ABSTRACT

Hydrilla and Eurasian watermilfoil (EWM) are invasive aquatic plants that aggressively compete with native plants, forming dense, monotypic stands. Previous studies have established the selectivity and concentration exposure time requirements for aquatic herbicides used to control hydrilla and EWM; therefore, the objective of this research was to conduct a comparative study evaluating fluridone, triclopyr, and penoxsulam absorption and translocation in these two aquatic plants. Using 14C-labeled herbicides, absorption and translocation were measured over a 192-h time course. On the basis of differences in lipophilicity among the three herbicides (fluridone >> penoxsulam > triclopyr), we expected fluridone to have the highest accumulation, with significantly lower accumulation for penoxsulam and triclopyr. Experimental results did not support this hypothesis. Triclopyr accumulation was highest in EWM and was equivalent to fluridone in hydrilla. Penoxsulam absorption and translocation was low in both species. In addition, accumulation after shoot exposure was approximately three times greater for EWM compared with hydrilla. Shoot-to-root translocation was limited, with a maximum of 12.5% of absorbed triclopyr reaching hydrilla roots 192 h after treatment.

Key words: fluridone, herbicide absorption and translocation, Hydrilla verticillata, log Kow, Myriophyllum spicatum, penoxsulam, triclopyr.

INTRODUCTION

Two commonly occurring submersed invasive species in the United States are Eurasian watermilfoil (Myriophyllum spicatum L.) (EWM) and hydrilla (Hydrilla verticillata (L.F.) Royle). Both species are invasive nonnatives that can severely affect aquatic ecosystems. They form dense, monotypic stands that can displace native species, drastically affecting water quality, light penetration, recreational use, water transfer, and human health (Grace and Wetzel 1978, Smith and Barko 1990, Langeland 1996, Haller 2014, Madsen 2014).

EWM was introduced from Eurasia. It was first reported in the United States in the 1940s (Madsen 2014) and is now reported in at least 45 states (USDA 2011b), thriving in temperate areas of the United States. Dioecious 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). Dioecious hydrilla was introduced in Florida as a result of the aquarium trade in the 1950s. It spread rapidly to infest many water bodies in Florida, and has since expanded its range.

To reduce the negative impacts of EWM and hydrilla, a range of mechanical, cultural, chemical, and biological methods have been implemented to control both species (Belaud 2014, Cuda 2014, Haller 2014, Netherland 2014). Although each of these methods is a useful tool in aquatic plant management, the most common, and often most cost-effective, control method is the use of aquatic herbicides. 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 (Netherland 2014). Each of these new products possesses different attributes that may be desirable on the basis of site conditions.

Both contact and systemic herbicides can be effective for submersed aquatic weed control, but contact herbicides are thought to have very limited to no translocation in aquatic plants. The current paradigm is that systemic herbicides are better able to translocate to roots, root crowns, rhizomes, and tubers of perennial species, such as EWM and hydrilla; however, data supporting this theory are limited. Fluridone, penoxsulam, and triclopyr are three systemic herbicides that have proven effective for aquatic weed control. All three herbicides can be used to control EWM, whereas only fluridone and penoxsulam are used for hydrilla control.

These herbicides have different modes of action and specific concentration exposure time (CET) requirements to be effective. Triclopyr is a synthetic auxin herbicide that affects a variety of plant processes normally under tight control by the level of the endogenous auxin, indole-3-acetic acid. CET requirements for triclopyr are in the range of 0.25 to 2.5 mg L−1 with an exposure time of hours to days. Penoxsulam is a potent inhibitor of the enzyme acetolactate synthase (ALS) involved in the synthesis of the branched-chain amino acids and is effective when applied via foliar spray. Fluridone is a synthetic auxin herbicide that affects a variety of plant processes normally under tight control by the level of the endogenous auxin, indole-3-acetic acid. CET requirements for fluridone are in the range of 0.25 to 2.5 mg L−1 with an exposure time of hours to days.
synthase, the first committed step in the production of the branched-chain amino acids valine, leucine, and isoleucine. Typical use rates for penoxsulam are in the range of 10–30 μg L⁻¹ with exposure times longer than 45 d. Fluridone stops carotenoid biosynthesis by inhibiting the enzyme phytoene desaturase and has similar CET requirements to penoxsulam.

Triclopyr, penoxsulam and fludicide differ in water solubility, with fludicide having relatively low water solubility (12 mg L⁻¹) and one of the higher log octanol–water partition coefficient ($K_{ow}$) values for an aquatic herbicide. Penoxsulam and triclopyr are examples of highly water-soluble herbicides with low log $K_{ow}$ values (negative values). Log $K_{ow}$ values for fluridone, penoxsulam, and triclopyr are 1.87, −0.35, and −0.44, respectively (Shaner 2014). On the basis of log $K_{ow}$ values, fludicide would be expected to accumulate to a much greater extent than penoxsulam or triclopyr (de Carvalho et al. 2007b).

Although sufficient herbicide accumulation is important, herbicide translocation to roots is equally important, and perhaps crucial for long-term control of perennial species, such as hydrilla and EWM. Therefore, the objectives of this research were to better understand absorption and translocation of three systemic herbicides in hydrilla and EWM by: 1) evaluating fluridone, penoxsulam, and triclopyr absorption and bioconcentration, 2) determining fluridone, penoxsulam, and triclopyr translocation to roots after shoot exposure, and 3) determining the effect of herbicide concentration in the water column on shoot absorption.

**MATERIALS AND METHODS**

**Plant material**

EWM fragments were collected from the Leggett Ditch near Boulder, CO in fall 2006 and maintained in two 1,000-L tanks as our standard Colorado biotype. The fluridone-susceptible dioecious hydrilla population used in these experiments was propagated from tubers collected from Saddle Creek, FL during spring 2009. The hydrilla population was also maintained year round in a 1,000-L tank. Plants were maintained in the greenhouse under 400-W sodium halide lamps, providing approximately 200 μmol m⁻² s⁻¹ of supplemental light. From mid-November until late January supplemental lighting was used to maintain a minimum day length of 10 h; however, from late January until mid-November day length varied between 10 and 15 h (normal seasonal variation in Colorado). The temperature was maintained year round at 24 C and 18 C for day and night, respectively.

**Herbicide absorption and translocation after shoot exposure**

To make direct comparisons between herbicides and plant species, we needed to simplify the plant’s structure. Since hydrilla and EWM will produce adventitious roots from stem cutting, 15-cm apical stem sections were removed from plants growing in large greenhouse tanks and transferred to 5-cm-diam by 9.5-cm glass jars. The most distal 3 cm of the shoot cutting was inserted into a glass jar containing topsoil amended with slow-release fertilizer (3 g L⁻¹) and 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 under growth chamber conditions (10 : 14 h day : night, 24 : 18 C day : night temperature) until they had produced roots (approximately 14 d). Before herbicide treatments, plants were removed from the glass jars to identify plants with adequate root systems, defined as plants having at least three adventitious roots. The most uniform plants were selected and these were repotted using washed silica sand amended with slow-release fertilizer (as previously described) in 5-cm-diam by 9.5-cm glass jars. The silica sand was saturated with distilled water and a layer of agarose gel (1.5% w/v) approximately 3 mm thick was placed on the surface of each jar to isolate shoot and root tissue. Three plants of each species were placed in 18 4-L polystyrene tanks containing 3 L of dechlorinated tap water and were allowed to equilibrate for 18 h before treatment. Plants were maintained at 23 C under fluorescent grow lights (approximately 200 μmol m⁻² s⁻¹) and a 10 : 14 h light : dark period. The experiment was conducted in a chemical flume hood using double containment as required by Colorado State University’s Radiation Control Office. The day length was intended to keep the plants in a vegetative growth stage.

Treatments included 10 μg L⁻¹ fluridone that contained 41.67 kBq of $^{14}$C-fluridone (1,357.9 kBq mg⁻¹ specific activity), 10 μg L⁻¹ penoxsulam containing 66.67 kBq$^{14}$C-penoxsulam (2,273.3 kBq mg⁻¹ specific activity), and 1 mg L⁻¹ triclopyr containing 66.67 kBq of $^{14}$C-triclopyr (2,869.5 kBq mg⁻¹ specific activity). Fluridone and penoxsulam were applied as $^{14}$C-labeled herbicide only, but the higher rate of triclopyr required the application of supplemental herbicide as technical-grade triclopyr. Three plants of each species were harvested randomly from each herbicide treatment 6, 12, 24, 48, 96, and 192 h after treatment (HAT). At each time point, glass jars were rinsed three times in distilled water, divided into shoot and root tissue, a water sample was taken, and the silica sand was washed to determine if any radioactivity was exuded from the root system. Plant tissue was then dried for 24 h at 60 C and dry biomass was recorded. Radioactivity was determined using liquid scintillation spectroscopy.

**Effect of concentration on herbicide absorption**

For each experiment, nine nonrooted apical sections, 15 cm in length, were excised from EWM and hydrilla that were propagated as previously described. Each fragment was placed in individual 75-ml glass vials containing 50 ml of water. Fluridone, penoxsulam, and triclopyr were applied at 10, 100, and 1,000 μg L⁻¹. Radiolabeled herbicide was applied at 0.33 kBq per vial, and was supplemented with formulated herbicide to reach the desired concentration. Plants were exposed to herbicide for 48 h and harvested. After harvest, plants were oven dried and analyzed for $^{14}$C as previously described.
Statistical analysis

There were three replications per treatment and all experiments were repeated. 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-to-root translocation study, SigmaPlot (ver. 10) was used to plot means, standard errors, and conduct nonlinear regression analyses. The model chosen for absorption data was a hyperbolic function, which is shown below in Equation 1.

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

On the basis of 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 to compare among plant parts, plant species, and herbicides.

In addition to nonlinear regression analyses, the percentage of total herbicide present in plant shoots and roots was 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.

\[ \text{PCF} = \frac{\text{Herbicide concentration in plant (ng/g fresh biomass)}}{\text{Herbicide concentration in water (ng/ml)}} \]  

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

Data from herbicide concentration experiments were analyzed using a linear regression model and plotted using SigmaPlot (ver. 10).

RESULTS AND DISCUSSION

Herbicide absorption and translocation after shoot exposure

Triclopyr had the highest shoot accumulation in hydrilla, followed by fluridone and penoxsulam 192 HAT (Table 1). For EWM, triclopyr also exhibited the greatest accumulation, again followed by fluridone and penoxsulam (Table 1). Root accumulation exhibited the same trend, but was significantly lower than shoot accumulation for all three herbicides (Figure 1). In all cases, the shoot accumulation by hydrilla was much less than accumulation by EWM. Hydrilla accumulated only 24, 35, and 39% of the radioactivity accumulated by EWM 192 HAT for fluridone, penoxsulam, 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. EWM has highly dissected leaves that provide more surface area for absorption than hydrilla, which has several compact leaves per whorl and entire margins (Sculthorpe 1967; Nielsen and Sand-Jensen 1989).

The rate at which herbicide accumulated in hydrilla varied for all three herbicides. Shoot accumulation based on predicted \(t_{90}\) values was rapid for fluridone, with 90% occurring by 76 HAT, whereas shoot accumulation rates for triclopyr and penoxsulam were slower, taking 113 HAT and 145 HAT to reach \(t_{90}\), respectively. Although \(t_{90}\) 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 EWM, fluridone had the fastest accumulation rate, with \(t_{90}\) occurring by 30 HAT, followed by triclopyr (73 HAT) and penoxsulam (110 HAT). In all cases, accumulation in EWM occurred more rapidly than in hydrilla (Table 1). Fluridone absorption by the submersed species sago pondweed (\(S\). \(p\)ectinata) and Richardson pondweed (\(P\). \(r\)ichardsonii) indicated that absorption was slow, continuing to increase for 336 HAT (14 DAT) (Marquis et al. 1981). Penoxsulam absorption by alligatorweed [\(A\). \(p\)hiloxeroides (Mart.) Griseb] continued to increase at 48

### Table 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).

<table>
<thead>
<tr>
<th>Species</th>
<th>Herbicide</th>
<th>Plant Part</th>
<th>PCF&lt;br&gt;192</th>
<th>(A_{192}) (pg/g)</th>
<th>(t_{90}) (h)</th>
<th>(a \pm SE)</th>
<th>(b \pm SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrilla</td>
<td>Fluridone</td>
<td>Aboveground</td>
<td>8.31 ± 0.66</td>
<td>0.76</td>
<td>76</td>
<td>0.054 ± 0.0129</td>
<td>0.066 ± 0.0200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belowground</td>
<td>0.01</td>
<td>163</td>
<td>0.001 ± 0.0003</td>
<td>0.003 ± 0.0026</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Penoxsulam</td>
<td>Aboveground</td>
<td>1.48 ± 0.17</td>
<td>0.13</td>
<td>145</td>
<td>0.002 ± 0.0008</td>
<td>0.010 ± 0.0072</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belowground</td>
<td>0.01</td>
<td>166</td>
<td>0.0001 ± 0.00003</td>
<td>0.002 ± 0.0019</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Triclopyr</td>
<td>Aboveground</td>
<td>9.52 ± 1.15</td>
<td>96.78</td>
<td>113</td>
<td>3.214 ± 0.7422</td>
<td>0.028 ± 0.0092</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belowground</td>
<td>41.70</td>
<td>161</td>
<td>0.384 ± 0.1449</td>
<td>0.004 ± 0.0041</td>
<td></td>
</tr>
<tr>
<td>EWM</td>
<td>Fluridone</td>
<td>Aboveground</td>
<td>19.97 ± 1.69</td>
<td>1.91</td>
<td>30</td>
<td>0.485 ± 0.1816</td>
<td>0.249 ± 0.1046</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belowground</td>
<td>0.27</td>
<td>85</td>
<td>0.016 ± 0.0058</td>
<td>0.054 ± 0.0254</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Penoxsulam</td>
<td>Aboveground</td>
<td>4.16 ± 0.48</td>
<td>0.37</td>
<td>110</td>
<td>0.013 ± 0.0042</td>
<td>0.030 ± 0.0133</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belowground</td>
<td>0.05</td>
<td>112</td>
<td>0.002 ± 0.0006</td>
<td>0.033 ± 0.0181</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Triclopyr</td>
<td>Aboveground</td>
<td>34.61 ± 5.61</td>
<td>397.09</td>
<td>73</td>
<td>30.659 ± 11.424</td>
<td>0.0716 ± 0.0331</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Belowground</td>
<td>71.42</td>
<td>14</td>
<td>44.435 ± 26.3682</td>
<td>0.617 ± 0.3908</td>
<td></td>
</tr>
</tbody>
</table>
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 EWM, respectively. Imazamox absorption by EWM 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 EWM within 12 h of being placed in clean water. This is important in areas where it is difficult to achieve long exposures because of water exchange. Previous CET studies illustrate this point, showing that maximum EWM control with triclopyr at 1 mg L$^{-1}$ was achieved with an exposure time of $\geq 96$ h (Netherland and Getsinger 1992). The same was true for fluridone, in which exposure at 12 $\mu$g L$^{-1}$ must be maintained for $\geq 60$ d for successful EWM 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 penoxsulam were applied at 10 $\mu$g L$^{-1}$, whereas triclopyr was applied at 1,000 $\mu$g L$^{-1}$, it is difficult to make a direct comparison among the three herbicides on the basis solely of the herbicide concentration in the plant without accounting for the herbicide concentration in the water column. Another method to evaluate differences in absorption is through bioconcentration values. In this case, bioconcentration values were based on PCF, shown in Equation 2. PCF is the herbicide concentration in the plant tissue relative to concentration in the water column. On the basis of the calculated PCF values, bioconcentration in EWM 192 HAT was greatest for triclopyr, followed by fluridone and penoxsulam (Table 1); however, for hydrilla, triclopyr and fluridone had similar PCF values, followed by penoxsulam. For fluridone and penoxsulam the PCF values are what would be predicted on the basis of their log $K_{ow}$, but the large amount of triclopyr accumulation by both species was unexpected. On the basis
of log \( K_{ow} \) values we would have expected similar PCF values for penoxsulam and triclopyr. The reasons for greater 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 et al. 1978), and no degradation was reported in sago pondweed and Richardson pondweed (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.

Pesticide accumulation in submersed aquatic plants has been examined from the standpoint of aquatic plants as bioaccumulators to remediate contaminated surface water (Crum et al. 1999, de Carvalho et al. 2007a,b). Previous work conducted by de Carvalho et al. (2007b) examined pesticide absorption by the submersed aquatic species Lagarosiphon as a function of log \( K_{ow} \), and their findings clearly demonstrate that compounds with higher log \( K_{ow} \) values have a greater affinity to accumulate in aquatic plants. While providing insights into the relationship between lipophilicity and bioaccumulation, their work focused on many highly lipophilic pesticides with log \( K_{ow} \) values > 2. There has been relatively little research examining the absorption of less lipophilic compounds with log \( K_{ow} \) < 2, which would be more indicative of most aquatic herbicides.

In research conducted by de Carvalho et al. (2007b), the pesticide that most closely resembled an aquatic herbicide included in our study was 4-chlorophenoxyleurea, with a log \( K_{ow} = 1.80 \), which is similar to that of fluridone. Observed PCF values for 4-chlorophenoxyleurea (PCF = 2.21) were lower than our maximum observed values for fluridone in hydilla and EWM (Table 1). Oxamyl (log \( K_{ow} = 0.47 \)), a nematicide/insecticide, has a log \( K_{ow} \) similar to those of penoxsulam and triclopyr; but the authors were not able to accurately predict bioaccumulation based on log \( K_{ow} \) because there was no predictable relationship between bioaccumulation and log \( K_{ow} \) for pesticides having log \( K_{ow} \) < 1. These differences may have been due to differences in experimental methods. de Carvalho et al. (2007b) used small plant fragments, whereas we used established, rooted plants.

Marquis et al. (1981) evaluated fluridone absorption and translocation by sago pondweed and Richardson pondweed. Although bioconcentration values for their 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 hydilla. As for the differences between hydilla and EWM, they may be due to differences in leaf shape and structure, as previously mentioned.

Shoot-to-root translocation in EWM 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. More translocation occurred in hydilla, 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 2). Little to no herbicide movement to roots of submersed aquatic species had been reported previously. Limited fluridone translocation to hydilla roots has been reported previously (Anderson 1979) and 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 d after treatment (DAT), respectively. Our results indicate slightly more translocation in hydilla 192 HAT.

Results reported by Willingham et al. (2008) showed 1.2% of absorbed penoxsulam present in the roots of alligatorweed 48 HAT. These results are similar to our observations for penoxsulam translocation in EWM, but translocation to hydilla 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 2); however, triclopyr translocation
in hydilla was greater, reaching 12.5% in roots 192 HAT. Triclopyr translocation to EWM roots reached a maximum by 12 HAT and then absorption decreased until 192 HAT (Figure 2). This peak during the first 12 HAT is likely an artifact of roots reaching equilibrium faster than shoot tissue, while shoots continued to absorb triclopyr (Figure 2). Because shoot absorption continues and root concentration remains constant, the proportion of herbicide in roots compared with total absorption appears to decrease. Results for EWM are similar to previous findings, but translocation to hydrilla roots was greater than previously reported values for all three herbicides.

Previous studies have indicated that roots of aquatic plants serve a similar function to those in terrestrial plants, being the main site of nutrient absorption (Wetzel 2001); however, submerged species can also obtain nutrients from the water column. For this reason, translocation to roots of aquatic plants might not be as extensive as translocation in terrestrial species. Although there is evidence that both acropetal and basipetal translocation occurs, acropetal translocation appears to be dominant (Wetzel 2001).

Radioactivity in the treatment tanks did not decrease significantly over the 8-d time course (data not shown). The water content and the relatively small volume of six 15-cm shoots, with which each herbicide equilibrated, were minimal compared with the water column (3 L); therefore, it was not possible to do a mass balance accounting in a manner similar to what is done with terrestrial plants. It is also important to account for any herbicide that might be exuded by the root system, because it also needs to be accounted for as part of translocation. In these experiments, the silica sand contained very little radioactivity, indicating little to no herbicide movement out of the root system (data not shown).

**Effect of concentration on herbicide absorption**

Linear regression analysis of herbicide absorption at 10, 100, and 1,000 \( \mu \text{g L}^{-1} \) after 48-h exposure indicated a strong linear relationship for all three herbicides in both species (Figure 3). This linear trend indicates that as herbicide concentration in the water column increases, the concentration in the plant tissue increases proportionally, providing strong evidence that absorption is due to diffusion as a result of a concentration gradient. Although this relationship was linear for the three herbicides, the slope of each linear regression was different. For example, increasing the external fluridone concentration had a much larger impact on the fluridone concentration in hydrilla compared with triclopyr. Interestingly, the reverse was true for EWM; increasing the external triclopyr concentration resulted in twice the triclopyr concentration in EWM compared with fluridone. Penoxsulam had a very limited response to increases in the external concentration for both species.

The main limitations of our research were the relatively short exposure time used to measure translocation for fluridone and penoxsulam and the fact that external herbicide concentrations were constant over the 8-d time course. Under field conditions, fluridone and penoxsulam would have exposure times of 45 to 60 d, which is just not feasible when using radiolabeled herbicides. Photo- and microbial degradation were also not accounted for under these laboratory conditions; however, these data provide an excellent relative comparison of absorption and translocation patterns for three important aquatic herbicides under controlled conditions. Major questions to be resolved include, does long-term exposure or a decrease in the external herbicide concentration significantly change translocation patterns? Highly water-soluble herbicides, like penoxsulam and triclopyr, would most likely desorb from shoots when external concentrations decrease, but would the same be true for fluridone, with its low water solubility and high \( \log K_{ow} \)? The mechanism leading to increased triclopyr absorption is also unknown, but it could be due to herbicide metabolism. Further studies should be conducted to examine triclopyr metabolism in submerged aquatic plants.

![Figure 3. Herbicide absorption by hydrilla (A) and Eurasian watermilfoil (B) after treatment with fluridone, triclopyr, and penoxsulam at concentrations of 10, 100, and 1,000 \( \mu \text{g L}^{-1} \). Data points represent the mean, and error bars represent the standard error of the mean (n = 6).](image-url)
SOURCES OF MATERIALS

1Osmocote 14-14-14, The Scotts Company, Marysville, OH 43041.
2Phytagar, Invitrogen Corp., Grand Island, NY 14072.
3Pantry 4.0 L, ClickClack, Palmerston North, New Zealand 4410.
4OX-500, R. J. Harvey Instrument Co., Tappan, NY 10983.
5OX-161, R. J. Harvey Instrument Co., Tappan, NY 10983.
6Packard 2500R, PerkinElmer, Waltham, MA 02451.
7Sonar AS, Galleon SC, Renovate 3, SePRO Corporation, Carmel, IN 46032.

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LITERATURE CITED

