ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2000 – December 31, 2000

PROJECT TITLE: The Microbial Degradation of Pesticides Important to Rice Culture

PROJECT LEADERS: Ronald S. Tjeerdema and Donald G. Crosby, Department of Environmental Toxicology, University of California, One Shields Avenue, Davis, CA 95616-8588

PRINCIPAL UC INVESTIGATOR: Ronald S. Tjeerdema, Department of Environmental Toxicology, UCD

CO-PRINCIPAL UC INVESTIGATOR: Mark R. Viant, Department of Environmental Toxicology, UCD

COOPERATORS: Kenley Ngim (UCD), Jack Williams (UC Cooperative Extension)

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

Objective I. To elucidate the potential role of microorganismal biodegradation in Delayed Phytotoxicity Syndrome (DPS) following the application of thiobencarb to rice fields. Emphasis was placed on identifying the importance of anaerobic versus aerobic microbes in the biodegradation, particularly dechlorination, of thiobencarb to form the highly toxic product implicated in DPS.

Objective II. To identify soil components critical for the dechlorination of thiobencarb to the toxic product and the onset of DPS. Emphasis was placed on the role of incorporated organic matter (e.g. straw).

Objective III. To identify optimal soil conditions for the microbial breakdown of the highly toxic dechlorinated product. Emphasis was to be placed on identifying the importance of anaerobic versus aerobic microbes in biodegradation, and on the role of incorporated organic matter.

Objective IV. To determine rice field management practices that will minimize the impact of DPS on rice production in the Central Valley.

SUMMARY OF 2000 RESEARCH ACCOMPLISHMENTS BY OBJECTIVE:

Objective I. It has long been recognized that organisms, from bacteria to vertebrates, have a tremendous capacity to degrade chemicals in the environment. Furthermore, both estuaries and wetlands generally represent areas of rich biological diversity. However in aquatic systems, such as flooded rice fields, most bacterial activity tends to be concentrated in the underlying soils, where dissolved oxygen content tends to be inversely proportional to depth; within a couple of centimeters soils become strongly anoxic. Therefore, we focused our search of the scientific literature on characterizing the importance of microorganisms in the degradation of thiobencarb (Bolero 10G) under both moist (aerobic) and flooded (anaerobic) rice field conditions. In particular, we concentrated on characterizing the relative importance of aerobic versus anaerobic microbes in the dechlorination of thiobencarb. From a number of previous investigations completed in Japan, it was quite obvious that production of deschlorothiobencarb was the result of anaerobic bacterial action, so a comparison of basic aerobic versus anaerobic soil conditions was not necessary. Also, it is apparent from the literature that dechlorination may result from the actions of several microbial species (a consortium of bacteria), so our focus moved to total dechlorination by soils and not to necessarily describing individual species. In addition, incorporated carbon (in the form of rice straw) was also considered to be a major factor in stimulating thiobencarb dechlorination and the onset of Delayed Phytotoxicity Syndrome (DPS) in rice. Therefore, our focus moved to investigating the role of incorporated rice straw in the microbial dechlorination of thiobencarb to the toxic product.

Objective II. Previous studies have suggested that organic matter (e.g. straw) incorporated into soil is an important factor in the microbial dechlorination of thiobencarb. Therefore, using environmentally controlled culture chambers (15 mL centrifuge tubes maintained under 100% nitrogen gas in a temperature-controlled plastic tent), we applied technical-grade thiobencarb to a San Joaquin grade soil known to be prone to DPS (provided by J. Williams). The soil was covered with 1 cm of well water to model flooded rice field conditions. Three soil amendments were modeled: (1) no straw amendment: (2) 0.33% rice straw amendment (to model a disked field), and (3) 2% rice straw amendment (to model a rolled field). All treatments were incubated for up to 60 days, and tubes were removed and sampled at the following time points: 0, 5, 11, 15, 20, 25, 35, 45, and 60 days. Parallel controls (autoclaved to kill soil bacteria) were also set up.

Upon sampling, the soils were centrifuged and extracted with methanol (to get complete wetting of the soils). The extracts were then solvent exchanged to hexane, dried, and analyzed for both parent thiobencarb and deschlorothiobencarb via gas chromatography-mass spectrometry (GC-MS). From pilot analyses thus far completed (please see attached figures), a number of observations can be made. First, sterile control soils showed virtually no deschlorothiobencarb, as would be expected (Fig. 1). However, anaerobic soils with varying amounts of rice straw incorporation showed significant dechlorination (Figs. 2-4), with the most deschlorothiobencarb production occurring with the highest straw content. Also, deschlorothiobencarb tends to appear between 10 and 20 days after application, which may correlate with the time needed for soils to become fully anoxic (as measured by the redox potential, Eh, in mV). Please remember these are pilot results; the remaining replicates are currently being analyzed.

Objective III. In recognition of the fact that the major dechlorinated product of thiobencarb is highly toxic to rice, we planned to conduct similar experiments to those described in Objective II using dechlorinated thiobencarb in place of thiobencarb. The goal was to determine whether anaerobic or aerobic microorganisms are involved in the breakdown of dechlorinated thiobencarb. However, since this was our first year of California Rice Research Board funding, research was not initiated until arrival of the award in June 2000. Therefore, we will incorporate this study into our proposed rice research for the 2000-2001 calendar year.

Objective IV. From our current results, it is apparent that the production of deschlorothiobencarb is an anaerobic process that is stimulated by the presence of soil-incorporated organic matter. While research is still ongoing, at this point it appears that conditions which either reduce the viability of anaerobic soil bacteria in rice fields or enhance the formation of aerobic conditions will potentially reduce the production of deschlorothiobencarb, thus reducing the potential for development of DPS. However, until a more comprehensive understanding of the influence of soil amendments is obtained, results and conclusions from this study should be considered to be preliminary.

PUBLICATIONS OR REPORTS: Since this report represents our first year of funding, no reports or publications have yet been prepared.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

1. An investigation into the cause of Delayed Phytotoxicity Syndrome (DPS) in rice was initiated. DPS has been recognized as a serious problem in some parts of the Central Valley of California.

2. DPS appears to be caused by deschlorothiobencarb, produced via the reductive dechlorination of the rice herbicide thiobencarb (Bolero). From previous investigations in Japan, anaerobic soil bacteria have been implicated in dechlorinating the herbicide.

3. In soils modeled after typical flooded field conditions, dechlorination of thiobencarb becomes apparent within 10 to 20 days after herbicide application. The delay in production appears to be correlated with the time required for the soils to become fully anoxic, thus capable of supporting a healthy population of anaerobic bacteria.

4. Soil amendments that enhance bacterial activity have been suspected to potentially increase production of deschlorothiobencarb. It appears that increased amounts of incorporated rice straw do indeed enhance production of the toxic product, with increased dechlorination resulting from increased straw content.

5. While results are still preliminary, it appears that conditions which either reduce the viability of anaerobic soil bacteria in rice fields or enhance the formation of aerobic conditions will potentially reduce the production of deschlorothiobencarb, thus reducing the potential for development of DPS.

Figure 1. Autoclaved Control

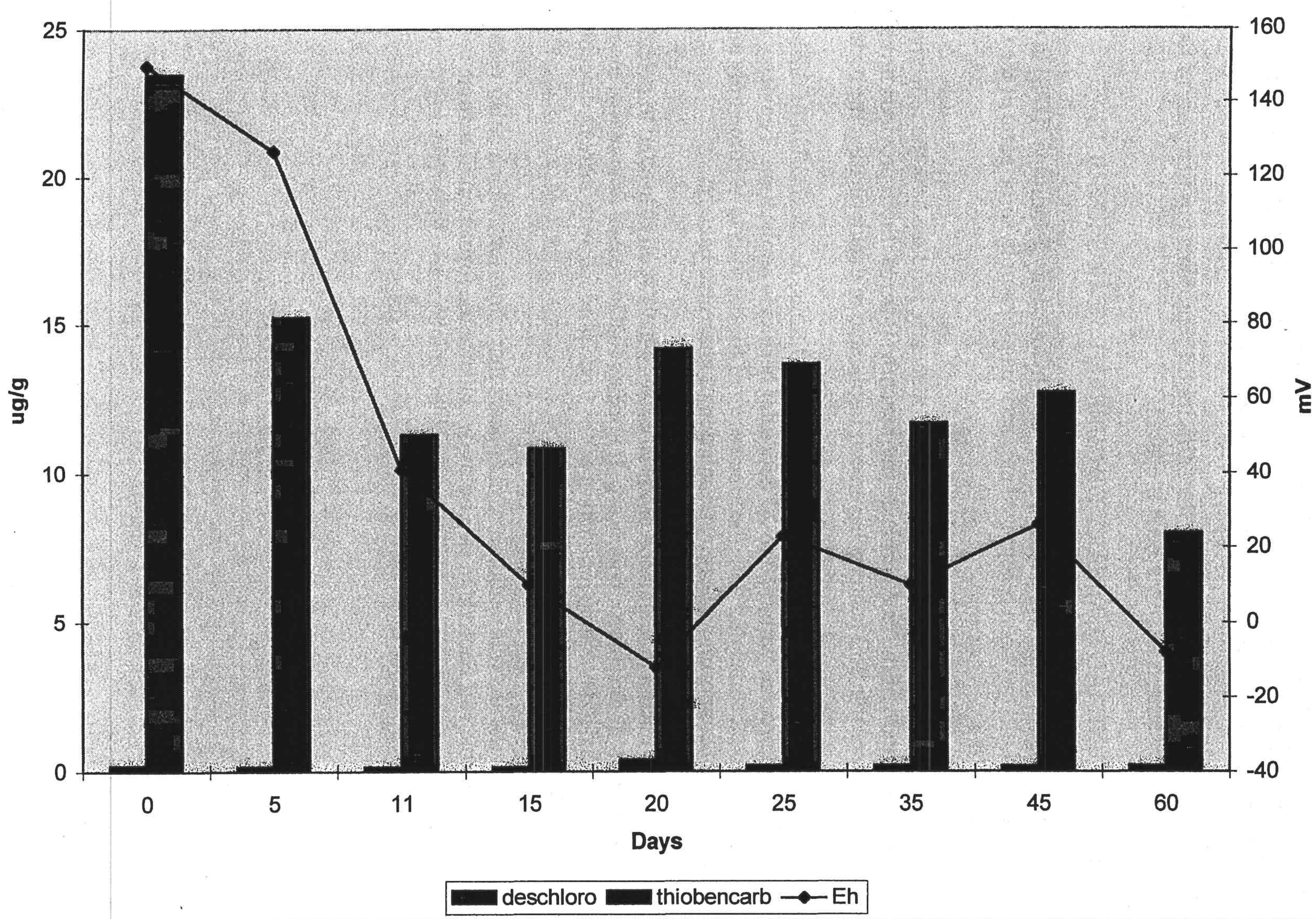
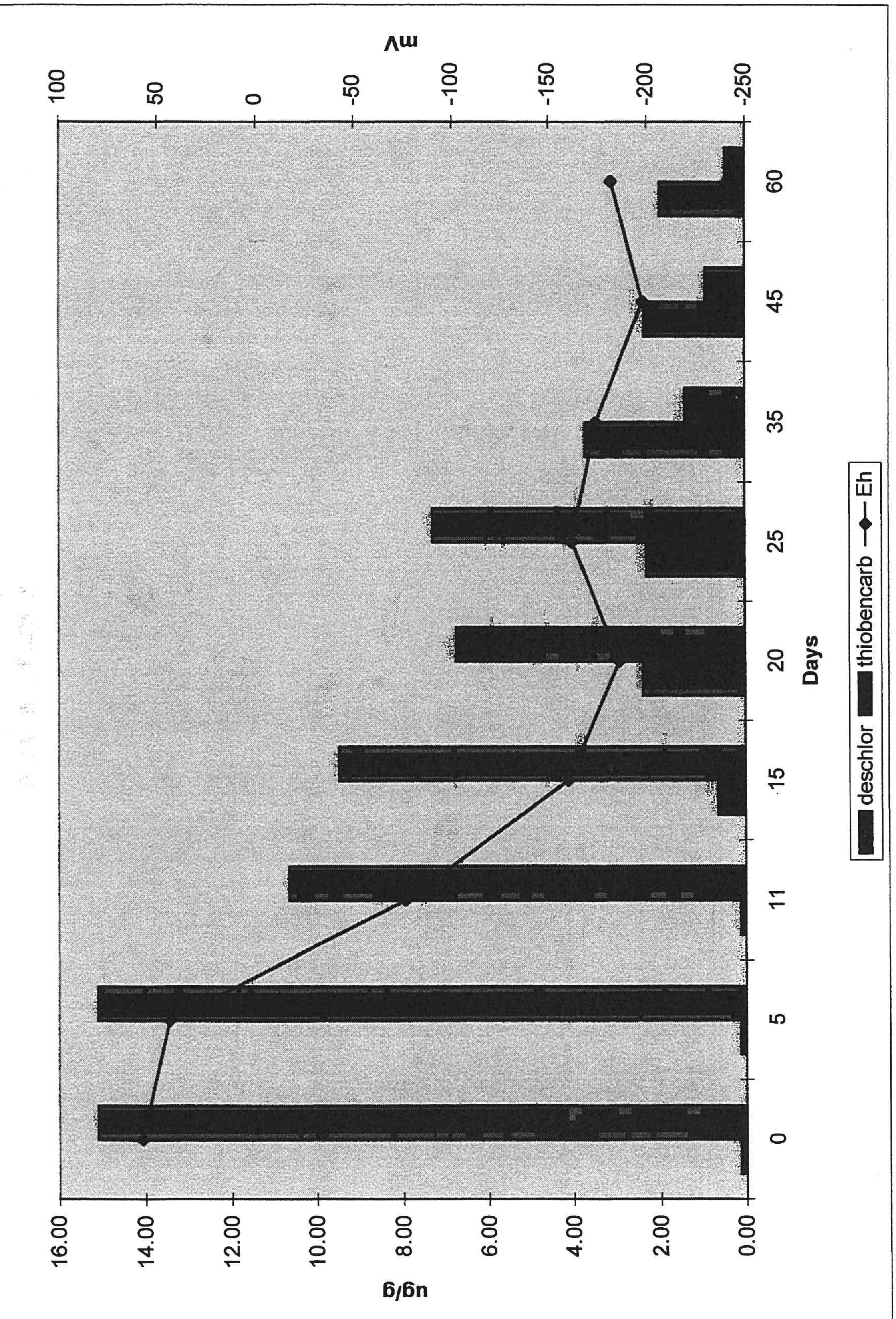


Figure 2. Rice Straw Removed From Field
(no added amendment, residual rice straw)



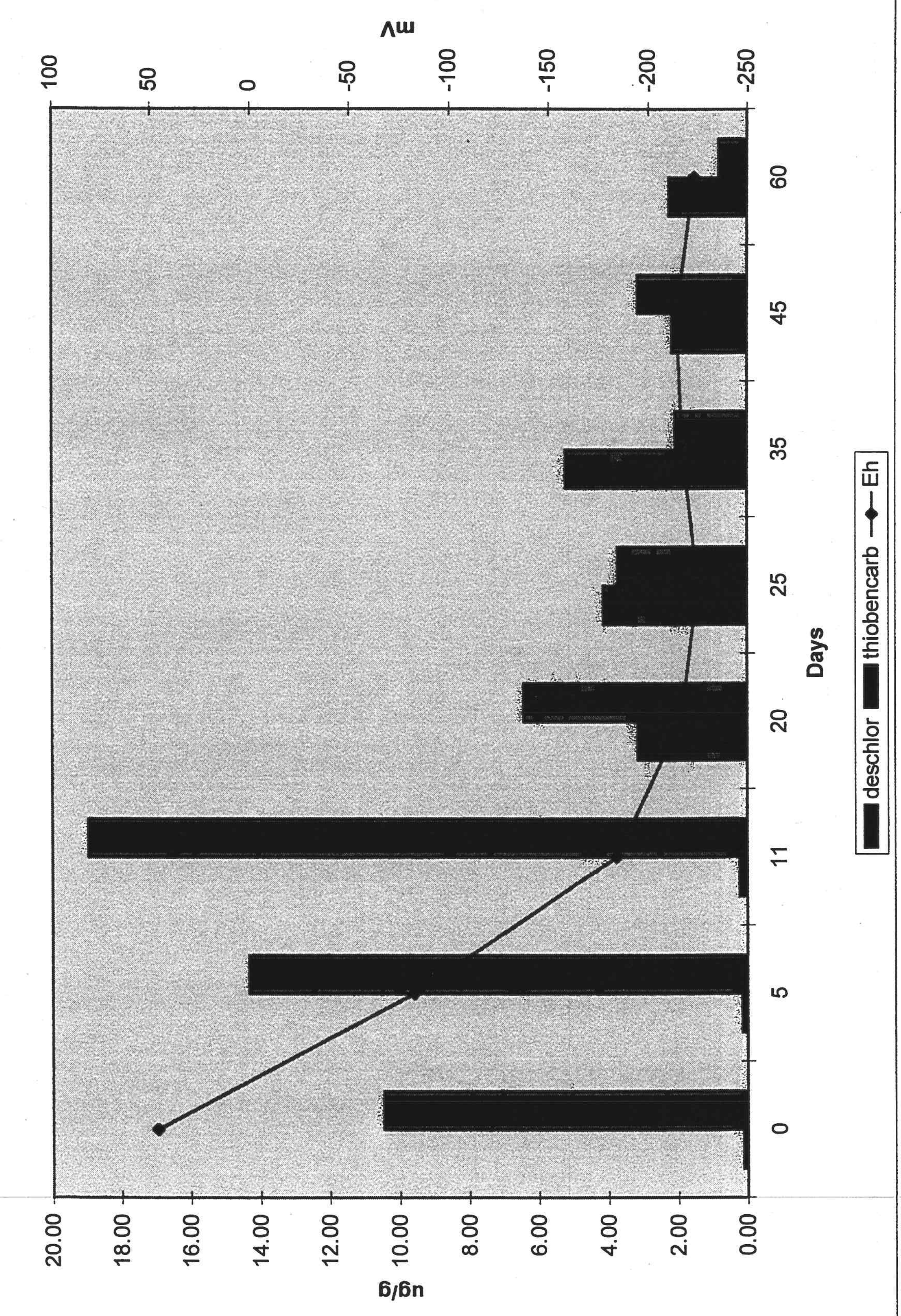


Figure 3. Rice Straw on Disked Field (0.33% amendment)

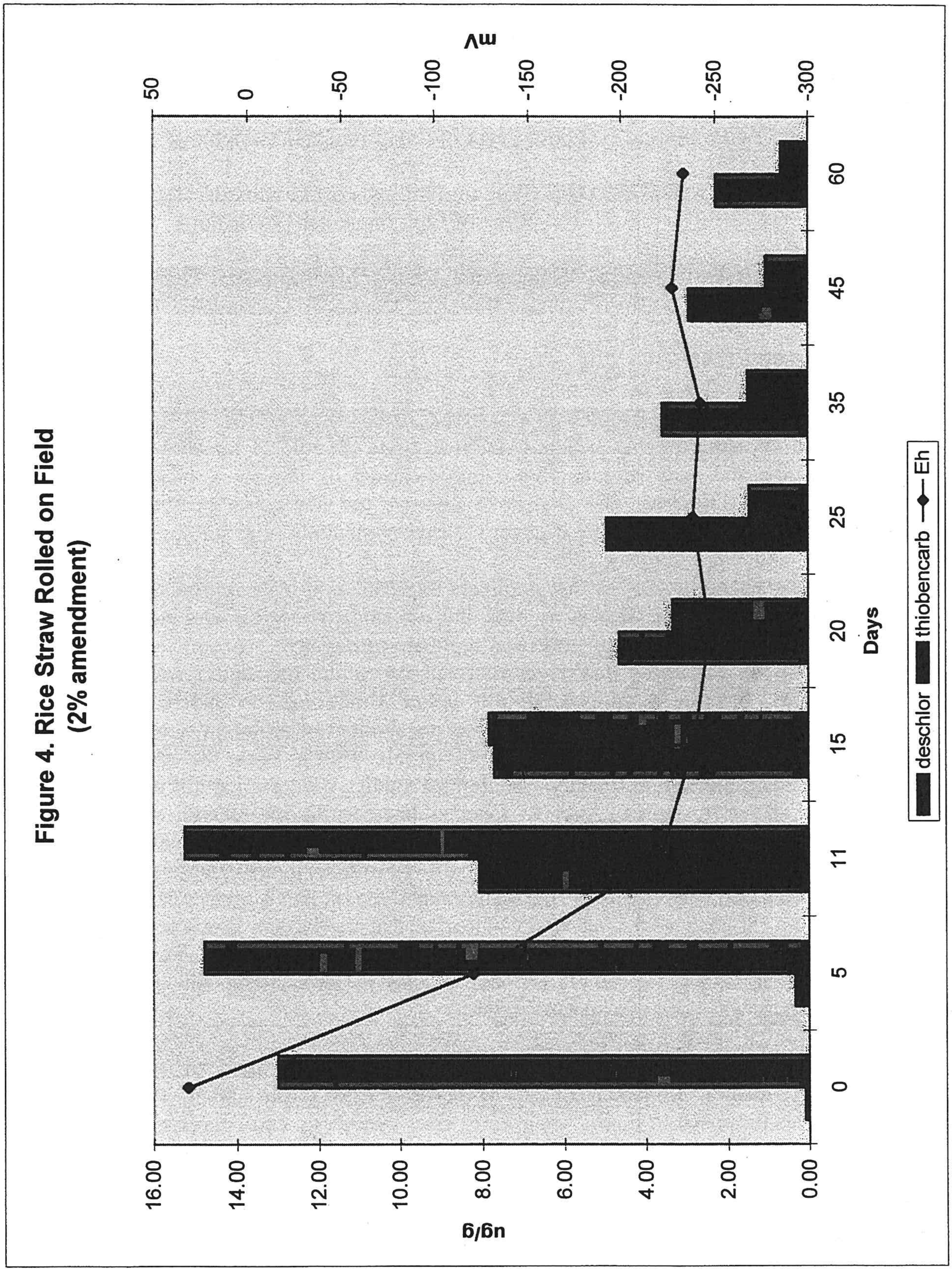


Figure 4. Rice Straw Rolled on Field (2% amendment)