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Triclopyr Absorption and Translocation by Eurasian Watermilfoil (*Myriophyllum spicatum*) Following Liquid and Granular Applications

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One method that appears promising for the treatment of Eurasian watermilfoil in areas of high water exchange is the use of herbicide-impregnated granules. Experiments were conducted using liquid triclopyr-triethylamine and granules impregnated with triclopyr-triethylamine to test this theory. Uniform, multistemmed Eurasian watermilfoil plants were selected for these experiments. Plants were treated in clear acrylic cylinders containing 7 L of water with 0.5 mg/L triclopyr as the liquid triethylamine plus 20 kBq ^{14}C-triclopyr or blank granules impregnated with triclopyr triethylamine plus 20 kBq of ^{14}C-triclopyr. Plants were harvested 6, 12, 24, 48, 96, and 192 h after treatment (HAT) and the radioactivity in the apical meristems, remaining shoot and root was determined with sample oxidation and liquid scintillation spectroscopy. There were no significant differences in overall herbicide absorption by Eurasian watermilfoil following liquid and granular triclopyr treatments; however, differences were observed between plant parts. Apical meristems accumulated the most radioactivity, whereas roots accumulated very little radioactivity following liquid treatment. Granular applications resulted in 7.5 times more radioactivity in the Eurasian watermilfoil roots then the liquid triclopyr application; therefore, long-term control of well-established Eurasian watermilfoil plants could improve with granular applications, especially in areas where rapid herbicide dilution could be an issue.

Nomenclature: Triclopyr; Eurasian watermilfoil, *Myriophyllum spicatum* L.

Key words: Aquatic applications, granular formulations, herbicide absorption, herbicide translocation, liquid formulations, triclopyr.

Eurasian watermilfoil is a submersed invasive species that occurs in at least 45 states across the United States (US Department of Agriculture [USDA] 2011). This nonnative species was first reported in the United States in the 1940s and has significantly expanded its range since the original introduction (Gettys et al. 2009). In addition to the dense infestations commonly occurring across the midwest, this species has also become established in many western states, infesting reservoirs, ponds, canals, and streams.

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

Herbicides are one of the most important management options for Eurasian watermilfoil control, and the selection of an appropriate herbicide must be determined based on individual site conditions and concentration exposure time (CET) requirements for that 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 d or more to provide Eurasian watermilfoil control (Netherland et al. 1993), whereas triclopyr required only 36 to 48 h of exposure time to provide similar control (Green and Westerdahl 1990; Netherland and Getsinger 1992).

CET is strongly influenced by the rate at which an herbicide dissipates, and the two main sources of dissipation in aquatic systems are herbicide

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degradation and dilution. Photolysis is the main degradation mechanism for many aquatic herbicides, including triclopyr (Gettys et al. 2009; Senseman 2007); therefore, parameters that influence ultraviolet light penetration, like water clarity and depth, will influence degradation rates. 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 degradation rates are more important for herbicides requiring longer exposure times, such as fluridone. For herbicides requiring shorter exposure times, such as triclopyr, dissipation as a result of dilution can have a greater impact on herbicide efficacy.

A small amount of dilution and mixing can be beneficial to distribute an herbicide evenly after application, but too much dilution will decrease herbicide concentrations before exposure-time requirements are met. For whole-lake treatments, these flows allow for herbicide equilibration throughout the water body; however, for spot treatments to smaller infestations, these flows can lead to rapid herbicide dilution.

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 the 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 in an attempt to improve herbicide performance in areas of high water exchange.

The proposed benefits of these granular formulations are similar to those for weighted trailing hose applications. Herbicides formulated on a solid carrier 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 absorption and translocation, but no published studies have addressed absorption and translocation trends following granular herbicide application.

Selecting the most appropriate herbicide formulation could have significant impacts on aquatic plant management and studies examining absorption and translocation for both formulations could provide additional insight into the benefits of granular herbicides. Therefore, the objective of this study was to compare triclopyr absorption and translocation in Eurasian watermilfoil following granular and liquid treatments.

Materials and Methods

Plant Materials. Eurasian watermilfoil fragments were collected from the Leggett Canal near Boulder, CO during the fall of 2006. Apical sections 15 cm in length were planted in topsoil and grown in the greenhouse until they were needed. While they were growing in the greenhouse, plants were maintained with a 10 : 14 h light : dark period with the temperature set at 24 and 18 C for day and night, respectively. Supplemental lighting was provided using 400-watt sodium halide lamps (~200 μmol m^{-2} s^{-1}). In order to produce uniform plant material for these experiments, 15-cm apical sections were excised from previously propagated plants and transplanted to 15-cm round pots containing topsoil amended with 3 g slow-release fertilizer (Osmocote 14–14–14, The Scotts Company, 14199 Industrial Parkway, Marysville, OH 43040).

These plants were allowed to grow under the greenhouse conditions mentioned above until they had produced 50 to 60 cm of top growth and several stems. At this time most of the shoot growth was removed, leaving approximately 5 cm of stem tissue and two leaf internodes above the crown. Plants were allowed to regrow from these internodes to approximately 50 cm, and 36 of the most uniform plants with two to four stems were selected for use in subsequent experiments. Prior to herbicide exposure, these established plants were removed from their original pots, had their roots rinsed with water to remove topsoil, and replanted in 9.5-cm-diam by 10-cm (708.5 cm^3) round pots in sand amended with 3 g slow-release fertilizer.

Plants were transferred to clear acrylic tubes (91.5 cm tall × 11.5 cm diam.) filled with 7 L of tap water, and were allowed to equilibrate for 24 h prior to herbicide applications. Eighteen cylinders were then treated with formulated triclopyr (Renovate® 3, SePRO Corp., 11550 North Meridian Street, Suite 600, Carmel, IN 46032) combined with ^{14}C-triclopyr (2.7 MBq/mg specific activity). The treatment solution for the liquid application was prepared by first diluting 194 µl of formulated triclopyr with 100 ml of water and then adding 500 kBq of ^{14}C-triclopyr. Each cylinder was then treated with 5 ml of treatment solution, containing 3.5 mg triclopyr and 20 kBq of ^{14}C-triclopyr to achieve a concentration of 0.5 mg/L in the water column.

Granular herbicide was formulated to simulate the granular triclopyr formulation Renovate OTF (SePRO Corp.) by starting with blank paper/clay-based granules and adding the appropriate amount of formulated triclopyr (Renovate 3) and ^{14}C-triclopyr. Although commercially available Renovate OTF contains 10% w/w acid equivalent triclopyr, granules used in this study were formulated at a concentration of 1% w/w acid equivalent triclopyr to provide better consistency in the relatively small water volumes. Seven grams of blank granules were treated with 2 ml of water containing 0.2 ml liquid triclopyr and 500 kBq of ^{14}C-triclopyr. Each of the 18 tubes received 350 mg of triclopyr and ^{14}C-triclopyr–impregnated granules. The granules were dropped through the water column, landing on the surface of the pot. The resulting concentration in the water column after the release of all the triclopyr was equivalent to the liquid application.

Following treatment, plants were harvested at 6, 12, 24, 48, 96, and 192 h after treatment (HAT). During the study, plants were maintained in the laboratory at 22 C, with a 10 : 14-h light : dark period, supplemented with fluorescent grow lights (approximately 200 µmol m^{-2} s^{-1}). Three replicates were harvested for each formulation at each time point, and the experiment was repeated. Upon harvest, each plant was separated into three samples; apical meristems (2 cm of each shoot apex), remaining 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 with a mortar and pestle to allow for complete sample oxidation. Ground plant samples were oxidized with the use of a biological sample oxidizer (OX500, R.J. Harvey Instrument Co., 11 Jane St., Tappan, NY 10983) and $^{14}CO_2$ was collected by a ^{14}C-trapping cocktail (R.J. Harvey Instrument Co). After oxidation, radioactivity was quantified by liquid scintillation spectroscopy (Packard 2500R, PerkinElmer, 940 Winter Street, Waltham, MA 02451).

Statistical Analysis. Levene's test for homogeneity of variance was used to determine whether repeated experiments could be combined for subsequent statistical analysis ($\alpha = 0.05$ level of significance). Data were plotted with the use of SigmaPlot (Systat Software, Inc., 1735 Technology Drive, Suite 430, San Jose, CA). Nonlinear regression analyses were also conducted to fit the following equation:

$$y = ax/(1 + bx) \qquad [1]$$

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} was the 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 part and herbicide formulation. Means and standard errors were calculated with the use of MS Excel (MS Office 2007) and plotted with nonlinear regression lines to provide a visual evaluation of how well the nonlinear regression fit these data; however, all data points were used for nonlinear regression analysis.

Results and Discussion

Levene's test for homogeneity of variance indicated data from repeated studies could be combined for statistical analysis ($\alpha = 0.05$) so combined data were used to examine triclopyr absorption on a whole-plant basis and by individual plant parts. Kniss et al. (2011) proposed a straightforward method to standardize nonlinear regression analyses for herbicide absorption studies that included a very useful parameter defined as A_{max} or maximum asymptote. Although the current studies were

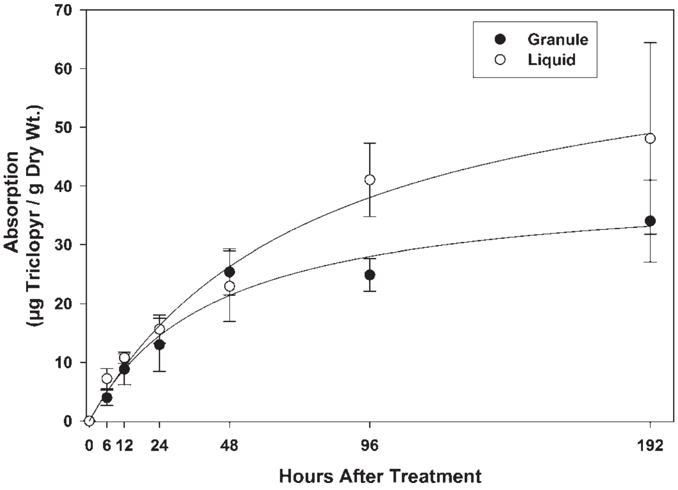

Figure 1. Total absorption of liquid and granular triclopyr expressed as µg g^{-1} dry weight, combined across all plant parts. Data points represent the mean, and error bars represent the standard error of the mean ($n = 6$). Nonlinear regression analysis was performed with the use of a hyperbolic regression equation (Equation 1), and all data points were included to construct the regression model.

conducted over a reasonably long time course of 192 h, triclopyr did not reach an A_{max} in most cases, so we substituted absorption at our last time point (A_{192}) for A_{max} and calculated the t_{90} values proposed by Kniss et al. (2011) based on A_{192} and not A_{max}. These minor modifications still provided useful parameters for comparing triclopyr absorption resulting from liquid and granular triclopyr applications.

The absorption rate was faster following liquid treatment (t_{90} = 120 HAT) than granular treatment (t_{90} = 138) (Figure 1), but regression analysis conducted on absorption combined across plant parts indicated that there was no significant difference in total plant absorption. Although there was no significant difference in whole-plant triclopyr absorption, there were differences in absorption when compared by plant part (Figure 2). Following liquid application, apical meristems accumulated significantly more radioactivity than shoots and roots; however, for the granular application there was no significant difference in accumulated radioactivity between apical meristems and shoots 192 HAT (Table 1).

Triclopyr accumulation by apical meristems following liquid treatment (94 ± 19.1 µg/g dry weight) was 1.7 times greater than with granular treatment (54.2 ± 4.6 µg/g dry weight), but there was no significant difference between treatments in shoot accumulation 192 HAT (Table 1). Although roots accumulated less herbicide than shoots and apical meristems with both treatments, there was 5.8 times more radioactivity in the root when plants were exposed to the granular triclopyr formulation compared to liquid formulation (24.1 ± 10.6 µg/g dry weight compared to 3.6 ± 0.8 µg/g dry weight). 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.

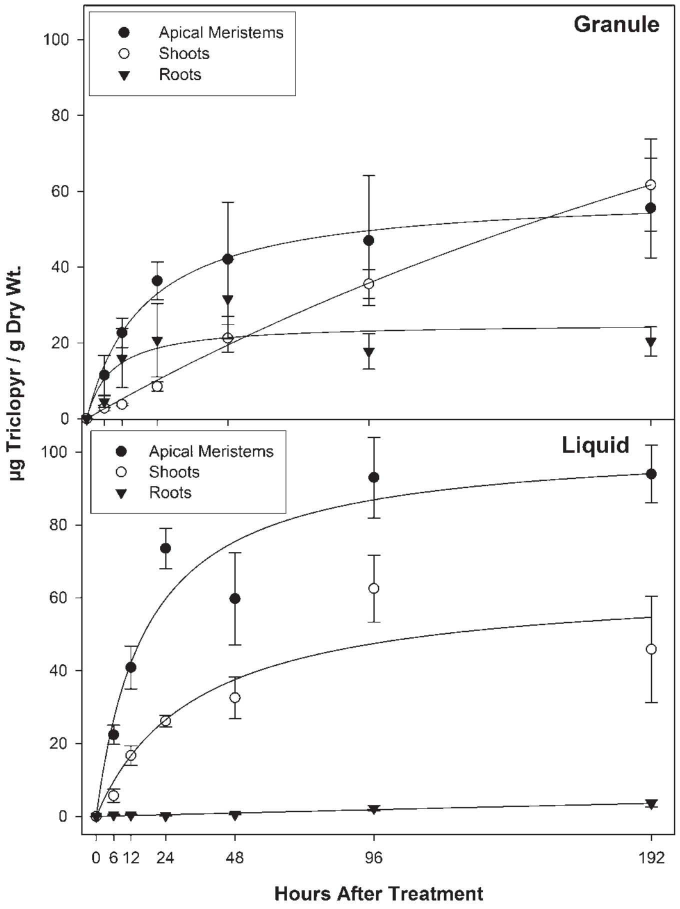

Figure 2. Herbicide absorption following treatment with granular (top) and liquid (bottom) triclopyr over a 192-h time course. Plotted points represent the mean, and error bars represent the standard error of the mean ($n = 6$). Regression parameters for the hyperbolic regression are shown in Table 1.

The accumulation rate, as determine by calculated t_{90} values, showed different trends following liquid and granular triclopyr treatments. Results indicate that with liquid treatment accumulation occurs faster in apical meristems (t_{90} = 81 HAT) and shoots (t_{90} = 111 HAT), but root accumulation was much slower (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) (Vassios et al. 2011a). Root absorption in an earlier study

Table 1. Predicted triclopyr absorption 192 HAT (A_{192}), the time required to reach 90% of A_{192} (t_{90}), 95% confidence interval, and regression parameters for hyperbolic regression separated by plant part.

		Predicted values and regression parameters				
Plant Part	Formulation	Tricolpyr A_{192}[a] (μg g^{-1} dry wt)	t_{90}[b] (h)	95% CI[c] (h)	$a \pm$ SE[d]	$b \pm$ SE[d]
Apical meristem	Liquid	94 ± 19.1	81	77–81	0.60 ± 0.17	0.06 ± 0.01
	Granular	54 ± 4.5	87	84–91	0.31 ± 0.04	0.05 ± 0.01
Shoot	Liquid	54.7 ± 17.6	111	105–126	0.19 ± 0.074	0.03 ± 0.02
	Granule	61.7 ± 3.2	166	165–168	0.04 ± 0.003	0.002 ± 0.001
Root	Liquid	3.6 ± 0.8	172	168–181	0.002 ± 0.001	0.0003 ± 0.002
	Granule	24.1 ± 10.6	53	51–72	0.3 ± 0.22	0.12 ± 0.1

[a] Predicted triclopyr absorption 192 HAT ± SE.
[b] Predicted time required to reach 90% of A_{192} value in hours
[c] 95% confidence interval for predicted t_{90} value in hours.
[d] Nonlinear regression parameters from Equation 1.

reached t_{90} by 14 HAT, and was probably due to limited translocation to shoots (Vassios et al. 2011b). In the current study, there was no isolation of the root system, so continued root accumulation likely occurred as a result of both translocation and herbicide diffusion into sediment pore water, leading to continued accumulation.

After a granular treatment, accumulation occurred rapidly in roots (t_{90} = 53 HAT) followed by apical meristems (t_{90} = 87 HAT), and shoot tissue (t_{90} = 166 HAT) (Table 1). Although accumulation in roots and apical meristems was relatively rapid, that was not the case in remaining shoot tissue, for which accumulation continued for the duration of the study (Figure 2). Rapid root accumulation after granular treatment is followed by accumulation in apical meristems, suggesting that most accumulation for granular applications occurs near the plant roots and that absorbed herbicide is translocated to apical meristems. After accumulation in the apical meristems reached 90% of A_{192} shoots continued to absorb herbicide for the duration of the study. These differences in accumulation rate and translocation based on herbicide formulation could impact overall triclopyr efficacy.

Observations at early time points following granular treatment showed approximately 60% of absorbed radioactivity present in roots and that concentration decreased between 24 and 192 HAT. This could be the result of continued triclopyr translocation to shoots, and the fact that significant root accumulation occurred rapidly after treatment. Following a liquid treatment, the amount of radioactivity found in the roots accounted for only 3.7 ± 1.7% of total absorption 192 HAT. The amount of radioactivity present in roots following granular treatment was much greater at 27.8 ± 11.1%, nearly 7.5 times the liquid treatment (Figure 3). These results provide additional support for the hypothesis that absorption following liquid treatment occurs primarily in the shoots, whereas root absorption is considerably more important for granular treatments. Although liquid treatments may provide good control the year of application, these results indicate that limited root translocation could limit 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 could provide advantages in achieving long-term Eurasian watermilfoil control, but the true implications of these findings may only

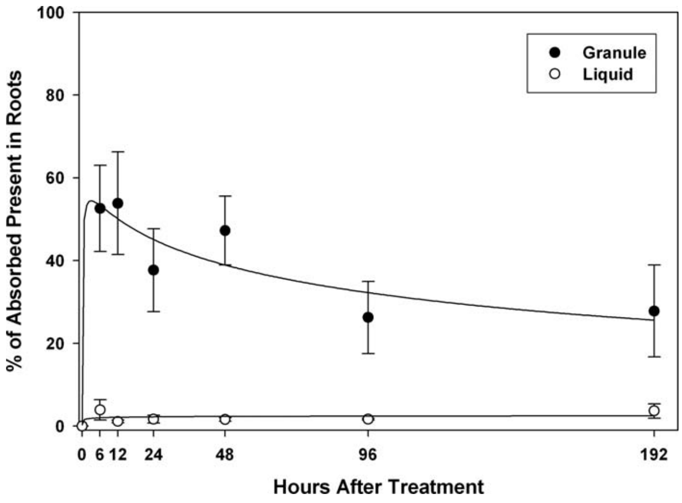

Figure 3. Translocation of radioactivity from roots to Eurasian watermilfoil shoots following exposure to liquid and granular triclopyr formulations. Data presented are means, and error bars are the standard error of the mean (= 6).

be realized after several years of intense field evaluations. Triclopyr CET experiments have been conducted with liquid formulations (Netherland and Getsinger 1992); however, additional studies should be conducted to evaluate the optimal CET relationship for granular formulations. Increased root absorption following granular applications could mean a reduction in the herbicide rate and/or exposure times. If reduced rates could provide equivalent control, this could reduce herbicide loading in the aquatic ecosystem. Reduced exposure times could result in more treatment flexibility and improve the success of spot treatments in high water exchange areas.

Field studies utilizing a granular triclopyr formulation 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 study, provides additional evidence that granular triclopyr formulations could be very useful for spot treatments.

It is important to note that this study does have limitations because of the experimental methods used. The current study used sand as the rooting media, whereas in a real-life setting there would be varying sediment types. If roots are an important absorption site 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 could be slower, reducing overall root absorption.

Other limitations of these studies were the fact that we used a static system and that herbicide exposures were maintained for the duration of the study; however, under field conditions the herbicide concentration would have decreased during the study as the herbicide was diluted or degraded (Getsinger et al. 2000). As the concentration in the water column drops, the herbicide may desorb from the plant in the absence of a strong external concentration gradient. This concept was previously illustrated by Vassios et al. (2011). Future studies should focus on examining these issues, and the resulting impact of absorption and translocation on Eurasian watermilfoil control.

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 the use of a liquid triclopyr formulation, apical meristems accumulated the most triclopyr, whereas root accumulation was very limited. For the granular formulation, triclopyr accumulation was more evenly distributed between plant parts with roots accumulating significantly more herbicide than with the liquid application. As a percentage of the total herbicide absorbed, there was approximately 11 times more herbicide present in roots following granular treatment compared to liquid treatment at the early sampling points. These results show definite differences in accumulation and distribution based on triclopyr formulation; however, there were several limitations to this study that should be addressed by future research.

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