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HERBICIDE EFFECTS ON TYPHA LATIFOLIA (LINNEAUS) GERMINATION AND ROOT AND SHOOT DEVELOPMENT

M.T. Moore[1]*, D.B. Huggett[1], G.M. Huddleston III[2], J.H. Rodgers, Jr.[2], and C.M. Cooper[3]

[1]Department of Biology, University of Mississippi, University, MS 38677 USA, [2]The Institute of Wildlife and Environmental Toxicology (TIWET), Clemson University, Pendleton, SC 29670, [3]USDA-ARS National Sedimentation Laboratory, Oxford, MS 38655 USA

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ABSTRACT

Aqueous 7-d germination and growth experiments were performed to compare responses of *T. latifolia* to exposures of atrazine (2-chloro-4-ethylamino-6-isopropylamine-*s*-triazine) and paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride). *T. latifolia* seed germination was < 50 % in concentrations ≥ 1.0 mg/L of paraquat dichloride. No observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for paraquat and root growth were 0.001 and 0.01 mg/L, respectively, while NOEC and LOEC for paraquat and shoot growth were 0.01 and 0.1 mg/L, respectively following 7-d exposures. Greater than 72 % of seeds germinated in each concentration up to 30 mg/L atrazine. After 7-d exposure, NOEC and LOEC for atrazine and root growth were 0.1 and 1.0 mg/L, while atrazine and shoot growth NOEC and LOEC values were 15 and 30 mg/L, respectively. This research provides data concerning relative sensitivity of *T. latifolia* seedlings to the herbicides atrazine and paraquat, as well as the potential use of *T. latifolia* as a representative plant test species.

* Author to whom all correspondence should be addressed. *All programs and services of the U.S. Department of Agriculture are offered on a non-discriminatory basis without regard to race, color, national origin, religion, sex, marital status, or handicap. Mention of a pesticide in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended. Names of commercial products are included for the benefit of the reader and do not imply endorsement or preferential treatment by the U.S. Department of Agriculture.*

INTRODUCTION

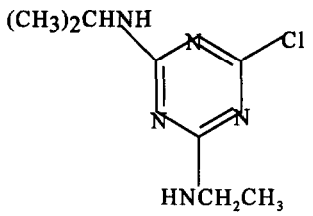
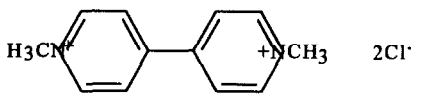
Evaluation of aquatic plant responses to herbicides is important because such plants are often exposed to herbicides by aerial drift or runoff events. *Typha latifolia* Linneaus (common cattail) represents a plant species commonly found in wetlands, as well as roadside and agricultural drainage ditches. Its reported distribution in North America ranges from central Alaska, throughout the continental United States, into Mexico [1]. Especially in areas of intensive agriculture, populations of *T. latifolia* may be exposed to a variety of agricultural pesticides, such as the herbicides atrazine (2-chloro-4-ethylamino-6-isopropylamine-*s*-triazine) and paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride). In development of new approaches in phytotoxicity testing, it is important to contrast responses between previously studied species and proposed species. It is likewise important to contrast toxicity results from different life stages of test species. Currently, little is known about the effects of atrazine and paraquat dichloride on *T. latifolia* seed germination and root and shoot growth. In this series of laboratory experiments, effects of the herbicides, atrazine (2-chloro-4-ethylamino-6-isopropylamine-*s*-triazine) and paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride), on *T. latifolia* seed germination, as well as root and shoot development of seedlings were determined following 7-d aqueous exposures. The purpose of this research was to determine the relative sensitivity of *T. latifolia* seedlings to two widely used herbicides, as well as suggest the use of *T. latifolia* as a representative plant test species.

When using aquatic macrophytes for toxicity tests, the question of which life stage of aquatic plants to use for testing (i.e. seeds, seedlings, or mature plants) is critical. Some researchers have suggested use of mature plants [2,3,4] while others have suggested use of seed germination and root elongation to determine effects from point and non-point source effluents [5,6]. For researchers proposing use of mature plants, plant size often precludes the use of larger macrophytes for toxicity evaluation (e.g. *Typha* and *Scirpus*) [4]. Relative sensitivity is also an important factor in determining which plant life stage to test. Seed germination and the first days of seedling growth are often the most sensitive stages of plant development [7]. Use of *Typha* seeds in testing effects of various environmental factors (e.g. pH, light, etc.) on germination and root elongation has been reported [8,9].

There are advantages to using seeds for toxicity testing. Most plant seeds can be stored from a few months to a year [6]. Their collection and storage, unlike most animal cultures, require little or no maintenance [6]. Additionally, natural genetic diversity found in seeds from the University of Mississippi Field Station (UMFS)-collected infloresences enables a more representative assessment of a toxicant's effects, since no known previous exposure to these pesticides has occurred.

Atrazine is one of North America's most commonly used herbicides, with 32-34 million kilograms of active ingredient being applied in 1993 alone [10]. Used for control of annual broadleaf weeds and grasses in corn, atrazine is sold as Aatrex™, Guardsman™, or Atrazine 4L™. Paraquat dichloride is a non-selective quaternary nitrogen herbicide used for broadleaf weed control. Commonly sold as Gramoxone Extra™, paraquat dichloride primarily destroys green plant tissue on contact (and by translocation within the plant), and it is currently used as a crop desiccant and defoliant. Atrazine and paraquat dichloride were used in these experiments as commercial agricultural preparations (Table 1).

Table 1. Physicochemical properties of atrazine and paraquat.

	Atrazine	Paraquat
		
Molecular weight (g/mol)[11,12]	215.7	257.2
Specific gravity (g/cm^3)[12,13]	1.187	1.24-1.26
Melting point (°C)[11]	175-177	300
Water solubility (mg/L)[12,13]	33	700,000
Vapor pressure (mm Hg 25°C)[10,18]	2.89×10^{-7}	$<1.0 \times 10^{-7}$
K_{ow}[11,14]	0.002	2.44
Water persistence ($T_{1/2}$)[11,15]	335 d	13.1 h
Soil persistence ($T_{1/2}$)[11,16]	12 d	480-4745 d
K_{oc}[11,17]	25-155	15473-51856

MATERIALS AND METHODS

Typha latifolia

Inflorescences were collected from wetland cells in March 1996 at the UMFS in Lafayette County, MS. Inflorescences were placed in plastic bags and transported to the University of Mississippi Department of Biology, where they were stored in a freezer (0°C) until use. Seeds were obtained from inflorescences by placing them in a commercial blender filled with Milli-Q™ water and blending for 20 seconds. According

to the methods of Rivard and Woodward [9], seeds sinking to the bottom were considered viable, while lighter non-viable seeds floated to the top. Viable seeds were collected in a 250 ml glass beaker for use in testing.

Experimental Design

Aqueous seed germination and seedling growth tests (7 d) were conducted with a 16 h light/8 h dark photoperiod, under 1500-3000 Lux fluorescent bulbs, at room temperature (20°C). Tests were initiated by adding fifteen *T. latifolia* seeds to each of three replicate beakers (50 ml) per concentration. Viable seeds were transferred to test vessels via glass pipettes. To maintain nominal aqueous herbicide concentrations, test chamber volumes were renewed every other day. Following 7 d exposures, *T. latifolia* seedlings were collected and stored in vials with 70 % ethanol. Measurements of root and shoot lengths were accomplished using a Videometric 150 Image Analyzer (American Innovision) with Videometric software (version 2.1). Seedlings without at least 5 mm root length were considered as not having germinated [19]. At the conclusion of each experiment, water temperature, pH, conductivity, dissolved oxygen, alkalinity and hardness were measured according to *Standard Methods* [19].

Herbicide Stock Solutions and Dilutions

Herbicide stock solutions for testing were prepared separately by dissolving Gramoxone Extra™ (37.05 % active ingredient paraquat dichloride) and Atrazine 4L™ (40.8 % active ingredient atrazine) in one liter of Milli-Q™ water. Dilution water was collected from springs at the UMBFS. Prior to use, water was filtered through MFS® 0.45 μm poly-membrane filters. Hardness and alkalinity of filtered water were adjusted with $NaHCO_3$ and $CaCl_2$(Fisher Scientific, Pittsburgh, PA) to values between 60-80 mg/L as $CaCO_3$. After stock solutions were mixed, dilution water and stock solutions were added to each of three replicate test beakers to obtain nominal exposure concentrations. Ranges in nominal aqueous exposure concentrations of atrazine and paraquat dichloride were 0.01-30.0 mg/L and 0.001-100 mg/L, respectively.

Exposure Verification

Ohmicron RaPID Assay™ was utilized to measure herbicide concentrations in aqueous exposure chambers by immunoassay [20]. Analytical ranges for paraquat were 50-500 ng/L and 0.1-5.0 μg/L for atrazine. If concentrations in amended spring water exceeded analytical ranges, dilutions were performed prior to repeated analyses. Samples were analyzed at 450nm with an Ohmicron RPA-1™ RaPID Photometer Analyzer.

Statistical Analyses

No observed effect concentrations (NOECs) and lowest observed effect concentrations (LOECs) for *T. latifolia* root and shoot growth were determined by statistically significant differences relative to controls ($P \leq 0.05$). One way analysis of variance (ANOVA) was performed with Dunnett's multiple range test to test for significance compared to controls ($P \leq 0.05$) [21]. If assumptions of a parametric ANOVA were not met, ANOVA on ranks with Dunn's multiple range test was performed.

RESULTS AND DISCUSSION

Exposure Verification

Recoveries of atrazine in exposure chambers ranged from 100 % (0.01 and 0.1 mg/L nominal concentrations) to 184 % (30.0 mg/L nominal concentration). In paraquat exposure chambers, recoveries ranged from 40 % (0.001 mg/L nominal concentration) to 210 % (10.0 mg/L nominal concentration) (Table 2). Point estimates (NOECs and LOECs) were not corrected for recovery. Ranges of percent recoveries were believed to be influenced by the difference in target nominal concentrations and limitations of immunoassay equipment. As previously stated, atrazine analytical limits ranged from 0.1-5.0 μg/L, while targeted nominal concentrations ranged from 10-30,000 μg/L (0.01-30 mg/L). Similarly for paraquat, analytical limits ranged from 0.05-0.5 μg/L (50-500 ng/L), while targeted nominal concentrations ranged from 1-100,000 μg/L.

Atrazine

In this study, germination was not significantly affected by exposure to concentrations of atrazine up to 30 mg/L as compared to controls (Table 3). Roots of *T. latifolia* were more sensitive to atrazine exposures than were shoots (Table 3). The *T. latifolia* 7-d NOEC for root growth was 0.1 mg/L, and the LOEC was 1.0 mg/L. The *T. latifolia* 7-d NOEC for shoot growth was 15 mg/L, and the LOEC was 30.0 mg/L. According to Esser et al. [30], plant roots grown in nutrient solutions containing atrazine, will readily uptake atrazine, which is then distributed via xylem into the shoot and other "aerial" portions of the plant. This supports current findings that roots are more sensitive to atrazine than were shoots. Similar studies which examined effects of metals on seedling growth also determined that root growth (elongation) was a more sensitive indicator of effects than was shoot growth [31,32].

Effects of atrazine on fish, benthos, zooplankton, phytoplankton, and some aquatic macrophytes have been previously examined [10]. Duckweed (*Lemna minor*) and the algal species *Selanastrum capricornutum* are typically two representatives of the plant kingdom used in toxicity testing. *L. minor* and atrazine had a 14-d EC50 of 8700 μg/L, with a NOEC of 10 μg/L and a LOEC of 100 μg/L (Table 4). The reported 7-d

Table 2. Atrazine and paraquat concentrations ($\bar{x}$) in exposure chambers during experiments (n=2).

Herbicide	Nominal concentrations (mg/L)	Mean measured concentrations (mg/L)	Percent recovery (%)
Atrazine	0.01	0.01	100
	0.1	0.1	100
	1.0	1.21	121
	10.0	14.82	148
	15.0	19.53	130
	30.0	55.1	184
Paraquat	0.001	0.0004	40
	0.01	0.006	60
	0.1	0.07	70
	1.0	0.80	80
	10.0	21.04	210
	100.0	71.12	71

atrazine and *S. capricornutum* EC50 was 214 μg/L (Table 4). Responses of other commonly tested aquatic animals to atrazine ranged from a 48-h LC50 of 720 μg/L (*Chironomus tentans*) to a 48-h LC50 of 33,000 μg/L (*Daphnia pulex*) (Table 4).

Paraquat

Unlike atrazine, paraquat exposures (0.1-100 mg/L) significantly decreased *T. latifolia* seed germination (as compared to controls) (Table 3). *T. latifolia* roots were also more sensitive to paraquat exposure than were shoots. Compared to atrazine exposures, paraquat elicited effects on both *T. latifolia* root and shoot systems at much lower exposure concentrations (Table 3). The *T. latifolia* 7-d NOEC for root growth was 0.001 mg/L, and the LOEC was 0.01 mg/L. The 7-d shoot growth LOEC was 0.1 mg/L, and the NOEC was 0.01 mg/L. Plants capability to translocate paraquat through roots, causing chlorosis and severe growth reduction in shoots was previously reported [13]. This translocation capability may account for observed sensitivity of both roots and shoots to paraquat in this study.

Table 3. 7-d root and shoot elongation ($\bar{x} \pm$ SD) and percent germination of *Typha latifolia* exposed to atrazine and paraquat (based on 3 independent experiments; n=3).

Herbicide	Nominal concentration (mg/L)	Root (mm)	Shoot (mm)	Seed germination	Percent germination
Control	0.00	15.2 (2.3)	7.9 (0.8)	114/135	84
Atrazine	0.01	14.5 (2.9)	7.6 (0.8)	110/135	82
	0.10	12.5 (1.6)	7.4 (0.5)	121/135	90
	1.0	10.0 (0.9)	7.6 (0.1)	97/135	72
	10.0	8.6 (1.1)	5.3 (3.0)	110/135	82
	15.0	8.8 (1.9)	6.6 (0.6)	108/135	80
	30.0	7.1 (0.3)	2.5 (0.7)	102/135	76
Paraquat	0.001	14.9 (3.2)	7.6 (0.2)	132/135	98
	0.01	9.0 (0.5)	7.5 (0.3)	109/135	81
	0.10	6.7 (0.6)	2.2 (1.4)	86/135	64
	1.0	5.5 (0.2)	0.0 (0)	44/135	33
	10.0	0.0 (0)	0.0 (0)	0/135	0
	100.0	0.0 (0)	0.0 (0)	0/135	0

CONCLUSIONS

Vascular plants not only aid in detection of herbicide toxicity, but they are also useful for toxicity assessments involving metals and other contaminants [5]. Atrazine and paraquat are two common and intensively used agricultural chemicals in the United States. Because of their agricultural use, there is potential for herbicide effects upon non-target areas (e.g. roadside ditches, wetlands adjacent to agricultural fields, etc.). *T. latifolia,* common inhabitants of such areas, may be affected by such application incidents. This research also aimed to evaluate *T. latifolia* germination and root/shoot growth as a bioassay technique, since the species could potentially be used in an ecological risk assessment. Seedlings are easily incubated and handled in the laboratory, and growth rates among seedlings were consistent under controlled conditions. While testing mature macrophytes having short life cycles (<2 months) may be beneficial in some cases, time

Table 4. Relative sensitivity of aquatic testing organisms to atrazine.

Organism	Test type	Concentration (μg/L)	Reference
Selanastrum capricornutum	7-d EC50	214	[22]
	5-d EC50	120	[23]
Lemna gibba	7-d EC50	180	[24]
Lemna minor	14-d EC50	8700	[25]
	NOEC	10	
	LOEC	100	
Daphnia pulex	48-h LC50	33,000	[26]
Daphnia magna	48-h LC50	9,400	[27]
Ceriodaphnia dubia	7-d NOEC	2,500	[28]
	7-d LOEC	5,000	
Chironomus tentans	48-h LC50	720	[29]
Pimephales promelas	96-h LC50	15,000	[29]

and space constraints may make seed germination and seedling growth a more practical indicator of effects on emergent macrophytes. For example, *T. latifolia* seeds are easily cultured (as described in this study), can be stored for several months, represent important macrophytes commonly inhabiting wetlands and ditches often impacted by contaminated runoff, require less space than mature plants, and exposure-response effects can be measured within 7 days. This research provides data on the relative sensitivity of *T. latifolia* seedlings to the herbicides atrazine and paraquat, as well as an evaluation of *T. latifolia* germination and root/shoot growth as a bioassay technique.

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