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Effects of 2,4-D with and without wiper-applied glyphosate on leafy spurge (*Euphorbia esula*) shoot, shoot regrowth, and root biomass

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Abstract

Outdoor studies were conducted to determine the extent of leafy spurge biomass reduction resulting from broadcast application of 2,4-D (2,244 g ae ha^{-1}) with and without wiper-applied glyphosate. Glyphosate (575 g ae L^{-1}) was applied at 0%, 33%, 50%, and 75% diluted concentrate with a wiper 24 h after 2,4-D was broadcast-applied. Injury estimates and shoot biomass did not differ between plants treated with 2,4-D only or when glyphosate was wiper-applied 21 d after treatment. Shoot regrowth biomass of plants treated with 2,4-D only was approximately 560% greater than nontreated plants 3 mo after treatment. Plants treated with wiper-applied glyphosate exhibited shoot regrowth biomass of less than 10% compared with nontreated plants 3 mo after treatment. Root biomass of plants treated with 2,4-D only (160% of nontreated plants) exhibited a similar pattern of shoot regrowth biomass. Root biomass of plants treated with wiper-applied glyphosate exhibited approximately 50% reductions compared with nontreated plants. All vegetative metrics were equally reduced with all tested concentrations of glyphosate; therefore, all labeled concentrations should be effective. The results of the experiment indicate that broadcast-applied 2,4-D is more effective at reducing leafy spurge biomass with the addition of wiper-applied glyphosate.

Introduction

Leafy spurge is a perennial broadleaf weed that inhabits various disturbed habitats, especially pasture and rangelands (Lym 1998). Management efforts need to be intensive and extensive to minimize its spread because leafy spurge can reproduce both vegetatively and through seed production (Lym 1998; Morrow 1979). Therefore, simply ceasing seed production may not always be effective if the underground rhizomes remain viable (Jacobs et al. 2006; Wicks and Derscheid 1964). Few herbicides applied alone are effective on leafy spurge; effective herbicides include aminocyclopyrachlor (categorized as a Group 4 herbicide by the Weed Science Society of America [WSSA]), imazapic (WSSA Group 2), and picloram (WSSA Group 4) (Lym 2014; Markle and Lym 2001). However, the effectiveness and sole reliance on these herbicides do not provide management longevity without the integration of other tactics (DiTomaso et al. 2017; Lym 1998). Although nonchemical tactics are important for successful leafy spurge management, herbicides remain the most efficient tactic (DiTomaso 2000; Nelson and Lym 2003). The herbicide 2,4-D (WSSA Group 4) is not effective alone in managing leafy spurge, but previous research has shown that 2,4-D in combination with other herbicides can additively increase its effectiveness (Al-Henaid et al. 1993; Gyl & Arnold, 1985; Lym 2000).

Glyphosate (WSSA Group 9) is a nonselective herbicide that controls a wide spectrum of weed species (Duke and Powles 2008). Due to its nonselectivity, this herbicide is rarely applied to pasture or rangeland because it may suppress or kill desirable grasses and forbs. Additionally, glyphosate applied alone is not recommended for leafy spurge management because the herbicide results in molecular changes that can also induce vegetative shoot and adventitious root growth when applied alone (Doğramacı et al. 2014, 2016; Maxwell et al. 1987). Mixing glyphosate and 2,4-D can be effective for managing leafy spurge, but desirable vegetation is injured or killed during broadcast sprays, which can contribute to economic and ecosystem services losses (Gyl & Arnold, 1985; Lym 2000). Wiper-applied herbicides are deployed to selectively manage weeds and allow higher herbicide concentrations to be applied to grasslands while reducing off-target injury to desirable vegetation (Grekul et al. 2005; Leif and Oelke 1990). Picloram has been applied with a wiper application to manage leafy spurge with success (Messersmith and Lym 1985). Wiper-applied glyphosate has also effectively managed Canada thistle (*Cirsium arvense* L.) in sensitive areas containing desirable vegetation (Krueger-Mangold et al. 2002). Because the desirable vegetation is not damaged, plants can still be competitive with later-emerging weeds (Lamb et al. 2024).

Despite the lack of efficacy against leafy spurge from broadcast-applied glyphosate, the greater herbicide concentrations associated with a wiper application as a follow-up could increase the longevity of management. Since 2,4-D effectiveness is largely dependent on being mixed with another herbicide, glyphosate could be sequentially applied with a wiper to manage leafy spurge. Because 2,4-D and picloram have been applied extensively to manage leafy spurge, the inclusion of glyphosate could provide an additional management tool and disrupt previous selection pressure. Because 2,4-D and glyphosate are both readily absorbed and translocated throughout treated leafy spurge plants, sequential applications of both herbicides could increase control of the weed (Doğramacı et al. 2014; Maxwell et al. 1987). The objective of this research was to determine leafy spurge biomass reductions, including treated shoots, and shoot and root regrowth resulting from a broadcast application of 2,4-D alone and in combination with sequential wiper-applied glyphosate at various concentrations.

Materials and Methods

Plant Establishment

Leafy spurge plants were collected from a field located at South Dakota State University in Brookings County, South Dakota (44.325677°N, 96.779732°W) in mid-June 2024. Plants were selected if yellow bracts were present and approximately 40 cm in height. Plants were carefully dug and transplanted into a 20-cm (6,280 cm^3) pot containing an equal mixture of Miracle-Gro (The Scotts Company, Marysville, OH) and field soil from the weed collection site (Marysland loam; a fine-loamy over sandy or sandy-skeletal, mixed, superactive, frigid Typic Calciaquolls). Plants were maintained outdoors under realized temperatures (average temperature: 27 C day/15 C night) and photoperiod (15-h day/ 9-h night) for the duration of the 4-mo study. Pots were watered to saturation daily for 2 wk. Watering of pots to saturation thereafter occurred approximately every 2 d for the duration of the study.

Broadcast and Wiper Application

Treatments were arranged as a randomized complete block design with three replications. The experiment was conducted twice, when the plant collection and run initiation were separated by 1 wk. After the plants were acclimated for 2 wk, they were treated (excluding nontreated controls) with 2,4-D ester (Weedone LV4 Solventless, 480 g ae L^{-1}; Nufarm, Cary, NC) applied at rate of 2,244 g ae ha^{-1}. The herbicide was applied using a CO_2-powered backpack sprayer at an output of 180 L ha^{-1} using Turbo TeeJet 8003 nozzles (TeeJet Technologies, Glendale Heights, IL) 50 cm above the target plan. Leafy spurge plants were treated when they were approximately 40 cm high and yellow bracts were present. The wiper-applied treatment occurred 24 h following the initial 2,4-D application. This delay was implemented to ensure the 2,4-D was absorbed into the plant and not transferred onto the wiper. The wiper applicator was positioned approximately halfway up the plant (20 cm) to simulate an application of herbicide above desirable vegetation growth height (Carlassare and Karsten 2002; Washburn and Seamans 2007). The upper portion of the plant was treated-to-wet prior to runoff. The frame of the wiper applicator was constructed with 1.9-cm PVC pipes with two 1.6-cm diameter cotton ropes (approximately 2.5 cm wide and 18 cm in length) affixed to the end of the frame (Figure 1). The glyphosate (Roundup Powermax 3, 575 g ae L^{-1}; Bayer Cropscience, St. Louis, MO)

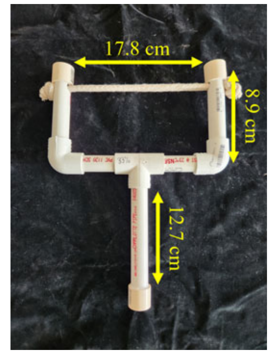

Figure 1. Wiper applicator schematic for the wiper-applied glyphosate experi.

concentrations included 0% (no glyphosate), 33%, 50%, and 75%, and the various concentrate dilutions were achieved by mixing glyphosate with distilled water. These concentrations were selected based on the herbicide label (Anonymous 2020). Separate wiper applicators were constructed for each glyphosate concentration tested. The wiper frames were disassembled prior to treatment and the wiper was submerged in a 300-mL solution of the respective concentrations until it was saturated.

Injury to leafy spurge was estimated 21 d after the 2,4-D treatment (DAT) using a rating scale of 0% to 100%; where 0% equals no injury observed and 100% equals plant death. After the injury evaluations, plants were excised at the surface of the potting media and weighed to collect the fresh biomass. The plant samples were then placed in paper bags and oven-dried at 50 C for 48 h. All plant samples were then weighed to collect the dry biomass in grams. Pots were maintained as described above for an additional 3 mo after 2,4-D treatment (MAT). Shoot regrowth was collected, dried, and weighed as described above. After shoot regrowth was collected, pots were not watered for 1 wk to dehydrate the soil. Roots were extracted from the dried potting media and additional potting media was cleaned from the roots via a water rinse. Roots were subsequently dried and weighed as described above. Dry biomass reduction for the treated shoot material (21 DAT), shoot regrowth (3 MAT), and roots (3 MAT) was calculated by dividing the dry biomass of the treated plants by dry biomass of the nontreated plants.

Statistical Analysis

Injury estimates and dry biomass reductions were subjected to ANOVA using the *Glimmix* procedure with SAS (v. 9.4; SAS Institute, Cary, NC) at a significance level of $\alpha = 0.05$. Glyphosate concentration was considered a main effect, whereas the replications and experimental runs were considered random effects. Replication and experimental run were considered random to allow inferences to be made across broader conditions (Blouin et al. 2011; Moore and Dixon 2015).

Concentration-response curves for injury estimates were fit with a three-parameter log-logistic equation with Sigmaplot 15 software (Grafiti LLC, Palo Alto, CA) as follows:

$$y = \frac{a}{\left[1 + \left(\frac{x}{x0}\right)^{b}\right]} \quad (1)$$

where a is the upper asymptote, x is the glyphosate concentration, $x0$ equals the effective concentration to cause 50% injury (EC_{50}), and b is the slope at $x0$.

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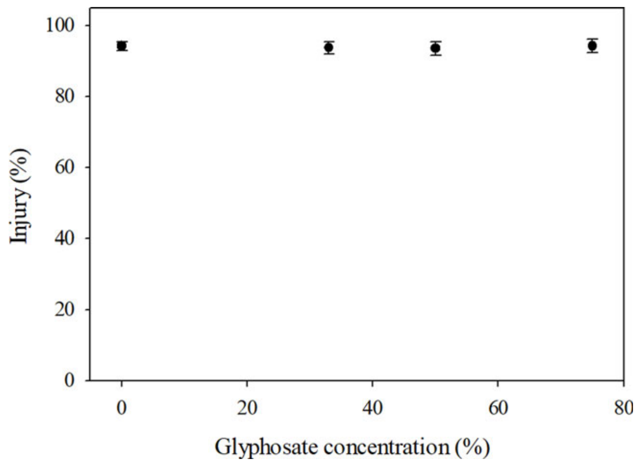

Figure 2. Injury estimates for leafy spurge treated with 2,4-D ester (0%) and the addition of various concentrations of wiper-applied glyphosate 21 d after treatment. Injury estimates could not be modeled across glyphosate concentrations due to a lack of differential response. The injury estimates of nontreated plants are not included. Error bars represent the standard error of the mean.

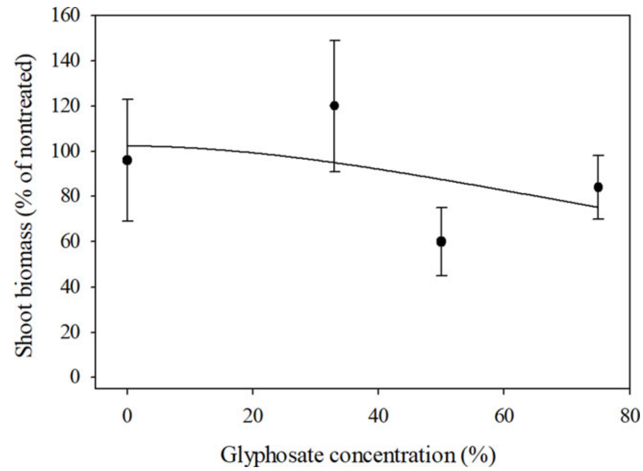

Figure 3. Concentration-response curve fit to a three-parameter log-logistic equation for shoot biomass of leafy spurge 21 d after being treated with 2,4-D and various concentrations of wiper-applied glyphosate. Error bars represent the standard error of the mean.

Glyphosate concentration-response curves for dry biomass reductions of shoot, shoot regrowth, and root were also fit with the three-parameter log-logistic equation with Sigmaplot 15 software where a is the upper asymptote, x is the glyphosate concentration, $x0$ equals the GR_{50} (concentration to reduce biomass by 50%]) rate, and b is the slope at $x0$. The GR_{90} (concentration to reduce biomass by 90%) values were derived from the respective equations.

Results and Discussion

Treated Shoot

At 21 DAT, glyphosate concentration did not influence injury estimates ($P = 0.97$) or shoot biomass ($P = 0.3$) of leafy spurge plants that had been treated with 2,4-D. Injury estimates were approximately 94% for all treatments, and therefore, an EC_{50} could not be modeled (Figure 2). All herbicide-treated shoot biomass ranged from 60% to 120% of nontreated plants on average (Figure 3). The GR_{50} value (129%) derived from the model was extrapolated outside of the tested concentrations and not achievable, and therefore it was not reliable (Table 1; Figure 2). The GR_{90} value could not be modeled due to the lack of response (Figure 3). These results suggest that 2,4-D applied alone as broadcast or in combination with wiper-applied glyphosate causes greater than 90% injury but it does not reduce shoot biomass of leafy spurge within 21 DAT.

Shoot Regrowth

Glyphosate concentration influenced shoot regrowth of leafy spurge 3 MAT ($P = 0.0012$). Leafy spurge shoot regrowth biomass treated with 2,4-D only was approximately 560% of the biomass of nontreated plants (Figure 4). Leafy spurge shoot regrowth was <10% of the biomass of nontreated plants when treated with 2,4-D and when combined with any of the tested wiper-applied glyphosate concentrations (Figure 4). The GR_{50} and GR_{90} values for shoot regrowth were obtained at glyphosate concentrations of 7% and 28%, respectively. (Figure 4; Table 1). These results suggest the addition of wiper-applied glyphosate to 2,4-D can significantly reduce leafy spurge regrowth, but there is no difference in biomass reduction between the glyphosate concentrations tested in these experiments (Figure 5).

Root Biomass

Glyphosate concentration influenced the root biomass of treated leafy spurge 3 MAT ($P = 0.0022$). The root biomass of plants treated with 2,4-D only was approximately 160% of the root biomass from nontreated plants. Herbicide-treated leafy spurge root biomass was between 35% to 49% of the root biomass of nontreated plants (Figure 6). The GR_{50} value occurred at a glyphosate concentration of 8%, whereas a GR_{90} value could not be calculated due to a lack of root biomass reductions (Figure 6; Table 1). Compared with nontreated plants, the root biomass of leafy spurge plants was decreased by at least 50% when the labeled concentrations of glyphosate were applied with a wiper (Figure 7).

The results of this experiment indicate that leafy spurge treated with 2,4-D and subsequently with or without wiper-applied glyphosate does incur injury, but shoot biomass is not reduced within 21 DAT. However, at 3 MAT, shoot and root regrowth were significantly increased when leafy spurge plants were treated with 2,4-D only compared with the regrowth exhibited by pants that received the other treatments. Although single applications of 2,4-D are generally not efficacious on leafy spurge, the integration of wiper-applied glyphosate does provide an additional herbicide that is rarely used in pasture/rangeland settings or around sensitive sites for targeted weed management (Gyl & Arnold, 1985; Krueger-Mangold et al. 2002). Although 2,4-D + glyphosate applied via broadcast is effective against leafy spurge, many land managers may not want to use this mixture due to undesirable vegetation injury or death of useful plants (Gylling and Arnold 1985; Lym 2000). Since the wiper provides a means of selective control with a nonselective herbicide, the leafy spurge plants are managed without injuring or killing desirable vegetation which leads to desirable vegetation competition, species richness, and increased land value (Krueger-Mangold et al. 2002; Lamb et al. 2024). Previous research has shown that leafy spurge management increases when desirable vegetation is competitive (Lym and Tober 1997). Since 2,4-D broadcast application followed by the wiper-applied glyphosate reduced leafy spurge shoot and root biomass, this protocol may be useful in slowing the spread of the infestation.

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Table 1. Parameter estimates from the three-parameter log-logistic equations for biomass of treated-shoots, shoot regrowth, and roots.[a]

	Regression parameters[b]					
	a	x^0	*b*	GR_{50}	GR_{90}	r^2
Shoot	102.3	129.4	1.9	129[c]	NA	0.2
Shoot regrowth	560	6.1	2.6	6	28	0.99
Root	160	7.6	0.5	8	NA	0.99

[a]Abbreviations: GR_{50}, concentration (% diluted concentrate) to reduce biomass by 50%; GR_{90}, concentration to reduce biomass by 90%; NA, not achieved; NA, not achievable.
[b]For regression parameters, *a* is the upper asymptote, x^0 equals the GR_{50}, and *b* is the slope at x^0.
[c]The GR_{50} value is not achievable, and therefore should not be considered reliable.

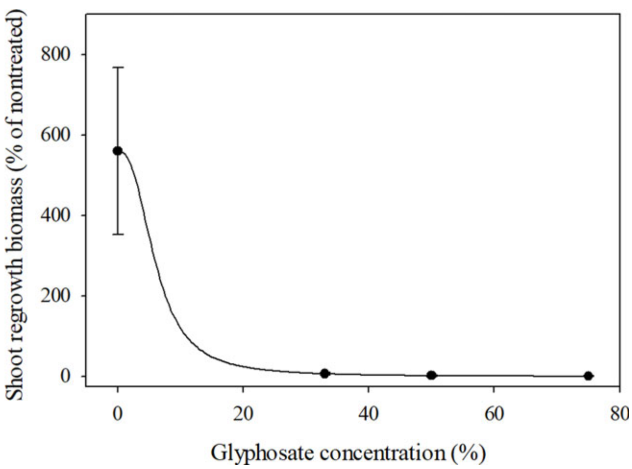

Figure 4. Concentration-response curve fit to a three-parameter log-logistic equation for shoot regrowth biomass of leafy spurge 21 d after being treated with 2,4-D and various concentrations of wiper-applied glyphosate. Error bars represent the standard error of the mean.

Figure 5. Visual representation of shoot regrowth of leafy spurge 3 mo after treatment that were (A) nontreated, (B) treated with 2,4-D, and (C) treated with 2,4-D followed by 33% glyphosate wiper-applied. Not shown is 2,4-D followed by 50% and 75% glyphosate wiper-applied because no regrowth occurred.

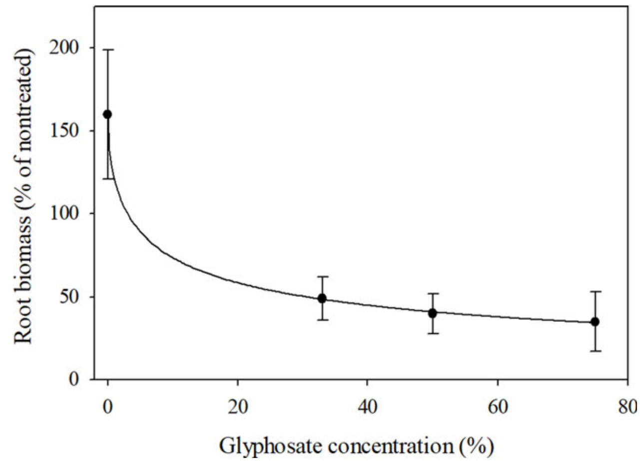

Figure 6. Concentration-response curve fit to a three-parameter log-logistic equation for root biomass of leafy spurge 3 mo after being treated with 2,4-D and various concentrations of wiper-applied glyphosate. Error bars represent the standard error of the mean.

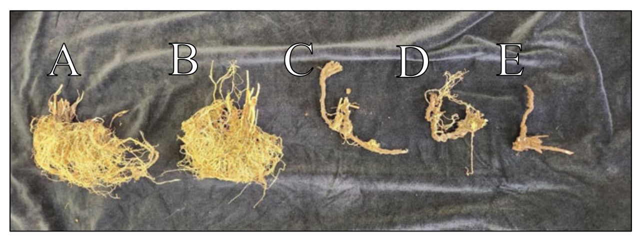

Figure 7. Visual representation of root biomass of leafy spurge shown 3 mo after treatment that were (A) nontreated, (B) treated with 2,4-D, and those treated with 2,4-D followed by (C) 33%, (D) 50%, and (E) 75% glyphosate wiper-applied.

While 2,4-D in addition to wiper-applied glyphosate was effective in this research, we caution not to overuse this tactic. Picloram has been extensively and intensively applied to manage leafy spurge; however, the effectiveness of this herbicide has gradually decreased, suggesting resistance evolution (Lym et al. 1996). Other weeds have evolved resistance to glyphosate through recurrent selection (Busi and Powles 2009; Zelaya and Owen 2005). Additionally, when new herbicides are used and applied recurrently, weed community shifts can occur (Culpepper 2006; Hodgskiss et al. 2022). This herbicide program using the combination of both herbicides should reduce selection pressure, but reliance should be avoided (Lake et al. 2023; Renton et al. 2024).

Even though broadcast applications of glyphosate are not effective and can increase vegetative growth, the results from this research suggest that the relatively great concentrations of glyphosate applied with a wiper may be more effective at managing leafy spurge. Glyphosate alone applied with a wiper should be evaluated to determine why the sequential applications described here were effective. Future research should investigate integrating wiper-applied glyphosate with other effective herbicides (i.e., picloram and imazapic) and nonherbicide tactics (i.e., biocontrol with the leafy spurge flea beetle [*Aphthona* spp.] and mowing). Research should investigate tandem broadcast and wiper applications on one unit to reduce the trips needed to manage weeds. Mixtures of 2,4-D (and related herbicides) and glyphosate applied with a wiper could be used in areas where sensitive forbs are desirable. Results from this research suggest that 2,4-D plus wiper-applied glyphosate is effective at reducing leafy spurge regrowth in comparison to 2,4-D applied alone, and further research will aim to validate these findings under field conditions. Abiotic and edaphic factors influence herbicide activity and plant growth; thus, realized conditions may affect the effectiveness of this herbicide program (Ganie et al. 2017; Hammerton 1967; Moxness and Lym 1989). The long-term aboveground and belowground regrowth should also be quantified to determine how often a

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follow-up tactic will need to be implemented. Since leafy spurge can be genetically diverse, this herbicide program should be tested on leafy spurge populations from varying genetically distinct populations and under site-specific production practices (Liu et al. 2023; Rowe et al. 1997).

Practical Implications

Leafy spurge is a perennial weed that is difficult to manage despite extensive efforts to effectively manage it. High levels of injury were observed with all herbicide treatments, but short-term (21 DAT) biomass reduction of leafy spurge with any treatment was not evident. At 3 MAT plants treated with broadcast-applied 2,4-D exhibited increased biomass compared with nontreated plants, whereas plants treated with broadcast-applied 2,4-D and wiper-applied glyphosate exhibited significant biomass reductions compared with nontreated plants. Since the various concentrations of glyphosate applied with the wiper resulted in similar treated shoot, shoot regrowth, and root biomass reductions, land managers can use the lower concentration (33%), which can decrease costs and the amount of herbicide being applied the environment. These results also indicate that leafy spurge treated with 2,4-D only can result in increased vegetative growth, which could exacerbate the spread of infestations. Therefore, providing more evidence that 2,4-D alone is not effective for managing leafy spurge. While 2,4-D in addition to wiper-applied glyphosate was effective in this research, caution must be taken not to overuse this tactic.

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