] Before clomazone addition, all air-dried soils were subjected to the addition of water at a specified water potential treatment shown in Figure 1. After 2 d of incubation at this water potential, clomazone was added to each treatment. One hundred grams of air-dried soil was treated with 3.51 kBq of ring-labeled clomazone, which accounted for approximately 1% of the total clomazone concentration. Technical-grade clomazone was added to each treatment such that the final concentration of clomazone in the final soil sample was 1.2 $\mu g\ g^{-1}$ of soil. This concentration represents a two-times rate of clomazone, assuming a 7.5-cm furrow slice. Clomazone was added to each soil in 99.8:0.2% water–methanol solution. Methanol was used in this mixture to aid in solubility. The soil was mixed with a laboratory spatula after clomazone addition to adequately distribute the herbicide in the sample. The incubation period began after two more days to allow clomazone to equilibrate with soil.

TASS, ACSS, and K_d were determined after the 48-h clomazone equilibration period. The equilibration temperature was 10 C to minimize degradation and weed seed germination in the soil. After equilibration, 20 g of treated soil was removed from each treatment and placed in a double-tube centrifugation apparatus similar to that described by Kobayashi et al. (1994) (Figure 2a). This apparatus consisted of a specially machined 20-mm–internal diameter (id), 75-mm stainless steel inner tube with a perforated end (Figures 2c and 2d). A 25-mm glass microfiber filter[3] (Figure 2f) was placed at the bottom of each tube before the soil being placed inside such that the soil solution would be free of particulates after centrifugation. At the opposite end of the tube, the outer diameter (od) of the tube was 28 mm such that the tube could be placed inside a 26-mm-id, 33-mm-od metal washer (Figure 2e) so as to suspend the stainless steel tube on top of a 28.6-mm-id by 114-mm Nalgene centrifuge tube[4] (Figure 2b) when the samples were centrifuged. The soil weight was adjusted to air-dry weight for each treatment based the soil type and the water retention results. Samples were centrifuged[5] at 13,000 × *g* for 30 min at a temperature of 20 C. This force was used to represent plant-available water (Kobayashi et al. 1994).

After centrifugation, extracted water at the bottom of the outer centrifuge tube was pipetted into a separate vessel and weighed to determine the volume of water extracted. Depending on the water potential, a minimum of 900 μl was removed from the extract and placed in a 7-ml scintillation vial[6] containing 5 ml of scintillation cocktail.[7] Radioactivity was quantified in each of the samples by liquid scintillation spectroscopy.[8] A concentration of radioactivity (dpm ml^{-1}) was calculated for each treatment. This information was used to calculate the TASS (ng g^{-1} soil) from the following equation:

$$\text{TASS} = \frac{\left\{(\text{RC})(\text{VSSE})\left[\frac{(\text{PNR})}{(\text{PR})}\right]\right\}}{[(\text{SA})(\text{MCS})]} \quad [2]$$

where RC is concentration of radioactivity (dpm ml^{-1}), VSSE is the volume of soil solution extracted from the sample (ml), PNR is percentage of nonradiolabeled clomazone added to the treatment (%), PR is the percentage of radiolabeled clomazone added to the treatment (%), SA is the specific activity of clomazone (dpm of radiolabeled clomazone ng^{-1}), and MCS is the mass of soil centrifuged (g).

The available concentration of clomazone (μM) in soil solution (ACSS) was calculated by the following equation:

$$\text{ACSS} = \frac{\left\{(\text{RC})\left[\frac{(\text{PNR})}{(\text{PR})}\right]\right\}}{[(\text{SA})(\text{MW})]} \quad [3]$$

where RC is concentration of radioactivity (dpm ml^{-1}), PNR is percentage of nonradiolabeled clomazone added to the treatment (%), PR is the percentage of radiolabeled clomazone added to the treatment (%), and SA is the specific activity of clomazone (dpm of radiolabeled clomazone μg^{-1}), and MW is the molecular weight of clomazone (239.7 $\mu g\ \mu M^{-1}$).

The partitioning coefficient (K_d) was then calculated for each treatment from the following equation:

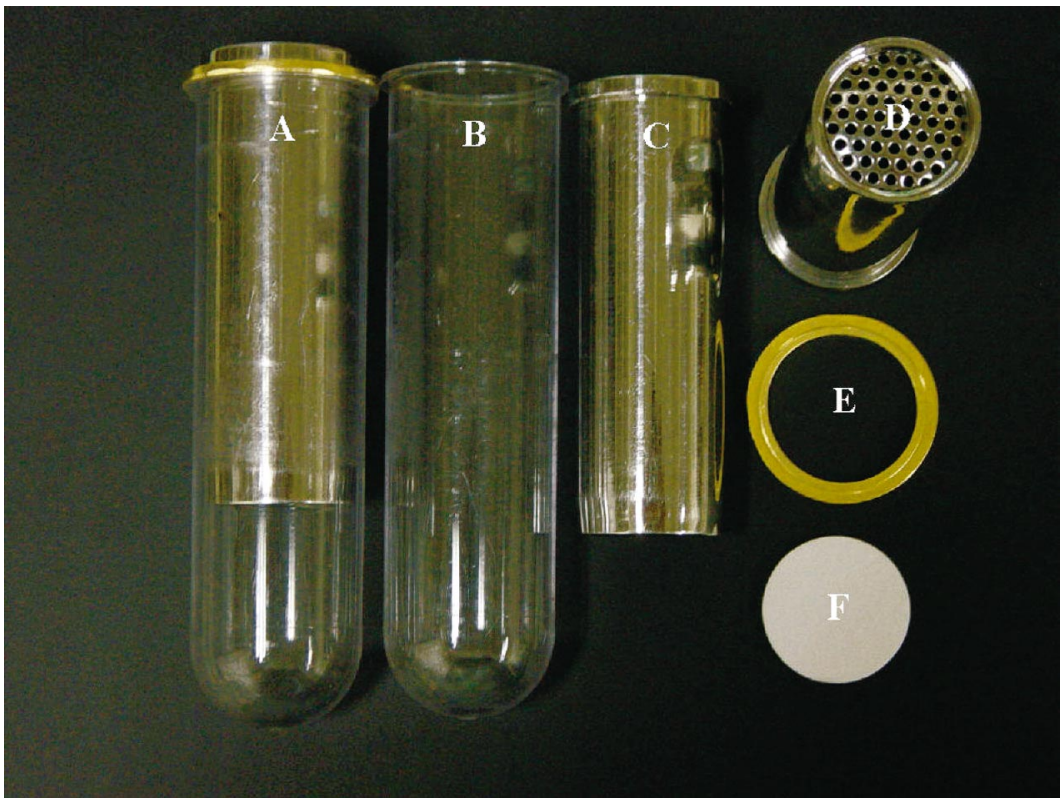

FIGURE 2. Centrifugation double-tube apparatus. (A) Assembled double-tube apparatus; (B) Outer Nalgene centrifuge tube; (C) side view of stainless steel inner tube; (D) end view of stainless steel inner tube showing perforated end where soil solution is dispensed; (E) metal washer that secures stainless steel inner tube when placed inside Nalgene outer centrifuge tube; (F) 25-mm glass microfiber filter that is placed at the bottom of the stainless steel inner tube to prevent soil particulate matter from getting into soil solution. Assembly of the apparatus is as follows: (1) the glass microfiber filter is placed at the bottom of the stainless steel inner tube before soil sample addition; (2) then the washer is placed over the stainless steel inner tube from the bottom and pushed to the top of the tube until it reaches the stop; and (3) the entire apparatus is placed inside the Nalgene centrifuge tube. The assembled units are then subject to 13,000 × *g* by centrifuge, which extracts available water for quantitation of herbicide and availability determinations.

$$K_d = \frac{\left[\frac{(RA_i - RA_{ac})(SA)}{MCS}\right]}{[(ACSS)(SA)]} \quad [4]$$

where K_d is the partitioning coefficient (ml g^{-1}), RA_i is amount of initial radioactivity (dpm), RA_{ac} is amount of radioactivity in soil solution after centrifugation (dpm), SA is specific activity (μg dpm^{-1}), MCS is the mass of soil that was centrifuged (g), and ACSS is the available concentration of clomazone in soil solution (dpm ml^{-1}).

Rice Plant Bioassay

Bioassay Conditions

Soil was treated with technical-grade clomazone as previously described in the plant-available clomazone experiment with the exception that no ^{14}C-clomazone was added to the soil samples. One hundred grams of air-dried soil was added to a 500-ml glass jar. Fungicide (mancozeb)-pretreated commercial rice seed of the 'Cocodrie' variety were pregerminated by soaking in water for 2 d at 30 C. The seed were then placed in a petri dish with the bottom covered with wet paper towels for 24 h at 30 C. Ten pregerminated rice seed were then placed approximately 2 mm below the soil surface inside the glass jars. Jars were covered with two layers of plastic wrap and placed in a growth chamber[9] set at 26 and 20 C day and night temperatures, respectively, with 12 h of light and 12 h of dark. Soil moisture was maintained gravimetrically. After 12 d of growth chamber incubation, 100 mg of leaf fresh weight from each treatment was removed and assayed for chlorophyll content. Untreated controls were also included to determine relative chlorophyll content when rice was grown without clomazone.

Determination of Chlorophyll Content

Chlorophyll content was determined for each set of treatments in the bioassay using the method similar to that described by Hiscox and Israelstam (1979). Leaf tissue was placed in a vial containing 7 ml of dimethyl sulfoxide[10] (DMSO) and extracted at 65 C for 1 h using a constant-temperature bath.[11] The samples were vortexed three times at 15-min intervals during the 1-h extraction. The liquid was decanted and brought to a 10-ml volume with DMSO in a graduated test tube. Each sample was vortexed again before reading on the spectrophotometer. An aliquot of each sample was analyzed using a Beckman DU530 UV-visible spectrophotometer.[12] Absorbance values were read simultaneously to quantify chlorophyll *a* (663 nm) and chlorophyll *b* (645 nm) against a DMSO blank. If absorbance values were greater than 0.7, then the samples were diluted by 50% with a 90% DMSO–10% water solution. Total chlorophyll content (chlorophyll *a* + chlorophyll *b*) (in μg ml^{-1}) was calculated using the following equation from Arnon (1949).

$$\text{Total chlorophyll}_{(a+b)} = 8.02A_{663} + 20.20A_{645} \quad [5]$$

TABLE 2. Total clomazone amount available in soil solution after 48-h equilibration period from four soils and four water potentials as determined by double-tube centrifugation.[a] Main effects are compared because soil by moisture interactions were not significant.

Water potential[b]	Crowley	Nada	Edna	Morey	Average
kPa	ng g^{-1} treated soil[c]				
− 90	107.9	98.6	59.6	35.9	75.5
− 75	132.4	115.6	80.0	43.5	92.9
− 33	160.5	141.2	85.9	75.9	115.9
0	181.3	173.9	130.1	115.4	150.2
Average	145.5	132.3	88.9	67.7	11.0[d]

[a] Centrifugation force was 13,000 × *g* and represented plant-available water as determined by Kobayashi et al. (1994).
[b] Water potential was determined by water retention analysis in Figure 1.
[c] Soil was treated with 1.2 μg g^{-1} clomazone to air-dried soil.
[d] LSD—Fisher's least significant difference at $\alpha = 0.01$ for main effects are $LSD_{soil\ (0.01)} = 11.0$, $LSD_{water\ potential\ (0.01)} = 11.0$.

where A_{663} is the absorbance at 663 nm for chlorophyll *a*, and A_{645} is the absorbance at 645 nm for chlorophyll *b* (Arnon 1949). These values were then converted to milligram of chlorophyll per gram of fresh weight.

Data Analysis

Plant-available clomazone and the bioassay were analyzed as randomized complete block designs with three replications. The experiments were repeated. The plant-available clomazone study was arranged in a 4 by 4 factorial arrangement with four different soils and four water potential levels. The bioassay experiment was also arranged in a factorial experiment with the same four soils and three water potential levels as a result of poor plant survival at the lowest water potential (− 90 kPa). Tests for heterogeneity between runs were not significant, therefore, runs were combined. Means were separated by Fisher's Protected LSD test at $\alpha = 0.01$ using SAS.[13] Comparisons were not orthogonal but chosen based on the objectives of the study.

Results and Discussion

Plant-Available Clomazone From Soil

The TASS of clomazone showed no significant interaction between water potential and soils after the 48-h equilibration. The two-way means for TASS are reported in Table 2. TASS was significantly greater for Crowley compared with the other soils. TASS in the Crowley soil was 11, 64, and 115% greater than Nada, Edna, and Morey soils, respectively. TASS was negatively correlated with percentage of organic carbon content ($r = 0.92$). Organic carbon content was a better predictor of TASS than both percentage of clay ($r = 0.87$) and percentage of sand ($r = 0.72$). These data indicate that the Crowley soil has the greatest opportunity to injure rice in a field situation at equivalent clomazone rates across all soils. Because TASS has been positively correlated with herbicide injury (Lee et al. 1998), the order of decreasing potential rice injury from clomazone would be Crowley > Nada > Edna > Morey.

Averaged across all soils, TASS was positively correlated with water potential ($r = 0.95$). The order of increasing TASS was − 90 < − 75 < − 33 < 0 kPa (Table 2). TASS values at 0 kPa were 33, 62, and 100% of the TASS at − 33, − 75, and − 90 kPa, respectively. Consequently, the higher moistures demonstrated the greatest opportunity for rice injury (Table 2).

TABLE 3. Available clomazone concentration in soil solution and K_d values for soils collected from Edna, Morey, Nada, and Crowley after 48-h equilibration period at four water potential levels.

Water potential[a]	Soil	Available concentration in soil solution	K_d[b]
kPa		μM	ml g^{-1}
− 90	Edna	5.0	0.96
	Morey	3.0	1.68
	Nada	7.1	0.65
	Crowley	7.5	0.62
− 75	Edna	4.4	1.10
	Morey	2.7	1.81
	Nada	5.4	0.88
	Crowley	6.0	0.79
− 33	Edna	3.6	1.35
	Morey	3.1	1.59
	Nada	5.2	0.91
	Crowley	5.8	0.82
0	Edna	3.5	1.36
	Morey	4.0	1.22
	Nada	4.8	0.98
	Crowley	5.4	0.88
$LSD_{0.01}$		0.9	0.26

[a] Refer to Figure 1 for water potential equations.
[b] Partition coefficient assuming unsaturated soil conditions.

Available clomazone concentration in soil solution (ACSS) and K_d values calculated after equilibration demonstrated an interaction between water potential and soil (Table 3). ACSS ranged from 2.7 to 7.5 μM of clomazone from the various soils and water potentials (Table 3). At the − 90-kPa water potential, the order of decreasing ACSS was Crowley = Nada > Edna = Morey. A similar trend was apparent at the other water potentials of − 75, − 33, and 0 kPa. K_d results showed the same trend as ACSS for the soils within each water potential. K_d values ranged from 0.6 to 1.8 ml g^{-1} (Table 3). The largest value came from the Morey soil (1.8 ml g^{-1}) at the − 75-kPa water potential (Table 3). These values are substantially lower than K_d values estimated by Weber et al. (2000) for clomazone that had been calculated from average K_{oc} values reported in the literature. Values obtained in their work ranged from 1.62 to 4.05, assuming 0.54 and 1.35% organic carbon, respectively. It is important to note that these determinations were made using a standard batch equilibrium technique and did not account for soil moisture changes.

For the Edna soil, the decreasing order of ACSS was 0 = − 33 < − 75 = − 90 kPa. Therefore, as soil moisture decreased, ACSS increased. The same trend occurred for Nada and Crowley soils. Herbicide concentration has been inversely correlated with moisture content for atrazine (Green and Obien 1969). Others have reported ACSS to remain constant across varying moisture content (Lee et al. 1996, 1998). However, ACSS for Morey decreased as water potential increased. The decreasing order was − 90 = − 75 < − 33 = 0 kPa. It is not clear as to the reason why Morey

TABLE 4. Total chlorophyll content of three- to four-leaf rice as affected by water potential 14 d after clomazone treatment represented by total chlorophyll by weight and chlorophyll percentage of untreated.

Water potential[a]	Soil	Total chlorophyll content[b]		Percentage of untreated[d]
		Untreated rice	Treated rice[c]	
kPa		— mg g^{-1} fresh weight[e] —		
− 75	Edna	1.4 ± 0.07	1.4 ± 0.17	100.0
	Morey	1.8 ± 0.07	1.4 ± 0.12	77.8
	Nada	1.8 ± 0.13	1.2 ± 0.08	66.7
	Crowley	1.8 ± 0.05	0.5 ± 0.08	27.8
− 33	Edna	1.6 ± 0.05	1.1 ± 0.08	68.8
	Morey	1.8 ± 0.07	1.0 ± 0.02	55.6
	Nada	1.6 ± 0.05	1.1 ± 0.11	68.8
	Crowley	1.9 ± 0.16	0.3 ± 0.09	15.8
0	Edna	1.4 ± 0.13	0.8 ± 0.04	57.1
	Morey	1.4 ± 0.18	0.9 ± 0.04	64.3
	Nada	1.7 ± 0.08	0.5 ± 0.04	29.4
	Crowley	1.5 ± 0.07	0.1 ± 0.04	6.7
$LSD_{0.01}$				9.3

[a] Refer to Figure 1 for water potential equations.
[b] Total chlorophyll content (Chlorophyll *a* + Chlorophyll *b*) = $8.02A_{663} + 20.20A_{645}$ by Arnon (1949).
[c] Clomazone treatment consisted of 1.2 ug g^{-1} clomazone in air-dried soil.
[d] Percentage of untreated − (total chlorophyll content of treated rice)/(total chlorophyll content of untreated rice) × 100.
[e] Mean ± standard deviation.

ACSS values showed different trends than the other soils. Green and Obien (1969) demonstrated the influence of organic matter on atrazine availability as organic matter decreased deeper in the soil horizon. In this case, decreasing organic matter caused a decreasing trend for available atrazine as moisture increased (Green and Obien 1969). Ultimately, they concluded that only on low-adsorptive soils would water content variations significantly alter herbicide concentration in soil solution (Green and Obien 1969). K_d values demonstrated essentially the same results that were determined from ACSS.

Total Chlorophyll Content From Bioassay

Results for total chlorophyll content from rice 14 d after clomazone (Table 4) addition agreed with results from plant-available clomazone estimations (Table 2). An interaction was found between water potential and soil. The total chlorophyll content as percentage of an untreated (TCPU) plant ranged from 6.7 to 100% for the treatments studied. The lowest TCPU value coincided with the most chlorophyll damage or bleaching and consequently, the greatest amount of clomazone injury (Table 4).

For any given soil, water potential was positively correlated with plant injury. For Edna, chlorophyll content decreased in the order of − 75 > − 33 > 0 kPa. The same trend occurred for the other soils. This agreed well with earlier data for soil characteristics and plant-available clomazone estimates where higher soil moistures and lower organic carbon and clay content provided more TASS. Based on plant-available clomazone estimates from TASS, Morey would have been expected to show the least clomazone injury; however, Edna had a substantial quantity of broadleaf signalgrass (*Brachiaria platyphylla* L.) seed in the soil samples, which germinated and absorbed substantial clomazone particularly at the − 75-kPa water potential (Figure 3; Table 4). These seedlings competed with rice for available water and ultimately available clomazone, which resulted in less chlorophyll damage than expected in this treatment.

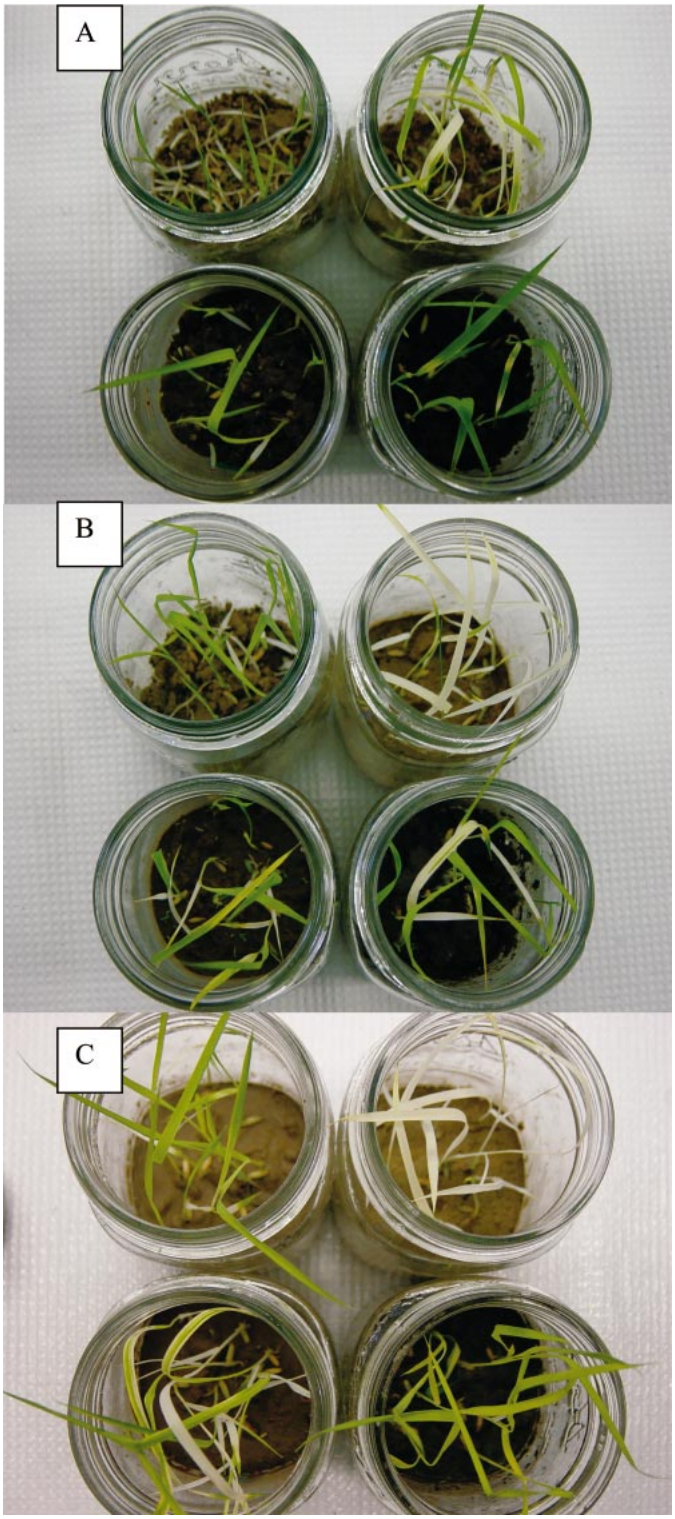

FIGURE 3. Bleaching patterns of rice shoots 14 d after clomazone treatment at (A) − 75 kPa, (B) − 33 kPa, and (C) 0 kPa. In each photograph, the soil samples are ordered as follows. Top left: Edna; right: Crowley; Bottom left: Nada; Bottom right: Morey.

TABLE 5. Correlation matrix for total chlorophyll, water potential, clay, sand, silt, organic carbon, K_d, total available amount of clomazone (TASS), and available clomazone concentration in soil solution (ACSS) across four rice soils.[a]

	% Total chlorophyll	Water potential	Clay	Sand	Silt	Organic carbon	K_d	TASS	ACSS
% Total chlorophyll	1.000	− 0.445***	0.403***	− 0.304**	0.151NS	0.589***	0.466***	− 0.713***	− 0.508***
Water potential	− 0.445***	1.000	0.000NS	0.000NS	0.000NS	0.000NS	− 0.036NS	0.522***	− 0.075NS
Clay	0.403***	0.000NS	1.000	− 0.949***	0.770***	0.939***	0.757***	− 0.632***	− 0.716***
Sand	− 0.304**	0.000NS	− 0.949***	1.000	− 0.932***	− 0.913***	− 0.649***	0.520***	0.590***
Silt	0.151NS	0.000NS	0.770***	− 0.932***	1.000	0.767***	0.444***	− 0.324**	− 0.370***
Organic carbon	0.589***	0.000NS	0.939***	− 0.913***	0.767***	1.000	0.774***	− 0.665***	− 0.769***
K_d	0.469***	− 0.036NS	0.757***	− 0.649***	0.444***	0.774***	1.000***	− 0.758***	− 0.956***
TASS	− 0.713***	0.522***	− 0.632***	0.520***	− 0.324**	− 0.665***	− 0.758***	1.000	0.756***
ACSS	− 0.508***	− 0.075NS	− 0.716***	0.590***	− 0.370***	− 0.769***	− 0.956***	0.756***	1.000

[a] Abbreviation: NS, not significant.
* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

FIGURE 4. Relationship between available clomazone concentration in soil solution (ACSS) and total available amount of clomazone in soil solution (TASS) after 48-h equilibrium. Data were modeled by linear regression for each water potential (− 90, − 75, − 33, and 0 kPa) and four representative rice soils (Morey, ●; Edna, ○; Nada, ▼; and Crowley, ▽).

At 0-kPa water potential, Morey and Edna showed the least chlorophyll damage, whereas Nada and Crowley had >70 and $>93\%$ chlorophyll loss, respectively (Figure 3; Table 4). Perhaps these differences at the 0-kPa water potential were due to some degradation and irreversible binding of clomazone during the 14-d period. Therefore, clomazone dissipation and recovery of rice in the Morey soil probably resulted in higher chlorophyll content at 0 kPa (Figure 3; Table 4). Higher organic carbon ($r = 0.59$) and clay content ($r = 0.40$) were associated with reduced chlorophyll damage (Table 5). Similar trends of chlorophyll damage occurred at the other soil moistures.

Critical TASS and K_d Estimation Based on Total Chlorophyll Content

The relationship between ACSS and TASS for all the soils at each water potential is shown in Figure 4. A strong linear relationship was determined for each water potential, with coefficients of determination ranging from 0.74 to 0.98. As TASS increased, ACSS was less sensitive to changes in water potential, which are indicated by gentler slopes at the higher water potentials. At − 90 kPa, ACSS reached a maximum, and the relationship between ACSS and TASS demonstrated the steepest slope of any of the other water potentials. However, at − 90 kPa the soil environment was too dry to sustain plant life and, therefore, may not be a particularly injurious treatment because of low plant uptake. In addition, the maximum endpoints for ACSS decreased as water potential increased, suggesting dilution of clomazone in soil solution. As water potential decreased, ACSS decreased from approximately 8 to 6 µM. However, at the same endpoints, TASS increased from approximately 125 to 240 ng g^{-1} as water potential increased. This trend of increasing TASS was consistent with increasing chlorophyll damage as water potential increased according to bioassay results (Tables 4). According to correlation statistics, TASS showed a higher correlation with chlorophyll content ($r = -0.71$) than ACSS with chlorophyll content ($r = -0.51$) (Table 5). TASS also had a stronger relationship to water potential ($r = 0.52$)

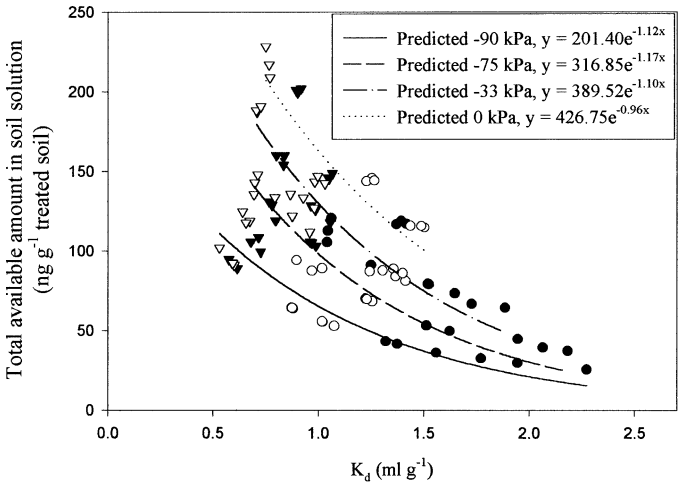

FIGURE 5. Relationship between total available amount of clomazone in soil solution (TASS) after 48-h equilibrium and soil affinity (K_d) of clomazone. Data were modeled using a first-order equation for each water potential (− 90, − 75, − 33, and 0 kPa) and four representative rice soils (Morey, ●; Edna, ○; Nada, ▼; and Crowley ▽).

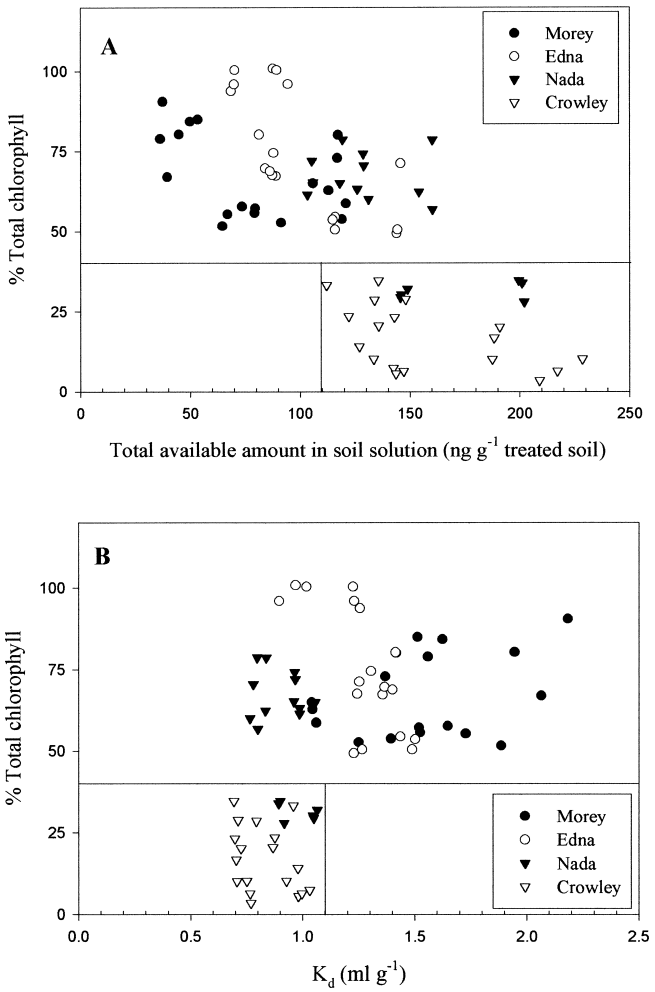

FIGURE 6. Relationship between percentage of total chlorophyll (chlorophyll *a* + *b*) with (A) total available amount of clomazone in soil solution (TASS) after 48-h equilibrium and (B) K_d. Data include three water potentials (− 75, − 33, and 0 kPa) and four representative rice soils (Morey, Edna, Nada, and Crowley). Water potentials have not been designated with a separate symbol.

than did ACSS ($r = -0.08$) (Table 5). These results are in agreement with earlier work by Lee et al. (1998), who stated that TASS was a better estimate of plant-available herbicide than ACSS.

Based on TASS being a better plant-available estimate than ACSS, it was deemed useful to describe the relationship of TASS to clomazone affinity (K_d) to soil (Figure 5). This would allow estimation of TASS for various soil types that would provide potential injury estimates across soil characteristics, particularly when combined with bioassay results (Figure 6; Table 5). TASS and K_d for each water potential were regressed using a first-order nonlinear model (Figure 5). Based on residual plot analysis, a good fit was determined at each water potential. As K_d increased, TASS decreased at all water potentials. A correlation between TASS and K_d was determined ($r = -0.76$).

Because K_d and TASS demonstrated a strong relationship, we plotted these two variables against total chlorophyll content to determine critical ranges that would be expected to cause significant chlorophyll damage (Figure 6). In studying the relationship of TASS (Figure 6a) and K_d (Figure 6b) to total chlorophyll, soil and moisture conditions that provided TASS values of > 110 ng g^{-1} and K_d values of < 1.1 ml g^{-1} were likely to demonstrate $> 60\%$ chlorophyll damage. Rice plants with this amount of chlorophyll damage may not recover if growing conditions are not optimal soon after clomazone uptake. Total chlorophyll reduction was greater than 65% for Crowley soil at all water potentials. Data for these soils had K_d values < 1.1 ml g^{-1} and TASS > 110 ng g^{-1} within the critical range. According to these data, the clomazone rate could be reduced to allow a safer application range because of the high availability of this compound in this soil.

Nada soil at 0 kPa also showed $> 70\%$ chlorophyll damage. Therefore, depending on water potential, the rate of clomazone may need to be reduced to allow safer application on the Nada soil. Some of Edna and Morey soils at high water potentials were within the critical range of TASS and K_d but did not show as much chlorophyll damage as Crowley and Nada soils (Figures 3 and 6). Perhaps, Edna and Morey soils have enough clay, organic carbon content, and microbial activity to reduce the quantity of available clomazone thereby reducing rice phytotoxicity within the 14-d incubation. Organic carbon ($r = 0.59$) and clay content ($r = 0.40$) were significantly correlated with total chlorophyll content (Table 5).

It is important to note that K_d values varied as much as 100% as soil moisture was altered. In other published work, researchers have used high solution–soil ratios of 2:1 (Kirksey et al. 1996), 5:1 (Mervosh et al. 1995), and 10:1 (Loux et al. 1989). Our data show that K_d was inversely correlated with water potential. Therefore, conventional batch equilibrium methods potentially underestimate plant-available herbicide. Because the double-tube technique can simulate a representative plant root–herbicide relationship by lowering solution–soil ratios < 0.33:1, we propose that this method provides a more accurate estimate of plant-available herbicide. Perhaps, this technique or a variation of it could be further developed such that clomazone rates could be more clearly defined particularly on lighter-textured soils. It might be possible to reduce the application rate to reduce TASS to < 110 ng g^{-1} thereby providing less potential injury to rice and yet still providing adequate weed control in these types of soils.

Clomazone ACSS was inversely correlated with water potential. In earlier work by Lee et al. (1996, 1998), ACSS remained relatively constant across soil moistures for thiobencarb, pretilachlor, cafenstrole, benfuresate, and simetryn. These conflicting results among compounds appear to be associated with varying water solubility. The water solubilities of the previously noted compounds are 30, 50, 2.5, 190, and 400 mg L^{-1}, respectively. Clomazone's solubility is 1,100 mg L^{-1} and at least 2.7 times greater than the highest water solubility of the previously mentioned moderately soluble compounds. Therefore, TASS may be a better predictor of plant-available herbicide than ACSS when evaluating highly water-soluble herbicides in a nonsaturated soil environment. Future studies are needed to evaluate more herbicides that encompass a wider range of pesticide properties.

As a method, the double-centrifuge technique is highly effective in quantifying differences in soil and plant-available clomazone. The technique proved to be relatively simple, rapid, and reproducible. Future applications of this technique could include plant-available nutrients as well as other herbicides. Also, adsorption data on agrochemicals collected using this type of technique or a variation would provide more accurate data for interpretation and modeling efforts because differences in adsorption can vary substantially with changes in soil types and moisture contents.

Sources of Materials

[1] Analytical clomazone, Chem Service, Inc., P.O. Box 599, West Chester, PA 19381-0599.

[2] Ring-labeled, radioactive clomazone, FMC Corporation, 1735 Market Street, Philadelphia, PA 19103.

[3] Millipore prefilter AP25, 25-mm, Millipore Corporation, 290 Concord Road, Billerica, MA 01821.

[4] Nalgene polycarbonate centrifugation tubes, Nalge Nunc International Corporation, 75 Panorama Creek Drive, Rochester, NY 14625-2385.

[5] IEC B-20A centrifuge, International Equipment Company, Needham Heights, MA 02194.

[6] Liquid scintillation vials, VWR Scientific Products, 1310 Goshen Parkway, West Chester, PA 19380.

[7] Liquid scintillation cocktail, Ecolite ICN, Costa Mesa, CA 92626.

[8] Beckman LS 6500 multi-purpose scintillation counter, Beckman Coulter, Inc., 4300 North Harbor Boulevard, Fullerton, CA 92634-3100.

[9] Growth chamber, Controlled Environments Limited, 590 Berry Street, Winnipeg, Manitoba, Canada R3H 0R9.

[10] Dimethyl sulfoxide, Fisher Scientific, P.O. Box 1546, 9999 Veterans Memorial Drive, Houston, TX 77251-1546.

[11] Blue M constant temperature water batch, Blue M Electric Company, 304 Hart Street, Watertown, WI 53094.

[12] Beckman-Coulter DU-530 UV-Visible Spectrophotometer, Beckman Coulter, Inc., 4300 North Harbor Boulevard, Fullerton, CA 92634-3100.

[13] SAS software, version 8.02, Statistical Analysis Systems Institute Inc., SAS Campus Drive, Cary, NC 27512.

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