

Article

# Efficacy of Various Herbicides for the Control of Perennial *Plantago* spp. and Effects on Alfalfa Damage and Yield

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**Abstract:** Broadleaf (*Plantago major* L.) and buckhorn plantain (*Plantago lanceolata* L.) are perennial weeds that are notoriously difficult to control in alfalfa cropping systems. Sharpen<sup>®</sup> (saflufenacil) herbicide has been registered for broadleaf weed control in dormant alfalfa, although it has not been evaluated on plantain control. Field and greenhouse experiments were conducted to determine the efficacy of saflufenacil on plantain control with assessments of damage to alfalfa and effects on yield. In the greenhouse, applications of saflufenacil alone caused greater injury to both broadleaf and buckhorn plantain compared to the non-treated control (NTC). Additionally, applications of saflufenacil in combination with other herbicides (imazethapyr or imazamox) caused the greatest amount of injury to both broadleaf and buckhorn plantain compared to all other commercially available herbicide treatments. However, this injury was not enough to effectively control the weeds and prevent recovery and regrowth over time. In the field, alfalfa did not exhibit damage symptoms, or have reduced yield when treated with saflufenacil compared to the NTC. This research indicates that applications of saflufenacil provided temporary injury throughout the duration of the study to both broadleaf and buckhorn plantain with few negative effects to alfalfa in the field.

**Keywords:** weed control; plantain forage; alfalfa; dormant application; herbicide injury; crop yield

## 1. Introduction

Alfalfa hay is the third most valuable cash crop in the United States [1] and is the most widely cultivated forage legume worldwide [2]. Additionally, the overall value of alfalfa hay is further enhanced by its essential contributions, as feed and forage, to livestock production (i.e., milk, meat, textiles). As crop production acreage and the availability of resources for management continue to decline, it is important to maximize yield and nutritive value of all alfalfa production as much as possible to meet the agricultural needs of producers, farmers, ranchers, livestock managers, and industry personnel, especially in light of the need to increase food production globally to meet the needs of an increasing population and climate change [3,4].

Managing weeds is a critical and ever-present component of successful alfalfa production. While weeds that emerge during the initial seeding stages of alfalfa typically have the greatest effect by competing for light, water, space, and nutrients, late-season weeds that populate established alfalfa fields can have a significant influence on yield through continued competition for resources throughout

the remaining and following growing seasons [5,6]. Additionally, the presence of annual and perennial weeds at any time can lower forage nutritive value, reduce stand longevity caused by premature plant loss or reduction, increase the incidence of disease and insect damage, and create detrimental harvesting issues [7–9].

Perennial weed populations are especially difficult to control in perennial crops, like alfalfa, because management practices have to address seed production and vegetative reproductive structures that allow the plant to survive from season to season. Simple perennial weeds, like plantain (*Plantago* spp.), have a hearty root system that allows the plant to die back and survive during non-ideal environmental conditions, proctoring tissue regrowth and re-establishment once conditions become ideal again [10,11]. Broadleaf plantain (*Plantago major* L.) and buckhorn plantain (*Plantago lanceolata* L.) are particularly difficult-to-control weeds whose infestations are widespread in alfalfa fields throughout the western U.S. [10,11]. Weed management of these simple perennial weeds must focus primarily on injury to the root system; however, it is difficult for herbicide active ingredients to move effectively enough within the entire plant to injure a hearty root system located deep within the soil [10]. Similarly, the use of selective herbicides to control broadleaf weeds, like plantain, in a broadleaf crop, like alfalfa, further complicates any effective management. As a result, there are only a few registered herbicide active ingredients, such as glyphosate (pre-plant burndown or Roundup® Ready systems), 4-(2,4-Dichlorophenoxy)butyric acid (2,4-DB amine), and 2-methyl-4-chlorophenoxyacetic acid (MCPA) that have been reported to cause injury to plantain in alfalfa fields [5,9]. Currently there are no herbicides labeled for use in alfalfa that will control plantain without multiple applications across several seasons [11,12]. Additionally, the broadleaf herbicide active ingredients labeled for use in alfalfa have never been evaluated for late-season broadleaf perennial weed control in dormant-season alfalfa. Furthermore, the continued use of these select few herbicide active ingredients to manage a specific population of weeds, like plantain in alfalfa, over time, can lead to the development of weed population shifts and herbicide resistance in the target weeds [13,14]. As a result, research to evaluate the effectiveness of newly registered herbicides with different active ingredients is greatly warranted for control of plantain in alfalfa.

Sharpen® (BASF Corporation, Research Triangle Park, NC, USA) has recently acquired a label for broadleaf weed control in dormant-season alfalfa in much of the western USA [15]. The active ingredient in Sharpen® is saflufenacil [N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide], which causes plant cell membrane damage and eventually plant death by inhibiting the production of protoporphyrinogen-oxidase (herbicide group 14 [16–18]). Specifically, saflufenacil can offer contact burn-down control of annual broadleaf weeds such as black nightshade (*Solanum nigrum* L.) and Palmer amaranth (*Amaranthus palmeri* S. Watson) [16,17,19], and perennial broadleaf weeds including, but not limited to, field bindweed (*Convolvulus arvensis* L.) and dandelion (*Taraxacum officinale* Weber ex Wigg) during limited (dormant) season growth of alfalfa [15,20]. Saflufenacil has yet to be studied as a potential herbicide option for broadleaf and buckhorn plantain control in alfalfa fields. The objectives of this study were to (1) compare the weed control performance of saflufenacil against commercially available herbicide standards under greenhouse conditions, and (2) evaluate the effects on alfalfa regarding damage symptoms and yield reduction resulting from the application of saflufenacil against commercially available herbicide products. Should results indicate that saflufenacil provides acceptable control on broadleaf and buckhorn plantain and equivalent crop safety compared to the commercial standards, actions will be taken to include plantain as a target weed in the most up-to-date product labels.

## 2. Materials and Methods

In 2017 and 2018, field research studies were established to evaluate the development of herbicide injury symptoms, as well as any negative impacts on yield, in a mature alfalfa stand. Due to the lack of a uniform infestation of the target weeds needed for a comparative research study in the fields of both NMSU Science Centers, research was also initiated in the greenhouse in the fall and winter of 2017 to evaluate the efficacy of saflufenacil on broadleaf and buckhorn plantain control compared to other industry standard herbicide active ingredients.

**Field Studies:** Field trials were initiated at New Mexico State University's (NMSU) Agricultural Science Center (ASC) at Los Lunas, NM (32°46.287' N, 106°45.578' W) in December 2017, and at the NMSU Leyendecker Plant Science Center (LSC) in Las Cruces, NM (32°12.131' N, 106°44.771' W) in December 2018 to evaluate any potential negative impacts of herbicide treatments to alfalfa growth and yield. The soil at the ASC site is a Vinton Series (sandy, mixed, thermic Typic Torrifluvents with 1.5% organic matter) [21], while the soil at the LSC site is an Armijo series (fine, smectitic, thermic Chromic Haplotorrerts with 1.3% organic matter) [22]. The fields at both locations had previously been prepared for alfalfa using typical procedures including tillage, seeding, irrigation, etc. The alfalfa at the ASC field was an established (6+ years), healthy stand of Reward II (Fall dormancy rating = 4, dormant) [23,24], and the alfalfa at the LSC field was an established (4+ years) healthy stand of TMA 990 Brand (Fall dormancy rating = 9, non-dormant) [24–29]. Neither alfalfa variety was Roundup Ready®. The herbicide treatments for the study are labeled for broadleaf weed control in dormant-season alfalfa growth. However, out of the chosen herbicide treatments, only Rhomene MCPA® lists plantain amongst the broadleaf weeds controlled according to the label [30], therefore, this herbicide was included in the treatments as a labeled comparison with Sharpen® and the other herbicide treatments for plantain control in alfalfa. While some of the other treatment herbicides labeled for general broadleaf weed control in alfalfa may injure plantain, limited research has been conducted to evaluate their efficacy on plantain in alfalfa fields. For example, glyphosate was evaluated for broadleaf weed control in alfalfa fields in Canada [31] and Connecticut [32], but provided inadequate control of broadleaf or buckhorn plantain [31]. As such, we felt it was important to test the most commonly used labeled herbicides [33] in alfalfa for comparison purposes to saflufenacil. Due to warm temperatures and long winter photoperiods throughout the southern regions where LSC is located, alfalfa tends to display slowed-growth effects rather than true dormancy; thus, herbicide applications must be made early enough for the alfalfa to recover during slowed fall-growth and spring re-growth [27,28]. As a result, herbicide treatment applications were initiated on 1 December 2017, in Los Lunas and 12 December 2018, in Las Cruces, after the final cutting and during a period of slowed growth for both fields starting in late November. This is also the best timing for herbicide control of perennial weeds like plantain since the redistribution of carbohydrates to the root system, in preparation for winter, allows for greater translocation of systemic herbicides, and more effective control [33–35].

The experimental design was a randomized complete block design with plot sizes of 3 × 3 m and four replications of 10 treatments. Treatments consisted of the following herbicides: saflufenacil (Sharpen®, at either 23 g ai (active ingredient) ha<sup>-1</sup> or 50 g ai ha<sup>-1</sup>, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709, USA); 2,4-DB amine (Butyrac 200®, 1684 g ae (acid equivalent) ha<sup>-1</sup>, Albaugh, LLC, 1525 NE 36th Street, Ankeny, IA 50021, USA); MCPA (Rhomene MCPA®, 518 g ae ha<sup>-1</sup>, Nufarm Inc., 11901 S. Austin Avenue, Alsip, IL 60803, USA); imazethapyr (Pursuit®, 104 g ae ha<sup>-1</sup>, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709, USA); imazamox (Raptor®, 54 g ae ha<sup>-1</sup>, BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709, USA); hexazinone (Velpar DF®, 3618 g ai ha<sup>-1</sup>, Bayer Environmental Science, 2 T. W. Alexander Drive, Research Triangle Park, NC, 27709); terbacil (Sinbar®, 1343 g ai ha<sup>-1</sup>, Tessenderlo Kerley, Inc. 2255 N. 44th Street, Phoenix, AZ 85008, USA). A non-treated control (NTC) was included for comparison for a total of 10 treatments. All treatments that contained saflufenacil were applied with the surfactant methylated seed oil at 1% v v<sup>-1</sup> (Firezone®, Helena Chemical Co. 225 Schilling Boulevard, Suite 300, Collierville, TN 38017, USA) [15,36,37], while applications of imazethapyr, imazamox, and hexazinone were applied with

non-ionic surfactant at 0.25%  $v v^{-1}$  (Induce<sup>®</sup>, Helena Chemical Co. 225 Schilling Boulevard, Suite 300, Collierville, TN 38017, USA) [37,38]. All treatments were applied with 2%  $w v^{-1}$  of soluble ammonium sulfate (21-0-0, United Suppliers, 30473 260th St., Eldora, IA 50627, USA). Treatments were applied using a CO<sub>2</sub>-powered backpack sprayer equipped with a 4-nozzle boom with 11002 VS TeeJet Flat-fan nozzles calibrated to deliver 187 l ha<sup>-1</sup> at 207 kPa. Air temperatures were 15 and 19 °C at the time of application at LSC and ASC, respectively, and winds averaged <9 km h<sup>-1</sup>. The alfalfa fields were not irrigated for approximately 24 h after the initial application to allow herbicide treatments to dry. Throughout the duration of the study, fields were irrigated as needed to maintain alfalfa growth and health.

At the ASC and LSC locations, alfalfa injury (%) due to herbicide applications was evaluated visually every two weeks for approximately 25 weeks after initiation of treatment (WAIT) at ASC, and 19 WAIT at LSC. Evaluations were assessed on a percent scale where 1 equaled no injury to alfalfa, and 100 equaled complete injury coverage of alfalfa plants. No broadleaf or buckhorn plantain weeds were located within either of the field trials; predominant weed species throughout the duration of the trials included annual sowthistle (*Sonchus oleraceus* L.) and prickly lettuce (*Lactuca serriola* L.) at ASC, and jungle rice (*Echinochloa colona* (L.) Link) and shepherd's purse (*Capsella bursa-pastoris* (L.) Medicus) at LSC. Field plots at both locations remained relatively weed-free throughout the duration of the trial. Alfalfa was harvested on May 22, 2018 (25 WAIT), and July 5, 2018 (31 WAIT), at ASC, and on 9 April 2019 (19 WAIT), and 6 June 2019 (24 WAIT), at LSC, to assess any treatment effects on yield. Alfalfa was harvested using hand-clipped fresh forage to collect weights from a 0.98 m<sup>2</sup> area within each plot. Samples from each plot were collected and weighed prior to drying in a forced-air oven at 52 °C until a constant weight to convert field weights to dry matter (DM) yield (g m<sup>-2</sup>). Temperature and precipitation data were collected from automated weather stations located within 1 km of the study field at each location.

**Greenhouse Studies:** Two runs of a climate-controlled greenhouse study were conducted in fall and winter 2017. Broadleaf and buckhorn plantain were seeded in potting soil at the LSC Greenhouse in Las Cruces, NM., Seedlings of both broadleaf and buckhorn plantain were transplanted 63 days after planting into individual 50-mm cone-tainers (Stuewe and Sons, Inc. 31933 Rolland Drive, Tangent, OR 97389, USA) and filled with potting soil (Lambert Peat Moss, Inc. 106, Chemin Lambert, Rivière-Ouelle, QC G0L 2C0, Canada) for further study. Plants were irrigated daily to allow for healthy growth and development until treatments were applied.

The experimental design for each run was four replications of 12 total treatments applied to each plantain species in a randomized complete block design with each cone-tainer having a single plant as the experimental unit. The same nine herbicide treatments and application rates that were used in the field trial were also applied in the greenhouse 93 days after planting (30 days after transplanting). Although neither test field was Roundup Ready<sup>®</sup>, a treatment of glyphosate (Roundup PowerMax<sup>®</sup>, 1734 g ai ha<sup>-1</sup>, Bayer Environmental Science, 2 T. W. Alexander Drive, Research Triangle Park, NC 27709, USA) was included in the greenhouse experiments to assess the potential control of plantain in Roundup Ready<sup>®</sup> alfalfa systems. Additionally, two herbicide treatments of saflufenacil (50 g ai ha<sup>-1</sup>) in combination with imazethapyr (104 g ai ha<sup>-1</sup>) or imazamox (54 g ai ha<sup>-1</sup>) were added to each of the replications in the greenhouse to explore the potential of tank-mixing herbicides for improved control of plantain weeds. A NTC was included for comparison for a total of 12 treatments. Treatments were applied using the same spray equipment and settings as the field study. Cone-tainers were not watered for 24 h following the treatment applications, and were then irrigated as needed throughout the study to maintain plantain growth and health. The greenhouse meteorological data (air temperature and relative humidity) was recorded throughout the study period using a HOBO Data Logger Model U14-002 Weather Station with HOBOWare Pro Version 2.7.18 software (Onset Computer Corporation, Bourne, MA, USA). The day/night temperatures and relative humidity averaged 26.7/24.3 °C, 31/26%. Due to data logger error, the daily light integral was not collected throughout the study period.

Beginning 1 WAIT, broadleaf and buckhorn plantain injury due to herbicide applications were evaluated visually once a week until 5 WAIT when plantain began to recover from herbicide injury. Evaluations were assessed on a percent scale where 1 equaled no injury to plantain, and 100 equaled death of plantain plants. Percent green cover based on digital image analysis was calculated using digital photographs. One photograph per plant was taken weekly until 5 WAIT using a constructed light box with mounted LED lamps to provide uniform lighting conditions and camera lens height for all the photographs taken [39]. A Canon PowerShot SX700 HS (Canon Inc., Tokyo, Japan) camera was set to a shutter speed of 1/10, an aperture of f/4.0, an ISO of 200, and a normal focus lens, and used to take digital images. Each cone-tainer was placed in a constructed red frame in order to isolate each plant from the surrounding area, which allows for the calculation of green cover only on the individual plant [40]. TurfAnalyzer software (<http://turfanalyzer.com> accessed on 12 August 2020) was used to calculate percent green cover following methods described by Richardson et al. [40]. The red frame was excluded from the entire picture and only the unselected pixels within the plant were used to calculate green cover [40]. Percent green cover was estimated by the amount of green pixels in each image, divided by the unselected pixels within the frame, and multiplied by 100 to determine the percentage of herbicide injury in comparison to healthy green plant tissue. Starting at 6 WAIT, above and below-ground tissue was harvested for both broadleaf and buckhorn plantain by cone-tainer. Below-ground tissue was washed to remove potting soil contaminants. Plant samples were then dried in a forced-air oven at 52 °C for one week and dry weights (g) were collected by container for both above- (plant) and below-ground (root) dry weights. Data ratings for both replications within each weed species were combined and analyzed as one data rating.

Statistical Analysis: Plantain injury, green cover, aboveground plant weight, and root weight data from the greenhouse study were analyzed using SAS PROC MIXED (version 9.4; SAS Institute Inc., Cary, NC, USA) for the main effects and all possible interactions involving run, plantain species, herbicide treatment, and weeks after treatment (WAIT), with significance defined at  $p \leq 0.05$ . Run  $\times$  rep  $\times$  plantain was considered random. Whenever a significant herbicide treatment effect or significant interaction was found, lsmeans (least squares means) were separated using least significant difference and the PDMIX800 macro [41], and the 5% LSD was calculated. If an interaction involving WAIT was significant, SAS PROC GLM (version 9.4; SAS Institute Inc., Cary, NC, USA) repeated measurements analyses were used to evaluate the interaction. In that case, if the interaction involved species, the analysis was sliced by species and means by species within WAIT were separated by LSD. The same procedures were used for weed coverage at Los Lunas and injury to the alfalfa, both over WAIT and yield over two harvests. Rep  $\times$  location was considered random. Injury to the alfalfa had to be analyzed by location due to differences in evaluation dates. Although yield was collected at different dates and intervals at each location, the data was collected from the first two harvests at the typical harvest time by location; consequently, data for yield were combined across harvests and locations and analyzed for the effects of location, harvest, and herbicide treatment and all possible interactions. As with the greenhouse data, the PDMIX800 macro [41] was used to separate lsmeans when there was a difference among herbicide treatments and for interactions involving WAIT, SAS PROC GLM repeated measurements analyses were used to explore the interaction.

### 3. Results

Greenhouse Studies: Although several effects involving run are significant, they are not considered biologically significant due to the controlled climate in the greenhouse, and were likely caused by subtle differences in position of the runs in the greenhouse among other factors. The date  $\times$  species  $\times$  treatment interaction was significant for herbicide injury (Table 1); therefore, broadleaf and buckhorn plantain weed injury and control data for each date are presented separately (Table 2).



**Table 1.** Results of statistical analysis from the greenhouse study evaluating the effect of various herbicides on broadleaf and buckhorn plantain at the NMSU Leyendecker Plant Science Center (LSC) greenhouse at Las Cruces, NM USA, during winter 2017–2018.

Effect	Greenhouse Study			
	Injury	Green Cover	Plant Wt.	Root Wt.
	Pr > F			
Run	0.1879	0.7307	0.0013	0.0538
Species	0.0003	<0.0001	0.8254	0.3545
Run × Species	0.0709	0.6937	0.0235	0.0260
Weeks after initial treatment (WAIT)	<0.0001	<0.0001	—	—
Run × WAIT	0.2150	0.1173	—	—
Species × WAIT	<0.0001	0.0235	—	—
Run × Species × WAIT	0.2878	0.4551	—	—
Treatment (TRT)	<0.0001	<0.0001	<0.0001	<0.0001
Run × TRT	0.0038	<0.0001	0.0305	0.3459
TRT × WAIT	<0.0001	0.8453	—	—
Run × TRT × WAIT	0.9915	0.9819	—	—
Species × TRT	<0.0001	<0.0001	0.7289	0.3216
Run × Species × TRT	<0.0001	<0.0001	0.6066	0.6998
Species × TRT × WAIT	<0.0001	0.1641	—	—
Run × Species × TRT × WAIT	0.9180	0.8519	—	—

**Table 2.** Percent herbicide injury observed visually over five weeks after initial herbicide treatment (WAIT) to broadleaf and buckhorn plantain in the NMSU Leyendecker Plant Science Center (LSC) greenhouse at Las Cruces, NM, during winter 2017–2018. Data are the means of two runs and four replications.

Treatment	Rate	Herbicide Injury (%)									
		1	2	3	4	5					
Broadleaf Plantain: Only the 1st order polynomial effect of WAIT × treatment was $p < 0.05$											
NTC <sup>1</sup>	—	1.0	E <sup>2</sup>	1.0	F	1.0	E	1.0	H	1.0	F
Saflufenacil	23 g ai ha <sup>-1</sup>	45.0	B	59.4	C	63.1	B	61.3	BCD	58.8	BC
Saflufenacil	50 g ai ha <sup>-1</sup>	50.0	AB	67.5	BC	71.9	AB	65.0	BC	66.3	AB
Saflufenacil + Imazethapyr	50 g ai ha <sup>-1</sup> + 104 g ai ha <sup>-1</sup>	58.8	A	71.3	AB	75.6	AB	73.1	AB	75.0	A
Saflufenacil + Imazamox	50 g ai ha <sup>-1</sup> + 54 g ai ha <sup>-1</sup>	63.8	A	77.5	A	80.6	A	77.5	A	76.9	A
2,4-DB amine	1684 g ae <sup>1</sup> ha <sup>-1</sup>	20.0	CD	31.3	D	47.5	C	52.5	DE	58.8	BC
MCPA	518 g ae ha <sup>-1</sup>	26.3	C	36.3	D	45.0	C	55.0	CD	61.3	ABC
Imazethapyr	104 g ae ha <sup>-1</sup>	10.6	CDE	13.8	E	21.3	D	20.0	H	22.5	E
Imazamox	54 g ae ha <sup>-1</sup>	8.8	DE	18.8	E	26.3	D	23.8	GH	35.0	DE
Hexazinone	3618 g ai ha <sup>-1</sup>	7.5	DE	18.1	E	40.6	C	35.0	FG	50.0	CD
Terbacil	1343 g ai ha <sup>-1</sup>	7.5	DE	20.0	E	46.3	C	35.0	FG	57.5	BC
Glyphosate	1734 g ai ha <sup>-1</sup>	12.5	DE	30.0	D	45.0	C	42.5	EF	46.3	CD
5% LSD <sup>1</sup>		15.6		8.6		12.8		12.3		16.1	
$p$ -value		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	
Buckhorn Plantain: Only the 1st order polynomial effect of WAIT × treatment was $p < 0.05$											
NTC <sup>1</sup>	—	1.0	G	1.0	I	1.0	F	1.0	F	1.0	D
Saflufenacil	23 g ai ha <sup>-1</sup>	70.0	A	62.5	BC	62.5	B	38.1	DE	41.3	C
Saflufenacil	50 g ai ha <sup>-1</sup>	72.5	A	72.5	AB	63.8	B	41.3	DE	43.8	C
Saflufenacil + Imazethapyr	50 g ai ha <sup>-1</sup> + 104 g ai ha <sup>-1</sup>	68.8	A	77.5	A	81.9	A	85.0	A	80.6	A
Saflufenacil + Imazamox	50 g ai ha <sup>-1</sup> + 54 g ai ha <sup>-1</sup>	65.0	AB	76.3	A	79.4	A	80.0	A	81.3	A
2,4-DB amine	1684 g ae ha <sup>-1</sup>	33.8	CD	37.5	EF	52.5	C	55.0	CD	70.0	AB
MCPA	518 g ae ha <sup>-1</sup>	55.0	B	52.5	CD	60.0	B	66.3	BC	76.9	A
Imazethapyr	104 g ae ha <sup>-1</sup>	22.5	DEF	28.8	FG	22.5	DE	32.5	E	43.8	C
Imazamox	54 g ae ha <sup>-1</sup>	25.0	CDE	20.0	G	21.3	DE	33.8	E	48.8	BC
Hexazinone	3618 g ai ha <sup>-1</sup>	11.3	F	15.0	H	19.4	E	26.3	E	32.5	C
Terbacil	1343 g ai ha <sup>-1</sup>	18.8	EF	22.5	GH	26.9	D	37.5	E	36.3	C
Glyphosate	1734 g ai ha <sup>-1</sup>	36.3	C	42.5	DE	58.8	BC	74.4	AB	82.5	A
5% LSD <sup>1</sup>		11.8		10.4		6.5		15.3		22.7	
$p$ -value		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	

<sup>1</sup> NTC = non-treated control; ai = active ingredient; ae = acid equivalent; LSD = least significant difference. <sup>2</sup> Means within a species and column followed by the same letter(s) are not significantly different based on the 5% LSD for that WAIT. Visual evaluations for herbicide injury were assessed on a percent scale where 1 equaled no plantain injury, and 100 equaled death of plantain plants.

Percent broadleaf plantain injury data indicated that applications of saflufenacil alone, or in combination with imazethapyr or imazamox provided significantly greater injury compared to the NTC and all other herbicide treatments, until 4 WAIT (Table 2). At 4 and 5 WAIT, only treatments including saflufenacil in combination with imazethapyr or imazamox continued to provide the greatest percentage of injury to broadleaf plantain. Saflufenacil alone at the high rate, or combined with imazethapyr or imazamox, still provided the highest levels of injury to the plantain at 5 WAIT. However, 2,4-DB amine, MCPA, hexazinone, and terbacil began exhibiting equal control to saflufenacil alone treatments. Results for percent buckhorn weed injury were similar to those for broadleaf plantain, with a few differences (Table 2). Generally, recovery from the application of saflufenacil alone was more rapid and the initial impact of applications (1 WAIT) of other herbicides was more pronounced, compared to the NTC, and more intense in progressive damage by 5 WAIT, except for imazamox, hexazinone, and terbacil (Table 2).

The species  $\times$  WAIT interaction was significant for percent green cover (Table 1) due to minor differences between species in the magnitude of change over time (data not shown). The species  $\times$  treatment interaction also was significant for percent green cover, but the species  $\times$  treatment  $\times$  WAIT was not (Table 1); therefore, broadleaf and buckhorn percent green cover data were averaged across WAIT (Table 3). Percent broadleaf plantain green cover indicated that, while all treatments, except imazethapyr and imazamox, had reduced green cover compared to the NTC, applications of saflufenacil alone or in combination with imazethapyr or imazamox provided significantly greater herbicide injury (less green cover) (Table 3). For buckhorn plantain, compared to the NTC, there was no reduction in plantain green cover due to any herbicide treatment, including those with saflufenacil (Table 3).

**Table 3.** Average percent green cover of broadleaf and buckhorn plantain after treatment with various herbicides in the NMSU Leyendecker Plant Science Center (LSC) greenhouse at Las Cruces, NM, USA, during winter 2017–2018. Data are the lsmeans (least squares means) of two runs and four replications.

Treatment	Rate	Broadleaf		Buckhorn	
NTC <sup>1</sup>	–	77.6	CDE <sup>2</sup>	75.1	DEF
Saflufenacil	23 g ai <sup>1</sup> ha <sup>−1</sup>	60.7	I	86.8	A
Saflufenacil	50 g ai ha <sup>−1</sup>	53.6	J	81.6	ABC
Saflufenacil + Imazethapyr	50 g ai ha <sup>−1</sup> + 104 g ai ha <sup>−1</sup>	49.3	JK	81.5	ABC
Saflufenacil + Imazamox	50 g ai ha <sup>−1</sup> + 54 g ai ha <sup>−1</sup>	46.3	K	82.8	ABC
2,4-DB amine	1684 g ae <sup>1</sup> ha <sup>−1</sup>	67.4	GH	80.4	BCD
MCPA	518 g ae ha <sup>−1</sup>	67.1	GH	84.1	AB
Imazethapyr	104 g ae ha <sup>−1</sup>	82.6	AB	80.3	BCD
Imazamox	54 g ae ha <sup>−1</sup>	74.1	EF	83.0	ABC
Hexazinone	3618 g ai ha <sup>−1</sup>	71.8	GF	82.4	ABC
Terbacil	1343 g ai ha <sup>−1</sup>	70.4	FGH	82.8	ABC
Glyphosate	1734 g ai ha <sup>−1</sup>	64.9	HI	81.6	ABC
5% LSD <sup>1</sup>		5.7			

<sup>1</sup> NTC = non-treated control; ai = active ingredient; ae = acid equivalent; LSD = least significant difference. <sup>2</sup> Means within the table followed by the same letter(s) are not significantly different at  $p < 0.05$ . Dark green color index, or the amount of green pixels in an image, was calculated by analyzing digital photographs with the TurfAnalyzer online software package.

There was no difference between species, and no species  $\times$  treatment interaction for plantain plant and root dry weight (Table 1); therefore, broadleaf and buckhorn data were combined for both plant and root weight (Table 4). Dried plant and root weight indicated that herbicide treatments, except imazethapyr or imazamox, provided significantly less plant weight, compared to the NTC; all herbicide treatments, except imazethapyr reduced root weight (Table 4).

**Table 4.** Main effect means for dried plant and root weights (g) of broadleaf and buckhorn plantain treated with selected alfalfa herbicides in the NMSU Leyendecker Plant Science Center (LSC) greenhouse at Las Cruces, NM, USA, during winter 2017–2018. Data are the lsmeans (least squares means) of two runs, two species, and four replicates.

Treatment	Rate	Dry Plant Weight (g)		Dry Root Weight (g)	
NTC <sup>1</sup>	–	4.97	A <sup>2</sup>	7.71	A
Saflufenacil	23 g ai <sup>1</sup> ha <sup>−1</sup>	4.07	B	5.88	CD
Saflufenacil	50 g ai ha <sup>−1</sup>	3.71	BC	6.04	BC
Saflufenacil + Imazethapyr	50 g ai ha <sup>−1</sup> + 104 g ai ha <sup>−1</sup>	3.56	BC	6.02	BC
Saflufenacil + Imazamox	50 g ai ha <sup>−1</sup> + 54 g ai ha <sup>−1</sup>	3.41	BCD	5.15	CD
2,4-DB amine	1684 g ae <sup>1</sup> ha <sup>−1</sup>	3.53	BC	5.72	CD
MCPA	518 g ae ha <sup>−1</sup>	3.79	BC	4.68	DE
Imazethapyr	104 g ae ha <sup>−1</sup>	4.91	A	7.18	AB
Imazamox	54 g ae ha <sup>−1</sup>	4.87	A	5.77	CD
Hexazinone	3618 g ai ha <sup>−1</sup>	3.18	CD	5.39	CD
Terbacil	1343 g ai ha <sup>−1</sup>	2.76	D	3.83	E
Glyphosate	1734 g ai ha <sup>−1</sup>	3.23	CD	3.86	E
5% LSD <sup>1</sup>		0.70		1.27	

<sup>1</sup> NTC = non-treated control; ai = active ingredient; ae = acid equivalent; LSD = least significant difference.

<sup>2</sup> Herbicide treatment means within a column followed by the same letter(s) are not significantly different at  $p < 0.05$ .

Visual ratings for herbicide injury data for both broadleaf and buckhorn plantain indicated that saflufenacil alone at both the low and high-labeled rates, with or without imazethapyr or imazamox provided weed injury significantly greater than that of the NTC, and comparable to or better than those of the industry standard herbicide treatments throughout the study (Table 2). In soybean systems, the efficacy of saflufenacil in controlling common lambsquarters (*Chenopodium album* L.) and common ragweed (*Ambrosia artemisiifolia* L.) was enhanced by tank-mixing with imazethapyr and dimethenamid; however, these applications were made as preemergence herbicides [42]. Postemergence control of broadleaf legumes such as coffeeweed (*Sesbania exaltata* P. Mill) was improved by tank-mixing saflufenacil with imazethapyr in rice fields in Texas [43]. Additionally, tank mixing saflufenacil with glufosinate enhanced the efficacy of broadleaf weed control in Florida citrus compared to applications of saflufenacil alone [44]. However, saflufenacil injury in this study, even with the tank mixes, was often not great enough to provide effective, long-term control with little regrowth or recovery of the weed over time. Similar observations were made with percent green cover in the broadleaf plantain, although the buckhorn plantain did not display a reduction in percent green cover due to any herbicide treatment compared the NTC (Table 3). As the trial progressed, very large NTC buckhorn plantain weeds continued to grow to the point where they were becoming root-bound within each cone-tainer. Therefore, the analysis software used to calculate percent green cover within an image was not able to distinguish differences between plant stress injury in the NTC treatments, and herbicide-related injury in the remaining treatments, to the above ground tissue (plant weight) of buckhorn plantain (Table 4) as effectively as our visual ratings were able to differentiate (Table 2). Additionally, applications of saflufenacil resulted in a reduction in plant and root dry weights (Table 4) compared to the NTC, although these reductions were not reflected in the buckhorn plantain green cover (Table 3) for the same reasons mentioned above.

Field Studies: Temperature and precipitation data through 17 WAIT for each location are presented in Table 5. The temperature data are consistent to the long-term averages for each location (data not shown) with cooler temperatures and more frequent freezes occurring at ASC than at LSC. Precipitation also is consistent as both locations have a continental precipitation patterns with dry winters.



**Table 5.** Average temperature (°C) and total precipitation (mm) by week after initial herbicide treatment (WAIT) during the winter study periods at Los Lunas (ASC) and Las Cruces (LSC), NM, USA. WAIT 0 represents the week preceding the treatment.

WAIT	Los Lunas, NM (2017–2018)		Las Cruces, NM (2018–2019)	
	Temperature	Precipitation	Temperature	Precipitation
0	8.2	0	7.5	8
1	−0.9	0	6.6	1
2	1.1	0	6.8	5
3	2.7	0	1.3	4
4	0.8	0	4.4	2
5	4.9	0	7.8	1
6	0.2	0	6.5	0
7	0.3	1	5.1	0
8	3.2	0	10.4	0
9	6.0	0	5.4	0
10	8.1	8	7.8	0
11	6.5	1	8.1	0
12	3.9	0	13.6	0
13	7.1	0	12.7	3
14	10.3	2	9.5	3
15	10.8	0	16.6	0
16	11.8	1	14.8	0
17	15.1	0	17.0	0

Herbicide damage ratings at LSC and ASC were analyzed separately because of the differences in rating dates. Additionally, there were no differences in ratings among treatments or WAIT or any significant interactions because no damage was observed (Table 6). The treatment  $\times$  WAIT interaction existed for percent herbicide damage to alfalfa at the LSC where percent herbicide damage to alfalfa was significantly greater than the NTC for all herbicide treatments until WAIT 17, except for imazethapyr, imazamox at WAIT 3 and 14. However, the saflufenacil treatments exerted significantly greater herbicide injury to the alfalfa compared to the other commercially available herbicide treatments for most of the study (Tables 6 and 7).

**Table 6.** Results of statistical analysis from field studies evaluating herbicide damage to alfalfa in winter 2017–2018 and yield of the first two alfalfa harvests in the following spring at the NMSU Agricultural Science Center (ASC) at Los Lunas, NM USA, and the NMSU Leyendecker Plant Science Center (LSC) at Las Cruces, NM USA.

Effect	Damage		Yield
	ASC	LSC	Both Locations
Pr > F			
Location	-----	-----	0.0002
Week after initial treatment (WAIT) or Harvest	1.000	<0.0001	<0.0001
Location $\times$ Harvest	-----	-----	<0.0001
Herbicide treatment (TRT)	1.000	<0.0001	0.3512
Location $\times$ TRT	-----	-----	0.3431
TRT $\times$ WAIT or Harvest	1.000	<0.0001	0.8611
Location $\times$ TRT $\times$ Harvest	-----	-----	0.1953

Damage ratings at Los Lunas and Las Cruces were analyzed separately because of differences in rating dates.

**Table 7.** Percent herbicide damage to alfalfa over 17 weeks after initial herbicide treatment (WAIT) during winter 2017–2018 shown in the table for the NMSU Leyendecker Plant Science Center (LSC) at Las Cruces, NM USA, and in the footnote for the NMSU Agricultural Science Center (ASC) at Los Lunas, NM, USA. Data are the means of four replications at each location.

WAIT × Treatment $p < 0.0001$ ; $p$ -Values for the 1st through 5th Order Polynomial Effects were $p < 0.01$														
Treatment	Rate	3	6	9	11	13	14	17						
NTC1	–	5	D <sub>2</sub>	0	E	5	E	0	D	0	D	5	D	5
Saflufenacil	23 g ai <sup>1</sup> ha <sup>−1</sup>	95	A	70	A	70	A	73	A	55	AB	31	A	5
Saflufenacil	50 g ai ha <sup>−1</sup>	95	A	74	A	71	A	76	A	58	A	33	A	5
2,4-DB amine	1684 g ae <sup>1</sup> ha <sup>−1</sup>	9	CD	15	CD	23	C	33	C	24	C	18	B	5
MCPA	518 g ae ha <sup>−1</sup>	31	B	30	B	38	B	58	B	45	B	29	A	5
Imazethapyr	104 g ae ha <sup>−1</sup>	8	D	13	D	14	D	23	C	15	C	10	CD	5
Imazamox	54 g ae ha <sup>−1</sup>	5	D	10	D	15	C	23	C	15	C	11	CD	5
Hexazinone	3618 g ai ha <sup>−1</sup>	13	CD	15	CD	19	C	23	C	13	C	10	CD	5
Terbacil	1343 g ai ha <sup>−1</sup>	20	BC	20	C	23	C	33	C	23	C	16	BC	5
5% LSD <sup>1</sup>		11		7		8		13		12		7		NS
$p$ -value		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		1.000

<sup>1</sup> NTC = non-treated control; ai = active ingredient; ae = acid equivalent; LSD = least significant difference. <sup>2</sup> Means within a column followed by the same letter(s) are not significantly different based on the 5% LSD for that date. For ASC in 2017–2018, no significant differences among treatments was observed for any measurement date because no injury was observed. Visual evaluations for herbicide injury were assessed on a percent scale where 1 equaled no alfalfa injury, and 100 equaled complete injury coverage of alfalfa plants.

When harvest events and locations are combined and analyzed together there were no differences in yield among herbicide treatments or compared to the NTC (Table 6). In research conducted in California, saflufenacil that was applied in dormant season alfalfa at least 90 days prior the first harvest event the following spring did not negatively affect yield [45]. The location × harvest interaction was significant because average first harvest yields at LSC were reduced compared to second harvest yields, while at ASC first harvest yields were greater than second harvest yields (91 vs. 117 g m<sup>−2</sup> for the first and second harvests at LSC, respectively, and 243 vs. 168 g m<sup>−2</sup> for the first and second harvests at ASC, respectively; LSD = 18), typical to the yield pattern of alfalfa in which yield declines across the growing season. The difference at LSC could have been due to the long recovery period required by the alfalfa throughout the winter that also would have utilized root energy needed for early spring growth. Additionally, frequent freezing temperatures throughout winter would have further depleted energy for that growth in spring, especially for the non-dormant variety grown at LSC (daily temperature data not shown) [27,28]. These freezes occurring shortly after the herbicide application and through WAIT 4 would have accounted for the observed damage to the NTC alfalfa (Table 7). Freezing or near freezing temperatures also occurred prior to the observations taken at WAIT 9 and 14, as well as within 10 days of the rating and first harvest taken at WAIT 17, when some damage was still observed for all treatments, including the NTC. Freezing temperatures also occurred throughout the winter of 2017–2018 at the ASC; however, no damage to the alfalfa was observed due to that or any herbicide treatment because the alfalfa was a dormant variety having little or no energy depletion throughout the study period [27,28] and the first harvest was taken later allowing more time for recovery after any late freezes.

#### 4. Discussion

At the ASC, there were no detectable visual differences amongst treatments for alfalfa injury or slowed growth as a result of herbicide applications throughout the duration of the study (Table 6 footnote). The glyphosate treatment was included in greenhouse component of this study to evaluate the potential for plantain control in Roundup Ready® systems.

Additionally, the injury observed at LSC is an indication that herbicide treatments were made while there was still active (although reduced) growth of the alfalfa [46,47]. As a result of the lack of

effect on alfalfa yield compared to the NTC, saflufenacil may be a viable candidate for late-season weed control when applied on slowed green growth of mature alfalfa crop stands during the fall months.

Applications of saflufenacil often displayed greater injury to both broadleaf and buckhorn plantain compared to the NTC with minimal negative effects on the alfalfa in the field. However, this injury was not enough to prevent weed recovery over time. This is not unusual since a single herbicide application seldom provides adequate injury to prevent recovery of mature difficult-to-control perennial weeds like plantain [5,33,48]. Both plantain species in the greenhouse studies were fully mature due to the presence of mature seed heads when herbicide treatments were applied 93 days after planting (personal observation). Additionally, the presence of organic matter in the potting mix used in the greenhouse cone-tainers could have further reduced the efficacy of all herbicide treatments, including saflufenacil [49,50]. Nonetheless, since saflufenacil has a different mode of action than the other comparable herbicides used in this study [15,16], it could be incorporated into an overall rotation for late-season broadleaf weed management in alfalfa to provide injury to plantain and to reduce the development of herbicide resistant weeds over time. This rotation would be especially impactful in Roundup Ready<sup>®</sup> systems where exponential increases in glyphosate resistant weeds have been observed [51–53]. Additionally, buckhorn plantain has been reported as resistant to 2,4-D in Indiana turfgrass management systems [54]. Applications of saflufenacil have also been observed to increase herbicide control of Palmer amaranth species resistant to other PPO-inhibiting herbicide active ingredients in the Southeast [19]. Therefore, any new mode of action herbicide that can be rotated into an herbicide application program for plantain in alfalfa is greatly warranted to also help limit any additional developments in herbicide resistant plantain.

## 5. Conclusions

This research has demonstrated that saflufenacil may provide comparable or greater injury to broadleaf and buckhorn plantain as the commercially available industry standard herbicides with minimal effects on the alfalfa yield. Even though the amount of visually observed injury often increased when saflufenacil was applied in combination with imazethapyr or imazamox, the injury was still not effective enough to prevent the eventual recovery of the target weeds. As a result, further research is needed to determine if additional combinations of saflufenacil with other commercially available herbicides, as well as sequential applications that cause additional and prolonged injury, can provide adequate control of plantain in dormant alfalfa. Similarly, additional research is needed to determine if the herbicide tank-mixes and sequential applications cause negative effects on alfalfa growth and yield over time. Both of these additional research hypotheses are currently being tested.

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