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Do applications of systemic herbicides when green fruit are present prevent seed production or viability of garlic mustard (*Alliaria petiolata*)?

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Research Article

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Abstract

Garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande] is a biennial invasive plant commonly found in the northeastern and midwestern United States. Although it is not recommended to apply herbicides after flowering, land managers frequently desire to conduct management during this timing. We applied glyphosate and triclopyr (3% v/v and 1% v/v using 31.8% and 39.8% acid equivalent formulations, respectively) POST to established, second-year *A. petiolata* populations at three locations when petals were dehiscing and evaluated control, seed production, and seed viability. POST glyphosate applications at this timing provided 100% control of *A. petiolata* by 4 wk after treatment at all locations, whereas triclopyr efficacy was variable, providing 38% to 62% control. Seed production was only reduced at one location, with similar results regardless of treatment. Percent seed viability was also reduced, and when combined with reductions in seed production, resulted in a 71% to 99% reduction in number of viable seeds produced per plant regardless of treatment. While applications did not eliminate viable seed production, our findings indicate that glyphosate and triclopyr applied while petals are dehiscing is a viable alternative to cutting or hand pulling at this timing, as it substantially decreased viable *A. petiolata* seed production.

Introduction

Garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara & Grande] is a biennial invasive forb originally from Europe (Nuzzo 2000). It was introduced into North America in the 1800s (Grieve 2013) and by 2000 had spread to 34 U.S. states and four Canadian provinces (Nuzzo 2000). The continued spread of *A. petiolata* is well documented, including more than 50,000 unique occurrences in the United States, with most occurring in the northeastern and midwestern United States (EDDMapS 2020). Although *A. petiolata* commonly infests forests in partial sunlight, it can also be present in a wide range of habitats, including railroad ballast, floodplains, and xeric ridgetops (Byers and Quinn 1998; Nuzzo 2000). Forest understories dominated by *A. petiolata* are associated with negative impacts to native ecosystems, including low richness and diversity of native herbaceous vegetation (Anderson et al. 1996; Nuzzo 2000) and reduced growth of tree seedlings that depend on arbuscular mycorrhizal fungi (Burke 2008; Stinson et al. 2006).

While a range of management activities are effective for *A. petiolata*, hand pulling is the most common method used to control adult (second-year) plants. Hand pulling is effective (Panke and Renz 2012; Shartell et al. 2012) and is typically conducted when plants are bolting to fruiting, but this technique requires more time and results in more soil disturbance compared with other control methods. Additionally, hand pulling requires annual efforts and multiple years to maintain high levels of control. Herbicides are also effective for controlling *A. petiolata*, with applications made to green rosettes during the fall, winter, or spring between seedling germination and flower stalk elongation providing high levels of control (Becker et al. 2013; Frey et al. 2007; Nuzzo 2000). While research has shown the period between fall and spring to be the ideal time for control and the prevention of seed production (Shartell et al. 2012), many obstacles prevent herbicide application during this time frame. Snow and excessive rain can limit site access, and cold temperatures can reduce effectiveness of herbicides (Bryson 1987, 1988; Reddy 2000; Roggenbuck et al. 1990). Additionally, competing priorities, including brush removal in fall and winter and prescribed fire in spring, conflict with timely herbicide application between fall and spring. Applications later in *A. petiolata*'s development would provide additional time to

Management Implications

Glyphosate and triclopyr POST applications to rosettes in the early spring are standard treatments used to manage *Alliaria petiolata* (garlic mustard). However, weather and other priorities limit the window for management, forcing field practitioners to utilize more labor-intensive methods such as hand pulling. It is not known how late in the development of *A. petiolata* these herbicides can be applied to prevent viable seed production. Because prevention of soil seedbank replenishment is a key management factor for effective long-term control of biennial invasive species, we hypothesized late spring foliar herbicide applications to second-year *A. petiolata plants* when flower petals were dehiscing could be an effective management tool if seed production or viability is eliminated. Our study indicated that glyphosate applications at this timing provided 100% control of *A. petiolata* plants by 4 wk after treatment at all locations, whereas triclopyr efficacy was inconsistent. Although both glyphosate and triclopyr decreased viable seed production to nearly zero at one of our three study locations, the same treatments produced significant amounts of viable seed at the other two locations. Our findings suggest late spring glyphosate and triclopyr applications should not be recommended over early spring applications to rosettes for *A. petiolata* management, as our late spring application timing did not prevent viable seed production, and may require multiple years of implementation to eradicate populations. Nonetheless, this application timing holds value in areas devoid of desirable understory vegetation compared with no management practices or mechanical management options, including hand pulling when fruit are present, as overall viable seed production was reduced to levels similar to those seen with these treatments.

Figure 1. Stage of *Alliaria petiolata* development during treatment application at all three sites. Petal dehiscence has initiated, and green fruit are present and developing.

manage populations when precipitation is less frequent, daytime maximum temperatures are optimal for uptake of systemic herbicides, and other land management priorities are reduced. If effective, this application timing could extend the management window for *A. petiolata*, but herbicide efficacy remains unknown, and production of viable seed is a concern when fruit are present during herbicide application.

We evaluated late spring herbicide applications when *A. petiolata* petals were dehiscing and green fruits were just beginning to develop. The objectives of this study were to evaluate the effectiveness of foliar glyphosate or triclopyr applications just after petal dehiscence to control *A. petiolata* and prevent viable seed production. Three field locations were established for 1 yr (two in Wisconsin and one in Illinois) in either 2017 or 2018 to evaluate the effectiveness of this timing on *A. petiolata* control, seed production, and seed viability.

Materials and Methods

Study Sites

The study was conducted at two different locations in Wisconsin (2017 and 2018) and at one location in Illinois (2018). The experiment conducted in 2017 was located near Prairie du Sac (Prairie), WI (43.352°N, 89.758°W), and the experiments conducted in 2018 were located near Fitchburg, WI (43.019°N, 89.454°W), and Dixon Springs, IL, at the Dixon Springs Agricultural Center (Dixon) (37.428°N, 88.664°W). All research sites consisted of forested areas with the understory having at least 75% cover of *A. petiolata* (Figure 1). Soil types were silt loams at both Wisconsin locations with 2% to 3% organic matter, whereas the Illinois site was a silt loam with 0.6% organic matter (USDA-NRCS 2020). Total rainfall at Prairie, Fitchburg, and Dixon during the experimental period (June and July at Prairie and Fitchburg; May and June at Dixon) was 46% to 75% above the 30-yr monthly average, among sites. However, monthly temperatures were similar to the 30-yr average during the experimental periods (Table 1).

Experimental Design, Measurements, and Analysis

Herbicides were applied at Prairie on May 27, 2017, Fitchburg on May 31, 2018, and at Dixon on May 7, 2018. All herbicides were applied POST to *A. petiolata* plants with green fruit present and dehiscing petals (Figure 1). Two herbicide treatments and one nontreated control were established in 1.5 by 5 m plots (7.5 m^2 plots) arranged in a randomized complete block design with three replications at Prairie and four replications at Fitchburg and Dixon. Herbicide treatments consisted of triclopyr (1% v/v of a 31.8% acid equivalent formulation; Garlon® 3A, Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN, USA) plus methylated seed oil (1% v/v) and glyphosate (3% v/v of a 39.8% acid equivalent formulation; Roundup PowerMax®, Monsanto Company, 800 N Lindbergh Boulevard, St Louis, MO, USA) plus ammonium sulfate (9.6 g L^{-1}). All herbicide treatments were applied to the foliage of individual plants until just before the point of runoff. Treatments were applied with a CO_2-pressurized backpack sprayer using a single-nozzle boom equipped with one TeeJet® 11002VS nozzle (TeeJet® Technologies, 200 W. North Ave, Glendale Heights, IL,

Table 1. Average monthly temperatures and precipitation during the experimental period at each research site with 30-yr monthly averages shown for comparison.[a]

		Prairie		Fitchburg		Dixon	
Year	Month	Average temp.C	Total precip. mm	Average temp. C	Total precip. mm	Average temp. C	Total precip. mm
2017	April	9.9	146	—	—	—	—
	May	13.4	92	—	—	—	—
	June	19.9	137	—	—	—	—
	July	20.8	258	—	—	—	—
	August	19.3	77	—	—	—	—
	September	18.2	40	—	—	—	—
	October	11.9	76	—	—	—	—
2018	April	—	—	3.1	66	8.9	124
	May	—	—	18.4	244	21.4	141
	June	—	—	20.4	280	24.1	280
	July	—	—	21.9	95	24.4	95
	August	—	—	21.9	289	23.6	75
	September	—	—	18.1	197	21.9	168
	October	—	—	9.2	156	13.7	69
30- yr avg.[b]	April	7.1	102	7.9	106	13.1	125
	May	13.9	105	14.3	108	17.9	135
	June	19.3	142	19.6	140	22.7	107
	July	21.4	111	21.7	117	24.8	114
	August	20.1	120	20.9	108	24.2	82
	September	15.7	89	16.4	91	20.0	84
	October	8.9	69	9.8	70	13.7	88

[a]Abbreviations: Prairie, Prairie du Sac; Dixon, Dixon Springs Agricultural Center; temp., temperature; precip., precipitation.
[b]Midwest Regional Climate Center (2020) 1987–2018 averages.

Table 2. Visual estimates of control and SE (in parentheses) at 2, 4, and 6 wk after treatment of *Alliaria petiolata*.[a]

	Prairie			Fitchburg			Dixon		
Treatments[b]	2 WAT	4 WAT	6 WAT	2 WAT	4 WAT	6 WAT[c]	2 WAT	4 WAT	6 WAT
	% control[d]								
Glyphosate (3% v/v)	78 a	100 a	100 a	91 a	100 a	—	98 a	100 a	100 a
Triclopyr (1% v/v)	20 b	43 b	73 b	29 b	38 b	—	48 b	62 b	94 a
Untreated	0 c	0 c	0 c	0 c	0 c	—	0 c	0 c	0 b
P-value	0.0003	0.0004	0.0001	0.0001	0.0001	—	0.0003	0.0005	0.0001

[a]Abbreviations: Prairie, Prairie du Sac; Dixon, Dixon Springs Agricultural Center; WAT, weeks after treatment.
[b]Treatments were applied when green fruits were present and petals were dehiscing. Glyphosate and triclopyr applications were made using 39.8% and 31.8% acid equivalent formulations, respectively.
[c]6 WAT control data were not collected at the Fitchburg site.
[d]Different lowercase letters within a column indicate differences in treatment LS means (P < 0.05, PDIFF method for multiple comparison).

USA) estimated to deliver 280 L of spray solution ha^{-1}. While applications targeted second year *A. petiolata* plants, spray solution also contacted rosettes underneath treated plants.

Effectiveness of treatments was evaluated by visually estimating control of *A. petiolata* and measuring seed production and viability. Visual estimates of control were performed 2, 4, and 6 wk after treatment on a rating scale of 0% to 100%, with 0% equivalent to no control and 100% equivalent to complete plant death. When *A. petiolata* siliques matured on the plant but before dehiscence between 6 and 8 wk after treatment, three plants were harvested from each plot. All seeds were removed from siliques, air-dried, pooled, and counted. Seed viability was estimated by subjecting subsamples of 50 seeds from each plot to a tetrazolium test (Sosnoskie and Cardina 2009). Tetrazolium-tested seeds were then dissected and analyzed using a dissecting microscope and classified as viable or nonviable using AOSA/SCST guidelines (AOSA/SCST 2010). Absolute number of viable seeds produced was estimated by multiplying the percentage of viable seed by the average number of seeds produced per plant for each treatment.

Initial analyses found a site by treatment interaction, and therefore each site was analyzed separately using PROC MIXED in SAS (SAS release 9.3, SAS Institute, 100 SAS Campus Drive, Cary, NC, USA). Treatments were considered fixed effects, whereas block was considered random. Differences were declared when P < 0.05, and mean separation was based on the PDIFF option of LSMEANS in SAS. Total seed production and seed viability data were square-root transformed to meet the ANOVA assumptions of normality and homoscedasticity; however, untransformed means are presented.

Results and Discussion

Control differed with respect to herbicide treatments at all research sites and evaluation timings (Table 2). Although our main study goal is to quantify the impacts of herbicide treatments on *A. petiolata* seed production and viability, visual assessment of control on the target weed species was collected as it is useful information for land managers (Enloe et al. 2016). At 2 and 4 wk after treatment, glyphosate provided *A. petiolata* control of 78% to 100% compared with triclopyr, which only provided 20% to 62% control at all locations (Table 2). By 6 wk after treatment, control was >90% with both treatments at Dixon; however, glyphosate maintained higher

Table 3. Total number of seeds produced per plant, percentage of viable seeds produced, and total number of viable seeds produced per plant of established *Alliaria petiolata* at 41–56 d after treatment.[a]

	Seed production[c]			Seed viability[c]			Seed viability[c]		
Treatments[b]	Prairie	Fitchburg	Dixon	Prairie	Fitchburg	Dixon	Prairie	Fitchburg	Dixon
	———no. seeds plant^{-1}———			———%———			———no. viable seeds plant^{-1}———		
Glyphosate (3 % v/v)	712	189	142 b	23.9 b	46.5 ab	1.9 b	170 b	88 b	2 b
Triclopyr (1 % v/v)	1,320	207	194 b	2.0 c	22.8 b	0.8 b	26 b	48 b	2 b
Untreated	1,600	385	540 a	77.6 a	77.9 a	81.6 a	1,240 a	300 a	440 a
P-value[d]	NS	NS	0.0013	0.0027	0.045	<0.0001	0.0017	0.0264	<0.0001

[a]Abbreviations; Prairie, Prairie du Sac; Dixon, Dixon Springs Agricultural Center.
[b]Treatments were applied when green fruit were present, and petals were dehiscing. Glyphosate and triclopyr were made using 39.8% and 31.8% acid equivalent formulations, respectively.
[c]Different lowercase letters within a column indicate differences in treatment LS means (P < 0.05, PDIFF method for multiple comparison).
[d]P-values listed as not significant if ≥0.10.

control than triclopyr at Prairie (100% vs. 73%, respectively) (Table 2). Others have found many active ingredients to be effective at controlling *A. petiolata* in addition to glyphosate and triclopyr, but applications are recommended during the rosette stage (Panke and Renz 2012). The poor control of adult *A. petiolata* plants by triclopyr could be explained by the late *A. petiolata* development stage at application, which could have substantial management implications, as Pardini et al. (2009) estimate that >85% control of adult plants is required annually to reduce or eliminate populations. Given that this relationship is density dependent (Pardini et al. 2009), more research is required to understand what threshold of control is required to obtain *A. petiolata* population reductions.

Effective long-term weed management strategies require understanding how management practices impact weed seed production and viability, especially for species like *A. petiolata* that rely on seed production to sustain populations. In our study, herbicide treatment effects on *A. petiolata* seed production per plant were identified at Dixon (P < 0.01); however, no difference among treatments was detected at Fitchburg or Prairie (P ≥ 0.10) (Table 3). At Dixon, glyphosate and triclopyr treatments provided equivalent reductions in *A. petiolata* seed production (74% and 64%) compared with nontreated plants (540 seeds plant^{-1}). These findings align with previous research that documents seed reductions when herbicides are applied to flowering weeds (Clay and Griffin 2000; Taylor and Oliver 1997; Walker and Oliver 2008; Menalled et al. 2018). However, at Prairie and Fitchburg, we did not detect a significant herbicide treatment effect compared with nontreated plants (Table 3; P > 0.05), suggesting reduction in seed production is highly variable. Other research has found that POST herbicide applications failed to reduce the number of seeds (Steckel et al. 1990) or that seed production per plant was more affected by crop canopy than herbicide treatments (Mosqueda et al. 2020). Thus, weed seed production responses to POST weed control strategies vary, as they can be impacted by different factors such as weed species, time of application, and other edaphoclimatic conditions.

Although herbicide applications did not result in significantly lower *A. petiolata* seed production in two of our three research locations, glyphosate and triclopyr applications decreased the percent viability and number of viable seeds per plant compared with the untreated control at all locations (P < 0.01, P < 0.05, and P < 0.01 at Prairie, Fitchburg, and Dixon, respectively) (Table 3). The greatest reduction in seed viability occurred at Dixon, where a >95% reduction in seed viability was observed with glyphosate and triclopyr applications (Table 3). The percent of *A. petiolata* seed viability was highly variable, as it depended on the site and herbicide treatment (<1% to 46.5%). Despite this variability, the total number of viable seeds produced decreased similarly across glyphosate and triclopyr treatments at each location. Glyphosate or triclopyr applications reduced the number of viable seeds anywhere from 71% to 99.5% compared with untreated plants (P < 0.05) but never eliminated viable seed (Table 3). This clearly demonstrates that late spring POST applications of glyphosate and triclopyr can result in production of viable seed; therefore, land managers should consider this before selecting this approach over earlier timings that would prevent seed production.

These results are similar to those seen with hand pulling of *A. petiolata* plants. Shartell et al. (2012) found a 76% reduction in adult *A. petiolata* density 1 mo after treatment, but hand-pulled plants can produce viable seed (Chapman et al. 2012). Despite similar results, hand pulling likely requires increased effort in moderate to large populations and has substantial soil disturbance that can increase the potential for establishment of *A. petiolata* seedlings compared with our approach. These differences in combination with the lack of control of seedling plants the following year (Shartell et al. 2012) highlight the benefits of glyphosate or triclopyr applications in late spring over hand pulling. However, substantial risk to desirable understory vegetation growing among *A. petiolata* exists with this approach. If spray solution contacts these desirable plants' leaves or stems, high levels of injury or even mortality may occur. This hazard may limit the benefit of this approach to areas devoid of desirable species in the forest understory at the time of application.

In summary, our findings indicate that even though late spring POST glyphosate and triclopyr applications decrease *A. petiolata* seed productivity and viability in the year of herbicide application, the later timing should not be recommended over early spring POST applications to rosettes, as the later timing did not completely prevent viable seed production. Glyphosate or triclopyr applied at this late stage of development is a suitable alternative to hand pulling, although both strategies require repeated implementation to reduce or eliminate *A. petiolata* populations. The value of this herbicide application timing may be particularly evident in large sites that have dense infestations of *A. petiolata* and lack desirable understory vegetation sensitive to glyphosate or triclopyr. In these areas, the increased efficiency of this herbicide application method relative to hand pulling could permit managers to manage more area in a given year.

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