ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2003 – December 31, 2003

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

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LEVEL OF 2003 FUNDING: $48,584

OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

Objective I. Determine the influence of copper and alternative terminal electron acceptors (phosphate) on the formation of deschlorothiobencarb (DTB) in California rice field soils. Results of last year's investigation of delayed phytotoxicity syndrome (DPS), in which the characteristics of DPS-resistant soils were compared to those of susceptible soils, indicated that exchangeable copper might inhibit those microbes that are responsible for the formation of DTB and subsequently, DPS. Copper has been used previously in rice fields as a fungicide and also for shrimp and algae control. Therefore, its use might be modified to inhibit microbes that are responsible for the development of DPS in susceptible soils.

Objective II. Compare toxicity of clomazone in rice versus watergrasses using both β-carotene levels and growth inhibition as endpoints. An active metabolite, ketoclomazone, is reponsible for clomazone's primary mode of toxicity, which is inhibition of carotenoid synthesis. Measurement of both the no-observable-effect-concentration (NOEC) and toxic effect (inhibition concentration, IC_{50}) for these two endpoints will give a comprehensive assessment of the selective toxicity of clomazone for watergrasses versus rice, and provide clear and useful endpoints to recognize herbicide injury.

Objective III. Compare the uptake and metabolism of clomazone by rice and both early and late watergrasses. Identify and quantify major metabolites, looking particularly for production of ketoclomazone. Relate phytotoxicity (from Objective II) to metabolic activity. An understanding of the comparative toxicity and metabolism of clomazone is an essential step towards development of safeners to use with the herbicide.

SUMMARY OF 2003 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE:

Objective I.

Introduction

The life cycle of the rice (*Oryza sativa*) requires initial propagation and subsequent growth in flooded soils. As measured by redox potential (Eh), water submerged soils become greatly reduced, and thus anoxic with time. Use of thiobencarb (TB) under these soil conditions has been found to be fatal to rice in some soils via delayed phytotoxicity syndrome (DPS). The occurrence of DPS as a result of TB use in rice fields was a serious problem in the Sacramento Valley during the late 1990s. Previous research on the occurrence of DPS found that dechlorinated TB (deschlorothiobencarb; DTB) was present in significant concentrations in rice fields exhibiting severe DPS symptoms (Ishikawa *et al.,* 1980). Subsequent work found that facultative anaerobic microbes might be responsible for production of DTB via reductive dechlorination (Moon and Kuwatsuka, 1985; Schmelzer *et al.,* 2003).

Related studies have described the reductive dechlorination of chlorinated contaminants such as trichloroethylene and chlorinated biphenyls under anaerobic soil conditions (Fava *et al.,* 2003; Kuipers *et al.,* 2003). Chemical dechlorination by anaerobic microbes is essential for energy utilization because accumulated electrons resulting from organic matter breakdown must be eliminated. Therefore, terminal electron acceptors are necessary for cell growth and reproduction. While in aerobic conditions oxygen is the preferential electron acceptor, under anoxic conditions alternate electron acceptors are utilized. These include, but are not limited to, chlorine (possibly from thiobencarb), nitrate, sulfate, iron, and manganese (Tanji *et al.,* 2003).

Based on the studies cited above, we explored if the production of DTB, and thus DPS, can be reduced by two mechanistic approaches: 1) inhibition (via copper) of microbes that utilize the reductive dechlorination of TB as part of cell growth and reproduction, and 2) use of alternate terminal electron acceptors (such as oxygen from phosphate) to prevent microbes from having to utilize chlorine from TB.

Methods

Soils. Anaerobic soil samples were collected from two rice fields in the Sacramento Valley, CA: Baggett and Mathews farms. Both farms have historically experienced severe DPS occurrences. All samples were collected during the growing reason when rice plants were in the early stages of growth. Four sampling locations (north, south, east, and west of field centerpoint) were randomly selected and four replicates were collected from each sampling location. The samples were collected by inverting a 50-mL Falcon polypropylene conical tube into the submerged soil and sealing in the water to maintain anaerobic conditions. Samples were transported and stored at 4°C until used. All samples for each field were mixed together, by a mechanical blender, in an inflatable plastic glove chamber with constant nitrogen flow to maintain anaerobic conditions, prior to using the soil in microcosm experiments. Moisture content of the soils was found to range between 30 and 50%. Characterization and elemental analysis was performed by the DANR Analytical Laboratory at UC Davis (http://danranlab.ucdavis.edu).

Microcosms. Approximately 6 g of soil were mixed with 10 mL of untreated well water (Placer County, CA) in 60 mL glass serum bottles that were sealed using butyl stoppers with aluminum

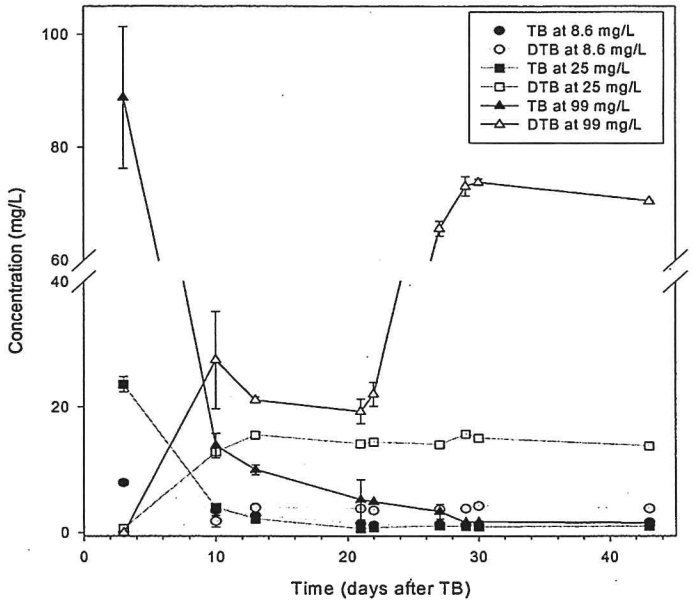

Figure 1. The dechlorination of TB (black symbols) at different concentrations and the subsequent formation of DTB (gray symbols) as observed in Baggett soil microcosm experiments in the dark at 30°C.

crimp caps. The samples were purged with nitrogen gas prior to sealing the vials to eliminate oxygen. The microcosms were allowed to incubate for seven days before adding TB to allow attainment of fully anaerobic conditions. Either copper(II) or phosphate (depending upon the experiment) was added at specific concentrations three days after incubation. All samples were allowed to incubate at 30°C in the dark without shaking to represent field conditions and eliminate abiotic breakdown (via photolysis) of TB. The vials were purged every 48 h to avoid pressure buildup. All experimental conditions were run in triplicate. Controls in which the soil and water mixtures were autoclaved prior to adding TB, were also run.

Extraction and organic compound sampling. The redox condition of each vial was measured and recorded on the day of extraction. Briefly, extractions were accomplished using 4 mL of methanol followed by two hexane extractions using 4 and 3 mL sequentially. For each hexane extraction, samples were briefly mixed via vortex and then mixed on a wrist-action shaker for approximately 20 min. They were then centrifuged at 3000 rpm for 10 min, and the supernatant (approx. 6 mL) was transferred into glass vials. The extracts were then concentrated to 5 mL under N_2. Molinate (4 mg/L) was used as an extraction surrogate. Analysis was conducted via GC-MS using methods established previously in our laboratory for TB and DTB.

Results and Discussion

We conducted microcosm experiments at different TB concentrations to determine the optimum TB dechlorination, and thus DTB formation, for the soils under investigation (Baggett and Mathews; Figure 1). An inverse relationship exists between TB dechlorination and DTB formation: as the concentration of TB decreased, DTB concentration increased. This can be explained by first-order kinetics, where DTB formation by microbial utilization of chlorine as a possible terminal electron acceptor is dependent on the concentration of freely available TB. Figure 2 shows this trend in more detail. At TB concentrations below 25 mg/L, a linear relationship between TB concentration and DTB formation exists as observed by the three low concentration data points. The ratio of TB to DTB at concentrations below 25 mg/L will be the same regardless of time. Such an observation could be explained by the capacity of the microcosmic system to reach equilibrium. For instance, TB will not be strongly withheld in

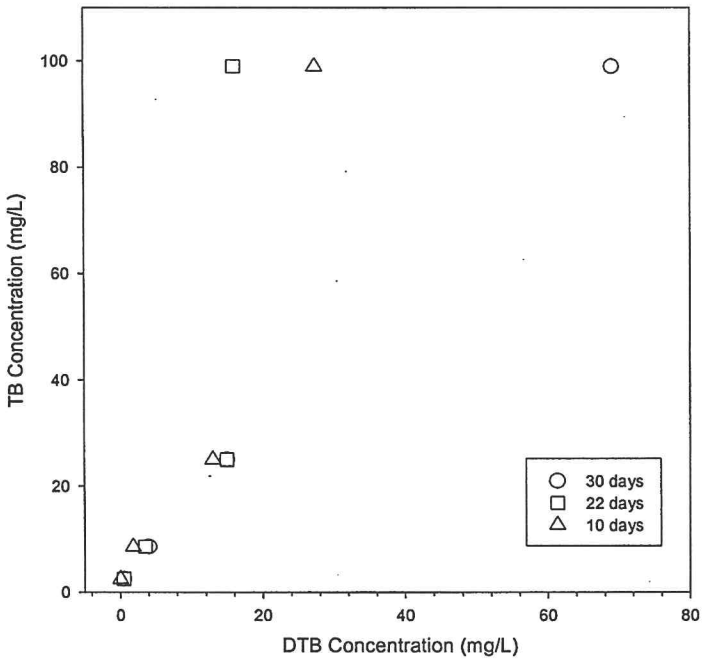

Figure 2. The relationship between TB and DTB in Baggett soil given different incubation times (days after TB had been added).

micropores of soil where their chlorine atoms are inaccessible to the microbes. Rather, they will be readily available to microbes for dechlorination in the soil solution matrix. However, at a high TB concentration (99 mg/L), time is an important factor in DTB formation. At this concentration, the amount of TB may exceed the needs of the microbial population. Therefore, it may take up to 30 days for the system to reach equilibrium between the microbes and dechlorination of TB.

Higher concentrations of TB will produce greater DTB concentrations, enhancing DPS (Figure 1). At TB concentrations of 8.6 and 25 mg/L, DTB formation reached a maximum concentration within 10 days, whereas at high TB concentrations (99 mg/L) a maximum trend began to occur only after 30 days. The specific reasons for such trends are not clear. However, at higher concentrations of DTB, microbial inhibition may have occurred; the low rate of DTB formation coincides with a reduced degradation rate of TB (observed by the slope of the two curves between 10 and 22 days at 99 mg/L TB). All concentrations produced DTB within 10 days after TB application. This is significant because state regulations require that pesticide applied rice field water must be held for at least 30 days before release to allow for herbicide dissipation and breakdown. Due to the rapid formation of DTB at 25 mg/L TB (Figure 3A) in flooded Baggett farm soils, this TB concentration was used in all subsequent experiments. Approximately 30% of the initial TB was lost due to abiotic degradation in Baggett soil over 43 days (Figure 3B). Similar results were observed for Mathews soil microcosms (not shown).

The rapid formation of DTB in anaerobic soils over a short time period, as observed in Figure 1, must be avoided to ensure that DPS does not occur and significantly affect rice field yields. In this study, two methods of reducing DTB formation were explored. The first approach attempted to use copper (Cu II) to inhibit those microbes that are responsible for DTB formation. Figure 4 illustrates the effects of adding $Cu(OH)_2$, the primary component of Kocide, at 2 mg/L a.i. Cu(II), on TB dechlorination in Baggett soil. The 2 mg/L concentration used in the microcosm study was five times the field-applied concentration of Kocide (4 fl oz a.i. Cu/100 lbs seed = 0.375 μg/g).

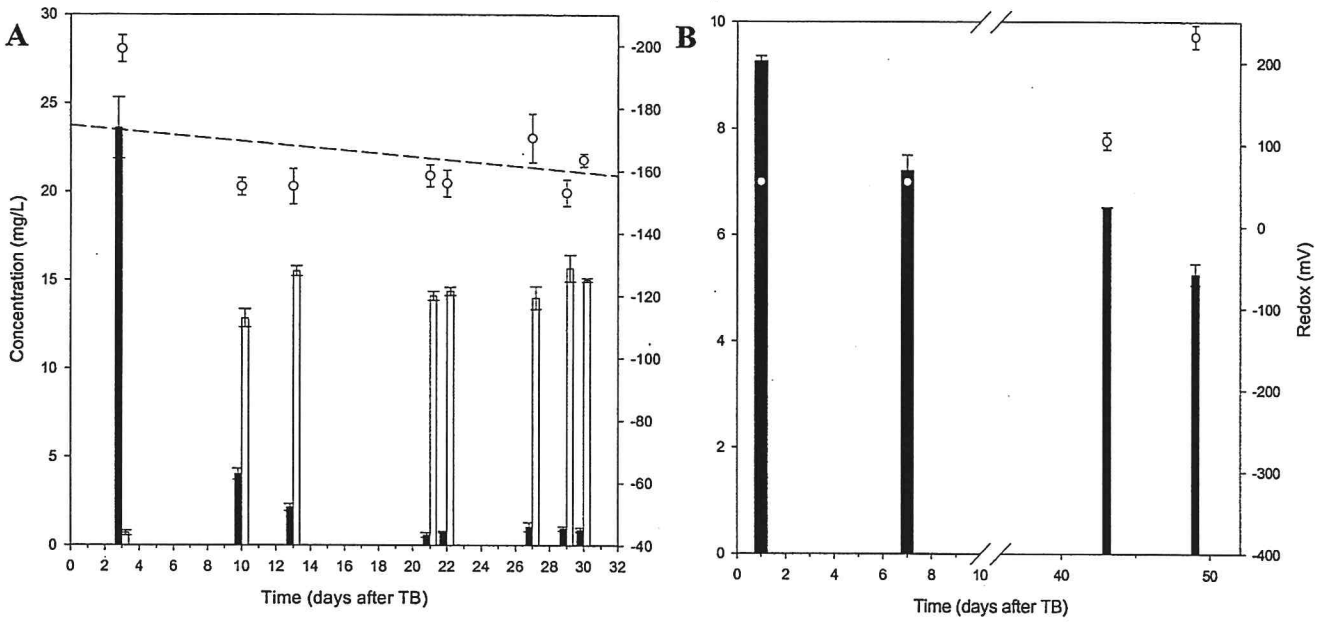

Figure 3. The dechlorination of TB (black bars) at 25 mg/L and subsequent formation of DTB (white bars) in reduced (circles indicate redox) flooded Baggett rice field soils (A), and its respective control (B). The dashed line shows the linear extrapolation of the redox points.

Our data showed that at the annual applied rate Kocide would not inhibit the microbes responsible for TB dechlorination in Baggett soil (Figure 4A). Formation of DTB from TB in copper-inoculated microcosms (Figure 4A) followed almost the exact trend of the corresponding control (Figure 4B). Thus, soil background concentrations of 68 ppm plus the applied concentration of 2 ppm $Cu(OH)_2$ in Baggett soil did not limit DTB formation. The same trend was seen for $CuSO_4$ in Baggett soil (Figure 5). Copper sulfate also did not inhibit the microbes responsible for dechlorination of TB to DTB in Baggett soil (Figure 5A), compared to the control (Figure 5B).

The same copper experiments were repeated in the Mathews soils that had a lower background copper concentration of 38 ppm. The effects of $Cu(OH)_2$ and $CuSO_4$ are presented in Figures 6 and 7, respectively. No difference in DTB production from TB dechlorination was observed between the copper-inoculated and control microcosms. Thus, annual application rates of Kocide (which are five times lower than the amount used in this study) will not inhibit DTB formation in Mathews soils. Also, DTB formation seems to be unrelated to background copper concentrations. For example, even through Baggett soil (68 ppm) has higher total background copper content than Mathews soil (38 ppm), DTB formation took longer to appear in Mathews (15 days) versus the Baggett (10 days) soils.

The second approach of eliminating DTB formation was the possibility that alternate electron acceptors can be provided to the microbes so that they do not need to utilize the chlorine atom on TB as a terminal electron acceptor during energy utilization. Phosphate has been shown to inhibit microbial respiration in anaerobic conditions at ≥20 mM, as Conrad *et al.* (2000) found that methane production, a major indication of anaerobic respiration, ceased upon the addition of phosphate concentrations greater than 20 mM. We tested if phosphate-derived oxygen can act as a terminal electron acceptor to those microbes that would otherwise use the chlorine of TB. It is well understood that microbes will preferentially utilize oxygen as a terminal electron acceptor over nitrate, sulfate, iron and other compounds (Paul and Clark, 1996). Experimental microcosms

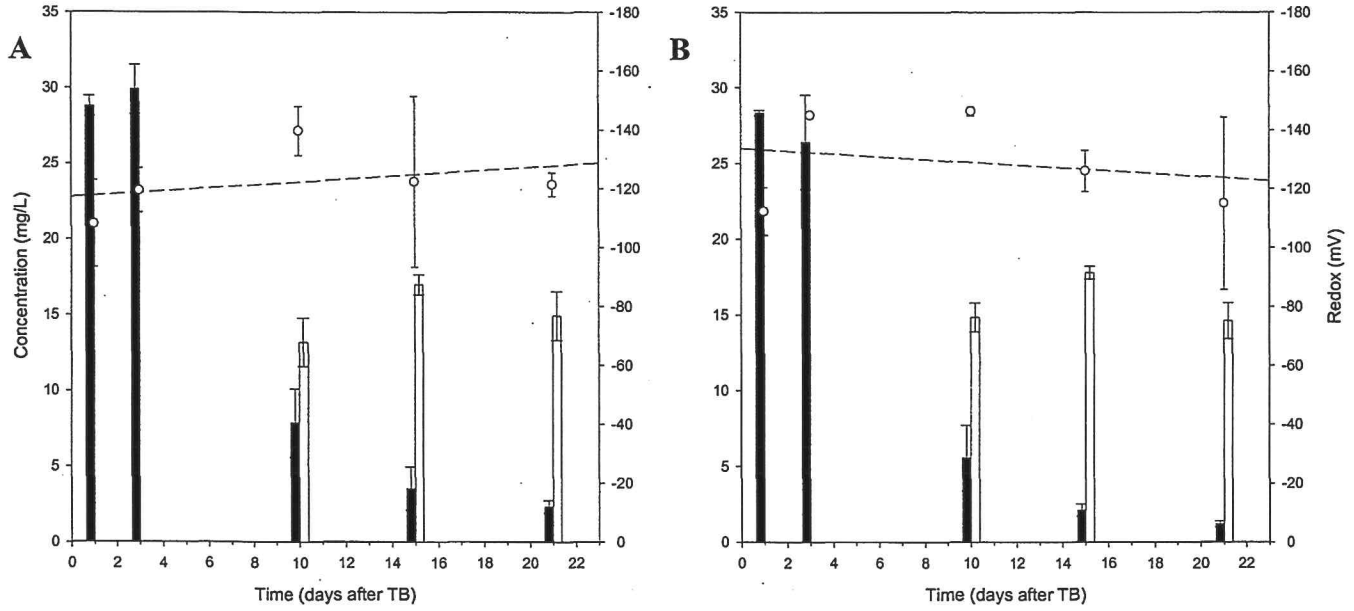

Figure 4. The effect of Kocide (copper hydroxide) at 2 mg/L on the (A) dechlorination of TB (black bars) in Baggett soil and subsequent formation of DTB (white bars) relative to the control (B) which has no copper added. Circles are a measure of redox and the dashed line shows the linear extrapolation of the redox points.

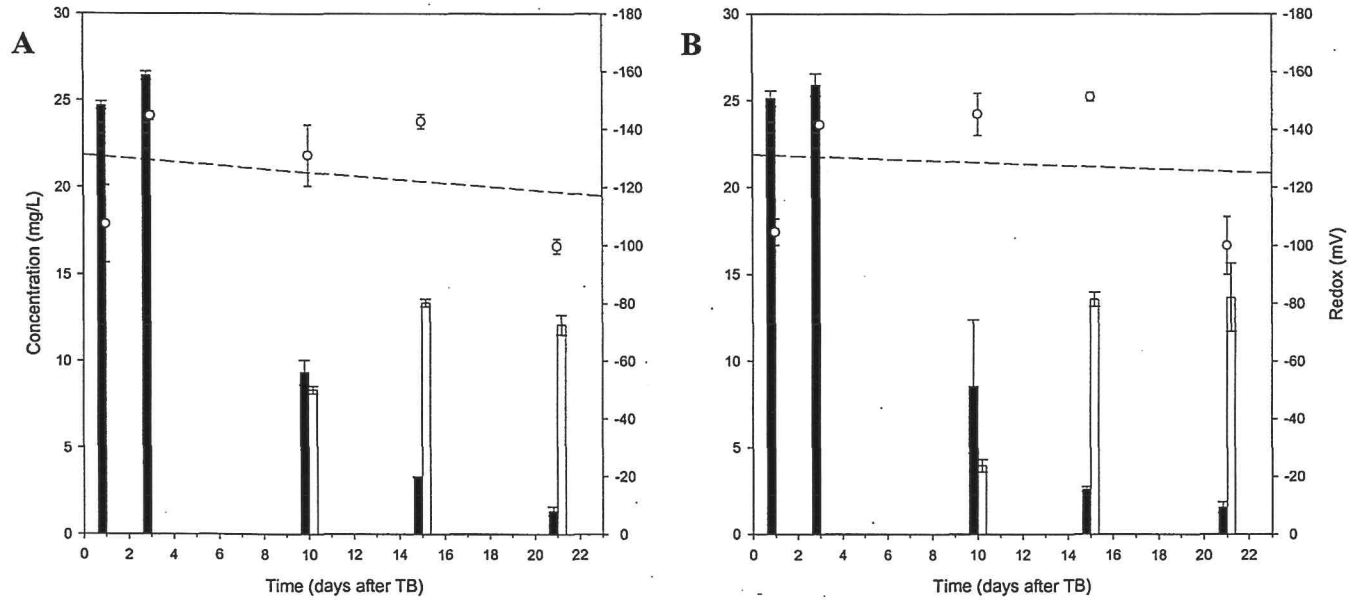

Figure 5. The effect of copper sulfate at 2 mg/L on the (A) dechlorination of TB (black bars) in Baggett soil and subsequent formation of DTB (white bars) relative to the control (B) which has no copper added. Circles are a measure of redox and the dashed line shows the linear extrapolation of the redox points.

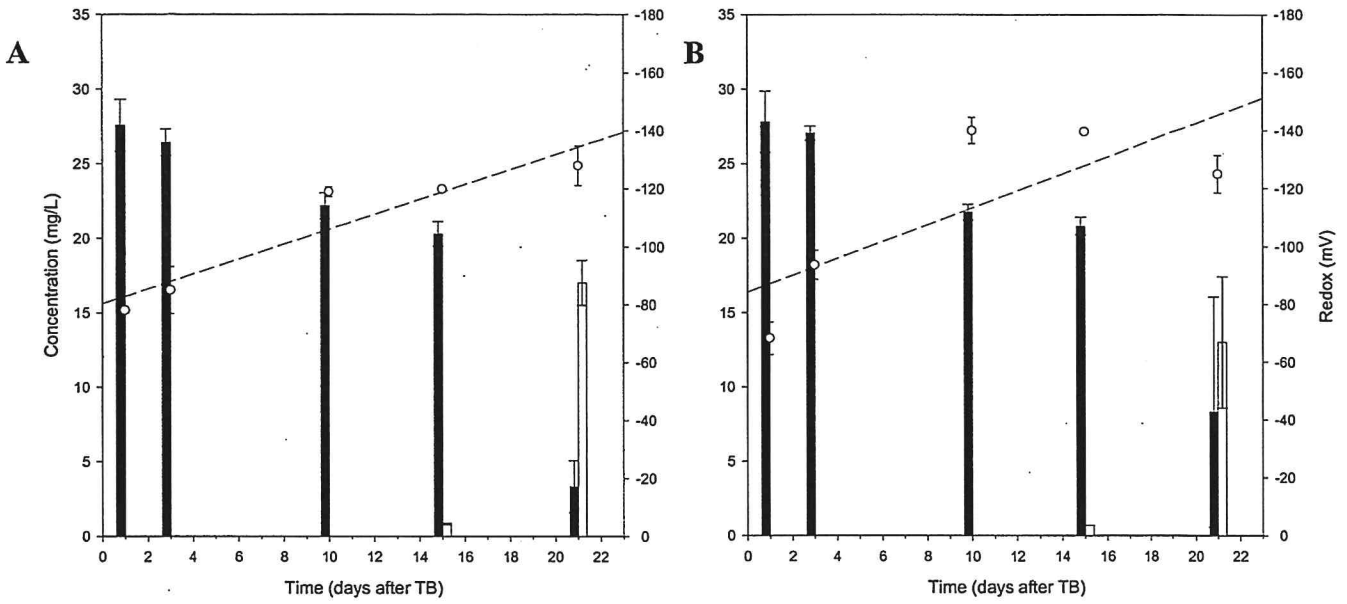

Figure 6. The effect of copper hydroxide at 2 mg/L on the (A) dechlorination of TB (black bars) in Mathews soil and subsequent formation of DTB (white bars) relative to the control (B) which has no copper added. Circles are a measure of redox and the dashed line shows the linear extrapolation of the redox points.

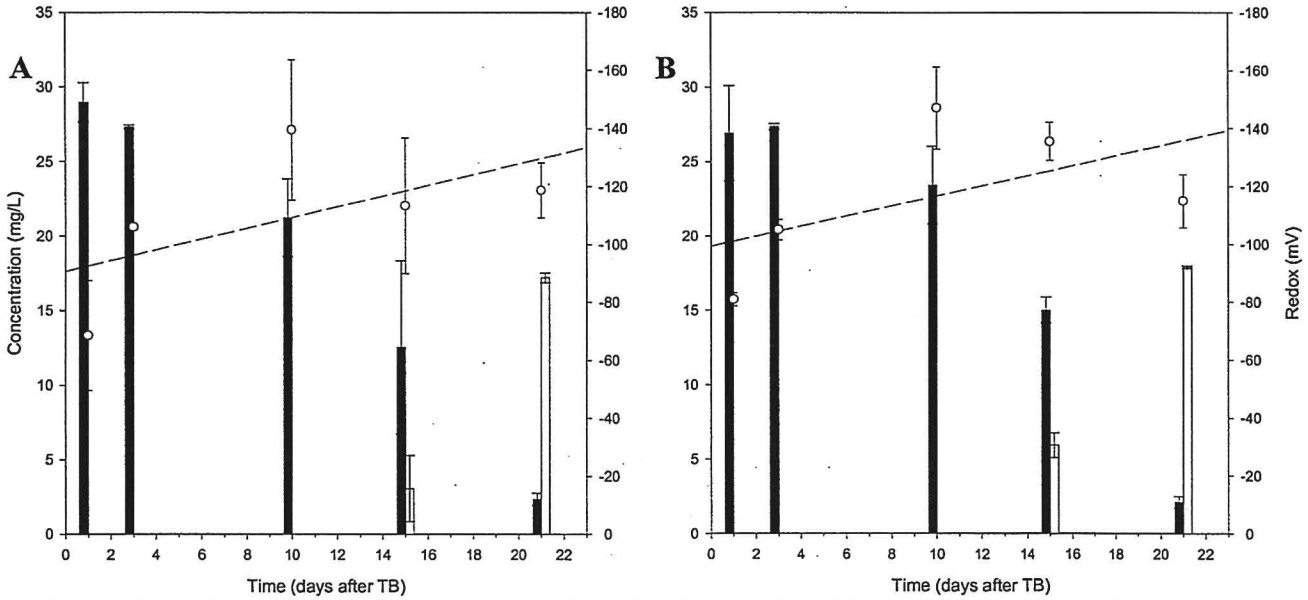

Figure 7. The effect of copper sulfate at 2 mg/L on the (A) dechlorination of TB (black bars) in Mathews soil and subsequent formation of DTB (white bars) relative to the control (B) which has no copper added. Circles are a measure of redox and the dashed line shows the linear extrapolation of the redox points.

with 2000 mg/L of phosphate were initiated with the Baggett and Mathews soil-water solutions with TB. Production of DTB was monitored (Figures 8 and 9). Figures 8A and 9A clearly show that no DTB is formed within 15 days after TB addition to either soil, even though there is approximately 8 mg/L of TB loss. In the controls, however (Figures 8B and 9B), DTB is formed at 15 days at a significant concentration: approx. 2 and 7 mg/L DTB for Baggett and Mathews soils, respectively. With a no-cost time extension of our current funding, we are extending the experimental microcosms studies shown in Figures 8 and 9 to include extractions at 22 and 30 days. Results will confirm the true, long-term effects of phosphate on DPS formation.

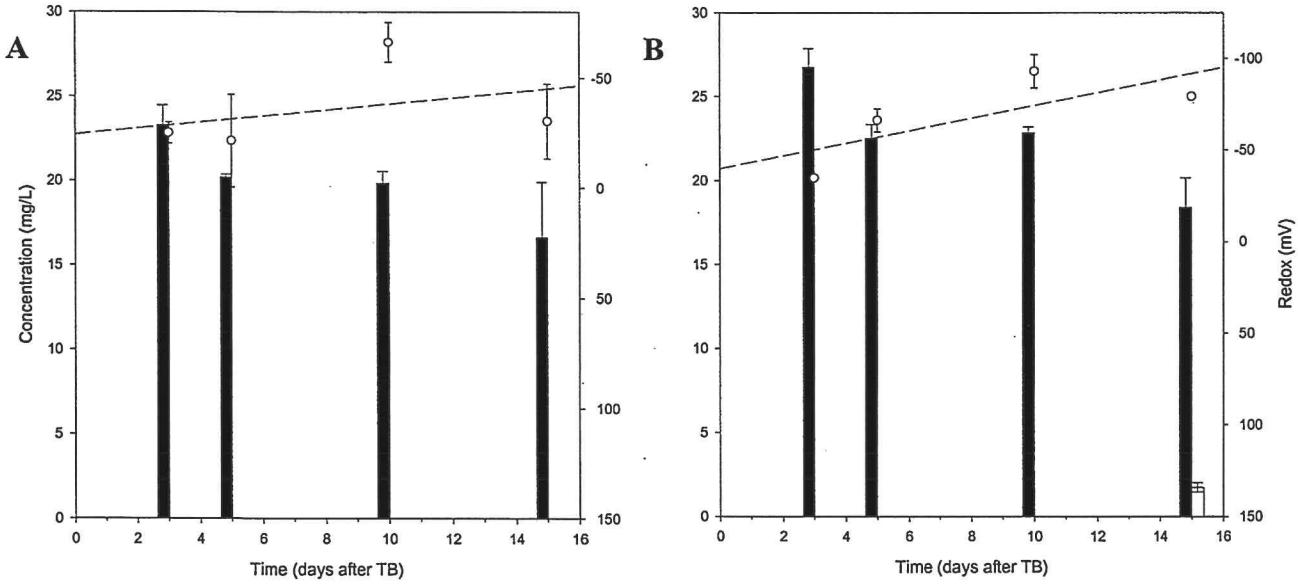

Figure 8. The effect of phosphate (2000 mg/L) on TB dechlorination and DTB formation in Baggett soil (A) and its corresponding control with no phosphate (B), in which DTB forms at 15 days. The dashed line shows the linear extrapolation of the redox points (circles).

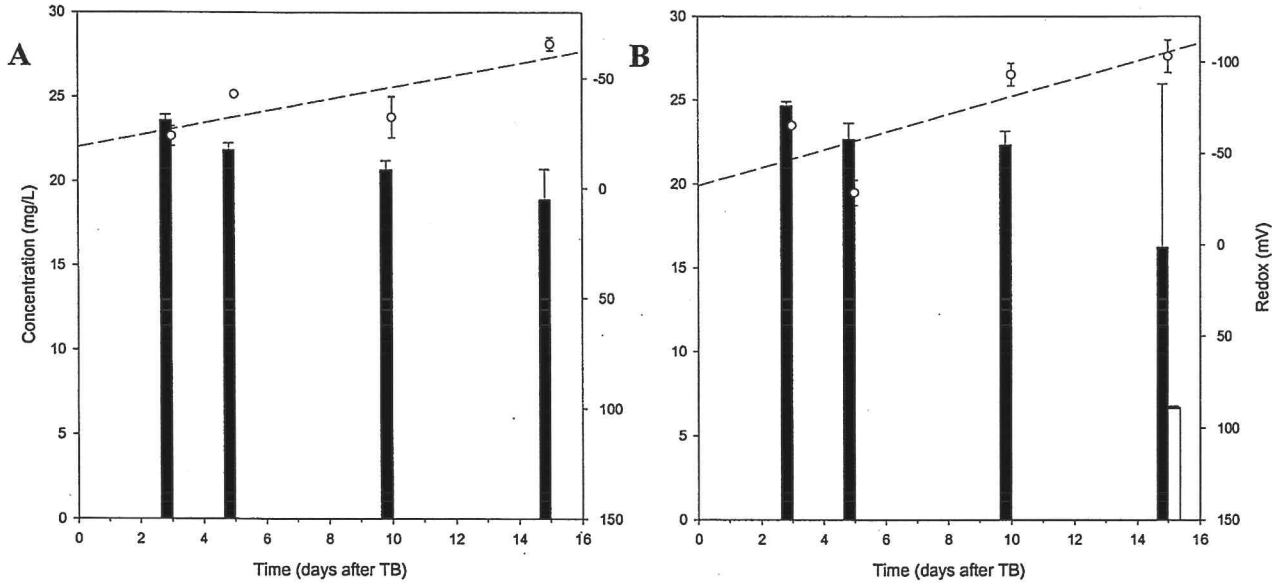

Figure 9. The effect of phosphate (2000 mg/L P) on TB dechlorination and DTB formation in Mathews soil (A) and its corresponding control with no phosphate (B), in which DTB forms at 15 days. The dashed line shows the linear extrapolation of the redox points (circles).

We are also currently repeating part of the copper study with slight modifications. Since Figures 4 through 7 did not show the expected inhibition of DTB as a result of the 2 mg/L copper concentration, we are testing if high copper concentrations may prevent DTB formation. Microcosm experiments are currently underway to determine if 100 mg/L of $Cu(OH)_2$ or $CuSO_4$ will inhibit microbes responsible for the dechlorination of TB. A 100 mg/L concentration was selected because it was found that some soils, such as Baggett, can have very high background copper concentrations (i.e. 68 mg/L). High background copper concentrations may play a role in the inhibition of microbes that utilize TB and reduce it to DTB, given that the copper in the soil is in an available form (not bound to organic matter or as a specific ion species; i.e., Cu^{+2}). These experiments are being conducted for both Baggett and Mathews soils.

In conclusion, the two objectives of this study have been met. We examined if inhibiting the microbes that utilize the reductive dechlorination mechanism as part of cell growth and reproduction would prevent DTB formation. At field application rates of Kocide, DTB will not be prevented. However, we are in the process of examining if higher copper concentrations will inhibit the microbes. We also examined if alternative, easily accessible, terminal electron acceptors can be used to prevent the microbes from having to utilize the reductive dechlorination mechanism. Initial data shows that phosphate may act as an alternative electron acceptor, inhibiting the formation of DTB, but data from extended time points are needed to confirm our hypothesis. Studies underway to confirm this are nearing completion.

Objective II.

Introduction

This investigation was designed to provide information regarding the comparative toxic actions of clomazone on β-carotene and growth in rice (*Oryza sativa*) and two watergrasses (*Echinochloa oryzoides* and *E. phyllopogon*). Growth is a general physiological endpoint that integrates numerous biochemical effects, while β-carotene provides a direct measure of the functioning of the non-mevalonate isoprenoid synthetic pathway.

Materials and Methods

Seeds of rice (variety M202) and resistant (R) and susceptible (S) varieties of *E. oryzoides* and *E. phyllopogon* were germinated for 7-10 d in deionized water at room temperature. Seeds were pre-treated with 3% bleach for 5 min and rinsed prior to germination. Seedlings were placed in test tubes (four per tube = replicate) containing 0.5X Hoagland's solution (pH 6.2 + 0.2) plus clomazone at concentrations ranging from 0.08-7.9 μM. Tubes were capped with polyurethane foam plugs and placed in a growth chamber (30°C day:16°C night; 14 h day: 10 h night; rel. humidity:30% day:80% night; maximum photosynthetically-active radiation = 800 nm). After 7-d exposure, plants were rinsed, blotted dry, weighed and frozen at –80°C prior to β-carotene analysis. Replicates (10-20) were analyzed at each exposure concentration for changes in growth (change in fresh weight), and three replicates were analyzed for β-carotene. Clomazone test solutions were extracted on C_{18} SPE columns and analyzed by HPLC to confirm concentrations.

Whole plants were extracted and pigment analysis was accomplished according to previously described methods with some modification (Takaichi 2000; AOAC 1990). Plants (four per replicate) were homogenized in 12.5 mL of a 7:2 acetone:methanol solution. Absorbance (436 nm) was recorded and the extinction coefficient of 0.196 mL μg^{-1} (AOAC 1990) was used to calculate β-carotene concentrations; extraction efficiency was 100 + 6.2%.

Linear interpolation was used to determine concentrations of clomazone producing 25 and 50% reductions in response compared to controls (IC25 and IC50, respectively); a bootstrapping technique was used to generate standard deviations for the IC values. ANOVA with Dunnett's post-test was used to determine no observed effect concentration (NOEC) values. Comparisons between IC values were made using ANOVA with Tukey-Kramer multiple comparison tests.

Results and Discussion

Table 1 shows IC25, IC50 and NOEC values calculated for both growth and β-carotene. To facilitate sensitivity comparisons between resistant and sensitive varieties, and between rice

Table 1. Clomazone toxicity endpoints (μM clomazone; mean ± SD).

Endpoint	*O. sativa*	Resistant *E. oryzoides*	Susceptible *E. oryzoides*	Resistant *E. phyllopogon*	Susceptible *E. phyllopogon*
ß-Carotene					
IC25	0.42 ± 0.26	0.08 ± 0.02	0.08 ± 0.02	0.33 ± 0.09	0.54 ± 0.15
IC50	1.9 ± 0.3	0.33 ± 0.20	0.33 ± 0.04	> 3.9	0.88 ± 0.15
NOEC	0.21	< 0.08	< 0.08	0.08	0.46
Growth					
IC25	5.6 ± 1.6	0.46 ± 0.06	0.42 ± 0.08	0.92 ± 0.451	0.79 ± 0.08
IC50	> 7.9	2.1 ± 0.8	1.2 ± 0.2	> 3.9	3.7 ± 0.2
NOEC	7.9	0.21	0.21	0.46	0.46

Table 2. Sensitivity comparisons

a. Ratios of IC25 values.

R/S	ß-carotene	Growth
E. oryzoides	1.0	1.1
E. phyllopogon	0.6*	1.2
***O. sativa* to:**		
Resistant *E. oryzoides*	5.0*	12.3*
Susceptible *E. oryzoides*	5.0*	13.5*
Resistant *E. phyllopogon*	1.3*	6.1*
Susceptible *E. phyllopogon*	0.8*	7.1*

b. Ratios of IC50 values.

R/S	ß-carotene	Growth
E. oryzoides	1.0	1.8*
E. phyllopogon	> 4.5	> 1.1
***O. sativa* to:**		
Resistant *E. oryzoides*	5.8*	> 3.8
Susceptible *E. oryzoides*	5.8*	> 6.6
Resistant *E. phyllopogon*	< 0.48	~ 2†
Susceptible *E. phyllopogon*	2.4*	> 2.1

c. Ratios of NOEC values.

R/S	ß-carotene	Growth
E. oryzoides	~1.0†	1.0
E. phyllopogon	0.17	1.0
***O. sativa* to:**		
Resistant *E. oryzoides*	> 2.6	37.6
Susceptible *E. oryzoides*	> 2.6	37.6
Resistant *E. phyllopogon*	2.6	17.2
Susceptible *E. phyllopogon*	0.46	17.2

* significant difference between numerator and denominator ($p < 0.01$)

† indeterminate values in numerator and denominator

and *Echinochloa* spp., ratios of IC25, IC50 and NOEC values were calculated and are shown in Tables 2a-c. For IC25 and IC50 ratios with determinate values in both the numerator and denominator, statistical significance is indicated. An R/S ratio greater than 1.0 indicates that the resistant variety is indeed more resistant to clomazone than the susceptible variety. Similarly, *O. sativa/Echinochloa* spp. ratios greater than 1.0 indicate that rice is more resistant than the *Echinochloa* spp.

Figs. 1 and 2 show dose-response curves for the ß-carotene and growth endpoints, respectively. At lower clomazone concentrations, the *E. oryzoides* R and S curves are similar for both endpoints, but they diverge slightly at higher clomazone concentrations for the growth endpoint. This is supported by the finding of a significant difference between R and S *E. oryzoides* for the growth IC50s (Table 2b), but not for the IC25s (Table 2a).

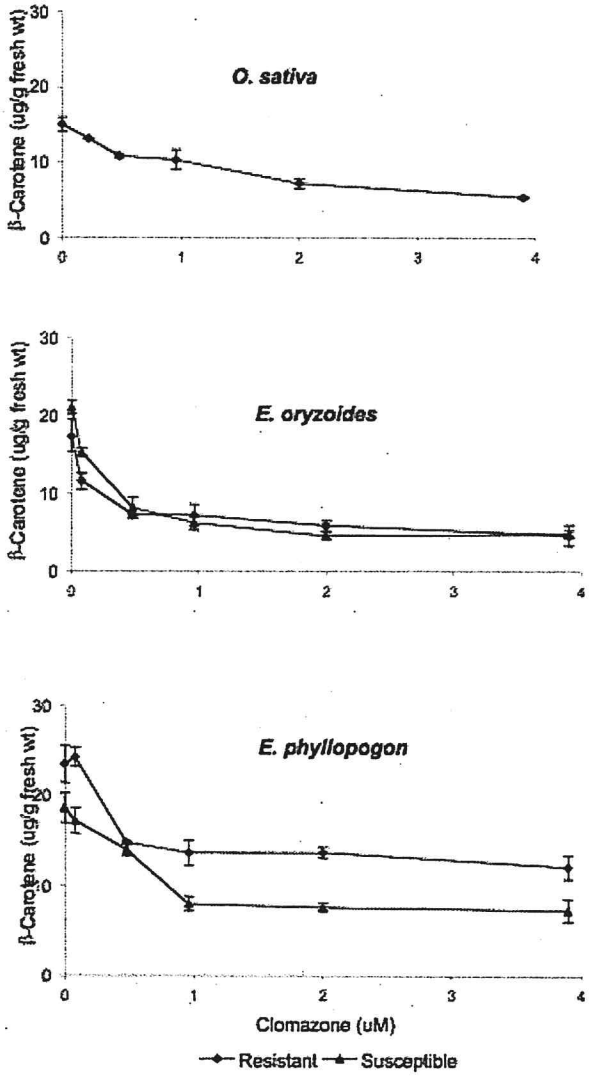

Figure 1. Dose-response curves for ß-carotene endpoint (mean ± se).

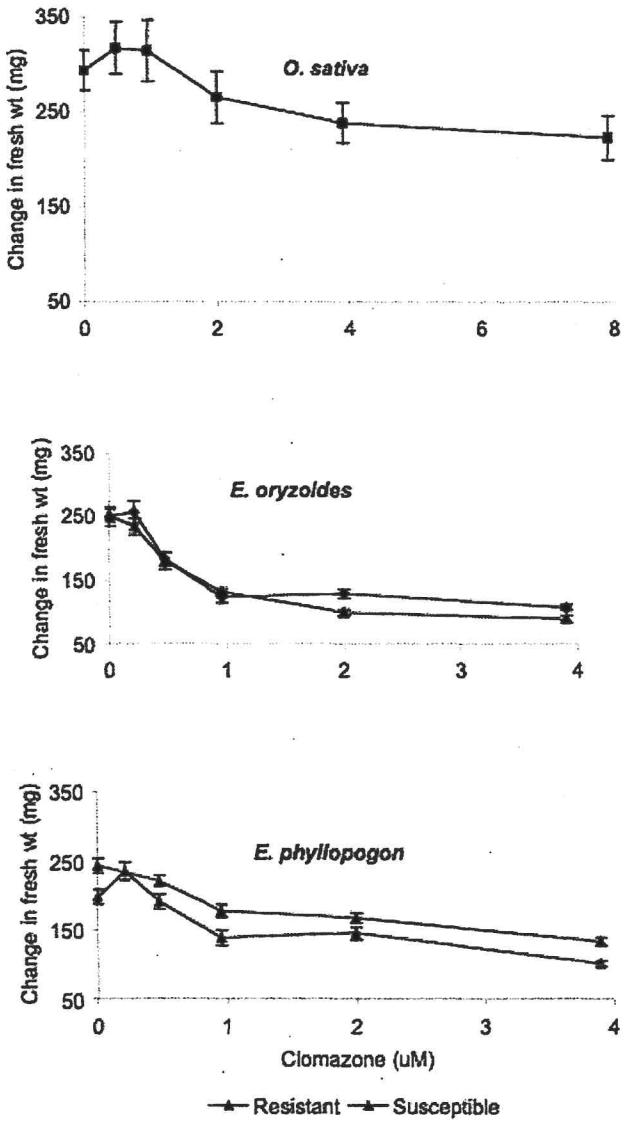

Figure 2. Dose-response curves for growth endpoint (mean ± se).

The R and S dose-response curves for *E. phyllopogon* diverge for both endpoints, with R responding less to higher clomazone concentrations (IC50 R/S ratios > 1.0; Table 2b.). However, at low clomazone concentrations, S responded significantly less to clomazone than the R variety (IC25 R/S ratio < 1.0; Table 2a.).

For rice no significant difference was observed for the growth endpoint at any of the concentrations tested (NOEC = 7.9 μM), but the β-carotene endpoint showed considerable sensitivity (NOEC = 0.21 μM). The ratios in Table 2 indicate that rice is considerably, and in many cases significantly, less sensitive to clomazone than either of the *Echinochloa* spp. for the growth endpoint. The β-carotene endpoint indicates that rice is less sensitive than *E. oryzoides* (R and S), but the results for *E. phyllopogon* vary with clomazone concentration. At low clomazone concentrations, rice is more sensitive than *E. phyllopogon* (S), but less sensitive than the R variety

(IC25 and NOEC ratios; Table 2a,c). At higher clomazone concentrations, rice is less sensitive than *E. phyllopogon* (S), but more sensitive than *E. phyllopogon* (R; IC50 ratios; Table 2b).

Clomazone reduced β-carotene levels in rice and *Echinochloa* spp. at very low concentrations. Effect on growth was less severe, and limited to *Echinochloa* spp. The recommended application rate of 0.67 kg ha^{-1} (FMC Corporation, 2001) would leave 2.3 μM clomazone in a paddy with a 10 cm flood, assuming all of the clomazone was in the water column. Reported K_d values range from 0.47-5.30 (Loux *et al.,* 1989; Mervosh *et al.,* 1995), thus with partitioning into sediment, there would be 0.36-1.6 μM clomazone in the water. These concentrations are above or near all of the IC25 and NOEC values determined in this study for β-carotene, and for *Echinochloa* spp. growth. However, rice growth would not be expected to be affected at those levels. That rice growth was not affected by clomazone could be due to its large seed relative to the seeds of *Echinochloa* species. Whether plants that have grown well, but have suffered clomazone bleaching, could recover and produce normal yields is an unexplored question.

Differential sensitivity to clomazone may be due to differential uptake, translocation, activation or detoxication. No differences in uptake, translocation or detoxication of clomazone were found in a study of soybean and velvetleaf (Weimer *et al.,* 1991). Clomazone detoxication through metabolism, particularly by conjugation with glutathione (Vencill *et al.,* 1990; Hall *et al.,* 2001) may also play a role in differential clomazone sensitivity. Several studies have shown that various plants are able to metabolize clomazone to different degrees (Norman *et al.,* 1990; Leibl and Norman, 1991; Weimer *et al.,* 1991; ElNaggar *et al.,* 1992; Weimer *et al.,* 1992).

The current study has shown that, with respect to production of β-carotene, rice and *E. phyllopogon* are quite similar in sensitivity to clomazone. Thus to be able to use clomazone for control of *E. phyllopogon* without injuring rice, a safening technique should be developed based on physiological and biochemical mechanisms of clomazone activity in plants.

Two promising avenues for further research are the mechanisms of clomazone transformation to the active 5-ketoclomazone, and of the interaction of 5-ketoclomazone with the enzyme 1-deoxyxylulose-5-phosphate synthase (DXS). First, activation of clomazone by a P450 enzyme is suggested by a study in which the organophosphates phorate and disulfoton (known P450 substrates) protected cotton from clomazone toxicity, but aldicarb (not a P450 substrate) did not (Culpepper *et al.,* 2001). Second, the enzyme DXS requires thiamine pyrophosphate (TPP) as a cofactor (Eubanks and Poulter, 2003). Structural similarities between 5-ketoclomazone and thiamine pyrophosphate suggest the possibility of competition at the DXS-TPP binding site. These mechanisms, if fully understood, may be exploited in development of chemical safeners for clomazone.

Objective III.

Introduction

Studies are underway to compare uptake and metabolism of clomazone by rice and early watergrass. These two plants showed the largest difference in sensitivity to clomazone (from Objective II) and thus were chosen for further study. In one first study, rice and early watergrass are being exposed to ^{14}C-labelled clomazone in a hydroponic system designed for mass balance determination. Metabolites will be extracted from plants and identified. In the second study, rice and early watergrass are being exposed to clomazone and 5-ketoclomazone (the active metabolite of clomazone) alone and in combination with piperonyl butoxide (PBO) and naphthalic anhydride

Table 1. Clomazone Fate in Hydroponic System

	Rice	Watergrass	
Solution	76.0%	92.5%	
Volatilized	0.2%	0.2%	
CO_2	1.5%	1.9%	
Plant	22.3%	5.4%	(p = 0.01)
Clomazone equivalents (umol/g plant)	93.4	83.3	(p > 0.05)

(NA). Evidence suggests (Culpepper *et al.*, 2001) that clomazone is transformed to the active 5-ketoclomazone by a P450 enzyme. PBO, a known P450 inhibitor, should protect plants from clomazone toxicity, while NA, a known P450 inducer, should synergize the toxicity of clomazone. Since 5-ketoclomazone is already in the active form, neither PBO nor NA should have an effect on its toxicity.

Methods

Study 1. Rice and early watergrass are being exposed to ^{14}C-labelled clomazone at 0.25 mg/L for 7 d. Radioactive residues in solution, as well as volatile residues (including CO_2) are being collected and quantified to provide a mass balance for clomazone in the system. Plant tissues will be homogenized and extracted for analysis of metabolites by HPLC and liquid scintillation counting.

Study 2. Using exposure systems and analytical techniques as described in Objective I, rice and early watergrass are being exposed to clomazone and 5-ketoclomazone in combination with PBO and NA. Effects are being measured by analysis of β-carotene levels after the 7-d exposure period.

Results and Discussion

These studies are ongoing and considerable tissue analysis has yet to be completed. However, preliminary results of the clomazone uptake study are presented here. Table 1 shows results of mass-balance analyses completed to date. Total uptake of clomazone by rice appears to be higher than that of early watergrass, however, when expressed as uptake per gram plant tissue, there is no statistical difference. Additional replication is being completed to determine if the trend is indeed statistically significant.

PUBLICATIONS OR REPORTS:

Manuscripts

1. Palumbo, A. J., TenBrook, P. L., Phipps, A., and Tjeerdema, R. S. Comparative toxicity of thiobencarb and deschlorothiobencarb to rice (*Oryza sativa*). *Bull. Environ. Contam. Toxicol.* (submitted for publication).

2. Schmelzer, K. R., Johnson, C. S., TenBrook, P. L., Viant, M. R., Williams, J. F., and Tjeerdema, R. S. Influence of organic carbon on the reductive dechlorination of thiobencarb (Bolero) in California rice field soils. *Pestic. Manage. Sci.* (submitted for publication).

3. TenBrook, P. L., and Tjeerdema, R. S. Comparative actions of clomazone on β-carotene and growth in rice and watergrasses (*Echinochloa* spp.). *Environ. Toxicol. Chem.* (submitted for publication).

4. TenBrook, P. L., Viant, M. R., Holstege, D., Williams, J. F., and Tjeerdema, R. S. Characterization of California rice field soils susceptible to delayed phytotoxicty syndrome after thiobencarb application. *Bull. Environ. Contam. Toxicol.* (submitted for publication).

Meeting Abstracts

1. TenBrook, P. L., and Tjeerdema, R. S., 2003. Comparative toxicity of clomazone to rice and watergrasses. *Proceedings of the Northern California Chapter of the Society of Environmental Toxicology & Chemistry*. Berkeley, CA.

2. TenBrook, P. L., Viant, M. R., Tjeerdema, R. S., Holstege, D., and Williams, J. F., 2003. Characterization of California rice field soils susceptible to delayed phytotoxicity syndrome. *Proceedings of the Northern California Chapter of the Society of Environmental Toxicology & Chemistry*. Berkeley, CA.

3. TenBrook, P. L., and Tjeerdema, R. S., 2003. Comparison of growth and β-carotene levels in *Oryza sativa, Echinochloa oryzoides* and *Echinochloa phyllopogon* exposed to clomazone. *Proceedings of the Society of Environmental Toxicology & Chemistry*. Austin, TX.

4. TenBrook, P. L., Viant, M. R., Holstege, D., Williams, J. F., and Tjeerdema, R. S., 2003. Characterization of California rice field soils susceptible to delayed phytotoxicty syndrome after thiobencarb application. *Proceedings of the Society of Environmental Toxicology & Chemistry*. Austin, TX.

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 from thiobencarb by anaerobic soil microbes. From our previous research, we found that DPS tends to occur on soils with high sand content, as well as low concentrations of copper and/or degraded organic carbon. The occurrence of DPS may also be enhanced by addition of raw organic matter (i.e. rice straw).

2. Experiments were conducted with two varieties of DPS-susceptible soils to determine if high copper concentrations can inhibit the anaerobic microbes responsible for producing deschlorothiobencarb. Concentrations higher than 100 ppm were found to reduce production of the DPS-producing toxic agent.

3. Experiments were also conducted with two varieties of DPS-susceptible soils to determine if high phosphate concentrations might inhibit microbial production of deschlorothiobencarb by providing oxygen to compete with the chlorine from thiobencarb as a terminal electron acceptor for essential energy metabolism. Initial results indicate this may be the case, and that high phosphate concentrations may avert DPS.

4. When tested for toxicity using two different endpoints (growth inhibition and reduction in β-carotene concentrations), rice was considerably, and in many cases significantly, less sensitive to clomazone than either of the watergrasses (*Echinochloa* spp.) for the growth endpoint. The more responsive β-carotene endpoint indicates that rice is less sensitive than *E. oryzoides*

(both resistant and sensitive varieties), but the results for *E. phyllopogon* vary with clomazone concentration.

5. Since a plant metabolite, ketoclomazone, has been hypothesizeded as the ultimate toxic form of clomazone, comparing differences in uptake and biotransformation of the herbicide between rice and watergrasses may lend insight as to the mechanism behind the selective toxicity of clomazone for rice versus watergrasses. Such information would facilitate our ongoing collaborative research to identify safeners to reduce injury to rice. Continuing is a study to determine differences in uptake and biotransformation of clomazone in rice and watergrasses.

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