ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2006 - December 31, 2006

PROJECT TITLE: Evaluation of additional alternative methods for managing algae in California rice fields.

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OBJECTIVES AND EXPERIMENTS CONDUCTED, BY LOCATION, TO ACCOMPLISH OBJECTIVES:

Objective 1. *Determine the effects of experimental compounds used for algae control and zinc sulfate on growth of Nostoc isolated from California rice fields under laboratory conditions*

We now have *Nostoc* from rice fields growing in a unialgal liquid culture in flasks at Dr. Lembi's laboratory at Purdue University. It will be used in the following types of experiments. Experiment 1: *Nostoc* will be exposed to a range of concentrations of two experimental chemicals that have been shown to be effective against algae by company and university tests. We will determine a dilution series (10 levels) based on data from other tests covering from 0 to 2X the range found to be effective in previous tests. The chemicals will be examined individually. Experiment 2: In separate experiments *Nostoc* will be exposed to thirteen concentrations of zinc sulfate. (Fifty pounds acre^{-1} was shown to inhibit algal growth in our 2005 studies.) We will run a similar concentration range using copper sulfate and untreated controls for comparison. Experiment 3: Once we have determined effective dosages of copper and zinc for killing *Nostoc*, we will set up experiments in which various amounts of rice straw are added incrementally to the cultures containing a known killing dose of either copper or zinc. If as we hypothesize copper or zinc are bound by the rice straw, we expect to see reduced efficacy of either copper or zinc as the amount of rice straw added increases. This type of experiment will answer questions about the relative binding capacity of rice straw residue for copper and zinc and may help explain field responses. Experiment 4: We will add 0, 1, 2, 5, and 10 ml of culture suspension of bacterium SG-3 to *Nostoc* cultures so that the final volume in each flask is 100 ml. In this experiment, the level of phosphorus in the culture medium will be varied (0.25, 0.5, and 1 X that of the basic medium). This experiment will provide information on how the susceptibility of *Nostoc* to this potential biological control bacterium changes as a function of different levels of a potentially limiting nutrient. The basic conditions for all of these experiments will be as follows. After treatment, the culture flasks (polycarbonate) will be placed in a controlled environment chamber at 100 µmol photons m^{-2} sec^{-1}, 25 C, and a 16:8 h light:dark photoperiod. After a two-week period, algae will be harvested, dried, and measured for dry weight and chlorophyll content (where appropriate). There will be three replicate flasks per treatment level. Data will be analyzed using analysis of variance and if appropriate summarized using probit analysis to determine the EC50 (concentration of product that causes a 50% reduction in dry weight) for the test algaecide. All statistical calculations will be done using SAS software.

Objective 2: *Determine the efficacy of zinc sulfate and barley straw extracts under field conditions for controlling species of algae that are known to occur in California rice fields, particularly those that are tolerant to copper.*

Field Experiment 1: We will also conduct field experiments by inserting sections of PVC pipe into rice field sediments so that a portion of the water column and associated algae are isolated. Each pipe section or ring will be considered a replicate. Prior to treatment we will collect algal samples to determine the species present and their biomass. We will also measure water temperature by inserting Tidbit data recorders within and adjacent to some of the rings. We will collect water samples to determine alkalinity and water hardness. We will use a YSI multiprobe instrument to measure pH, dissolved oxygen, and conductivity. There will be three replicate rings for each treatment. The treatments will consist of levels of the products shown to be effective in the laboratory test conducted for objective 1. Following application of the treatments we will measure algal dry weight after seven days by harvesting the algal material in each of the cores. We will establish this basic experiment in at least three fields, more if sufficient cooperators can be found. (The experimental design may be expanded to using 5m x 5m plots along the edges of rice fields if sufficient sites are available. Treatments and procedures would be the same as above.) We will use analysis of variance with planned orthogonal contrasts to test hypotheses about efficacy of the new algaecide relative to copper sulfate. In addition we will assess any injury caused to rice seedlings by the treatments using a visual rating system.

Field Experiment 2: To test the hypothesis that barley straw extracts produced during the natural decomposition of barley straw in rice fields will inhibit *Nostoc* and other algae we will set up a field experiment. In this experiment barley straw wattles (i.e., mesh bags loosely filled with barley straw) and similar wattles filled with rice straw will be deployed in an alternate pattern along the edges of selected rice fields. The rice straw wattles will serve as controls. The wattles will be placed (staked) prior to the introduction of water so that they are 25 meters apart and alternate with each other. Three weeks after the field is filled with water and for the next two weeks, we will collect algal biomass samples at 0.5 m intervals along a 5-meter long transect parallel with the shore extending from the pillow in both directions. If barley straw releases an algaecidal product, we expect to see decreased algal biomass nearer the wattle. Data from the transects from the barley wattless will be compared with those from control pillows containing rice straw using analysis of variance.

SUMMARY OF 2006 RESEARCH (major accomplishments), BY OBJECTIVE:

Objective 1. *Determine the effects of experimental compounds used for algae control and zinc sulfate on growth of Nostoc isolated from California rice fields under laboratory conditions.*

The microbial products, EM-1 and EM-5, did not reduce algal growth relative to untreated controls when exposed for 7 days at dilutions equal to 1:100, 1:500, 1:1000, and 1:10000 at Davis. In fact, algal growth was stimulated at the 1:100 dilution in an experiment with EM-1.

Results from experiments with three ALS (acetolactate synthase) inhibitors, bispyribac, imazamox, and penoxsulam, at 0, 100, 200, or 500 ppb (parts per billion) did not show consistent reduction in growth of *Nostoc* isolated from California rice fields. In contrast, *Nostoc* growth was reduced by two protox (protoporphyrinogen oxidase) inhibitors, flumioxazin and carfentrazone. Growth was reduced by approximately 50% at 1000 ppb for each compound. However, carfentrazone has been used to control weeds in rice fields and may produce injury to rice at 200 ppb. Flumioxazin is an herbicide used in peanut production and has not been used in rice culture. Thus, its impact on rice is not known.

In additional experiments, zinc reduced algal dry weight when it was applied at 20 ppm. The effects of zinc were detectable, as reduced chlorophyll reflectance, within 48 hours of treatment. Zinc at 20 ppm reduced algal dry weight more than the standard copper treatment at 1 ppm. When a commercially available liquid zinc sulfate solution was used to produce a range of zinc concentrations (0, 7.9, 15.9, and 31.7 ppm), algal dry weight was reduced by 50% at the highest zinc concentration. The results of these three experiments are consistent with the data we obtained during 2005. They may partially explain the anecdotal reports that fields that have zinc sulfate added at 50 lbs / acre (i.e., 20 ppm Zn) as a fertilizer treatment often have less severe algal problems.

We were unable to obtain the bacterium SG-3 and thus did not conduct experiments to determine its impact on *Nostoc.*

Results from experiments with combinations of copper sulfate or Cutrine Ultra and rice straw indicate that rice straw ameliorates the toxic properties of copper ions (Cu^{2+}) by binding the copper ions (Figure 1).

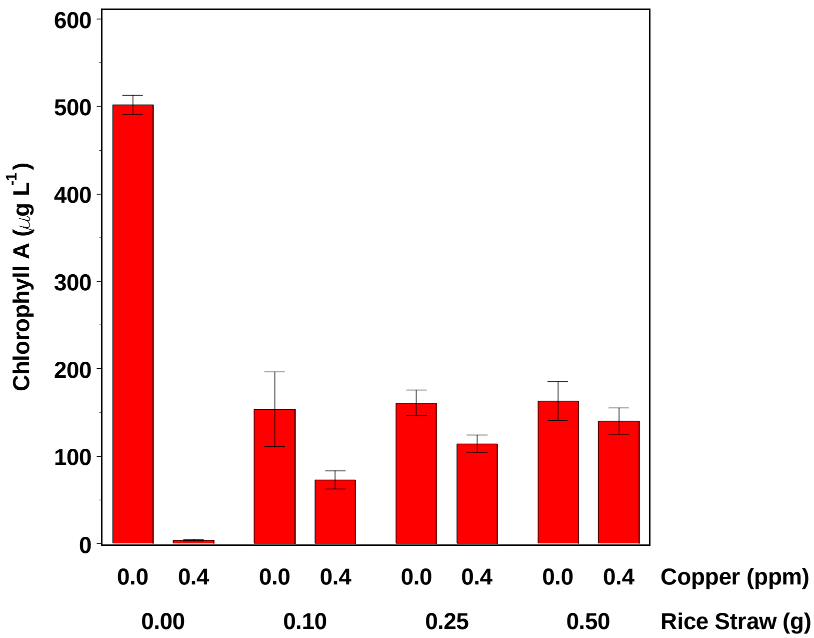

Figure 1. Interaction between rice straw and copper sulfate. Values are the mean $\pm$ 1 standard error.

In a separate experiment, the amount of rice straw was held constant and the concentration of copper was increased from 0.4 ppm to 10 ppm. Results indicated that the ameliorating effect of rice straw was completely reduced when the copper concentration was 8 ppm (Figure 2). These results are consistent with the idea that rice straw binds copper ions and prevents them from interacting with *Nostoc*.

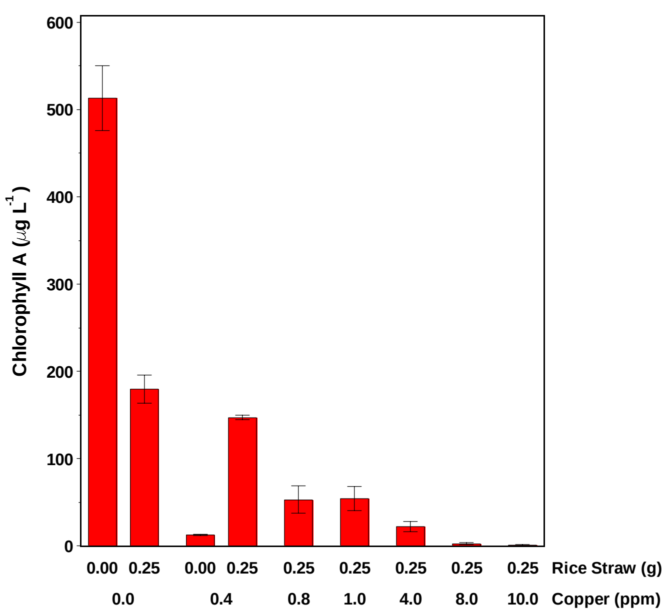

Figure 2. Response of *Nostoc* to 0.25 g rice straw (RS) and increasing amounts of copper sulfate. Values are the mean $\pm$ 1 standard error. This graph shows that it takes 8 ppm copper sulfate to overcome the ameliorating effect of 0.25 g or rice straw.

Results of experiments with soils collected from five separate rice fields indicate that burying the phosphorus fertilizer at 1 inch deep in the soil reduces the level of phosphorus in the overlying water to levels similar to soils that received no added phosphorus (Figure 3). Burying the phosphorus greatly reduced algal growth (Figure 4).

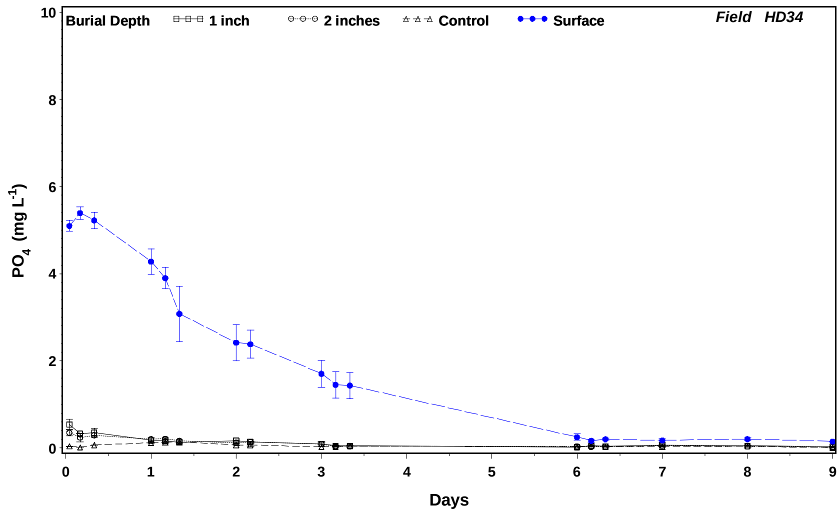

Figure 3. Time course of inorganic phosphorus concentration in the water following introduction of water into containers filled with rice field soil and with the phosphorus fertilizer place either on the surface, 1 inch below the surface, or 2 inches below the surface. The “control” containers had no phosphorus fertilizer added to them. Values are the mean $\pm$ 1 standard error.

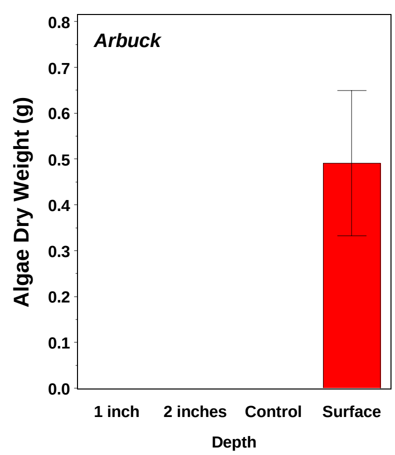

Figure 4. Dry weight of algae produced after 10 days in the containers used for the fertilizer burial experiment. Values are the mean $\pm$ 95% confidence interval.

Objective 2. *Determine the efficacy of zinc sulfate and barley straw extracts under field conditions for controlling species of algae that are known to occur in California rice fields, particularly those that are tolerant to copper.*

Field Experiment 1

We were unable to secure a field site for this experiment and thus unable to accomplish this objective.

Field Experiment 2

Dry weight of algae collected on June 1 and June 15, two and four weeks after the rice field was flooded were not significantly reduced by the presence of barley straw wattles compared to control wattles filled with rice straw (Figure 5). Mean water temperatures at three sites in this field were greater than 68 F which according to reports is sufficient to insure barley straw decomposition and the release of algaecidal components. Algae samples collected on June 21 were dominated by *Hydrodictyon* and *Anabaena.*

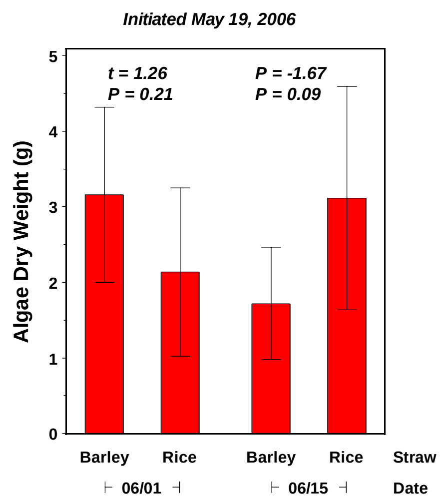

Figure 5. Dry weight of algae collected on two dates from sample points next to barley straw and rice straw wattles. Values are the mean ± 95% confidence interval.

The results from two additional experiments conducted at Davis, California indicate that a commercially available products known as "barley straw pellets" did not suppress growth of algal cultures containing *Nostoc* collected from this field when applied at the maximum recommended rate of 5 ounces per 80 gallons of water (P > 0.5).

PUBLICATIONS OR REPORTS:

Spencer, D. F., C. A. Lembi, and R. R. Blank. 2006. Spatial and temporal variation in the composition and biomass of algae present in selected California rice fields. *Journal of Freshwater Ecology* 21: 649-656.

D. Spencer and C. Lembi, 2006. Spatial and temporal variation in the composition of algae present in California rice fields, Western Aquatic Plant Management Society, March, San Diego, CA. (poster presentation)

Oral Report at the Big Valley Rice Round Table, Jan. 11, 2006, Richvale, California

Oral Reports at UC Cooperative Extension 2006 Annual Rice Grower Meeting
Jan. 25, 8:30 am, Evangelical Church, 5219 Church St., Richvale
Jan. 25, 1:30 pm, Glenn Pheasant Hall, 1522 Highway 45, south of Glenn
Jan. 26, 8:30 am, Cachil Dehe Tribal Village Community Center, South of Colusa Casino, 3730 Highway 45, Colusa
Jan. 26, 1:30 pm, Sutter Co. Agricultural Bldg., 142-A Garden Highway, Yuba City

Oral Report at the San Joaquin and Stanislaus Rice Growers Meeting, Feb. 21, 8:30 am, Nathaniel's Restaurant, Escalon

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

The microbial products, EM-1 and EM-5, did not reduce algal growth relative to untreated controls when exposed for 7 days at recommended treatments. In fact, algal growth was stimulated at the 1:100 dilution in an experiment with EM-1. Results from experiments with proposed new aquatic herbicides (bispyribac, imazamox, and penoxsulam) did not show consistent reduction in growth of algae. In contrast, *Nostoc* growth was reduced by proposed new aquatic herbicides (flumioxazin and carfentrazone). Growth was reduced by approximately 50% at the highest concentration tested. However, one of the herbicides, carfentrazone, has been used to control weeds in rice fields and may produce injury to rice at levels lower than those which inhibited the algae. The other herbicide is used in peanut production and has not been used in rice culture. Thus, its impact on rice is not known. In additional experiments, zinc sulfate reduced algal dry weight when it was applied at 20 ppm. The effects of zinc were detectable, as reduced chlorophyll reflectance, within 48 hours of treatment. Zinc sulfate at this concentration reduced algal dry weight more than the standard copper treatment at 1 ppm. When a commercially available liquid zinc solution was used to produce a range of zinc concentrations (0, 7.9, 15.9, and 31.7 ppm), algal dry weight was reduced by 50% at the highest zinc concentration. The results of these three experiments are consistent with the data we obtained during 2005. They may partially explain the anecdotal reports that fields that have zinc sulfate added at 50 lbs / acre as a fertilizer treatment often have less severe algal problems. Results from experiments with combination of copper sulfate or Cutrine Ultra and rice straw indicate that rice straw ameliorates the toxic properties of copper ions (Cu^{2+}) by binding the copper ions. Results of experiments with soils collected from five separate rice fields indicate that burying the phosphorus fertilizer at 1 inch deep in the soil reduces the level of phosphorus in the overlying water to levels similar to soils that received no added phosphorus. The result is that there was greatly reduced algal growth. A field test conducted in an active rice field with barley straw showed that algae dry weight was not significantly reduced by the presence of barley straw wattles compared to control wattles filled with rice straw. This is even though water temperatures in the field were greater than 68 F which according to reports is sufficient to insure barley straw decomposition and the release of algaecidal components.