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Comparisons of Herbicide Toxicity Using *In Vitro* Cultures of *Myriophyllum spicatum*

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ABSTRACT

In vitro cultures of the aquatic plant *Myriophyllum spicatum* L. were used to determine the effects of the herbicides 2,4–D (dichlorophenoxyacetic acid), atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) and glyphosate (N-[phosphonomethyl] glycine) and the leaf defoliant thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) on plant development. The developmental response measured percent reduction of new branches relative to controls. Linear regressions of percent branch number reduction (BNR) as a function of the log (toxin concentration+1) were highly correlated and statistically significant. The plant growth regulator 2,4-D had the greatest effects on development of branches with a 50% BNR of 0.04 mg/l, followed by glyphosate (1.6 mg/l), atrazine (3.7 mg/l), and thidiazuron (9.8 mg/l). The short 5-day time period required for these assays and ability to determine dose–response relationships supports the use of *in vitro* culture of *Myriophyllum spicatum* as a bioassay system.

Key words: atrazine, 2,4-D, glyphosate, thidiazuron, bioassays.

INTRODUCTION

Bioassays play a significant role in toxicological research. Bioassay data may be used to determine effects of environmental pollutants or the design of application protocols for aquatic weed control. Bioassay organisms are particularly useful if they grow rapidly, are easy to culture and are important in the environments of interest (Rand and Petrocelli 1985).

In vitro culture of aquatic plants offers advantages for bioassays. Methods have now been developed for culture of a number of aquatic plants *in vitro* (Kane *et al.* 1991, Jenks *et al.* 1990, Kane and Gilman 1991). Such cultures are axenic, hence microflora do not affect toxin chemistry or concentrations. Growth under *in vitro* culture is rapid. Aquatic plant cultures can be easily maintained in the laboratory.

Recently, Kane and Gilman (1991) demonstrated that *in vitro* cultures of *Myriophyllum* spp. show decreases in growth as a function of Cycocel concentrations, a growth retardant. They used shoot length and percent dry weight as measures of growth. We examined the effects of several herbicides and thidiazuron on organogenesis in nodal cultures of *Myriophyllum spicatum* L. grown in liquid medium (Christopher and Bird 1992). The development of new branches appeared to be a reliable indicator of toxin effects over a short period of 5 days. In this paper, I examine whether branch development can be modeled to determine dose–response relationships of several herbicides and a defoliant.

MATERIALS AND METHODS

Liquid stock cultures of *Myriophyllum spicatum* were propagated in a Murashige and Skoog salt-based medium (Kane and Gilman 1991). Axenic stock cultures of *M. spicatum* were obtained from M. Kane, University of Florida. Bioassays were performed on three node segments in which the foliar portions of the middle node were excised. Each segment was cultured in 10 ml of the bioassay medium and the nominal concentration of herbicide (see Christopher and Bird 1992 for details). The three herbicides were 2,4-D, atrazine and glyphosate. The defoliant was thidiazuron. Atrazine was tested over a concentration range of 0.1 to 100 mg/l, 2,4-D over 0.02 to 0.1 mg/l, glyphosate from 0.5 to 10 mg/l and thidiazuron from 1.0 to 50 mg/l. Controls containing no toxin were included in each experimental run. After 5 days, the segments were removed and the number of new branches at the middle node tabulated. For statistical purposes, three experimental runs were performed for each type of toxin and five replicates were used at each concentration.

Statistical analyses were run on the mean values of the data from the three replicate experiments for each toxin. The number of branches produced in the controls was compared with the numbers produced at each concentration of toxin to determine the percent of branch number reduction (BNR). The concentrations of the toxins were transformed using the log of concentration+1 (conc+1). Probability plots of the percent of BNR were examined to determine whether data were normally distributed using Fastat (Systat 1989). The data were checked for homogeneity for variance (Steel and Torrie 1960). Regressions and 95% confidence intervals were determined for each toxin using Fastat (Systat 1989). Regression

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equations were used to determine the concentration at which a 50% reduction in new branches occurred.

RESULTS

There were strong correlations between the log (conc+1) transformed concentrations of the toxins and percent of branch number reduction (Figure 1). The herbicide 2,4-D caused branch number reduction (BNR) over a concentration range of 0.02 to 0.1 mg/ml. The percent of BNR was linear as a function of the transformed concentrations (log [conc+1]). Analysis of variance (ANOVA) indicated that the regression was highly significant ($p = 0.002$, $r = 0.96$) with a 50% BNR at a concentration of 0.04 mg/l of 2,4-D.

Atrazine also resulted in significant percent BNR, although there was more variability in the data than for the other toxins. Despite some variability, the regression was highly significant as determined by ANOVA ($p = 0.024$, $r = 0.82$) with a 50% BNR of 3.7 mg/l.

Glyphosate treatment resulted in a significant BNR, particularly at concentrations of 1.0 mg/l and higher. The regression was highly significant as determined by ANOVA ($p = 0.003$, $r = 0.93$). A 50% BNR of 1.6 mg/l was determined from the regression equation.

Thidiazuron caused a reduction in branch number in concentrations ranging from 1 to 50 mg/l. The regression of percent BNR on log (conc+1) was linear and highly significant as determined by ANOVA ($p = 0.003$, $r = 0.92$). The regression equation was used to calculate a 50% BNR of 9.8 mg/l.

DISCUSSION

In our prior paper (Christopher and Bird 1992), comparisons of concentration effects for these toxins were made using empirical data and statistical comparisons of means. In that paper, we suggested that the order of greatest developmental inhibition of *Myriophyllum spicatum* was 2,4-D

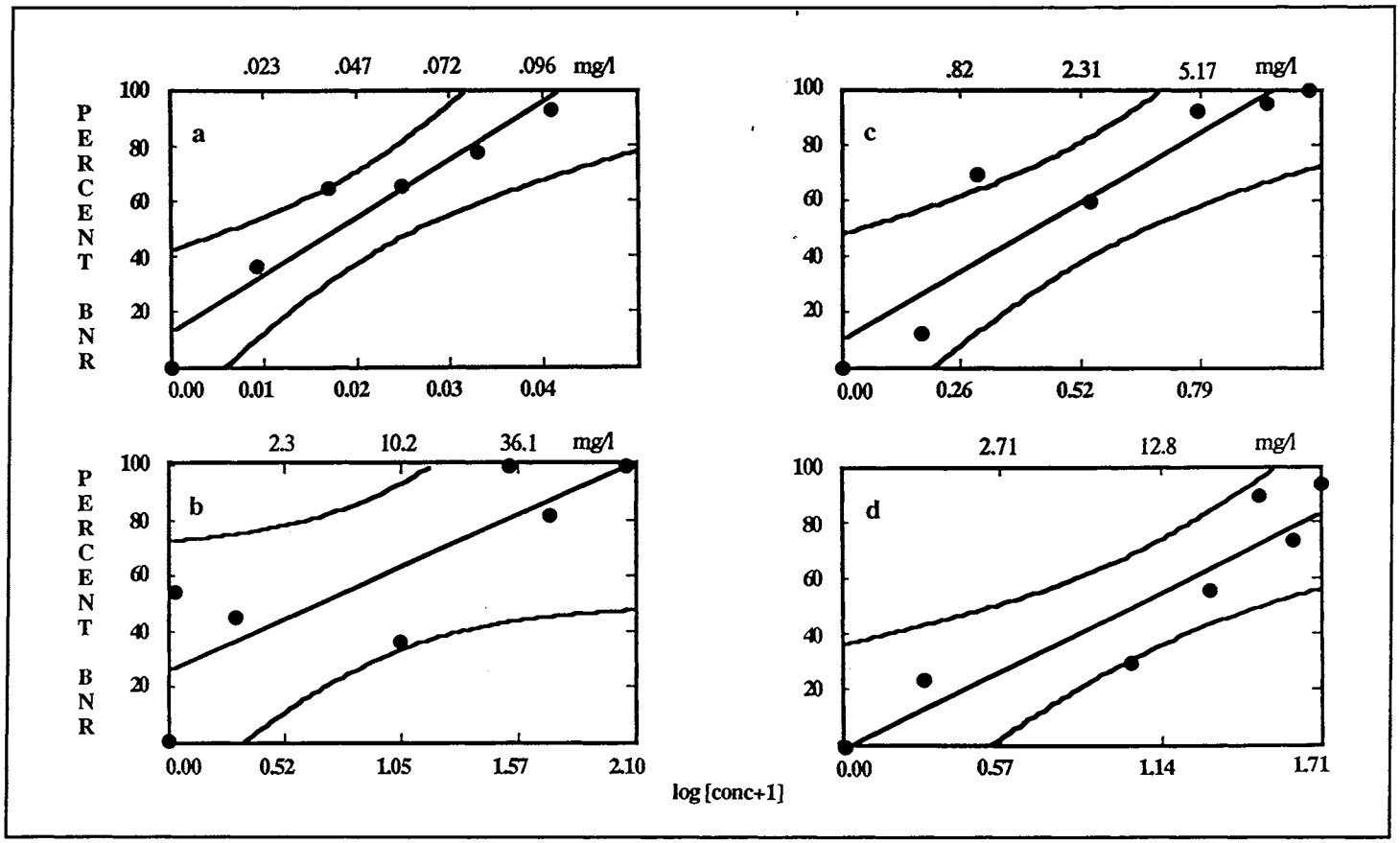

Figure 1. Linear regressions of percent branch number reduction (BNR) of *Myriophyllum spicatum* determined as a function of the log (concentration+1) of a) 2,4-D, b) atrazine, c) glyphosate and d) thidiazuron. Lower axes show log [conc+1] and upper axes show untransformed concentrations of these toxins (in mg/l) for comparison. The regression equations are: a) percent BNR = 12.4+2095.9(log[conc+1]), $r = 0.96$, $p = 0.002$; b) percent BNR = 25.6+36.2(log[conc+1]), $r = 0.82$, $p = 0.024$; c) percent BNR = 9.8+95.1(log[conc+1]), $r = 0.93$, $p = 0.003$; d) percent BNR = 1.7+50.0(log[conc+1]), $r = 0.92$, $p = 0.003$. Each datum point represents 15 replicates.

>atrazine >glyphosate >thidiazuron. Use of regression analyses for prediction of 50% BNR suggests that 2,4–D >glyphosate >atrazine >thidiazuron.

Myriophyllum spicatum appears to be extremely sensitive to 2,4–D. When the concentrations which caused a 50% BNR are compared, the amount of 2,4-D required (0.04 mg/l) was approximately 100 times less than the amounts of the other toxins, which ranged from 1.6 to 9.8 mg/l. Van *et al.* (1986) found that a concentration of 0.08 mg/l was low enough to control *M. spicatum* growth in a flow-through growth system. This concentration is close to that reported for effective control of *M. spicatum* in Kitty Hawk Bay, North Carolina (Getsinger *et al.* 1982). Similarly, Bergquist (1971) found that levels of 1 mg/l 2,4-D caused significant morphological changes. The effective concentration of 0.04 mg/l 2,4-D for 50% BNR in this analysis is in close agreement with these other studies.

This bioassay system was rapid and based on developmental parameters (numbers of branches). Such a system may be more sensitive than measuring mortality, changes in weight or growth. In addition, use of branch number reduction as a parameter provided data that could be used to develop concentration dependent linear regression analyses. Such analyses are key components of bioassay systems (Finney 1978). These analyses support the suggestion of Kane and Gilman (1991) that *in vitro* cultures of *M. spicatum* provide useful systems for toxicity bioassays. *In vitro* cultures of other aquatic plant species should also be useful in toxicological research.

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