J. Aquat. Plant Manage. 28: 50-51

NOTES

An Aquatic Bioassay of Herbicide Bleaching in the Charophyte Sporeling, *Nitella Furcata*

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INTRODUCTION

Aquatic angiosperms are sensitive to fluridone [1-methyl-3-phenyl-5-[3-[trifluoromethyl)-phenyl]-4-(1H)-pyridinone] at the labelled rate[(2)] of 0.1 mg/1. Fluridone blocks biosynthesis of carotenoids in newly developing tissues. Without the protection of carotenoids, chlorophyll is bleached by its own excitation products (Bartels and Watson 1978, Moreland 1980) The extent of bleaching is dose-proportional in cucumber (Drexler and Fletcher 1981) and in wheat (Shea and Weber 1983).

Species of unidentified charophytes are reported from field surveys to be unaffected by fluridone, while angiosperms are destroyed[(2)]. This observation prompted an examination of bleaching of chlorophyll in charophytes by fluridone. Subsequently, a convenient bioassay was developed for the detection and quantification of fluridone bleaching on charophyte sporelings.

The charophyte genera, *Chara* and *Nitella* are common constituents of rooted vegetation in the littoral of lakes. Generally regarded as pioneer species, they grow rapidly and have many qualities desired in lake management (Crawford 1979). In clear lakes, charophytes also grow in vast sublittoral meadows (Kasaki 1964, Stross et al. 1988). Shallower members of the meadow, such as *Nitella furcata*, are annuals and reproduce by oospores, which may accumulate in the sediments. The buried oospores undergo an annual cycle of dormancy, analogous to weed seeds in arable soils and germinate when exposed to light (Sokol and Stross 1986). Dormant oospores require afterripening at low temperatures for up to three months.

MATERIALS AND METHODS

Sporelings for the bioassay were prepared in advance. Tubes of sporelings were developed from oospores that were germinated, as described by Sokol and Stross (1986), in acid washed pyrex tubes (25 x 95mm). Each tube was prepared by layering 5.0 ml base of bactoagar (Difco; 1.2 % w/w in glass distilled water) with 20 ml of filtered water from L. George. Each tube was inoculated with 10 oospores and incubated for 14 days in a walk-in growth chamber (Percival) at 19.0 C under daylight fluorescent lamps at 30 uM/m²/s and a daylength of L15:D9.

Stocks of oospores (*N. furcata* subsp. *megacarpa* (Allen emend. Wood)) were collected at night from the sediments of L. George in the spring when they are non-dormant. The oospores were stored at 4.0 C in total darkness, which maintained them in a germination-ready state (Sokol and Stross 1986).

An aqueous suspension of fluridone (4 A.S.) was diluted with glass distilled water. Stock solutions were prepared by serial dilution with filtered (0.8 um Diaflo, Amicon) water from Lake George (NY). One ml of a fluridone stock solution was added to each assay tube of sporelings.

Five concentrations of the herbicide were tested: 0.0 (control), 0.01, 0.1, 1.0 and 10.0 mg/1, a range that included the labelled rate (0.1 mg/1) and the near maximum solubility of fluridone in water (12.0 mg/1).

Bleaching sensitivity was assayed in each of four treatments. Each treatment of five herbicide concentrations (x 6 replicates per concentration) was exposed to a photon flux density (PFD) of 130 or 500 uM/m²/s for an interval of 2.0 or 6.0 days. The PFDs chosen for this study simulate expected maxima for the species in its native environment.

Removal of fluridone from the sporelings was accomplished by fushing the contents of each tube with jets of filtered lake water. Unattached sporelings were captured on a nylon screen. The sporelings in the tube and on the screen were rinsed five times with 10 ml each of filtered lake water. The unattached sporelings were returned to the tube, which was refilled with filtered lake water. The sporelings were incubated for an additional 14 days before examination, as a test for persistence of effect.

Chlorophyll content of each sporeling was first rated visually (not shown) as described by Banks and Merkle (1979). A quantitative test of chlorophyll was obtained from 20 sporelings, chosen randomly from each treatment. The chlorophyll was extracted with 5.0 ml of a chloroform-methanol mixture (2:1) as described by Wood (1985). The concentration of chlorophyll *a* was measured in a Turner Fluorometer and converted to micrograms per sporeling with a coefficient prepared from pure chlorophyll *a*.

The mean effective concentration (EC_{50}) of fluridone that bleached one-half of the chlorophyll from each sporeling was estimated as follows. The means of measured chlorophyll content for each fluridone concentration were subjected to a least squares regression following a log-log transformation. The acute effective concentration (EC_{50})

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[2]Schmitz, D.C. 1985. Fluridone Literature Review. Florida Dep. Nat. Res., Bur. Aquat. Plant Control. Tallahassee, FL. Unpublished Report.

was estimated with the "X" from "Y" technique, described by Sokal and Rohlf (1981).

RESULTS AND DISCUSSION

Mean amounts of chlorophyll *a* in untreated sporelings of *N. furcata* ranged from 8.3 to 9.2 ug per sporeling. Exposure to 0.01 ug/l fluridone for 6 days at 'high' light failed to influence chlorophyll content, which was 9.2 ug per sporeling. However, at the same concentration but with 2-days exposure at 'low' light, mean chlorophyll was increased to 14.9 ug per sporeling.

Fluridone at 0.1 mg/l bleached chlorophyll from *Nitella* sporelings at a PFD of 500 uM/m²/s. Bleaching was more than 80 % complete, relative to minimum concentration, following exposure to the herbicide for 2 days (2.8 ug per sporeling) and complete (0.4 ug) with 6 days of exposure.

The dose of 0.1 mg/l produced other effects. Rooting was prevented in 40 % of the sporelings. However, in a separate study, when fluridone was added to tubes of oospores, their germination was unaffected, even at the maximum dose of 10.0 mg/l.

The effectiveness of fluridone as a bleaching agent increased with concentration, duration of exposure, and PFD (Fig. 1). Irradiance and exposure duration appeared to be additive at the recommended dose of 0.1 ug/l. Regression slopes ranged from -0.38 to a -0.45 for the four combinations (duration X irradiance) shown in Fig 1. The slopes were not significantly different, and the data for each concentration of fluridone were pooled for further analysis.

In *Nitella* sporelings, the acute effective concentration (EC_{50}) for the pooled data was 0.02 mg/l or 20 ppb. In other words, the bleaching of chlorophyll in sporelings of *Nitella* in response to fluridone approximates the sensitivity reported for the cucumber cotyledon (Drexler and Fletcher 1981). The charophyte assay of fluridone could measure the residual level of herbicide in water of lakes treated to remove nuisance plants. The assay would precede "reseeding" with the oospores of charophytes, which are intended to establish a cover crop following the removal of undesired plants, as suggested by Crawford (1979) and Clayton (unpubl).

ACKNOWLEDEMENTS

B. Rottier, R. Sokol and L. Wood assisted in various ways. V. W. Proctor and F. K. Daily verified the charophyte taxa. Eli Lilly & Co. kindly supplied the herbicide. Supported in part by New York Adirondack Park Agency.

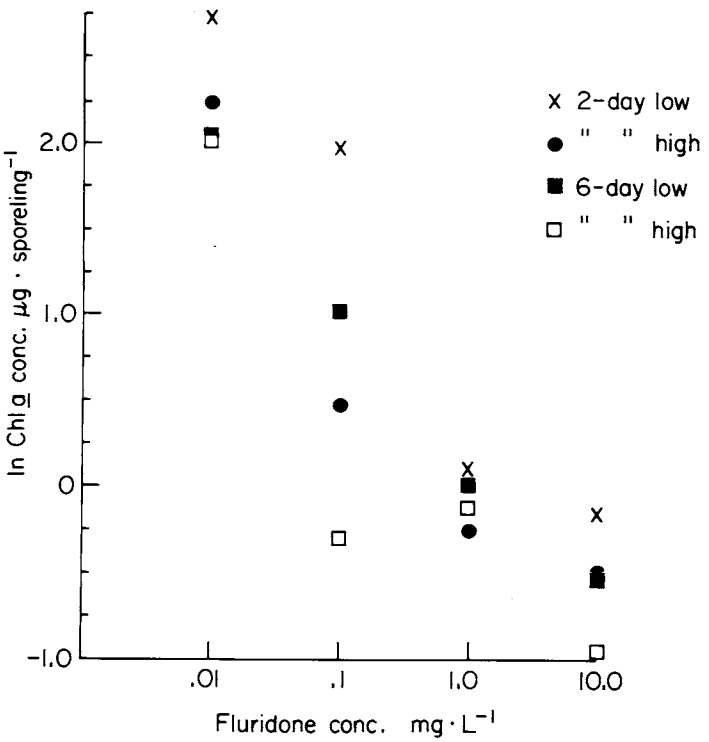

Figure. 1. Chlorophyll *a* in sporelings of *Nitella furcata* in relation to fluridone concentration at each of four combinations of PFD and duration of exposure; 'low' = 130 uM/m²/s; 'high' = 500 uM/m²/s.

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