

Comparison of Imazapyr and Imazamox for Control of Parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.)

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INTRODUCTION

Parrotfeather (*Myriophyllum aquaticum* (Vell. Verdc) is an invasive aquatic plant to the United States that is native to South America. *Myriophyllum aquaticum* is described as "stout, stems moderately elongate, partially submersed but with portions of leafy branches emersed (Godfrey and Wooten 1981). Emergent leaves are whorled, stiff, usually with 20 or more linear filiform divisions, appearing feather-like and grayish green. Submersed shoots are comprised of whorls of four to six filamentous, pectinate, red or orange, leaves arising from each node. Flowers are all pistillate, borne in the axils of unreduced leaves (Godfrey and Wooten 1981). *Myriophyllum aquaticum* is dioecious, however only pistillate plants are found outside of its native range (Sutton 1985). In fact, staminate plants are rare even in native populations of South America (Orchard 1981). For this reason, seed production is not known to occur and reproduction is exclusively vegetative through fragmentation of emergent shoots, submersed shoots, and stolons (Aiken 1981, Orchard 1981). Additionally, *M. aquaticum* lacks structures for storage, dispersal, and perennation (e.g., tubers, turions, and winter buds) and therefore stolons are believed to serve all these functions (Sytsma and Anderson 1993).

Myriophyllum aquaticum grows rapidly and can persist as a submersed plant or more commonly grows as an emergent creeping stoloniferous perennial. The creeping growth has caused major problems in water-bodies in the United States, where such infestations of have reduced access, use, and runoff in ditches, streams, ponds, and shallow lakes (Sutton 1985). The creeping growth of *M. aquaticum* enables it to cover large areas of a water-body in a short period of time which impedes navigation, stream flow, and runoff to such an extent that flooding of adjacent lands occurs (Sutton 1985). *Myriophyllum aquaticum* also provides mosquito larvae a refuge from predation and can indirectly aid in the spread of insect born diseases (Orr and Resh 1989). The problems posed by *M. aquaticum* are often perpetuated as this species is widely cultivated and sold in the United States via the water garden industry (Aiken 1981, Sutton 1985).

Myriophyllum aquaticum is difficult to control and once established, it is capable of thriving in a variety of environmental conditions in addition to the deployment of management

techniques (Moreira et al. 1999). To date, chemical control has been the most effective method for controlling infestations of *M. aquaticum*. Contact herbicides such as diquat (6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazinedium dibromide) and endothall (7-oxabicyclo (2.2.1) heptane-2,3-dicarboxylic acid) have been evaluated with mixed results (Moreira et al. 1999, Westerdahl and Getsinger 1988). Contact herbicides are typically effective for short-term control, but significant regrowth of *M. aquaticum* typically occurs and multiple applications are necessary (Moreira et al. 1999). Therefore, the use of a systemic herbicide may be more effective in controlling this species.

Imazapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imazol-2-yl]-3-pyridinecarboxylic acid) is a systemic herbicide that has been labeled for aquatic use and has shown promise in controlling smooth cordgrass (*Spartina alterniflora* Loisel) (Patten 2003) and torpedograss (*Panicum repens* L.) (Hanlon and Langeland 2000). Imazamox, 2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H imidazol-2-yl)-5-(methoxymethyl)-3-pyridinecarboxylic acid, is a new herbicide currently being evaluated for use in aquatic systems under an experimental use permit (EUP) from the United States Environmental Protection Agency. Both imazapyr and imazamox belong to the imidazolinone herbicide family. The site of action of the imidazolinone herbicides is the acetohydroxyacid synthase (AHAS) enzyme, the first step in the biosynthesis of the essential branched chain amino acids isoleucine, leucine and valine (WSSA 2002). Imidazolinone herbicides inhibit the production of AHAS enzymes resulting in a lethal decrease in protein synthesis (Shaner and Mallipudi 1991). Both imazapyr and imazamox are rapidly absorbed into the foliage and translocated through the target plants via phloem and xylem tissues (Shaner and Mallipudi 1991, WSSA 2002). Both herbicides inhibit plant growth within the first 24 hours after application; however, with visual symptoms typically appear at least one week after treatment (WSSA 2002). The meristematic regions of the plant are targeted with symptoms of slow foliar chlorosis and necrosis (WSSA 2002).

To date, there have been no published data on the use of imazapyr for control of *M. aquaticum* and no published data on the use of imazamox for the control of any aquatic plants. The objective of this study was to evaluate the efficacy of various rates of imazapyr and imazamox for the control of *M. aquaticum*. The systemic properties of these herbicides may provide control of the entire *M. aquaticum* plant, not just the emergent portion that is typically affected by application of contact herbicides.

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MATERIALS AND METHODS

The study was conducted in an outdoor mesocosm facility at the R. R. Foil Plant Research Station, Mississippi State University, Starkville, Mississippi, for 14 weeks beginning in August 2006 and ending November 2006. The experimental design consisted of a randomized complete block design with three rates of imazapyr, three rates of imazamox, and an untreated control. Each treatment was replicated three times in 378 L tanks. *Myriophyllum aquaticum* used in this study was cultured from greenhouse stock by planting 20 cm apical shoots into 3.78 L plastic pots. Pots were filled with super soil potting medium (a mixture of top soil, loam, and masonry sand) and amended with 2 g L⁻¹ of 19-6-12 Osmocote® fertilizer³ and placed into the tanks. Four pots were placed into every tank for a total of 84 pots. *Myriophyllum aquaticum* was allowed to grow in the tanks for approximately four weeks until the emergent growth covered the water surface. After four weeks, prior to treatment, one pot of *M. aquaticum* was harvested from each tank by cutting plant mass at the sediment surface. Plants were dried to a constant mass and weighed to assess pre-treatment biomass.

Following the growth period, foliar applications of imazapyr (1123, 584, 281 g ai ha⁻¹) and imazamox (561, 281, 140 g ai ha⁻¹) were made to *M. aquaticum* using a CO₂-pressurized sprayer at a spray volume of 187 L ha⁻¹. A non-ionic surfactant (Dyne-Amic®⁴) was added to the spray mixture at a rate of 0.25% v.v. Barriers were placed around each tank during applications to prevent herbicide drift and cross contamination between treatments. A spray volume of 187 L ha⁻¹ was used to simulate a low volume application. Imazapyr applied at 187 L ha⁻¹ controlled torpedograss (*Panicum repens* L.) in 10 of 26 plots in Florida (Hanlon and Langeland 2000). The reduced level of control was attributed to differences in environmental conditions at treatment sites, and the presence of thatch in the treatment areas that inhibited herbicide contact with target plants (Hanlon and Langeland 2000). Gly-

phosate applied at 187 L ha⁻¹ increased herbicide retention on waterhyacinth (*Eichhornia crassipes* (Mart.) Solms.) leaves resulting in greater herbicide efficacy (Van et al. 1986). Imazamox was examined at spray volumes of 47, 94, and 190 L ha⁻¹ for control of 'Valley' oat (*Avena sativa* L.), foxtail millet (*Setaria italica* (L.) P. Beauv.), and proso millet (*Panicum miliaceum* L.) (Ramsdale and Messersmith 2001). In these trials, efficacy was not affected by changes in spray volume and efficacy was similar across all volumes (Ramsdale and Messersmith 2001).

Visual injury ratings of percent control were recorded weekly after treatment (WAT) for ten weeks. *Myriophyllum aquaticum* control was assessed on a scale of 0 to 100, where 0 = no control and 100 = complete plant mortality. At 10 WAT viable *M. aquaticum* was harvested, dried to a constant mass, weighed, and compared to the control plants 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 biomass between herbicide treatments. A one-way ANOVA with repeated measures was conducted on the visual ratings. All analyses were conducted at a p = 0.05 level of significance using Statistix 8.0 (Analytical Software 2003).

RESULTS AND DISCUSSION

At 4 WAT, imazapyr at 1123 and 584 g ai ha⁻¹ rates and imazamox at 561 and 281 g ai ha⁻¹ controlled *M. aquaticum* 73.6, 80.0, 63.3, and 78.3%, respectively (Table 1). However, by 6 WAT regrowth of plant tissue in the imazamox treatments was observed and control decreased to 63.3 and 56.7% for the 561 and 281 g ai ha⁻¹ rates, respectively. Additionally, *M. aquaticum* treated with imazapyr at 1123 and 584 g ai ha⁻¹ was controlled 90.0% 6 WAT with an increase to 100.0% for both treatments by 8 WAT. At the conclusion of the study *M. aquaticum* treated with the two highest rates of imazapyr was controlled 100.0%. It was observed that beyond 5 WAT, *M. aquaticum* control with any imazamox rate was significantly less than all imazapyr treatments except imazapyr at 281 g ai ha⁻¹. The 281 g ai ha⁻¹ rate of imazapyr and 140 g-ai ha⁻¹ rate of imazamox were not effective for controlling *M. aquaticum*.

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TABLE 1. PERCENT CONTROL (± 1 SE) OF PARROTFEATHER (*MYRIOPHYLLUM AQUATICUM*) BIOMASS FOLLOWING HERBICIDE TREATMENT WITH IMAZAPYR AND IMAZAMOX.

Herbicide (g ai ha ⁻¹)	Weeks after treatment (percent control)									
	1	2	3	4	5	6	7	8	9	10
Untreated reference	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Imazapyr										
1123	13.3 ± 1.6 b	23.3 ± 1.6 b	65.0 ± 7.6 b	73.3 ± 6.6 b	86.7 ± 3.3 b	90.0 ± 0.0 b	95.0 ± 0.0 b	100.0 ± 0.0 b	100.0 ± 0.0 b	100.0 ± 0.0 b
584	15.0 ± 0.0 b	21.7 ± 3.3 b	48.3 ± 14.2 b	80.0 ± 0.0 b	86.7 ± 3.3 b	90.0 ± 0.0 b	96.7 ± 1.6 b	100.0 ± 0.0 b	100.0 ± 0.0 b	100.0 ± 0.0 b
281	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	6.7 ± 1.6 a	10.0 ± 0.0 a	10.0 ± 0.0 a
Imazamox										
561	13.3 ± 1.6 b	15.0 ± 2.8 b	20.0 ± 2.8 b	63.3 ± 8.8 b	76.7 ± 3.3 bc	63.3 ± 6.6 c	63.3 ± 6.6 c	63.3 ± 6.6 c	63.3 ± 6.6 c	53.3 ± 6.6 c
281	8.3 ± 3.3 b	16.7 ± 3.3 b	41.7 ± 16.4 b	78.3 ± 4.4 b	71.7 ± 1.6 bc	56.7 ± 6.6 c	60.0 ± 10.0 c	60.0 ± 10.0 c	60.0 ± 10.0 c	46.7 ± 12.0 c
140	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	8.3 ± 0.0 a	8.3 ± 0.0 a	8.3 ± 0.0 a	8.3 ± 0.0 a

^aHerbicide treatments applied at 187 L ha⁻¹ using a CO₂ pressurized backpack sprayer.

^bMeans in a column followed by the same letter do not differ significantly at an 0.05 level of significance according to Fisher's LSD.

Imazamox at the two highest rates caused necrosis of the apical meristems of *M. aquaticum*, however once this occurred, new emergent shoots began to grow from the nodes beneath the dead apical shoots; an observation typically called witches broom. Witches broom is a deformity in the growth of plants as a result of disease or damage to the apical meristems. Witches broom can be caused by several factors most notably by fungi, insects, mistletoe, mites, nematodes, and viruses, however, any damage to the apical meristem such as herbicide injury could cause the symptoms. In a study conducted by Boutin et al. (2000) witches broom was observed for two wetland plant species, monkey-flower (*Mimulus ringens* L.) and bur-marigold (*Bidens cernua* L.), and three terrestrial plant species beans (*Phaseolus vulgaris* L.), wild mustard (*Sinapis arvensis* L.), and barnyardgrass (*Echinochloa crusgalli* L.). Witches broom was induced through exposure of the plant species to 1% and 10% label rate of the sulfonyleurea herbicide metsulfuron methyl (2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl) benzoate). *Bidens cernua* plants sprayed with the 10% label rate resulted in mortality of flower buds and unnaturally bushy plants. *Phaseolus vulgaris* plants sprayed with the 1% label rate appeared to grow more lateral branches than control plants. *Echinochloa crusgalli* plants sprayed with the 10% label rate were shorter than control plants and produced numerous small tillers in response to exposure to metsulfuron methyl (Boutin et al. 2000).

Witches broom is characterized by the release of apical dominance and subsequent outgrowth of lateral buds, symptoms that can be duplicated by treating seeds and seedlings with cytokinins (Murai et al. 1980). Plant hormones (gibberellins, cytokinins, and abscisic acid) are typically produced in the meristematic regions of the plant and enforce apical dominance; treating a plant with an imidazolinone herbicide results in the death of the apical tip of a plant shoot that results in a decrease in the rate of auxin production and the subsequent release of apical dominance (Shaner 1991). Apical dominance has been reported in some grass species where applications of imidazolinone herbicides on susceptible grasses will kill the main culm releasing tillers from apical dominance (Little and Shaner 1991). The tillers are not dependent on the main culm for photosynthates and do not import the imidazolinones from the main culm unless the initial dose reaching the tillers prior to the death of the apical meristem is sufficient to cause death (Little and Shaner 1991). Therefore, the loss of apical dominance could be an explanation or a secondary sub-lethal effect of imazamox causing the observed shoot growth of *M. aquaticum*. The initial treatment of imazamox in this study may have only been enough to kill the apical tips of the emergent shoots or imazamox was not translocated in high enough concentrations throughout the rest of plant, which allowed new emergent shoots to grow. However, once these new shoots appeared, subsequent growth stalled with no additional growth or signs of herbicide injury for the remainder of the study. Other work has reported this phenomenon with imidazolinone herbicides where plant tissue remained green for a long period of time and plants remained in an arrested state of growth for weeks after treatment prior to the herbicides achieving control (Shaner 1991).

Percent control observed in this study was supported by plant biomass collection of the treated and untreated plants at the conclusion of the study (Figure 1). *Myriophyllum aquaticum* biomass was significantly ($F = 32.7$, d.f. = 62, $p \leq 0.001$) reduced when treated with rates of 1123 and 584 g ai ha⁻¹ of imazapyr and 561 and 281 g ai ha⁻¹ rates of imazamox. However, imazapyr performed better than imazamox in this study as reflected by reductions in *M. aquaticum* biomass. Plants were not removed from the tanks after herbicide application; therefore, injury and reductions in biomass may have occurred through herbicide uptake via submersed tissue. However, imazapyr is not labeled for application to the water column and must be applied as a foliar application to actively growing emergent plants. If imazapyr at the highest rate were sprayed on the water surface of the tanks, in the absence of plant cover, the resulting concentration would have been approximately 0.3 µg/L, which is not a lethal concentration. Patten (2003) reported that initial imazapyr concentrations in sea water after a foliar application to *Spartina alterniflora* Loisel was only 3.4 µg/ml. Additional water samples taken minutes later just outside the application area had imazapyr concentrations that were reduced by 99.0% (Patten 2003). Also, Patten (2003) reported that when imazapyr was applied directly to Japanese eelgrass (*Zostera japonica* Aschers) it resulted in complete mortality of the plants, however, when a film of water was over the canopy of the *Z. japonica* imazapyr had no effect on the plants. Therefore, it appears that imazapyr does not have a long residence time, does not have activity in water, and would not significantly impact submersed tissues of *M. aquaticum* in this study.

Imazamox can be applied to the water column at effective concentrations of 5 to 200 µg/L; however, if imazamox at the highest rate was sprayed on the water surface of the tanks, in the absence of plant cover, the resulting concentration would have been approximately 0.1 µg/L, a concentration well below the label minimum effective rate. *Myriophyllum aquaticum* covered the water surface of the tanks, so it is unlikely that large amounts of the spray solution entered the water column. The small amount of spray solution that did enter the water column likely would not have been at a high enough concentration to confound the results of this study. Therefore, the difference in efficacy between imazapyr and imazamox observed in this study may be attributed to the differential metabolism of the herbicides by *M. aquaticum* to

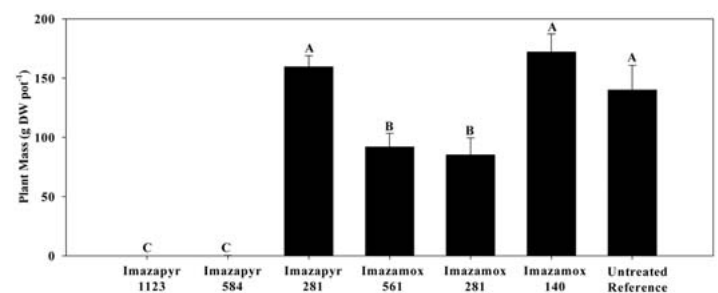


Figure 1. Mean (± 1 SE) biomass of parrotfeather (*Myriophyllum aquaticum*) 10 weeks after treatment with imazapyr (g ai ha⁻¹) and imazamox (g ai ha⁻¹) herbicides. Bars sharing the same letter do not differ significantly at a 0.05 level of significance according to Fisher's LSD.

non-toxic metabolites. Differential metabolism is common with the imidazolinone herbicides and has been documented with imazapyr, imazethapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid), imazamethabenz-methyl (methyl 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-4 (or 5)-methylbenzoate), and imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid) (Shaner and Mallipudi 1991). Imazamox is readily metabolized in jointed goatgrass (*Aegilops cylindrica* L.) and feral rye (*Secale cereale* L.) (Pester et al. 2001). It appears that *M. aquaticum* is better able to metabolize the active ingredient in imazamox which would explain the reduced efficacy and subsequent regrowth as imazamox was not fully translocated throughout the plant.

Reduced control and regrowth of *M. aquaticum* following herbicide applications as observed with the imazamox treatments in this study is not uncommon as this species has shown resiliency towards most herbicides and application methods. Gray et al. (2007) reported that *M. aquaticum* control was only 70% when carfentrazone-ethyl (ethyl α , 2-dichloro-5-[4-(difluoromethyl)-4, 5-dihydro-3-methyl-5-oxo-1*H*-1, 2, 4-triazol-1-yl]-4-fluorobenzenepropanoate) was applied to the water column. In a similar study, control of *M. aquaticum* was only 29 to 54% when carfentrazone-ethyl was applied to the water column (Glomski et al. 2006). In Portugal, foliar treatments of glyphosate N- (phosphonmethyl) glycine, and diquat were not effective for controlling *M. aquaticum* and often permitted rapid re-infestation (Moreira et al. 1999). In New Zealand, foliar applications of clopyralid (3,6-dichloropicolinic acid), fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone), triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid), glyphosate, endothall, and dichlobenil (2,6-dichlorobenzonitrile) were evaluated in contained mesocosm studies, while triclopyr, dichlobenil, and endothall were evaluated in field studies (Hofstra et al. 2006). Fluridone and clopyralid were not successful in controlling *M. aquaticum* in these studies and glyphosate showed initial efficacy, however, significant regrowth was reported. Triclopyr, dichlobenil, and endothall were effective in controlling *M. aquaticum* in mesocosm trials. Triclopyr was effective at reducing *M. aquaticum* percent cover in field situations, but regrowth did occur (Hofstra et al. 2006). The auxin herbicides clopyralid and dichlobenil are not labeled in the United States for aquatic use and this leaves 2, 4-D (2,4-dichlorophenoxy) acetic acid, triclopyr, and imazapyr as potential herbicides to treat *M. aquaticum* infestations as foliar applications.

Based on the results of this study, imazapyr at the rates of 1123 and 584 g ai ha⁻¹ were effective as a foliar application for control of *M. aquaticum*, as there was complete control of the plant after 10 weeks with no regrowth.

Imazapyr and imazamox, both relatively new chemistries to aquatics, were evaluated as potential systemic herbicides for control of *M. aquaticum*. Both herbicides show promise as new tools for aquatic plant management. This study indicates that imazapyr was most effective at controlling and maintaining control of *M. aquaticum* for at least 10 weeks. Imazamox activity was observed on *M. aquaticum*; however, regrowth was evident and a second application may be necessary to

achieve complete control. The delay in regrowth in the imazamox treatments suggests that the herbicide may be still active in the plants and higher sprayer volumes, higher rates, or use of two herbicides with different modes of action may be an effective alternative to re-application.

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