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Differential Response of Four *Trifolium* Species to Common Broadleaf Herbicides: Implications for Mixed Grass-Legume Swards

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Clovers are commonly included as utility plants within mixed grass swards, such as pastures and roadside right-of-ways. As such, they provide supplemental nitrogen, quality forage, and insect habitat. Yet weed control within mixed swards is often hampered by the lack of selective herbicides that are tolerated by clovers. Differential tolerance of legumes to common row-crop and pasture herbicides has previously been reported, yet little information is available that is specific to clover species. Herbicide injury of clover is often inconsistent, hypothetically due to differential species tolerance. Field and greenhouse experiments were conducted with the objective of testing differential tolerance amongst four clover species. Our experiments suggest varying tolerances amongst clover species and common broadleaf herbicides. Only imazaquin control differed due to species; however, treatment by clover interactions were further demonstrated due to variable reductions in clover height. Imazaquin, 2,4-D, 2,4-DB, and triclopyr height reductions differed due to clover species. Differential clover response to herbicide treatment should be an important consideration when managing mixed grass–clover swards and should be accounted for in future research. On a more practical level, our experiments demonstrate a range of herbicides that effectively control clover species, including atrazine, dicamba, clopyralid, 2,4-D, triclopyr, metsulfuron, and trifloxysulfuron. However, results suggest that 2,4-DB, imazethapyr, and bentazon are candidate herbicides for weed control in scenarios in which clover is a desirable crop.
Nomenclature: 2,4-D; 2,4-DB; atrazine; bentazon; clopyralid; dicamba; imazaquin; imazethapyr; MCPA; metsulfuron; triclopyr; trifloxysulfuron; ball clover, *Trifolium nigrescens* Viv.; crimson clover, *Trifolium incarnatum* L. TRFIN; small hop clover, *Trifolium dubium* Sibth. TRFDU; white clover, *Trifolium repens* L. TRFRE.
Keywords: Biodiversity, grass-clover swards, herbicide tolerance, legume inclusion.

Los tréboles son comúnmente incluidos como plantas útiles dentro de zonas con coberturas mixtas de zacates, tales como pastizales y bordes de caminos. De tal forma, que brinden nitrógeno suplementario, calidad de forraje y hábitat para insectos. Sin embargo, dentro de esas zonas de cobertura mixta, el control de malezas se ve frecuentemente obstaculizado por la ausencia de herbicidas selectivos que sean tolerados por los tréboles. La tolerancia diferencial de leguminosas a herbicidas para cultivos extensivos y pasturas ha sido reportada anteriormente, aunque hay poca información disponible que sea específica para especies de trébol. El daño causado por los herbicidas es usualmente inconsistente, hipotéticamente debido a las diferencias en tolerancia entre especies. Se realizaron experimentos de campo y de invernadero con el objetivo de evaluar la tolerancia diferencial entre cuatro especies de trébol. Nuestros experimentos sugieren que existe variación entre especies de trébol en la tolerancia a herbicidas de hoja ancha comunes. Solamente el control con imazaquin difirió debido a las especies, aunque interacciones entre tratamiento y especie de trébol fueron demostradas debido a reducciones variables en la altura del trébol. Las reducciones en altura, producto del efecto de imazaquin, 2,4-D, 2,4-DB y triclopyr, variaron según la especie de trébol. La respuesta diferencial de los tréboles a los tratamientos con herbicidas debería ser una consideración importante cuando se manejan áreas con coberturas mixtas de zacates y tréboles y debería ser incluida en investigaciones futuras. A un nivel más práctico, nuestros experimentos muestran un rango de herbicidas que efectivamente controlan especies de trébol, incluyendo atrazine, dicamba, clopyralid, 2,4-D, triclopyr, metsulfuron, and trifloxysulfuron. Sin embargo, los resultados sugieren que 2,4-DB, imazethapyr y bentazon son herbicidas candidatos para el control de malezas en escenarios en los cuales el trébol es un cultivo deseable.

Clovers (*Trifolium* spp.) are routinely included within pastures and low-maintenance turf as utility plants. These legumes provide important ecosystem services, such as nitrogen (N) fixation (Ledgard and Steele 1992; McNeill and Wood 1990; Whitehead 1995) and insect habitat (Abraham et al. 2010; Rogers and Potter 2004). Clovers, like many legumes, increase forage yields and quality as well as decrease N fertilizer requirements (Hoveland 1989; Rao et al. 2007). When included within low maintenance turf, clovers improve sward color by contributing N to associated grasses (Sincik and Acikgoz 2007) and have proven useful for maintaining roadside slopes maintained as turf (Roberts and Bradshaw 1985).

Herbicidal weed control is critical to maximizing forage yields (DiTomaso 2000; Seefeldt et al. 2005) and is often required during clover establishment because seedlings are not competitive with many weeds and grasses (Carlisle et al. 1980; Evers et al. 1993; Young et al. 1992). Weeds compete with desirable species for nutrients and resources and are often toxic to grazing animals (Carlisle et al. 1980; Marten and Andersen 1975; Vengris et al. 1953).

Selective weed control in grass–clover swards is hampered by the lack of effective herbicides that are tolerated by clovers. Many effective broadleaf herbicides are reported to control clover, including 2,4-D, carfentrazone, clopyralid, dicamba, and triclopyr (MacRae et al. 2005; Neal 1990; Neal and

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Table 1. Four clover species and their respective harvest and transplant dates. Plants were harvested and allowed to mature in a greenhouse setting. Plants were then subject to selection for uniform size and maturity followed by random assignment to either field or greenhouse experiments.

Year	Clover	Harvest date	Growth cycle	Transplant date	Treatment date	Flowering stage at treatment[a]	Leaves per plant at treatment
2010	White	January 19	Perennial	February 10	March 10	Vegetative	10–20
	Small hop	January 19	Annual	February 10	March 10	Early-flowering	20–30
	Crimson	January 20	Annual	February 10	March 10	Early-flowering	10–20
	Ball	January 19	Annual	February 10	March 10	Early-flowering	15–25
2011[b]	White	February 11	Perennial	February 15	February 22	Vegetative	10–20
	Small hop	February 11	Annual	February 15	February 22	Early-flowering	20–30
	Crimson	February 11	Annual	February 15	February 22	Early-flowering	10–20
	Ball	February 10	Annual	February 15	February 22	Early-flowering	20–30
2012[c]	White	February 11	Perennial	February 15	February 22	Early-flowering	10–20
	Small hop	February 11	Annual	February 15	February 22	Late-flowering	20–30
	Crimson	February 10	Annual	February 15	February 22	Early-flowering	20–30
	Ball	February 10	Annual	February 15	February 22	Mid-flowering	20–30

[a] Flowering stage is indicated as either early (blooms present but remaining un-opened or slightly opened), mid (having bloomed but no signs of flower senescence), or late (flower keels having more than roughly 25% discoloration due to senescence).

[b] 2011 dates refer to both field and greenhouse studies.

[c] 2012 dates refer to greenhouse studies only.

Mascianica 1988; Willis et al. 2007). Yet few herbicides are labeled for postemergence application to various clover species, and most are restricted to states where clovers are cultivated for seed production or forage.

Furthermore, differential herbicide tolerance of legume cultivars and species has previously been reported, including differential reductions in seed yield, biomass, and N input for subsequent crops (Beran et al. 1999; Bowran 1993; Young et al. 1992). Understanding differential herbicide treatment effects upon clover species may advance efforts for selective weed control within grass–clover swards as well as increase clover control options within grass monocultures.

Experiments were conducted to identify herbicides tolerated by utility clovers and to evaluate the potential for differential clover response to common herbicide treatments. Due to previous reports of differential herbicide tolerance amongst other legume species, researchers postulated that clover response to herbicides would differ by species. Emphasis was placed upon determining herbicide tolerance of four clover species endemic amongst the local flora, including: white clover, small hop clover, crimson clover, and ball clover. We report differential responses of these species to a range of broadleaf herbicides.

Materials and Methods

Field and greenhouse experiments were repeated for 2 yr to evaluate clover response to a range of common broadleaf herbicides. Field experiments were conducted during 2010 and 2011 at the Auburn University Turfgrass Research Unit (32°34′40″N, 85°29′57″W) in Auburn, AL.

Cool-season legumes (Table 1) were collected to a depth of 7.6 cm using a 10.8-cm-diameter golf-green cup-cutter (Par Aide Product Company, Lino Lakes, MN) between January 19 to 22, 2010, and February 1 to 18, 2011. Plants were collected at a single site from a Marvyn sandy loam (fine-loamy, kaolinitic, thermic Typic Kanhapludult) soil with pH 6.3 (1 : 1 soil : H_2O) and were allowed to mature in a greenhouse setting until subject to selection for uniform size and maturity.

Plants were transplanted into field conditions 10 February 2010 or 15 to 21 February 2011. The transplant site was a hybrid bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy] sward maintained at 5-cm mowing height without supplemental fertility. Soil at the transplant site was a Marvyn sandy loam soil similar to that found at the collection site where plants originated. The site was not mown or fertilized during studies, but was hand-watered to prevent clover wilt. Plants were clipped with shears to identical height (8 cm) and diameter (11 cm) 2 d prior to treatment. Further information concerning collection date, stage of growth, and transplant date is presented in Table 1.

The field study was conducted as a split-plot design with the four clover species as randomized subunits within herbicide main plots (three replications). Herbicide treatments and application rates (Table 2) included commonly applied broadleaf herbicides or were chosen based upon labeling for leguminous crops. Treatments included a non-treated control. All treatments included a 0.25% v/v nonionic surfactant (Induce, Helena Chemical Company, Collierville, TN). Herbicides were applied at 280 L ha^{-1} spray volume on March 10, 2010, or February 22, 2011, via a CO_2 pressurized back-pack sprayer equipped with four TeeJet XR8002 flat fan nozzles (Spraying Systems Co., Wheaton, IL).

During field experiments, clover control was visually assessed 6 wk after treatment relative to the nontreated control, where 100% control equaled complete plant death. Control was based upon a combination of herbicide injury and plant health. Control assessments did not account for height reductions. However, plant height from the soil surface was sampled twice by lifting the two tallest foliar meristems, whether inflorescence or leaf, and measuring to the uppermost point.

Supplemental greenhouse experiments were conducted during 2011 and 2012 at the Auburn University Weed Science Greenhouse, (32°35′12″N, 88°29′15″W) in order to evaluate herbicide effects upon clover biomass. Plants were

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Table 2. Herbicide rates and formulations applied in field and greenhouse experiments to four clover species. All treatments included a 0.25% v/v nonionic surfactant. Herbicides were applied at 280 L ha^{-1} spray volume. Experimental rates were chosen based upon common labeled rates and unpublished studies where legume tolerance had been observed.

Mechanism of action[a]	Common name	Trade name	Formulation	Rate 100 m^{-2}	Manufacturer	City, state	Website
Synthetic auxins	2,4-D	Amine 400	Dimethyl amine salt	15.8 g ae	PBI Gordon	Kansas City, MO	www.pbigordon.com
	2,4-DB[b,c]	Butyrac 200	Dimethyl amine salt	15.8 g ae	Albaugh	Ankeny, IA	www.albaughinc.com
	Dicamba	Banvel	Dimethyl amine salt	11.2 g ae	Arysta LifeScience	Cary, NC	www.arystalifescience.com
	MCPA[b]	MCPA Ester 4	Ethylhexyl ester	5.2 g ai	Albaugh	Ankeny, IA	www.albaughinc.com
	Clopyralid	Lontrel Turf and Ornamental	Monoethanolamine salt	4.2 g ai	Dow AgroSciences	Indianapolis, IN	www.dowagro.com
	Triclopyr	Turflon Ester Ultra	Butoxyethyl ester	5.6 g ai	Dow AgroSciences	Indianapolis, IN	www.dowagro.com
Photosystem II inhibitors	Atrazine	AAtrex 4L	—	22.4 g ai	Syngenta Crop Protection	Greensboro, NC	www.syngenta.com
	Bentazon[b,c]	Basagran	Sodium salt	11.2 g ai	Arysta LifeScience	Cary, NC	www.arystalifescience.com
Acetolactate synthase inhibitors	Imazaquin[b,c]	Scepter 70 DG	Free acid	5.6 g ai	BASF	Research Triangle Park, NC	www.basf.com
	Imazethapyr[b,c]	Pursuit	Ammonium salt	0.7 g ai	BASF	Research Triangle Park, NC	www.basf.com
	Metsulfuron-methyl	MSM Turf	—	0.2 g ai	FarmSaver	Raleigh, NC	www.farmsaver.com
	Trifloxy-sulfuron	Monument 75 WG	Sodium salt	0.3 g ai	Syngenta Crop Protection	Greensboro, NC	www.syngenta.com

[a] According to Senseman (2007).

[b] Commonly labeled for use within forage and pasture legumes.

[c] Labeled for use within soybean production (*Glycine max*).

collected February 1 to 18, 2011, and January 13 to 20, 2012 (Table 1) identically to those of the field experiments. To prevent sample erosion and to facilitate sample randomization, greenhouse plants were placed in pots (11-cm diameter, 730-cm^3 volume). Greenhouse air temperature was maintained between 23 and 25 C. Plants were subject to normal daytime irradiance (less than 350 μmol m^{-2} s^{-1} at foliage height) and were watered via overhead mist irrigation twice daily. Herbicide treatments were identical to those applied in field experiments (Table 2). Treatments were applied in an enclosed research spray cabinet applying 280 L ha^{-1} through a single TeeJet TP8002EVS nozzle (Spraying Systems Co.). The study was conducted as a completely randomized design with three replications and one pot per experimental unit. Plants were randomized daily to account for variations within the greenhouse microclimate. Foliage was harvested at the soil surface and oven dried at 50 C for 72 hours to ascertain above ground biomass.

Height and biomass responses are based upon percent reduction relative to the nontreated control. All data were subject to analysis of variance (ANOVA) within SAS procedure GLIMMIX using mixed model methodology (SAS® Institute v. 9.2, Cary, NC). Field and greenhouse data were analyzed separately. Treatment was considered a fixed effect in the model. Year, replication (nested within year), and iterations containing these effects were considered random in the model and were nonsignificant for all response variables (Carmer et al. 1989; Hager et al. 2003). Basic model assumptions were confirmed. Means were separated based upon adjusted 95% confidence intervals, which allows for multiple comparisons by protecting family-wise error rate (Littell et al. 2006).

Table 3. ANOVA results and source sum of squares (SS) relative to the total SS for field and greenhouse experiments 6 wk after treatment (WAT).

Experiment	Field[a]		Greenhouse[b]
Source	Control[c]	Height[d]	Biomass[d]
Herbicide	0.0001[e]	0.0001	0.0028
Species	0.5752	0.0001	0.0001
Herbicide × species	0.0081	0.0001	0.0695

[a] Field experiments were conducted during winters 2010 and 2011 and did not include biomass analysis.

[b] Supplemental greenhouse experiments were conducted during winters 2011 and 2012 and evaluated biomass.

[c] Control was visually assessed on a percent scale 6 WAT relative to the nontreated control.

[d] Height and biomass responses were calculated based upon percent reduction relative to the nontreated control 6 WAT.

[e] P > F values obtained within SAS Proc MIXED.

Results and Discussion

ANOVA indicated that year and year by treatment interactions were not significant ($P > 0.05$; Table 3). Therefore, experiments were pooled across years with respect to growing condition (e.g., field or greenhouse). Precedence was given to field data, with greenhouse biomass reductions presented separately. Of the field data, priority was given to percent control, with relative height discussed as supporting evidence. Studies indicated varying control and height reductions due to species by herbicide interactions. Interaction effects were given precedence to main effects.

Field Experiments. ANOVA (Table 3) indicated significant herbicide by species interaction effects upon control and height data of field experiments (Table 4). 2,4-D control did not differ due to species and was ≥ 88% for all clovers. However, 2,4-D reduced small hop clover height greater than

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Table 4. Control and height reductions of four clover species measured 6 wk after treatment (WAT) in field studies. Effects were restricted to $P \leq 0.05$ level of significance. Effects were combined across years. Model validity ($P > F$) is provided for significant species by herbicide interaction.

		% Control[a]			% Height reduction[b]		
Herbicide	Clover	Mean[c]	±95% CI[d]	P > F	Mean	±95% CI	P > F
2,4-D	Ball	88	8	NS[d]	−64 ab	34	0.049
	Crimson	91	8		−63 ab	27	
	Small hop	95	8		−97 a	27	
	White	91	8		−41 b	27	
2,4-DB	Ball	18	28	NS	−12 bc	12	< 0.001
	Crimson	30	28		+2 c	12	
	Small hop	58	26		−27 ab	12	
	White	28	28		−50 a	12	
Dicamba	Ball	99	1	NS	−100	0	NS
	Crimson	100	1		−100	0	
	Small hop	100	1		−100	0	
	White	100	1		−100	0	
MCPA	Ball	86	31	NS	−23	32	NS
	Crimson	58	27		−11	32	
	Small hop	56	25		−67	32	
	White	78	25		−51	39	
Clopyralid	Ball	100	3	NS	−100	0	NS
	Crimson	100	3		−100	0	
	Small hop	95	3		−100	0	
	White	100	3		−100	0	
Triclopyr	Ball	88	11	NS	−17 c	21	< 0.001
	Crimson	81	12		−22 bc	21	
	Small hop	92	12		−61 ab	21	
	White	88	11		−91 a	21	
Atrazine	Ball	100	1	NS	−100	21	NS
	Crimson	100	1		−100	17	
	Small hop	100	1		−100	17	
	White	98	1		−86	17	
Bentazon	Ball	9	11	NS	−17	22	NS
	Crimson	15	10		−2	22	
	Small hop	4	11		−3	22	
	White	5	10		+30	27	
Imazaquin	Ball	80 ab	19	0.033	−47 b	20	0.012
	Crimson	62 ab	19		−38 b	24	
	Small hop	91 a	21		−88 a	20	
	White	50 b	19		−36 b	20	
Imazethapyr	Ball	7	13	NS	−12	26	NS
	Crimson	15	13		+9	26	
	Small hop	10	13		−33	26	
	White	10	13		−45	32	
Metsulfuron	Ball	90	12	NS	−79	24	NS
	Crimson	93	11		−78	24	
	Small hop	93	11		−97	24	
	White	88	11		−82	24	
Trifloxy-sulfuron	Ball	92	14	NS	−84	35	NS
	Crimson	95	14		−70	29	
	Small hop	80	14		−91	29	
	White	89	15		−45	35	

[a] % Control was visually assessed 6 WAT relative to the nontreated control.

[b] % Height and biomass reductions are relative to the nontreated control. Negative numbers indicate height reduction.

[c] Mean separations were performed using 95% confidence intervals. Overlapping intervals signify a lack of difference between means of the same herbicide treatment. Letters are presented as a method of easily distinguishing significant differences amongst herbicide treatment.

[d] Abbreviations: 95% CI, 95% confidence interval; NS, nonsignificant.

that of white clover (97% vs. 41%) and reduced ball and crimson clover heights 64 and 63%, respectively. Herbicide effects on plant height are likely of biological importance to plant survival and stand resilience. However, reductions in size may be linked to more than just herbicide induced plant injury. Fletcher and Raymond (1956) first demonstrated that phenoxy hebicides, like 2,4-D, reduced the success of *Rhizobium trifolii* to form symbiotic relationships with white clover, subsequently reducing N fixation. More recent studies have demonstrated that various herbicides directly damage both host plant and symbiotic rhizobium (Clark and Mahanty 1991). Herbicide effects upon rhizobium, nodulation, and N fixation were not examined within these experiments. However, future research should focus upon plant competitiveness, rather than simply plant survival.

Since the 1950s legume tolerance to butyric acid compounds, such as 2,4-DB and MCPB, has been linked to reduced beta-oxidation within tolerant species (Wain and Wightman 1954). Within our own experiments, 2,4-DB was moderately tolerated by all clovers, and control did not differ due to species (≤ 58% control; Table 4). However, 2,4-DB did affect clover heights differently. 2,4-DB did not affect crimson and ball clover heights (+2 and 12%, respectively) relative to the nontreated control; however, 2,4-DB did reduce small hop clover height 27%, which was similar to ball and white clover height reductions but greater than that of crimson clover. 2,4-DB reduced white clover height 50%, which was greater than ball and crimson clover height reductions and similar to height reductions observed due to 2,4-D. Differential response to 2,4-DB in leguminous pasture species has previously been reported. Mulholland et al. (1989) demonstrated differential *Medicago* species responses, while Young et al. (1992) reported that *Medicago doliata* Carmign. var. *muricata* Heyn and *T. subterraneum* L. were more tolerant of 2,4-DB than *M. truncatula* Gaertn.

MCPA is applied alone and in commercially available herbicide mixtures for pasture and rangeland management but may lack selectivity for many pasture legumes (Conrad and Stritzke 1980; Evers et al. 1993). Our experiments demonstrated this lack of tolerance amongst four clover species. MCPA controlled clovers between 56 and 86% and reduced heights between 11 and 67%. An alternative to MCPA not included amongst our treatments was the butyric acid compound MCPB, which has utility within leguminous crops (Senseman 2007) and has previously been demonstrated safe upon white clover (Elliot 2006).

Clopyralid and dicamba effectively controlled all clovers (≥ 95%; Table 4) and completely reduced heights across species. Triclopyr control was similar to that of clopyralid (≥ 81%); however, triclopyr affected clover heights differently. Triclopyr failed to reduce ball clover height relative to the nontreated and reduced crimson clover height only 22%. Small hop clover height was reduced 61%, which was similar to reductions in crimson clover height but greater than that of ball clover. Triclopyr reduced white clover height 91%, which was greater than ball and crimson clover height reductions. It is noteworthy that herbicides from the same family (e.g., clopyralid and triclopyr) did not exhibit similar efficacy in this experiment.

Atrazine effectively controlled all clovers (≥ 98%) and reduced clover heights ≥ 86% (Table 4). On the contrary, bentazon was well tolerated by all clover species (≤ 15% control and ≤ 17% height reduction). In fact, a 30% increase

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Table 5. Herbicide main effects upon clover biomass reductions measured 6 wk after treatment (WAT) during greenhouse experiments.

Herbicide	Greenhouse	
	% Biomass reduction	
	Mean[a]	±95% CI
2,4-D	−85 abc	9
2,4-DB	−45 ef	10
MCPA	−50 ed	9
Dicamba	−92 ab	9
Clopyralid	−98 a	9
Triclopyr	−89 ab	9
Atrazine	−98 a	9
Bentazon	−36 f	9
Imazaquin	−73 bc	9
Imazethapyr	−28 f	9
Metsulfuron	−84 abc	9
Trifloxysulfuron	−68 cd	9

[a] Mean separations were performed using 95% confidence intervals. Overlapping intervals signify a lack of difference between means of the same herbicide treatment. Letters are presented as a method of easily distinguishing significant differences amongst herbicide treatment.

in white clover height was observed due to bentazon application. Other researchers have previously reported similar responses to bentazon. Ceballos et al. (2004) reported increases in red clover (*T. pratense* L.) plant height (70 and 48% for 12 and 24 g 100 m^{-2} rates) at the expense of roots, which were reported to have decreased 42% by 20 d after treatment. Root biomass was not measured during our experiments.

Only imazaquin resulted in differential clover control. Imazaquin controlled small hop clover greater than white clover (91% vs. 50%; Table 4). Ball and crimson clover control (80 and 62%, respectively) were similar to that of other clovers. Imazaquin reduced small hop clover height 88%, which exceeded height reductions measured among other clovers ($\leq$ 47%). Differential soybean [*Glycine max* (L.) Merr.] cultivar responses to imazaquin have been reported (Kent et al. 1988). More recently, differential responses to acetolactate synthase (ALS) inhibitors, such as imazaquin, have been attributed to resistance mechanisms (Tranel and Wright 2002). However, ALS resistance has not been confirmed amongst *Trifolium* spp. (International Survey of Herbicide Resistant Weeds, 2012).

Imazethapyr was well tolerated by all clover species. Imazethapyr controlled clovers $\leq$ 15% (Table 4). Crimson clover height (+9%) did not differ from that of the nontreated. Small hop and white clovers were reduced in height 33 and 45%, respectively, while ball clover height was reduced 12%. Previous research has demonstrated imidazolinone herbicides, such as imazethapyr, can be used for promoting the establishment of certain legumes within tall-grass prairies (Beran et al. 1999).

Metsulfuron and trifloxysulfuron herbicides are highly effective against many broadleaf weeds found within mixed grass swards, yet knowledge of differential tolerance among legume species is limited. Our results did not suggest differential tolerance, with metsulfuron and trifloxysulfuron having controlled and reduced heights similarly across clovers. Metsulfuron controlled all clover species $\geq$ 88% and reduced clover heights $\geq$ 78% (Table 4). Similarly, trifloxysulfuron controlled clovers $\geq$ 80% and reduced clover heights $\geq$ 45%.

Greenhouse Experiments. Supplemental greenhouse experiments evaluated biomass harvests (Table 5). Biomass reductions differed due to herbicide treatment as well as clover species but did not differ due to herbicide by species interaction. Biomass reductions are important considerations when managing mixed grass–clover swards for forage.

Clopyralid and atrazine reduced clover biomass 98%, similar to 2,4-D (85%), dicamba (92%), triclopyr (89%), and metsulfuron (84%), but greater than those of all other treatments (Table 5). Imazaquin reduced clover biomass 73%, similar to 2,4-D, dicamba, triclopyr, metsulfuron, and trifloxysulfuron (68%). MCPA reduced clover biomass 50%, similar to 2,4-DB (45%), bentazon (36%), and imazethapyr (28%).

White clover biomass was reduced less than crimson and hop clovers (58% vs. 72%), but equal to that of ball clover (61%; data not shown). Species main effects are important in several contexts. Foremost, labels do not always clearly define species for which herbicides are tolerated. These results suggest that clovers vary in herbicide susceptibility. Secondly, labels may ambiguously emphasize hop clover control. Yet there are at least three *Trifolium* spp. that are generically called "hop clovers" (Plants Database 2012; Weed Science Society of America 2012), some of which differ dramatically in phylogeny (Ellison et al. 2006).

Implications for Management. On a practical level, our results demonstrate potential herbicide options for maintaining mixed grass-clover swards. Candidate herbicides include bentazon, 2,4-DB, and imazethapyr. These herbicides are commonly labeled for use within leguminous crops as well as forage and rangeland legumes. Bentazon and 2,4-DB have proven to be moderately tolerated by subterranean (*T. subterraneum* L.) and arrowleaf (*T. vesiculosum* Savi.) clovers (Hawton et al. 1990; Smith and Powell 1979). The relative tolerance of clover species to these candidate herbicides is further evidence of their value within certain scenarios. Yet, it is difficult to foresee herbicide applicators choosing these herbicides without further evidence of weeds controlled, costs, and effects upon mixed swards. There are undoubtedly many herbicides that are tolerated by clover species, yet questions remain about application rates and timing.

Our experiments suggest varying tolerances amongst clover species and common broadleaf herbicides. This agrees with previous research of differential herbicide tolerance amongst other pasture and forage legumes (Bowran 1993; Mulholland et al. 1989; Young et al. 1992). However, to our knowledge, this is the first report of differential tolerance solely amongst *Trifolium* spp. This supposition has broad impacts within agronomic scenarios. Pasture and rangeland managers have long sought herbicidal weed control without harming utility clover species, with limited success. Clover seed producers may benefit from the knowledge that certain clovers may be preferentially favored by differential herbicide responses. Additionally, legumes such as clovers have application within mixed turf swards. Legume species and varieties continue to

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be developed and improved for various agronomic applications (Rajeev et al. 2009). However, herbicide labels often fail to clearly define the clover species for which an herbicide is intended (whether for selective weed control or for tolerance). As the number of species, varieties, and uses of clovers increase, label statements must more precisely scrutinize species tolerance in order to increase the viability and profitability of biodiverse agricultural scenarios.

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