**REPORT HEADERS.**

PROJECT NO. _14_

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2008 - December 31, 2008

PROJECT TITLE: Assessing alternative methods for managing algae in California rice fields.

PROJECT LEADER (include address): David F. Spencer, USDA-ARS, Associate in the A.E.S., Plant Science Department, Mail Stop 4, One Shields Ave, Davis, CA 95616

COOPERATORS: Carole A. Lembi, Professor, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

LEVEL OF 2008 FUNDING: $14,160

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

Objective 1. *Determine the effects of rice field water quality parameters and algicides on growth of Nostoc spongiaeforme isolated from California rice fields under laboratory conditions.*

We now have *Nostoc spongiaeforme* from rice fields growing in a unialgal liquid culture in flasks at the USDA ARS Exotic & Invasive Weeds Research Unit facility in Davis, California. We also have considerable data on water quality parameters (chloride, sulfate, calcium, magnesium, sodium, and potassium) for rice field water samples. This information will be used in experiments where the effects of selected water quality parameters, e.g., sodium, on *Nostoc spongiaeforme* can be tested. For example, *Nostoc spongiaeforme* will be exposed to a range of sodium concentrations at 25 C, 13:11 h light:dark cycle, 400 $\mu M\ m^{-2}\ s^{-1}$ for one week. There will be four replicate flasks at each of the following sodium concentrations: 0, 8.6, 25.8, 43.0, 60.2, 77.4, and 94.6 mg $L^{-1}$. These sodium concentrations are based on the water quality data which indicate that sodium concentrations in rice field water ranged from 3 to 96 mg $L^{-1}$. After one week, 10 ml of culture medium will be collected and the chlorophyll content determined following extraction with DMSO. The logarithms (base 2) of starting and ending chlorophyll concentrations will be used in linear regression versus time to determine the growth rate, yielding growth rates which have units of doublings $day^{-1}$. The effects of the tested parameter will be assessed using graphical and statistical methods (liner or nonlinear regression). All statistical calculations will be done using SAS software. Additional parameters to be tested in this type of experiment will be calcium, magnesium, inorganic carbon, zinc, and the herbicides Londax and Shark, alone and in combination. We will also assess the influence of temperature on *Nostoc spongiaeforme* growth.

We will use this approach and previously described outdoor "bucket" experiments to evaluate the algicidal properties of new aquatic herbicides that may enter the market.

*Objective 2: Determine the efficacy of zinc sulfate under field conditions for controlling species of algae in California rice fields.*

Field Experiment:

We will sample algal abundance in three rice fields. We will treat either entire fields or portions of fields with a solution of zinc sulfate so that the treatment is equivalent to 20 parts per million (ppm) zinc. This is the concentration equivalent to 50 pounds per acre of zinc sulfate which is currently used as a fertilizer treatment for some fields. Following treatment we will sample the fields for algal abundance at 2 to 3 day intervals for up to two weeks. Multiple samples will be collected within each field by collecting all of the algal material within 45 cm diameter tubes placed randomly in the area where algae are present. Dry weight will be determined (24 h at 80 C). Sub-samples of the algal mats will be collected, preserved in Lugol's solution, returned to the laboratory for species identification (in some cases identification will be determined only to genus). We will be especially alert to the presence of *Nostoc* spp. Algal species identification will be determined by Dr. Carole Lembi (Purdue University). We will compare before and after treatment mean algal biomass values to determine the effectiveness of this treatment.

Our ability to accomplish this experiment is dependent upon finding a grower willing to cooperate in this experiment.

*Objective3: Determine the effectiveness of incorporating phosphorus fertilizer at 1 inch deep in the soil for reducing algal growth.*

Field Experiment:

We have met with growers. At least one of them has agreed to apply phosphorus containing fertilizer in spring prior to the beginning of ground preparation. This will result in the phosphorus fertilizer being incorporated into the soil as the ground is prepared for planting. In adjacent fields the phosphorus containing fertilizer will be applied directly to the surface and there will be no attempt to incorporate it into the soil. Following flooding, we will sample the fields for algal abundance at 2 to 3 day intervals for up to two weeks. We will also collect water samples for phosphate analysis. Biomass samples will be collected and processed as described above. We will compare algal biomass in the surface applied and incorporated fields to determine the effectiveness of this approach. In addition, we will deploy 18 PVC rings (10 inches deep and 18 inches in diameter) along the edges of the fields. Twelve rings will have lime added to them at one of two levels. The highest lime addition level will simulate levels in the water column at normal agricultural use rates. The remaining six will serve as controls as no lime will be added to them. We will monitor algal growth in these rings by collecting digital photographs of them at 2 to 3 day intervals and by collecting the algal biomass after one week. We will also collect water samples for phosphate analysis. Studies from lakes indicate that lime additions will likely cause phosphate in the water to precipitate which should lead to reduced algal growth within the treated rings. This experiment will test this hypothesis. Data will be analyzed statistically with analysis of variance.

<u>Our ability to accomplish this experiment is dependent upon finding a grower willing to cooperate in this experiment, but as of this date we have relatively strong commitments from at least one grower.</u>

SUMMARY OF 2008 RESEARCH (major accomplishments), BY OBJECTIVE:

Please note some experimental designs and procedures were modified from the original proposal as we were not always able to obtain field sites for some of the proposed work.

Objective 1. *Determine the effects of rice field water quality parameters and algicides on growth of Nostoc spongiaeforme isolated from California rice fields under laboratory conditions.*

*Nostoc spongiaeforme* exhibited significantly reduced chlorophyll reflectance and biomass at harvest relative to untreated cultures when exposed to Rice Cop 5 (Figure 1).

Results from experiments with various levels of sodium indicated that *Nostoc spongiaeforme* achieved maximum growth at 1.98 mg $L^{-1}$ sodium. Sodium levels measured in water samples from 89 rice fields measured in 2007 indicated that the lowest sodium value was 3.0 mg $L^{-1;}$ the highest value was 94.1 mg $L^{-1}$; and the mean value was 15.0 mg $L^{-1}$. Thus it does not appear that sodium is likely to be a limiting factor for *Nostoc spongiaeforme* (Figure 2).

*Nostoc spongiaeforme* exhibited significantly reduced biomass at harvest relative to untreated cultures when exposed to elevated levels of iron applied as ferrous sulfate ($FeSO_4 \cdot 7H_2O$). The response was adequately described by statistically significant linear regression equations which were similar for three separate experiments (Figure 3).

There is very little published information on growth requirements for *N. spongiaeforme*. We grew *Nostoc spongiaeforme* in BG-11 medium at 24 combinations of light *(*22, 87, 162, and 400 μM $m^{-2}$ $s^{-1}$) and temperature (10, 15, 20, 25, 30, and 35 C). Results indicate that optimal growth occurred at 26 C and 227 μM $m^{-2}$ $s^{-1}$. The data indicate that *N. spongiaeforme* grows well at warm water temperatures and low irradiances, similar to those measured in rice fields during the critical thirty days following initial flooding and rice seeding. The data can be described by a quadratic equation relating light and temperature to growth. The equation is: $Z = a_0 + a_1X + a_2Y - a_3X^2 - a_4 XY - a_5 Y^2$, where Z = relative growth rate, X = irradiance, and Y = temperature. The values for the coefficients are: $a_0 = -0.848$, $a_1 = 0.0035$, $a_2 = 0.0896$, $a_3 = -0.0000073$, $a_4 = -0.000061$, $a_5 = -0.0017$. All coefficients are significant at the $P < 0.0001$, except $a_4$ which was not significantly different from zero, $P = 0.26$. The $R^2$ of the regression is 45.1%. The total number of relative growth rates used to calculate this response surface was 890.

For three fields, the water temperature records (Figure 5) include more than 2700 measurements collected over 57 to 59 days. For these fields, mean water temperatures were very similar (24.3, 24.6, and 25 C). The field with the lowest mean temperature varied from 8.3 to 43.4 C. The field with the intermediate temperature varied from 9.1 to

42.2 C, and the field with the highest mean value varied from 10.4 to 41.3 C. For a fourth field, data were only collected over a 15 day period. This field had a mean water temperature of 22.8 C and varied from 11.2 to 37.1 C. When the other fields were analyzed for the same 15-day period, their mean water temperatures were 21.5, 20.9, and 22.1 C. There did not appear to be very large differences in water temperature in these fields. An important factor contributing to water temperature variation is the addition of water to the field by pumping from surface or groundwater sources or by transferring water from one field to another.

Light levels (Figure 6) at the soil surface of field JSA1 were consistently less than 400 $\mu M\ m^{-2}\ s^{-1}$. Light levels at the soil surface for field K30 were frequently at or less than 400 $\mu M\ m^{-2}\ s^{-1}$, but there were some days with higher values later in the recording period. Rice field light levels at the soil surface may be influenced by several factors, including water level fluctuations, the movement of floating algal mats from place to place by intermittent winds, and the suspension of soil particles on windy days. Soil particles can remain suspended for some time afterward. In addition rice plants will affect light levels by shading as they grow taller.

The water temperature and light levels at the soil surface collected during 2008 indicate that the light levels and temperatures chosen for the laboratory studies were ecologically reasonable. Water temperatures in rice fields reached 41 to 43 C for brief periods on some days. Thus the maximum temperature used for the laboratory studies, 35 C, was less than those observed at the field sites.

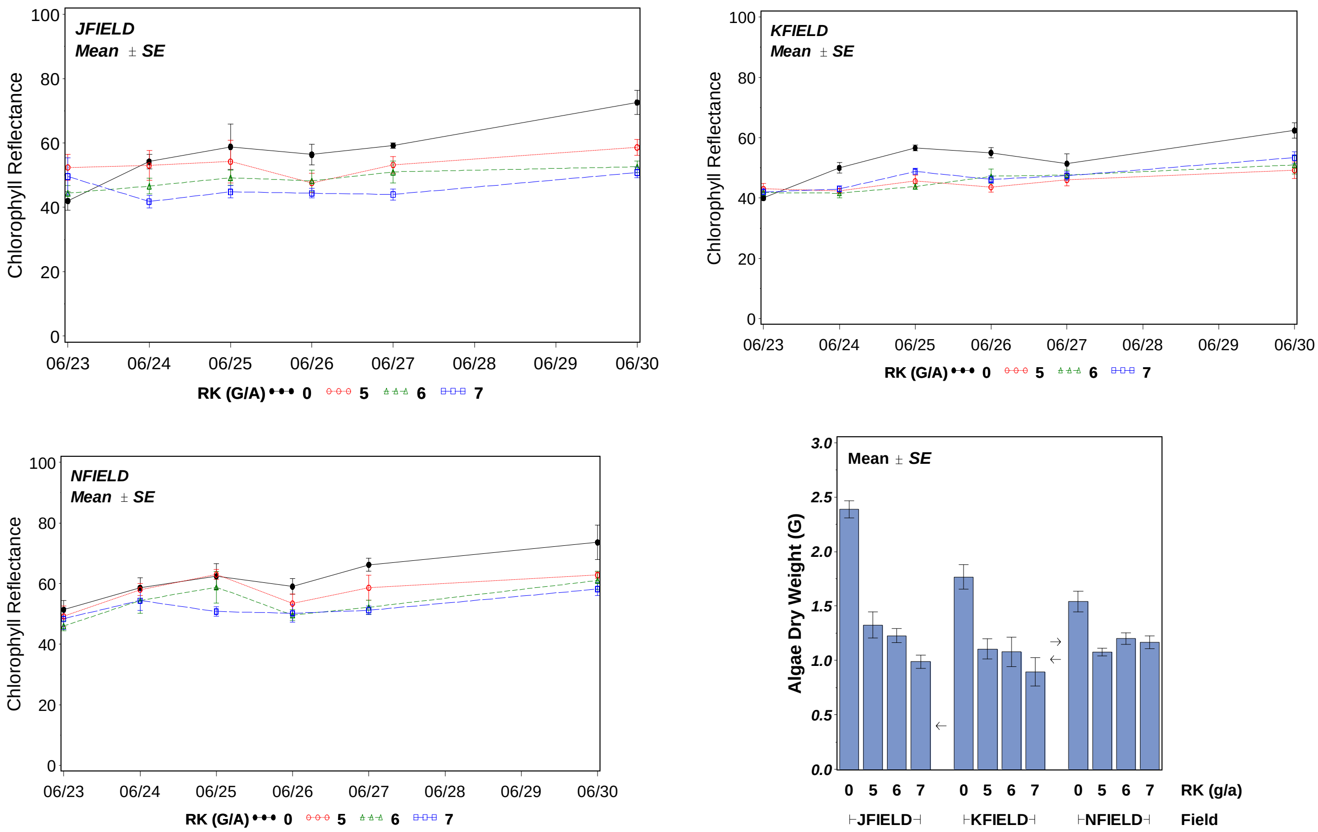



Figure 1. Response of field collected mixed algae (including *Nostoc spongiaeforme*) to treatment with the algicide, Rice Cop 5. The graphs at the top of the figure and the one in the lower left show chlorophyll reflectance measure on six days of the experiment. The values are the mean and standard error (SE) based on 5 replicates. The graph in the lower left hand corner shows the algal biomass at the end of the experiment. The small arrows represent the average starting biomass when the experiment was initiated. The number of rice cop 5 gallons per acre (G/A) is given in the legend for the figures.

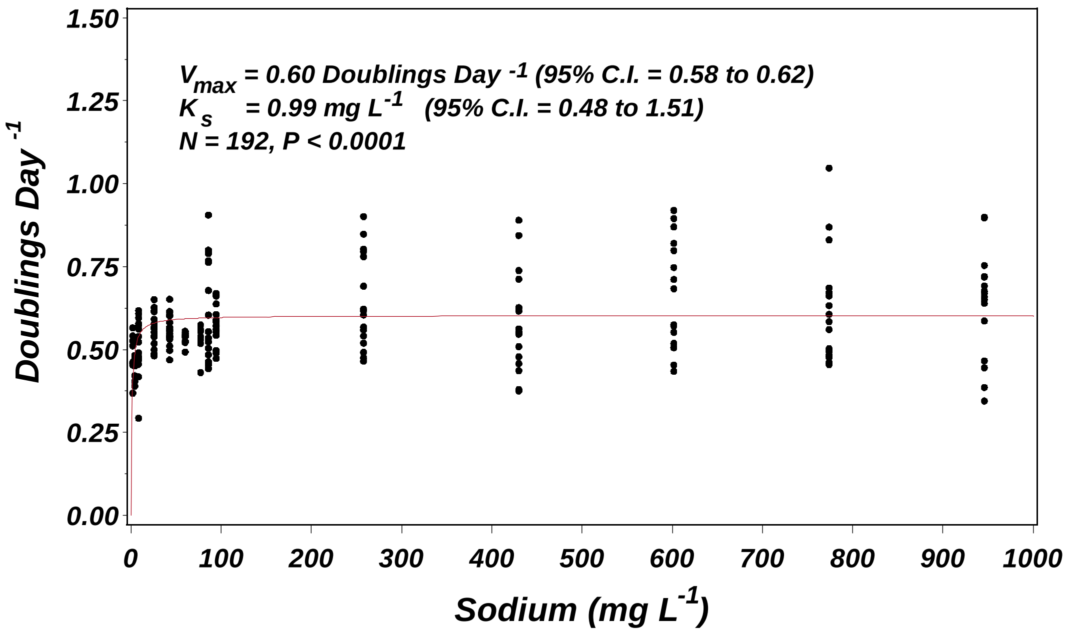



Figure 2. Upper graph depicts growth rates for *Nostoc spongiaeforme* versus sodium concentration in the culture medium. There were 8 or 16 replicate flasks at each of the following sodium concentrations: 2.15, 4.3, 8.3, 25.8, 43, 60.2, 77.4, 86, 94.6, 258, 430, 602, 774, or 946 mg $L^{-1}$. Values are the mean $\pm$ 1 standard error.

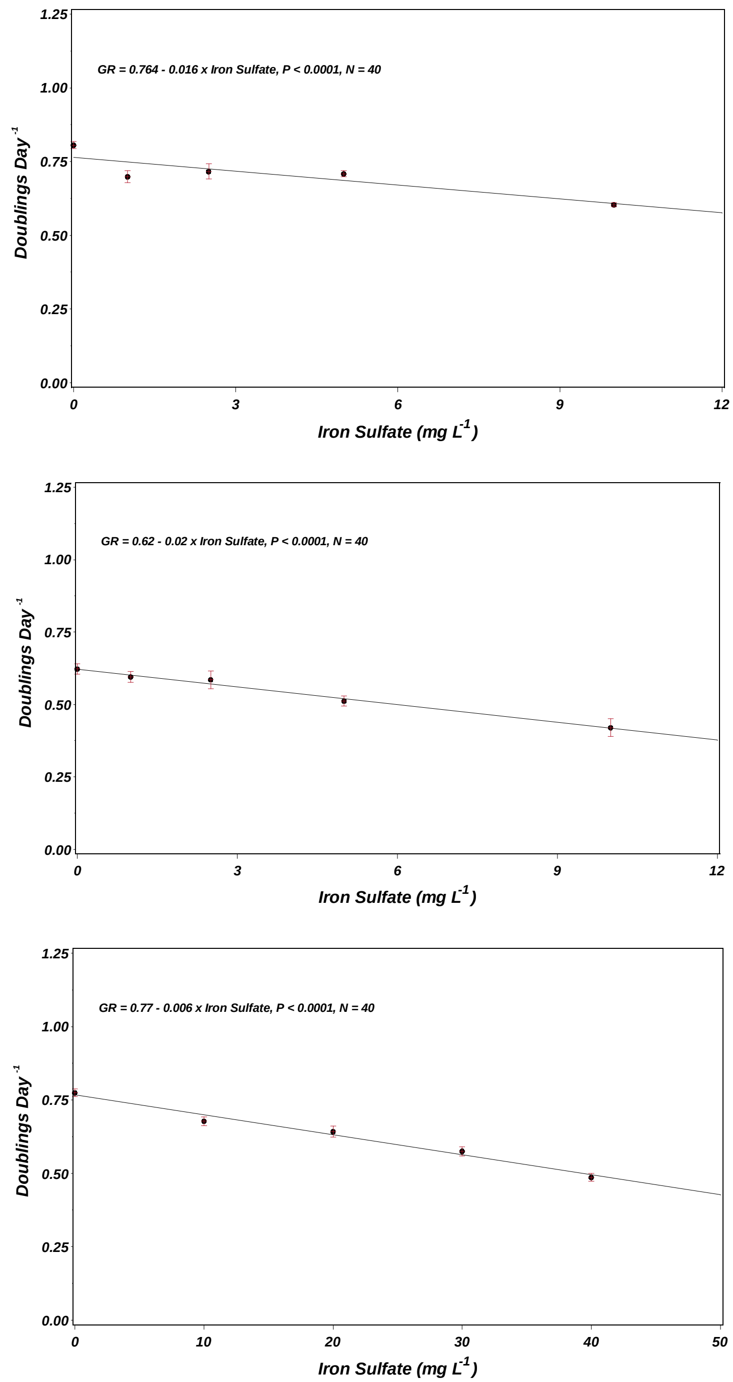



Figure 3. Response of *Nostoc spongiaeforme* (aka “black algae” or “elephant hide algae”) to different concentrations of  iron sulfate in the culture medium.

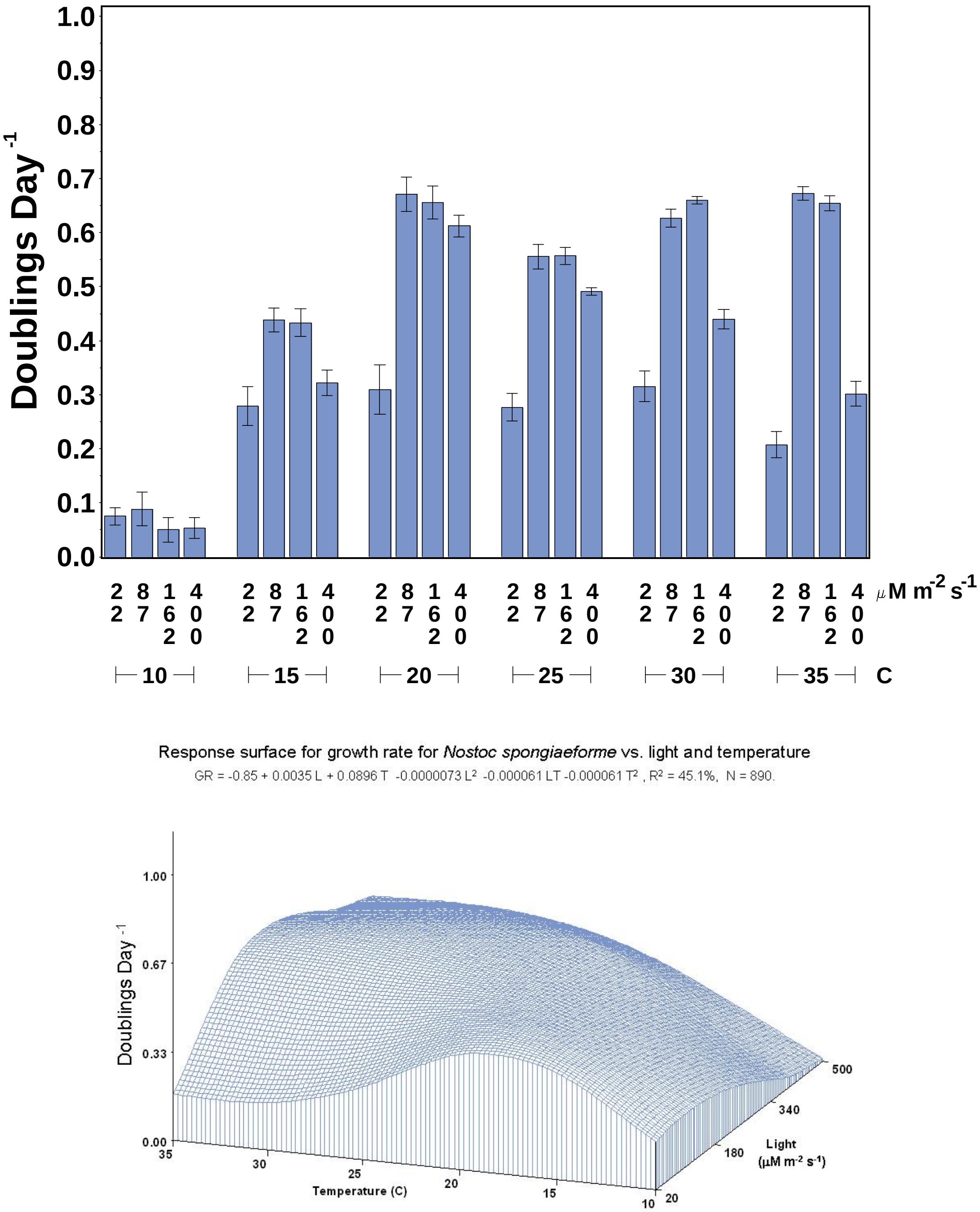



Figure 4. Upper graph shows relative growth rate (RGR; doublings day$^{-1}$) for *Nostoc spongiaeforme* as functions of temperature and irradiance. Values are the mean of 32 to 42 replicates. Error bars are the standard error of the mean. Lower graph shows a response surface relating light (L) and temperature (T) to the growth rate of *Nostoc spongiaeforme.* The equation for this surface is shown in the graph.

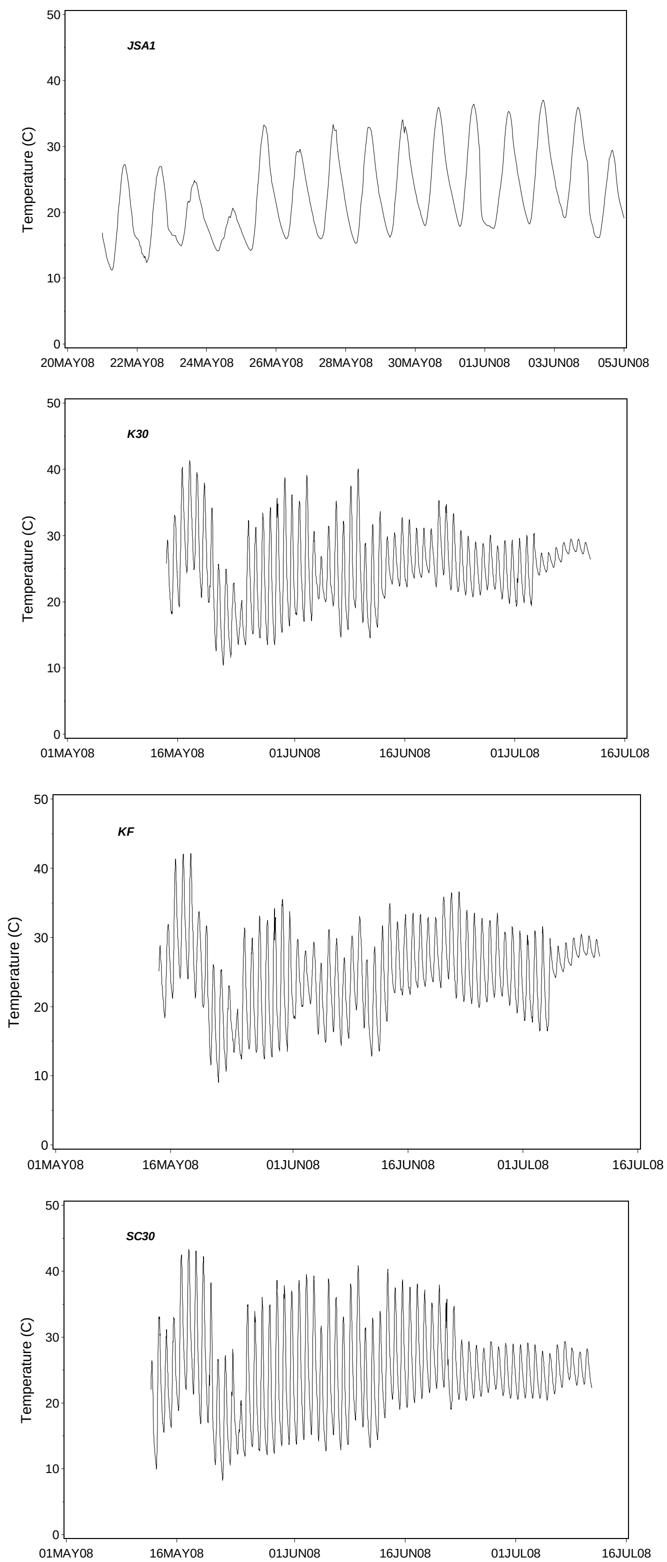



Figure 5. Daily temperature (C) variations in four northern California rice fields. Measurements were collected at 0.5 h intervals.

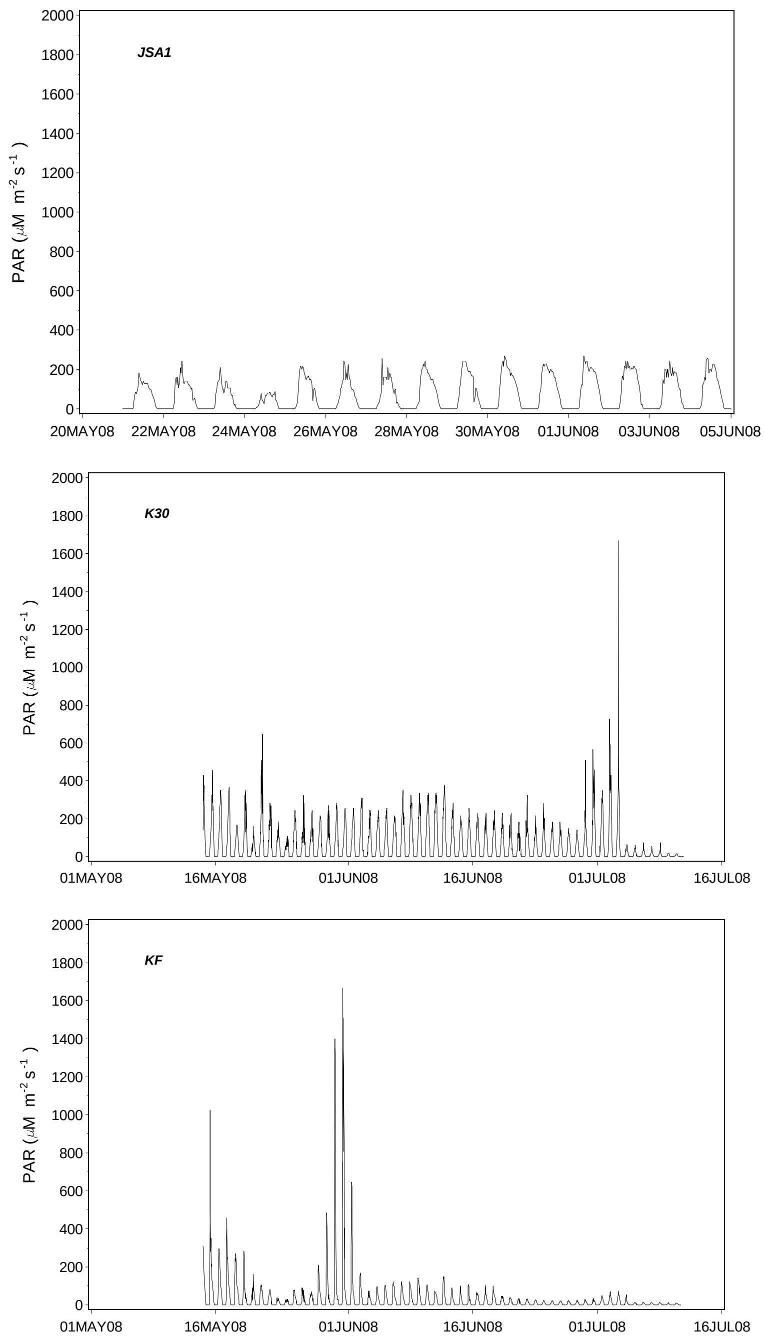



Figure 6. Daily irradiance (μM $m^{-2}$ $s^{-1}$) fluctuations in three northern California rice fields. Measurements were collected at 0.5 h intervals.

*Objective 2: Determine the efficacy of zinc sulfate under field conditions for controlling species of algae in California rice fields.*

We were unable to accomplish this objective.

*Objective3: Determine the effectiveness of incorporating phosphorus fertilizer at 1 inch deep in the soil for reducing algal growth.*

This study was modified from the original design included with the research proposal so a brief description of the methods is included here.

In this field study, we compared phosphate levels in water samples, algal biomass, and the potential of rice field water to be phosphate limited for samples from fields that received one of the following phosphate fertilizer regiments: conventional, i.e., liquid P applied directly to the surface followed by a roller; spring applied incorporated, i.e., phosphate fertilizer applied in the spring and incorporated as part of the spring ground work up; fall applied, i.e., phosphate fertilizer applied in the preceding fall and incorporated as part of the spring ground work up; and thirty-day delay, i.e., phosphate fertilizer applied thirty days after initial flooding of the field. These treatments were applied to the fields of three separate growers.

We collected water samples for phosphate determination at weekly intervals. Some fields were dry for an extended period due to an herbicide application. Near the end of the initial 30 day period we collected algal biomass using an 18-inch diameter PVC pipe. This was randomly placed near the edge of a field and all of the algae within it was collected and dried at 65 C for 48 h. Biomass values were converted to grams dry weight per square meter. The potential for phosphate limitation was evaluated by collecting water samples from each field within one or two days of flooding. The sample was returned to Davis where it was filtered using a glass fiber filter. Samples were frozen and later used in bioassay experiments. These experiments consisted of growing algae under standard conditions (see above). The “treatments” were: filtered rice field water with no added nutrients, filtered water with all of the BG-11 nutrients added except phosphate, except that various levels of phosphate were added (0.12, 0.5, 11, or 22 mg $L^{-1}$). Nine replicate flasks received each treatment and after one week algal biomass was estimated as chlorophyll A content. In fields of one of the growers we collected digital GPS located photographs along the entire perimeter of each of the “treatment” fields at weekly intervals. These photographs were examined and if any floating algal mats were present the GPS location was scored as a 1. If no floating algal mats were present the GPS location was scored as a 0. Based on these data we determined algal abundance over time by calculating the percentage of all photographs which had algae present.

Results of these measurements indicate that phosphate water concentration was lower in fields that received either the spring applied incorporated treatment or the thirty day delayed phosphate application (Figure 7). The thirty day delayed phosphate application had the lowest values. The fields which received the fall applied and the surface applied phosphate treatments at one grower’s site were essentially dry for most of the initial thirty days so no water samples were collected and those that were, were not comparable with the other fields.

Algal biomass was lowest for fields which received the thirty day delayed treatment (Figures 8, 9). These fields had from 72% to 88% less algal biomass than fields which received the conventional phosphate application, i.e., surface application of a liquid phosphate fertilizer followed by a roller.

Water samples from fields which received the thirty day delayed treatment were clearly phosphate limited for growth of *Nostoc spongiaeforme* while samples from the other treatments were less so (Figures 10, 11, 12).

The results of these measurements clearly show that phosphate water concentrations, algal biomass, and growth of *Nostoc spongiaeforme* (aka “black algae” or “elephant hide algae”) was greatly reduced by the thirty day delayed phosphate application scheme.

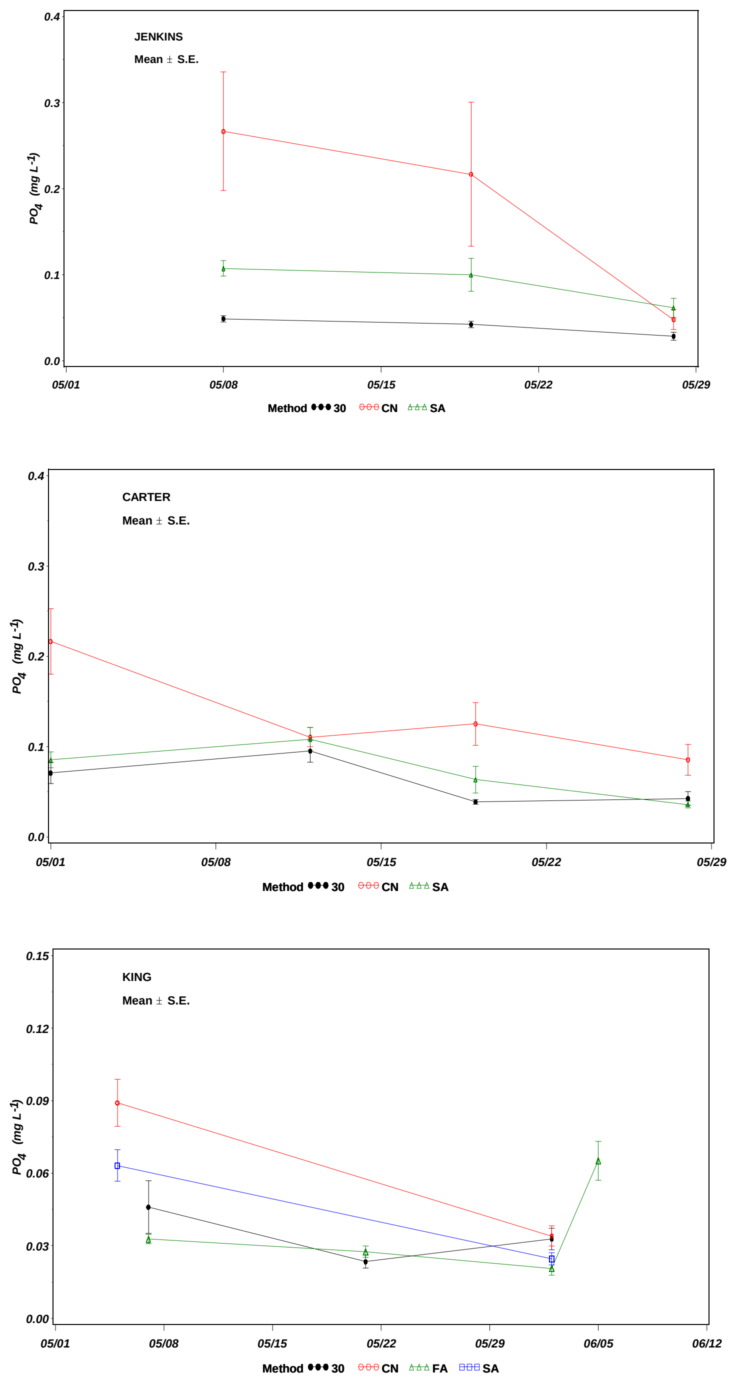



Figure 7. Phosphate concentration in water samples from rice fields that received alter phosphate fertilizer applications: 30 is phosphate applied 30 days after flooding; CN is surface applied liquid phosphate fertilizer followed by a roller; SA is phosphate fertilizer applied in the spring and incorporated into the soil as part of the spring ground work up; FA is fall applied phosphate fertilizer applied in the fall and incorporated into the soil as part of the spring ground work up.

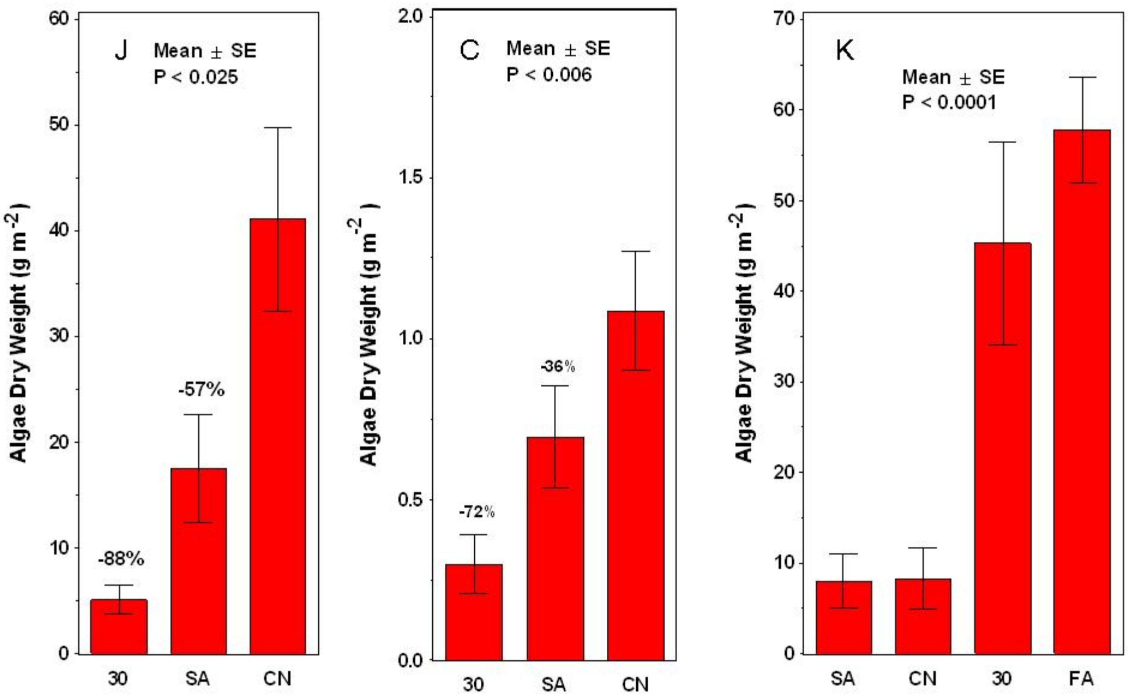



Figure 8. Algal biomass from rice fields that received altered phosphate fertilizer applications: 30 is phosphate applied 30 days after flooding; CN is surface applied liquid phosphate fertilizer followed by a roller; SA is phosphate fertilizer applied in the spring and incorporated into the soil as part of the spring ground work up; FA is fall applied phosphate fertilizer applied in the fall and incorporated into the soil as part of the spring ground work up. Different growers are denoted as “J”, “C”, or “K”.

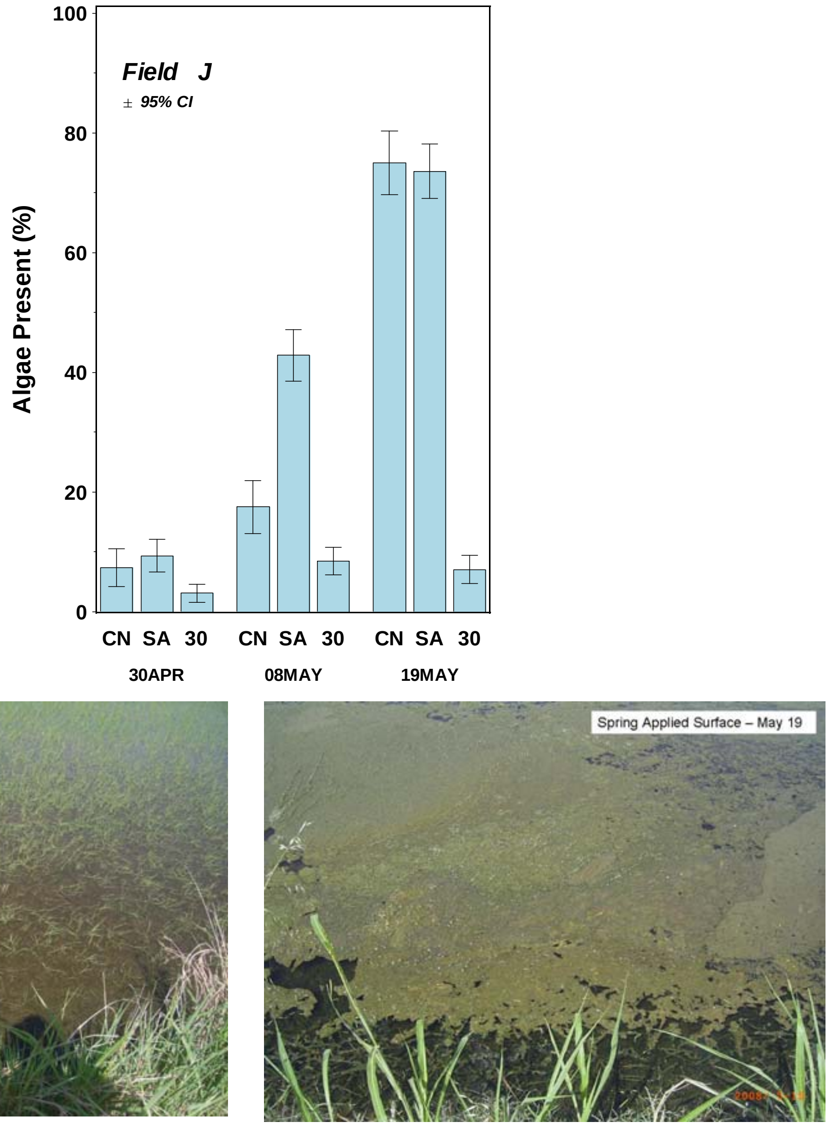
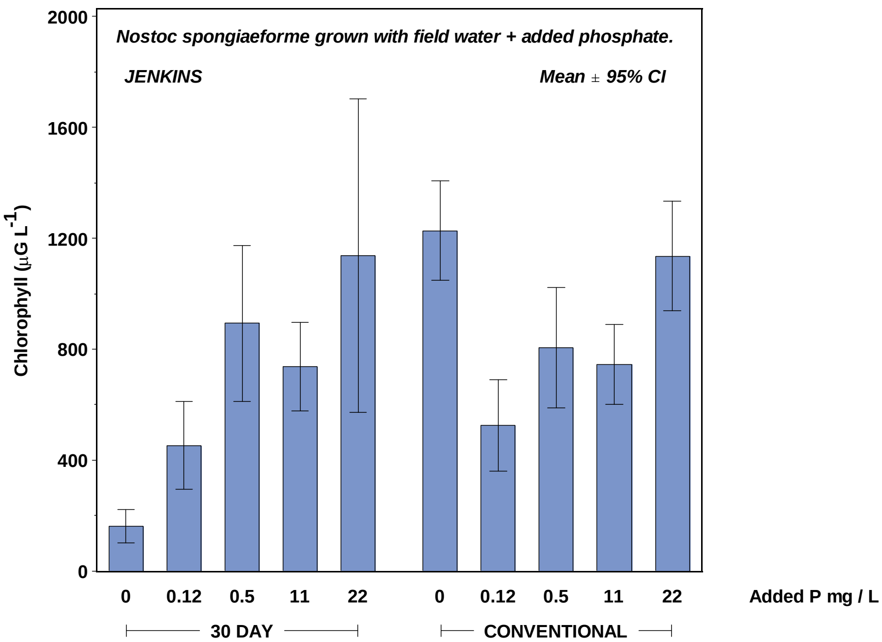



Figure 9. Changes over time in the abundance of floating algal mats in rice fields that received alter phosphate fertilizer applications: 30 is phosphate applied 30 days after flooding; CN is surface applied liquid phosphate fertilizer followed by a roller; SA is phosphate fertilizer applied in the spring and incorporated into the soil as part of the spring ground work up; FA is fall applied phosphate fertilizer applied in the fall and incorporated into the soil as part of the spring ground work up. The bottom pictures are examples of photographs from two treatments.



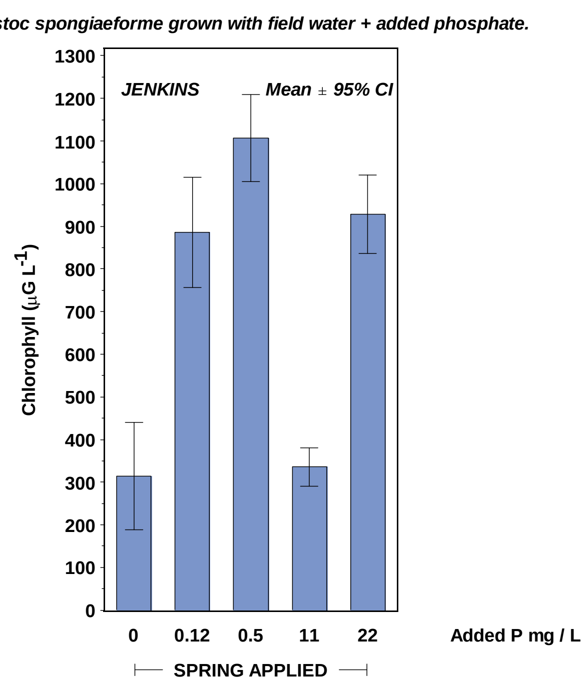
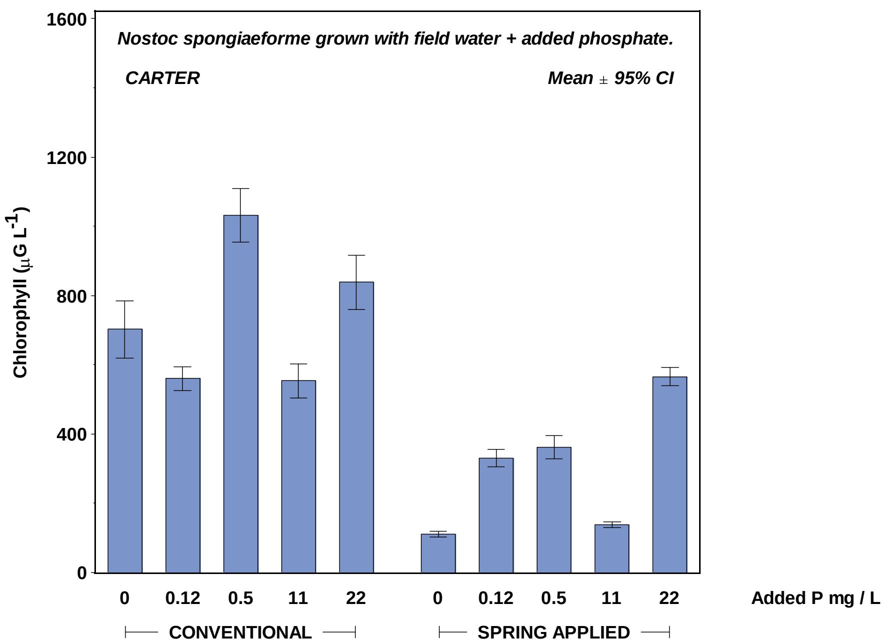




Figure 10. Results from nutrient limitation experiments for water from rice fields that received altered phosphate fertilizer applications: 30 Day is phosphate applied 30 days after flooding; Conventional is surface applied liquid phosphate fertilizer followed by a roller; Spring Applied is phosphate fertilizer applied in the spring and incorporated into the soil as part of the spring ground work up.



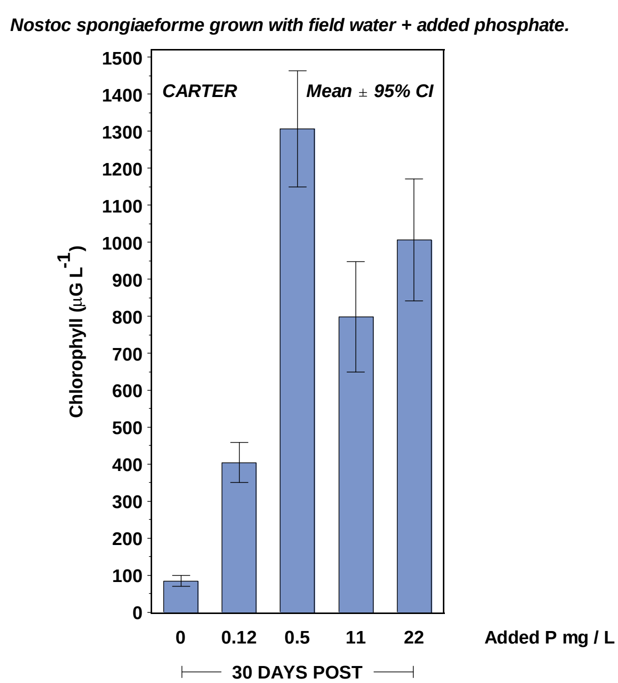




Figure 11. Results from nutrient limitation experiments for water from rice fields that received altered phosphate fertilizer applications: 30 Days Post is phosphate applied 30 days after flooding; Conventional is surface applied liquid phosphate fertilizer followed by a roller; Spring Applied is phosphate fertilizer applied in the spring and incorporated into the soil as part of the spring ground work up.

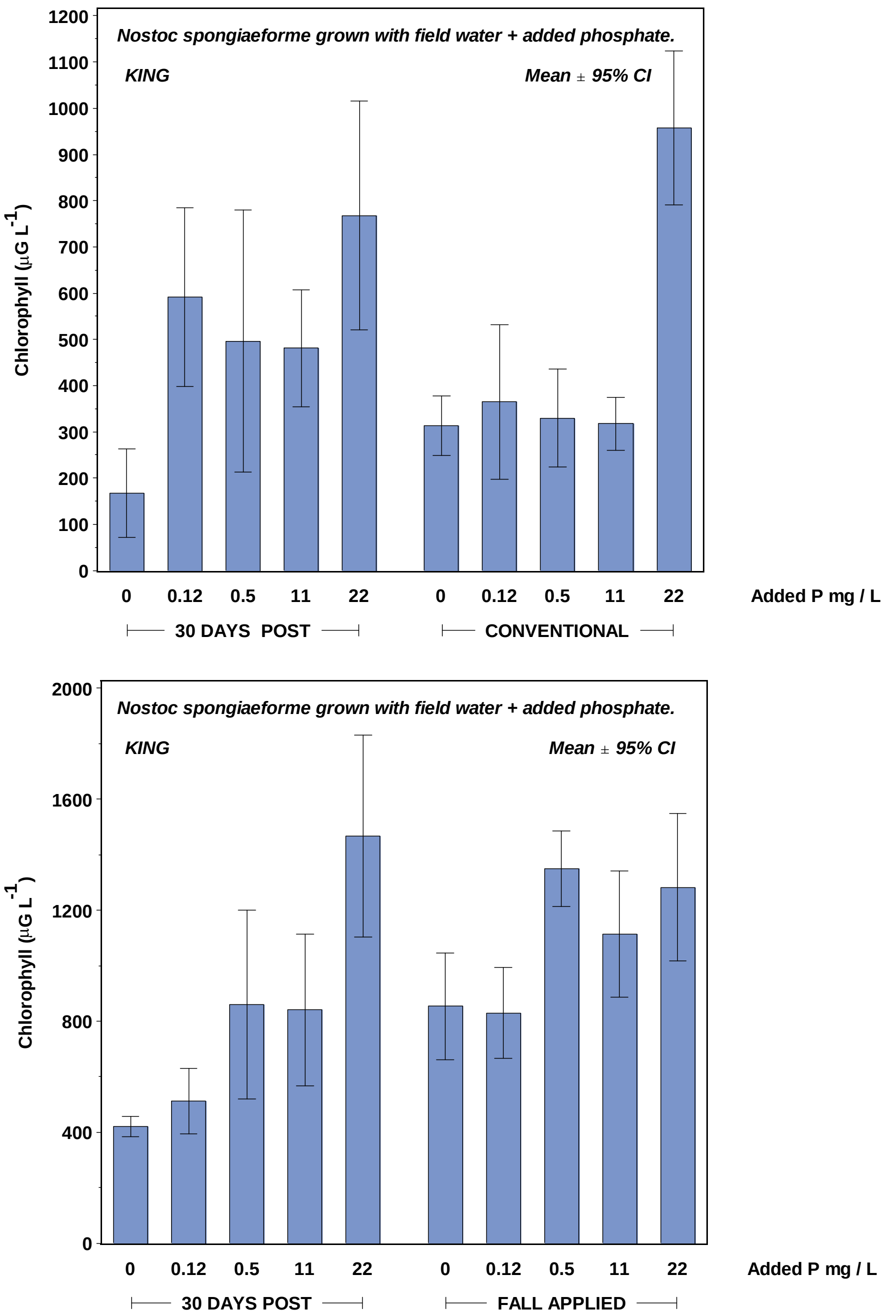



Figure 12. Results from nutrient limitation experiments for water from rice fields that received altered phosphate fertilizer applications: 30 Day Post is phosphate applied 30 days after flooding; Conventional is surface applied liquid phosphate fertilizer followed by a roller; FA is fall applied phosphate fertilizer applied in the fall and incorporated into the soil as part of the spring ground work up. The 30 Day Post data in the upper and lower graphs are from two separate checks, each of which received the 30 Day Post treatments.

PUBLICATIONS OR REPORTS:

- D. F. Spencer, C. A. Lembi, P. S. Liow, and D. D. Lubelski. 2008. Interactions between rice straw and copper: implications for algae management. 32nd Rice Technical Working Group, February 18-21, 2008, San Diego, California (poster presentation).

- Oral Report at California Rice Research Board Meeting, December 1, 2008, Davis, California.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

*Nostoc spongiaeforme* exhibited significantly reduced biomass at harvest relative to untreated cultures when exposed to Rice Cop 5. The effect of Rice Cop 5 appears to be algistatic. Results from experiments with various levels of sodium indicated that *Nostoc spongiaeforme* achieved maximum growth at 1.98 mg $L^{-1}$ sodium. Sodium levels measured in water samples from 89 rice fields measured in 2007 indicated that the lowest sodium value was 3.0 mg $L^{-1}$. Thus it does not appear that sodium is likely to be a limiting factor for *Nostoc spongiaeforme*. *Nostoc spongiaeforme* exhibited significantly reduced biomass at harvest relative to untreated cultures when exposed to elevated levels of iron applied as ferrous sulfate.

We grew *Nostoc spongiaeforme* in BG-11 medium at 24 combinations of light and temperature. Results indicate that optimal growth occurred at 26 C and 227 $\mu M\ m^{-2}\ s^{-1}$, suggesting that *Nostoc spongiaeforme* grows well at warm water temperatures and low irradiances, similar to those measured in rice fields during the critical thirty days following initial flooding and rice seeding. Data from three fields, collected over 57 to 59 days indicate that mean water temperatures were very similar (24.3, 24.6, and 25 C). The field with the lowest mean temperature varied from 8.3 to 43.4 C. The field with the intermediate temperature varied from 9.1 to 42.2 C, and the field with the highest mean value varied from 10.4 to 41.3 C. Light levels at the soil surface of one field were consistently less than 400 $\mu M\ m^{-2}\ s^{-1}$. Light levels at the soil surface for a second field were frequently at or less than 400 $\mu M\ m^{-2}\ s^{-1}$, but there were some days with higher values later in the recording period. The water temperature and light levels at the soil surface collected during 2008 indicate that the light levels and temperatures chosen for the laboratory studies were ecologically reasonable. Water temperatures in rice fields reached 41 to 43 C for brief periods on some days. Thus the maximum temperature used for the laboratory studies, 35 C, was less than those observed at the field sites.

Results of measurements from field study comparing four different phosphate fertilizer application methods (phosphate applied 30 days after flooding; surface applied liquid phosphate fertilizer followed by a roller; phosphate fertilizer applied in the spring and incorporated into the soil as part of the spring ground work up; or phosphate fertilizer applied in the fall and incorporated into the soil as part of the spring ground work up) indicate that phosphate water concentration was lower in fields that received either the spring applied incorporated treatment or the thirty day delayed phosphate application. The thirty day delayed phosphate application had the lowest values. The fields which received the fall applied and the surface applied phosphate treatments at one grower's site were essentially dry for most of the initial thirty days so no water samples were collected and those that were, were not comparable with the other fields. Algal biomass was lowest for fields which received the thirty day delayed treatment. These fields had from 72% to 88% less algal biomass than fields which received the conventional phosphate application, i.e., surface application of a liquid phosphate fertilizer followed by a roller. Water samples from fields which received the thirty day delayed treatment were clearly phosphate limited for growth of *Nostoc spongiaeforme* while samples from the other treatments were less so.

The results of these measurements clearly show that phosphate water concentrations, algal biomass, and growth of *Nostoc spongiaeforme* (aka "black algae" or "elephant hide algae") were greatly reduced by the thirty day delayed phosphate application method.