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Mesocosm evaluation of triclopyr on Eurasian watermilfoil and three native submersed species: The role of treatment timing and herbicide exposure

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ABSTRACT

Early-season recommendations to improve herbicide efficacy and selectivity when targeting Eurasian watermilfoil (*Myriophyllum spicatum* L.) have been adopted by many aquatic plant managers in the upper Midwest. To address the role of treatment timing and exposure, two mesocosm studies were conducted on plants established the prior fall to evaluate short- and long-term exposure scenarios when the auxin mimic herbicide triclopyr ([3,5,6-trichloro-2-pyridinyl)oxy]acetic acid) is used for selective control of Eurasian watermilfoil (EWM). The first study included liquid and granular triclopyr applied at 1.5 mg L^{-1} in late February and late April 2009 under high–water-exchange conditions (12-h half-life). The second trial included static liquid exposures for early March and late April 2011 treatments at use rates of 0.25, 0.5, and 1.5 mg L^{-1} and liquid and granular treatments at 1.5 mg L^{-1} under high–water-exchange conditions (5-h half-life). The second study also included the native species American pondweed (*Potamogeton nodosus* Poir.), Illinois pondweed (*P. illinoensis* Morong), and vallisneria (*Vallisneria americana* Michx.). In the first study, February triclopyr treatments with liquid and granular formulations did not reduce biomass compared to the untreated plants during a May harvest, whereas both April treatments resulted in complete control of EWM. Results of the second trial indicated that treatment timing (March vs. April) was not a significant factor for static treatments (0.25 to 1.5 mg L^{-1}) and near 100% EWM control was achieved. In contrast, under high flow conditions, the March liquid treatment did not differ from the untreated reference plants. The granular treatment reduced EWM by 55% compared to the untreated reference, yet it still increased in biomass by 4-fold compared to the initial biomass. The April treatments were more effective than the March applications under conditions of high water exchange. The native plants evaluated were not impacted by treatment timing, rate, formulation, or exposure scenario. Results demonstrate early-season treatments were effective regardless of treatment timing under extended triclopyr exposure periods; however, in areas of high–water-exchange early treatments of EWM resulted in reduced plant control. Managers can use this information to determine if early- or later-season treatments are warranted based on the likely exposure scenario.

Key words: aquatic herbicides, invasive aquatic plants, SAV, Submersed Aquatic Vegetation.

INTRODUCTION

Prior research on the selective use of herbicides for control of the invasive plants Eurasian watermilfoil (EWM) and curlyleaf pondweed (*Potamogeton crispus* L.) have focused on factors such as water temperature (Netherland et al. 2000), treatment timing (Poovey et al. 2002, Skogerboe and Getsinger 2006, Skogerboe et al. 2008), concentration and exposure time (CET) scenarios (Getsinger et al. 2002, Madsen et al. 2002, Poovey et al. 2004), and plant hybridity (Poovey et al. 2007, Slade et al. 2007, Glomski and Netherland 2010). In addition, efficacy and selectivity in small field plots (Parsons et al. 2001, Poovey et al. 2004, Wersal et al. 2010) impacts on native emergent species (Glomski and Nelson 2008, Glomski et al. 2009), field dissipation of herbicides (Getsinger et al. 2000, Fox et al. 2002, Netherland et al. 2002, Koschnick et al. 2010), and combining different herbicide modes of action (Skogerboe and Getsinger 2006) have been studied. One common focus of this research has been to improve species-selective control by changing herbicide treatment timing to expose invasive plants early in the season, when native plants are presumably dormant. This philosophy has led to an increasing number of operational recommendations that call for early-season applications of 2,4-D ([2,4-dichlorophenoxy] acetic acid), triclopyr, or endothall (7-oxabicyclo[2.2.1]heptanes-2,3-dicarboxylic acid) for EWM control in the upper Midwestern states.

Recent efforts to monitor the dissipation of herbicide concentrations following a wide range of use patterns in glacial lakes of the Midwest has led to new insights regarding the role of treatment timing, exposure requirements, and potential for impacts on selectivity. Specifically, two distinct dissipation patterns of auxin-mimic herbicides and endothall have been noted following early-season applications for EWM control. The first pattern includes small-scale treatments that often result in a short exposure period

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(hours) characterized by rapid herbicide dispersion from the treatment zone (Poovey et al. 2004, Wersal et al. 2010). This pattern of exposure is typical of many spot or narrow shoreline applications. Prior CET research captured many of these scenarios where higher concentrations are required for short exposure times (Green and Westerdahl 1990, Netherland and Getsinger 1992). In contrast, some documented applications have resulted in an extended exposure (several weeks and longer) to low but potentially active concentrations of herbicide (Parsons et al. 2001, Glomski and Netherland 2010). This pattern of exposure is characteristic of large-block applications that can result in lake-wide (or enclosed bays) exposure to sustained low concentrations, which is similar to the recommended use pattern of fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4[1H]-pyridinone) (Getsinger et al. 2002).

These treatment scenarios have challenged traditional assumptions from earlier CET studies, and have led us to ask the following questions about early-season applications: 1) is there a link between initial EWM biomass and exposure-time requirements, 2) do native plants respond differently when exposed early in the growing season versus later in the season, 3) do native plants respond differently to short-term high-dose exposures versus long-term low-dose exposures, and 4) does efficacy or selectivity vary between granular and liquid applications in high–water-exchange environments?

Early-season applications have become widely accepted for both EWM and curlyleaf pondweed control based on the premise of increased efficacy and improved selectivity; nonetheless, native plants may be vulnerable early in the season at a particularly sensitive life stage. Likewise, EWM is sparse early in the season and may lack enough actively growing biomass to absorb and translocate the herbicide to large carbohydrate reserves in the root crown, resulting in treatment failures of early spot applications. Although there have been numerous positive outcomes following early-season applications, we wanted to address the questions noted above to ensure that current recommendations maximize both the efficacy and selectivity of auxin-mimic herbicides when used for control of EWM. Study objectives were to determine the effect of treatment timing of liquid and granular triclopyr formulations under static and high–water-exchange conditions on EWM and three native species.

MATERIALS AND METHODS

Two studies were conducted at the U.S. Army Engineer Research and Development Center, Lewisville Aquatic Ecosystem Research Facility (LAERF) located in Lewisville, TX. Both studies were conducted in 6,700-L fiberglass mesocosms filled with Lake Lewisville water to determine the role of treatment timing and exposure on triclopyr efficacy. The first study was conducted on EWM only and the second study included EWM and three species of native submersed plants.

Study 1

EWM was collected from Lake Minnetonka, MN. Two apical stem sections were planted per pot (3.78-L) filled with LAERF pond sediment amended with 3 g L^{-1} Osmocote® (18–6–12).[1] Six pots of EWM were placed in each mesocosm in late September 2008 and allowed to overwinter. Establishing plants in September allowed for rapid growth during the early fall months in Texas; therefore, February treatments were conducted on well-established plants that were emerging from winter senescence. The first set of mesocosms was treated on February 27 and the second set on April 23, 2009. For clarity in discussing results the February 27 treatment will be referred to as early season and the April 23 treatment will be referred to as the late-season application. Given latitudinal differences the treatment timing utilized for these studies in Texas was based on early onset of new EWM growth and the objective was to mimic early spring and late spring applications in the upper Midwest. Rates for each set of mesocosms included 1.5 mg L^{-1} (1,500 μg L^{-1}) triclopyr liquid (Renovate 3™)[2] and granular (Renovate OTF™)[3] and an untreated control. Treatments were replicated three times and randomly assigned. Untreated water was circulated through the tanks at a rate to provide a nominal 12-h water-exchange half-life. Extensive monitoring of spot herbicide applications in the upper Midwest has shown that many of the larger spot applications produce product half-lives in this range.

Water samples were collected at 1, 4, and 8 h and 1, 2, 3, 10, and 14 d posttreatment to determine triclopyr degradation following February and April applications. Triclopyr concentrations were measured via use of an enzyme-linked immunoassay[4] (Fox et al. 2002).

Temperature loggers were placed in the mesocosms prior to application and initial water temperature for the February 27 and April 23 treatments was 17.5 ± 0.2 and 22.0 ± 0.1 C (63.5 and 71.6 F), respectively.

Prior to treatment, three pots of EWM were collected to determine initial dry weight. Viable biomass was harvested and dried at 65 C for the early-season treatment tanks at 8 and 12 wk after treatment (WAT) and the late-season treatments were harvested at 4 and 8 WAT. Three pots were randomly selected and harvested from each tank at each sample time.

Plant dry weight data were subjected to one-way analysis of variance (ANOVA) and means separated via the Student-Newman-Keuls method (SNK; $P < 0.05$). When necessary, data were square-root transformed to meet the assumptions of normality and equal variance. Nontransformed data are presented. Herbicide concentration data for the liquid applications were subjected to an exponential decay model to allow determination of triclopyr half-lives in the mesocosm tanks.

Study 2

EWM was collected from a LAERF pond. Two apical stem sections of EWM were planted per pot (3.78 L) filled with LAERF pond sediment amended with 3 g L^{-1} Osmocote® (18–6–12), and four pots were placed in each of 30 mesocosms filled with Lake Lewisville water in September 2010. Each mesocosm also included two 1.9-L pots each of American pondweed, Illinois pondweed, and vallisneria.

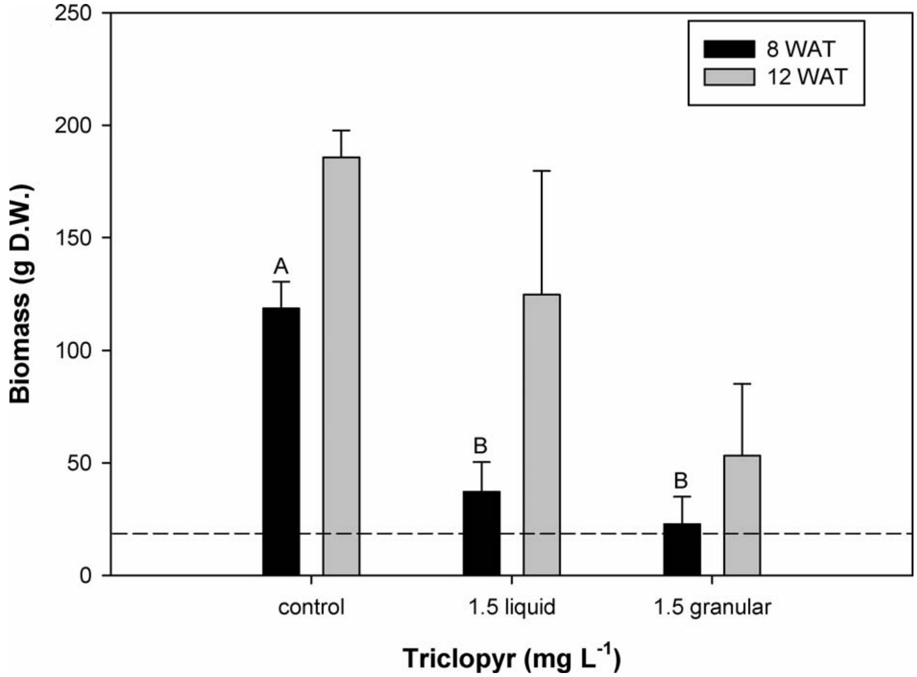

Figure 1. Mean (± SE) dry weight (g) of Eurasian watermilfoil shoot biomass 8 and 12 wk after treatment following early-season liquid and granular triclopyr applications on February 27. Each bar represents the average of three replicate treatments. Bars sharing the same letter do not differ (Student-Newman-Keuls; $P < 0.05$). The dashed line represents pretreatment biomass.

The first set of mesocosms was treated on March 3 and the second set on April 18, 2011. As described above, for clarity in reporting the results, these treatments will be referred to as early-season and late-season applications. The timing of the early March application was based on visual observation of numerous red apical shoots of EWM growing from established root crowns, thus indicating the plants were actively growing. Treatment rates were selected to mimic low-dose and whole-lake static exposures and to challenge the native plants with an extended exposure to a higher dose. Moreover, in high–water-exchange conditions, efficacy of liquid and granules was compared at different stages of EWM and native plant growth. The six treatments included static exposures of 0.25, 0.5 and 1.5 mg L^{-1} (250, 500, and 1,500 µg L^{-1}) triclopyr liquid, 1.5 mg L^{-1} (1,500 µg L^{-1}) triclopyr liquid and granular with a 5-h water-exchange half-life and an untreated control. Whole-lake applications typically produce a pattern of extended exposure to lower herbicide concentrations (Glomski and Netherland 2010), whereas small-scale spot applications often result in product half-lives of 5 h and less.

Water samples were collected at 1, 3, 5, 7, 14, and 21 d posttreatment for the 1.5 mg L^{-1} static treatment to determine degradation patterns of the static treatments following early- and late-season applications. For tanks that were flowing, water samples were collected at 1, 3, 5, 24, and 48 h. Triclopyr concentrations were analyzed as described above, and product half-lives were calculated.

Temperature loggers were placed in the mesocosms prior to application and initial water temperature for March and April treatments was 16.3 ± 0.2 and 21.1 ± 0.3 C (61.3 and 70 F respectively), respectively. Prior to treatment, three pots of EWM were randomly collected to determine initial dry weight. Viable shoot biomass from all tanks was harvested and dried at 65 C on June 28. This single harvest date was conducted at 15 WAT for the early-season application and ~ 9 WAT for the late-season application.

The early- and late-season treatments were replicated three times. Data were subjected to a two-way ANOVA and means separated via the SNK method ($P < 0.05$). Where no interaction was detected, data were pooled. In addition, the data for the static liquid application at 1.5 mg L^{-1} and the data for the flow-through liquid application at 1.5 mg L^{-1} were subjected to an exponential decay model to allow determination of triclopyr half-lives in the mesocosm tanks.

RESULTS AND DISCUSSION

Study 1

Following the early-season application, EWM displayed initial triclopyr symptoms; however, biomass remained at or above pretreatment levels during the first harvest and was not different from untreated references by the second harvest (Figure 1). In contrast, although the late-season treatment started with three times more initial EWM biomass, both the liquid and granular applications resulted in no viable plants at either harvest (Figure 2). For both treatments, EWM was actively growing and the temperature difference on the date of application was 4.5 C. Water sampling indicated that the herbicide exposures for the early- and late-season applications were similar (Figure 3); however, the treatment effect was very different. An exponential decay model indicated that half-lives of the

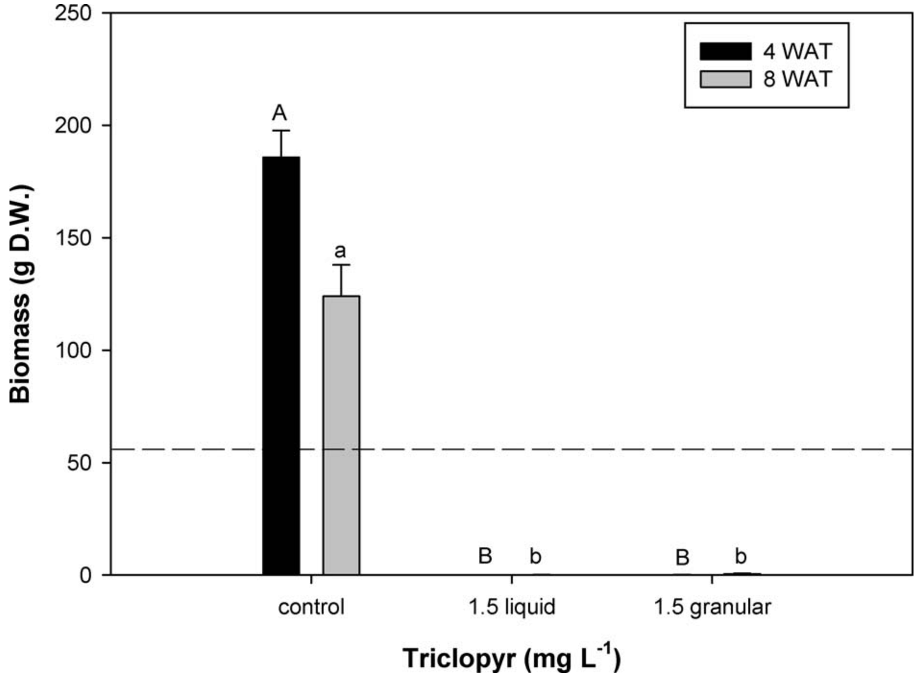

Figure 2. Mean (± SE) dry weight (g) of Eurasian watermilfoil shoot biomass 4 and 8 wk after treatment following late-season liquid and granular triclopyr applications on April 23. Each bar represents the average of three replicate treatments. Bars sharing the same letter do not differ (Student-Newman-Keuls; $P < 0.05$). Nontransformed data are presented. The dashed line represents pretreatment biomass.

liquid triclopyr applications for early- and late-season applications 11.7 and 13.2 h, respectively (Figure 3). One key difference between the two treatments was initial biomass, which was 18.2 ± 1.5 g prior to the early-season application and 55.1 ± 5.6 g for the late-season application. This trial provides evidence that early-season spot applications with an auxin-mimic herbicide in areas of high water exchange may be negatively impacted when EWM biomass is low. Prior research evaluating contact herbicides on curlyleaf pondweed showed only a slight increase or no impact on endothall and diquat activity when temperatures were increased from 16 to 20 C (Poovey et al. 2002); however, in the current study the early-season treatments at ~ 17 C allowed significant recovery whereas the late-season treatments at ~ 21 C resulted in near complete EWM control. This study did not allow for determination of whether biomass or metabolic activity was the key factor in dictating differences in efficacy. The link between temperature, biomass, and metabolic activity has been previously described by Barko and Smart (1981) under greenhouse conditions and a similar trial could be conducted to determine the putative role of biomass and temperature following a short-term auxin-mimic exposure.

Plant managers are often encouraged to implement treatment when the target species are small and more susceptible, but this study suggests that increased EWM biomass (and presumably increased metabolic activity) may improve herbicide uptake and control when short exposure periods are expected. Although the explanation for reduced control requires further evaluations, these results suggest that early-season treatments with triclopyr or other auxin mimic herbicides may have significant drawbacks when targeting small areas that contain limited EWM biomass early in the growing season.

Study 2

As noted in Study 1, for the early- and late-season treatments the EWM was actively growing and the difference in water temperature between the treatments

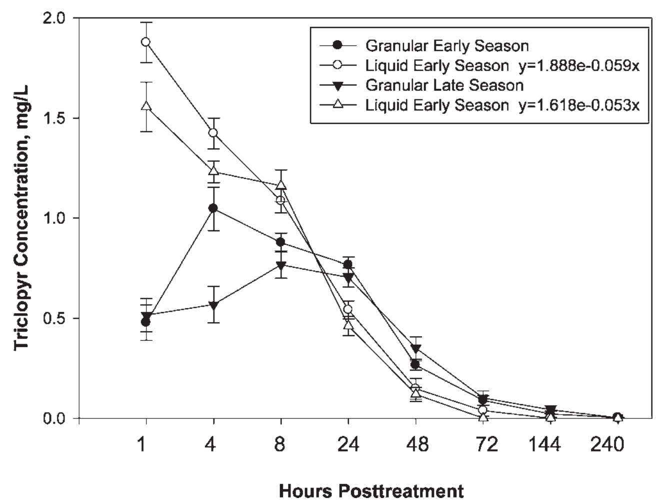

Figure 3. Triclopyr concentrations following liquid and granular applications at a nominal application rate of 1.5 mg L^{-1} in flowing mesocosms set to yield a product half-life of 12 h. First- order dissipation half-life values for the liquid triclopyr applications were calculated at 11.7 h for the early-season February 27 application and 13.1 h for the late-season April 23 application. Half-lives were not calculated for the granular applications.

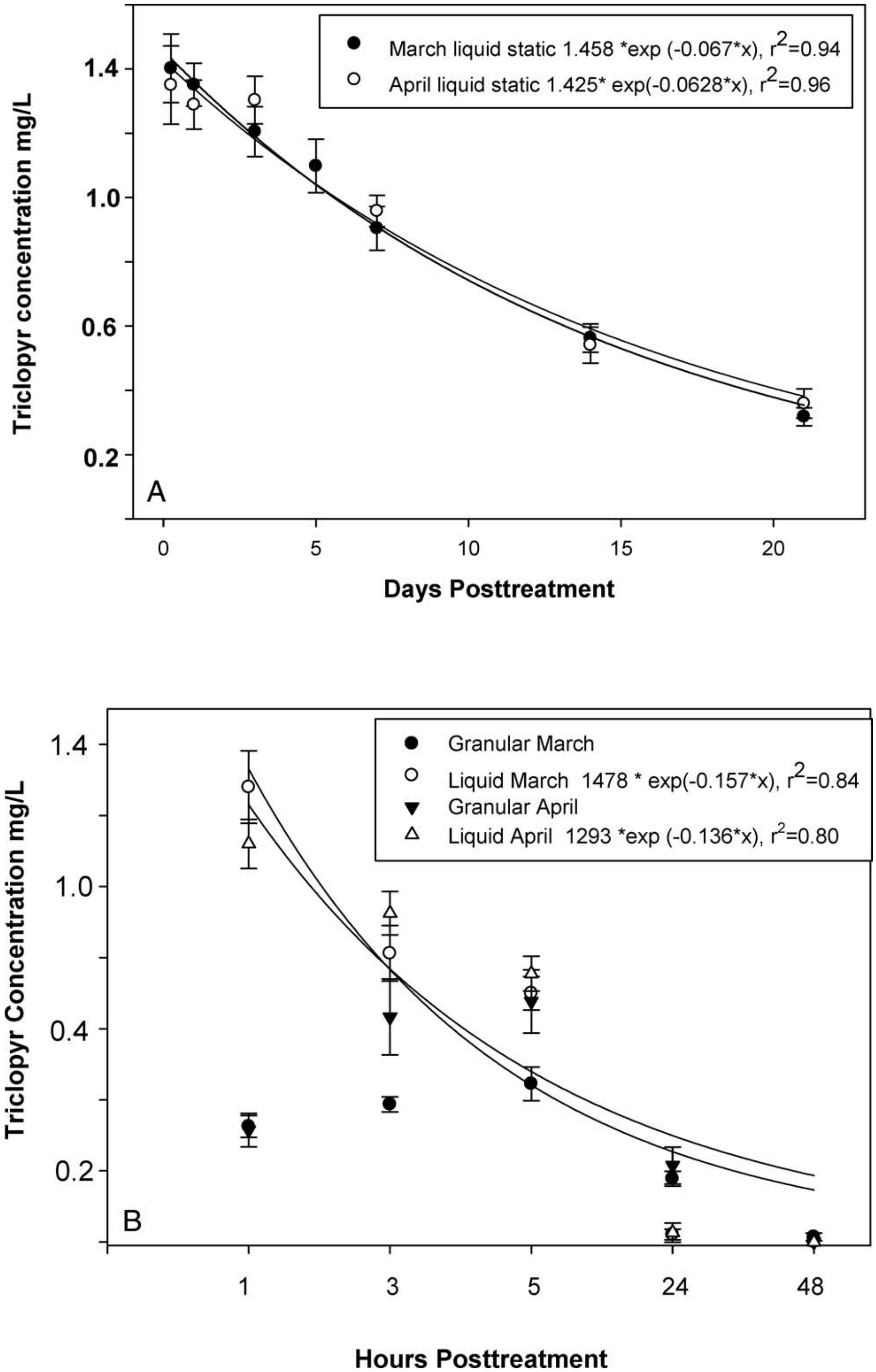

Figure 4. (A) Triclopyr concentrations following a liquid application at a nominal concentration of 1.5 mg L^{-1}. First-order dissipation half-life values were calculated as 10.4 and 11.2 d for the early-season March 3 and late-season April 18, 2011, treatments, respectively. (B) Triclopyr concentrations following liquid and granular treatments at a nominal concentration of 1.5 mg L^{-1} in flowing mesocosms. First-order dissipation half-life values of 4 and 5 h were determined for the early- and late-season treatments, respectively.

was 4.8 C. Furthermore there was an approximate 3-fold difference between initial EWM biomass for the early- and late-season treatments (7.8 ± 2.3 g and 25.1 ± 4.3 g, respectively).

Herbicide concentration analyses indicated that half-lives of static triclopyr treatments were 10 and 11 d for the early- and late-season treatments, respectively (Figure 4A). Given these half-life projections, plants were exposed to

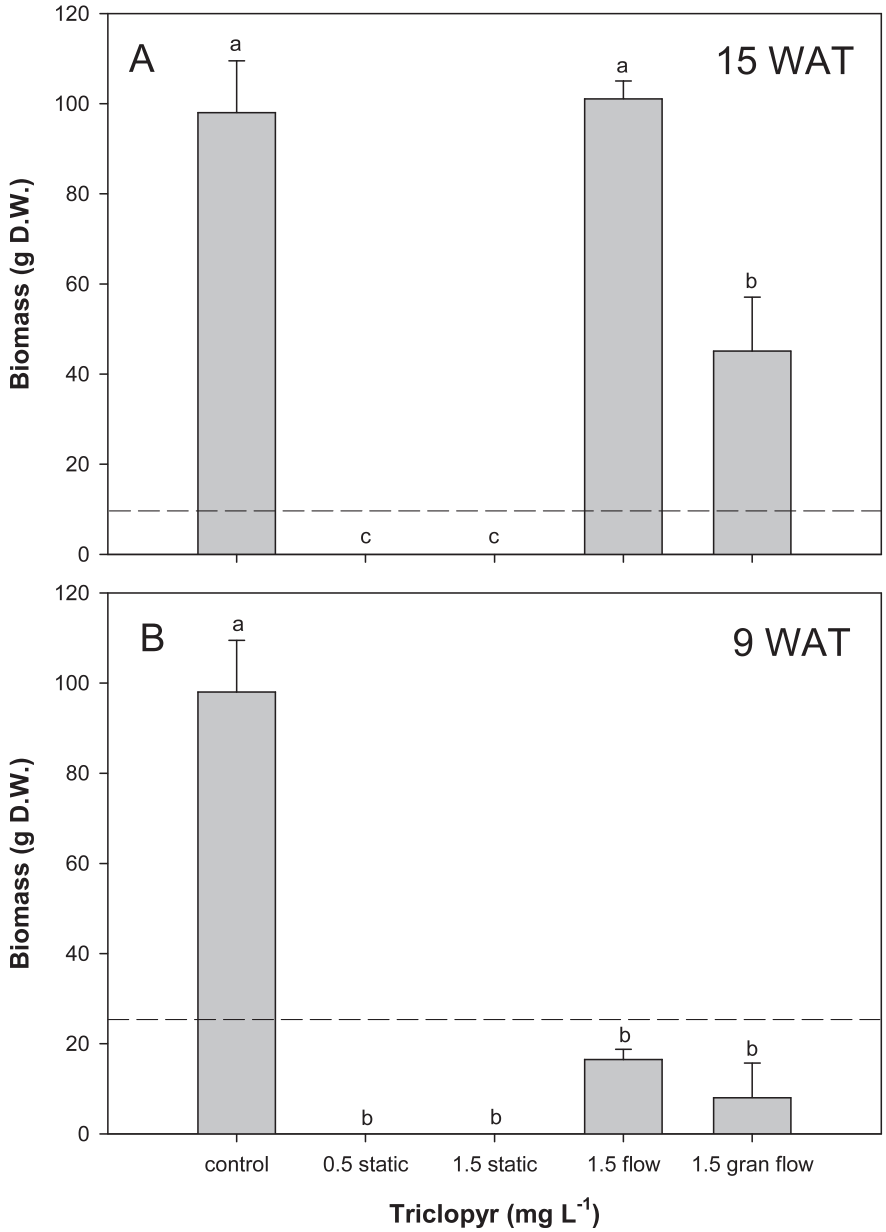

Figure 5. Mean (± SE) dry shoot weight of Eurasian watermilfoil treated with triclopyr following a June 28 harvest at 15 wk after treatment (WAT) following an early-season March 3 application (A) and at 9 WAT following a late-season April 18 application. (B) Treatments included static exposures to liquid triclopyr at 0.5 and 1.5 mg L^{-1} and liquid and granular applications at 1.5 mg L^{-1} in flowing mesocosms set to yield a half-life of 5 h. Bars sharing the same letter do not significantly differ from each other based on the Student-Newman-Keuls; $P < 0.05$. The dashed line represents pretreatment biomass.

triclopyr concentrations above 0.1 mg L^{-1} (see Glomski and Netherland 2010) for several weeks following the 1.5 mg L^{-1} static treatments but for just over 2 wk following the 0.25 mg L^{-1} static treatment. In essence, at different concentrations, the various static treatments provided a range of exposure periods to triclopyr. Water samples in the high-exchange tanks demonstrated rapid clearance of herbicide concentrations following the liquid applications, with half-lives of the liquid treatments calculated as 4 and 5 h for the early- and late-season treatments, respectively (Figure 4B). Granular applications provided lower initial triclopyr concentrations on the first day of sampling, but a higher triclopyr concentration was noted by 1 d postapplication. Given the delayed release of the granular products, we did not calculate half-lives of triclopyr for these applications. Overall, results suggest the CET profiles for the early- and late-season treatments were very similar.

There was a significant interaction between concentration and treatment time for EWM. For both the early- and late-season applications, the static exposures of 0.5 and 1.5 mg L^{-1} resulted in complete control of EWM (Figure 5). The early-season treatment of 0.25 mg L^{-1} also provided 100% control of EWM (data not shown). These results indicate a high level of EWM sensitivity to static exposures at low triclopyr concentrations, which further supports the laboratory results of Glomski and Netherland (2010). For

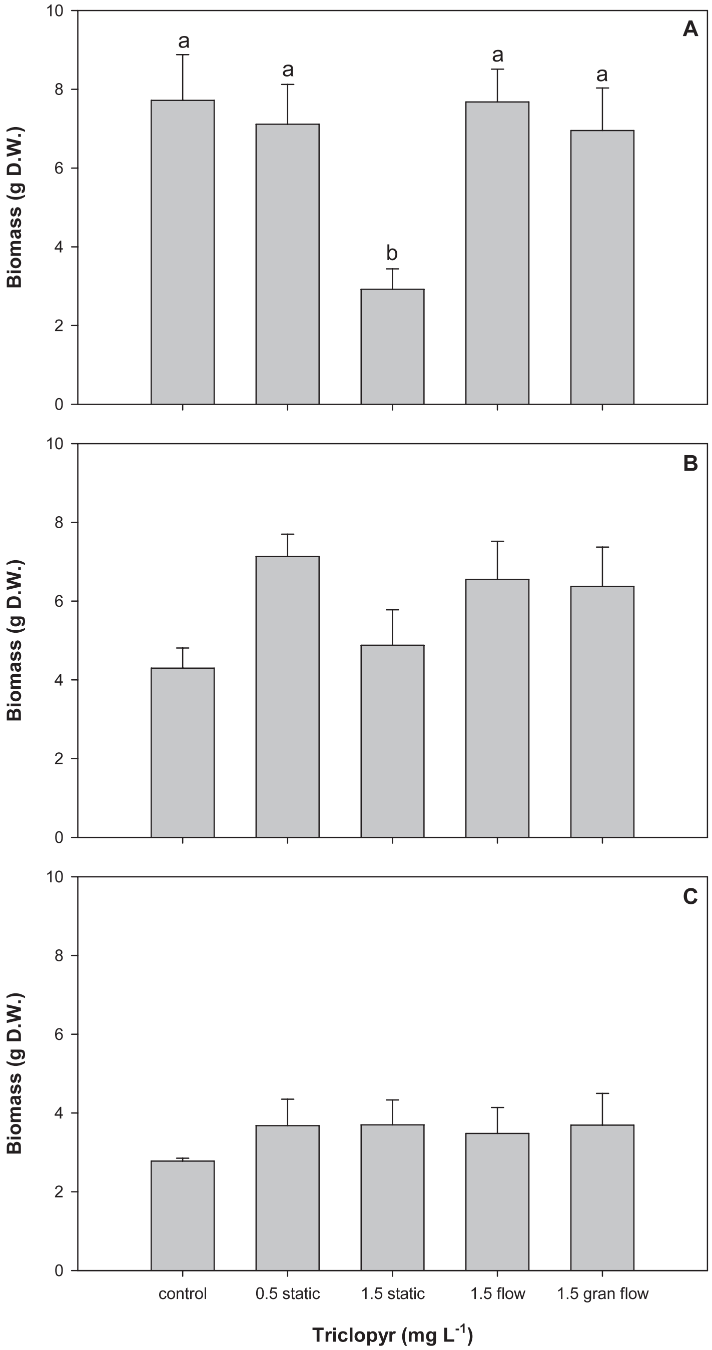

Figure 6. Mean (± SE) dry shoot weight of (A) American pondweed, (B) Illinois pondweed, and (C) vallisneria treated with triclopyr on March 3 and April 18, 2011. Treatments included static exposures to liquid triclopyr at 0.5 and 1.5 mg L^{-1} and liquid and granular applications at 1.5 mg L^{-1} in flowing mesocosms set to yield a half-life of 5 h. No significant interaction was detected between rate and timing of application; therefore, March and April data were pooled for each species. Bars sharing the same letter are not different (Student-Newman-Keuls; $P < 0.05$). When no letters are present, no significant differences were detected among treatments (Student-Newman-Keuls; $P < 0.05$).

the static treatments, neither timing nor use rate had an impact on EWM efficacy. These results are in direct contrast to Study 1 (conducted under flowing conditions) (Figure 1), and despite lower initial biomass and metabolic activity of EWM in the early season, the low-rate static treatments were highly effective in providing control.

Both the liquid and granular treatments under high–water-exchange scenarios (5 h) had regrowth present at the time of harvest. Late-season applications resulted in harvest of four to five times less biomass than early-season applications. The early-season granular applications were more effective at reducing EWM biomass compared to the liquid application (Figure 5). Biomass following late-season applications remained below pretreatment levels at the time of harvest; however, the biomass that was present was healthy and recovering from herbicide exposure. Although the early-season applications provided a longer period of recovery, the decision to harvest at a single date was based on the desire to mimic an operational outcome. Visual observations suggested limited impacts following the early-season applications, whereas the late-season treatments showed a more characteristic collapse of biomass following the application.

There was no interaction between rate and time for any of the native species, and therefore, biomass data were pooled for the early- and late-season treatments. For American pondweed, only the static treatment of 1.5 mg L^{-1} was different than the untreated control (Figure 6A). There were no differences detected between any treatments for either Illinois pondweed (Figure 6B) or vallisneria (Figure 6C). The ability of these natives to withstand a static treatment of 1.5 mg L^{-1} following both early- and late-season applications would suggest a high level of tolerance to triclopyr regardless of the stage of vegetative growth. Although none of these species is recognized as being highly susceptible to triclopyr, these results confirm that tolerance to high concentrations and sustained exposures of triclopyr was consistent between newly emerging growth and more established plants. It is recommended that future research in this area include species that show greater susceptibility to triclopyr.

Prior laboratory and mesocosm trials have generally relied on evaluating herbicide impacts on plants that were recently established from apical shoots (Netherland and Getsinger 1992), and it has been questioned whether these plants might be more sensitive to herbicides than established plants. Treatment of well-established EWM in mesocosms indicates that plants remained highly sensitive to triclopyr treatment concentrations as low as 0.25 mg L^{-1}. Nonetheless, the ability of EWM to recover from several of the early-season applications under short contact-time (flowing) conditions does suggest that plant phenology (biomass, metabolic activity, leaf surface area available for uptake) may play a key role in response to herbicide treatments. The increased leaf and shoot surface available for herbicide uptake following short-term exposures would potentially result in improved translocation of herbicide to the root crown. In this study, plants were established several months prior to herbicide exposure, and therefore root crowns were well developed at the time of application. To our knowledge, this study design is unique in linking submersed plant phenology, treatment timing, multiple exposure scenarios, and multiple species. It represents a first step in refining and tailoring herbicide CET relationships to address complex factors driven by seasonal ambient conditions. The general lack of response of the native plants across a broad range of triclopyr concentrations provides evidence that early-season treatment strategies would have limited impact on selectivity for species that have a higher tolerance for triclopyr. Additional studies on native species with a greater level of sensitivity to triclopyr should be conducted to describe the relationship between treatment timing and selectivity further.

Results from this study suggest that early-season treatment strategies with triclopyr can be effective for EWM control across a broad range of triclopyr concentrations when extended exposure (> 1 wk) to the herbicide is likely. Early-season applications of triclopyr in areas where the herbicide is subject to rapid clearance from the application site may risk compromising efficacy against EWM.

SOURCES OF MATERIALS

[1]Osmocote®, The Scotts Company, PO Box 606, Marysville, OH 43040.

[2]Renovate® 3, SePRO Corporation, 11550 North Meridian Street Suite 600, Carmel, IN 46032.

[3]Renovate® OTF, SePRO Corporation, 11550 North Meridian Street Suite 600, Carmel, IN 46032.

[4]Strategic Diagnostics Inc., 111 Pencader Drive, Newark, DE 19702-3322.

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