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Sensitivity of Wild Rice (*Zizania palustris* L.) to the Aquatic Herbicide Triclopyr

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ABSTRACT

A primary goal of managing invasive aquatic plants is to selectively control the target plant without impacting more desirable plant species. A native plant of concern is wild rice (*Zizania palustris* L.). Wild rice provides habitat for fish and wildlife, is a food source for waterfowl, and is utilized by humans as a cereal grain. The objective of this study was to examine the sensitivity of three wild rice growth stages, seedling, young, and mature, to aqueous concentrations (0, 0.75, 1.5, and 2.5 mg ae L^{-1}) of triclopyr. Plant control was rated on a 0 to 100% scale each week after treatment. At four weeks post treatment, plants were harvested, measured for height (cm), number of seedheads and tillers, and dried at 70 C to a constant mass. Wild rice exposed to the highest concentration of herbicide exhibited reduced height and mass regardless of life stage. The lowest triclopyr concentration did not significantly reduce height, biomass, seedhead density, or tiller formation. Wild rice seedlings exhibited the greatest sensitivity to triclopyr. Young and mature plants were less sensitive than seedling plants to triclopyr applications of 1.5 mg ae L^{-1}. A triclopyr concentration of 0.75 mg ae L^{-1} should have a negligible effect on all wild rice growth stages.

Key words: Eurasian watermilfoil management, flowering, growth stage, Renovate®3, tiller, seed head, selectivity.

INTRODUCTION

The management of invasive aquatic plants is more than just removing the target species; a major concern is the effect of herbicide treatment on nontarget plants. A species of concern in the northern United States is wild rice (*Zizania palustris* L.). In North America, three species of wild rice, *Z. aquatica* L., *Z. palustris* L., and *Z. texana* Hitchc., can be found with all three species and associated varieties being referred to as wild rice (Aiken et al. 1988, Oelke 1993, Duvall 1995). *Zizania palustris* L. will hereafter be referred to as wild rice. Wild rice is the only cereal grain indigenous to North America (Counts and Lee 1987). It is described as an annual grass with short roots that can be uprooted easily; stems are slender to thick, reaching a height of 4 m; leaves flat, often floating when young; spikelets in branched panicles; flowers are unisexual, upper parts of the panicle are pistillate, and spikelets of the lower panicle are staminate; staminate flowers are typically red, green, or yellow; lemmas of pistillate spikelets have long awns (Crow and Hellquist 2000). Wild rice inhabits shallow portions of lakes and slow-moving rivers of the north central and northeastern portions of North America (Aiken et al. 1988). Ecologically, wild rice provides food and shelter for fish and wildlife, most notably, migratory waterfowl (Baldasserre and Bolen 1994). This species was important to Native Americans and early European settlers of the Great Lakes region (Chamblis 1940). In recent times, wild rice has been exploited both as a subsistence food and as a cash crop (Counts and Lee 1987, Aiken 1988, Oelke 1993). At present, wild rice populations are increasingly being threatened by invasion of non-native aquatic plants.

One common invasive species in northern waters is Eurasian watermilfoil (*Myriophyllum spicatum* L.), which can occur in wild rice habitat, and management often includes the use of herbicides. One herbicide shown to be efficacious against Eurasian watermilfoil while leaving most of the surrounding native submersed plant community intact is triclopyr (triethylamine (TEA) salt of [(3,5,6-trichloro-2-pyridinyl) oxy]acetic acid) (Getsinger et al. 1997, 2000). The TEA salt formulation of triclopyr, registered in 2002 by the U.S. Environmental Protection Agency for use in aquatic sites (Petty et al. 2003), is a pyridinecarboxylic acid compound that generally provides selective control of broadleaf (dicotyledon) plants with little injury to most grass (monocotyledon) species (Petty et al. 2003). Triclopyr is a systemic herbicide that is absorbed through the roots, stems, and leaf tissues and translocated via apoplastic and symplastic processes, accumulating in the growing points (Vencill 2002). This herbicide acts as a synthetic auxin, producing an auxin overdose 1000 times the natural level, which disrupts the hormonal balance and interferes with growth (Ganapathy 1997). Although the site of action of triclopyr has not been identified, it is thought that triclopyr acidifies the cell wall resulting in cell elongation, rapid cell division, and eventual tissue destruction (Vencill 2002). Symptoms of triclopyr exposure are typical of other auxin-type herbicides (i.e., 2,4-D; Parsons et al. 2001), where ethylene and protein production in the plant increases, resulting in epinastic bending and twisting of stems and petioles, stem swelling at the nodes, leaf elongation, and leaf cupping and curling (Ganapathy 1997, Vencill 2002). Susceptible plants usually die within 3 to 5 weeks (Vencill 2002).

While triclopyr is labeled for use in production of domestic rice (*Oryza sativa* L.), little data exist on the effects of this herbicide on wild rice populations. Chemical control of giant burreed (*Sparganium eurycarpum* Engelm. Ex Gray) in *Z. palustris* has been investigated using other auxin mimicking herbicides: 2,4-D (dimethylamine salt of ((2,4-dichlorophenoxy))

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acetic acid); bentazon (3-(1-methylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide); propanil (*N*-(3,4-dichlorophenyl) propanamide); and 2,4-D + crop oil (0.50% v:v; Clay and Oelke 1990). Herbicide rates above 1.1 kg ha^{-1} injured wild rice and reduced yields. In addition, the authors reported that none of the herbicide treatments adequately controlled giant burreed without injury to the wild rice (Clay and Oelke 1990). Nelson et al. (2003) found that wild rice injury to the aquatic herbicides 2,4-D, diquat (6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazinediium dibromide), endothall (the dipotassium salt of 7-oxabicyclo [2.2.1] heptane-2,3-dicarboxylic acid), and fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone) varied with plant growth stage. Flowering of mature plants was not significantly affected by herbicide applications; though plants treated at younger growth stages (seedling or early tillering stages) were sensitive to herbicide applications (Nelson et al. 2003). Exposure of wild rice to triclopyr has yet to be investigated. Therefore, the objective of this study was to evaluate the sensitivity of wild rice at different growth stages to varying aqueous concentrations of triclopyr.

MATERIALS AND METHODS

This study was conducted in an outdoor mesocosm facility located at the R.R. Foil Plant Research Center, Mississippi State University, Starkville, Mississippi, beginning in April 2006 and ending in June 2006. The study was conducted as a completely randomized design using 48, 1100-L tanks with four triclopyr concentrations, three wild rice growth stages, and four replications per treatment. Aqueous concentrations of triclopyr (formulated as Renovate®3[4]) were 0, 0.75, 1.5, and 2.5 mg ae L^{-1}. The highest concentration used in this study is the maximum concentration allowed on the label. Growth stages of wild rice consisted of the seedling (floating leaf) stage, young (one or two emergent leaves) stage, and mature (flowering) stage (Nelson et al. 2003). Wild rice seed[5] was purchased commercially and propagated in shallow trays in a greenhouse. After seed germination, sprouted wild rice plants were planted (approximately five plants per pot) in 3.78-L pots filled with a potting mixture of top soil, loam, and masonry sand) and amended with 2 g L^{-1} of 19–6–12 Osmocote® fertilizer[6]. Fifteen pots were placed into each tank for a total of 720 pots. Water was supplied to each tank from an irrigation reservoir adjacent to the mesocosm facility. Water was filtered via a basket strainer and a sand filter before filling the tanks. Air was supplied to tanks using 2.5-cm stone diffusers and a PVC lift pipe outfitted in each tank. A 30% shade cloth was placed over the tanks to mitigate heat effects on the wild rice plants.

Wild rice plants were allowed to grow until the desired growth stage was reached. Prior to herbicide treatment plants were thinned so each pot contained two wild rice plants. Seedling stage plants were treated with triclopyr concentrations on 18 April 2006, young stage plants were treated on 8 May 2006, and mature plants treated on 22 May 2006. A concentrated aqueous solution was applied to each tank such that, when diluted with water in the tank, it provided the desired herbicide concentration. Water samples were collected 30 min and 72 hrs post treatment. Water samples were frozen and shipped to SePRO Corporation (Whitakers, NC) for immunoassay analyses to verify triclopyr concentrations (USEPA 1997, Poovey et al. 2004). Wild rice plants were exposed to treatments for 72 hours, after which water from each tank was drained and refilled with fresh water three times to remove triclopyr residues. After refilling the tanks, water was then made to slowly flow through each tank for one week to ensure no triclopyr residues remained. The draining and refilling procedure was also done to the reference tanks.

Visual ratings of percent control were recorded weekly after treatment for four weeks. Wild rice was assessed on a scale of 0 to 100% in 5% increments, where 0 = no control and 100 = complete plant mortality. At four weeks after treatment (WAT), viable wild rice plants were harvested by cutting shoots and tillers at the sediment surface and plant height (cm); tiller density (N pot^{-1}) and seedhead density (N pot^{-1}) were recorded. Plants were then dried at 70 C to a constant mass, weighed, and compared to the untreated reference to assess herbicide efficacy. A one-way Analysis of Variance (ANOVA) with a Fisher's LSD post hoc analysis was used to assess differences in shoot biomass, plant height, tiller density, and seed head number between herbicide treatments within a given growth stage. A one-way ANOVA with a Fisher's LSD post hoc analysis was conducted within week and within growth stage for visual ratings. All analyses were conducted at the $p = 0.05$ level of significance using Statistix 8.0 (Analytical Software 2003).

RESULTS AND DISCUSSION

Triclopyr residue analyses of water sampled 30 min post treatment indicated nominal concentrations were near target concentrations (Table 1). Triclopyr degradation was 39% at the 0.75 mg ae L^{-1} concentration followed by 26% for the 1.5 mg ae L^{-1} concentration, and 33% for the 2.5 mg ae L^{-1} concentration after 72 hours. Field studies indicate that triclopyr applied to natural waters degrades relatively quickly depending on the degree of water exchange (Woodburn et al. 1993, Petty et al. 2003). Triclopyr concentrations used in this study are typical concentrations used to control Eurasian watermilfoil in field situations (Poovey et al. 2004).

Triclopyr effects on wild rice were most pronounced on seedling plants, most notably at concentrations of 1.5 and 2.5 mg ae L^{-1}. At four WAT, plant mass was reduced ($p < 0.01$) when plants were exposed to the highest triclopyr concentrations (Figure 1A). Plant height was reduced by 69% when exposed to 2.5 mg ae L^{-1} (Figure 1B). Seedhead density was

Table 1. Mean (± 1 SE) concentrations of triclopyr residues in water sampled 30 minutes and 72 hours post treatment (n = 36, mean of 9 replicates per concentration).

Target triclopyr concentration (mg L^{-1})	30 minute post treatment (mg L^{-1})	72 hour post treatment (mg L^{-1})	% Degradation
0	0 ± 0	0 ± 0	0
0.75	0.72 ± 0.07	0.44 ± 0.04	39
1.5	1.54 ± 0.06	1.15 ± 0.04	26
2.5	2.48 ± 0.11	1.91 ± 0.13	33

[4]SePRO Corporation, Carmel, Indiana.
[5]Wildlife Nurseries, Oshkosh, Wisconsin.
[6]Scotts-Sierra Horticultural Products Company, Marysville, Ohio.

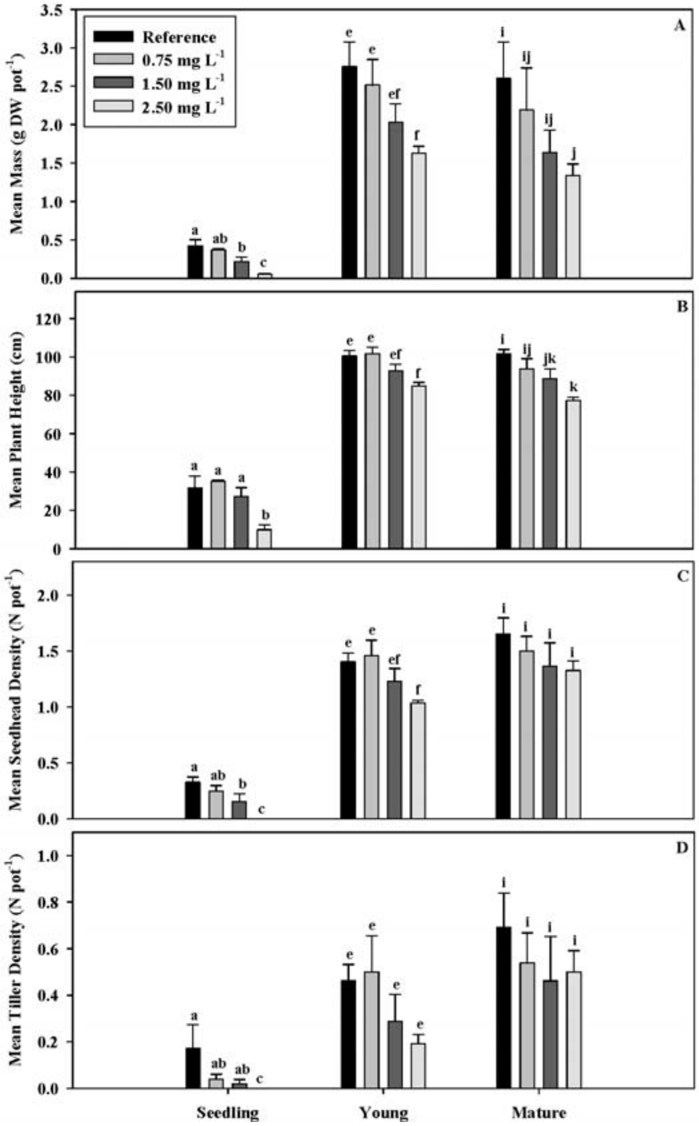

Figure 1. Mean (± 1 SE) plant mass (A), plant height (B), seedhead density (C), and tiller density (D) of wild rice (*Zizania palustris* L.) four weeks after treatment to varying concentrations of triclopyr. Bars sharing the same letter do not differ significantly at p = 0.05 according to Fisher's LSD. Analyses were conducted within growth stage.

reduced by ($p < 0.01$) four WAT when plants were exposed to 1.5 and 2.5 mg ae L^{-1} concentrations respectively (Figure 1C). Seedling plants exposed to the 0.75 mg ae L^{-1} did not experience any significant reductions in plant height, plant mass, seedhead density (Figure 1A-C), or visual control (Table 2). However, tiller density was reduced by 82% compared to reference plants (Figure 1D). Control ratings further indicate the sensitivity of seedling plants to triclopyr. Control was evident 1 WAT with 20% control when exposed to the 2.5 mg ae L^{-1} concentration (Table 2). Wild rice control was not different four WAT between reference plants and those exposed to 0.75 mg ae L^{-1} triclopyr for the seedling growth stage.

Young wild rice plants exposed to the 2.5 mg ae L^{-1} concentration experienced reductions in plant mass ($p = 0.04$; Figure 1A). Plant height ($p < 0.01$) and seedhead density ($p = 0.04$) were also reduced at the 2.5 mg ae L^{-1} triclopyr concentration (Figure 1B, C). Tiller density of young wild rice plants was not effected ($p = 0.17$) when exposed to 2.5 mg ae L^{-1} (Figure 1D). Young wild rice was not injured at 0.75 mg ae L^{-1} when compared to reference plants (Table 2). Control ratings for plants exposed to 1.5 and 2.5 mg ae L^{-1} were 10 and 15%, respectively (Table 2). In a similar study, the younger growth stages (i.e., seedling and young) of wild rice were also most sensitive to 2,4-D, diquat, endothall, and fluridone exposure as compared to mature plants (Nelson et al. 2003).

Mature wild rice plants were less sensitive to triclopyr than younger growth stages. Wild rice mass was reduced by 48% when exposed to 2.5 mg ae L^{-1} (Figure 1A). Plant height was reduced ($p < 0.01$) when exposed to the two highest triclopyr concentrations (Figure 1B). Seedhead density and tiller density were not affected by triclopyr at any concentration. There were no adverse effects of triclopyr exposure to mature plants at the 0.75 mg ae L^{-1} concentration. Control ratings were only 10 and 15%, respectively, for the 1.5 and 2.5 mg ae L^{-1} concentrations four WAT and correspond to the significant reductions in plant height (Table 2). Control ratings at 0.75 mg ae L^{-1} were similar to reference plants.

Plant age and stage of development can influence susceptibility to herbicides (Ross and Lembi 1998), as indicated by the sensitivity of seedling and young wild rice to triclopyr observed in this study and those observed by Nelson et al. (2003). In general, annual plants are more susceptible to herbicide applications when in the seedling stage, and susceptibility tends to decrease with maturity (Anderson 1996). Furthermore, young actively growing plants are more susceptible to herbicide injury than mature plants (Anderson 1996, Radosevich et al. 1997, Ross and Lembi 1998). Younger plants typically are in a stage of rapid growth as compared to mature plants, resulting in a greater susceptibility to herbicide injury through increased herbicide uptake (Radosevich et al. 1997). Younger plants have lower carbohydrate reserves than that of mature plants, making them less likely to recover from herbicide injury (Ross and Lembi 1998). Likewise, plant morphology or growth habit can determine the degree of sensitivity to some herbicides (Anderson 1996, Radosevich et al. 1997). The seedling stage of wild rice was characterized by having floating leaves, resulting in the entire plant being in contact with both the water and triclopyr for the entire exposure time, which may have attributed to the greater sensitivity. In contrast, the young (one or two emergent leaves) stage, and mature (flowering) stage plants were growing out of the water, limiting the contact of triclopyr to submersed tissues.

Herbicide metabolism has been examined extensively in agricultural settings, but little data exist for metabolism of aquatic herbicides in plants. The metabolism of herbicides is considered to be the most important mechanism of herbicide degradation in terrestrial plants (Devine et al. 1993), a mechanism that should apply to aquatic systems as well. Metabolism of herbicides may also increase as a plant species matures. Efficacy of trifloxysulfuron (*N*-

TABLE 2. PERCENT CONTROL RATINGS OF THREE GROWTH STAGES OF WILD RICE (*Zizania palustris* L.) FOLLOWING 72 HOUR EXPOSURE TO TRICLOPYR.

Growth stage	Triclopyr concentration mg L^{-1}		Weeks after treatment[ab] 1	2	3	4
			%			
Seedling	0.00		0 a	0 a	0 a	0 a
	0.75		15 ab	15 a	15 a	20 a
	1.50		10 ab	50 b	50 b	55 b
	2.50		20 b	80 c	80 c	90 c
		LSD	18	21	23	26
Young	0.00		0 a	0 a	0 a	0 a
	0.75		0 a	0 a	0 a	1 a
	1.50		0 a	0 a	5 b	10 b
	2.50		0 a	5 b	5 b	15 c
		LSD	0	2	3	4
Mature	0.00		0 a	0 a	0 a	0 a
	0.75		0 a	0 a	0 a	0 a
	1.50		0 a	10 b	10 b	10 b
	2.50		0 a	5 b	10 b	15 b
		LSD	0	4	5	5

[a]Means in a column followed by the same letter do not differ significantly at p = 0.05 according to Fisher's LSD.
[b]Analyses were conducted within weeks and growth stages.

[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(2,2,2-trifluoroethoxy)-2-pyridinesulfonamide) and glyphosate (N-(phosphonomethyl) glycine) decreased in some weed species when applications were delayed from the four-leaf stage to the six-leaf stage (Singh and Singh 2004, Grichar and Minton 2007). In addition, gramineous plants have higher metabolic activity than other plants; rice (*Oryza* sp.) in particular has a higher metabolic activity than other plants to detoxify herbicides (Usui 2001). The role of metabolism should be investigated further with wild rice and aquatic herbicides.

Our data suggest that wild rice is tolerant to triclopyr concentrations typically used in Eurasian watermilfoil control. These concentrations usually fall between 0.75 and 1.5 mg ae L^{-1} (Poovey et al. 2004). A minimum exposure time of 42 hours is required for >85% Eurasian watermilfoil control at 0.75 mg ae L^{-1} (Netherland and Getsinger 1992). A minimum exposure time of 30 hours is required for >85% Eurasian watermilfoil control at a triclopyr concentration of 1.5 mg ae L^{-1}. Eighteen hours is required for similar Eurasian watermifoil control at 2.5 mg ae L^{-1}. Based on these relationships, control of Eurasian watermilfoil using the rates from this study can be achieved at exposure times much less than 72 hours, which may result in reduced susceptibility to wild rice, even at higher triclopyr concentrations. Maintaining higher concentrations for 72 hours under field conditions is likely unrealistic due to herbicide degradation and dissipation. It is unclear however, if lower triclopyr concentrations at longer exposure times would impact wild rice. A triclopyr concentration of 0.25 mg ae L^{-1} at a 72 to 84 hour exposure time resulted in >85% control of Eurasian watermilfoil (Netherland and Getsinger 1992). In this study, the 0.75 mg ae L^{-1} concentration did not adversely impact growth of wild rice. In fact, after 72 hours there was still 0.44 mg ae L^{-1} remaining in the tanks, which would have resulted in control of Eurasian watermilfoil at 48 hours. Our results suggest that lower concentrations at prolonged exposure times may not impact wild rice, but more data at longer exposures are needed.

Sensitivity of wild rice to triclopyr is dependent upon the growth stage of the plants, with the seedling stage exhibiting the greatest sensitivity to different concentrations. This difference in sensitivity among the growth stages could be due to plant development, increased herbicide metabolism in older plants, decreased translocation in aerial portions, differences in plant morphology, or a combination of any or all of these. A triclopyr concentration of 0.75 mg ae L^{-1} should have a negligible effect on wild rice at all growth stages, based on those evaluated in this study.

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