DISSERTATION

HERBICIDE ABSORPTION AND TRANSLOCATION BY EURASIAN WATERMILFOIL AND HYDRILLA

Submitted by

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ABSTRACT

HERBICIDE ABSORPTION AND TRANSLOCATION BY EURASAIN WATERMILFOIL AND HYDRILLA

Hydrilla and Eurasian watermilfoil are submersed invasive species that occur commonly across the US. These species are aggressive competitors, and form dense, monotypic stands. Dense stands of these species form mats on the surface, and impact water flow, as well as the economic and ecological value of water bodies. With the severe impact of these species, many control methods have been implemented to restore value to infested areas. The systemic herbicides fluridone, penoxsulam, and triclopyr are registered for aquatic use. While all three herbicides can be used for Eurasian watermilfoil control, only fluridone and penoxsulam can be used for hydrilla. The rates and selectivity for these herbicides have been documented, but little work has been completed to characterize their absorption and translocation in submersed aquatic species. The goals of this research were to (1) evaluate herbicide absorption and translocation following shoot exposure, (2) evaluate herbicide absorption and translocation following root exposure, and (3) evaluate triclopyr absorption and translocation in Eurasian watermilfoil following liquid and granular treatments.

Previous work established linear relationships between herbicide lipophilicity (as determined by log K_{ow}) and bioaccumulation in both terrestrial and aquatic species. Based on the differences in lipophilicity among the three herbicides tested (fluridone>>penoxsulam>triclopyr) we expected fluridone to accumulate the most in these species, with signfincantly lower accumulation of penoxsulam and triclopyr. Bioaccumulation following root exposure followed this trend, with significantly greater fluridone accumulation than penoxsulam or triclopyr; however, following shoot exposure, triclopyr accumulation was greatest in both species 192 HAT, followed by fluridone and penoxsulam. Overall accumulation was similar for both species following root exposure, but accumulation following shoot exposure was approximately three times greater for Eurasian watermilfoil.

Translocation was limited following both root and shoot exposures. The translocation following root treatment was greater than shoot treatment in nearly all cases, with up to 27% of absorbed herbicide present in shoots 192 HAT. Translocation following shoot treatment showed a maximum of 12.5% of absorbed herbicide present in roots 192 HAT. These findings are consistent with previous work that indicated there was more acropetal than basipetal translocation in submersed species.

There were no significant differences in overall absorption by Eurasian watermilfoil following liquid and granular triclopyr treatments; however, differences were observed between plant parts. Apical meristems accumulated the most triclopyr following liquid treatment, and root accumulation was greatest following granular treatment. Distribution at the whole plant level resulted in 11 times more herbicide in roots following granular treatment when compared to the liquid treatment. These results indicate that granular formulations may provide better control of Eurasian watermilfoil roots, compared to an equal rate of a liquid formulation.

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Introduction

Two commonly occurring submersed invasive species in the US are Eurasian watermilfoil (*Myriophyllum spicatum* L) and hydrilla (*Hydrilla verticillata* (L.F.) Royle). Both of these species are invasive, non-natives, and can have severe impacts on aquatic ecosystems. They form dense, monotypic stands that can displace native species, drastically impacting water quality, light penetration, recreational use, water transfer, and human health (Gettys 2009; Grace 1978; Langeland 1996; Smith and Barko 1990). In order to mediate these impacts, many strategies have been implemented to control current infestations and avoid spread to new sites.

Eurasian watermilfoil is native to Eurasia, and while most agree that Eurasian watermilfoil was introduced to the US, there is some debate as to when. This species was first reported in the US in the 1940's (Gettys 2009). There are many *Myriophyllum* species present in the US, but currently only Eurasian watermilfoil and parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.) are invasive (Gettys 2009). Although it is a perennial, it exhibits an annual growth habit, growing to the water surface early in the growing season, flowering once it has reached the surface, and fragmenting. Following the fragmentation process, plants regrow later in the season and subsequent years growth occurs from plant fragments or established root crowns. While vegetative fragments are the primary source of reproduction for Eurasian watermilfoil, it can also produce viable seed (Smith and Barko 1990). Because vegetative fragments are the main means of reproduction, inadvertent spread within or between water bodies by humans is common.

Eurasian watermilfoil has been reported in at least 45 states (USDA 2011b), and thrives in temperate areas of the US. While Eurasian watermilfoil commonly occurs in waters 1 to 4 m deep, it can establish in waters greater than 10 m deep if water clarity is sufficient (Smith and Barko 1990). Eurasian watermilfoil has been shown to photosynthesize at lower temperatures than some native species, such as vallisneria (*Vallisneria americana* Michx.). This ability to thrive in low temperature systems allows it to become established early in the growing season and contributes to its competitiveness over native species (Barko 1981).

Hydrilla has been reported in at least 19 states and is widespread across much of the South, the East Coast, California, and Washington (USDA 2011a). There are two distinct biotypes of hydrilla that have been introduced into the US, one monecious and one dioecious (Langeland 1996). Dioecious hydrilla was the first of the two biotypes introduced, and was introduced in Florida as a result of the aquarium trade in the 1950's. It spread rapidly to infest many water bodies in Florida, and has since expanded its range. To date, only the female form of dioecious hydrilla has been found in the US, preventing sexual reproduction. Monoecious hydrilla populations were first reported in the Potomac River in the 1970's. While dioecious hydrilla thrives in warmer climates, the monoecious biotype is common in temperate areas. In laboratory studies, production of viable monoecious hydrilla tubers has been documented, but has not been documented in natural systems (Gettys 2009). Hydrilla is also a perennial species, and like Eurasian watermilfoil, can reproduce through stem fragments, which are the most prevalent method of long-distance dispersal. A single node of a hydrilla stem is enough to start a new plant. In addition to vegetative fragments, hydrilla also produces rhizomes, resulting in a creeping growth habit. Subterranean tubers also form on rhizomes, allowing hydrilla to survive seasonal water drawdown and overwinter in temperate climates. These tubers can survive for several days out of water, and 3-5 years in moist sediments, making long-term control difficult. Tuber formation in dioecious hydrilla occurs in response to short daylength in the Southeastern US, and the monoecious biotype acts similar to an annual, producing tubers in late summer into early fall. The final method of vegetative reproduction in hydrilla is through modified stem tissue known as turions that form at nodes along the stem. Tuber and turion production can be significant, with one study reporting 6,000 tubers and 2,803 turions per square meter formed from a single tuber. Although it has been shown that monoecious hydrilla may be capable of producing viable seed, vegetative methods contribute most to hydrilla establishment and spread. (Langeland 1996)

Hydrilla is also able to grow at lower light levels compared to many native species, allowing it to begin photosynthesizing earlier in the morning, taking advantage of higher CO_2 levels. It can grow in deeper water than some native species, giving it another advantage. Water quality can also limit the establishment and growth of native species, but hydrilla is capable of growing in a wide range of conditions, including high nutrient loads and high salinity. (Langeland 1996)

A range of mechanical, cultural, physical, and biological methods implemented for the control of Eurasian watermilfoil and hydrilla. While each of these methods are useful tools in the management of submersed aquatic species, the most common, and often most cost-effective method for control of these submersed species is through the use of aquatic herbicides. For many years, there were a limited number of herbicides registered for aquatic use, compared to the number of herbicides available for terrestrial applications. With the discovery of herbicide resistance in hydrilla (Albrecht 2004), there has been a push to identify and register new aquatic herbicides. As a result of this renewed effort, seven new active ingredients have been registered for aquatic use since 2000 (Gettys 2009). Each of these new products possesses different attributes that may be desirable based on site conditions. In general, contact herbicides are faster acting and require a shorter contact time to achieve control. These herbicides are ideal for areas where it is difficult to maintain treatment concentrations, including areas with high water exchange, areas requiring spot treatments in larger water bodies where dilution may impact contact time, or flowing water systems. In contrast, most systemic herbicides are slower acting, require longer contact times, and are ideal for systems with slower water exchange or fully contained systems, such as lakes and ponds.

There are various contact and systemic herbicides that can be used for Eurasian watermilfoil control. Contact herbicides include copper (as copper sulfate or chelated copper), diquat and endothall, and systemic herbicides that are commonly used include 2,4-D, triclopyr, and fluridone. A range of herbicides are also available for hydrilla control. These include the contact herbicides copper, diquat, and endothall. Systemic herbicides that can be used for hydrilla control include penoxsulam and fluridone. Each of these herbicides can be useful depending on site conditions, selectivity, and herbicide residence time. (Gettys 2009)

Both contact and systemic herbicides can be effective for submersed aquatic weed control, but contact herbicides are not readily translocated, and will often only control aboveground growth. Contact herbicides may provide good control of annual species, but systemic herbicides that translocate to the roots are ideal for control of perennial species, such as hydrilla and Eurasian watermilfoil, and fluridone, penoxsulam, and triclopyr are three systemic herbicides that have proven effective for aquatic weed control. All three herbicides can be used to control Eurasian watermilfoil, only fluridone and penoxsulam have activity on hydrilla. Each of these herbicides has a different mode of action, but all are useful tools for aquatic plant management.

There have been several publications that examine pesticide accumulation in submersed aquatic plants, but a majority have examined bioaccumulation as a method for removal of organic pollutants from surface water (Crum et al. 1999; de Carvalho et al. 2007a; de Carvalho et al. 2007b). Previous work conducted by de Carvalho (2007b) examined the absorption of various pesticides by the submersed aquatic species curly waterweed (*Lagarosiphon major* (Ridley) Moss) as a function of log K_{ow}. Log K_{ow}, or log of the octanol/water partitioning coefficient, is an excellent parameter to describe a compounds lipophilicity. Based on these values, herbicides with higher log K_{ow} values have a greater affinity for octanol over water, indicating they are more lipophilic and less polar. In general, their findings indicated that the greater the log K_{ow} value, the more herbicide accumulates in plant tissue. While this previous work provided insight into the relationship between herbicide lipophilicity and bioaccumulation, their work focused on many highly lipophilic pesticides, with log K_{ow} values >2. There has been relatively little work examining accumulation of less lipophilic aquatic herbicides (log K_{ow}<2) that are commonly used for aquatic plant management. While there is a significant amount of information on selectivity and mode of action for these herbicides, there has been little work conducted to characterize their absorption and translocation in submersed aquatic species.

Fluridone has one of the highest log K_{ow} values of any aquatic herbicides, and penoxsulam and triclopyr are examples of highly water-soluble herbicides with relatively low log K_{ow} values. Log K_{ow}values for fluridone, penoxsulam, and triclopyr are 1.869, -0.354, and -0.444, respectively (Senseman 2007). Based on previous findings, water solubility, and log K_{ow}

values, fluridone would be expected to accumulate to a much greater extent than penoxsulam or triclopyr (de Carvalho et al. 2007b).

While sufficient herbicide accumulation is important, herbicide translocation to roots is fairly important, and probably crucial for long-term control of perennial species, such as hydrilla and Eurasian watermifoil. Previous studies have indicated roots of aquatic plants serve a similar function to those in terrestrial plants, being the main site of nutrient absorption (Wetzel 2001). Roots are the main nutrient absorption site, but submerged species can also obtain nutrients from the water column. For this reason, translocation to shoots of aquatic plants might not be as extensive as translocation in terrestrial species, where there is a greater demand for nutrients and water to maintain growth and turgor pressure. There has also been evidence that both acropetal and basipetal translocation occur, with acropetal translocation being dominant (Wetzel 2001). With the use of systemic herbicides, such as those included in these studies, one would expect basipetal translocation following herbicide exposure in the water column.

To better understand absorption and translocation of systemic aquatic herbicides in hydrilla and Eurasian watermilfoil, we aimed to: (1) evaluate absorption and fluridone, penoxsulam, and triclopyr bioconcentration (herbicide concentration inside the plant compared to herbicide concentration in the water column), (2) evaluate fluridone, penoxsulam, and triclopyr translocation following shoot exposure to these herbicides, and (3) evaluate the effect of herbicide concentration on bioaccumulation. Based on the trends of these studies, we will try and determine the extent of translocation, herbicide partitioning behavior, and relationship between herbicide log K_{ow} and bioaccumulation.

Materials and Methods

Plant Material

Eurasian watermilfoil fragments were collected from the Leggett Ditch near Boulder, CO in Fall 2006. Fluridone-susceptible, dioecious hydrilla fragments were collected from Saddle Creek, FL during Spring 2009. Following collection, 15 cm apical fragments were cut and planted in topsoil until needed. Plants were maintained in the greenhouse under 400-watt sodium halide lamps (approximately 200 mE m^{-2} s^{-1}) with a 10:14 h light:dark period. Temperature was set at 24 C and 18 C for day and night, respectively. If plants grew too large to use in experiments, apical sections were removed and replanted as previously described. During experiments, plants were maintained at room temperature, under fluorescent grow lights (approximately 200 mE m^{-2} s^{-1}) and a 10:14 h light:dark period.

Herbicide Absorption and Translocation Following Shoot Exposure

Prior to treatment 15 cm apical sections were removed and transferred to 5 cm dia by 9.5 cm glass jars. Each jar contained topsoil amended with slow-release fertilizer (3g/L) (Osmocote 14-14-14, The Scotts Company, Marysville, OH). Following planting, a sand cap was placed on the surface to avoid suspension of sediment in the water column. Plants were transferred to 30 L tanks filled with tap water and allowed to grow until they had produced roots (approximately 14 d). Prior to experiments, plants were removed from the tank, and water removed from the surface of the sand. At this time, a layer of agarose gel (1.5% v/v) (Phytagar, Invitrogen Corp., Grand Island, NY) was placed on the surface to isolate aboveground and belowground portions of the plant. Three plants of each species were placed in 4 L plastic tanks containing 3 L of tap water and were allowed to equilibrate for 18 h before treatment. Treatments included 10 µg/L fluridone that contained 41.67 KBq of ^{14}C-fluridone (1,357.9 KBq/mg specific activity), 10 µg/L penoxsulam containing 66.67 KBq ^{14}C-penoxsulam (2,273.3 KBq/mg specific activity), and 1 mg/L triclopyr containing 66.67 KBq of ^{14}C-triclopyr (2,689.5 KBq/mg specific activity).Fluridone and penxosulam were applied as ^{14}C labeled herbicide only, but the higher rate of triclopyr required the application of supplemental herbicide as the formulated product (Renovate 3, SePRO Corporation, Carmel IN). Three plants of each species were harvested at 6, 12, 24, 48, 96 and 192 hours after treatment (HAT). Upon harvest, plant tissue was collected, divided into aboveground and belowground sections. Tissue was then dried for 24 h at 60 C to obtain constant moisture. Dry biomass was recorded and radioactivity was determined using biological oxidation (OX500, R.J. Harvey Instrument Co., Tappan, NY) with $^{14}CO_2$ collected in 10 mL of ^{14}C trapping oxidizing cocktail (OX-161, R. J. Harvey Instrument Co., Tappan, NY). Radioactivity was then quantified using liquid scintillation spectroscopy (Packard 2500R, PerkinElmer, Waltham, MA).

Effect of Concentration on Herbicide Absorption

For each experiment, nine apical sections, 15 cm in length, were excised from plants propagated as previously described. Each fragment was placed in individual 75 mL glass vials containing 50 mL water. Fluridone, penoxsulam, and triclopyr were applied at 10, 100, and 1,000 µg/L. Radiolabeled herbicide was applied at 0.33 KBq per vial, and was supplemented with formulated herbicide (Sonar AS, Galleon, Renovate 3, SePRO Corporation, Carmel, IN) to reach the desired concentration. Plants were exposed to herbicide for 48 h and harvested. After harvest, plants were analyzed for ^{14}C as previously described.

Statistical Analysis

For all studies, Levene's test for homogeneity of variance was used to determine if data from repeated studies could be combined for statistical analysis. Means and standard errors for each experiment were calculated using MS Excel (MS Office, 2007). For the shoot exposure study, SigmaPlot (Ver. 10) was used to plot means, standard errors, and conduct non-linear regression analyses. The model chosen for absorption data was a hyperbolic function, which is shown below in Equation 1.

$$y = \frac{ax}{1 + bx} \qquad [1]$$

Based on the predicted values from the hyperbolic model, two other values were calculated for interpretation (A_{192} and t_{90}). Predicted absorption at 192 HAT (A_{192}) was calculated using the model, as was the time required for absorption to reach 90% of the A_{192} value (t_{90}). These calculated values provide a method of comparison between plant parts, plant species, and herbicides used.

In addition to non-linear regression analyses, the percentage of total herbicide present in aboveground and belowground portions of the plant were calculated to determine translocation, and the plant concentration factor (PCF) was calculated to determine bioconcentration. The equation used to calculate PCF was adapted from de Carvalho et al. (2007b) and can be defined using the formula shown below in Equation 2.

$$PCF = \frac{\text{Herbicide concentration in plant (ng/g Fresh Biomass)}}{\text{Herbicide concentration in water (ng/mL)}} \qquad [2]$$

This formula provides a bioconcentration factor to describe herbicide partitioning into plant tissue, which can be related to other herbicide properties.

Data from concentration experiments were analyzed using a linear regression model and plotted using SigmaPlot (Ver. 10). For all experiments, treatments were replicated three times and each study was repeated.

Results and Discussion

Herbicide Absorption and Translocation Following Shoot Exposure

In hydrilla 192 HAT, triclopyr shoot accumulation was the greatest (95.17 ± 11.46 µg/g), followed by fluridone (0.83 ± 0.07 µg/g), and penoxsulam (0.15 ± 0.02 µg/g). For Eurasian watermilfoil, triclopyr exhibited the greatest accumulation (346.09 ± 56.15 µg/g), again followed by fluridone (2.00 ± 0.17 µg/g) and penoxsulam (0.42 ± 0.05 µg/g). Root accumulation exhibited the same trend, but was significantly lower than shoot accumulation for all three herbicides. In all cases, the shoot accumulation by hydrilla was much less than accumulation by Eurasian watermilfoil. Hydrilla accumulated only 24, 35, and 39% of accumulation by Eurasian watermilfoil 192 HAT for fluridone, penxosulam, and triclopyr, respectively. Although the exact cause of this difference is unknown, it may be due to structural differences between species, including leaf shape and surface area. Eurasian watermilfoil has highly dissected leaves that provide more surface area for absorption than hydrilla, which have several compact leafs per whorl and entire margins.

The rate at which herbicide accumulation occurred varied for all three herbicides. Shoot accumulation based on predicted t_{90} values was rapid for fluridone in hydrilla, with 90% occurring by 76 HAT, but shoot accumulation rates for triclopyr and penxosulam were slower, taking 113 HAT and 145 HAT to reach t_{90}, respectively. While t_{90} occurred by 145 HAT for these herbicides, absorption of the contact herbicide endothall by hydrilla was slower, continuing for 288 HAT (12 DAT) (Haller and Sutton 1973).

In Eurasian watermilfoil, fluridone had the fastest accumulation rate, with t_{90} occurring by 30 HAT, followed by triclopyr (73 HAT) and penxosulam (110 HAT). In all cases, accumulation in Eurasian watermilfoil occurred more rapidly than in hydrilla (Table 1.1). Fluridone absorption by the submersed species sago pondweed and Richardson pondweed indicated that absorption was slow, continuing to increase for 336 HAT (14 DAT) (Marquis et al. 1981). Examination of fluridone absorption in terrestrial species indicated that a majority occurred in the first 5 DAT in the susceptible species corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.), but absorption continued to increase to 168 HAT (7 DAT) (Berard et al. 1978). Compared to these terrestrial studies, fluridone absorption by hydrilla and Eurasian watermilfoil was faster, with t_{90} occurring by 76 and 30 HAT, respectively. Triclopyr absorption by the susceptible species chickweed (*Stellaria media*) was rapid, with a majority occurring the first 24 HAT (Lewer and Owen 1990); however, absorption by hydrilla and Eurasian watermilfoil was slower than for chickweed, taking 113 and 73 HAT to reach t_{90}, respectively. Penoxsulam absorption by alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.) continued to increase at 48 HAT (Willingham et al. 2008). This is similar to penoxsulam absorption in these studies, where t_{90} occurred by 145 and 110 HAT for hydrilla and Eurasian watermilfoil, respectively. Absorption of imazamox, another ALS inhibiting herbicide, by Eurasian watermilfoil continued to increase for 72 HAT; however, approximately 50% occurred during the first 24 HAT (Vassios et al. 2011).

Even though herbicide absorption occurs fairly rapidly, it is important to note that herbicides may readily move out of the plant if transferred to untreated water. Vassios (2011) demonstrated that approximately 45% of absorbed imazamox moved out of Eurasian watermilfoil within 12 h of being placed in clean water. This is important in areas where it is difficult to achieve long exposures due to water exchange. Previous concentration exposure time studies (CET) illustrate this point, showing that maximum Eurasian watermilfoil control with triclopyr at 1 mg/L is achieved with an exposure time of >36 h (Netherland and Getsinger 1992). The same is true for fluridone, in which exposure at 12 µg/L must be maintained for >60 d for successful Eurasian watermilfoil and hydrilla control (Netherland et al. 1993). Most herbicide absorption occurs during the first few days after treatment; however, those concentrations must be maintained to achieve control.

Because fluridone and penoxsualm were treated at 10 µg/L and triclopyr at 1,000 µg/L, it is difficult to make a direct comparison between the three herbicides; however, another method to evaluate differences in absorption is through bioconcentration values. In this case, bioconcentration values were based on PCF, shown above in Equation 2, which can be described as the herbicide concentration in the plant tissue relative to concentration in the water column. Based on the calculated PCF values, bioconcentration in both hydrilla and Eurasian watermilfoil 192 HAT was greatest for triclopyr, followed by fluridone and penoxsulam (Table 1.1). These values indicate that herbicide bioconcentration was occurring in hydrilla shoots, with the concentration in plant tissue being greater than the water column for all three herbicides. The values for fluridone and penoxsulam are similar to what would be expected based on log K_{ow} values, but the large amount of triclopyr accumulation by both species was unexpected. Based on log Kow values we would have expected similar results for penoxsulam and triclopyr. The reasons for large amounts of triclopyr accumulation are unknown, but one possible explanation could be rapid triclopyr metabolism. Previous studies indicated little to no metabolism of fluridone by a range of crop species (Berard 1978), and no degradation was reported in sago pondweed (*Stuckenia pectinata*) and Richardson pondweed (*Potamogeton richardsonii*) (Marquis et al. 1981). Penoxsulam metabolism was slow in susceptible barnyardgrass (*Echinochola crus-galli*), with a half-life of 106 HAT (Kramer and Schirmer 2007); however, triclopyr metabolism in the susceptible species chickweed (*Stellaria media*) was much more rapid, with a half-life of 48 h, and only 40% remaining intact at 72 HAT (Lewer and Owen

1990). Rapid triclopyr metabolism could help maintain a concentration gradient, allowing for continued triclopyr accumulation. Triclopyr metabolism may not result in complete deactivation, but could be amino acid conjugation. Conjugation with aspartate was previously reported in susceptible chickweed. If the primary metabolite is an amino acid conjugate, the conjugation would likely be reversible, allowing for release of triclopyr at a later time (Lewer and Owen 1990). This is an aspect of triclopyr absorption that needs to be studied in more detail.

As previously mentioned, others have examined pesticide biococentration in submersed aquatic studies, including de Carvalho et al. (2007b) who evaluated pesticide absorption by curly waterweed. The pesticide that most closely resembled a herbicide included in our study was 4-chlorophenylurea (log K_{ow} = 1.80), which is similar to fluridone (log K_{ow} = 1.87). Observed PCF values for 4-clorophenylurea (2.12) were significantly lower than our maximum observed values for hydrilla (8.31) and Eurasian watermilfoil (19.97). These differences may have been due to differences in experimental methods. De Carvalho et al. (2007b) used small plant fragments, and we used established, rooted plants. Oxamyl (log K_{ow} = -0.47) has a log K_{ow} similar to penoxsulam and triclopyr, but the authors were not able to accurately predict bioaccumulation based on log K_{ow}, as the authors did not observe a linear relationship of bioaccumulation for pesticides with log K_{ow}<1. Marquis et al. (1981) evaluated fluridone absorption and translocation by the submersed species sago pondweed and Richardson pondweed. While biococentration values for the study were based on plant dry biomass, when converted to fresh weight (assuming 90% water content), the bioconcentration values (sago pondweed PCF = 9.46, Richardson pondweed PCF = 9.35) were very similar to our results for hydrilla. As for the differences between hydrilla and Eurasian watermilfoil, they may be due to differences in leaf shape and structure, as previously mentioned.

Shoot to root translocation in Eurasian watermilfoil was extremely limited with only 2.6 ± 0.3, 2.0 ± 0.4, and 1.3 ± 0.3% of total absorbed herbicide found in roots 192 HAT for triclopyr, fluridone, and penoxsulam, respectively. Slightly more root translocation occurred in hydrilla, with 12.5 ± 2.9, 9.0 ± 2.2, and 6.1 ± 1.5% of total absorbed radioactivity present in the roots 192 HAT for triclopyr, fluridone, and penoxsulam, respectively (Figure 1.2). Little to no herbicide movement to roots of submersed aquatic species had been reported previously. Limited fluridone translocation to hydrilla roots has been reported previously (Anderson 1979). Marquis et al. (1981) reported limited fluridone translocation to sago and Richardson pondweed roots, with only 0.4% and 0.3% present in roots 14 DAT, respectively. Our results indicate slightly more translocation with 9% present in hydrilla roots 192 HAT. Results reported by Willingham et al. (2008), showed 1.2% of absorbed penoxsulam was present in the roots of alligatorweed 48 HAT. These results are similar to our observations of penoxsulam translocation in Eurasian watermilfoil, but translocation to hydrilla roots was greater, with 6.1% present in roots 192 HAT. Triclopyr translocation to roots for the terrestrial species horsenettle (*Solanum carolinense* L.) reached a maximum of 3.6%, similar to triclopyr translocation in our studies (2.1% present in roots, Figure 1.2); however, triclopyr translocation in hydrilla was greater, reaching 12.5% in roots 192 HAT. It appears that there is a large amount of translocation to roots of Eurasian watermilfoil, reaching a maximum by 12 HAT. After this point, the amount of herbicide in Eurasian watermilfoil roots as a percent of total absorption decreases until 192 HAT (Figure 1.2). This peak during the first 12 HAT is likely an artifact of roots reaching an equilibrium faster than shoot tissue, while shoots continued to absorb triclopyr (Figure 1.1). Because shoot absorption continues and roots concentration remains constant, the proportion of herbicide in roots compared to total absorption appears to decrease. Results for Eurasian watermilfoil are similar to previous findings, but translocation to hydrilla roots was greater than previously reported values for all three herbicides.

Effect of Concentration on Herbicide Absorption

Linear regression analysis of herbicide absorption at 10, 100, and 1,000 µg/L following 48 h exposure indicated a strong linear relationship for all three herbicides in both species (Figure 1.3). This linear trend indicates that as herbicide concentration in the water column increases, concentration in the plant tissue increases proportionally. This direct linear relationship between internal and external herbicide concentration provides strong evidence that absorption is due to diffusion as a result of a concentration gradient. The herbicide concentrations included in this study were well below the water solubility values for all three herbicides. The fact that the herbicide was soluble at these concentrations, paired with the fact that submersed aquatic species, such as Eurasian watermilfoil and hydrilla, have little to no cuticle (Sculthorpe 1967), provide evidence that herbicide diffusion into plant tissue could easily occur. These findings are similar to the relationship found for imazamox in Eurasian watermilfoil (Vassios et al. 2011). The relative difference in absorption by both species correspond to those seen 48 HAT in the whole plant absorption studies we conducted, with fluridone having the greatest accumulation, followed by penoxuslam and triclopyr in hydrilla, In Eurasian watermfilfoil, triclopyr accumulation was greatest, followed by fluridone and penxosualm.

Herbicide absorption by Eurasian watermilfoil and hydrilla was rapid, with a majority occurring during the first 100 HAT. Fluridone and penxosulam absorption were similar to what would have been predicted based on log K_{ow} values; however, triclopyr absorption was much greater than would have been predicted based on log K_{ow} values. The cause of increased triclopyr absorption is unknown, but it could be due to herbicide metabolism, and further metabolism studies should be conducted to examine triclopyr metabolism in submersed aquatic species. Translocation to roots was relatively limited, with a maximum of 12.5% of absorbed triclopyr present in hydrilla roots 192 HAT. Herbicide absorption across a range of concentrations exhibited a linear relationship, indicating that absorption was likely driven by diffusion due to a concentration gradient between the water column and plant tissue. It is important to note that although herbicide absorption is rapid, concentrations must be maintained for extended periods of time to achieve control. These data provide insight into herbicide absorption and translocation in submersed aquatic species.

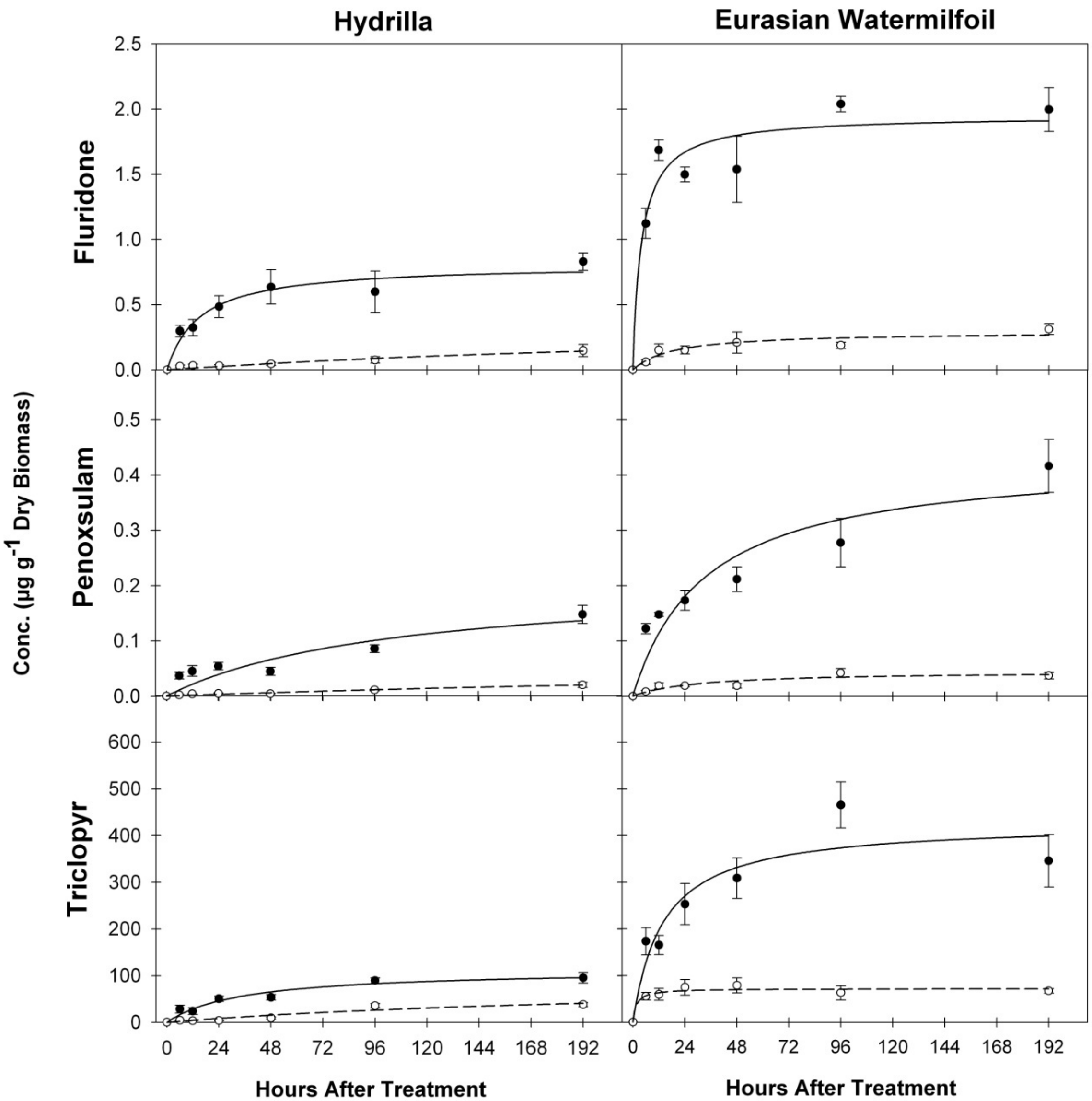

Figure 1.1: Herbicide absorption following shoot exposure. Open circles are the herbicide concentration in the roots, and closed circle are herbicide concentration in the shoots. Datapoints represent the mean, and error bars represent the standard error of the mean. (n = 6)

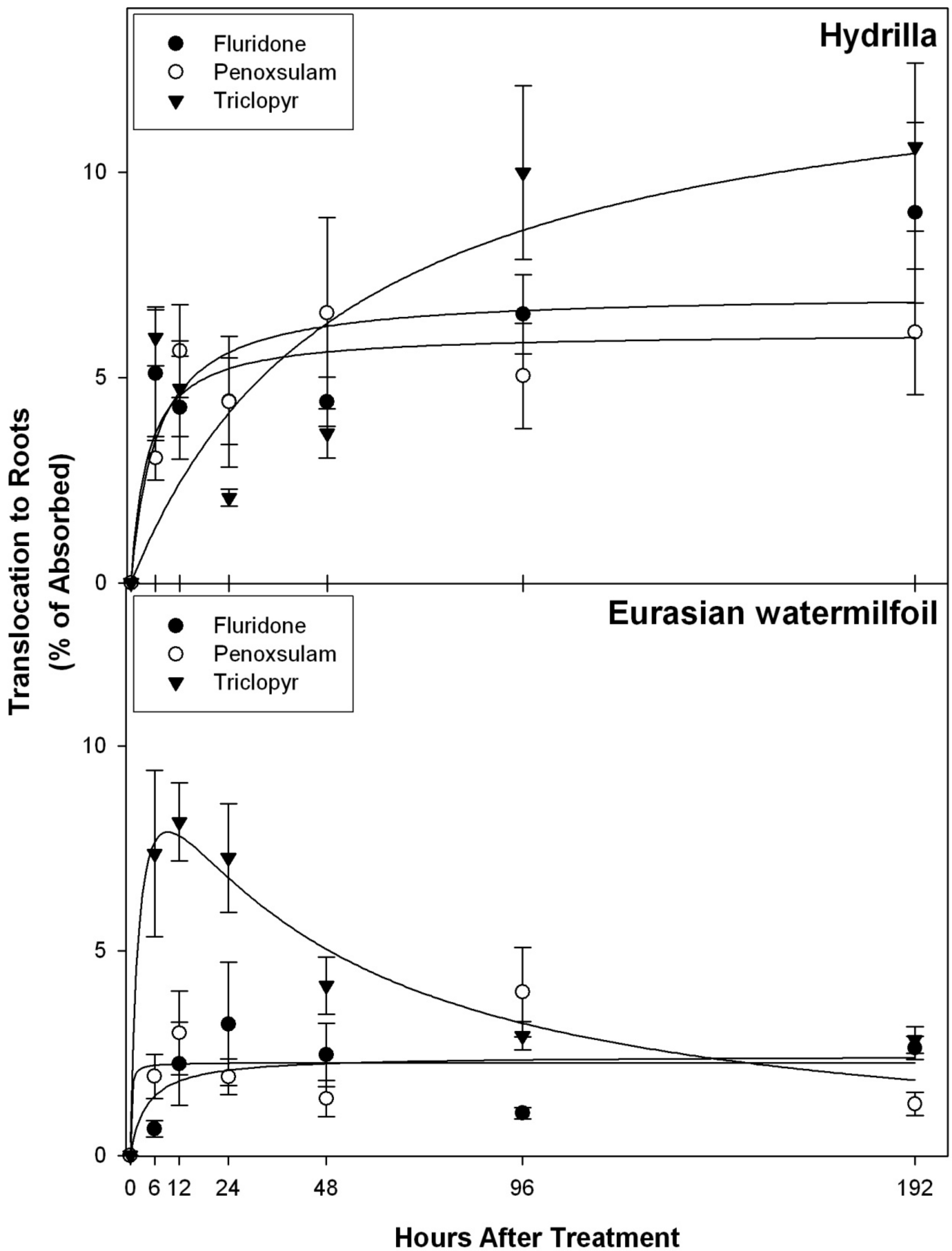

Figure 1.2: Herbicide translocation to roots following shoot herbicide exposure as a percentage of total absorbed herbicide. Datapoints represent the mean, and error bars represent the standard error of the mean. (n = 6)

Table 1.1: Plant Concentration Factor (PCF), parameters, and calculated values based on hyperbolic regression analyses. Values represent the mean, and error terms represent the standard error of the mean. (n = 6)

				Parameters and Estimates Based on Hyperbolic Regression Analyses			
Species	**Herbicide**	**Plant Part**	PCF_{192}	A_{192} **(μg/g)**	t_{90} **(h)**	***a*± SE**	***b*± SE**
Hydrilla							
	Fluridone	Aboveground	8.31 ± 0.66	0.76	76	0.054 ± 0.0129	0.066 ± 0.0200
		Belowground		0.01	163	0.001 ± 0.0003	0.003 ± 0.0026
	Penoxsulam	Aboveground	1.48 ± 0.17	0.13	145	0.002 ± 0.0008	0.010 ± 0.0072
		Belowground		0.01	166	0.0001 ± 0.00003	0.002 ± 0.0019
	Triclopyr	Aboveground	9.52 ± 1.15	96.78	113	3.214 ± 0.7422	0.028 ± 0.0092
		Belowground		41.70	161	0.384 ± 0.1449	0.004 ± 0.0041
EWM							
	Fluridone	Aboveground	19.97 ± 1.69	1.91	30	0.485 ± 0.1816	0.249 ± 0.1046
		Belowground		0.27	85	0.016 ± 0.0058	0.054 ± 0.0254
	Penoxsulam	Aboveground	4.16 ± 0.48	0.37	110	0.013 ± 0.0042	0.030 ± 0.0133
		Belowground		0.05	112	0.002 ± 0.0006	0.033 ± 0.0181
	Triclopyr	Aboveground	34.61 ± 5.61	397.09	73	30.659 ± 11.424	0.0716 ± 0.0331
		Belowground		71.42	14	44.435 ± 26.3682	0.617 ± 0.3908

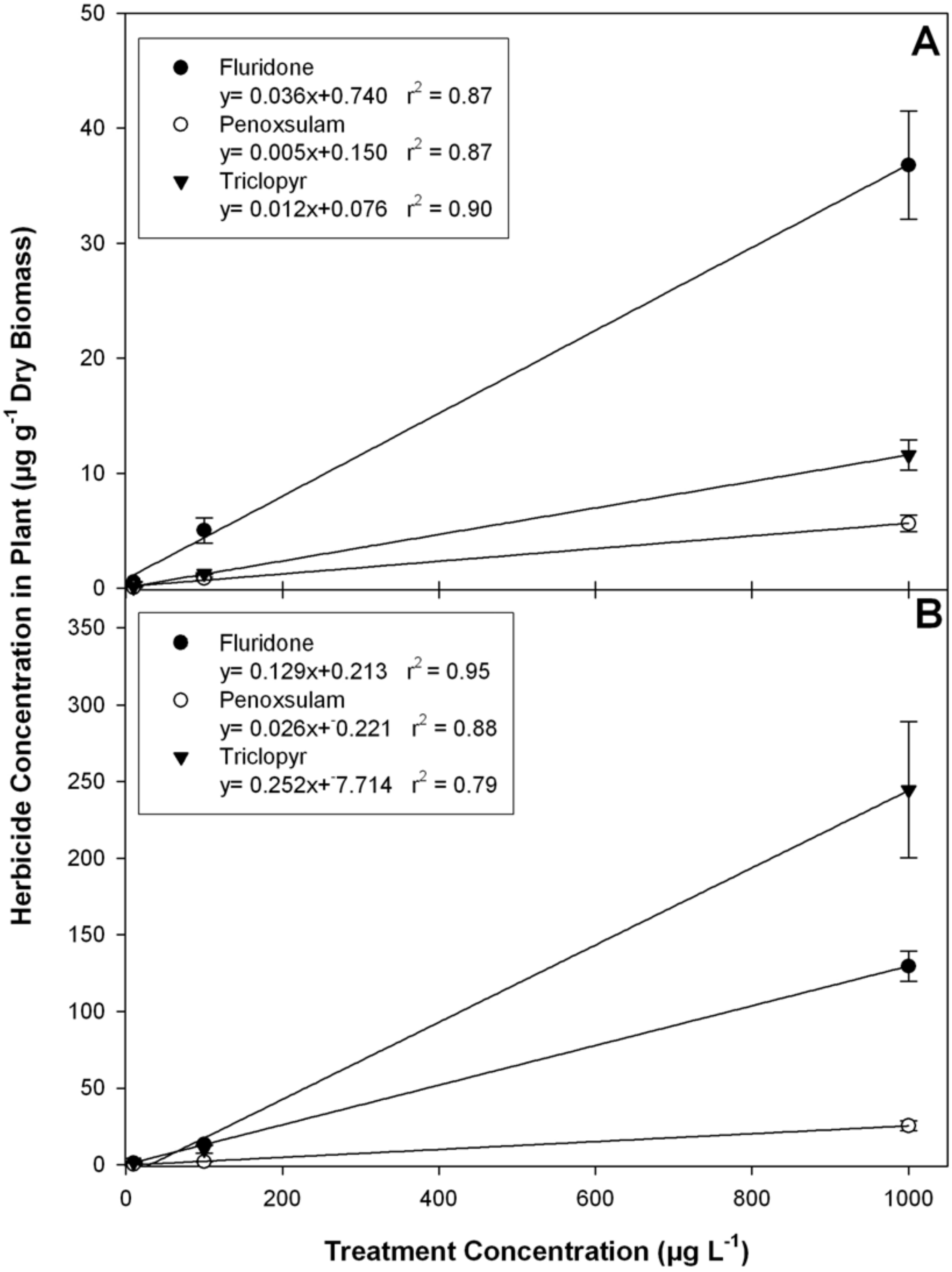

Figure 1.3: Herbicide absorption by hydrilla (A) and Eurasian watermilfoil (B) at treatment concentrations of 10, 100, and 1000 µg/L. Datapoints represent the mean, and error bars represent the standard error of the mean. (n = 6)

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Chapter 2: Root Absorption and Translocation of Fluridone, Penoxsulam, and Triclopyr by Eurasian watermilfoil and Hydrilla

Introduction

Hydrilla (*Hydrilla verticillata* (L.F.) Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) are submersed, non-native invasive species. These species form dense monotypic stands that can alter aquatic ecosystems by displacing native species, impacting water quality, and reducing light penetration. In addition to these ecological impacts, they can affect the economic value of water bodies by impacting recreational activities, water flow, human health, and aesthetic value (Gettys 2009; Grace 1978; Langeland 1996; Smith and Barko 1990). A range of control methods have been developed to alleviate these impacts, and prevent the spread of current infestations within and between water bodies.

Eurasian watermilfoil was first reported as a problem in the US during 1940's (Gettys 2009). Since first reported in the US, it has expanded its range to include at least 45 states (USDA 2011b). Eurasian watermilfoil is a perennial species that produces new vegetative growth each year. As water warms in the spring, Eurasian watermilfoil grows quickly toward the surface. Upon reaching the surface, the plants will branch, flower, and fragment. Regrowth later in the growing season or the following year occurs from fragments that fall to the bottom of the water column and become established, or from previously established root crowns. Root crowns and stolons contribute to short-range expansion, and vegetative fragments are the most prominent method of long-distance dispersal (Grace 1978; Smith and Barko 1990).

Hydrilla was first reported in the US in Florida in 1960. It was likely introduced as a result of the aquarium trade (Gettys 2009), and since its introduction it has spread to at least 19 states (USDA 2011a). At least two separate hydrilla introductions have occurred, because there are two distinct biotypes present in the US, one dioecious and one monoecious. While the dioecious biotype is primarily limited to warmer areas of the Southeastern US, the monoecious biotype grows in more temperate areas, such as North Carolina and Virginia (Langeland 1996). Plants may form many tubers and turions, although vegetative fragments are the most common source for long-distance dispersal (Gettys 2009; Langeland 1996).

Both Eurasian watermilfoil and hydrilla possess several characteristics that allow them to outcompete native species. Both have a very plastic growth habit, allowing them to tolerate a large range of water chemistry parameters, such as salinity and pH. In addition, hydrilla is able to begin photosynthesis earlier in the day, taking advantage of higher CO_2 concentrations present in the morning (Langeland 1996). Eurasian watermilfoil can grow at a wide range of temperatures, with growth starting at temperatures as low as 15 C, with maximum growth occurring at 32 C (Smith and Barko 1990). Rapid growth of both species leads to formation of dense mats on the surface, shading out native species. These characteristics give Eurasian watermilfoil and hydrilla a competitive advantage over many native species (Langeland 1996; Smith and Barko 1990). The highly competitive growth habit of these species can lead to dense infestations, requiring management to return value to infested water bodies.

The most common, and often most cost-effective, control method for Eurasian watermilfoil and hydrilla is the use of aquatic herbicides. In the past, options for chemical control of Eurasian watermilfoil and hydrilla were relatively limited, and many available herbicides had minimal selectivity. Since the first reports of fluridone resistant hydrilla in 1999 (Albrecht 2004), there has been a renewed effort for the development on additional aquatic herbicides. These efforts have resulted in seven new active ingredients registered for aquatic use since 2000 (Gettys 2009). Each of these new herbicides have unique characteristics and spectra of plants controlled, and they each have a fit in aquatic plant management.

Systemic herbicides translocate throughout the plant, and are ideal for control of perennial species. In perennial species, translocation can result in better long-term underground structure control. In general, these herbicides require longer exposure times and are slower acting than contact herbicides. Fluridone, penoxsulam, and triclopyr are systemic herbicides registered for aquatic use. Penoxsulam and fluridone are used for hydrilla control, but all three herbicides provide Eurasian watermilfoil control (Gettys 2009). Even though all three herbicides are systemic, they each have a different mode of action, and may provide control depending on individual site conditions.

A number of studies have attempted to predict systemic aquatic herbicide behavior by examining the relationship between herbicide accumulation and lipophilicity (as determined by log K_{ow}). Log K_{ow} is the partitioning coefficient for octanol/water, and can be used as an indicator of a herbicides lipophilicity. A larger log K_{ow} value indicates that a herbicide is more lipophilic, as it has a greater affinity for octanol over water. Studies conducted on aquatic plants have focused mainly on the potential of aquatic plants as bioaccumulators for phytoremediation (Crum et al. 1999; de Carvalho et al. 2007a; de Carvalho et al. 2007b). Additional research was conducted on barley (*Hordeum vulgare)* root accumulation in a hydroponic system (Briggs et al. 1982; Briggs et al. 1987). These studies were conducted on the shoots and roots of aquatic plants, and the roots of barley, but studies found similar trends.

Similarities between studies indicate that aquatic shoots, aquatic roots, and terrestrial roots exhibit similar accumulation of organic compound as a function of log K_{ow}. In all cases, as log K_{ow} increased, so did accumulation. There was a strong linear relationship between log K_{ow}

and bioaccumulation when the compound's log K_{ow}>1; however, the authors were unable to develop a linear relationship between log K_{ow} and bioaccumulation for compounds with a log K_{ow}<1 (Briggs et al. 1982; Briggs et al. 1987; de Carvalho et al. 2007a; de Carvalho et al. 2007b).

There have been relatively few studies conducted to examine absorption and translocation of the highly water soluble herbicides that are used for aquatic plant management. Their performance and selectivity has been documented and evaluated in laboratory, field, and greenhouse studies, but little work has been completed to examine herbicide absorption and translocation in submersed aquatic plants. Based on this relationship, we would expect the greatest accumulation of fluridone (log K_{ow} = 1.869), and significantly less penoxsulam (log K_{ow}= -0.354) and triclopyr (log K_{ow}= -0.444) accumulation (log K_{ow} values obtained from Senseman (2007)).

The ability of an aquatic plant to accumulate sufficient amounts of herbicide is important for control, translocation within the plant is also important, especially for perennial species. Roots of aquatic plants appear to function similarly to those of terrestrial plants, being the main site of nutrient absorption; however, aquatic plants are also able to absorb nutrients from the water column through their foliage. Because submersed aquatic plants are able obtain nutrients from the water column, it may not be necessary for them to translocate as much throughout the plant. Even though there is evidence that both basipetal (from shoots to roots) and acropetal (from roots to shoots) translocation occur in submersed aquatic plants, there is usually more acropetal translocation (Wetzel 2001). The systemic herbicides used in this study would be expected to translocate throughout the plant, and because acropetal translocation has been shown to be greater, we would expect more translocation following root exposure than translocation seen following shoot exposure (Table 1.1). The objectives of this study were to (1) examine fluridone, penoxsulam, and triclopyr root accumulation in hydrilla and Eurasian watermilfoil, and (2) to evaluate translocation to shoots following root exposure. These data provide a basis for comparison between herbicides, insight into their translocation trends following root exposure, and will allow for examination of the relationship between log K_{ow} and herbicide bioaccumulation.

Materials and Methods

Plant Materials

Eurasian watermilfoil and dioecious hydrilla fragments were collected from the Leggett Ditch near Boulder, CO, and Saddle Creek, FL, respectively. Hydrilla fragments were fluridone-susceptible and collected in Spring 2009. Fifteen cm apical sections were excised and planted in topsoil until needed for experiments. Plants were maintained in 300 L fiberglass tanks in the greenhouse with a 10:14 h light:dark period. Supplemental lighting was provided by overhead, 400-watt, sodium halide lamps (approximately 200 mE m^{-2} s^{-1}), and temperature set at 24 C and 18 C for day and night periods, respectively. If plants grew too large for use before experiments were conducted, 15 cm apical sections were again excised and replanted as previously mentioned.

Herbicide Absorption Following Root Exposure

Prior to experiments, 15 apical sections, 15 cm long were removed from previously propagated plants, and were transplanted into 3 x 3 x 6 cm pots in topsoil amended with 3 g/L slow release fertilizer (Osmocote 14-14-14, The Scotts Company, Marysville, OH). Plants were placed in tap water in 40 L aquaria and allowed to grow until they had produced roots (approximately 14 d). At this time, plants were removed from pots, roots rinsed to remove soil, and transplanted into 25 x 99 mm glass vials (66011-165, VWR International, Radnor, PA), using a method adapted from Marquis et al. (1981). Each vial contained 35 mL of a 1/5th strength Hoagland's solution (H2395, Sigma Aldrich, St. Louis, MO), and once the plant was placed in the vial, a layer of low melting-point eicosane wax (219274, Sigma Aldrich, St. Louis, MO) was added to each vial to isolate roots and shoots. After transplanting, plants were allowed to equilibrate for 5 d in the nutrient solution. At this time, herbicide was applied using a syringe and needle to penetrate the eicosane wax layer. Each vial received a herbicide treatment containing 3.33 KBq of ^{14}C-fluridone (1,357.9 KBq/mg specific activity, final concentration 70.1 μg/L), ^{14}C-penoxsulam (2,273.3 KBq/mg specific activity, final concentration 42.1 μg/L), or ^{14}C-triclopyr (2,689.5KBq/mg specific activity, final concentration 25.7 μg/L). After herbicide was applied, all vials were transferred to 40 L aquaria with recirculating pumps. Each tank also contained a charcoal filter to remove any herbicide from the water column to prevent contamination of the water column. During the study, plants were maintained at room temperature, and supplemental light was provided by fluorescent grow lights with a 10:14 h light:dark period.

Plants were harvested at 12, 24, 48, 96, and 192 HAT. Upon harvest, plants were separated into aboveground and belowground portions, and dried for 48 h at 60 C to obtain constant moisture. Dry biomass was recorded and radioactivity determined using biological oxidation (OX500, R.J. Harvey Instrument Co., Tappan, NY). $^{14}CO_2$ was collected in 10 mL of ^{14}C trapping oxidizer cocktail (OX161, R.J. Harvey Instrument Co., Tappan, NY), and quantified using liquid scintillation spectroscopy (Packard 2500R, PerkinElmer, Waltham, MA). Each treatment was replicated three times, and each study was repeated.

Statistical Analysis

For each study, Levene's test for homogeneity of variance was used to determine if data from repeated studies could be combined for statistical analysis ($\alpha = 0.05$). Means and standard errors were calculated for each study using MS Excel (MS Office 2007). SigmaPlot (Version 10) was used to plot means and standard errors. Non-linear regression analyses were also conducted in SigmaPlot, and data were fitted to the hyperbolic function shown below in Equation 1:

$$y = \frac{ax}{1 + bx} \qquad [1]$$

Based on the predicted values from the hyperbolic model, two additional values were calculated (A_{192} and t_{90}). Predicted absorption at 192 HAT (A_{192}) was calculated using this model. Time required for absorption to reach 90% of the A_{192} value (t_{90}), was also calculated. These calculated values provide parameters for comparison between herbicides and species.

In addition to absorption analysis, translocation to shoots was calculated as a percentage of total absorbed herbicide. Root concentration factor (RCF) was also calculated as a measure of bioconcentration. The formula used to calculate RCF is similar to a formula used by De Carvalho et al. (2007b), and is shown in Equation 2:

$$RCF = \frac{\text{Herbicide concentration in roots (ng/g Fresh Biomass)}}{\text{Herbicide concentration in water (ng/mL)}} \qquad [2]$$

Calculated RCF values provide biococentration values that can be compare herbicides.

Results and Discussion

Herbicide Absorption Following Root Exposure

In both species, fluridone had the highest bioaccumulation. The fluridone concentration was 9.52 ± 2.23 μg/g dry biomass, and 8.99 ± 1.92 μg/g dry biomass in Eurasian watermilfoil and hydrilla roots, respectively (Figure 2.1). Penoxsulam and triclopyr accumulation were similar, and there was slightly more accumulation in Eurasian watermilfoil for both herbicides. Penoxsulam accumulation was not significantly different between hydrilla (0.25 ± 0.03 μg/g dry biomass) and Eurasian watermilfoil (0.34 ± 0.07 μg/g dry biomass) (Figure 2.1); however, triclopyr accumulation was significantly higher in Eurasian watermilfoil (0.40 ± 0.04 μg/g dry biomass) than hydrilla (0.26 ± 0.02 μg/g dry biomass) (Figure 2.1). Unlike bioaccumulation following shoot exposure, where Eurasian watermilfoil accumulated herbicide faster than hydrilla (Table 1.1), there was little to no difference in absorption between hydrilla and Eurasian watermilfoil following root exposure. The difference in absorption between species was attributed to differences in surface area or leaf morphology (Chapter 1). Unlike shoot accumulation (Chapter 1), root accumulation in both species did not show large differences, likely due to the fact that root morphology (surface area) was similar for both species at this early growth stage.

Based on calculated t_{90} values, the rate of accumulation was more rapid in hydrilla for all three herbicides (Table 2.1). In both species, fluridone accumulation was slowest, reaching t_{90} 131 HAT in hydrilla, and 141 HAT in Eurasian watermilfoil. Based on results from shoot exposure studies, we expected fluridone to accumulate rapidly in root tissue, as accumulation of fluridone was fastest in the previous shoot exposure studies (Table 1.1), which reached t_{90} by 76 HAT. However, observed root accumulation took approximately twice as long as shoot accumulation (Table 2.1, Table 1.1). Fluridone accumulation in both species (reaching t_{90} by

141 HAT) was more rapid than fluridone accumulation by sago pondweed (*Stuckenia pectinata* (L.) Borner) roots, which continued to increase 336 HAT (Marquis et al. 1981). Fluridone accumulation was also faster than endothall absorption by hydrilla shoots, which continued to increase to 288 HAT (Haller and Sutton 1973). In contrast, fluridone absorption by Richardson pondweed (*Potamogeton richardsonii* (Benn.) Rydb.) roots reached a maximum 48 HAT, and continued to decrease to 336 HAT (Marquis et al. 1981). Fluridone root absorption was also evaluated in several terrestrial crop species. In cotton (*Gossypium hirsutum* L.), soybean (*Glycine max* (L.) Merr.), and corn (*Zea mays* L.), most absorption occurred by 120 HAT, which is similar to accumulation rate by Eurasian watermilfoil and hydrilla (Berard et al. 1978).

Triclopyr absorption was slower following root exposure in Eurasian watermilfoil (t_{90} = 95 HAT) than shoot exposure, which reached t_{90} 73 HAT (Table 1.1). For hydrilla, root absorption (t_{90} = 43 HAT) was faster, than shoot absorption (t_{90} = 113 HAT, Table 1.1). Penoxsulam root absorption was faster than shoot absorption for both species, with hydrilla and Eurasian watermilfoil reaching t_{90} 90 HAT and 67 HAT (Table 2.1), respectively, while shoot absorption reached t_{90} 110 HAT and 145 HAT (Table 1.1) for Eurasian watermilfoil and hydrilla, respectively.

Penoxsulam root absorption by both species was similar to shoot absorption by the emergent aquatic species alligatorweed (*Alternantera philoxeroides* (Mart.) Griseb.), which continued to absorb herbicide 48 HAT (Willingham et al. 2008). There were many differences between shoot (Table 1.1) and root absorption rates, but there was little difference in the rate of root accumulation between Eurasian watermilfoil and hydrilla (Table 2.1).

Because each of the three herbicides used were applied at different rates, it is difficult to compare total absorption using the concentration in plant tissue alone. Instead, we can use a bioconcentration factor, which compares herbicide concentration in the plant to the water column concentration. The term used in this case was defined as RCF, and was calculated using Equation 2. As previously mentioned, we would have expected the greatest accumulation of fluridone, and much less penoxsulam and triclopyr accumulation based on their relative log K_{ow}. Fluridone had the greatest accumulation in both species 192 HAT; however, there was no significant difference between hydrilla and Eurasian watermilfoil. The herbicide that exhibited the next highest accumulation was triclopyr (Table 2.1). Because it is difficult to predict accumulation behavior for pesticides with a log $K_{ow}<1$, small differences may occur between actual values and predicted biococentration based on log K_{ow}, as is the case with penoxsulam (log K_{ow} = -0.35) and triclopyr (log K_{ow} = -0.44). For triclopyr, there was a significant difference in accumulation between species, with accumulation by Eurasian watermilfoil being approximately 150% of hydrilla accumulation (Table 2.1).

Penoxsulam accumulated least of all three herbicides in hydrilla and Eurasian watermilfoil root tissue, with no significant difference in accumulation between the two species (Table 2.1). Triclopyr and fluridone plant tissue concentrations were greater than the water column concentration (RCF $\geq$ 1), but for penoxsulam, concentration in plant tissue was less than the water column concentration (RCF $<$1) (Table 2.1). Previous research suggests that when aquatic plant RCF $<$ 1, diffusion into plant cell water is likely the driving force for accumulation, and when RCF $>$ 1, partitioning onto plant solids is responsible for accumulation (de Carvalho et al. 2007a). Shoot exposure studies (Table 1.1) indicated greater triclopyr accumulation than would have been predicted based on log K_{ow} values; however, that was not the case with root absorption. The amount of herbicide absorbed by Eurasian watermilfoil and hydrilla roots was similar to what would have been predicted by herbicide log K_{ow} values.

Bioconcentration has been used to compare root accumulation in both terrestrial and aquatic species (Briggs et al. 1982; de Carvalho et al. 2007a). deCarvalho et al. 2007a) evaluated bioaccumulation of many pesticides in the emergent aquatic species parrotfeather (*Myriophyllum aquaticum* (Vell.) Verdc.), as well as the relationship between log K_{ow} and pesticide biococentration. Results from previous work conducted on barley roots were similar to those reported in emergent parrotfeather (Briggs et al. 1982), with bioconcentration increasing as log K_{ow} increased. Both of these studies were conducted in hydroponic systems similar to ours, and it is important to note that both studies used only non-ionized compounds, where pH would not effect bioconcentration. Two compounds included in their work had similar lipophilicity to the herbicides used in our study. The organic compound 4-chlorophenylurea (log K_{ow} = 1.80) was similar to fluridone, and oxamyl (log K_{ow} = -0.47) was similar to penoxsulam and triclopyr. The root bioconcentration of 4-chlorophenylurea was much lower in parrotfeather (RCF = 2.01)(de Carvalho et al. 2007a) and barley (RCF = 2.00) (Briggs et al. 1982) than flurdione bioconcentration in our studies, where RCF values for hydrilla and Eurasian watermilfoil were 12.84 ± 2.74 and 13.58 ± 3.18, respectively (Table 2.1). These differences could be due to differences in experimental methods, plant structure, or plant physiology. Previous studies were unable to accurately predict RCF values for oxamyl, due to its low lipophilicity. In studies on parrotfeather and barley, it was impossible to establish a linear relationship between log K_{ow} and RCF for herbicides with log K_{ow}<1 (Briggs et al. 1982; de Carvalho et al. 2007a).

The relationship between bioconcentration and log K_{ow} was determined using non-ionized organic compounds; however, additional studies were conducted to evaluate the impact of pH on 3,5-D (an analogue of the herbicide 2,4-D) root accumulation in barley (Briggs et al. 1987; de Carvalho et al. 2007a) and 3,5-D shoot accumulation in curly waterweed (*Lagarosiphon major*

(Ridley) Moss) (de Carvalho et al. 2007b). The pKa of 3,5-D is 2.84, which is very similar to triclopyr (2.68) (Senseman 2007). As water pH increased from 4 to 8, bioconcentration (PCF or RCF) decreased. Above the pKa of 2.84, the dissociated form would be dominant, but below the pKa, the undissociated form would be. Presence of the undissociated form would allow acid trapping to occur, resulting in more bioconcentration. Some aquatic herbicides, including triclopyr, are ionizable; however, the pKA of triclopyr is below physiological pH range, and typical water pH values. While the pKa of triclopyr would not allow for increased bioconcentration as a result of acid trapping, a pKa in the physiological range could result in pH dependent biococentration. This is a factor that will need to be considered in the future as additional aquatic herbicides are developed.

A study conducted by Marquis et al. (1981) examined fluridone root absorption from a hydroponic solution in submersed sago and Richardson pondweeds. The bioconcentration values presented by these authors were based on plant dry weight, so approximate fresh weight values were calculated on the assumption plants were 90% water prior to drying. Accumulation by the roots of sago pondweed (Bioconcentration = 3.84) and Richardson pondweed (Bioconcentration = 3.10) 288 HAT was significantly less than absorption by hydrilla and Eurasian watermilfoil (Table 2.1), but did show a higher concentration in plant tissue compared to the water column (Bioconcentration $\geq$ 1). These results were lower than root absorption in our studies, but they were similar to the values for 4-chlorophenylurea in parrotfeather (RCF = 2.01) and barley roots (RCF=2.00) (Briggs et al. 1982; de Carvalho et al. 2007a). As previously mentioned, the difference between previous studies and our current research could have been due to growth stage, experimental methods, or species.

Evaluation of acropetal translocation following hydroponic root exposure in parrotfeather and barley indicated that compounds with a log K_{ow} of 1.8 should result in the greatest translocation (Briggs et al. 1982; de Carvalho et al. 2007a). Based on these observations, we would have expected fluridone to have the greatest acropetal translocation (log K_{ow} = 1.869). Fluridone translocation to shoots was only 2.13-5.25% of absorbed herbicide, and penoxsulam and triclopyr translocation was greater than 15% 192 HAT.

Greater acropetal translocation would be anticipated based on observations of nutrient translocation in submersed aquatic species (Wetzel 2001). This was not the case for fluridone in hydrilla, where translocation following shoot exposure resulted in 9.0% ± 2.2 of absorbed herbicide in roots, and with root treatment only 2.13% ± 0.92 of absorbed herbicide translocated to shoots (Figure 2.2, Figure 1.2). For both penoxsulam and triclopyr in hydrilla, there was more extensive acropetal translocation following root exposure than basipetal transocation to roots following shoot exposure 192 HAT (Figure 2.2, Figure 1.2).

In Eurasian watermilfoil, acropetal translocation following root exposure was greater than basipetal translocation following shoot exposure for fluridone, penoxsulam, and triclopyr 192 HAT (Figure 2.2, Figure 1.2). Fluridone translocation from roots to shoots was previously reported in hydrilla, but not quantified (Anderson 1980). Acropetal translocation of fluridone was reported for sago pondweed and Richardson pondweed, with 5.2% and 2.3% of absorbed herbicide moving to shoots 336 HAT, respectively (Marquis et al. 1981). These values are similar to our results that indicated 2.13% ± 0.92 and 5.25% ± 2.56 of absorbed fluridone moving to shoots 192 HAT for hydrilla and Eurasian watermilfoil, respectively (Figure 2.2).

Several previous studies reported varying amounts of acropetal translocation for different aquatic species, but these studies used outdated techniques, such as autoradiography, to monitor translocation. The few studies that did quantify translocation reported similar results to fluridone in our studies. The amount of absorbed herbicide translocating to shoots was less than 30%, and there was significantly more acropetal translocation than basipetal translocation for all herbicides in both species, except for fluridone in hydrilla. Translocation in these aquatic species appears to be very limited compared to terrestrial species. Hydroponic fluridone root exposure in several crop species resulted 14, 33, 35, and 81% of absorbed herbicide moving to shoots of cotton, soybean, corn, and rice (*Oryza sativa* L.) 144 HAT, respectively (Berard et al. 1978). Greater translocation to the shoots of these terrestrial species was likely the result of greater transipriation and acropetal movement compared to aquatic species, which would have less demand for water movement to shoots.

Penoxsulam and triclopyr root absorption by hydrilla and Eurasian watermilfoil was rapid, but fluridone absorption was slower than absorption from shoot exposure (Table 1.1, Table 2.1). Bioaccumulation of these herbicides, calculated as RCF values, was similar to what we predicted based on herbicide lipophilicity (log K_{ow}), with fluridone having the greatest accumulation, and penoxsulam and triclopyr having significantly lower accumulation. In contrast to the shoot exposure studies (Chapter 1), there was little to no difference in root bioaccumulation between hydrilla and Eurasian watermilfoil, likely due to similar root morphology at the early growth stage used in these studies.

Acropetal herbicide translocation following root exposure was greater than basipetal translocation in all cases, except for fluridone in hydrilla, where basipetal translocation was greater. Penoxsulam acropetal translocation was greatest, with approximately 27% of absorbed herbicide present in shoots 192 HAT. Absorption occurs rapidly during the first 192 HAT, but if these concentrations are not maintained, there is a possibility that herbicide could move out of the plant and back into the water column, as demonstrated by Vassios et al. (2011). It is important to note that our studies were performed in a somewhat artificial system, where accumulation and translocation occurred under ideal conditions. Herbicide chemical properties and soil binding may affect herbicide availability in the root zone. Evidence of greater net acropetal translocation supports the development of new herbicide application methods and formulations that could be used to exploit a plants own transport system to increase root loading, especially in areas of high water exchange, increasing efficacy.

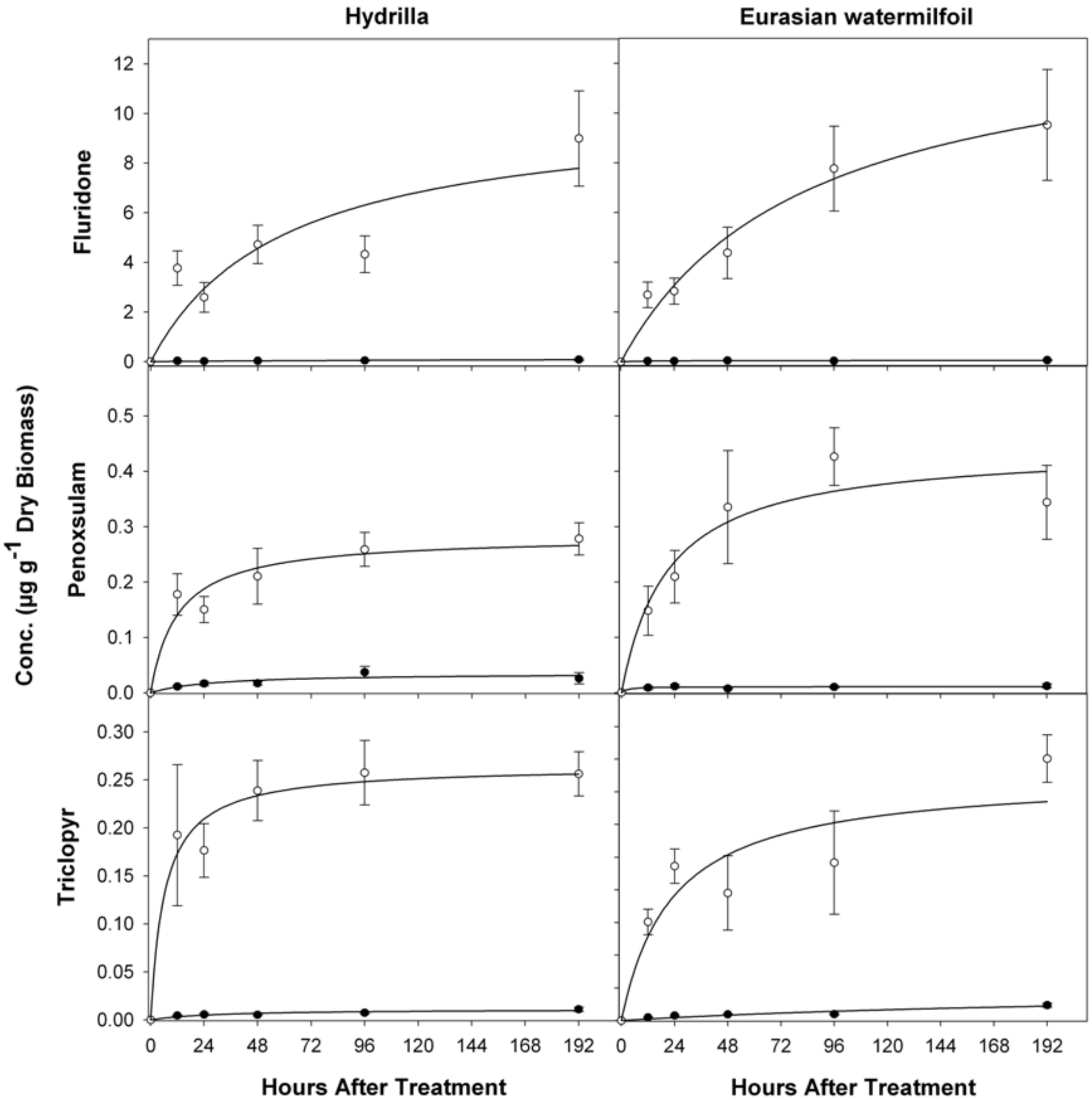

Figure 2.1: Herbicide absorption following root exposure. Open circles are the herbicide concentration in the roots, and closed circle are herbicide concentration in the shoots. Datapoints represent the mean, and error bars represent the standard error of the mean. (n = 6)

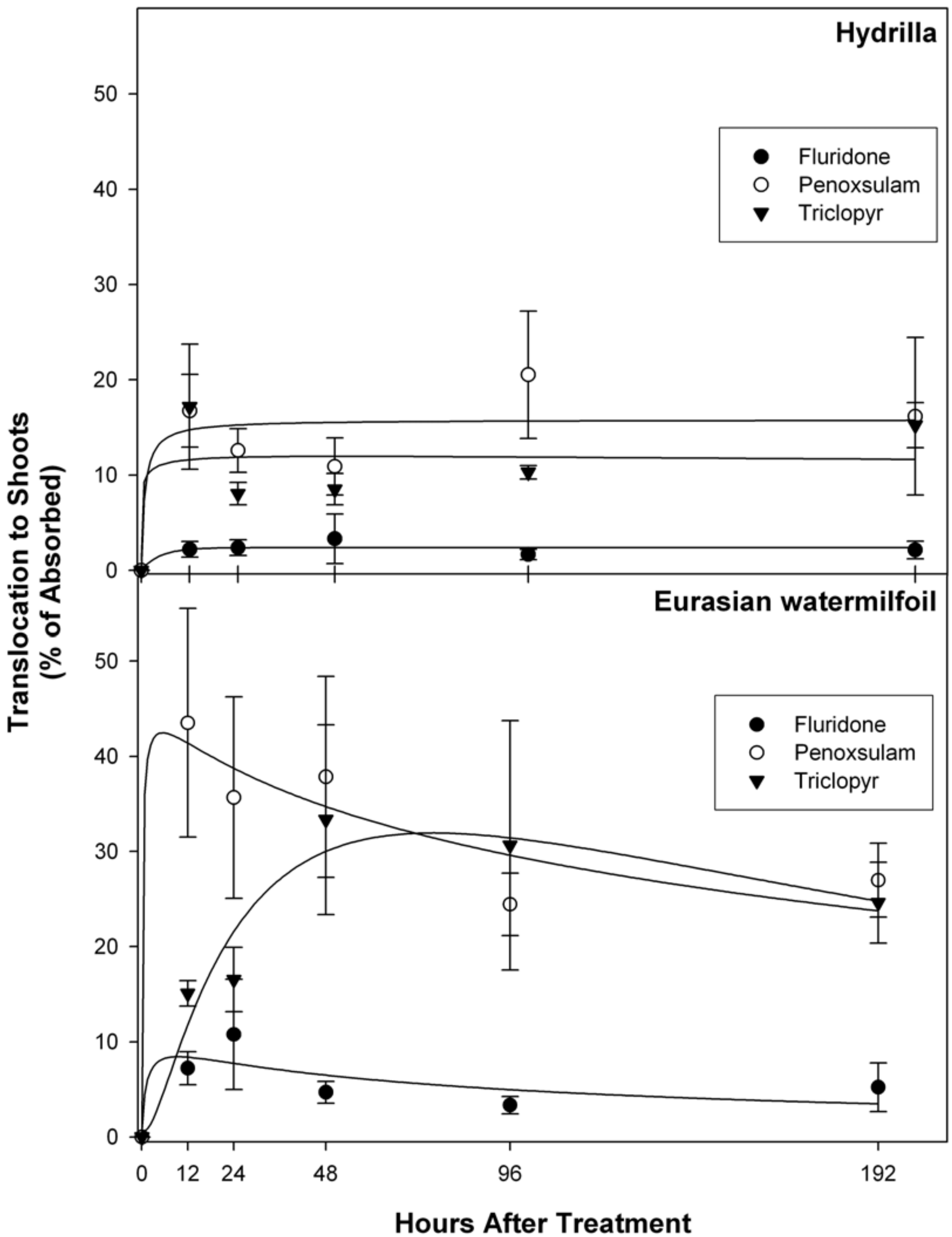

Figure 2.2: Herbicide translocation to shoots following root herbicide exposure as a percentage of total absorbed herbicide. Datapoints represent the mean, and error bars represent the standard error of the mean. (n = 6)

Table 2.1: Plant Concentration Factor (PCF), parameters, and calculated values based on hyperbolic regression analyses. Values represent the mean, and error terms represent the standard error of the mean. (n = 6)

				Parameters and Estimates Based on Hyperbolic Regression Analyses			
Species	**Herbicide**	**Plant Part**	PCF_{192}	A_{192} **(μg/g)**	t_{90} **(h)**	***a*± SE**	***b*± SE**
Hydrilla							
	Fluridone	Aboveground		0.07	156	0.0008 ± 0.0003	0.0057 ± 0.0048
		Belowground	12.84 ± 2.74	7.80	131	0.1726 ± 0.0939	0.0169 ± 0.0142
	Penoxsulam	Aboveground		0.03	101	0.0013 ± 0.0007	0.0374 ± 0.0274
		Belowground	0.66 ± 0.07	0.27	67	0.0233 ± 0.0084	0.0825 ± 0.0358
	Triclopyr	Aboveground		0.01	105	0.0004 ± 0.0002	0.0336 ± 0.0187
		Belowground	1.00 ± 0.09	0.26	43	0.0418 ± 0.0143	0.1582 ± 0.0615
EWM							
	Fluridone	Aboveground		0.05	74	0.0040 ± 0.0021	0.0700 ±0.0448
		Belowground	13.58 ± 3.18	9.59	141	0.1651 ± 0.0293	0.0120 ± 0.0036
	Penoxsulam	Aboveground		0.01	20	0.0045 ± 0.0066	0.4148 ± 0.6550
		Belowground	0.82 ± 0.16	0.40	90	0.0211 ± 0.0073	0.0477 ± 0.0211
	Triclopyr	Aboveground		0.02	160	0.0002 ± 0.00008	0.0042 ± 0.0043
		Belowground	1.56 ± 0.14	0.34	95	0.0164 ± 0.0084	0.0436 ± 0.0292

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Chapter 3: Triclopyr Absorption and Translocation Following Liquid and Granular Applications

Introduction

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is a submersed invasive species that occurs in at least 45 states across the US (USDA 2011b). It is a non-native that was first reported in the US in the 1940's, and since that time has expanded its range (Gettys 2009). While dense infestations commonly occur across the Midwest, this species has also become established in Colorado, infesting various reservoirs, ponds, canals and streams along the Front Range.

Eurasian watermilfoil exhibits several characteristics that allow it to be a competitive invader. The first of these is its growth habit. It will grow early in the spring before many native species. When shoots reach the surface, they branch profusely, forming dense mats. These dense mats can affect water quality, habitat, and shade out native species (Barko et al. 1982). Eurasian watermilfoil can produce viable seeds, but the ability to spread through vegetative fragments also contributes to its invasiveness. As little as one node on a stem fragment is enough to start a new plant. Human activities, wildlife, and water flow move these fragments, which are the main source of long-distance dispersal (Barko et al. 1982; Gettys 2009).

While Eurasian watermilfoil is a competitive invader, during the past century there have been many control strategies implemented to limit its spread and control current infestations (Gettys 2009). Systemic herbicides are one control method that can effectively provide long-term Eurasian watermilfoil control. Unlike contact herbicides, systemic herbicides are translocated throughout plants, making them ideal for control of perennial species. Several herbicides are currently registered for aquatic that provide Eurasian watermilfoil control.

Triclopyr is an auxinic herbicide that can be used for selective control of dicot species. Triclopyr's structure is similar to the plant hormone indoleacetic acid (IAA). It works as a plant growth regulator, and at high doses leads to epinasty and plant death. Triclopyr is an ideal aquatic herbicide, due to its minimal toxicity, and a relatively short half life (~6.5 d) in aquatic systems (Petty et al. 2003). As a result of its water solubility (Senseman 2007), there is little accumulation in aquatic sediments following application (Petty et al. 2003). Triclopyr has been used for many years in terrestrial systems; however, the triethylamine salt of triclopyr was not registered for aquatic use until 2002 (Gettys 2009). Recommended triclopyr application rates for submersed species are 0.75-2.5 µg/L (Anonymous 2010), with higher rates recommended for areas where contact time is limited or there is a high dilution potential.

Selection of an appropriate herbicide must be determined based on individual site conditions, and concentration exposure time (CET) requirements for the herbicide. CET studies for Eurasian watermilfoil have been conducted for several aquatic herbicides (Green and Westerdahl 1990; Netherland and Getsinger 1992; Netherland et al. 1993). Studies with the aquatic herbicide fluridone, a slower-acting carotenoid biosynthesis inhibitor, indicated concentrations must be maintained for 60 days or more to provide Eurasian watermilfoil control (Netherland et al. 1993). Triclopyr required only 36-48 h exposure time to provide similar control (Green and Westerdahl 1990; Netherland and Getsinger 1992). These values represent minimum contact times required for Eurasian watermilfoil control, but many factors can influence contact time.

Factors that will affect herbicide concentration and exposure time will vary with each water body, and may lead to rapid dissipation resulting in decreased control. The two main sources of dissipation in aquatic systems are herbicide degradation and dilution. There can be several types of degradation in water; while, photolysis is the main degradation mechanism for many aquatic herbicides, including triclopyr (Gettys 2009; Senseman 2007). Because ultraviolet light leads to photolytic degradation, water clarity and depth will affect degradation rates. Clarity may be affected by suspended solids and dissolved organic carbon. Plant canopy cover and water column depth may also impact light penetration. Any decrease in light penetration will reduce degradation rates, and prolong herbicide exposure; however, clear water and good light penetration may increase herbicide degradation rates (Koschnick et al. 2010). Although degradation rates can impact contact time, the rate of degradation is more important for herbicides requiring longer exposure times, such as fluridone. For herbicides require shorter exposure times, such as triclopyr, dissipation as a result of dilution can have a greater impact on herbicide efficacy.

Herbicide dilution has a significant impact on exposure times, and the speed of dilution is most often affected by water flow. A small amount of dilution and mixing can be beneficial to evenly distribute the herbicide after application, but too much dilution will decrease herbicide concentration before exposure time requirements are met. Water flow can vary based on the individual water bodies, and applicators must be aware of flow patterns prior to treatment. Canals, rivers, and streams have an obvious water flow patterns, but larger water bodies can also have water movement that can be difficult to detect. When making whole-lake treatments, these flows allow for herbicide equilibration throughout the water body; however, when spot treatments are applied to smaller infestations in a large system, these flows can lead to rapid dissipation and result in herbicide dilution. Protected coves may have little dilution from water movement, but applications to small areas or areas along unprotected, exposed shoreline are more susceptible to dilution (Gettys 2009). In addition to treatment location within a water body, environmental factors also impact dilution. Increased wave action and wind can promote water mixing and dilution, with the effects being more pronounced in exposed areas. Also, surface runoff from snowmelt or precipitation events can increase water flow (Gettys 2009; Koschnick et al. 2010). All of these factors affect herbicide dilution, especially following spot treatments, and can impact treatment efficacy.

In order to maximize herbicide effectiveness, several application methods have been developed for aquatic treatments. The first of these is the use of weighted trailing hoses, which place herbicide into plant beds, increasing the herbicide concentration near plants, and improving contact time (Koschnick et al. 2010). Fox et al. (2002) observed that the use of weighted trailing hoses provided more uniform treatment throughout the water column, and maintained a longer exposure time compared to a surface application. Subsurface applications of liquid herbicides have become very common, and in recent years many manufacturers have developed granular formulations.

Herbicide including copper (Harpoon Granular ®), triclopyr (Renovate® OTF), 2,4-D (Sculpin G®, Navigate®), endothall (Aquathol® Super K, Hydrothol® Granular), and fluridone (Sonar® SRP, PR, Q, and SonarOne®) have all been formulated as granules. The proposed benefits of these granular formulations are similar to those for weighted trailing hose applications. Each of these herbicides is formulated on a solid carrier, which will carry the herbicide to the bottom of the water column, coming in contact with the sediment/water interface. After falling to the bottom, these herbicides are fast-, slow-, or controlled-release formulations, helping maintain the treatment concentration near the plants for their required exposure times. The release of product over time provides additional protection against dilution, compared to liquid formulations, which can be diluted immediately following application.

Herbicide placement at the sediment/water interface may also result in decreased dilution as a result of less water movement within the plant beds, and even less at the sediment/water interface due to the present of a benthic boundary layer (Wetzel 2001). In addition to dilution protection, the granules may result in higher concentrations near the sediment/water interface, potentially increasing herbicide loading into plants. There have been many anecdotal reports and speculation as to the benefits of granular herbicides in respect to their absorption and translocation, but there have been no studies published to address absorption and translocation trends following granular herbicide application.

The triethylamine form of triclopyr is available for aquatic use as a liquid (Renovate® 3) and a granule (Renovate® OTF). The liquid formulation contains 359 g/L acid equivalent, and the granular formulation contains 10% acid equivalent triclopyr on a clay/paper based biodegradable carrier. While the entire amount of triclopyr is immediately available following liquid treatment, a laboratory study with Renovate® OTF indicated that herbicide release from the granule is slower, with 94% being released the first 4 HAT, and 99% release by 24 HAT (Koschnick et al. 2010). A study examining the relationship between liquid triclopyr and Rhodamine WT dye, indicated there was a direct relationship between dye and triclopyr concentrations (Fox et al. 2002). For this reason, Koschnick et al. (2010) used Rhodamine WT as a substitute for liquid triclopyr, and conducted a study to evaluate dissipation rates of Rhodamine WT and Renovate® OTF following a spot treatment. Concentrations reported by Koschnick et al. (Koschnick et al. 2010) did not indicate unusually high or long-duration concentrations at the sediment/water interface, and vertical distribution reached equilibrium by 28 HAT. The half-life following granular application was approximately 2.2 times the Rhodamine WT half-life. Increased exposure time in treated areas may support the hypothesis of increased herbicide loading into plants following granular application. In addition to increased exposure time, being able to maintain a concentration longer could reduce the amount of herbicide needed to achieve control. These hypotheses could have significant impacts for aquatic herbicide treatments, and studies examining absorption and translocation for both formulations could provide additional insight into the benefits of granular herbicide.

The objectives of this study were to compare triclopyr absorption and translocation in Eurasian watermilfoil following granular and liquid treatments. Treatments were made using formulated triclopyr (Renovate® 3), and granular triclopyr, both supplemented with ^{14}C-triclopyr.

Materials and Methods

Plant Materials

Eurasian watermilfoil fragments were collected from the Leggett Canal near Boulder, CO during Fall 2006. Apical sections 15 cm in length were planted in topsoil and grown out in the greenhouse until they were needed for experiments. While growing in the greenhouse, plants were maintained using a 10:14 h light:dark period with the temperature set at 24 C and 18 C for day and night, respectively. Supplemental lighting was provided by 400-watt sodium halide lamps (~200 mE/m^2/s). Prior to use in experiments, 15 cm apical sections were excised from previously propagated plants and transplanted to 15 cm round pots containing topsoil amended with 3 g/L slow release fertilizer (Osmocote 14-14-14, The Scotts Company, Marysville, OH). These plants were allowed to grow under the greenhouse conditions mentioned above until they had produced 50-60 cm of topgrowth and several stems. At this time plants were clipped back to their root crowns, leaving approximately 5 cm stems above the crown. After clipping, plants were allowed to grow to approximately 50 cm and the most uniform plants with 2-4 stems were selected for use in subsequent experiments. Prior to herbicide exposure, these established plants were removed from their pots, roots were rinsed with water to remove topsoil, and potted in 9.5 cm round pots in sand amended with 3 g/L slow release fertilizer.

Liquid Herbicide Treatment

Eighteen potted plants were transferred to individual clear acrylic tubes (91.5 cm tall x 11.5 cm wide) in 7 L of tap water, and were allowed to equilibrate for 24 h prior to treatment. Each cylinder was then treated with 25 KBq of ^{14}C-triclopyr (2,689.5 KBq/mg specific activity) supplemented with formulated triclopyr (Renovate® 3, SePRO Corp., Carmel, IN) to achieve a total concentration of 0.5 µg/L in the water column. Following treatment, plants were harvested at 6, 12, 24, 48, 96, and 192 HAT. During the study, plants were maintained in the laboratory at room temperature, with a 10:14 h light:dark period, with supplement lighting provided by fluorescent grow light (approximately 200 $mE/m^2/s$). Three replicates were harvested at each time point, and the experiment was repeated. Upon harvest, each plant was separated into three samples; apical meristems, shoots, and roots. After separation, plants were dried at 60 C for 48 h to achieve constant moisture. Dried samples were then ground to a fine powder using a mortar and pestle to allow for easy sample oxidation. Ground plant samples were oxidized using a biological sample oxidizer (OX500, R.J. Harvey Instrument Co., Tappan, NY) and $^{14}CO_2$ was collected using a ^{14}C-trapping cocktail (OX161, R.J. Harvey Instrument Co., Tappan, NY). After oxidation, radioactivity was quantified using liquid scintillation spectroscopy (Packard 2500R, PerkinElmer, Waltham, MA).

Granular Herbicide Treatment

Granular herbicide was formulated to simulate the granular triclopyr Renovate OTF formulation. While commercially available Renovate OTF contains 10% w/w acid equivalent triclopyr, granules used in this study were formulated to a concentration of 1% w/w acid equivalent triclopyr to provide better consistency in the relatively small water volumes used in this study. Formulated granules consisted of blank paper/clay based granules, formulated triclopyr (Renovate 3, SePRO Corp., Carmel, IN), and ^{14}C-triclopyr (2,689.5 KBq/mg specific activity).

Again, 18 potted plants were transferred to individual clear acrylic tubes (91.5 cm tall x 11.5 cm wide) in 7 L of tap water, and allowed to equilibrate overnight prior to treatment. For the granular herbicide treatment, each tube received 0.350 g of ^{14}C-triclopyr granules, which contained 1% w/w acid equivalent triclopyr. These granules were dropped through the water column and placed on the surface of the sand. The added granules contained 30 KBq of ^{14}C-triclopyr, and resulted in a water column concentration of 0.5 µg/L in each cylinder. Plants were harvested over a time course at 6, 12, 24, 48, 96, and 192 HAT. For the duration of the study, plants were maintained and harvested as previously described.

Statistical Analysis

Data analysis from both studies used Levene's test for homogeneity of variance, which indicated variance from repeated experiments was not significantly different, and could be combined for subsequent statistical analysis ($\alpha = 0.05$ level of significance). Means and standard errors were calculated using MS Excel (MS Office 2007), and data plotted in SigmaPlot (Version 10). Non-linear regression analyses were also conducted in SigmaPlot and fitted to the following equation:

$$y = \frac{ax}{1 + bx} \qquad [3.1]$$

This equation is similar to the function used by Kniss et al. (2011), which was useful in analyzing herbicide absorption in terrestrial species. From the parameter estimates of this model, two additional values were calculated, A_{192} and t_{90}. A_{192} is the amount of herbicide absorption predicted based on regression analysis 192 HAT, and t_{90} represents the amount of time required to achieve 90% of A_{192}. For this study, these terms can be used to evaluate difference in herbicide absorption by plant parts and herbicide formulations. In addition to non-linear regression analysis for absorption, biococentration values were calculated using the following equation, which was adapted from previous work conducted by de Carvalho et al. (2007b):

$$PCF = \frac{\text{Herbicide concentration in plant (ng/g Fresh Biomass)}}{\text{Herbicide concentration in water (ng/mL)}} \qquad [3.2]$$

Biococentrations provide a value to compare herbicide accumulation in plant tissue relative to the concentration in the water column.

Results and Discussion

For both liquid and granular experiments, Levene's test for homogeneity of variance indicated that data from repeated studies could be combined for statistical analysis at the $\alpha = 0.05$ level of significance. The absorption rate was faster following liquid treatment ($t_{90} = 120$ HAT) than granular treatment ($t_{90} = 138$) (Table 3.1, Figure 3.1), but regression analysis conducted on absorption combined across plant parts indicated that there was no significant difference in absorption based on overlapping 95% confidence intervals, and absorption standard error 192 HAT (Table 3.1). These results suggest that there was no advantage to using granular formulations in terms of overall triclopyr absorption.

There was no significant difference in whole plant absorption; however, there were differences in absorption by plant part (Figure 3.2). Accumulation following liquid treatment was greatest in apical meristems, followed by shoots and roots. In contrast, there was no significant difference between apical meristems and shoots following granular treatment, but the amount of radiolabeled herbicide in these two plant parts were both were significantly higher than the amount in the roots 192 HAT (Table 3.2). Roots accumulated less herbicide than shoots and apical meristems with both treatments, but there was significantly more root accumulation when plants were exposed to the granular formulation (2.04 ± 0.39 μg/g fresh weight). There was 5.8 times more herbicide in roots for granular treatment compared to liquid treatments (0.36 ± 0.10 μg/g fresh weight) (Figure 3.2). Accumulation by apical meristems following liquid treatment (9.40 ± 0.79 μg/g fresh weight) was 1.7 times greater than granular treatment (5.56 ± 1.31 μg/g fresh weight), but there was no significant difference between treatments in shoot tissue 192 HAT. Although there was greater accumulation in apical meristems following liquid treatment, greater root accumulation following granular treatment may result in increased root crown control for a perennial species, such as Eurasian watermilfoil.

When PCF values were calculated for each treatment, bioconcentration following liquid treatments was similar to previous shoot and root absorption studies (Chapter 1, Chapter 2), with shoots accumulating a majority of absorbed herbicide. PCF values for previous shoot and root studies were 34.61 ± 5.61 and 1.56 ± 0.14 192 HAT, respectively (Table 1.1, Table 2.1). The previous studies were also treated with liquid triclopyr, and their PCF values were greater than PCF values for apical meristems (18.79 ± 1.58), shoots (9.17 ± 2.92), and roots (0.71 ± 0.19) following liquid treatments in the present studies (Table 3.2). The greater absorption observed in previous studies compared to liquid treatments here could have been due to differences in plant growth stage. Previous studies used young plants with smaller, compact shoots, while the current studies used larger, established plants. These larger plants had elongated internodes compared to the younger plants, and leaves were less dense. This may have resulted in a lower surface area to biomass ratio than studies using small, newly established plants. Significant differences between liquid and granular treatments were also seen (Table 3.2), with differences in root absorption being the most pronounced. Following liquid treatment, the calculated PCF value was only 0.71 ± 0.19 192 HAT, granular treatment resulted in a PCF of 4.09 ± 0.77 (Table 3.2). As previously mentioned, this additional accumulation by plant roots could result in better root crown control.

The accumulation rate, as determine by calculated t_{90} values showed different trends following liquid and granular triclopyr treatments. Results indicate that liquid treatment accumulation occurs faster in apical meristems (t_{90} = 81 HAT) and shoots (t_{90} = 111 HAT), and there is a lag of more than 2 d in root equilibration (t_{90} = 172 HAT). For these liquid treatments, most accumulation occurs in the aboveground portions of the plant, with the accumulation rate being similar to previous shoot exposure studies (shoot t_{90} = 73 HAT). Root absorption in earlier studies reached t_{90} by 14 HAT, and was likely an artifact of little translocation to roots. In the current study, there was no isolation of shoots so continued root accumulation likely occurred as a result of both translocation and diffusion of herbicide into sediment pore water, leading to continued accumulation in root tissue.

After a granular treatment, equilibration occurred fastest in roots (t_{90} = 53 HAT) followed by apical meristems (t_{90} = 87 HAT), and shoot tissue (t_{90} = 166 HAT) (Table 3.2). While accumulation in roots and apical meristems appeared to reach an equilibrium, that was not the case in remaining shoot tissue, for which accumulation continued for the duration of the study (Figure 3.2). Rapid accumulation by roots following granular treatment followed by accumulation in apical meristems suggests that most accumulation for granular applications occurs near the roots of plants, and that absorbed herbicide is translocated to meristems. After accumulation in the apical meristems reaches equilibrium, and the vertical herbicide distribution in the water column begins to equilibrate, shoots continue to absorb herbicide for the duration of the study. Differences in accumulation rate and translocation based on herbicide formulation could impact overall efficacy following a triclopyr application.

There were definite differences in total accumulation and accumulation rate for plant parts following granular and liquid treatments, and there were also significant differences at the whole plant level. Observations at early time points following granular treatment appear to show approximately 60% present in roots, and that concentration decreased between 24 and 192 HAT. This is likely an artifact of continued triclopyr accumulation by shoots, and the fact that large amounts of root accumulation occurred early after treatment. Following a liquid treatment, the percentage found in the roots accounted for only 3.67 ± 1.74% of total absorption 192 HAT, which was similar to previous shoot exposure experiments (Figure 1.2) in roots 192 HAT. The amount present in roots following granular treatment was much greater at 27.82 ± 11.08%, nearly 7.5 times liquid treatment accumulation (Figure 3.3). This value was very similar to herbicide distribution 192 HAT in previous root exposure studies, where 24.62 ± 4.24% was present in roots. These results, and their correlation with other shoot and root treatments, provide additional support to our hypothesis that absorption following liquid treatment occurs primarily in the shoots, and absorption for granular treatments primarily occurs via roots. Although liquid treatments may provide good control the year of application, these results indicate that limited root translocation could limit their long-term success. With a larger amount of herbicide present in roots following granular treatment, more translocation throughout the plant could result in better long-term Eurasian watermilfoil control.

Our results appear to support the theory that granular formulations provide advantages in achieving long-term Eurasian watermilfoil control, but the true implications of these finding are still unknown. Currently published triclopyr CET experiments were conducted with liquid formulations (Netherland and Getsinger 1992). For this reason, additional studies should be conducted to evaluate the optimal CET relationship for granular formulations. Increased root absorption and faster root absorption following granular applications could reduce required rates and exposure times. If reduced rates could provide equivalent control, herbicide loading in the aquatic ecosystem. Reduced exposure times could result in more treatment flexibility and improve the potential for success, especially for spot treatments in high water exchange areas.

Field studies utilizing a granular triclopyr formulation (Renovate® OTF) resulted in a half-life that was approximately twice as long as a liquid formulation (Koschnick et al. 2010).

This increased triclopyr half-life following a spot treatment, paired with the increased root absorption observed in the current studies, provide additional evidence that granular triclopyr formulations are superior for spot treatments. The half-life was longer in this field study, but it is important to note that the the study was conducted in a secluded cove and water flow out of the treatment area was relatively limited. Additional studies should focus on triclopyr release rates and dissipation, and how they are affected by different environmental factors.

Factors affecting granular release rate may include water temperature and flow, with higher temperature and flow increasing release rates. Other factors that could contribute to triclopyr availability would include suspended solids, sediment organic matter, and pH. While these each may impact triclopyr availability, the effects would be minor given the high water solubility (430 mg/L), low lipophilicity (log K_{ow}), and a pKa (2.68) (Senseman 2007) of triclopyr that is well below the pH of most water bodies that can support plant growth. While each of these may impact triclopyr half-life and availability, the increased root absorption following granular application may still increase control over liquid formulations.

It is important to note that this study does have some limitations due to the experimental methods used. These limitations require additional research to determine their impact on the absorption and translocation observations. Previous studies of shoot and root exposure (Chapter 1, Chapter 2) isolated roots and shoots, but not in this study. For this reason it is difficult to determine if herbicide presence is a result of absorption or translocation. Additional studies isolating shoots and roots isolated would provide additional insight to the site of absorption. Another limitation is the effect of sediment type. Our studies used sand as a sediment source. In a real-life setting, there would be varying types of sediment present. If the roots are the main site of absorption for granular applications, most exposure would occur through triclopyr diffusion into pore water near the sediment/water interface. In fine textured soils, pore spaces would be smaller, and this diffusion would be slower. Slowed diffusion could reduce overall root absorption.

Another limitation of these studies was the fact that static systems were used, and herbicide exposures were maintained for the duration of the study. In a field setting, the herbicide concentration would have decreased during the study as the herbicide was diluted or degraded. A drop in herbicide concentration in the water column could eliminate the concentration gradient that drives herbicide absorption. As the concentration in the water column drops, herbicide may be able to desorb from the plant in the absence of a strong concentration gradient. This concept was previously illustrated by Vassios et al. (2011), where approximately 50% of absorbed imazamox desorbed once plants were transferred to untreated water. Future studies should focus on examining these issues, and the resulting impact on absorption, translocation, and Eurasian watermilfoil control.

Our studies only compared two triclopyr formulations; however, different trends could emerge with examination of other active ingredients formulated as granules. Significant differences could occur with other systemic herbicides possessing different chemical properties, such as water solubility and lipophilicity. Also, different absorption and translocation trends could occur for contact herbicides that do not readily translocate through plants.

In conclusion, the current comparison between liquid and granular triclopyr treatments showed several trends that provide supporting evidence for the use of granular formulations. No significant difference in overall absorption was observed over the 192 h time course; however, there were significant difference in herbicide accumulation by plant part, with apical meristems accumulating the most triclopyr following treatment with liquid triclopyr, and roots accumulating most following granular treatment with simulated Renovate® OTF granules. At the whole plant level, differences in distribution 192 HAT were also seen, with approximately 11 times as much herbicide present in roots following granular treatment when compared to the liquid treatment. These results show definite differences in accumulation and distribution, but there were several limitations to this study. Additional research could help to quantify the impact of each limiting factor.

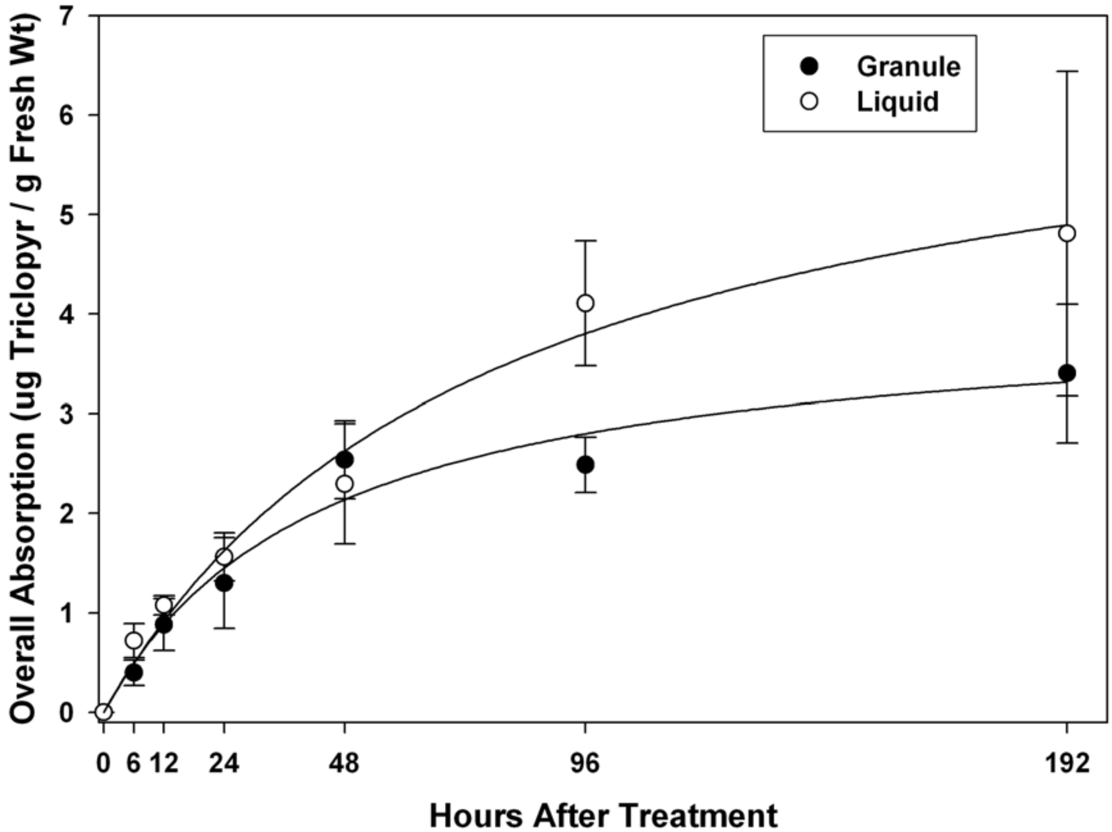

Figure 3.1: Total absorption of liquid and granular triclopyr, combined across all plant parts. Datapoints represent the mean, and error bars represent the standard error of the mean. (n = 6)

Table 3.1: Plant Concentration Factor (PCF), parameters, and calculated values based on hyperbolic regression analyses for herbicide absorption combined across all plant parts (Figure 3.1). Values represent the mean, and error terms represent the standard error of the mean. (n = 6)

	Parameters and Estimates Based on Hyperbolic Regression Analyses			
Formulation	$\boldsymbol{A_{192}}$ ***(μg/g)***	$\boldsymbol{t_{90}}$ ***(h)***	***a*** **± SE**	***b*** **± SE**
Granular	4.90	138	0.094 ± 0.018	0.023 ± 0.007
Liquid	3.32	120	0.089 ± 0.011	0.013 ± 0.003

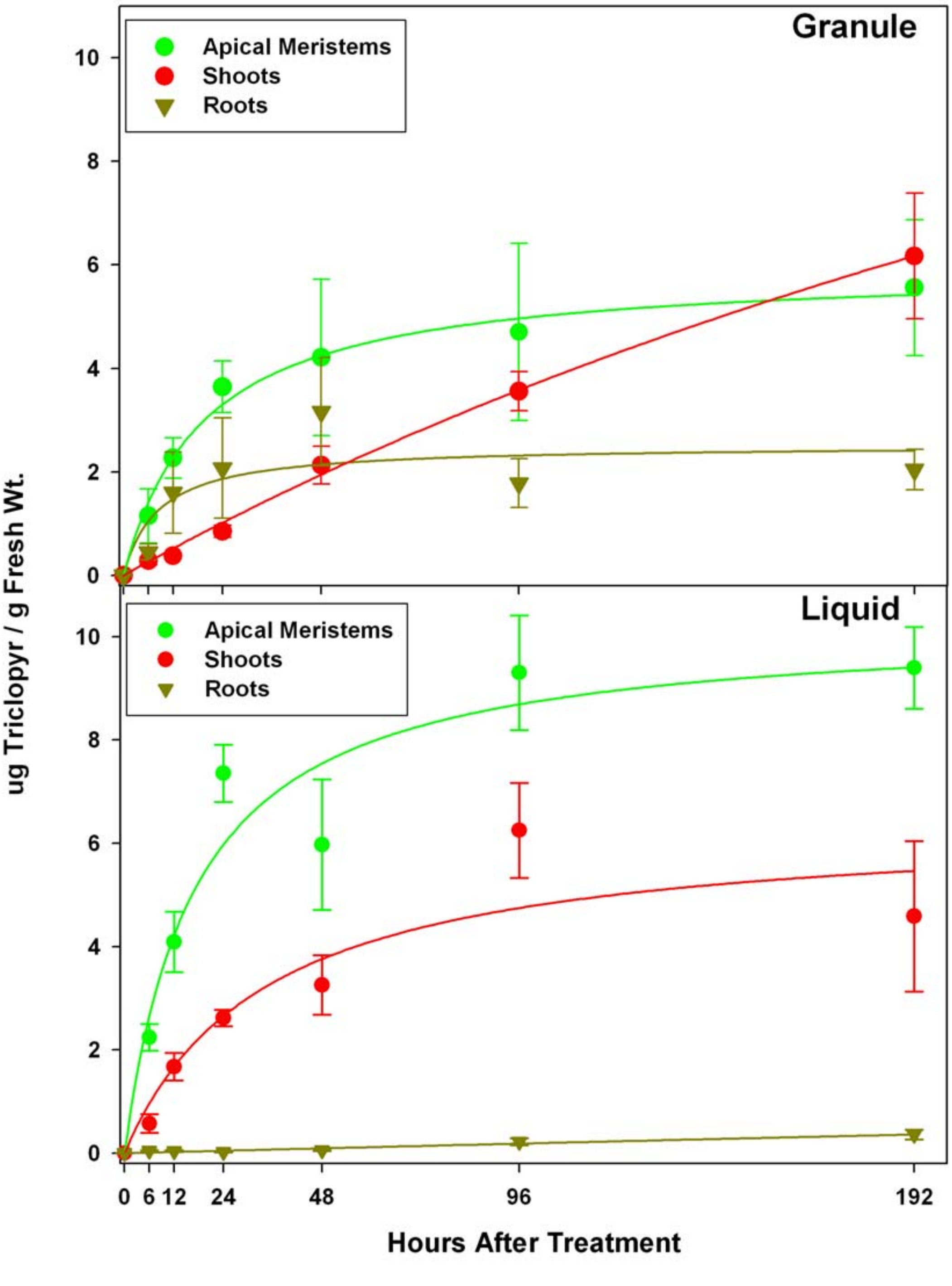

Figure 3.2: Herbicide absorption following treatment with granular (top) and liquid (bottom) triclopyr over a 192 h time course. Plotted points represent the mean, and error bars represent the standard error of the mean. (n = 6)

Table 3.1: Plant Concentration Factor (PCF), parameters, and calculated values based on hyperbolic regression analyses for herbicide absorption. Values represent the mean, and error terms represent the standard error of the mean. (n = 6)

			Parameters and Estimates Based on Hyperbolic Regression Analyses			
Formulation	**Plant Part**	PCF_{192}	A_{192} **(μg/g)**	t_{90} **(h)**	***a*± SE**	***b*± SE**
Granular						
	Apical	13.24 ± 2.43	5.42	87	0.308 ± 0.035	0.052 ± 0.008
	Shoot	11.12 ± 2.63	6.17	166	0.044 ± 0.003	0.002 ± 0.001
	Root	4.09 ± 0.77	2.41	53	0.299 ± 0.221	0.119 ± 0.104
Liquid						
	Apical	18.79 ± 1.58	9.40	81	0.596 ± 0.167	0.058 ± 0.012
	Shoot	9.17 ± 2.92	5.47	111	0.188 ± 0.074	0.029 ± 0.016
	Root	0.71 ± 0.02	0.36	172	0.002 ± 0.001	0.0003 ± 0.002

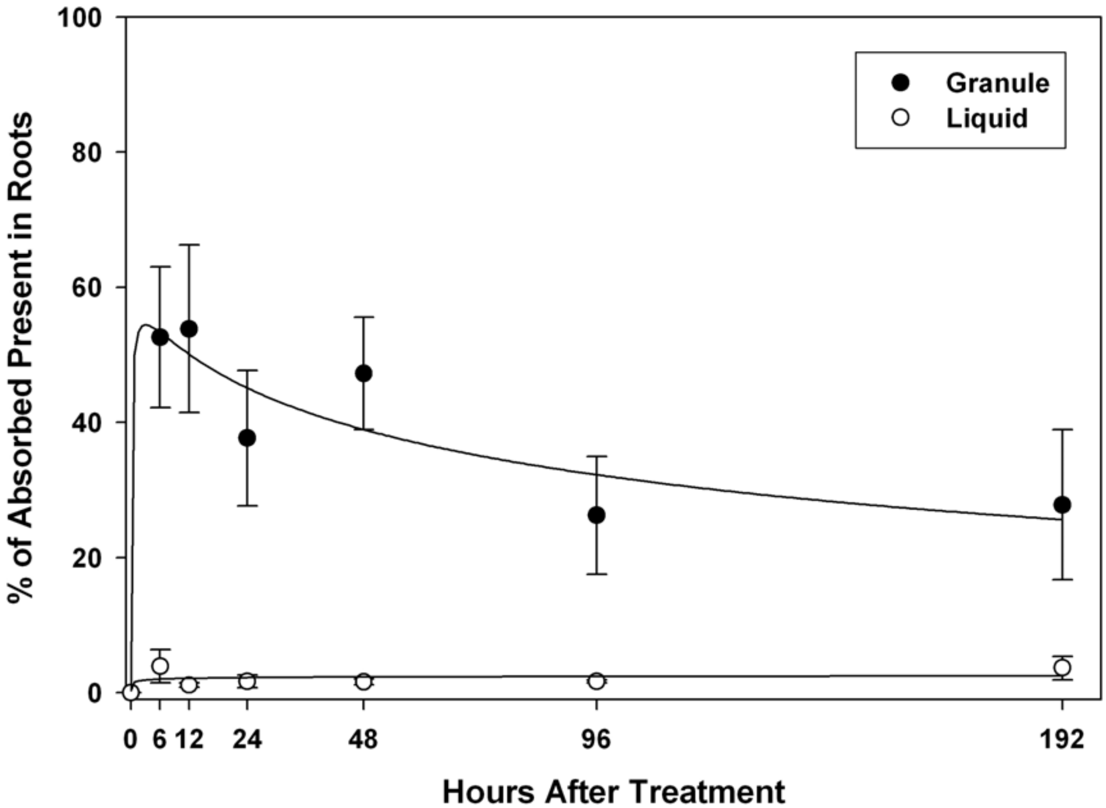

Figure 3.3: Translocation to roots of Eurasian watermilfoil following water column exposure to liquid and granule triclopyr formulations. Data presented are means, and error bars are the standard error of the mean. (n = 6)

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