ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2002 – December 31, 2002

PROJECT TITLE: The Environmental Fate of Pesticides Important to Rice Culture

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

Objective I. Determine the level of toxicity of deschlorothiobencarb (DTB) to rice plants in a hydroponic system. Studies to date have correlated production of DTB with Delayed Phytotoxicity Syndrome (DPS); however, no studies have determined what level of DTB causes DPS. This study will determine an EC_{50} (concentration at which 50% of plants exhibit DPS symptoms) and a no-observable-effect-concentration (NOEC) for DTB in rice.

Objective II. Determine statistically significant correlations between soil characteristics and DTB production. While DPS appears to be positively correlated with organic carbon content, other soil characteristics may also influence its development.

Objective III. Compare uptake and metabolism rates of clomazone in rice versus an important, susceptible weed species. Determine uptake and metabolism rates of clomazone by rice at normal and elevated temperatures. Identify and quantify major rice and weed metabolites, looking particularly for production of ketoclomazone. Relate phytotoxicity to metabolic activity.

SUMMARY OF 2002 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

Objective I.

The toxicity study was expanded to a comparative study between thiobencarb (TB) and DTB so that significance could be assigned to the toxicity values obtained for DTB; in essence, how much more toxic it DTB to rice than the parent herbicide TB.

Methods

Rice plants were grown from seeds in a hydroponic culture system. Briefly, one seed was placed in each 25 mL test tube and filled with 15 mL (4" depth) of de-ionized water. The tubes were

	Set	NOEC (µg/L)	IC_{50} (µg/L)
Deschlorothiobencarb	1	15.6	34.1
	2	16.2	70.7
	3	23.4	66.6
	Mean	**18.4**	**57.1**
Thiobencarb	1	158	529
	2	244	511
	Mean	**201**	**520**

Table 1. Comparison of the toxicity of thiobencarb and deschlorothiobencarb.

maintained in a controlled environment chamber throughout the subsequent experiment. The growth chamber had a 14-h photoperiod and temperature ranged from 30°C to 16°C. After one week the growing solution was changed to Hoagland's nutrient solution. Exposure to TB or DTB began at the growth period when either TB is typically applied, or DTB is expected to appear in the field (6 days after TB application, and when the plants were 16 days old and at the 4 leaf stage). Dosing solutions of TB were prepared within a range of 0.05 to 10.0 mg/L (ppm), while those for DTB were in a range of 0.01 to 3.0 mg/L (ppm) in Hoagland's nutrient solution; controls contained only plants and nutrient media. Chemical exposure was for 8 days, and dosing solutions were renewed every two days to maintain constant concentrations.

Exposure concentrations were confirmed via hexane extraction followed by GC-MS, the methods having been previously developed in our laboratory (Schmelzer, *et al.*, in preparation). Briefly, extractions were performed on 2.5 mL samples of each of the solutions just before dosing the plants and on the after they had just served as the nutrient solution for 2 days. To each solution, 1 mL of methanol (pesticide grade) and 20 ng molinate (as a surrogate standard) were added. The solutions were vortexed for 1 min, 4 mL of hexane was then added, and the solution was again vortexed for 1 min. The supernatant was then collected. Solutions were re-extracted twice more with 4 mL of hexane and the extracts were combined, dried over anhyd. magnesium sulfate, and concentrated to 0.5 mL under nitrogen gas.

Analyses were performed on each extract, in duplicate, via GC-MS. The GC was equipped with a ZB-50 capillary column (30 m x 0.25 mm id, 25 μ film thickness). Helium (0.6 mL/min) was the carrier gas, the injector temperature was 250°C, and the detector temperature was 280°C. The GC oven was initially set at 100°C for 1 min, then ramped at 20°C/min to 270°C and held for 1 min. The MS was operated in the selective ion monitoring mode.

Results

Preliminary studies showed that dwarfing was the major symptom observed in hydroponic exposures to either TB or DTB. No appreciable blue-green color, tillering, or curling of leaves was observed. Therefore, shoot growth during the exposure period was selected for use in calculating the NOEC and IC_{50} values; IC_{50} describes a 50% inhibition of growth, which was more useful than a typical EC_{50} (Table 1).

FIELD	SERIES[1]	TEXTURAL CLASSIFICATION[2]
Dennis	Willows	Clay
Lamalfa	West Stockton	Clay Loam
Lauppe	Galt	Loam
Maben	Willows	Loam
Maltby	Harrington	Silty Clay
Mathews	San Joaquin	Loam
Swanner	Plaza	Silty Clay Loam
Vogt	Exeter	Sandy Loam

Table 2. The soils used in the characteristics comparison.

Shoot heights of the plants were recorded at the beginning and end of the 8-day exposure period and the difference in growth of the shoots during the exposure period were used to assess toxicity. IC_{25} and IC_{50} values were calculated using linear interpolation with ToxCalc (Tidepool Scientific Software) toxicology software. The IC_{25} was used to estimate the NOEC (USEPA). Results to date clearly show that *DTB is 10 times more toxic to rice than the parent TB* (Table 1). One more dataset for TB is in progress and will be completed before December 31, 2002.

Objective II.

To date little has been done to try to correlate soil characteristics with DPS. Moon and Kuwatsuka (1984) made a few soil measurements in conjunction with a study of TB dechlorination in a variety of Japanese rice soils. However, they measured only 12 characteristics without replication, which precluded any kind of statistical analyses. Of 17 soils tested, only 2 were found to produce DTB, and only one of those was tested for chemical characteristics. This study was designed to determine physical-chemical characteristics that may be conducive to development of DPS in California rice field soils. The study design of 5 replicate samples from each of 8 rice fields allowed statistical comparisons to be made.

Materials and Methods

Five replicates each of 8 soils were collected in the Spring of 2002 from rice fields in Northern California. Three of the soils (Lauppe, Mathews, Vogt) were from areas that have experienced incidents of DPS, while 5 others (Dennis, Lamalfa, Maltby, Maben, Swanner) were from areas that have never experienced DPS. Table 2. provides soil series and textural classifications for all the soils used in the comparison.

Soils were analyzed for 24 chemical/physical parameters by the Division of Agriculture and Natural Resources (DANR) Laboratory at UCD. They were sieved to 2 mm and dried for at least 48 h prior to analysis. The Carlo Erba Combustion Method (Dumas, 1981) was used to determine total nitrogen and carbon (%). Briefly, samples were flash combusted and gaseous products (N_2, NO_x, CO_2 and H_2O) were analyzed by gas chromatography with a thermal conductivity detection system (GC/TCD). Method detection limit is 0.01% for carbon and 0.04% for nitrogen and is reproducible within 5%.

Total Kjeldahl Nitrogen (TKN) was determined by wet oxidation of soil organic matter using a micro-Kjeldahl procedure with sulfuric acid and digestion catalyst. Ammonium was determined by the diffusion-conductivity technique (Bremner and Mulvaney, 1982; Isaac and Johnson, 1976).

Nitrate (NO_3-N) was extracted from soil using an equilibrium extraction with 2.0 N KCl solution. Nitrate was determined by reduction to NH_4-N via a granular zinc reactor and was calculated by difference. Ammonium (NH_4-N) and nitrate were measured by the diffusion-conductivity method (Carlson *et al*, 1990).

Extractable phosphorus (Olsen-P) was determined by alkaline extraction with 0.5 N $NaHCO_3$ and subsequent analysis of reduced phosphomolybdate complex by flow infection analysis (Olsen and Sommers, 1982; Prokopy, 1995).

Exchangeable cations were determined by equilibrium extraction of soil for exchangeable potassium, sodium, calcium and magnesium using 1 N ammonium acetate (pH 7.0) and subsequent determination by atomic absorption/emission spectrometry (Thomas, 1982). Zn, Mn, Cu and Fe were determined by equilibrium extraction of soil using diethylenetriaminepentaacetic acid (DTPA) and subsequent determination by atomic absorption spectrometry (Lindsay and Norvell, 1978). Soil pH was determined by the saturated paste method using a pH meter. The method is generally reproducible within 0.2 pH units (U.S. Salinity Laboratory Staff, 1954).

Estimated soluble salts were determined by the saturated paste method using a conductivity meter (Rhoades, 1982). Cation exchange capacity (CEC) was determined by the barium saturation and calcium replacement method (Janitzky, 1986).

A modified Walkley-Black method was used to determine soil organic matter (OM) and organic carbon (C-org). Briefly, organic carbon was reduced by potassium dichromate and measured spectrophotometrically (Nelson and Sommers, 1982).

Percent sand, silt and clay were determined in soil supspension by hydrometer. The method has a detection limit of 1% sand, silt and clay (dry soil basis) and is generally reproducible within 8% (Gee and Bauder, 1982).

Soils were grouped according to whether or not DPS had ever been observed in the field. Lauppe, Mathews and Vogt were in the DPS group, while Dennis, Lamalfa, Maben, Maltby, and Swanner were in the non-DPS group. Data were analyzed by ANOVA followed by unpaired t-tests. Supervised and unsupervised Principle Component Analysis (PCA) were used to reveal patterns in the data.

Results

Figure 1 shows significant differences between DPS and non-DPS soils for most of the characteristics measured, while complete results are shown in Table 3. Extremely significant differences ($p \leq 0.001$) were found between DPS and non-DPS-soils for C-tot, OM, C-org, TKN, N-tot, sand, clay, X-Ca, X-K, X-Mg, CEC, EC, X-Na, NO_3-N. Highly significant differences ($p \leq 0.01$) were found for silt. Significant differences ($p \leq 0.05$) were found for Mn, pH and NH_4-N. No significant differences were found for Zn and Olsen-P.

Compared to non-DPS soils, soils from fields that have experienced DPS are characterized by high sand, high Mn, low clay, low OM, low carbon (total and organic), low nitrogen (organic and inorganic) low CEC, low EC, low exchangeable cations, low pH and low Cu.

To date, DPS has been observed in fields on the east side of the Sacramento River, which includes the Lauppe, La Malfa, Mathews and Vogt fields. However, La Malfa has never experienced DPS, similar to the fields on the west side of the SR (Dennis, Maben, Maltby, Swanner).

The measured chemical characteristics, together with the geographical information, present a few possible explanations for development of DPS. First, the non-DPS soils, with higher OM, may simply bind DTB making it unavailable to rice plants. La Malfa, located on the east side of the SR, is similar in texture to the soils from the west side.

Non-DPS soils have higher levels of macro- and most micronutrients, suggesting that there is some other limiting or inhibiting factor. For example, copper is higher in the non-DPS soils, suggesting that copper may be inhibiting anaerobic bacteria. This is further supported by the observation that the soil from the La Malfa, which is geographically closer to all of the DPS has never experienced DPS symptoms, and had the highest copper levels measured in this study.

PCA analysis revealed that DPS soils do group together in multivariate analysis. Further, La Malfa had characteristics that distinguished it from all other soils. The high levels of OM, copper and zinc in La Malfa suggest that further studies could be focussed on the effects of those factors in the formation of DTB.

Objective III.

As a first step in studying metabolism of clomazone in rice and watergrasses, this study was done to determine comparative toxicity of clomazone to these plants. Rice (*Oryza sativa* variety M202), early watergrass (*Echinochloa oryzicola*, resistant and susceptible varieties) and late watergrass (*Echinochloa oryzoides*, resistant and susceptible varieties) were exposed to a range of concentrations of clomazone. Change in wet weight over 7 days was measured and used to determine NOECs and inhibition concentrations having 50% effect (IC_{50}) for clomazone.

Methods

Seedlings (7-10 d old) were pre-weighed and then exposed to clomazone concentrations ranging from 0 to 1.9 mg/L. Plants were exposed hydroponically in 0.5X Hoagland's solution in test tubes (25 X 200 mm with Teflon lined caps). The experimental unit was one tube containing 36 mL test solution and 4 plants; for all concentrations tested n=10. Experiments were conducted in a controlled-environment chamber (day: 30°C, 30% relative humidity, 14 h; night: 16°C; 80% relative humidity; 10 h). After 7 days, plants were harvested and weighed and NOEC and IC50 values were determined using ToxCalc (Tidepool Software).

Results

Figures 2-4 show results of the toxicity tests. In addition to the growth endpoint, these figures indicate observational bleaching data. Extent of bleaching was not quantified, but notes were

Table 3. Results of soil analyses.

	DPS				non DPS					
	Lauppe	Mathews	Vogt	DPS Mean	Dennis	Lamalfa	Maben	Maltby	Swanner	non DPS Mean
N (Total, %)	0.11 ± 0.00	0.12 ± 0.00	0.08 ± 0.00	0.08 ± 0.01	0.21± 0.00	0.17 ± 0.00	0.15 ± 0.00	0.16 ± 0.00	0.20 ± 0.00	0.18 ± 0.00
TKN (%)	0.095 ± 0.003	0.094 ± 0.004	0.065 ± 0.010	0.084 ± 0.005	0.174 ± 0.005	0.168 ± 0.007	0.129 ± 0.004	0.135 ± 0.003	0.174 ± 0.003	0.156 ± 0.005
NO3-N (ppm)	7.0 ± 0.7	4.23 ± 0.2	4.2 ± 0.1	5.1 ± 0.4	12.8 ± 0.2	6.4 ± 0.1	9.6 ± 0.3	9.7 ± 0.2	8.0 ± 0.3	9.3 ± 0.4
NH4-N (ppm)	12.7 ± 0.8	8.1 ± 0.4	6.2 ± 0.5	9.0 ± 0.8	14.8 ± 0.4	17.1 ± 0.8	7.4 ± 0.4	11.9 ± 0.4	8.6 ± 0.2	12.0 ± 0.8
C (Total, %)	1.32 ± 0.04	1.44 ± 0.03	0.80 ± 0.02	1.19 ± 0.08	2.51 ± 0.04	2.04 ± 0.04	1.76 ± 0.06	1.89 ± 0.03	2.19 ± 0.05	2.08 ± 0.06
C-Org (%)	1.05 ± 0.01	1.25 ± 0.03	0.62 ± 0.01	0.97 ± 0.07	1.83 ± 0.03	1.84 ± 0.04	1.56 ± 0.02	1.48 ± 0.03	1.79 ± 0.02	1.70 ± 0.03
OM (%)	1.81 ± 0.02	2.16 ± 0.05	1.07 ± 0.01	1.68 ± 0.12	3.16 ± 0.05	3.16 ± 0.07	2.68 ± 0.03	2.56 ± 0.04	3.09 ± 0.03	2.93 ± 0.06
Olsen-P (ppm)	12.0 ± 0.5	13.1 ± 0.5	11.3 ± 0.4	12.1 ± 0.3	11.2 ± 0.2	29.4 ± 0.5	3.3 ± 0.2	9.6 ± 0.2	21.0 ± 0.4	14.9 ± 1.9
X-Ca (meq/100g)	7.9 ± 0.0	5.5 ± 0.1	2.9 ± 0.0	5.4 ± 0.6	19.2 ± 0.1	11.4 ± 0.0	8.3 ± 0.0	12.1 ± 0.1	12.7 ± 0.0	12.9 ± 0.8
X-K (ppm)	97 ± 0	79 ± 1	66 ± 3	81 ± 4	358 ± 2	148 ± 4	127 ± 3	208 ± 6	186 ± 2	205 ± 17
X-K (meq/100g)	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.9 ± 0.0	0.4 ±0.0	0.3 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
X-Mg (meq/100g)	6.32 ± 0.06	1.44 ± 0.05	1.56 ± 0.04	3.11 ± 0.61	17.6 ± 0.5	6.82 ± 0.02	6.86 ± 0.04	10.0 ± 0.1	10.9 ± 0.0	10.4 ± 0.8
X-Na (meq/100g)	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	2.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	1.1 ± 0.0	0.3 ± 0.0	0.9 ± 0.2
X-Na (ppm)	56 ± 4	26 ± 2	98 ± 4	60 ± 8	510 ± 7	68 ± 4	79 ± 6	259 ± 2	62 ± 1	196 ± 35
Cu (DTPA, ppm)	8.4 ± 0.3	10.9 ± 0.2	3.0 ± 0.1	7.5 ± 0.9	12.6 ± 0.6	30.1 ± 3.8	16.9 ± 2.6	23.6 ± 1.9	15.2 ± 0.4	19.7 ± 1.7
Fe (DTPA, ppm)	223 ± 4	179 ± 4	136 ± 6	179 ± 10	108 ± 4	179 ± 2	172 ± 16	183 ± 2	225.2 ± 5.2	173.6 ± 8.4
Mn (DTPA, ppm)	129 ± 3	182 ± 6	59 ± 4	123 ± 14	101 ± 3	136 ± 2	33 ± 2	98 ± 1	65 ± 3	36 ± 7
Zn (DTPA, ppm)	3.9 ± 0.1	6.1 ± 0.2	5.2 ± 0.1	5.1 ± 0.3	4.7 ± 0.2	35.7 ± 1.4	3.7 ± 0.2	6.8 ± 0.8	3.0 ± 0.3	10.8 ± 2.6
CEC (meq/100g)	15.1 ± 0.1	7.6 ± 0.2	5.5 ± 0.1	9.4 ± 1.1	40.9 ± 0.6	19.3 ± 0.0	16.1 ± 0.1	24.1 ± .01	24.7 ± 0.1	25.0 ± 1.8
EC (mmhos/cm)	0.44 ± 0.02	0.48 ± 0.02	0.63 ± 0.03	0.52 ± 0.03	1.46 ± 0.03	0.63 ± 0.03	0.69 ± 0.02	0.84 ± 0.03	0.46 ± 0.01	0.81 ± 0.07
pH	4.8 ± 0.1	5.2 ± 0.0	5.3 ± 0.2	5.1 ± 0.1	5.9 ± 0.2	4.8 ± 0.2	5.3 ± 0.1	5.4 ± 0.0	5.6 ± 0.0	5.4 ± 0.1
Clay (%)	24 ± 0	19 ± 0	11 ± 0	18 ± 1	55 ± 1	38 ± 1	23 ± 0	40 ± 0	34 ± 0	38 ± 2
Sand (%)	44 ± 1	35 ± 1	60 ± 0	46 ± 3	9 ± 0	23± 1	30 ± 0	16 ± 0	18 ± 0	19 ± 1
Silt (%)	32 ± 1	46 ± 1	29 ± 1	36 ± 2	36 ± 1	39 ± 0	47 ± 1	44 ± 0	48 ± 0	43 ± 1

Plant	NOEC (mg/L)	IC_{50} (mg/L)
Rice, M202	> 1.9 (p = 0.01)	> 1.9
Early Watergrass, Resistant	0.112 (p = 0.01), 0.052 (p = 0.05)	0.21
Early Watergrass, Susceptible	0.112 (p = 0.01)	0.29
Late Watergrass, Resistant	0.112 (p = 0.01)	> 1.0
Late Watergrass, Susceptible	0.47 (p = 0.01), 0.112 (p = 0.05)	0.81

Table 4. NOEC and IC_{50} values for clomazone.

made regarding the number of replicates that had plants with any degree of bleaching. Table 4 shows NOEC and IC_{50} values for the growth endpoint for each test.

Clomazone had no significant effect on growth of rice in the highest concentration tested. However, bleaching did occur at the 0.112 mg/L level. Large variability in rice growth in controls and in the test solutions made clomazone effects difficult to detect.

Early watergrasses were most sensitive to clomazone with the resistant and susceptible varieties having similar sensitivities (IC_{50}s of 0.21 mg/L and 0.29 mg/L, respectively, and NOECs of 0.112 mg/L (p = 0.01) for both). Bleaching was observed in all replicates in the lowest concentration tested, although the degree of bleaching was reduced. Late watergrasses showed intermediate sensitivity, with IC_{50}s of >1.0 mg/L and 0.81 mg/L for the resistant and susceptible varieties, respectively. The NOECs were 0.112 mg/L for the resistant variety, and 0.47 (p = 0.01) or 0.112 (p = 0.05) for the susceptible variety. Both watergrasses were more sensitive to clomazone than rice for the growth endpoint. Further work to measure the effects of clomazone on β-carotene levels will provide better understanding of relative sensitivities.

The recommended application rate of clomazone at 0.6 lb ai/acre would result in a water concentration of about 0.6 mg/L, if all of the clomazone were dissolved in the water. The K_d (ratio of concentration in soil to concentration in water) for clomazone is around 1.0, indicating that about half of the clomazone would be expected to partition into the soil, leaving 0.3 mg/L in the water. This level is high enough to negatively affect growth of early watergrasses, but not late watergrasses or rice. However, it is high enough to cause some degree of bleaching in all of the plants tested.

PUBLICATIONS OR REPORTS:

1. Schmelzer, K. R., C. S. Johnson, M. R. Viant, J. F. Williams, and R. S. Tjeerdema. Influence of organic carbon in the reductive dechlorination of TB in California rice field soils. In preparation for *J. Agric. Food. Chem.*

2. TenBrook, P.L., D. Holstege, J. F. Williams and R. S. Tjeerdema. Delayed phytotoxicity syndrome and soil characteristics. In preparation for *Weed Sci.*

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

1. Investigation into the cause of DPS in rice was continued, as it has been a serious problem in rice fields of the eastern Sacramento Valley of California for many years. DPS is caused by

deschlorothiobencarb, which is produced by anaerobic soil bacteria. Production of deschlorothiobencarb is enhanced by addition of organic carbon (straw).

2. The toxicity (via growth inhibition) of deschlorothiobencarb was compared to that of the parent herbicide thiobencarb in rice plants grown in a hydroponic culture system and deschlorothiobencarb was found to be over 10 times more toxic. This helps to confirm that deschlorothiobencarb is indeed the toxic agent causing DPS.

3. A comparison the characteristics of soils known to be susceptible to DPS versus those resistant to DPS was performed. In general, compared to non-DPS soils, DPS-susceptible soils are characterized by high sand, high magnesium, low clay, low organic matter, low carbon (total and organic), low nitrogen (organic and inorganic) low cation exchange capacity, low exchangeable cations, low pH and low copper.

4. In DPS-susceptible soils, organic carbon may be a limiting factor such that addition of rice straw stimulates anaerobic bacterial action. Conversely, the high levels of exchangeable copper found in DPS-resistant soils may serve to inhibit bacterial action. The actions of copper on inhibiting development of DPS should be investigated further, as it is already applied to rice fields to control tadpole shrimp and algae and might also be used to inhibit DPS.

5. When tested for toxicity (growth inhibition), early watergrasses were most sensitive to clomazone, while late watergrasses were intermediate in sensitivity. Clomazone had no significant effect on growth of rice at even the highest concentration tested. However, substantial bleaching was observed and may be a more sensitive and reliable indicator of toxic injury. Development of a method to measure bleaching in rice plants and determination of the clomazone level responsible for bleaching should be investigated further.

6. An understanding of the mechanism behind the selective toxicity of clomazone for rice versus watergrasses will facilitate the development of safeners to reduce the injury caused to rice upon application in the field. A plant metabolite, ketoclomazone, has been suggested as the toxic agent. It should be confirmed so that safening agents can be developed to target it.

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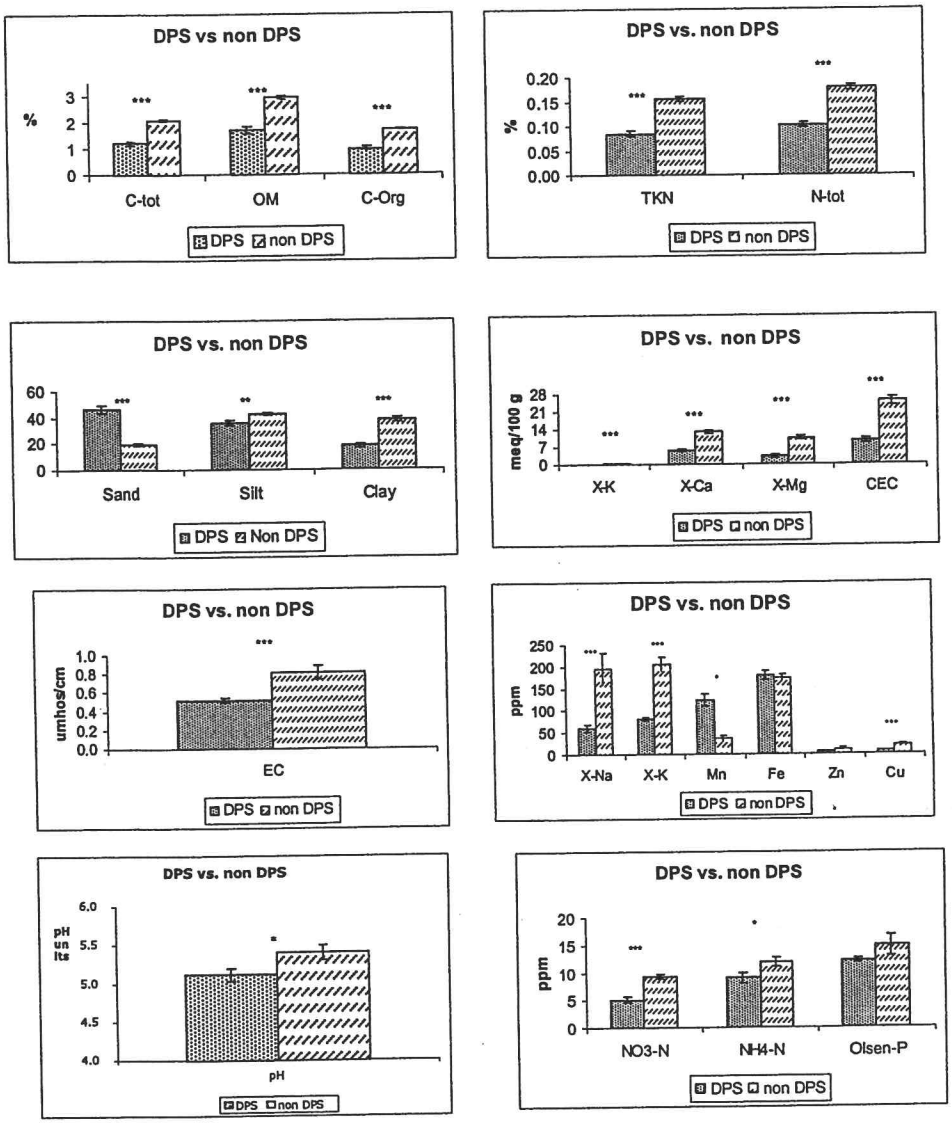

Figure 1. Differences between DPS and non-DPS soils. * = significant; ** = highly significant; *** = extremely significant.

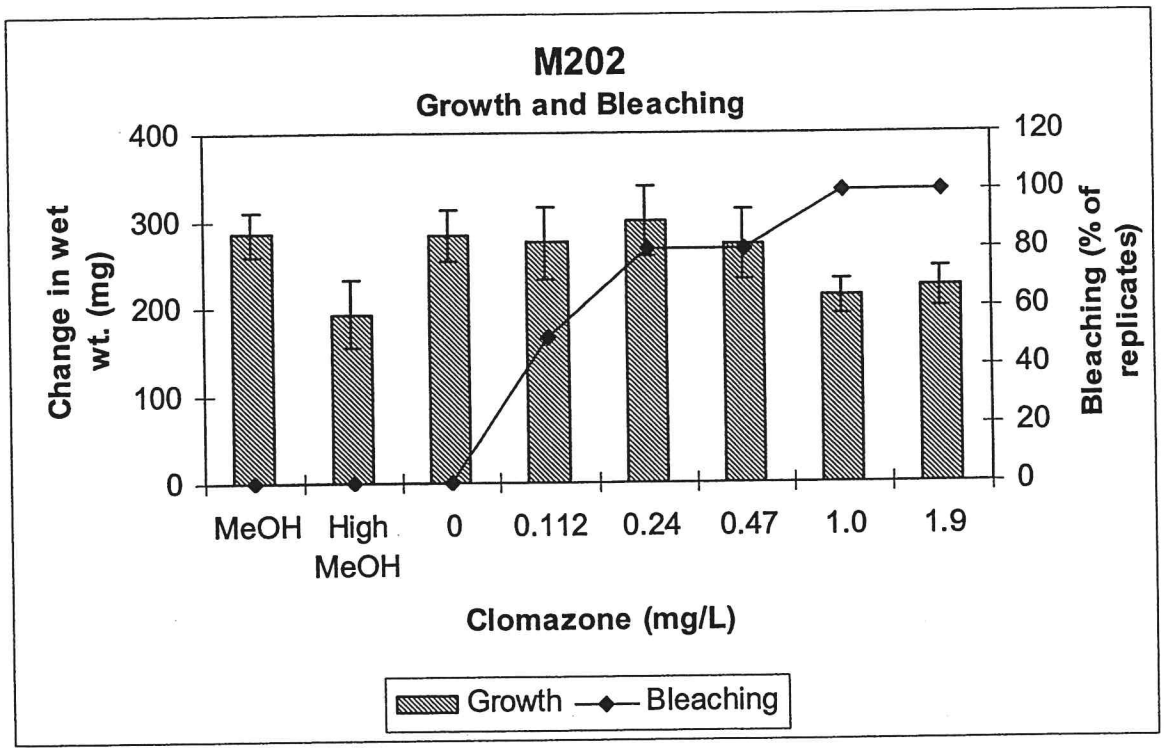

Figure 2. Effects of clomazone on growth and bleaching in rice plants.

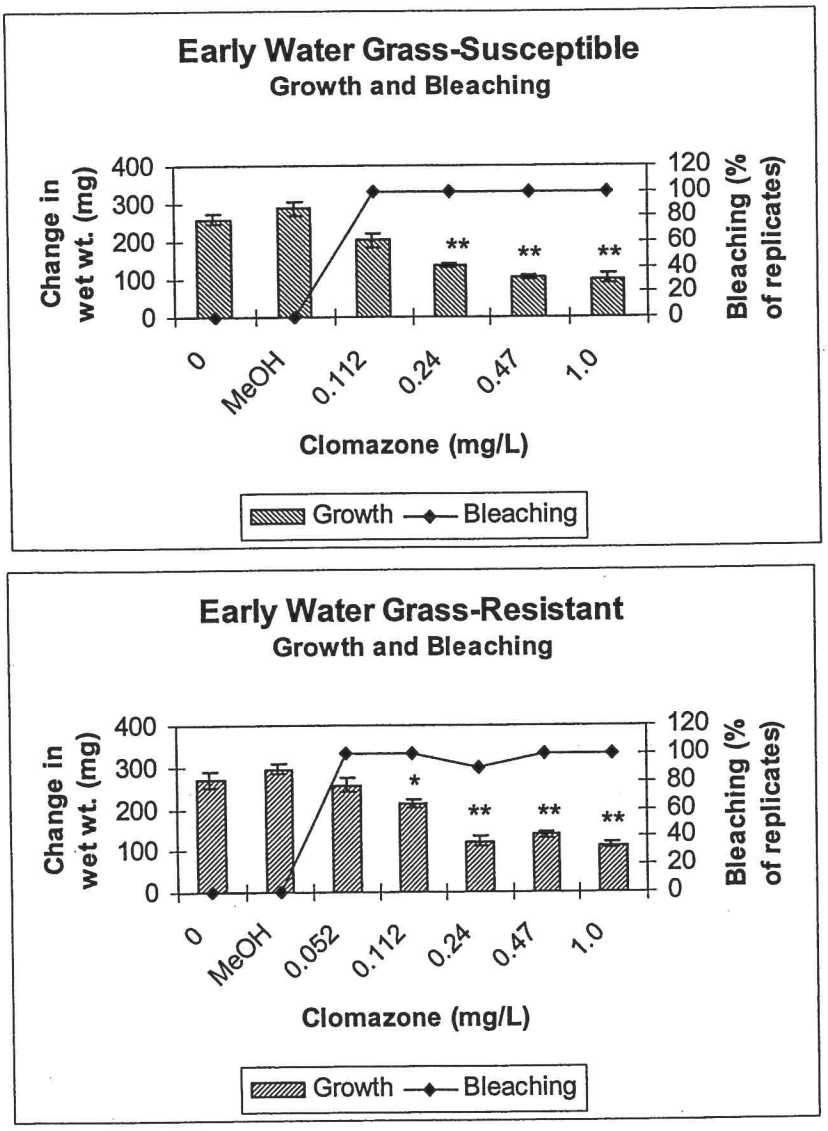

Figure 3. Effects of clomazone on growth and bleaching in early watergrasses.

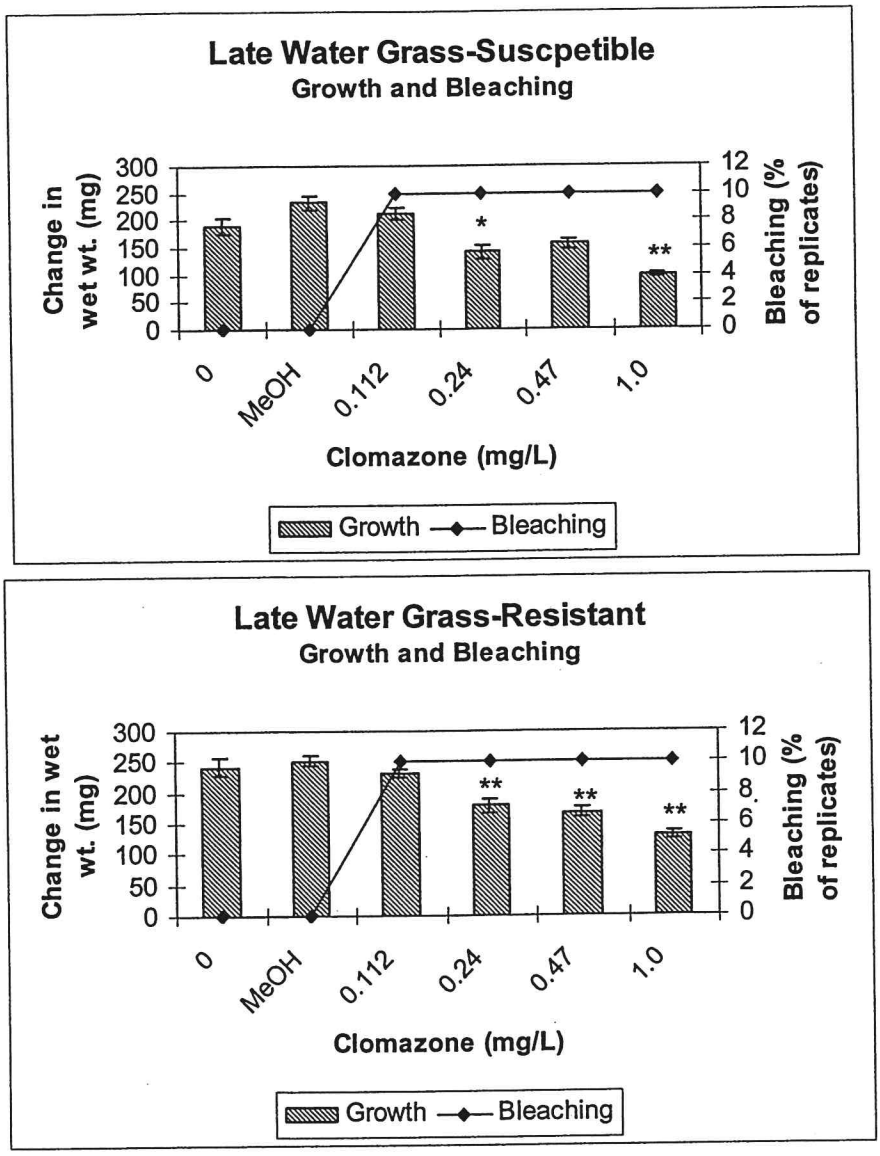

Figure 4. Effects of clomazone on growth and bleaching in late watergrasses.