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TECHNICAL REPORT

Influence of a non-copper algicide on the cyanobacterium, *Nostoc spongiaeforme*, and the green alga, *Hydrodictyon reticulatum*, in field and laboratory experiments

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Abstract Cyanobacteria and algae grow in California rice fields where they form large mats that may smother seedlings or cause them to dislodge, resulting in reduced rice stand establishment and potential yield loss. The most troublesome species of cyanobacteria is *Nostoc spongiaeforme*. It is very difficult to control using currently accepted methods, i.e., aerial applications of copper sulfate. A non-copper algicide, the mono (*N*,*N*-dimethylalkylamine) salt of endothall has been suggested as an alternative method for controlling nuisance cyanobacterial and algal growths in California rice fields. The purpose of the experiments described here was to evaluate the effect of the mono (*N*,*N*-dimethylalkylamine) salt of endothall on growth of *N. spongiaeforme* and the green alga, *Hydrodictyon reticulatum*. In laboratory experiments, the mono (*N*,*N*-dimethylalkylamine) salt of endothall reduced *N. spongiaeforme* growth at 0.3 mg L^{-1}. This effect was removed when rice straw was added to the growth medium, indicating that the rice straw may have introduced bacteria capable of degrading the mono (*N*,*N*-dimethylalkylamine) salt of endothall. In outdoor experiments, which used rice field water containing decomposing rice straw, the mono (*N*,*N*-dimethylalkylamine) salt of endothall concentrations between 0 and 5 mg L^{-1} had little effect on *N. spongiaeforme*. In contrast, *H. reticulatum* exhibited injury symptoms at 1 mg L^{-1} or greater. However, *H. reticulatum* recovered by the end of the 7-day exposure. It is not clear how this algicide will be useful in the management of *N. spongiaeforme* or *H. reticulatum* in California rice fields.

Keywords Algae management · Algicide · Rice straw · Microbial degradation · Hydrothol 191

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Introduction

Excessive algae biomass may lead to yield reductions in California rice fields. The traditional approach to managing algae involves aerial applications of copper sulfate. There are environmental concerns about copper accumulation in rice field soils and the long-term sustainability of rice culture (Hill et al. 2006), and growers have reported that copper sulfate treatments have recently (within the last 10 years) not provided adequate control (Spencer et al. 2006). Thus, a search for alternative and effective methods for managing algae (primarily *Nostoc spongiaeforme*) has been initiated by the California Rice Research Board. This report covers evaluation of the effects of the mono (*N*,*N*-dimethylalkylamine) salt of endothall (Hydrothol 191) on the cyanobacterium, *N. spongiaeforme*, and the green alga, *Hydrodictyon reticulatum*, in field and laboratory experiments.

Hydrothol 191 (hereafter referred to as H191) is a US EPA registered algicide, although it is not yet registered for use in California rice fields. According to the product label, rates of 0.05–0.3 parts per million (mg L^{-1}) are effective for algae control, although environmental conditions and types of algae being treated may influence susceptibility. In California, H191 is permitted for the management of macroscopic green algae, *Cladophora*, *Pithophora*, *Spirogyra*, and *Chara*. The active ingredient is a mono (*N*,*N*-dimethylalkylamine) salt of endothall (7-oxabicyclo[2,2,1]heptane-2,3-dicarbolylic acid). It is quite soluble in water and has a short persistence is aquatic environments. It does not accumulate in sediments or bioaccumulate and half-lives from 1–8 days have been reported (Westerdahl and Getsinger 1988). Degradation by the action of microorganisms appears to be the dominant process which controls its persistence in aquatic systems (Hiltibran 1962; Westerdahl and Getsinger 1988). Because other management approaches have not proven effective for the control of *N. spongiaeforme* in California rice fields (Spencer and Lembi 2007; Spencer et al. 2009), we performed field and laboratory experiments to test the hypothesis that H191 would have an impact on *N. spongiaeforme* and/or *H. reticulatum*, both of which may be problematic for California rice production.

Materials and methods

We tested a range of H191 concentrations, including values greater than the highest concentration listed for use on the H191 label, which is 5 mg L^{-1}, for their effect on *N. spongiaeforme*. The experiments were conducted adjacent to two rice fields. One field (N) was located ~5 km west of Gridley, California and the second (R) was ~11 km north of Gridley, California. These experiments were conducted from June 3 to July 1, 2009. This period is part of the 30 days following initial flooding of the rice fields, which is the period when excessive algal growth is most problematic for growers. In these experiments, 3.78 L of water from the adjacent rice field (or its source water) was put into each of 24, 19-L white plastic buckets. The water in field N originally came from ground water and the water in field R was originally from an irrigation canal. Water from field R had a mean pH of 6.9 with range 5.8–8.2; mean total alkalinity was 23.3 mg L^{-1} $CaCO_3$ with a range of 4–100 mg L^{-1} $CaCO_3$, mean PO_4-P was 0.13 mg L^{-1} and the range was 0.01–1.68 mg L^{-1} based on 51 measurements from this field. For water from field N, the mean pH was 7.2 with a range of 6.5–8.2; mean total alkalinity was 24 mg L^{-1} $CaCO_3$, range 6–58 mg L^{-1} $CaCO_3$, mean PO_4-P was 0.38 mg L^{-1} and the range was 0.02–2.57 mg L^{-1} based on 33 water samples collected from the field during the duration of these experiments. Samples of algae (almost entirely *N. spongiaeforme*, but other species were present as well) were collected from either field N or R and placed in the buckets. Initial algal dry weights of 5 similar samples were determined for each experiment. For these experiments, the mean starting weight of algae per bucket was 0.7 g dry weight ($N = 64$, $SD = 0.6$). This corresponds to 10 g m^{-2} dry weight, which is 31 % of the mean algal biomass value of 32.7 g m^{-2} dry weight reported by Spencer et al. (2006) for northern California rice fields. The appropriate dose of H191 was added using an automatic pipette. There were three sets of experiments (four experiments per set) each covering a different range of H191 concentrations. In one set the final concentrations were 0, 0.05, 0.1, 0.2, 0.3, and 0.5 mg L^{-1}. A second set used 0, 1, 2, 3, 4, and 5 mg L^{-1}; and a third set used 0, 6, 7, 8, 9, and 10 mg L^{-1}. For each experiment, buckets serving as controls (0 mg L^{-1}) did not receive H191. There were four buckets which received each treatment (6 treatments,

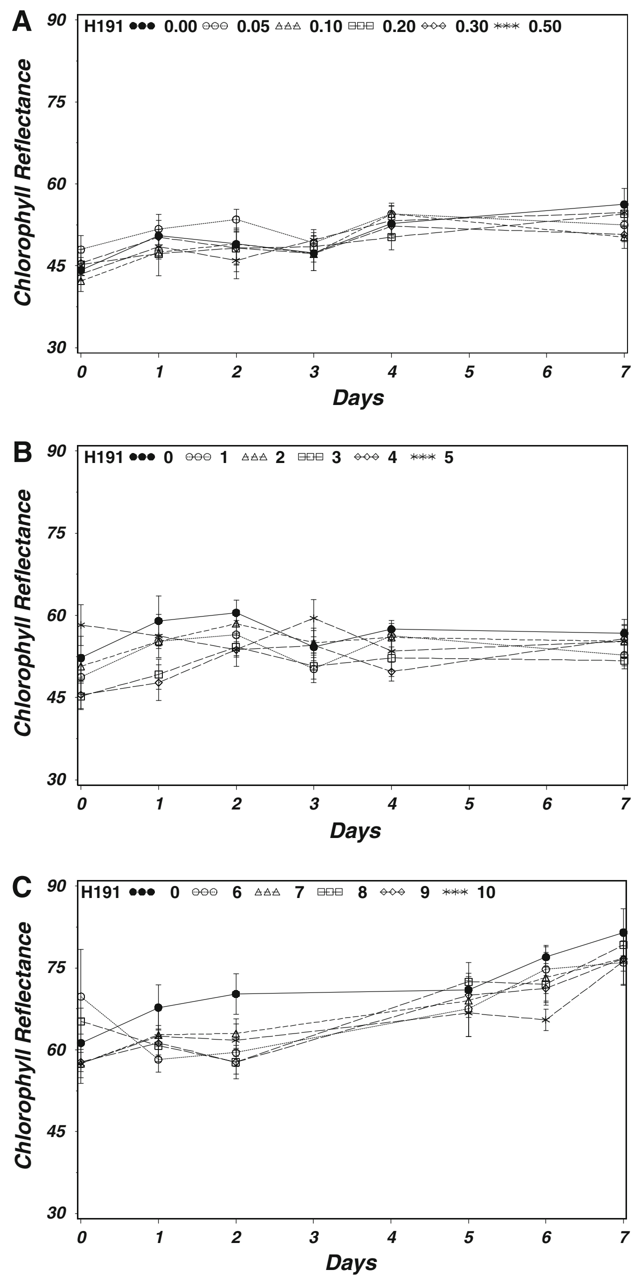

Fig. 1 Chlorophyll reflectance measurements for field-collected *Nostoc spongiaeforme* exposed to various concentrations (**a** 0–0.5 mg L^{-1}, **b** 0–5 mg L^{-1}, **c** 0–10 mg L^{-1}) of Hydrothol 191 (H191). Plotted values are the mean ± standard error, based on four replications. Experiments were conducted adjacent to field N. The *horizontal axis* is in days with day zero being the day of treatment

see above, per experiment) so each experiment used a total of 24 buckets. The buckets were left in place for 1 week. The four experiments within each of the three sets of experiments were run consecutively, so 4 weeks were required to complete each set. We used a Field Scout CM1000 chlorophyll meter (Spectrum Technologies, Inc., Plainfield, Illinois) to measure the chlorophyll reflectance from the algae daily except over the weekend. At the end of each experiment, the contents of each bucket was filtered through a 6-cm diameter metal strainer (1-mm mesh size), and the weight of algae was determined after drying at 65 °C for 48 h. We also set up a separate bucket which contained 3.78 L of water only. In this bucket we placed a UA-002-64 Hobo Temperature/Light Pendant® data logger (Onset, Bourne, Massachusetts) to record water temperature.

We used the MIXED procedure (Litell et al. 2006) to calculate a repeated measures analysis of variance for chlorophyll reflectance values, considering H191 levels and days as fixed effects and bucket as a random effect for each experiment. The covariance structure was modeled as autoregressive (1) and the denominator degrees of freedom were estimated by the Kenward–Roger procedure. The GLM procedure was used to calculate an analysis of variance for the algal dry weights at the end of the 1-week exposure for each experiment (SAS Institute Inc. 2004). Tests were judged as significant at significance levels less than 0.05, but exact probability levels are presented in the results.

In addition, we conducted experiments using the green alga, *H. reticulatum.* Algal material was collected from a rice field where we have previously observed it to grow and cause a considerable problem to the grower. These experiments were conducted outdoors in Davis, California at the Exotic and Invasive Weeds Research Unit facilities. In this study, the algae were grown in buckets filled with ground water from a local well instead of rice field water. Water from this source typically has the following characteristics: pH 7.8, PO_4-P 0.15 mg l^{-1}, K, 2 mg L^{-1}, SO_4 20 mg L^{-1}, Ca, 34 mg L^{-1}, Mg, 34.2 mg L^{-1}, total alkalinity 215 mg L^{-1} (Spencer and Rejmanek 2010). For the three sets of experiments, H191 treatment concentrations and number of replications were as described above; however, each experiment was only repeated twice, resulting in six individual experiments. Chlorophyll reflectance was measured each day of the experiment. Final algal dry weights were determined as described above. For these experiments, the starting weight of algae per bucket was 0.6 g dry weight ($N = 15$, SD = 0.11). This corresponds to 8 g m^{-2} dry weight, which is 25 % of the mean value of 32.7 g m^{-2} dry weight reported by Spencer et al. (2006) for northern California rice fields. Statistical analyses were as described above.

To better understand the interaction between H191 and *N. spongiaeforme*, we conducted two additional sets of laboratory experiments. In these experiments *N. spongiaeforme* was grown in 500-mL glass flasks containing 250 mL of sterile BG-11 culture medium (without the nitrate component since *N. spongiaeforme* fixes atmospheric nitrogen), which had been pH adjusted to 6.8. In the first set of 2 experiments, flasks were randomly assigned to one of the following H191 treatment levels: 0, 0.1, 0.3, 0.5, 1, 3, 5, 6, 8, and 10 mg L^{-1}. Then a 2-mL inoculum of *N. spongiaeforme* was added to each flask. Flasks were placed on a shaker table (ca 25 rpm) in a growth chamber maintained at 25 °C, 400 μM m^{-2} s^{-1}, and a 12:12 h L:D cycle. The cultures were allowed to grow for 1 week, at which time two 10-mL samples were collected from each flask which had been swirled to mix the contents just before sample collection. Each 10-mL aliquot was filtered through a glass fiber filter. Chlorophyll content was determined following extraction with DMSO (Spencer and Ksander 1987). Growth rates were calculated by linear regression of the $\log_2$ of the initial and final chlorophyll values versus time in days (SAS Institute Inc. 2004). Initial chlorophyll values were based on determinations from five separate aliquots collected from the stock culture at the

Table 1 Mixed model analysis of variance results for chlorophyll reflectance for field-collected *Nostoc spongiaeforme* exposed to various concentration of Hydrothol 191

Experiment	Hydrothol range (mg L^{-1})	Field	Source	Num DF	Den DF	*F* value	Prob.
1	0–0.5	N	H191	5	17.7	0.50	0.77
			Days	5	66.6	13.86	<0.0001
			H191 * days	25	70	0.76	0.77
3	0–5	N	H191	5	18	1.92	0.14
			Days	5	61.1	5.50	0.0003
			H191 * days	25	67.6	1.51	0.09
12	0–10	N	H191	5	18.8	0.98	0.46
			Days	5	66.4	17.88	<0.0001
			H191 * days	25	70.5	1.44	0.12

For experiment 1, concentrations were 0, 0.05, 0.1, 0.2, 0.3, 0.5 mg L^{-1}. For experiment 3, concentrations were 0, 1, 2, 3, 4, 5 mg L^{-1}. For experiment 12, concentrations were 0, 6, 7, 8, 9, 10 mg L^{-1}

beginning of the experiment. An additional set of two experiments was performed using the same procedures except that the experiments followed a two-way analysis of variance design with four replicate flasks at each combination of the main effects, H191 (0, 0.1, 0.3, 3, and 8 mg L^{-1}) and rice straw (0.5 g L^{-1}).

Results and discussion

Results from the twelve experiments with *N. spongiaeforme* were similar; results from three of them are shown here (Fig. 1; Table 1). Based on the chlorophyll reflectance measurements, H191 did not have a detrimental effect on *N. spongiaeforme* by the end of the 7-day exposures used in these experiments. There was a slight decrease in chlorophyll reflectance after 1–3 days exposure at the highest concentrations examined (Fig. 1). However, *N. spongiaeforme* chlorophyll reflectance then began to increase. Results of the statistical analysis (Table 1) indicate that there was a significant increase in chlorophyll reflectance over time, but it was not attributable to the H191 treatment. The lack of effect of H191 exposure was also reflected in final algal dry weights (data not shown), which were significantly reduced in only two experiments at H191 concentration $\geq$6 mg L^{-1}. However, only algae grown in water from one of the fields (Field R) were affected.

Hydrodictyon reticulatum chlorophyll reflectance showed a similar decrease during the first 3–5 days following exposure to H191. This was most pronounced at H191 concentrations above 1 mg L^{-1} (Fig. 2). However, *Hydrodictyon reticulatum* chlorophyll reflectance values were the same as those for the untreated controls by the end of the 7-day experiment (Fig. 2). This pattern of changing effects for the algicide treatment over time is reflected in the significant statistical interaction terms (between H191 concentration and time) present in the mixed model analysis of variance results (Table 2). The observation of reduced effect at the lower H191 concentrations agrees with findings of Wells and Clayton (1993) who reported that *H. reticulatum* displayed no injury symptoms when exposed to H191 at 1 mg L^{-1}.

Results of the laboratory experiments showed that *N. spongiaeforme* growth rates declined significantly when the H191 concentration was 0.3 mg L^{-1} or higher (Fig. 3; Table 3). Ruzycki et al. (1998) reported that growth rates of two planktonic cyanobacteria, *Microcystis aeruginosa* and *Phormidium inundatum*, were suppressed to almost zero by H191 concentrations less than 0.2 mg L^{-1}. Thus, *N. spongiaeforme* appears to be more tolerant of H191 than *M. aeruginosa* and *P. inundatum*. Differences in growth form between the planktonic species and *N. spongiaeforme*, as well as different experimental conditions may also contribute to the different responses to H191.

In this study, addition of 0.5 g L^{-1} of rice straw to the culture medium resulted in *N. spongiaeforme* growth rates that were not reduced at 0.3 mg L^{-1} of H191 as indicated by the significant rice straw by H191 interaction term (Table 4).

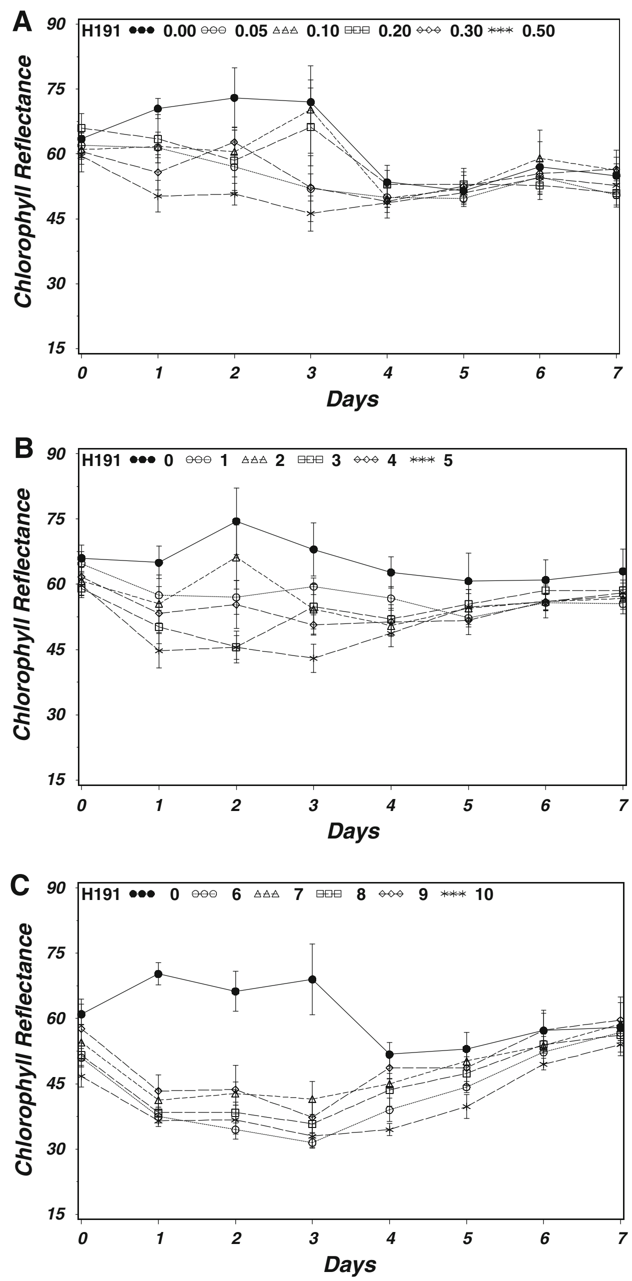

Fig. 2 Chlorophyll reflectance measurements for field-collected *Hydrodictyon reticulatum* exposed to various concentrations (**a** 0–0.5 mg L^{-1}, **b** 0–5 mg L^{-1}, **c** 0–10 mg L^{-1}) of Hydrothol 191 (H191). Plotted values are the mean $\pm$ standard error, based on four replications. The horizontal axis is in days with day zero being the day of treatment. These experiments were conducted at Davis, California

Table 2 Mixed model analysis of variance results for chlorophyll reflectance for field-collected *Hydrodictyon reticulatum* exposed to various concentration of Hydrothol 191 (H191)

Experiment	Hydrothol range (mg L^{-1})	Source	Num DF	Den DF	*F* value	Prob.
1	0–0.5	H191	5	18	1.20	0.35
		Days	7	103	10.92	<0.0001
		H191 * days	35	103	2.32	0.0006
2	0–5.0	H191	5	18	2.69	0.06
		Days	7	103	6.02	<0.0001
		H191 * days	35	103	2.50	0.0002
3	0–10.0	H191	5	18	8.76	0.0002
		Days	7	101	44.38	<0.0001
		H191 * days	35	102	6.55	<0.0001

The concentrations, "0–0.5 mg L^{-1}" = 0, 0.05, 0.1, 0.2, 0.3, 0.5 mg L^{-1}; "0–5" = 0, 1, 2, 3, 4, 5 mg L^{-1}; and "0–10" = 0, 6, 7, 8, 9, 10 mg L^{-1}

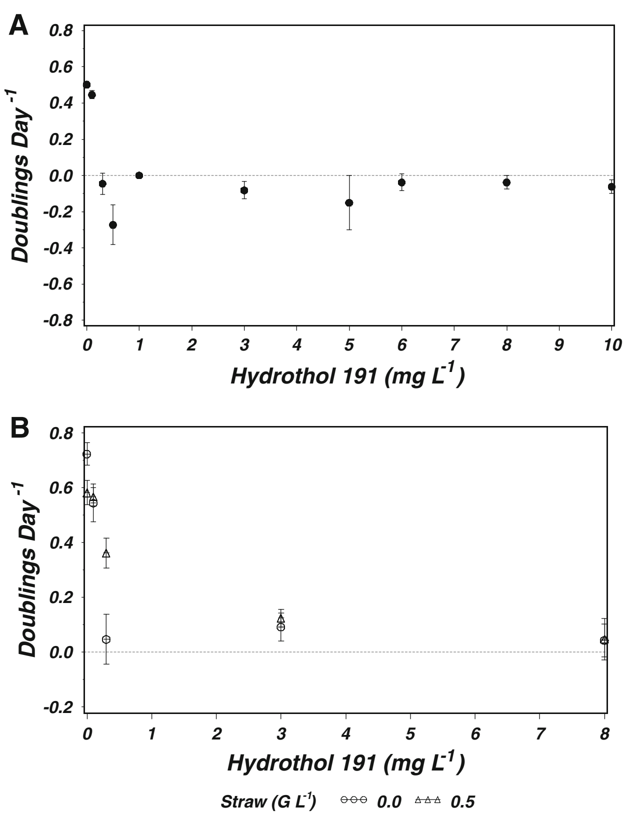

Fig. 3 Upper graph (**a**) shows the effect of Hydrothol 191 on *Nostoc spongiaeforme* relative growth rates (doublings day^{-1}) in laboratory cultures. Lower graph (**b**) shows the effect of Hydrothol 191 on *N. spongiaeforme* growth rates in laboratory cultures with 0.5 g L^{-1} rice straw added. Plotted values are the mean ± standard error, based on four replications. A negative growth rate indicates that *N. spongiaeforme* biomass decreased during the 1-week experiment, while a positive growth rate indicates that *N. spongiaeforme* biomass increased

Table 3 Analysis of variance results for *Nostoc spongiaeforme* growth rates versus concentration of Hydrothol 191 (H191) in a growth chamber experiment

Source	DF	SS	*F* value	Prob.
H191	9	2.23	13.38	<0.0001
Error	30	0.55		

The H191 concentrations were 0, 0.1, 0.3, 0.5, 1, 3, 5, 6, 8, and 10 mg L^{-1}

Table 4 Results of two-way analysis of variance for *Nostoc spongiaeforme* growth rates versus Hydrothol 191 (H191) and rice straw (0.5 g L^{-1})

H191	4	2.40	44.62	<0.0001
Rice straw	1	0.0220	1.64	0.21
Rice straw × H191	4	0.220	4.10	0.009
Error	30	0.403		

H191 concentrations were 0, 0.1, 0.3, 0.5, 1, 3, 5, 6, 8, and 10 mg L^{-1}

This clearly indicates that the addition of rice straw reduced the effect of H191 on *N. spongiaeforme*. The introduction of rice straw may have introduced bacteria or promoted the growth of bacteria capable of breaking down H191. The major influence on H191 persistence in the field is microbial degradation (Westerdahl and Getsinger 1988). Such a mechanism, a reduction in the half-life of H191 due to enhanced microbial activity, could also explain the temporary reduction in chlorophyll reflectance and recovery observed in the bucket experiments as rice straw and other debris present in the rice fields were also present in the buckets.

Other test conditions may also have influenced results of the bucket experiments. For example, fluctuating temperatures, sometimes briefly in excess of 40 °C, in the buckets (Table 5) may have enhanced microbial breakdown of H191 (Keller et al. 1988). Temperature fluctuations in the test buckets were quite similar to those recorded for seven rice fields in 2008, which had average temperatures of 21, 22, 23, 24, 24, 25, and 25 °C, respectively (Spencer et al. 2011). Results of the bucket tests may also have been influenced by water quality. McGaughey (1983) presented data showing that Aquathol K (potassium salt of endothall) was most effective against *Hydrilla* when the pH was 7 or higher and when the water was described as "soft". The pH

Table 5 Daily temperature data inside test buckets for field experiments with Hydrothol 191

Experiment (s)	Date	Mean daily temperature (°C)	Minimum daily temperature (°C)	Maximum daily temperature (°C)
1	03JUN2009	28.2	19.9	36.6
1	04JUN2009	22.0	14.7	31.3
1	05JUN2009	19.0	12.7	29.8
1	06JUN2009	21.7	14.4	31.9
1	07JUN2009	22.9	13.4	36.3
1	08JUN2009	23.1	13.3	37.9
Overall mean		22.8		
3	08JUN2009	23.1	13.3	37.9
3	09JUN2009	20.8	12.2	33.6
3	10JUN2009	21.4	13.5	35.6
3	11JUN2009	22.7	13.4	37.6
3	12JUN2009	21.8	12.4	37.7
3	13JUN2009	21.5	12.8	36.1
3	14JUN2009	22.0	11.9	40.0
3	15JUN2009	21.9	12.6	35.3
Overall mean		21.9		
12	22JUN2009	23.6	12.7	42.9
12	23JUN2009	25.4	13.7	45.3
12	24JUN2009	27.0	15.1	42.5
12	25JUN2009	25.4	14.7	39.5
12	26JUN2009	25.5	13.5	40.9
12	27JUN2009	28.0	16.7	44.3
12	28JUN2009	29.4	19.0	45.5
12	29JUN2009	28.5	18.1	45.7
12	30JUN2009	23.3	14.5	40.6
12	01JUL2009	17.7	16.0	19.9
Overall mean		25.4		

values in the bucket experiments with *N. spongiaeforme* might have been very slightly more acidic than optimum. Although we did not measure water hardness, the values of total alkalinity for the bucket experiments were typical of those associated with soft water by the USGS (http://water.usgs.gov, accessed 11/22/10). In any case, water temperature, pH, and alkalinity values in these tests were within the range of values reported for these parameters from California rice fields by Spencer et al. (2006). It is interesting, however, that water from the bucket tests with *H. reticulatum* (which was more susceptible than *N. spongiaeforme*) had higher alkalinity values, which would be associated with hard water conditions. This appears at variance with McGaughey (1983).

Results of the outdoor experiments indicate that H191 did not consistently kill the cyanobacterium, *N. spongiaeforme*, even at concentrations greater than the maximum labeled rate of 5 m L^{-1}. In contrast, its effect on the green alga, *Hydrodictyon* sp., was more pronounced at concentrations above 1 mg L^{-1}. Wells and Clayton (1993) also reported that *H. reticulatum* was not injured by H191 concentrations less than 1 mg L^{-1}. The present results would also support their conclusion that *H. reticulatum* was more tolerant to H191 than other species of filamentous green algae listed on the label. Due to the H191 concentrations required for growth inhibition of *N. spongiaeforme*, concentrations that are above currently labeled values, it is not clear how H191 will fit into algal control strategies for California rice fields. These results underscore, however, the importance of developing novel algicides for use in these systems and testing them under realistic water quality conditions.

Conclusions

The cyanobacterium, *Nostoc spongiaeforme*, is very difficult to control in California water seeded rice production systems using currently accepted methods. We evaluated the effect of the mono (*N*,*N*-dimethylalkylamine) salt of endothall (H191) on growth of *N. spongiaeforme* and the green alga, *H. reticulatum.* In laboratory experiments, H191 reduced *N. spongiaeforme* growth at 0.3 mg L^{-1}. This effect was removed when rice straw was added to the growth medium, indicating the rice straw may have introduced bacteria capable of degrading H191. In outdoor experiments, which used rice field water containing decomposing rice straw, H191 concentrations between 0 and 5 mg L^{-1} had little effect on *N. spongiaeforme.* In contrast, *H. reticulatum* exhibited injury symptoms at 1 mg L^{-1} or greater. However, *H. reticulatum* recovered by the end of the 7-day exposure. These results emphasize the need for alternative algal management techniques and point out the importance of evaluating their impacts using realistic water quality conditions, reflective of those in California rice fields.

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