### ANNUAL REPORT COMPREHENSIVE RESEARCH ON RICE January 1, 2010 - December 31, 2010

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

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LEVEL OF 2010 FUNDING: \$14,160

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

Objective 1. Determine the effects of rice field water quality parameters and algaecides 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. We also have considerable data on water quality parameters (pH, chloride, sulfate, calcium, magnesium, and potassium) for rice field water samples. This information will be used in experiments where the effects of selected water quality parameters, e.g., sulfate concentration on *Nostoc spongiaeforme* growth can be tested. The basic experimental design will involve growing *Nostoc spongiaeforme* under a range of concentrations of the chosen water quality parameter or algaecide at 25 C, 13:11 h light:dark cycle, 400  $\mu$ M m<sup>-2</sup> s<sup>-1</sup> for one week. There will be four replicate flasks at each of the test concentrations. These test concentrations will be based on the water quality data measured in rice field water samples in 2007. 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<sup>-1</sup>. 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.

We will use this approach and previously described outdoor "bucket" experiments to evaluate the algicidal properties of Hydrothol 191 and other new aquatic herbicides that may enter the market.

# *Objective 2: Determine the effectiveness of four phosphorus fertilizer application methods for reducing algal growth.*

#### Field Experiments:

Data from the 2008 field experiment indicates that algal growth was limited when the phosphorus fertilizer was applied 30 days after the initial flooding of the field. We need to obtain additional results from a greater variety of fields both geographically and in terms of spring soil phosphorus levels. We

have met with growers. At least one of them has agreed to apply phosphorus containing fertilizer in the following manner: P-fertilizer applied when the rice plants have emerged or shortly thereafter; surface applied liquid P fertilizer followed by a roller; and P fertilizer applied in the spring and incorporated into the soil as part of the spring ground work up. Following flooding, we will sample the fields for algal abundance at 2 to 3 day intervals for up to four weeks using a GPS camera. We will also collect water samples for phosphate analysis. Biomass samples will be collected and processed as described above. We will also use filtered rice field water in bioassay experiments to determine if phosphorus is limiting to the growth of *Nostoc spongiaeforme*. We will compare algal biomass in the four phosphorus fertilizer application methods to determine the effectiveness of this approach in a greater diversity of rice fields than we used in 2008. Where appropriate, data will be analyzed statistically with analysis of variance.

A second experiment will be conducted in cooperation with Dr. Bruce Lindquist, UC Davis, Department of Plant Sciences. This experiment will be conducted in part of a check where P fertilizer application has been delayed. Microcosms (metal rings inserted into the soil) will be installed. All of the microcosms will thus initially contain water with phosphate concentrations that reflect only the background levels. Four microcosms will be assigned to each of the following treatments: no added P fertilizer, P fertilizer added 7 days after flooding, P fertilizer added 14 days after flooding, P fertilizer added 21 days after flooding, and P fertilizer added 28 days after flooding. Each day the surface of each microcosm will be photographed using a time lapse camera. These images will be used to determine the abundance of floating algae and cyanobacteria. One week after each P fertilizer addition, the biomass of all of the algae/cyanobacteria in each microcosm will be harvested and dried at 80 C for 48 hours. Periodically, water samples will be collected for phosphate-P analysis and rice leaf samples analyzed for tissue P content. At the end of the growing season rice yield will be determined. Where appropriate, data will be analyzed statistically with analysis of variance. If sufficient sites are found we will replicate this entire experiment in additional checks.

Our ability to accomplish the above experiments is dependent upon finding additional growers willing to cooperate in this experiment, but as of this date we have relatively strong commitments from at least one grower.

#### SUMMARY OF 2010 RESEARCH (major accomplishments), BY OBJECTIVE:

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

We conducted a total of four "bucket" experiments to evaluate the algicide CopCheck. The range of CopCheck concentrations used were 0, 0.18, 0.36, 0.72, and 1.08 parts per million (ppm) as copper. Results from these experiments indicate that CopCheck exposure significantly affected final algal dry weights in two experiments (Figures 1 and 4 and Table 1). Examination of the data does not show a consistent response to the amount of copper in the test. In experiments 5 and 9, there was no effect on final algal dry weight (Figures 2 and 3 and Table 1). The results of these outdoor experiments indicate that CopCheck at concentrations up to 1 ppm did not consistently kill *N. spongiaeforme*. At present this algaecide is not labeled for use in California rice fields and it is not clear how it may fit into algal control strategies for these systems.

Table 1. Results of analysis of variance for testing the null hypothesis that Copcheck did not affect growth
of rice field algae (measured as dry weight after one week) in four separate outdoor bucket experiments.
There were five CopCheck concentrations (0, 0.18, 0.36, 0.72, and 1.08 ppm, parts per million as copper)
with four replicate buckets randomly assigned to each treatment for each experiment. "DF" indicates the
degrees of freedom, "SS" is the Sum of Squares, and "Pr > F" is probability of obtaining a larger F-value
as calculated by Proc GLM in SAS (2004) <sup>A</sup> .

Experiment	Source	DF	SS	F-value	PR > F
3	CopCheck	4	1.53	4.11	0.02
3	Error	15	1.39		
5	CopCheck	4	0.17	1.79	0.18
5	Error	15	0.35		
9	CopCheck	4	0.28	2.24	0.11
9	Error	15	0.47		
13	CopCheck	4	0.26	6.18	0.004
13	Error	15	0.16		

<sup>A</sup> SAS Institute, Inc. 2004. SAS OnlineDoc® 9.1.3. SAS Institute, Inc., Cary, NC.

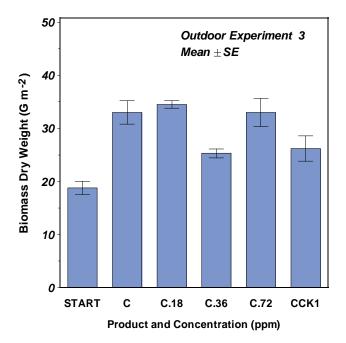


Figure 1. Biomass of rice field algae in outdoor experiment 3. Means are based on four replicates. The control or untreated is labeled as "C", CopCheck concentrations were 0.18, 0.36, 0.72, and 1.08 ppm (parts per million) as copper. They are labeled as "C.18", "C.36", "C.72" and "CCK1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

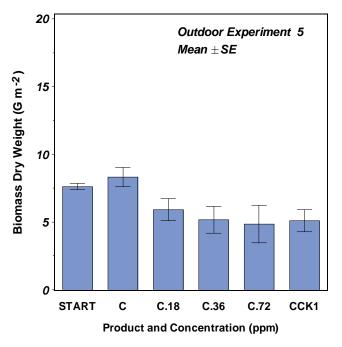


Figure 2. Biomass of rice field algae in outdoor experiment 5. Means are based on four replicates. The control or untreated is labeled as "C", CopCheck concentrations were 0.18, 0.36, 0.72, and 1.08 ppm (parts per million) as copper. They are labeled as "C.18", "C.36", "C.72" and "CCK1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

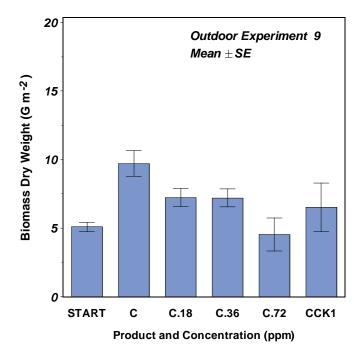


Figure 3. Biomass of rice field algae in outdoor experiment 9. Means are based on four replicates. The control or untreated is labeled as "C", CopCheck concentrations were 0.18, 0.36, 0.72, and 1.08 ppm (parts per million) as copper. They are labeled as "C.18", "C.36", "C.72" and "CCK1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

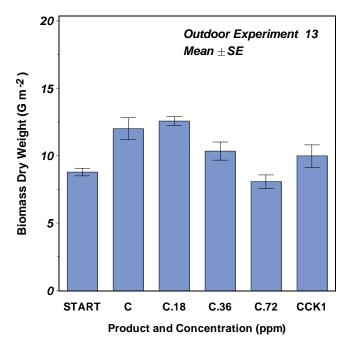


Figure 4. Biomass of rice field algae in outdoor experiment 13. Means are based on four replicates. The control or untreated is labeled as "C", CopCheck concentrations were 0.18, 0.36, 0.72, and 1.08 ppm (parts per million) as copper. They are labeled as "C.18", "C.36", "C.72" and "CCK1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

We conducted an additional eight "bucket" experiments with the algicides, KOMEEN, K-TEA, NAUTIQUE, compounds 41, and 70. The algae used in these experiments were primarily *Nostoc spongiaeforme*, but other species were present as well. Algae and water used in these experiments were collected from rice fields near the site where the buckets were placed. Experiments were conducted at a site about 5 miles north of Gridley, California. The materials were added to the buckets at concentrations of 0, 0.5 and 1 parts per million (ppm) as copper. Results from the Komeen, K-Tea, and Nautique experiments indicate that the treatments significantly affected final algal dry weights in two experiments (Figures 1 and 4 and Table 2). Examination of the data does not show a consistent response to the amount of copper in the test. However, there appeared to be increased impact when the starting biomass was lower. In two other experiments there was no effect on final algal dry weight (Figures 2 and 3 and Table 1); however in these experiments indicate that these materials at concentrations up to 1 ppm did not consistently kill *N. spongiaeforme*. At present these algicides are not labeled for use in California rice fields and it is not clear how it may fit into algal control strategies for these systems.

Table 1. Results of analysis of variance for testing the null hypothesis that various algaecides did not affect growth of rice field algae (measured as dry weight and expressed as grams per square meter, after one week) in outdoor bucket experiments. There were four experiments (numbers 1, 2, 4, and 12) where the treatments were KOMEEN (0.5, 1 ppm), K-TEA (0.5, 1 ppm), and NAUTIQUE (0.5 and 1 ppm) plus untreated controls. There were four experiments (numbers 6, 8, 10, and 14) where the treatments were SP4041 (0.5, 1 ppm) and SP4170 (0.5 and 1 ppm) plus untreated controls. Four replicate buckets were randomly assigned to each treatment for each experiment. "DF" indicates the degrees of freedom, "SS" is the Sum of Squares, and "Pr > F" is probability of obtaining a larger F-value as calculated by Proc GLM in SAS (2004)<sup>A</sup>.

Experiment	Source	DF	SS	F-value	PR > F
KOMEEN	, K-TEA, AND N	IAUTIQUE			
1	Treatment	6	1.48	0.77	0.60
1	Error	21	6.71		
2	Treatment	6	2.52	1.07	0.41
2	Error	21	8.28		
4	Treatment	6	0.65	6.75	0.0004
4	Error	21	0.34		
12	Treatment	6	0.73	5.57	0.0014
12	Error	21	0.46		
SP4041 and SP4	170	_			
6	Treatment	4	0.18	0.9	0.49
6	Error	15	0.75	0.9	0.12
8	Treatment	4	0.19	5.35	0.007
8	Error	15	0.13		
10	Treatment	4	0.57	36.24	0.0001
10	Error	15	0.06		
14	Error	15	0.37		

<sup>A</sup> SAS Institute, Inc. 2004. SAS OnlineDoc® 9.1.3. SAS Institute, Inc., Cary, NC.

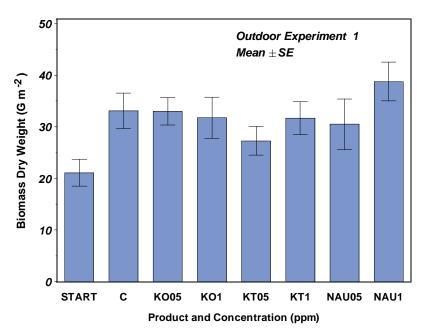


Figure 5. Biomass of rice field algae in outdoor experiment 1. Means are based on four replicates. The control or untreated is labeled as "C". KOMEEN concentrations were 0.5 and 1.0 ppm (parts per million) as copper. They are labeled as "KO05" and "KO1", respectively. K-TEA concentrations were 0.5 and 1.0 ppm as copper. They are labeled as "KT05" and "KT1", respectively. NAUTIQUE concentrations were 0.5 and 1.0 ppm. They are labeled as "NAU05" and "NAU1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

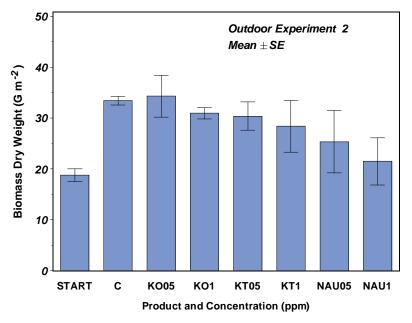


Figure 6. Biomass of rice field algae in outdoor experiment 2. Means are based on four replicates. The control or untreated is labeled as "C". KOMEEN concentrations were 0.5 and 1.0 ppm (parts per million) as copper. They are labeled as "KO05" and "KO1", respectively. K-TEA concentrations were 0.5 and 1.0 ppm. They are labeled as "KT05" and "KT1", respectively. NAUTIQUE concentrations were 0.5 and 1.0 ppm. They are labeled as "NAU05" and "NAU1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

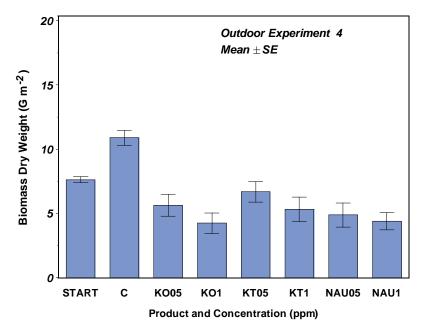


Figure 7. Biomass of rice field algae in outdoor experiment 2. Means are based on four replicates. The control or untreated is labeled as "C". KOMEEN concentrations were 0.5 and 1.0 ppm (parts per million) as copper. They are labeled as "KO05" and "KO1", respectively. K-TEA concentrations were 0.5 and 1.0 ppm. They are labeled as "KT05" and "KT1", respectively. NAUTIQUE concentrations were 0.5 and 1.0 ppm. They are labeled as "NAU05" and "NAU1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

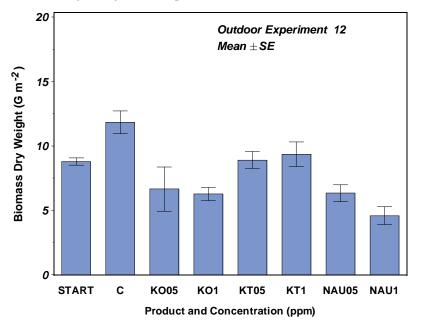


Figure 8. Biomass of rice field algae in outdoor experiment 2. Means are based on four replicates. The control or untreated is labeled as "C". KOMEEN concentrations were 0.5 and 1.0 ppm (parts per million) as copper. They are labeled as "KO05" and "KO1", respectively. K-TEA concentrations were 0.5 and 1.0 ppm. They are labeled as "KT05" and "KT1", respectively. NAUTIQUE concentrations were 0.5 and 1.0 ppm. They are labeled as "NAU05" and "NAU1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

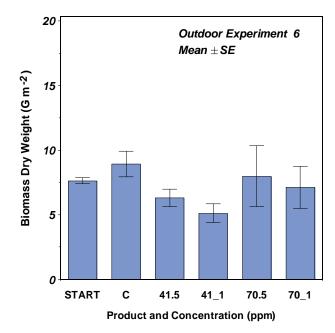


Figure 9. Biomass of rice field algae in outdoor experiment 6. Means are based on four replicates. The control or untreated is labeled as "C". SP4041 concentrations were 0.5 and 1.0 ppm (parts per million) as copper. They are labeled as "41.5" and "41\_1", respectively. SP4170 concentrations were 0.5 and 1.0 ppm. They are labeled as "70.5" and "70\_1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

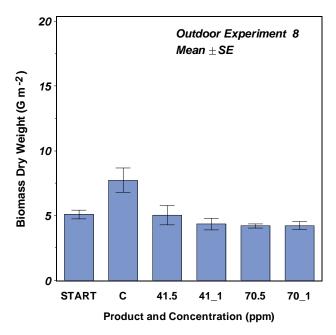


Figure 10. Biomass of rice field algae in outdoor experiment 6. Means are based on four replicates. The control or untreated is labeled as "C". SP4041 concentrations were 0.5 and 1.0 ppm (parts per million) as copper. They are labeled as "41.5" and "41\_1", respectively. SP4170 concentrations were 0.5 and 1.0 ppm. They are labeled as "70.5" and "70\_1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

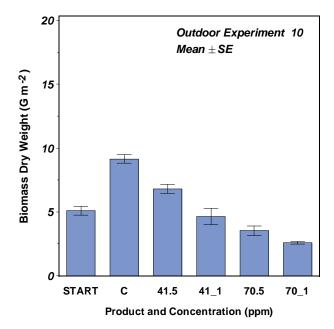


Figure 11. Biomass of rice field algae in outdoor experiment 6. Means are based on four replicates. The control or untreated is labeled as "C". SP4041 concentrations were 0.5 and 1.0 ppm (parts per million) as copper. They are labeled as "41.5" and "41\_1", respectively. SP4170 concentrations were 0.5 and 1.0 ppm. They are labeled as "70.5" and "70\_1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

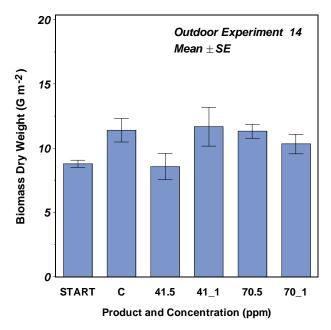


Figure 9. Biomass of rice field algae in outdoor experiment 6. Means are based on four replicates. The control or untreated is labeled as "C". SP4041 concentrations were 0.5 and 1.0 ppm (parts per million) as copper. They are labeled as "41.5" and "41\_1", respectively. SP4170 concentrations were 0.5 and 1.0 ppm. They are labeled as "70.5" and "70\_1", respectively. The bar labeled "START" shows the amount algae present at the beginning of the experiment.

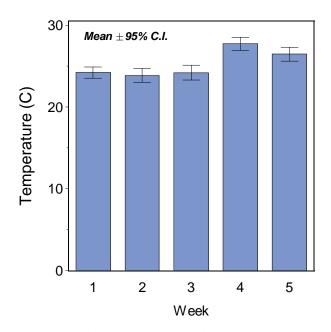


Figure 10. Weekly mean temperatures in the buckets used in the outdoor experiments. Experiment 1 was conducted during week 1. Experiment 2 was conducted during week 2. Experiments 4 and 6 were conducted during week 3. Experiments 8 and 10 were conducted during week 4. Experiments 12 and 14 were conducted during week 5. Experiment 3 was conducted during week 2. Experiment 5 was conducted during week 3. Experiment 9 was conducted during week 4. Experiment 13 was conducted during week 5. The mean temperatures are based on temperature readings collected every 30 minutes during each week using a HOBO Pendant data logger which was placed in a bucket containing the same amount of water as the treatment buckets.

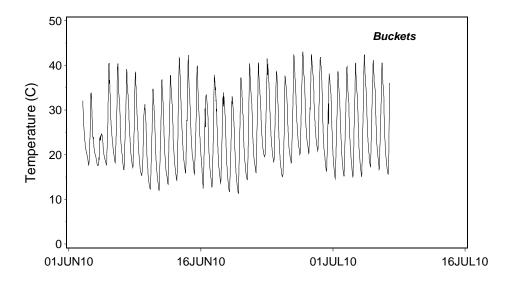


Figure 11. Water temperatures in the buckets used in the outdoor experiments. The temperature readings were collected every 30 minutes using a HOBO Pendant data logger which was placed in a bucket containing the same amount of water as the treatment buckets.

We also conducted laboratory experiments to test the effect of cyanophage (virus) on growth of *Nostoc spongiaeforme*. Cyanophage were obtained from American Type Culture Collection (ATCC 29106-B1 and 27893-B16). They were grown in a host strain of *Anabaena* sp from Pasteur Culture Collection (PCC 7120) at 30 C. *Anabaena* sp. cultures were inoculated with cyanophage at mid-exponential growth stage. After 2 to 3 days the supernatant from the *Anabaena* sp. cultures were used to inoculate *Nostoc spongiaeforme* cultures. These procedures were adapted from a paper by Baker et al. (2006, Applied and Environmental Microbiology 72: 5713 – 5719). Baker et al (2006) developed molecular markers for these cyanophages (B1 and B16) and stated that these freshwater cyanophages are virulent myoviruses that are infectious to the filamentous cyanobacterial genera *Anabaena* and *Nostoc*.

We conducted several experiments in which the cyanophage were added alone or in combination. In every case we found that there was no effect on *Nostoc spongiaeforme* growth rates which were in the typical range of values that we have observed under the conditions that we used, 0.5 to 0.7 doublings day<sup>-1</sup>. This lack of effect required that we check the presence of cyanophage in the supernatant of the *Anabaena* sp. cultures which we used for to inoculate the *Nostoc* cultures. In cooperation with Dr. M. Sudarshana (USDA ARS plant pathologist, Davis, California) molecular techniques as well as staining and observation with fluorescence microscopy are being employed to quantify the level of cyanophage in the inoculums. These studies are underway at this writing so we cannot yet make a definitive conclusion about the effect that cyanophage have on growth of *Nostoc spongiaeforme*.

#### *Objective 2: Determine the effect of P fertilizer on algal growth.*

We secured access to two rice fields located north and west of Gridley, California (Figure 12). Flooding began in one field around May 13, 2010 and around May 26, 2010 in the other. The field that flooded earlier had low water temperatures during the two week period after flooding (Figure 13). As a result no floating algal mats were present during the first phase of the study in this field. Results of an analysis of variance for the experiment designated "RYSTR" indicate that adding P fertilizer significantly increased biomass of algae and cyanobacteria present in the rings (Figure 15). This agrees with previous work on the relationship between rice field algae and phosphate water concentration in rice field water samples. The day that the samples were collected either 7 or 14 days after P fertilizer addition also had a significant effect on biomass of algae and cyanobacteria. This is likely due to increased water temperatures over time. A similar response was observed for the statistical analysis of the data from the experiment designated "MYERS." However, in this case there were additional significant effects. The significant effect "DATE ADDED" and the interaction between "DATE ADDED" and "DAY" reflect the lack of algae in the rings which received the P fertilizer treatment immediately after the field was flooded. This was likely due to the lower water temperatures in the rings due to weather conditions (Figure 13).

One other aspect of the biomass results that deserves consideration relates to the similarity in biomass for the 14 day samples in the "RYSTR" experiment (Figure 15). Between June 17 and June 20 while this experiment was still in progress, the water level in the field (and thus the rings) dropped considerably, to the point that bare soil was exposed (Figure 16). This likely would have altered both algal growth and phosphate availability. This information was available to us because of the "spy" cameras that had been installed above the rings and which recorded a photograph every hour. In future experiments it may be worthwhile to install this type of monitoring system especially in rice fields which often undergo "unpredictable" water level fluctuations.

The mean phosphate ( $PO_4$ -P) concentrations in water samples from the rings were higher shortly after addition of the P fertilizer (Figure 14). Water phosphate concentrations were either 21 or 31 times greater than those in untreated rings in samples collected the same day that the fertilizer was added. Mean water phosphate concentrations decreased markedly after one week. This was likely due to uptake by algae which grew during the period. The high levels of phosphate in treated rings with available information, and are strong evidence that the well-known relationship between phosphate concentration and algal biomass applies to rice fields as well as other types of aquatic habitats. The management implication from these experiments is that reducing phosphate concentrations in rice field water will result in reduced algal biomass. Previous work in other rice fields indicate that this reduction can be accomplished by either incorporating P fertilizer into the soil or by delaying its application until rice plants are well above the water's surface.



Figure 12. Layout of field experiments at two rice fields. Note the presence of "spy" cameras which were above the treated rings for 14 days following application of the P fertilizer.

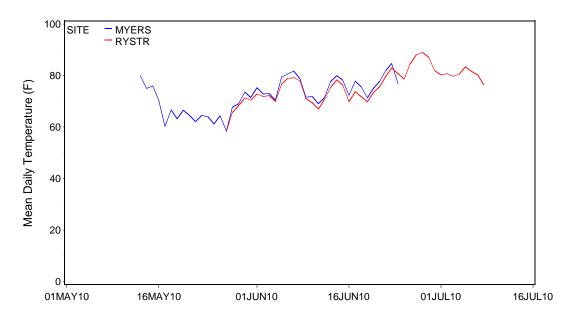


Figure 13. Mean daily water temperature (F) in 29.5 inch diameter rings used in the field experiments at two sites.

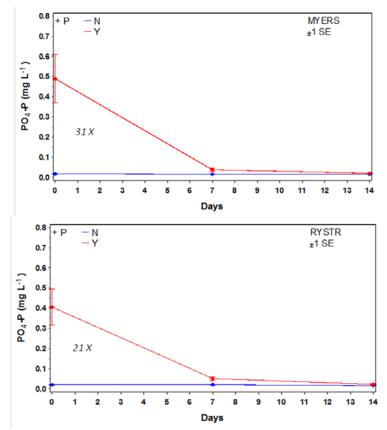


Figure 14. Mean  $PO_4$ -P concentration of water inside rings used in the field experiments at two sites. Day 0 is the day that the P fertilizer was applied.

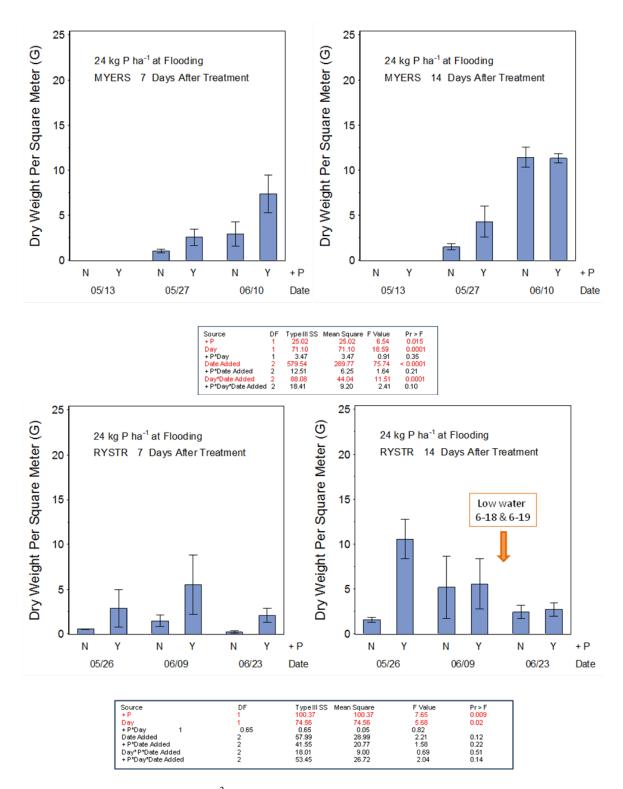


Figure 15. Mean algal biomass (G m<sup>-2</sup>) and statistical analysis for field experiments at two sites. "Date" refers to the date the P fertilizer was added. A "Y" indicates that P was added. A "N" means that P was not added to the rings.

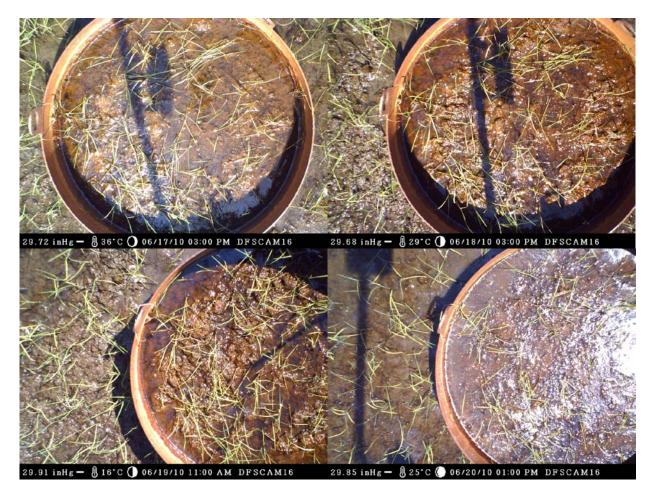


Figure 16. Time sequence of photographs or rings in the "RYSTR" experiment showing a drop in the water level between June 17 and June 20, 2010.

### PUBLICATIONS OR REPORTS:

- D. F. Spencer, P. S. Liow, C. A. Lembi, 2009. Effect of a combination of two rice herbicides, Londax and Shark, on the cyanobacterium, *Nostoc spongiaeforme*. *Journal of Aquatic Plant Management* 47: 145-147.
- Spencer, D. F. P-S Liow, and C. A. Lembi. 2010. Influence of Hydrothol 191 on the cyanobacterium, *Nostoc spongiaeforme*, and the green algal, *Hydrodictyon* sp., in field and laboratory experiments" Journal of Aquatic Plant Management (submitted)
- Spencer, D. F. P-S Liow, and C. A. Lembi. 2010. Growth response to temperature and light in *Nostoc spongiaeforme* (Cyanobacteria)" Journal of Freshwater Ecology (submitted)
- Presented a poster at the UC Rice Field Day, August 25, 2010 at the Rice Experiment Station, Lundy, M. E., D. F. Spencer, C. van Kessel and B. A. Lindquist. "Managing Phosphorus Fertilizer to Reduce Algae, Maintain Water Quality and Sustain Yields in Direct-Seeded Rice."
- Oral Report, Lundy, M. E., D. F. Spencer, C. van Kessel and B. A. Lindquist. "Managing Phosphorus Fertilizer to Reduce Algae, Maintain Water Quality and Sustain Yields in Direct-Seeded Rice" presented at the 2010 International Annual Meetings of the American Society of Agronomy, Crop Science Society of America, and the Soil Science Society of America, October 31 to November 4, 2010, Long Beach, California.
- Oral report, Managing *Nostoc* in rice fields, March 8, 2010, Big Valley Ag Services PCA Meeting, Gridley, California.
- Presented an invited talk on "Managing algae and cyanobacteria in California rice fields." California Weed Science Society, January 12, 2010, Visalia, CA
- Presented an invited oral report at the University of California 2010 Annual Rice Grower Meetings, "Assessing alternative methods for managing algae in rice." There were three meetings, January 25, 8:30 AM at Richvale, January 25, at 1:30 PM at Yuba City, and January 26, at Glenn, CA.
- Oral Report at California Rice Research Board Meeting, December 1, 2010, Davis, California.

## CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

We conducted four experiments with CopCheck concentrations up to 1 part per million (ppm). Algae and water used in these experiments were collected from rice fields near the site where the buckets were placed. Experiments were conducted at a site about 5 miles north of Gridley, California. The results indicate that CopCheck at these concentrations did not consistently kill *N. spongiaeforme*. We conducted an additional eight experiments with KOMEEN, K-TEA, NAUTIQUE, compound 41, and compound 70 at concentrations of 0, 0.5, or 1 ppm. These materials did not consistently kill *N. spongiaeforme*. Results are likely related to rice field water quality, especially the presence of decomposing rice straw from the previous growing season. At present these algicides are not labeled for use in California rice fields and it is not clear how they may fit into algal control strategies for these systems.

We also conducted laboratory experiments to test the effect of viruses on growth of *Nostoc spongiaeforme*. Preliminary results indicate no effect on growth rates. However, this interpretation requires additional test to confirm the presence of the virus in the algal cultures. These procedures are still underway at this writing, so it is not possible to conclude that the viruses did or did not affect of *Nostoc spongiaeforme*.

Results from two field experiments in which phosphate containing fertilizer was either added or not added to large metal rings in rice fields indicate that adding P fertilizer significantly increased the amount of algae. Water phosphate concentrations in rings that received the P fertilizer were either 21 or 31 times greater than those in rings that did not for samples collected the same day that the fertilizer was added. These results agree with previous work on the relationship between rice field algae and phosphate water concentration in rice field water samples. The management implication from these experiments is that reducing phosphate concentrations in rice field water will result in reduced algal biomass. Previous work in rice field systems indicate that this reduction can be accomplished by either incorporating P fertilizer into the soil or by delaying its application until rice plants are well above the water's surface.