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NOTES

Effect of a Combination of Two Rice Herbicides on the Cyanobacterium, *Nostoc spongiaeforme*

DAVID F. SPENCER[1], P.-S. LIOW[1] AND C. A. LEMBI[2]

INTRODUCTION

Floating algal mats are a significant problem in California rice fields. Cyanobacteria fix atmospheric nitrogen and are thus considered beneficial in some rice production systems. California rice fields are planted from seeds not transplants, and rice seedlings are adversely impacted by floating algal mats. The algal mats cause two significant problems that lead to yield reduction: floating mats can entrap and uproot seedlings; and large thick mats may accumulate in windward areas and sink, smothering seedlings. These problems are most critical during the 30 days following initial flooding of the fields because seedlings are less impacted after they emerge from the water. California rice fields typically have shallow water depths (<15 cm) and relatively high levels of nitrogen and phosphorus (Spencer et al. 2006). The high nutrient levels are believed to improve rice seedling establishment and survivorship, but they provide ideal conditions for growth of algae as well as rice (Chapman et al. 1972).

The most problematic cyanobacterium was identified by Spencer et al. (2006) as *Nostoc spongiaeforme*, described by Desikachary (1959). *Nostoc spongiaeforme* has been very difficult to control using currently accepted methods (i.e., aerial applications of copper sulfate at 1 mg L^{-1}) during the critical 30-day period following spring flooding of fields. Two herbicides commonly used in rice production are Londax (bensulfuron-methyl (methyl 2[[[[[(4,6-dimethoxy-2pyrimidnyl)amino] carbonyl]amino]sulfonyl]methyl]benzoate)) and Shark (carfentrazone-ethyl (ethyl α,2-dichloro-5-[4-(difluoromethyl)- 4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl] 4fluoro-benzenepropanoate)). They may be used in combination to increase the spectrum of rice field weeds controlled with a single application (Hill and Fisher 1999). Discussions with pest control advisors indicated that less severe algal problems were observed in fields with combinations of these herbicides. The purpose of the experiments described here was to evaluate the effect of a Londax-Shark mixture on *N. spongiaeforme*.

[1]USDA ARS Exotic and Invasive Weeds Research Unit, Department of Plant Sciences, Mail Stop 4, 1 Shields Avenue, Davis, CA 95616.

[2]Department of Botany and Plant Pathology, 915 West State Street, Purdue University, West Lafayette, IN 47907. Received for publication April 17, 2009 and in revised form August 17, 2009.

MATERIALS AND METHODS

Stock cultures of *N. spongiaeforme* were grown in BG-11 medium without nitrate at 25 C, 13:11 h light:dark cycle, 400 μM m^{-2} s^{-1} in a reach-in growth chamber. The initial pH of the culture medium was adjusted to either 6.7 or 7.1 using 1 N NaOH or 1 N HCl because the pH of rice field water varies (Spencer et al. 2006). Following inoculation with 2 ml into each test flask, *N. spongiaeforme* was allowed to grow for 7 days. At that time, two 10-ml aliquots were withdrawn from each flask and 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 beginning of the experiment. We repeated the experiment four times. There were four replicate flasks at each of the following Londax-Shark mixture concentrations: 0, 0.041, 0.104, 0.207, and 0.414 mg L^{-1}. These rates were chosen because 0.630 kg ha^{-1} (i.e., 0.414 mg L^{-1} if water depth is 15 cm at the time of application) is the normal use rate for this combination of herbicides, and because we were especially interested in knowing if lower rates would be effective. Lower rates may reduce injury to rice when applied early in the season for algal control. The Londax-Shark mixture we used was prepared by mixing 8 parts Shark to 1 part Londax, reflecting individual herbicide use rates. It was formulated and supplied by Big Valley Ag Services, Gridley, California.

RESULTS AND DISCUSSION

In three of the four experiments, the treatments had no statistically detectable influence on *N. spongiaeforme* growth rates (Figure 1). In one experiment, there was a statistically significant effect; however, growth rate decreased by only 12% at the highest level of the Londax-Shark mixture examined. Initial pH of the culture medium did not influence the impact of the Londax-Shark mixture. Thus, it does not appear that this herbicide mixture will be useful in the management of *N. spongiaeforme* in California rice fields.

These results contrast with earlier reports that bensulfuron-methyl (the active ingredient in Londax) was associated with reduced growth and reproduction of several submersed

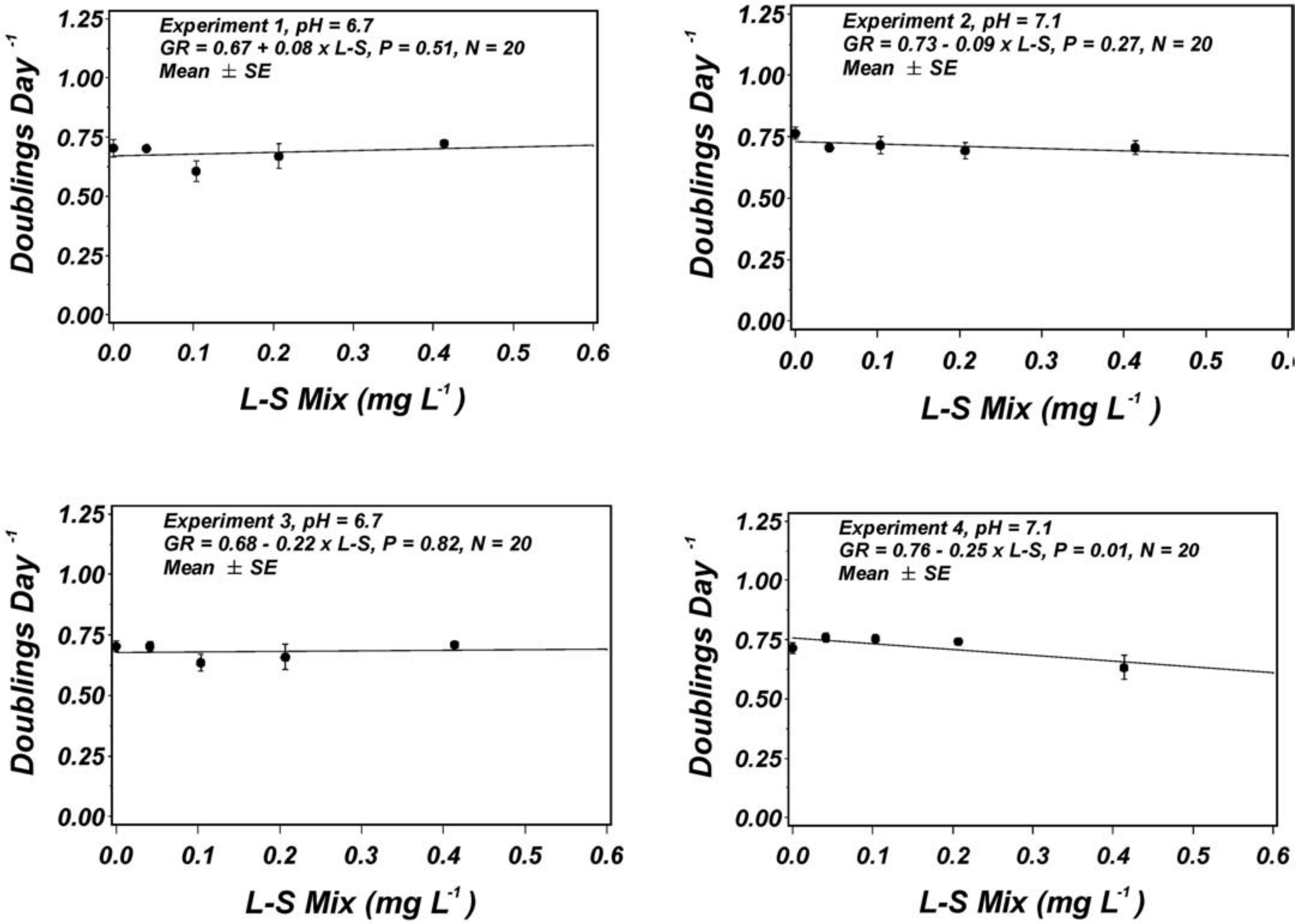

Figure 1. Response of *Nostoc spongiaeforme* to different concentrations of a Londax-Shark mixture (L-S Mix).

flowering aquatic plants: *Myriophyllum papillosum* Orchard, *Elodea canadensis* Rich.; *Vallisneria gigantea* Graebner; *Potamogeton tricarinatus* F. Muell. & A. Benn ex A. Benn (1892) (Bowmer et al. 1992); *Hydrilla verticillata* L. f. (Royle) (Haller et al. 1992, Langeland and Laroche 1992, Van and Vandiver 1992, 1994); *M. spicatum* L. (Nelson et al. 1993, Getsinger et al. 1994); *V. americana* Michx.; and *P. nodosus* Poiret (Getsinger et al. 1994). More recent reports indicated that carfentrazone-ethyl (the active ingredient in Shark) treatments also reduced growth of the flowering aquatic plants *Eichhornia crassipes* (Mart.) Solms-Laub., *Pistia stratiotes* L., and *Landoltia punctata* (G. Mey.) Les & D. J. Crawford (Koschnick et al. 2004); *M. spicatum* L., *M. aquaticum* (Vell.) Verdc., *Stukenia pectinata* (L.) Boerner (Glomski et al. 2006, Gray et al. 2007), and *M. heterophyllum* Michx. (Glomski and Netherland 2007, 2008). Two fern species are also sensitive to carfentrazone-ethyl: *Salvinia minima* Baker (Koschnick et al. 2004) and *S. molesta* D. S. Mitchell (Glomski and Getsinger 2006).

Little research has been conducted on the effects of either herbicide on algal or cyanobacterial species. To our knowledge, no published studies report the effects of carfentrazone-ethyl alone or in combination with bensulfuron-methyl on growth of cyanobacteria or algae. Ma et al. (2002) reported that the EC_{50} (the concentration required to reduce growth by 50%) for bensulfuron-methyl, the active ingredient in Londax, was 17.96 mg L^{-1}, for the green alga *Chlorella vulgaris*. Another active ingredient with the same mode of action, nicosulfuron, had an EC_{50} of 4.33 mg L^{-1} for *C. vulgaris*. Kim and Lee (2006) reported that bensulfuron-methyl inhibited photosynthesis of the cyanobacteria *Anabaena variabillis* and *N. commune* by 50% at a concentration of 8 to 10 mg L^{-1}. Chen et al. (2007) reported that the 96-h EC_{50} for *Nostoc* spp. exposed to bensulfuron-methyl was 40.6 mg L^{-1}. The highest mixture concentration used in our present experiments was equivalent to 0.147 mg L^{-1} carfentrazone-ethyl (Shark) and 0.028 mg L^{-1} bensulfuron-methyl. Thus, algae and cyanobacteria appear to be susceptible to bensulfuron-methyl only at concentrations much higher than those used in rice fields. The lack of response to the mixture could also be because *N. spongiaeforme* is composed of cells arranged as filaments embedded in a gelatinous matrix that may interfere with entry of these herbicides into the cells.

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