THESIS

EVALUATION OF HERBICIDES FOR CONTROL OF EURASIAN WATERMILFOIL AND SAGO PONDWEED

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY JOSEPH D. VASSIOS ENTITLED EVALUATION OF HERBICIDES FOR CONTROL OF EURASIAN WATERMILFOIL AND SAGO PONDWEED BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

EVALUATION OF HERBICIDES FOR CONTROL OF EURASIAN WATERMILFOIL AND SAGO PONDWEED

The aquatic species Eurasian watermilfoil (*Myriophyllum spicatum*) and sago pondweed (*Stuckenia pectinata*) can act in an invasive manner, and when present can negatively impact wildlife habitat. Eurasian watermilfoil is a submersed perennial noxious weed species that is widespread across the United States. Sago pondweed is a submersed perennial species that is a native to all 50 states. Although sago pondweed is a native, it thrives and can become troublesome in irrigation canals. Experiments were conducted to evaluate herbicides to control both species.

Imazamox is a newly registered aquatic herbicide that can be used to control Eurasian watermilfoil. Three laboratory experiments were conducted to examine the response of Eurasian watermilfoil to imazamox. ^{14}C –imazamox was used to evaluate imazamox absorption rate, the influence of external imazamox concentration on absorption, imazamox desorption when plants were transferred to clean water, and imazamox absorption. Imazamox absorption by Eurasian watermilfoil was low. The weed absorbed only 0.5% of the herbicide applied 24 HAT, and reached a maximum of 0.97% 72 HAT. External concentration affected imazamox absorption, where plants absorbed 1.05 μg per plant at a treatment concentration of 200 μg L^{-1}, while at 800 μg L^{-1}

absorption was 4.06 μg per plant. The percent of applied imazamox absorbed was the same regardless of the external concentration, indicating that absorption was the result of simple diffusion driven by a concentration gradient. Desorption after plants were placed in clean water was rapid, reaching equilibrium by 12 hours with 46% of absorbed imazamox having moved into the surrounding water. The metabolism study indicated that 144 HAT; 69.04% of absorbed ^{14}C-imazamox was found in the bound fraction, 11.52% as soluble metabolites and 21.44% remained as imazamox. In addition to laboratory experiments, three whole lake treatments were applied and imazamox dissipation was monitored.

Three greenhouse experiments on sago pondweed were conducted to evaluate herbicide control when applied pre-emergence to a soil surface simulating a dewatered irrigation canal treatment. Herbicides evaluated included imazamox, imazapyr, fluridone, penoxsulam, flumioxazin, pyroxasulfone, dimethenamid, and metolachlor. In addition to herbicide control, the effect of incorporation using simulated rainfall was evaluated. Rainfall incorporation did not have a significant effect, and all treatments resulted in a biomass reduction on 70% or greater when compared to the untreated control. In addition to greenhouse studies, four field studies were conducted. Herbicide residues were quantified in canal sediments and canal water for all sites.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1: Imazamox absorption, desorption, and metabolism by Eurasian watermilfoil.

ABSTRACT

Eurasian watermilfoil (*Myriophyllum spicatum*) is a submerged invasive species currently infesting 45 states, including Colorado. Eurasian watermilfoil negatively impacts recreation, wildlife habitat, and the efficiency of water delivery. Several laboratory experiments were conducted to determine the response of Eurasian watermilfoil to imazamox. Experiments were: 1) imazamox absorption rate using ^{14}C-imazamox, 2) the influence of external imazamox concentration on absorption, 3) imazamox desorption when plants were transferred to clean water, and 4) imazamox metabolism over a six day time course. Imazamox absorption by Eurasian watermilfoil 24 HAT was only 0.5% of the herbicide applied, and absorption increased to 0.97% 72 HAT. External imazamox concentration affected imazamox absorption. At 200 µg L^{-1} imazamox, Eurasian watermilfoil plants absorbed 1.05 µg per plant, while at 800 µg L^{-1} absorption increased to 4.06 µg per plant. The percent of applied imazamox absorbed was the same regardless of the external concentration, indicating that absorption was the result of simple diffusion driven by a concentration gradient. Desorption occurred rapidly, reaching equilibrium 12 hours after plants were transferred to clean water with 46% of absorbed imazamox moving into the surrounding water column. The metabolism study indicated 69.04% of absorbed ^{14}C-imazamox was found in the bound fraction 144

HAT, while 11.52% appeared as soluble metabolites and only 21.44% as intact imazamox.

INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a submersed aquatic macrophyte that is considered invasive across much of the United States. While the upper Midwest has some of the most significant infestation, heavy infestations can be also be found in lakes along Colorado's Front Range, as well as in irrigation canals. Eurasian watermilfoil can drastically impact recreation, aquatic vertebrate habitat, and the ability to efficiently deliver water.

Although it is a perennial, Eurasian watermilfoil has an annual growth pattern. When waters warm up in spring, single shoots will grow rapidly toward the surface. Once shoots near the water surface they will branch profusely and form large, dense mats. After branching at the water surface, plants will flower and fragment. These shoot fragments will then fall to the bottom of the water body, and the cycle starts over. Although Eurasian watermilfoil does produce viable seeds, the main method of spread and reproduction is through vegetative fragments (Smith and Barko, 1990). Eurasian watermilfoil thrives in waters 1-4 m deep (Nichols and Shaw, 1986), but in water with greater water clarity, it can grow from a depth of 10 m (Aiken et al., 1979). Maximum growth is achieved at 30-35ºC, which also corresponds to the temperature range for maximum photosynthetic activity (Smith and Barko, 1990).

Eurasian watermilfoil has several characteristics that contribute to its invasiveness. It often establishes early in the growing season when water temperatures are relatively low (Barko et al., 1982), shading native competitors. Since light is a major limiting factor in aquatic systems, this can make it difficult for native species to establish and can lead to dense monocultures of Eurasian watermilfoil. Also, colonizing through

fragments allows Eurasian watermilfoil to be spread easily by animals, human activities, and flowing water.

Due to Eurasian watermilfoil's aggressive nature a variety of strategies have been implemented to control this invasive species. Biological, mechanical, cultural, and chemical control methods are available. Biological controls for Eurasian watermilfoil include a native milfoil weevil (*Euhrychiopsis lecontei*), which has been shown to provide some control (Roley and Newman, 2006). The milfoil weevil feeds only on plants in the *Myriophyllum* genus, and prefers Eurasian watermilfoil to hybrid milfoil (Roley and Newman, 2006) and other native species including *Myriophyllum sibiricum* (Newman, 2004). Another option for biological control is grass carp (*Ctenopharyngodon idella*). Even though grass carp can provide control, they are generalist feeders and prefer feeding on many of the native submerged species (McKnight and Hepp, 1995). Eurasian watermilfoil can be managed with mechanical harvesting. Mechanical harvesters cut plants several feet below the surface; however, since it spreads mainly through vegetative fragments, harvesting can actually contribute to spreading Eurasian watermilfoil if all fragments are not collected. Mechanical harvesting can provide temporary control, but is not a practical long-term solution.

Herbicides represent a more long-term management strategy for Eurasian watermilfoil. Contact herbicides labeled for aquatic use include endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid), diquat (6,7-dihydrodipryido[1,2-α:2',1'-c]pyrazinediium ion), and copper. An advantage of using contact herbicides is that they may require a shorter contact time than systemic herbicides, but they may only provide temporary control. Systemic herbicides currently labeled for Eurasian

watermilfoil control include 2,4-D ((2,4-dichlorophenoxy)acetic acid), triclopyr ([(3,5,6-trichloro-2-pryidinyl)oxy]acetic acid), and fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone) (Petty, 2005; Vencill, 2002). Systemic herbicides may provide more long-term control than contact herbicides, but require a longer exposure time in order to be effective. There are many options for the Eurasian watermilfoil management; however, they have limitations.

Imazamox [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo- 1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid] (Vencill, 2002) is a newly registered herbicide that inhibits acetolactate synthase (ALS), the first committed step in branched chain amino acid biosynthesis. Imazamox is effective on a variety of emergent and submersed species, including Eurasian watermilfoil. Imazamox also has a favorable environmental profile, which has led to it being granted a tolerance exemption from the US EPA, as well as minimal irrigation restrictions on turf and crops.

Previously published research that has focused on pesticide absorption in aquatic plants has presumed that aquatic plants are bioaccumulaors of very lipophilic pesticides including atrazine (log K_{ow} 2.34), linuron (log K_{ow} 3.00), and diazinon (log K_{ow} 3.81) (Crum et al., 1999; de Carvalho et al., 2007). The most lipophilic herbicide currently labeled for aquatic use is fluridone (log K_{ow} 1.87). Very little information is available regarding the absorption (bioaccumulation) of highly water-soluble compounds such as 2,4-D (log K_{ow} 0.18) and triclopyr (log K_{ow} -0.44). Imazamox more closely resembles these water-soluble herbicides with a log K_{ow} of 0.73; therefore, previous studies with highly lipophilic pesticides do not accurately reflect absorption for a highly water-soluble compound such as imazamox. A lipophilic compound would likely accumulate in plant

tissue, but for a more water-soluble compound, absorption driven by a concentration gradient between the water column and water in the plant may be the main route of uptake.

Currently there is no information available regarding the behavior of imazamox in aquatic plants. Therefore, the objectives of this project were 1) to examine imazamox absorption and desorption; 2) to determine the effect of imazamox concentration in the water column on absorption, and 3) to determine the rate of imazamox metabolism by Eurasian watermilfoil.

MATERIALS AND METHODS

Plant Materials

Eurasian watermilfoil shoot fragments were collected from a single population in the Leggett Ditch north of Boulder, CO (40°13' N, 105°08' W) in Fall 2006. The fragments were then cut into 15 cm pieces and the distal end was planted in 5 cm diameter by 10 cm deep plastic cups filled with fine sand. Each pot was fertilized with 0.5 g of slow release fertilizer (Osmocote Classic 14-14-14, The Scotts Company, USA) at planting to maintain active growth. Plants were grown in a 1.2 x 2.4 x 0.3 meter fiberglass tank in the greenhouse until they produced roots. The photoperiod was 10:14 h light:dark cycle with natural light supplemented with 400-watt sodium halide light bulbs. Temperature in the greenhouse was set at a 24°C during the day and 18°C at night. Plants that grew too large for use in laboratory experiments were recycled by removing the apical 15 cm of each plant and replanting as previously described. Unless otherwise noted, potted plants for laboratory experiments were removed from the fiberglass tank and placed in 1.2 L glass cylinders and submerged in 1 L of tap water. After transferring the plants to cylinders, they were allowed to equilibrate in a growth chamber for 24 h prior to treatment with ^{14}C imazamox. Following treatment, the cylinders and plants were moved to a growth chamber with a 10:14 hour light:dark cycle and temperature set at 20°C during the light period and 10°C during the dark period, with a light intensity during the light period of 250 μmoles/m^2•s.

Determination of ^{14}C in Plant Samples

Unless otherwise noted, for all experiments plants were harvested, divided into aboveground and belowground parts, dried to a constant weight at 60°C for 24 hours and

absorbed ^{14}C was determined by biological oxidation (OX500, R.J. Harvey Instrument Co., USA) with 10mL of ^{14}C trapping cocktail (OX-161, R. J. Harvey Instrument Co., USA). To confirm the amount of ^{14}C-imazamox present in the treatment solutions, 100 µL water samples were collected using a pipette and samples were transferred to 20 mL scintillation vials. Scintillation cocktail (10 ml) was then added to each vial (6013371, Ultima Gold LLT, PerkinElmer, USA). Radioactivity for both plant and water samples was then quantified using a liquid scintillation spectroscopy (LSS) (Packard 2500R, PerkinElmer, USA).

Imazamox Absorption Rate

Fifteen rooted plants were treated with 200 µg L^{-1} imazamox that contained 21.7 KBq of ^{14}C imazamox (specific activity 1,850 KBq/mg). The plants were harvested at 6, 12, 24, 48, and 72 hours after treatment (HAT). Three plants were harvested at each time point and samples were analyzed as described above. Three plants were randomly selected for harvest at each time point, each plant representing one replication. The study was repeated.

Influence of External Concentration on Imazamox Absorption

Once placed in nine glass cylinders, rooted plants received one of three treatments: 1) 200 µg L^{-1} + 16.7 KBq, 2) 400 µg L^{-1} + 33.3 KBq, or 3) 800 µg L^{-1} + 66.7 KBq of formulated imazamox plus ^{14}C imazamox, respectively. Three plants were treated at each concentration harvested 24 HAT and analyzed for ^{14}C as described above. The study was repeated.

Imazamox Desorption

To determine imazamox desorption rate, three rooted plants were first treated with 800 ng L^{-1} imazamox concentration that contained 216.7 KBq of ^{14}C imazamox. Plants were allowed to absorb imazamox for 24 hours. After 24 hours plants were triple rinsed in clean water and were then placed in jars that contained 50 mL of tap water. The amount of imazamox desorbing from treated plants was determined by taking 1 mL water samples at 0, 1, 2, 4, 6, 12, 24, 48 and 72 HAT and radioactivity was determined using LSS. After 72 hours in the clean water, whole plants were harvested, dried and oxidized to determine the amount of ^{14}C remaining in the plant. There were three replicate water samples taken per time point. The study was repeated.

Imazamox Metabolism

Plants were placed in 250 ml jars containing 200 mL of water and an 800 ng/mL imazamox concentration that contained 90 KBq of ^{14}C imazamox. Plants were then harvested at 24, 48, 72 and 144 HAT. Shoot material was placed in 50 mL test tubes and 10 mL of an acetone:water (9:1 v/v) solution were added. Tissue was ground using a mechanical tissue homogenizer (302968, Tempest, VirTis, USA) and the homogenate was transferred to 50mL centrifuge tubes with 0.45 micron filter inserts (6831-0409, VectraSpin 20, Whatman, England). Samples were centrifuged for 15 minutes at 3,000 RPM. Next, the filter was rinsed using 2 mL of the acetone:water solution, and then centrifuged for 5 minutes at 3,000 RPM. This was repeated twice and the filtrate was transferred to clean 50 mL glass centrifuge tubes. Samples were then concentrated using a sample evaporator (Rapidvap, Labconco Corp., USA) until most of the acetone was removed. The remaining liquid was then transferred to 2 mL centrifuge tubes with 0.45 μm filter inserts (Costar Spin-x 8170, Corning Inc., USA) and centrifuged for 10 minutes.

The filtrate was then removed and placed in 0.4 mL inserts and ^{14}C imazamox was determined with reverse phase HPLC using a C8 2.1mm x 150mm column (Zorbax, USA). The injection volume was 100 μL. Imazamox eluted at 14 minutes using the following gradient: 89.95% water:10% acetonitrile:0.05% phosphoric acid solution to a 69.95% water: 30% acetonitrile: 0.5% phosphoric acid solution over 25 minutes with a flow rate of 0.3 mL/minute. Radioactivity was quantified using an inline radioactive detector (β-Ram Radioactivity Detector Model 2B, IN/US, USA). All material that was retained by the centrifuge filters were dried and oxidized as previously described. Three replicates (plants) were harvested at each time point. The study was repeated.

Data Analysis

Levene's test for homogeneity of variance was conducted using JMP (Version 7.0.1, SAS Institute, 2007) to determine if data from repeated experiments could be combined. Regression analyses were performed and data plotted using SigmaPlot (Version 9, SYSTAT, 2005).

RESULTS AND DISCUSSION

Imazamox Absorption Rate

Based on results of Levene's test for homogeneity of variance, data from repeated experiments were combined for statistical analyses for all studies. Imazamox absorption over a 72-hour time course was low compared to the amount applied (Figure 1). The function that best described imazamox absorption by Eurasian watermilfoil was:

$$y = \frac{a}{1 + e^{\left(\frac{-(x - x_0)}{b}\right)}} \quad [1]$$

where a=1.51, b=33.93, and x_0=52.37. Only 0.5 % ± 0.06 of applied imazamox was absorbed in the first 24 HAT and by 72 HAT the maximum amount absorbed was 0.97% ± 0.12. These results indicate that 50% of the imazamox absorption occurs in the first 24 HAT and the remaining 50% occurs over the next 48 HAT. This is in sharp contrast to terrestrial species like jointed goatgrass (*Aegilops cylindrical*) and feral rye (*Secale cereale* L.), which absorbed 58% and 44% of applied imazamox by 24 HAT, respectively (Pester et al., 2001). Low imazamox absorption by Eurasian watermilfoil was very similar to herbicide absorption in other submerged macrophytes. Sago pondweed (*Stuckinea pectinatus (L.) Böerner*) and Richardson pondweed (*Potamogeton richardsonii (Benn.) Rydb.*) absorbed only 0.4% and 0.7% of applied fluridone at the end of a 14 day time course, respectively . Due to the high water solublility and low Log K_{ow} of imazamox we would have expected the concentration of imazamox in the plant to be nearly equal to the external concentration, but the actual concentration inside of the plant was 6.93 times the external concentration 72 HAT, based on total radioactivity. Carvalho

et. al. (2007) suggested that more lipophilic compounds would easily permeate membranes, while more water soluble compounds may be absorbed by acid trapping. We predict that the likelihood of this happening with a water-soluble compound like imazamox would be less likely when macrophytes, such as Eurasian watermilfoil, are present. Photosynthesizing aquatic plants can significantly increase water pH, working against this acid trapping hypothesis (Sculthorpe, 1967). Of the previous work, imazamox would be most similar to the uptake of 3,5-D by *Lagarosipon major*, which showed decreased absorption as pH increased. The dissociated form of 3,5-D has a log K_{ow} of 0.25, and more accurately represents the same trends in absorption demonstrated by our research with imazamox (Carvalho, 2007).

Influence of External Concentration on Imazamox Absorption

Imazamox absorption was strongly correlated with external herbicide concentrations over a range of 200 to 800 µg L^{-1} (Figure 2). This relationship appears to be linear with a corresponding function of:

$$y = y_0 + a * x \quad [2]$$

where y_0=0.015 and a=0.005. When treatment concentration increased from 200 µg L^{-1} to 400 µg L^{-1}, the amount of imazamox absorbed increased from 1.05 µg per plant to 1.99 µg per plant and when the concentration increased from 400 µg L^{-1} to 800 µg L^{-1} the amount of imazamox absorbed on a whole plant basis increased from 1.99 µg per plant to 4.06 µg per plant. The amount of imazamox absorbed was approximately 0.5% of the amount applied. The direct linear relationship between external concentration and the amount of imazamox absorbed indicates that absorption was driven by the concentration gradient between the water column and plant. Eurasian watermilfoil possesses a very

thin cuticle, which offers little resistance to diffusion into plant tissue (Sculthorpe, 1967). Given imazamox's high water solubility (4,413 mg L^{-1}) (EPA, 1997), and Eurasian watermilfoil's greatly reduced cuticle, it seems likely a water soluble compound such as imazamox would easily diffuse into plant tissue and partition into water filled free space, eventually coming to equilibrium with the surrounding water column if the plants were exposed over a longer period.

There is very little evidence of significant translocation from the shoot to root tissue. The shoot accounted for approximately 98% of absorbed imazamox, while the root accounted for only 2% (Table 1). This partitioning in shoot and root biomass remained consistent across all three treatment concentrations, indicating little or no translocation to roots. This lack of basipetal translocation has also been observed in Sago pondweed and Richardson's pondweed when shoots were treated with fluridone (Marquis et al., 1981). While this appears to hold true in other aquatic species, it is a sharp contrast to what has been seen for imazamox in terrestrial species. Pester et al. (2001) found that 96 HAT, 27% and 20% of absorbed imazamox had translocated to the roots in feral rye and jointed goatgrass, respectively. So, even though imazamox is readily translocated in terrestrial species, it is similar to fluridone in its behavior in aquatic species.

Imazamox Desorption

Imazamox was rapidly desorbed when treated plants were transferred to tap water with no herbicide. The amount desorbed was determined as a percentage of total imazamox absorbed on a whole plant basis (Figure 3). Imazamox desorption can be described by the function:

$$y = a(1 - e^{(-bx)}) \quad [3]$$

where a=46.188 and b=0.905. In the first 12 HAT 46% of absorbed imazamox moved out of the plant and into the surrounding water column. Imazamox readily moved out of the plant and eventually reached equilibrium with the surrounding water column by the end of the 72 hour time course. We did not continue the desorption process by continually exposing plants to clean water, so there is no way to determine if some portion of the radioactivity remaining in the plant was bound and not easily desorbed. These data do support the theory that imazamox absorption and desorption are driven mainly by a concentration gradient and that there is a dynamic equilibrium established between the water column and aquatic vegetation. Our observed rapid photolysis of imazamox in whole lake treatments, with a half-life of less than 10 days (data not shown), would suggest that the maximum concentration in the plant will occur soon after application and will decline primarily due to decreasing external concentrations (assuming no water movement).

Imazamox Metabolism

Imazamox metabolism was determined by dividing radioactive fractions into three categories: intact imazamox, soluble metabolites, and bound metabolites (Figure 3). No attempt was made to identify metabolites. Intact imazamox was identified as the radioactive peaks corresponding to retention time of the imazamox standard. Predicted imazamox metabolism rates can be described by the power function:

$$y = ax^{b} \quad [4]$$

where a=50.347 and b=-0.298.

Other radioactive peaks that did not correspond to the retention time of the standard were considered soluble metabolites. This fraction can be described by the following exponential rise to max function to obtain predicted values:

$$y = a(1 - e^{(-bx)}) \quad [5]$$

where a=21.021 and b=0.026.

Bound metabolites were determined by oxidizing the remaining dried plant material following extraction, and were assumed to be bound to plant tissue. This fraction was then estimated using Equation 5 when a=68.948 and b=0.249.

Approximately 70% of the absorbed imazamox was found in the bound fraction 24 HAT, while 10% appeared to be soluble metabolites, Only 19% ± 2.47 remained as intact imazamox. The percent of absorbed radioactivity found in the bound fraction remained constant from 24 to 144 HAT. Over the same time period, the soluble metabolites increased to 21.44% ± 2.88 by 144 HAT, while intact imazamox decreased to 11.52% ± 1.02. Imazamox metabolism appears to occur very rapidly in Eurasian watermilfoil compared to jointed goatgrass and feral rye. In these terrestrial species 75% of the imazamox remained intact 24 HAT and even at 96 HAT 25-50% remained intact. Based on predicted values the half-life of imazamox in Eurasian watermilfoil was short (7.65 h) compared to feral rye (42 h) or jointed goatgrass (84 h) (Pester et al., 2001). Considering the internal concentration found in the absorption study that was 6.93 times the external concentration, and percentage of that reamianed as intact imazamox 72 HAT (13.35%), the concentration of imazamox inside of the plant was nearly equal to the external concentration. This provides additional support that imazamox absorption is driven by a concentration gradient. It appears that a significant amount of absorbed

imazamox is quickly bound to plant tissue within 24 HAT and this fraction remains steady at around 70%, at later time points the remaining intact imazamox slowly decreases, while the amount of soluble metabolites slowly increases. These bound residues could be conjugated to lignins, or cell wall constituents. While bound metabolites are probably not phytotoxic, there is evidence from terrestrial species that hydroxylated metabolites of many imidazolinones remain phytotoxic, but do not translocate (Shaner and Mallipudi, 1991). In aquatic applications, where the entire aboveground portion of the plant is exposed to the herbicide at one time, translocation may be less important.

Our field studies evaluating Eurasian watermilfoil control show that imazamox can provide multiple season control at concentrations of 100 – 200 μg L^{-1} in whole lake treatments (data not shown). It appears that rapid imazamox absorption does occur and absorption is driven by a concentration gradient. Although absorption driven by a concentration gradient allows for relatively fast absorption, this can also be a disadvantage in a system where imazamox concentration in the water column may drop quickly. If the external concentration were to drop, the herbicide appears to quickly diffuse out of the plant. Imazamox metabolism also occurs rapidly, with only about 20% of imazamox remaining intact by 24 HAT. Maintaining a treatment concentration can be difficult in flowing water, and may not allow for sufficient absorption, and herbicide diffusion out of the plant may not provide adequate exposure time for control. Ongoing research is investigating optimal imazamox concentration, exposure time, and application timing for the maximum efficacy on Eurasian watermilfoil.

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Table 1.1. Partitioning of imazamox into aboveground and belowground biomass expressed as a percent of total absorbed imazamox following a 24-hour exposure time.

Treatment Concentration	% in Shoot	% in Root	Standard Error
200 ng/mL	97.99	2.01	0.75
400 ng/mL	97.79	2.21	0.93
800 ng/mL	97.56	2.44	0.64

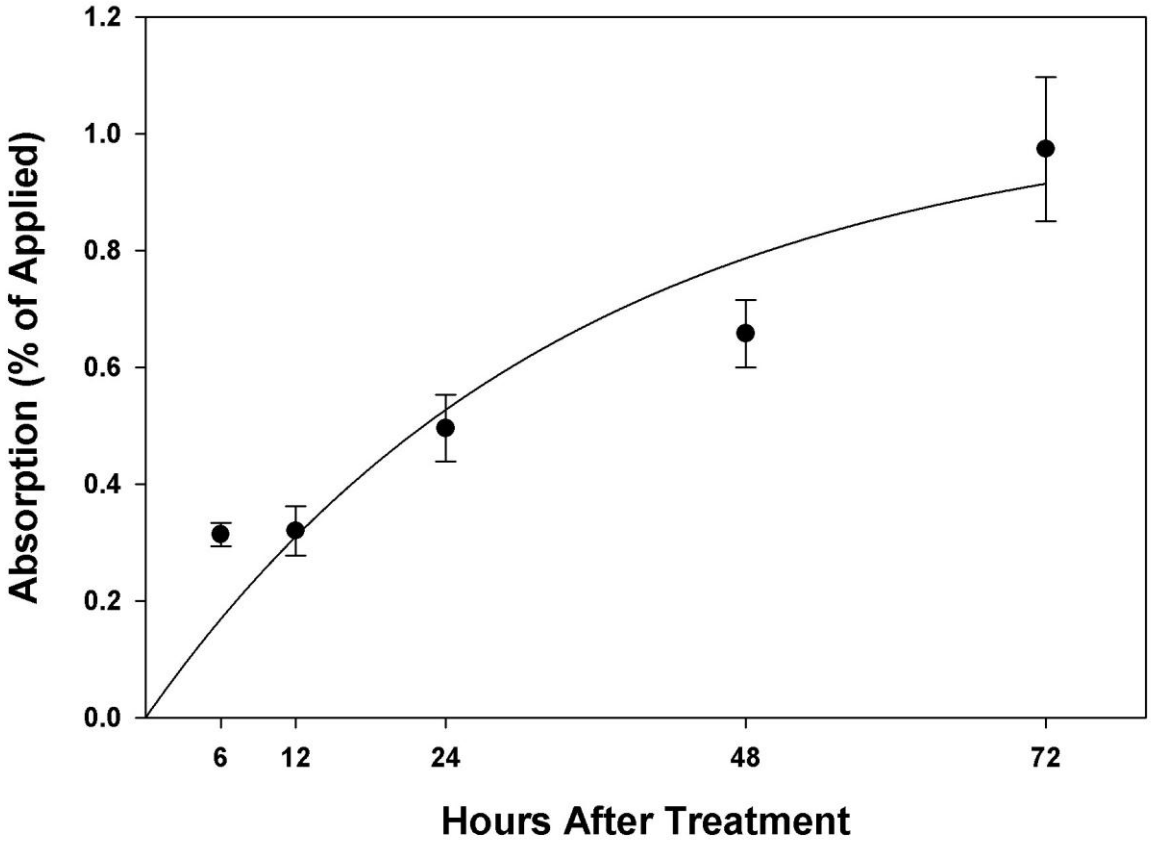

Figure 1.1. Imazamox absorption over a 72-hour time course at a 200 μg L-1 treatment concentration as a percentage of total applied showing the regression line as calculated using SigmaPlot (Equation 1).

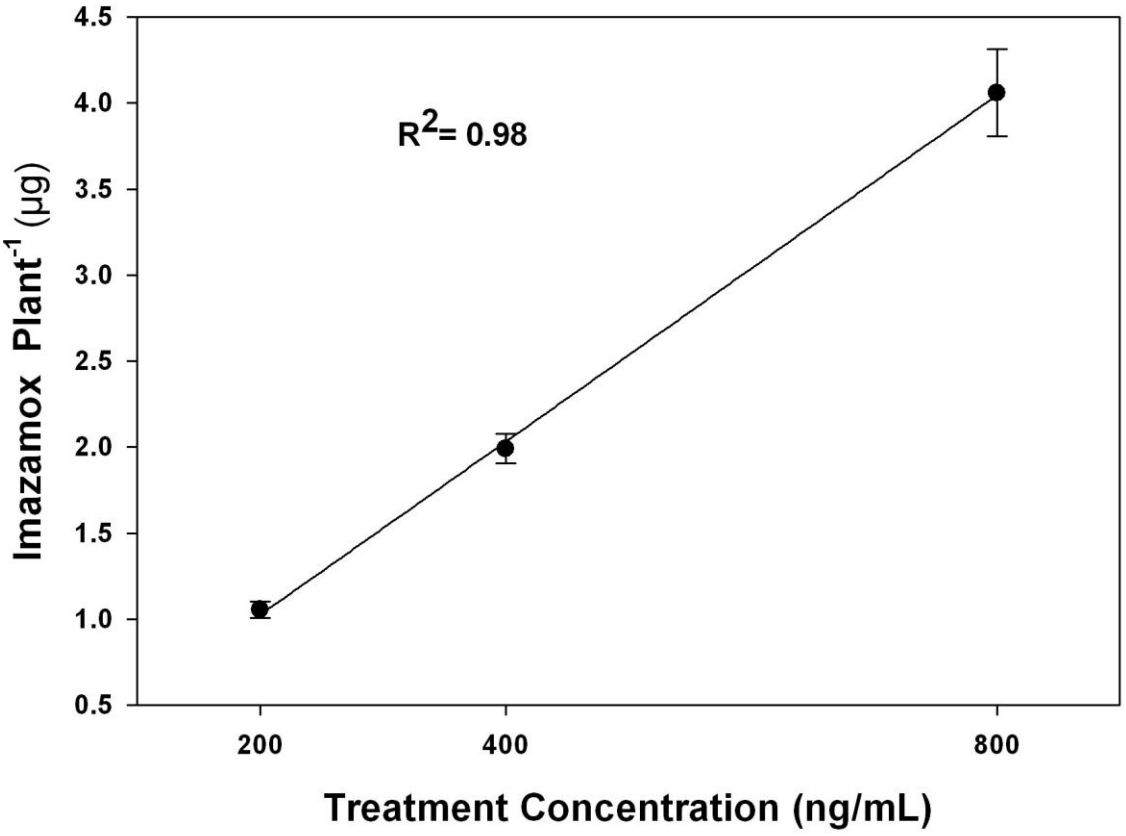

Figure 1.2. Total amount of imazamox absorbed per plant at treatment concentrations of 200, 400, and 800 µg L^{-1} following a 24-hour exposure period.

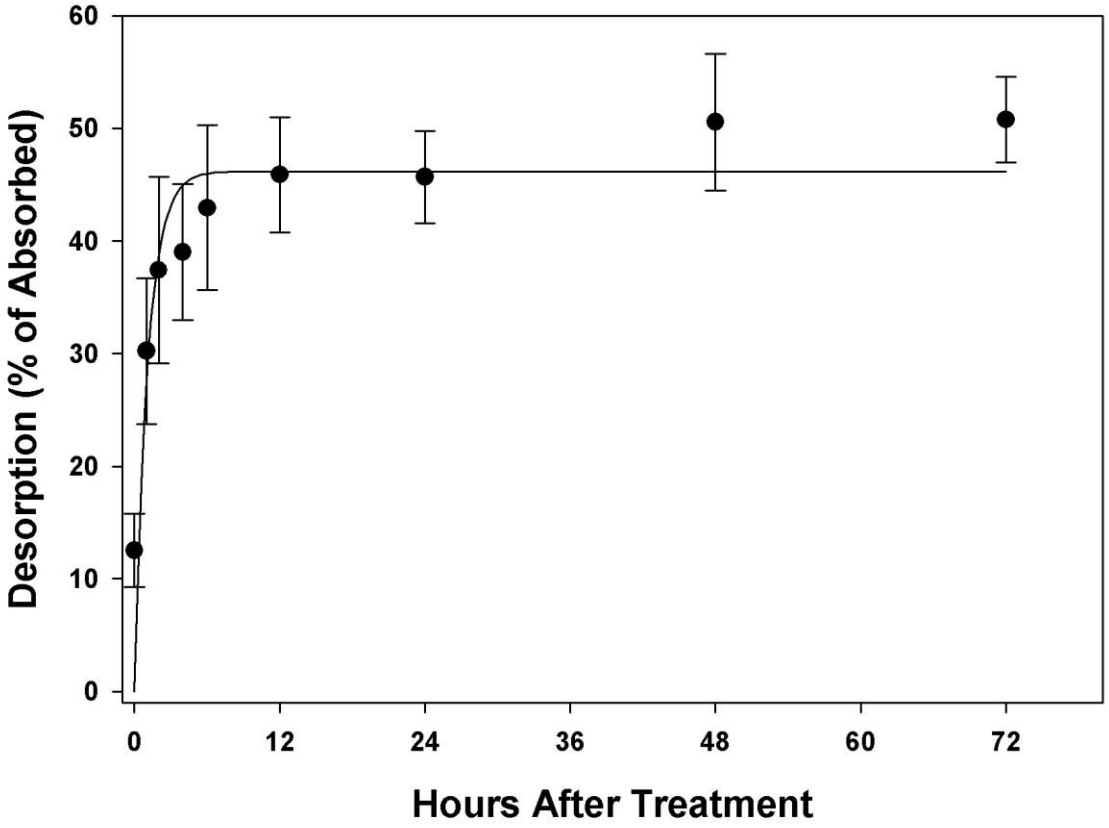

Figure 1.3. Desorption of ^{14}C as a percentage of total absorbed ^{14}C following a 24-hour treatment period with an initial treatment concentration of 200 µg L^{-1}. Only total ^{14}C was measured and likely represents remaining imazamox and soluble metabolites. Regression line indicates predicted values as calculated using Equation 3.

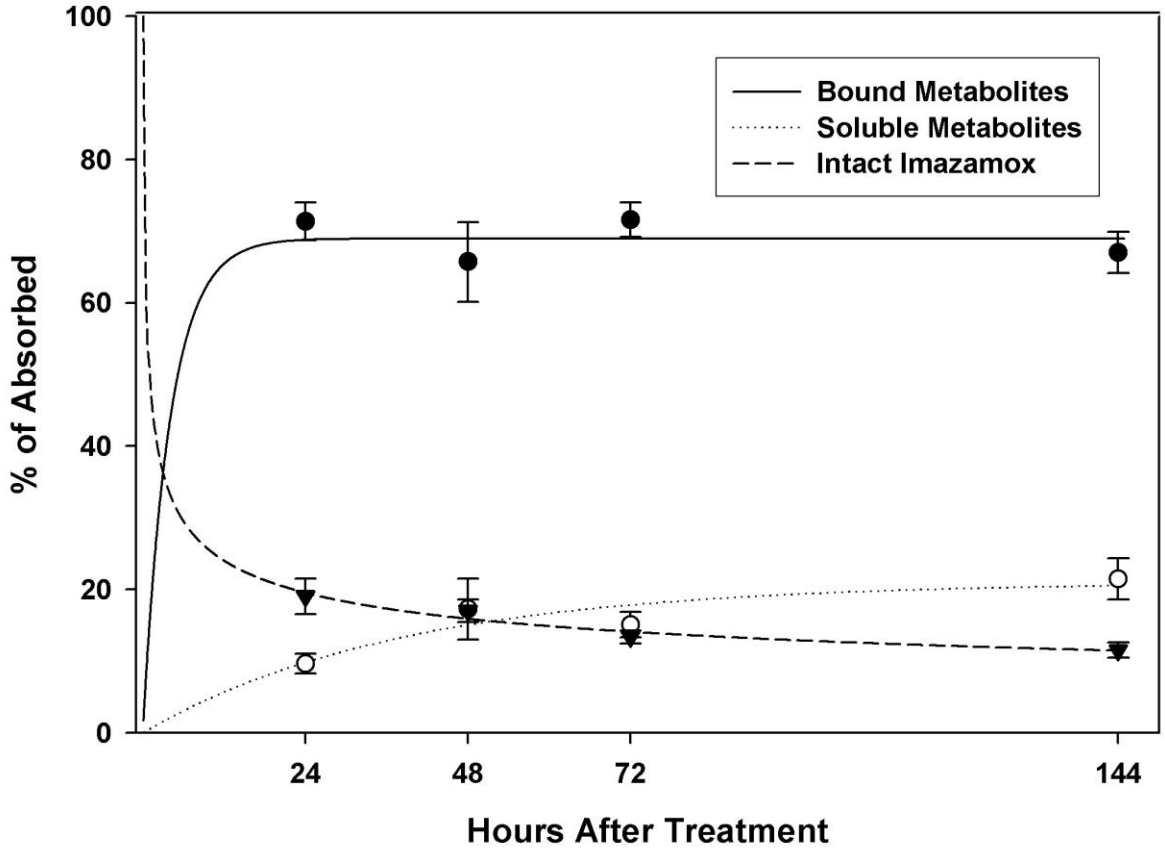

Figure 1.4. Imazamox metabolism as a percentage of total absorbed imazamox over a 144-hour time course that was separated into three fractions; 1) Bound Metabolites (Equation 4), 2) Soluble Metabolites (Equation 5) and 3) Intact imazamox (Equation 5).

Chapter 2: Sago pondweed control in simulated dewatered irrigation canals using herbicide

INTRODUCTION

Sago pondweed (*Stuckenia pectinata* (g.) *Boerner*) control in flowing water has proven to be difficult with very limited options to manage top growth and no options for proven long-term control. Sago pondweed is a perennial, submersed, aquatic plant. It is native in all 50 states (http://plants.usda.gov). It provides an important food source for many waterfowl and usually does not cause problems in still water ponds; however, it thrives in flowing waters, decreasing the efficiency of irrigation canals (Sprecher et al., 1998).

Sago pondweed reproduces primarily by tubers developed from nodal and internodal tissues on branches, but seed can also contribute to its spread. Tuber production can be extremely high, with one study reporting 2,380 tubers forming from a single tuber over a six-month period. Tubers are oval shaped and can grow up to 1.5 cm in length, and weighing up to approximately 1 g. Tubers can appear alone, or in connected chains of as many as five tubers. Sago pondweed can produce tubers at depths up to 45 cm, with deeper tubers being larger in perennial populations. Although tubers are present at these depth, plants growing from 30 cm or deeper are less vigorous. Tubers are the main means of sago pondweed reproduction in irrigation canals, allowing plants

to survive “dewatering” or canal drawdown during the winter, which is common in Colorado. Although they do not contribute as much to reproduction in irrigation canals, sago pondweed does produce viable seed that can contribute to reproduction in wetlands.(Yeo, 1965)

Sago pondweed shoot growth occurs once water temperatures reach 10 ºC, with more vigorous growth occurring as water temperature and light intensity increases (Yeo, 1965). When this vigorous growth occurs, plants can quickly reach the surface, slowing water-flow in the canals and impeding water delivery.

Since flowing water is where troublesome infestations of sago pondweed occur, achieving adequate control with traditional water column treatments can be difficult. There are two main mechanical removal methods that can help provide sago pondweed control, but these methods will provide only temporary control. The first mechanical control method is to dredge the canal bottom. Using this method, a backhoe or similar implement is used to remove aboveground biomass and several inches of sediment. This will temporarily remove aboveground biomass, but will have little impact on the tuber bank in the sediment. The other mechanical method is known as “chaining”. This method uses a large section of chain attached to tractors on opposite sides of a canal. The tractors drive the length of the canal, dragging the chain along the canal bottom. This removes aboveground biomass, but has no impact on tubers, and plants grow back quickly.

Chemical control in flowing water systems can be difficult, as dilution and water movement make it difficult to achieve the required exposure times at concentrations needed for control. One commonly used chemical control method involves treatments

using the contact herbicide acrolein (2-propenal) (MAGNACIDE H, Baker Petrolite). Since this is a contact herbicide, it provides only temporary control. Acrolein is a restricted use pesticide that requires careful handling by the applicator. Two other contact herbicides are active on sago pondweed are diquat (6,7-dihydrodipyrido[1,2-α:2',1'-c]pyrazinediium ion) and endothall (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) (Vencill, 2002) and they can be applied as flowing water treatments to provide temporary control. Even though flowing water treatments using contact herbicides can provide adequate temporary control, a herbicide that could provide long-term control and potentially reduce tuber densities would be of great value to irrigation districts.

Previous studies that examined sago pondweed control when herbicides were applied to the water column found that a wide range of compounds control sago pondweed. Canal treatments with acrolein provided good initial control, but resulted in biomass reductions of only 40-60% and did not reduce the tuber numbers (Bentivegna et al., 2004). Sago pondweed biomass was significantly reduced when endothall was applied to a flowing irrigation canal at 0.30 mg L^{-1} for 84 hours, (Sisneros et al., 1998). Previous work with fluridone showed that 12 mg L^{-1} fluridone with a 24-48 hour exposure time and 4 mg L^{-1} fluridone with continuous exposure resulted in a 75% reduction in sago pondweed biomass (Irigoyen and Brevedan, 1983). Tank studies with endothall resulted in >90% biomass reduction when applied at >2 mg L^{-1} for >12 hour exposure time (Slade et al., 2008). Westerdahl and Hall (1983) found that treatment with 0.25 mg L^{-1} 2,4-D resulted in a 60% biomass reduction. Diquat has also been shown to significantly reduce sago pondweed biomass. One study found that 0.5 mg L^{-1} of diquat resulted in 100% sago pondweed control with as little as one hour exposure, and 0.1 mg

L^{-1} and 0.2 mg L^{-1} resulted in a significant reduction at exposure times ranging from 1-168 hours. The authors did note that the speed of regrowth varied depending on exposure time (Skogerboe et al., 2006). Even though these trials indicate a wide range of contact and systemic herbicides may provide submerged sago pondweed, none have previously examined control in dewatered irrigation canals.

Three herbicides are labeled for use in dewatered irrigation canals; fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone), penoxsulam (2-(2,2-difluoroethoxy)-N-(5,8-dimethoxy[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide), and imazapyr ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo1H-imidazol-2-yl]-3-pyridinecarboxylic acid) (Vencill, 2002). There are no published reports on sago pondweed control using these systemic herbicides. The aim of this project was to examine sago pondweed control in irrigation canals following pre-emergence herbicide application and to determine the importance of rainfall for incorporation. Herbicides evaluated included those that are currently labeled as well as several other herbicides that have shown activity on other aquatic plant species.

MATERIALS AND METHODS

Plant Materials and Herbicide Application

Sago pondweed tubers were collected from the Western Ditch (40º 18' 59.98" N, 104º 45' 27.12" W) near LaSalle, CO in November 2007 and stored in cold storage at 2.2ºC until they were needed for use in greenhouse trials. Tubers were potted in 7.5 cm square pots using field soil collected from the same site. Each pot constituted one replication. Treatments were applied pre-emergence to the soil surface with no aboveground biomass present. Herbicide treatments were applied using an overhead track sprayer calibrated to deliver 187 l ha^{-1} using a 11002EVS flat fan nozzle (TeeJet). Herbicides used included fluridone, penoxsulam, imazapyr, imazamox (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo- 1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid, ammonium salt), flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione), pyroxasulfone (3-[5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl)pyrazol-4-ylmethylsulfonyl]-4,5-dihydro-5,5-dimethyl-1,2-oxazole), dimethenamid (2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-thien-3-yl)-acetamide), and metolachlor (2-chloro-n-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide) (Vencill, 2002). Incorporation treatments received 1 cm of simulated rainfall immediately following herbicide application. The rainfall incorporation was applied using an overhead track sprayer. All studies were repeated.

Study #1

Imazamox was applied at 0.28, 0.42, and 0.56 kg ai ha^{-1}, while imazapyr was applied at 1.12 and 1.68 kg ai ha^{-1}. In addition to herbicide treatments an untreated

control treatment was also included. Eight pots were treated at each herbicide rate. After herbicide application half of the pots received simulated rainfall to determine if incorporation had a significant effect on sago pondweed control.

Study #2

Herbicide treatments included an untreated control, fluridone at 2.2 kg ai ha^{-1}, penoxsulam at 0.2 kg ai ha^{-1}, fluridone 2.2 kg ai ha^{-1} + penoxsulam 0.2 kg ai ha^{-1}, flumioxazin at 0.39 kg ai ha^{-1}, and imazamox at 0.56 kg ai ha^{-1}. This study was conducted in the same manner as Study #1, except only four replications were included and all treatments received rainfall incorporation.

Study #3

No previous studies had been conducted to evaluate sago pondweed control using pyroxasulfone, dimethenamid, or metolachlor. To evaluate sago pondweed efficacy using these herbicides, a dose response study was initiated. Herbicides were applied at 0X, 0.25X, 0.5X, 1X, 2X, and 4X, where X represents the highest labeled rate used for terrestrial applications (pyroxasulfone 336 g ai ha^{-1}, dimethenamid 1.7 kg ai ha^{-1}, and metolachlor 1.6 kg ai ha^{-1}). Four replications were conducted for each treatment and all treatments were incorporated.

Grow Out Conditions

Following treatment and incorporation (if applicable), pots were placed in cold storage at 2.2 °C for 14 days to simulate overwintering. Following this simulated overwintering, pots were submerged in 90 L plastic tanks at the Colorado State University Greenhouse Facility, and allowed to grow for 30 days. Greenhouse conditions were maintained at a 16-hour light: 8-hour dark photoperiod, with natural sunlight

supplemented with 430 watt HID lights. Temperature was maintained at 25 °C. Plastic tanks were aerated during the study using a commercial air compressor.

Plant Harvest and Statistical Analyses

At the end of the 30 day grow out, whole plants were harvested, dried for 48 hours at 60 °C, and whole plant (aboveground and belowground) dry biomass recorded. Data in Study #1 were subjected to a two-way ANOVA with herbicide treatment and rainfall incorporation as variables. Data from Study #2 were subjected to a one-way ANOVA with herbicide treatment as the variable. Mean comparison was conducted for both studies using a Tukey HSD test in JMP (Version 7.0.1, SAS Institute, 2007). For Study #3 regression analyses were performed and data plotted using SigmaPlot (Version 9, SYSTAT, 2005). For all studies, Levene's test for homogeneity of variance was used to determine if data from repeated studies could be combined.

RESULTS

Study #1

Levene's test for homogeneity of variance indicated that data from repeated studies could be combined for Study #1, Study #2, and Study #3. Incorporation did not have a significant effect on herbicide efficacy (p=0.34), so data were combined to compare sago pondweed biomass from herbicide treatments to the biomass produced by control plants (Table 2.1a). Herbicide treatment was highly significant (p=<0.001). Based on Tukey's HSD, all herbicide treatments resulted in a significant reduction compared to the untreated control, but there was no significant differences between herbicides or a rate response (Table 2.1b). Therefore, data were combined and compared across herbicides and rates. Imazamox and imazapyr reduced sago pondweed biomass an average of 74.1% (±2.4 SE) compared to control plants.

Study #2

Since Study #2 included more lipophilic herbicides than Study #1, incorporation was included for all treatments, as the effect of incorporation on these herbicides was not known. Herbicide treatment was highly significant (p=<0.001) (Table 2.2). Based on Tukey's HSD, all herbicide treatments resulted in a significant reduction compared to the untreated control, but there was no significant differences between herbicides or a rate response (Table 2.2). The average reduction in sago pondweed biomass for all treatments was 76.5% (±2.4 SE).

Study #3

All herbicide treatments in Study #3 resulted in significant biomass reduction (p=0.0076) compared to the untreated control. Data were plotted and regression analyses

were performed using SigmaPlot. Exponential decay regression curves were fit for each herbicide using the following equation:

$$y = y_0 + ae^{-bx}$$

Data and parameter estimates are shown in Figure 2.1. Calculated GR_{50} values based on parameter estimates were 78 g ai ha^{-1}, 109 g ai ha^{-1}, and 192 g ai ha^{-1} for pyroxasulfone, dimethenamid, and metolachlor, respectively. Although all three herbicides resulted in a significant decrease in biomass when compared to the untreated control, there was a significant difference in the response of the three herbicides. Pyroxasulfone had the lowest GR_{50} value, but provided a maximum reduction in biomass of approximately 70%, while dimethenamid and metolachlor had higher GR_{50} values and provided a biomass reduction of greater than 90%.

DISCUSSION

All treatments examined in these three studies resulted in a biomass reduction of 70% or greater. Although there have been no other published data on sago pondweed control using dry ground applications, these results indicate that this type of application may provide a level of control equal to or greater than that of currently available flowing water treatments. Currently used control methods may only provide temporary sago pondweed control, and repeat applications often have to be made during a single growing season. Treatments with acrolein can be dangerous to applicators and mechanical methods can be costly and time consuming. Achieving high levels of control with currently available flowing water treatments can also be challenging with control varying based on water quality. Also, depending on canal conditions it may be difficult to maintain the proper concentration and exposure time needed for sago pondweed in flowing water. If proven effective in the field, dewatered canal treatments could provide another control option that may be safer, more cost effective, and provide more long-term control than current methods.

Since the herbicides included in this study encompass several modes of action, as well as a wide range Log K_{ow} values, they may provide different management options based on irrigation demands and sago pondweed growth patterns across the United States. For example, herbicides that are more lipophilic may provide longer soil residual, less movement in the soil profile, and possibly slower depuration into the water column when the canal is flooded. Having multiple modes of action available would provide options that would allow for rotation to minimize the possibility of developing herbicide resistance in sago pondweed. Given that sago pondweed can reproduce by seeds, it is

possible that this species could develop herbicide resistance. Given its prolific tuber production, a single resistant plant could yield thousands of resistant tubers and contribute to the spread of these resistant biotypes. Further studies are needed to evaluate control under field conditions. Other factors that will need to be examined in future studies include application rate, application timing, and water quality. Since applications are made to the exposed soil in canal beds, studies are also needed to determine the effects of soil texture, pH, and soil organic matter on sago pondweed control.

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Table 2.1: ANOVA tables and mean separation for Study #1 indicating that incorporation and incorporation*treatment interaction were not significant (2.1a). Data were combined and mean separation conducted using Tukey's HSD (2.1b).

a.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	11	1.1550055	0.1050005	4.0715206	<0.0001
Error	84	1.98575405	0.02578901	0.00010427	
C. Total	95	3.14075955			

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	5	5	1.08676017	8.42808638	<0.0001
Incorporation	1	1	0.02155726	0.83590881	0.3634
Treatment*Incorporation	5	5	0.0085717	0.06647561	0.9968

b.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.1240745	0.2248149	9.2526281	<0.0001
Error	90	2.01668505	0.02429741		
C. Total	95	3.14075955			

Treatment	Mean Dry Biomass (g)
Untreated	0.453 a
Imazamox 0.28	0.101 b
Imazamox 0.42	0.101 b
Imazamox 0.56	0.100 b
Imazapyr 1.12	0.153 b
Imazapyr 1.68	0.133 b

* Means not sharing the same letter are significantly different at the $\alpha = 0.05$ level of significance based on Tukey's HSD (q=2.92).

Table 2.2: ANOVA and Tukey's HSD mean separation for Study #2.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	5	1.1240745	0.2248149	9.2526281	<0.0001
Error	90	2.01668505	0.02429741		
C. Total	95	3.14075955			

Treatment	Mean Dry Biomass (g)
Untreated	0.453 a
Imazamox 0.28	0.101 b
Imazamox 0.42	0.101 b
Imazamox 0.56	0.100 b
Imazapyr 1.12	0.153 b
Imazapyr 1.68	0.133 b

* Means not sharing the same letter are significantly different at the $\alpha = 0.05$ level of significance based on Tukey's HSD (q=3.05).

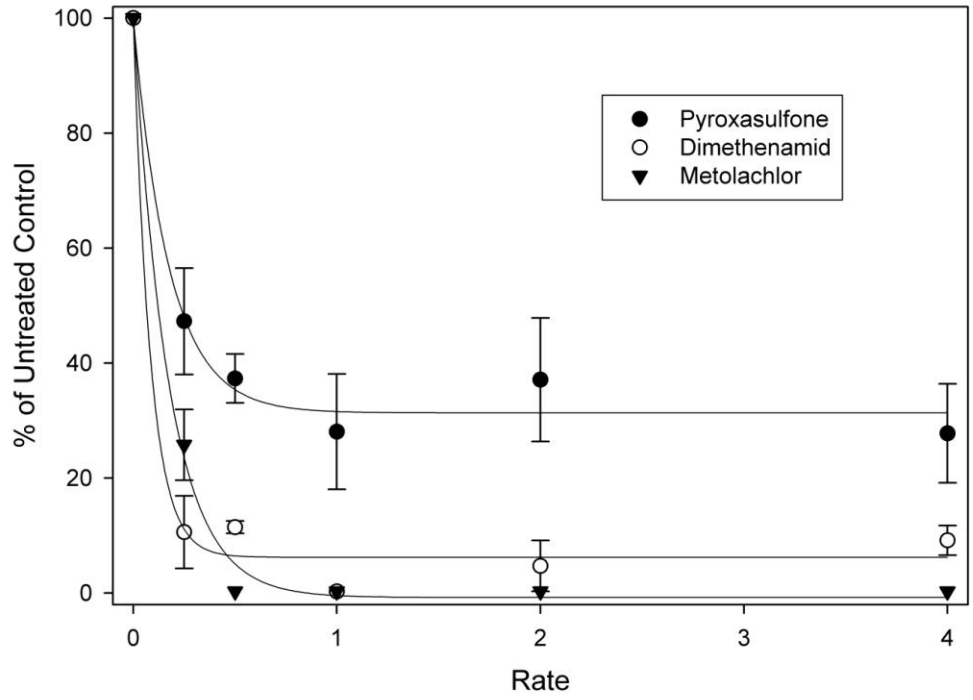

Figure 2.1. Dose response regression of sago pondweed to pyroxasulfone ($y=31.34+68.59e^{-5.66x}$), dimethenamid ($y=6.22+93.77e^{-11.81x}$), and metolachlor ($y=-0.76+101.03e^{-5.7x}$) where X represents a typical terrestrial field rate of 336, 1,681, and 1602 g ai ha^{-1}. Expressed as a percentage of the untreated control at harvest 30 DAT.

Appendix 1: Imazamox Whole Lake Treatments

In the summer of 2006, three lakes with dense infestations of Eurasian watermilfoil or Northern watermilfoil were chosen for whole lake applications of imazamox. Bass Lake and West Lake in Wheat Ridge, CO were both infested with Eurasian watermilfoil. West Lake was nearly 100 percent infested, and had reached the water surface (topped out) each of the previous four years. Bass Lake had a smaller infestation in approximately 25% of the lake. Raccoon Creek Lake is located in Littleton, CO and is an irrigation storage pond for the Raccoon Creek Golf Course. The lake at Raccoon Creek was nearly 100 percent infested with Northern watermilfoil. Each of the three lakes was treated with different application rates and timings to evaluate herbicide degradation under different treatment regimes. Lake attributes and treatment specifications are listed in Table 3.1.

Following herbicide treatments, six 30 mL water samples were periodically taken from each lake to confirm treatment concentration and monitor herbicide dissipation. Following collection, water samples were stored at -20°C until analysis was performed. Sample preparation prior to analysis was conducted by passing 1.5 mL of each sample through a 0.45 μm syringe filter (6779-1304, 13mm Disposable Filter Device, Whatman, England). Herbicide residues were analyzed using reverse phase HPLC using a C8 4.6 mm x 250 mm column (Zorbax, USA). The injection volume was 100 μL. Imazamox eluted at 11.50 minutes using the following gradient: 89.5% water:10% acetonitrile:0.05% phosphoric acid solution to a 69.95% water: 30% acetonitrile: 0.5% phosphoric acid solution over 25 minutes with a flow rate of 1.4 mL/minutes with a wavelength of 250 nm. Data were entered into Microsoft Excel and data plotted using SigmaPlot. Results for West Lake, Bass Lake, and Raccoon Creek Lake are shown

below in Figures 3.1, 3.2, and 3.3, respectively.

Table 3.1: Lake size and application rate for imazamox whole lake treatments.

Location	Surface Acres	Average Depth	Application Rate
West Lake	22	6	2 applications of 100 ppb
Bass Lake	8	5	4 applications of 25 ppb
Raccoon Creek	17	5	1 application of 200 ppb

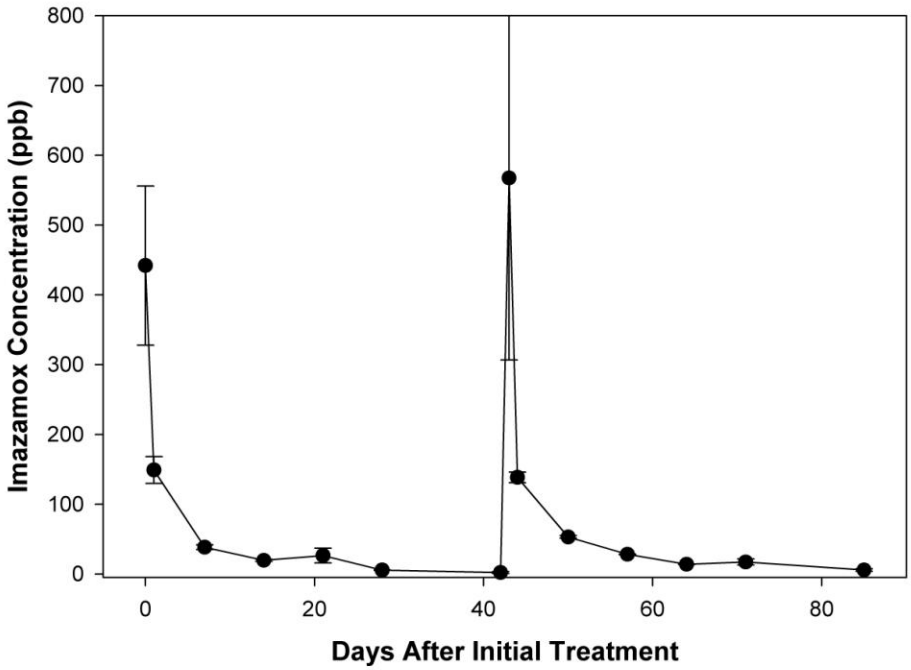

Figure 3.1: Imazamox dissipation in West Lake following two applications of 100 ppb applied on May 19, 2006 and June 30, 2006.

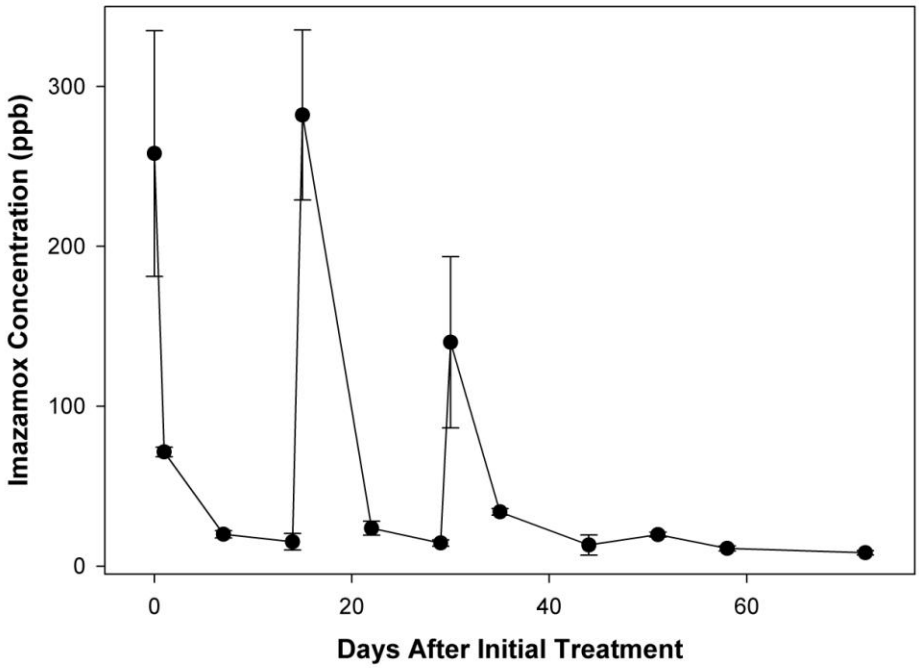

Figure 3.2: Imazamox dissipation in Bass Lake following four treatments of 25 ppb each applied every 14 days starting on May 19, 2006.

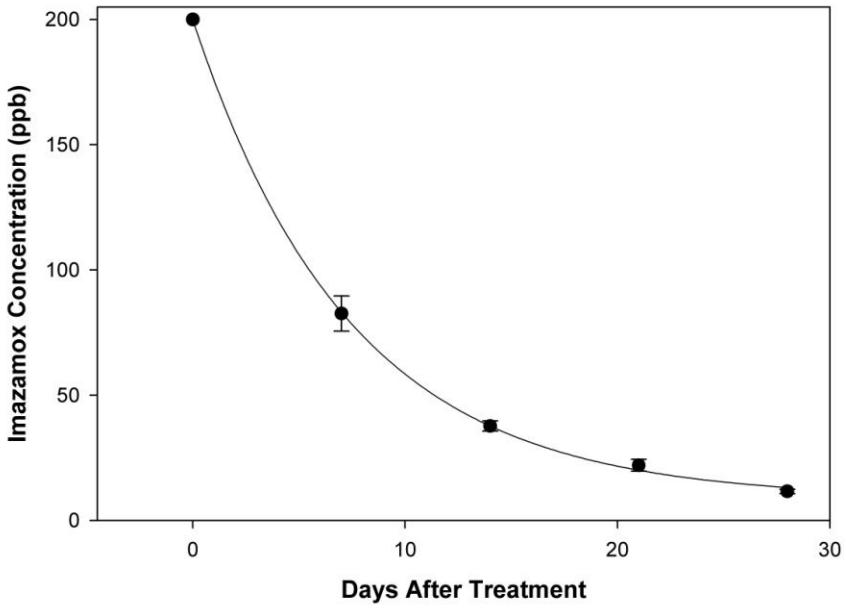

Figure 3.3: Imazamox dissipation in Raccoon Creek Lake following an application of 200 ppb on June 16, 2006.

Appendix 2: Sago Pondweed Dewatered Canal Treatments

Canal Herbicide Applications

Field studies were conducted between 2006 and 2008 at four sites to evaluate herbicide persistence and sago pondweed control using a range of pre-emergence treatments. Herbicide treatments included imazamox, imazapyr, fluridone and penoxulam. Both fall and spring treatments were included to determine the role of application timing on sago pondweed efficacy and dissipation. Plot size varied between studies based on size and accessibility of infested canals. Location and application information for all studies are shown in Table 4.1. Following herbicide application, studies were monitored and efficacy was determined using visual assessments.

Determination of Herbicide Residue in Canal Sediment Samples

Sediment samples were taken from Site 1 24 hours prior to the canals being flooded. Sediment samples were collected from 0-6 inches deep, and three samples were taken from each plot. The three samples from each plot were then combined and thoroughly mixed. Sediment samples of the top three inches were collected 24 hours prior to flooding in Study 3 were collected in the same manner.

For analysis of residues, imazamox and imazapyr were extracted into water by placing a 10 g aliquot of each sediment sample in a 50 mL plastic centrifuge tube. Next, 10 mL of distilled water was added to the samples, which were then shaken for 1 hour. After shaking the samples were then centrifuged at 3,000 rpm for 15 minutes. Water was then poured into clean 50 mL centrifuge tubes. Water was then passed through a 0.45 μm syringe filter (6779-1304, 13mm Disposable Filter Device, Whatman, England) and into a vial. Herbicide concentration was then determined using reverse phase HPLC using a C8 4.6 mm x 150 mm column (Zorbax, USA). The injection volume was 100 μL.

Imazapyr eluted at 9 minutes and imazamox at 12 minutes using the following gradient: 89.5% water: 10% acetonitrile: 0.05% phosphoric acid solution to a 69.95% water: 30% acetonitrile: 0.05% phosphoric acid over 25 minutes with a flow rate of 1 mL/minute. Data were then entered into Microsoft Excel and standard errors calculated.

Fluridone and penoxsulam sediment samples from Site 2 were analyzed using HPLC by the SePro Corporation, entered into Microsoft Excel, and standard errors were calculated.

Determination of Herbicide Residue in Canal Water Samples

For Site 1, Site 2, and Site 3 herbicide residue in water was analyzed. Samples were collected at the downstream end of each study. Three 30 mL water samples were collected at 0, 24, and 48 hours after flooding (HAF). Samples were then prepared using a 0.45 μm syringe filter (6779-1304, 13mm Disposable Filter Device, Whatman, England) and placed into a clean vial. Samples were then analyzed using the same reverse phase HPLC method as was used for analysis of sediment samples. Data were entered into Microsoft Excel and standard errors were calculated.

Table 4.1: Application information for Sago pondweed herbicide trials in dewatered irrigation canals.

	Site 1	**Site 2**	**Site 3**	**Site 4**
Location	LaSalle, CO	Lucerne, CO	Platteville, CO	Lucerne, CO
Plot Size (ft)	14 X 60	10 X 40	6.7 X 30	10 X 50
Replications	3	3	3	3
Application Volume	27 GPA	20 GPA	20 GPA	20 GPA
Nozzle	XT024 Boom Xtender	11002 Flat Fan	11002 Flat Fan	11002 Flat Fan
Treatment Timings	Fall - Nov. 25, 2006 Spring- Mar. 22, 2007	Fall - Nov. 19, 2007 Spring 1 - Apr. 18, 2008 Spring 2 - May 9, 2008	Fall - Nov. 19, 2007 Spring - Apr. 3, 2008	Fall - Oct. 30, 2007 Spring 1 - Apr. 2, 2008 Spring 2 - Apr. 18, 2008
Treatments	Imazamox 48 oz/A Imazamox 64 oz/A Imazapyr 64 oz/A Imazapyr 96 oz/A	Fluridone 2 qt/A Penoxsulam 11.6 oz/A	Imazamox 64 oz/A Imazapyr 96 oz/A	Imazamox 64 oz/A Imazapyr 96 oz/A

Table 4.2: Herbicide residue in canal sediment prior to flooding for Site 1.

Herbicide (oz/A)	Fall 2006 (µg/L)	Spring 2007 (µg/L)
Imazamox (64)	28 ± 12	67 ± 43
Imazapyr (96)	57 ± 75	320 ± 45

Table 4.3: Fluridone and penoxsulam concentrations (ppb) and standard error for sediment one day prior to canal flooding for each of the three application timings for Study 2.

Herbicide	Fall	Spring 1	Spring 2
Fluridone	0.53 ± 0.29	0.82 ± 0.37	1.25 ± 0.55
Penoxsulam	--	0.14 ± 0.07	0.07 ± 0.05

Table 4.4: Herbicide concentration and standard errors (ppb) of water at 0, 24 and 48 HAF for Sites 1, 3, and 4.

Herbicide: HAF	Site 1	Site 3	Site 4
Imazamox: 0	19.7 ± 1.1	5.1 ± 0.5	10.7 ± 0.5
Imazapyr: 0	55 ± 4.7	4.5 ± 0.2	3.5 ± 0.1
Imazamox: 24	24 ± 6.4	0	0
Imazapyr: 24	0	0	0
Imazamox: 48	0	0	0
Imazapyr: 48	0	0	0