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Nontarget Woody Plant Responses to Broadcast Herbicide Treatment for Mesquite and Pricklypear Control[☆]

R. James Ansley^{1,*}, Megan Clayton², William E. Pinchak³

¹ Professor and Department Head, Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK 74078-6013, USA

² Professor & Extension Range Specialist, Texas A&M AgriLife Extension Service, Uvalde, TX 78801, USA

³ Professor, Texas A&M AgriLife Research, Vernon, TX 76384, USA

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ABSTRACT

Aerial spraying of herbicides is an option for treating undesirable woody species on grasslands and rangelands, but few studies have determined effects of these products on nontarget woody plants important to wildlife. A recently introduced herbicide containing a mixture of clopyralid and aminopyralid (CA) is thought to be specific to honey mesquite (*Prosopis glandulosa*) control. Our objective was to document effects of CA alone and mixed with other brush herbicides, including picloram and triclopyr, on two target species, honey mesquite and pricklypear (*Opuntia* spp.), and two nontarget woody plants, lotebush (*Zizyphus obtusifolia*) and hackberry (*Celtis laevigata* var. *reticulata*). Treatments were 1) CA, 2) CA + triclopyr (CA + Tr), 3) CA + picloram (CA + Pc), and 4) clopyralid + triclopyr (Cp + Tr). We applied aerial spray treatments on four, 4-ha replicated plots of mature mesquite thickets that also contained pricklypear in each of 3 consecutive yr in north-central Texas and evaluated plots at 1 yr and 2 yr post treatment (YPT). We developed a tolerance-rating model with five levels (highly tolerant, tolerant, moderately tolerant, moderately susceptible, and susceptible) that integrated stand-level percent whole plant mortality (root-kill) and percent canopy reduction of surviving plants. Mesquite was susceptible to all treatments in all spray years. Pricklypear was susceptible to CA + Pc (root-kill more than doubled [33–84%] from 1 to 2 YPT) but highly tolerant of the other treatments. Lotebush was highly tolerant or tolerant of all treatments. Hackberry was tolerant of CA and Cp + Tr but susceptible to CA + Pc. The negative effect of CA + Pc on hackberry was greater when hackberry was drought stressed. We recommend inspection of drought status, foliage condition, and abundance of nontarget woody species before broadcast spraying for control of targeted woody species or cacti.

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Introduction

Woody plant encroachment (WPE) has occurred in savanna and grassland systems over the past century throughout the world (Archer et al. 1995; Scholes and Archer 1997; Van Auken 2000; Roques et al. 2001; Fensham et al. 2005; Stevens et al. 2017). WPE often can reduce understory grass and forb production and species diversity (Stuart-Hill and Tainton 1989; Dalle et al. 2006; Eldridge

et al. 2011; Ratajczak et al. 2012; Sala and Maestre 2014; Archer et al. 2017). Well-known examples are the expansion of the deciduous woody legume honey mesquite (*Prosopis glandulosa* Torr.) in the Southern Great Plains (SGP) region of the United States (Archer 1989; Asner et al. 2003; Hughes et al. 2006; Mirik and Ansley 2012) and velvet mesquite (*P. velutina*) in the southwestern United States (Cable 1977; McClaran et al. 2008; Brunelle et al. 2014). Studies in the SGP have shown that as honey mesquite density and canopy cover increases, grass production decreases due to competition for water, light, and other resources (Bedunah and Sosebee 1984; Ansley and Castellano 2006). This can negatively impact cattle production and grassland wildlife habitat because some of the most productive C₄ grass species that provide forage for cattle and structural and thermal cover for wildlife habitat are most vulnerable to increasing honey mesquite cover (Tanner et al. 2017; Tomecek et al. 2017; Carroll et al. 2018; Ansley et al. 2023).

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* Correspondence: R. James Ansley, Natural Resource Ecology and Management Dept, 008C, Agricultural Hall, Oklahoma State University, Stillwater, OK 74078-6013, USA.

E-mail address: jim.ansley@okstate.edu (R.J. Ansley).

Most early studies designed to control mesquite focused on grass responses following treatments because of concerns related to cattle forage. Treatment of mesquite with a variety of aerial sprayed herbicides, including clopyralid, triclopyr, and picloram has been shown to reduce mesquite density or cover and increase grass growth (Dahl et al. 1978; Bedunah and Sosebee 1984; Ansley et al. 2004). These chemicals are known to control certain dicotyledonous species but generally do not harm grasses (Hart et al. 2012; Lyons et al. 2020).

In recent years land ownership values in the SGP have shifted from primarily livestock production to livestock and wildlife production mainly to earn additional income from wildlife hunting leases. An elevated level of plant species diversity is a common goal of wildlife habitat management as it satisfies wildlife dietary or shelter needs throughout different seasons and weather conditions (Fulbright 1996; Linex 2014). Encroaching woody and cactus species such as mesquite and pricklypear (*Opuntia* spp.) provide some benefits to wildlife, but as their size and density increase, the amount and diversity of understory grasses and forbs for wildlife habitat needs declines (Price et al. 1985; Ansley and Castellano 2006; Archer et al. 2017). However, diversity of associated woody species that are valuable for wildlife may increase (Archer et al. 1988; Franco-Pizaña et al. 1995; Ramirez et al. 1997; Fernandez and Guthery 2012).

Several studies have quantified effects of broadcast herbicide treatments designed to control invasive shrubs or forbs on nontarget forb species (Arnold and Santelmann 1966; McDaniel et al. 1982; Meyer and Bovey 1985; Rice et al. 1997; Sheley and Denny 2006; Fuhlendorf et al. 2009). However, more studies are needed to determine the effect of broadcast herbicide applications designed to control unwanted woody species on nontarget woody species valuable for wildlife that reside beneath or near the overstory of targeted woody species (Bovey et al. 1970; Whisenant 1987).

A recently introduced herbicide that includes a mixture of clopyralid and aminopyralid (hereafter “CA”) is thought to be specific to mesquite control with minor damage to associated nontarget plants. In some situations where pricklypear plants dominate the understory beneath mesquite, an aerial spray treatment that targets both mesquite and pricklypear is desired. Picloram is typically an effective chemical treatment for pricklypear control (Price et al. 1985; Peterson et al. 1988), but effects on nontarget woody species need further investigation. Our objective was to document effects of CA alone and mixed with other herbicides, including picloram and triclopyr, on target species mesquite and pricklypear and nontarget woody species that occur within dense mesquite thickets in northern Texas. In this paper, we focus on two nontarget woody species, lotebush (*Zizyphus obtusifolia*) and netleaf hackberry (*Celtis laevigata* var *reticulata*) (hereafter: hackberry). Lotebush provides excellent resting and escape cover for bobwhite quail (*Colinus virginianus*) (Renwald et al. 1978; Fernandez and Guthery 2012) and nesting sites for nongame birds (Renwald 1978). Browse and/or fruits of both species are important food sources for bobwhites, deer, antelope, coyotes, wild turkeys, songbirds, and small mammals (Hatch and Pluhar 1993; Tyrl et al. 2008; Ramirez et al. 1997; Linex 2014).

Methods

Research was conducted on three sites in north-central Texas: Smith-Walker Experimental Ranch near Vernon, Texas (lat 34.035502°; long -99.243138°; elev. 365 m; hereafter: Vernon); Moore Creek Ranch near Hamlin (32.959810°; -100.278529°; elev. 548 m; hereafter: Hamlin); and Hart Ranch near Baird (32.445747°; -99.263454°; elev. 436 m; hereafter: Baird). The 30-

yr (1981–2010) mean annual rainfall is 708, 591, and 706 mm at Vernon, Hamlin, and Baird, respectively (NOAA-NCEI 2022).

Soils at the Vernon site are mostly composed of the Rotan (52%), Vernon (27%), and Wichita (11%) soil series. The Ecological Sites are Clay Loam (R078CY096TX) for Rotan and Wichita and Clay Prairie (R078CY112TX) for the Vernon series. Soils at Hamlin are mostly of the Tillman (73%) and Bippus (15%) soil series. The Ecological Sites are Clay Loam (R078CY096TX) for Tillman and Loamy Bottomland (R078BY080TX) for Bippus. Soils for one replicate at Baird are of the Throck-Owens-Lueders Association (50%) and the Throck-Callahan-Owens Association (43%). The Ecological Sites are Rocky Hill (R078AY123TX) for Throck-Owens-Lueders, and Clay Slopes (R078AY120TX) for Throck-Callahan-Owens. Soils for the second replicate at Baird are of the Frio-Gageby Association (68%) and Gageby loam series (32%). The Ecological Site is Loamy Bottomland (R078AY121TX) for both of these types (USDA-NRCS 2022).

Vegetation at all three sites consists of a multistemmed 3- to 5-m tall, 30- to 40-year-old honey mesquite overstory, 40–60% canopy cover, ~500 trees·ha⁻¹, and a herbaceous mixture of C₃ and C₄ perennial grasses. Mesquite accounted for > 90% of total woody cover in all plots. The primary C₃ perennial grass is Texas wintergrass (*Nassella leucotricha* [Trin. and Rupr.] Pohl.) that occurs mainly beneath mesquite canopies. C₄ midgrasses include sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.), vine mesquite (*Hoplia obtusa* [Kunth] Zuloaga & Monroe), and sand dropseed (*Sporobolus cryptandrus* [Torr.] A. Gray). Common C₄ short-grasses are buffalograss (*Buchloe dactyloides* [Nutt.] Engelm.) and curly mesquite (*Hilaria belangeri* [Steud.] Nash). C₃ annual grasses include Japanese brome (*Bromus japonicus* Thunb. ex Murray) and little barley (*Hordeum pusillum* Nutt.). Perennial forbs include western ragweed (*Ambrosia psilostachya* DC.) and silverleaf nightshade (*Solanum elaeagnifolium* Cav.). Common annual forbs are annual broomweed (*Amphichrysis dracunculoides* DC.) and maretail (*Conyza canadensis*), [L.] Cronquist (Hatch and Pluhar 1993; Stubbendieck et al. 2017). Before and during the study period, cattle freely grazed at a moderate stocking rate of 6 to 8 ha·animal unit⁻¹·yr⁻¹ at Vernon and Baird. Livestock grazing was excluded at Hamlin for 5 yr before the study. Nontarget shrub species differed at each site, but the most common species at all three sites were lotebush and hackberry. Lotebush occurred in much greater numbers in each plot than any other nontarget shrub.

We applied four broadcast aerial spray herbicide treatments via commercial helicopter on two, 4.05-ha randomly selected plots that contained a dominant mesquite overstory at each of two locations in Central Texas in early July 2013, 2014, and 2015 (four total replicate plots per treatment each year). Thus, in each spray year we treated a new cohort of plots and any one plot was treated only one time in 2013, 2014, or 2015 (the study was essentially duplicated in 3 consecutive yr). Treatments were 1) clopyralid+aminopyralid at 2.046 l·ha⁻¹ (CA), 2) CA at 2.046 l·ha⁻¹+triclopyr at 0.585 l·ha⁻¹ (CA+Tr), 3) CA at 2.046 l·ha⁻¹+picloram at 2.338 l·ha⁻¹ (CA+Pc), and 4) clopyralid at 0.782 l·ha⁻¹+triclopyr at 0.585 l·ha⁻¹ (Cp+Tr) (technical descriptions, Table 1). We selected untreated areas located near previously treated areas for treatment each year. Treatments were applied at the Vernon site in all 3 yr. We applied the same treatments at Hamlin in 2013 and 2014 and at Baird in 2015 as the Hamlin site had an insufficient amount of untreated mesquite in 2015. Thus, we established 48 total plots (2 sites/yr × 4 treatments/site × 2 replicates/treatment × 3 yr).

We applied each treatment during early to mid-July each year. Before spraying, we constructed shape files of plot locations on aerial images via ArcMap GIS. These files were uploaded to the helicopter computer system. The helicopter system tracked spray swaths in each plot via Global Positioning System (GPS), and we

Table 1

Herbicide mixtures and rates aerially applied directly above mesquite canopies during 2013–2015, Central Texas. Total spray volume was 46.8 l·ha⁻¹ (5 gal·ac⁻¹) with nonionic seed-oil spray adjuvant. Each replicate plot was 4.05 ha (usually 100.6 × 402 m) and consisted of seven 14.4-m wide straight-line spray swaths along the long direction. Some plots were shorter in length but wider and remained 4.05 ha in total size. Texas A&M AgriLife employees supervised the mixing of each treatment load.

Treatment ¹	Herbicide and rate ²
CA	Clopyralid + aminopyralid at 2.046 l·ha ⁻¹ (0.69 kg·ha ⁻¹) ³ . Rate per component: clopyralid = 1.681 l·ha ⁻¹ (0.56 kg·ha ⁻¹) (82.1%); aminopyralid = 0.365 l·ha ⁻¹ (0.12 kg·ha ⁻¹) (17.9%) (equivalent to Trade Label 4, 2020).
CA + Tr	CA at 2.046 l·ha ⁻¹ + Triclopyr-2-butoxyethyl ester at 0.585 l·ha ⁻¹ (0.28 kg·ha ⁻¹) (equivalent to Trade Label 3, 2020).
CA + Pc	CA at 2.046 l·ha ⁻¹ + Picloram potassium salt at 2.338 l·ha ⁻¹ (0.56 kg·ha ⁻¹) (equivalent to Trade Label 2, 2019).
Cp + Tr	Clopyralid monoethanolamine salt at 0.782 l·ha ⁻¹ (0.28 kg·ha ⁻¹) (see Trade Label 1, 2012) + Triclopyr at 0.585 l·ha ⁻¹ (0.28 kg·ha ⁻¹) (equivalent to Trade Label 3, 2020).

¹ The CA treatment (and as a portion of the other mixtures) was applied at the recommended rate (Lyons et al. 2020).

² English unit rates: CA = 2.046 l·ha⁻¹ (28 oz·ac⁻¹); triclopyr = 0.585 l·ha⁻¹ (8 oz·ac⁻¹); picloram = 2.338 l·ha⁻¹ (32 oz·ac⁻¹); clopyralid = 10.7 oz·ac⁻¹ (0.782 l·ha⁻¹).

³ Acid equivalent (kg·l⁻¹): CA = 0.336 (clopyralid 0.276; aminopyralid 0.60); triclopyr = 0.479; picloram = 0.24; clopyralid = 0.359.

Table 2

Soil temperature and weather conditions during treatment applications, 2013–2015, Central Texas.

Site	Spray date	Spray time	Soil Temperature at 46 cm (°C) ¹	Air temperature (°C)	Relative humidity (%)	Wind speed and direction (m·sec ⁻¹)
Vernon	10July13	6:55-8:20	32.2	26.1-28.9	39-45	0.89-2.24 SSW
Hamlin	05July13	6:45-8:15	30.6	20.6-26.1	38-42	0.89-3.13 SW
Vernon	10July14	6:30-7:20	28.9	23.9-26.1	50-56	0.45-1.79 SW
Hamlin	11July14	7:10-8:00	28.3	22.2-25.6	60-64	0.45-1.34 W
Vernon	19July15	6:45-8:15	30.6	23.3-26.7	50-61	0-0.89 SSW
Baird	13July15	7:15-8:30	26.7	23.9-25.6	78-82	2.24-4.92 SSW

¹ Soil temperature of at least 23.9°C but preferably closer to 26°C at 46 cm depth was needed for best results with 2,4,5-T and picloram foliar herbicides on mesquite (Dahl et al. 1971; Sosebee et al. 1973). This has been assumed to apply to other mesquite foliar herbicides (Hart et al. 2012).

downloaded those data for use during evaluations. Spray conditions in all years were appropriate for minimizing spray drift or evaporative loss (Lyons et al. 2020; Table 2). The total volume of each treatment mixture in each helicopter load was equal to that needed to cover the land area in each replicated plot for each treatment. We documented that the helicopter load tank was empty upon each return to the mixing platform. This information plus the GPS tracking confirmed that total load content was delivered evenly over each designated plot.

The region had near-average precipitation in spring 2010, but this was followed by a period of extreme drought and heat from fall 2010 through the first half of 2013 (Figs. 1, S1, S2, available online at <https://doi.org/10.5061/dryad.tdz08kq65>). We sprayed our first set of treatments in early July 2013 before the region received rainfall that was 3 × the average for that month. The yr 2014 had a dry spring but above-average precipitation in June and July during our treatment applications. The yr 2015 received > 3 × average precipitation in May and close to average precipitation in June and July before our 2015 treatment applications.

We evaluated target mesquite and pricklypear and nontarget lotebush and hackberry at 1 yr and 2 yr post treatment (YPT) at the individual plant (or motte for pricklypear) level for whole-plant mortality (hereafter: root-kill). If plants were not root-killed, we estimated percent canopy reduction of individual plants compared to the original canopy stem and branch (or motte) frame. We then converted these values to percentages of the total population per species per plot.

Mesquite and lotebush were found in all plots during each evaluation period. Pricklypear was found in at least 2 of the 4 replicate plots, while hackberry presence was highly variable. We evaluated approximately 50–150 mesquite, 5–70 lotebush, 0–40 hackberry plants, and 0–90 pricklypear mottes in each plot. Over the 4-yr period that included 1 and 2 YPT after each spray application (2014–2017), we evaluated 8 971 mesquite, 2 650 lotebush, and 565 hackberry plants and 1 800 pricklypear mottes. No plants were marked, so each evaluation included a potentially distinct set of individuals of each species in each plot. We transferred the GPS-linked map of spray swaths in each plot from the helicopter system

Table 3

P values for PROC MIXED analysis of mesquite, pricklypear, lotebush, and hackberry root-kill (RK), canopy reduction (CR), and tolerance rating (TLR) as affected by herbicide treatment (TRT), spray yr (YEAR), and yrs post treatment (YPT). Any P value < 0.05 is shown in bold.

Effect	Df ²	RK	CR	TLR	Detail
---Mesquite---					
TRT	3	<.0001	<.0001	<.0001	
YEAR	2	<.0001	<.0001	<.0001	
YPT	1	0.6134	0.6107	0.2514	
TRT · YEAR	6	0.0019	0.0004	0.0007	Figure 2
TRT · YPT	3	0.9093	0.6318	0.2944	
YEAR · YPT	2	0.4365	0.5073	0.5783	
TRT · YEAR · YPT	6	0.9681	0.8795	0.7423	
---Pricklypear---					
TRT	3	<.0001	<.0001	<.0001	
YEAR	2	0.0571	0.0371	0.1196	
YPT	1	0.0032	0.0266	0.2825	
TRT · YEAR	6	0.0634	0.1722	0.4407	
TRT · YPT	3	<.0001	<.0001	0.0005	Figure 3
YEAR · YPT	2	0.0298	0.0687	0.2820	
TRT · YEAR · YPT	6	0.0340	0.0168	0.1443	
---Lotebush---					
TRT	3	0.3420	0.0369	0.0254	Table 4
YEAR	2	0.4056	0.0003	0.0007	Table 4
YPT	1	0.4672	0.0116	0.0275	Table 4
TRT · YEAR	6	0.6199	0.6311	0.6713	
TRT · YPT	3	0.2391	0.4888	0.5399	
YEAR · YPT	2	0.0260	<.0001	<.0001	Figure 4
TRT · YEAR · YPT	6	0.6837	0.3655	0.4260	
---Hackberry ¹ ---					
TRT	1	0.0004	<.0001	<.0001	Table 5
YEAR	1	0.3492	0.3601	0.4285	
YPT	1	0.8815	0.0894	0.0450	Table 5
TRT · YEAR	1	0.6055	0.6878	0.8230	
TRT · YPT	1	0.7765	0.8056	0.5797	
YEAR · YPT	1	0.5252	0.1687	0.1311	
TRT · YEAR · YPT	1	0.8837	0.6021	0.4455	

¹ Hackberry analysis included only treatments CA and CA + Pc and only 2013 and 2015 spray yrs due to lack of replicates in CA + Tr at 2 YPT after the 2014 spray yr, CA + Pc at 1 YPT after the 2014 spray yr, and Cp + Tr at 1 YPT after the 2013 spray yr.

² Df indicates degrees of freedom; Detail, comparisons shown in Figures 2–4 and Tables 4 and 5.

Table 4

Lotebush percent root-kill (RK), percent canopy reduction (CR), and color-coded tolerance rating (TLR) as affected by main effects of herbicide treatment, yr of application, and yrs post treatment (YPT) based on analysis from Table 3. Means (\pm least squares standard error) with similar letters within each column are not significantly different at $P \leq 0.05$.

Effect	RK (%)	CR (%)	TLR ¹
CA	2.09 \pm 0.89 a	9.52 \pm 3.29 b	86.73 \pm 4.16 a
CA+Tr	1.94 \pm 1.16 a	17.63 \pm 5.52 a	75.23 \pm 6.55 b
CA+Pc	4.08 \pm 1.35 a	18.35 \pm 5.46 a	74.82 \pm 6.53 b
Cp+Tr	1.99 \pm 0.61 a	13.58 \pm 2.61 ab	80.33 \pm 3.19 a
2013	2.13 \pm 0.97 a	11.52 \pm 2.48 b	83.18 \pm 3.07 a
2014	3.44 \pm 1.07 a	21.85 \pm 5.38 a	70.63 \pm 6.51 b
2015	2.00 \pm 0.67 a	10.95 \pm 1.55 b	84.02 \pm 2.21 a
1 YPT	2.17 \pm 0.67 a	11.68 \pm 1.91 b	82.76 \pm 2.56 b
2 YPT	2.88 \pm 0.85 a	17.86 \pm 3.92 a	75.79 \pm 4.76 a

¹Blue indicates highly tolerant (80–100); green, tolerant (60–79.9).

CA indicates clopyralid + aminopyralid; CA + Tr, CA + triclopyr; CA + Pc, CA + picloram; Cp + Tr, clopyralid + triclopyr.

onto our cell phones or tablets to monitor our location in each plot during evaluations. We observed during evaluations that mesquite in unsprayed 50-m wide buffers between plots were undamaged; this indicated that spray drift outside plots was minimal.

In addition to root-kill and canopy reduction, we developed a model that provided a tolerance rating of each species to each treatment that integrated population-level root-kill and canopy reduction responses of each species within each plot. We determined the percentage of individual plants of each species that occurred in each of six categories: 1) live, original canopy unaffected; 2) live, original canopy reduced 1–25%; 3) live, original canopy reduced 26–50%; 4) live, original canopy reduced 51–75%; 5) live, original canopy reduced 76–100% (if 100%, there was evidence of basal sprouting); and 6) root-killed (i.e., whole plant mortality). Foliage from basal sprouting was not included in the canopy reduction estimates. Percentages in each category were multiplied by a tolerance multiplier that generated a tolerance rating within a range of 0–100 that was divided into five tolerance categories: highly tolerant (rating 80–100), tolerant (60–79.9), moderately tolerant (40–59.9), moderately susceptible (20–39.9), and susceptible (0–19.9).

The tolerance multiplier value was progressively lower as the level of plant damage increased (1.0, 0.75, 0.35, 0.15, 0.01, and 0 for categories 1 through 6 listed earlier, respectively). This approach ensured that any rating classified as highly tolerant or tolerant did not include a high percentage of severely damaged or root-killed plants. We used an iterative process to develop the multiplier values and tolerance categories by revisiting plots after our evaluations to see if the calculated tolerance ratings were compatible with what we would qualitatively observe for a particular species in a plot (Table S1, available online at <https://doi.org/10.5061/dryad.tdz08kq65>).

To demonstrate how a tolerance rating was determined, we include two examples based on actual data collected. In the first ex-

ample, the plot evaluation for a particular species yielded 55.6%, 27.8%, 3.7%, 5.6%, 3.7%, and 3.7% of trees in each of the six original categories in the order listed earlier. Using the multiplier values, the tolerance rating was 78.6 (i.e., $[55.6 \cdot 1.0] + [27.8 \cdot 0.75] + [3.7 \cdot 0.35] + [5.6 \cdot 0.15] + [3.7 \cdot 0.01] + [3.7 \cdot 0.0]$). The species in this plot would be included in the tolerant category. In contrast, evaluation of a different species yielded 0%, 0%, 2.5%, 5.1%, 44.3%, and 48.1% of trees in order of the six original categories and a tolerance rating of 2.1. This species would be in the susceptible category. The second example illustrates why we included a progressively decreasing tolerance multiplier value. Even though more than half the population survived, the tolerance rating remained in the lowest category because root-kill was 48.1% and 44.3% of surviving plants had > 75% canopy reduction.

Analysis

We used the SAS PROC MIXED procedure (SAS 9.4, SAS Institute Inc., Cary, NC) to test main effects of treatment (4 levels), spray year (3 levels), and YPT (2 levels: 1 and 2 YPT) and their interactions on percent root-kill, percent canopy reduction, and tolerance rating for each of the species studied. There were four replicate plots for each herbicide treatment (two per site each spray year). However, because individual pricklypear and hackberry plants were not found in some plots, some of the analyses for these two species had fewer than four replicates. For example, hackberry response was limited to the CA and CA + Pc treatments due to no replicates in Cp + Tr at 1 YPT after the 2013 spray year and in CA + Tr at 2 YPT after the 2014 spray year.

Because one of the spray sites was moved from Hamlin to Baird in the third spray year (2015), we did not include “site” as a main effect in the model described earlier that included all 3 spray years. However, we conducted an additional 4-way, site \times treatment \times spray year \times YPT, analysis comparing the Vernon

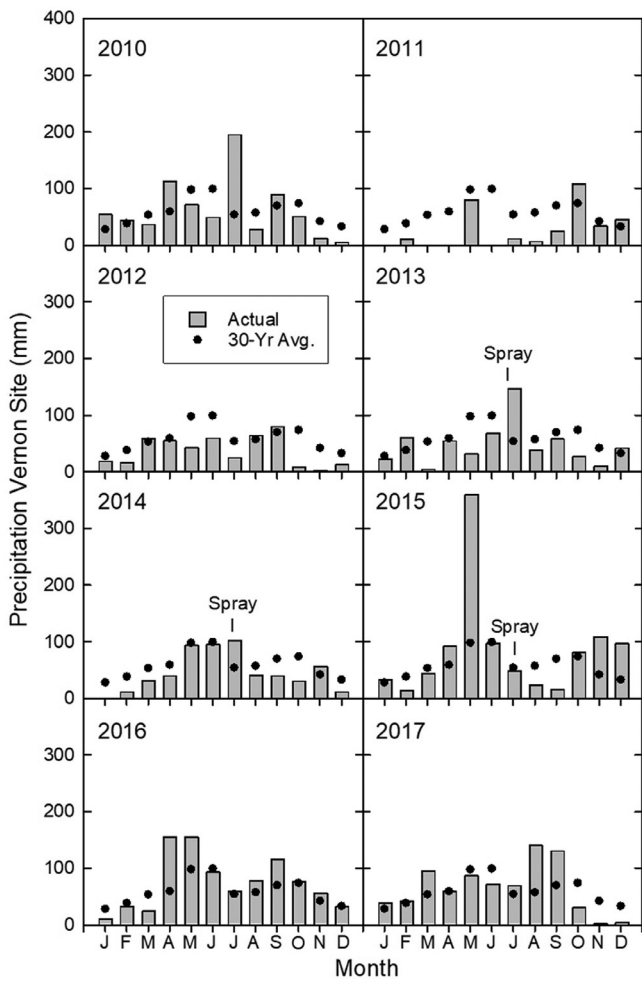


Figure 1. Monthly precipitation at the Vernon site compared with 30-yr average, 2010–2017. Treatment spray events occurred in July 2013–2015. Precipitation patterns for Hamlin and Baird are in Figures S1 and S2.

and Hamlin sites in the first 2 spray years (2013, 2014) and a 3-way, site × treatment × YPT, analysis comparing the Vernon and Baird sites in the final spray year (2015). These analyses were only possible for mesquite and lotebush as these species had sufficient representation in each replicate plot of both sites in each spray year.

We used the SAS Least Squares procedure for mean separations at $P \leq 0.05$ (hereafter, “significant” means $P \leq 0.05$). Data were not transformed before analysis (Warton and Hui 2011; Ribeiro-Oliveira et al. 2018).

We evaluated responses of many other nontarget shrub species using the same methodology described earlier (complete list in Table S2, available online at <https://doi.org/10.5061/dryad.tdz08kq65>). However, the number of individuals of each of those species was considerably lower than the nontarget species reported here, thus limiting statistical analysis. These results will be reported in a future publication.

Results

In the three-way analysis of treatment × spray year × YPT, mesquite had significant main effects of treatment and spray year and a significant treatment × treatment year interaction for all three response variables (Table 3). This occurred because root-kill and canopy reduction were lower and tolerance rating score was higher in the Cp + Tr treatment following the 2015 spray year

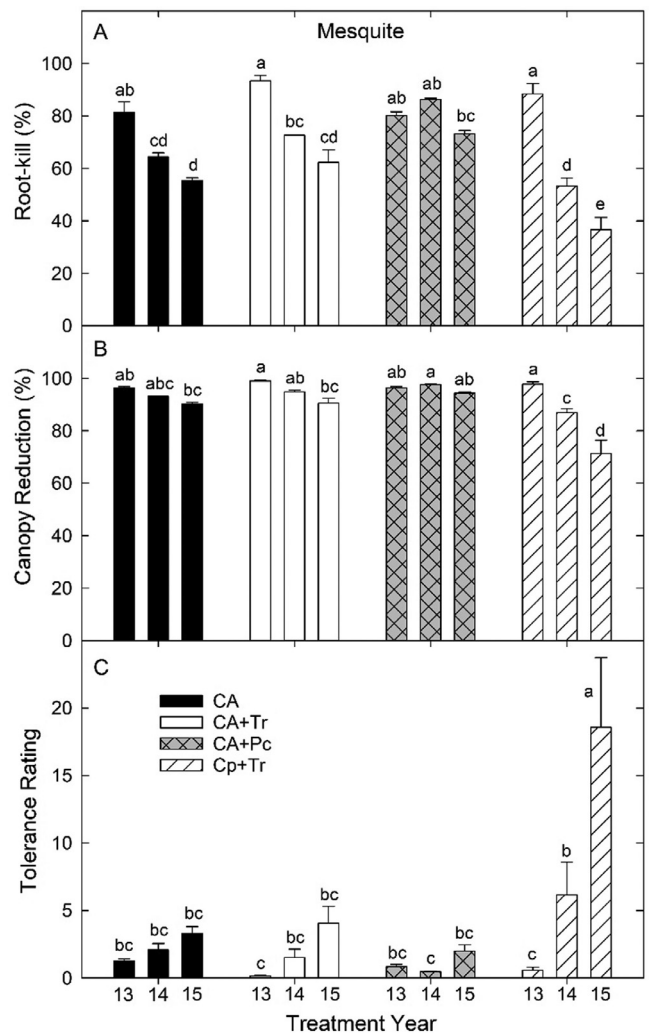


Figure 2. Mesquite root-kill (A), canopy reduction (B), and tolerance rating (C) in response to the treatment × treatment yr (13 = 2013, 14 = 2014, 15 = 2015) interaction from Table 3 analysis (1 and 2 YPT pooled). Vertical lines are standard error. Means with similar letters within each panel are not significantly different at $P \leq 0.05$. CA indicates clopyralid + aminopyralid; CA + Tr, CA + triclopyr; CA + Pc, CA + picloram; Cp + Tr, clopyralid + triclopyr.

(Fig. 2). However, tolerance rating remained in the susceptible category (< 20).

Pricklypear had significant treatment × YPT interaction for all three response variables, and several other significant main effects and interactions (see Table 3). The significant treatment × YPT interaction occurred because root-kill and canopy reduction increased from 1 to 2 YPT in the CA + Pc treatment but not in the other treatments (Fig. 3). This reduced the tolerance rating from moderately susceptible (20–39) to susceptible (< 20) from 1 to 2 YPT in the CA + Pc treatment.

Lotebush had significant treatment, year, and YPT effects on canopy reduction and tolerance rating and a significant year × YPT interaction on all three response variables (see Table 3). Lotebush canopy reduction was greater in the CA + Tr and CA + Pc treatments than in the CA treatment, which lowered the tolerance rating of these treatments compared with the CA treatment from highly tolerant to tolerant (Table 4). The spray year × YPT interaction occurred because root-kill, canopy reduction, and tolerance rating responses from 1 to 2 YPT were different after the 2013 compared with the 2014 treatments (Fig. 4). In all comparisons, lotebush tolerance rating remained in the highly tolerant (> 80) or

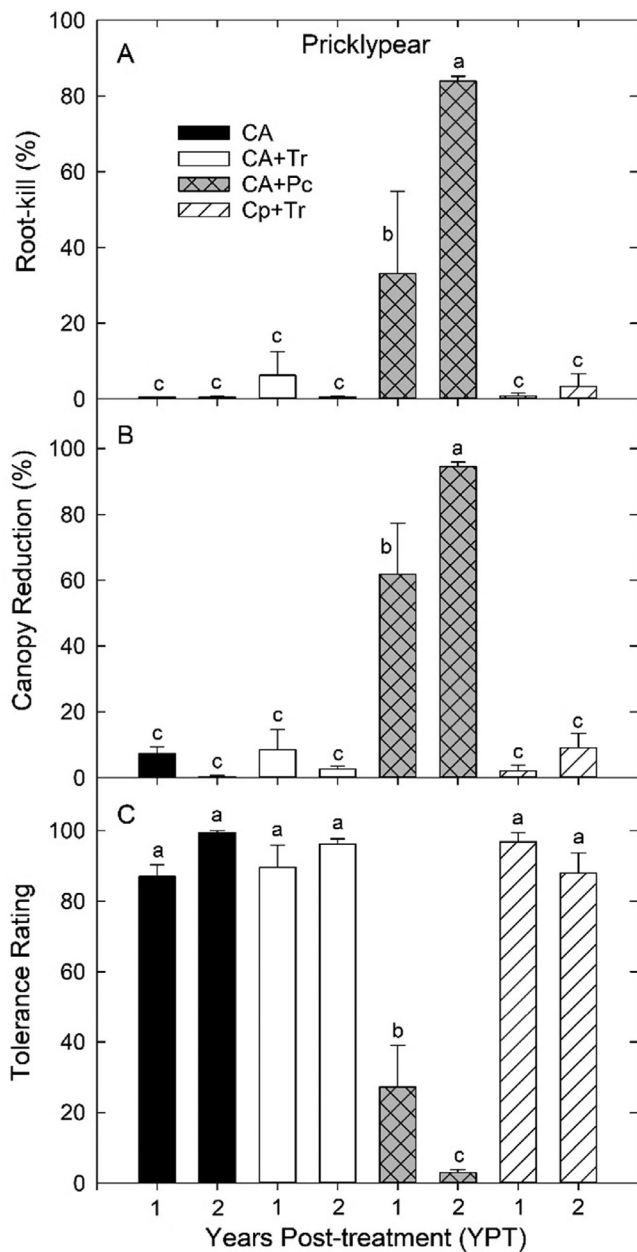


Figure 3. Pricklypear root-kill (A), canopy reduction (B), and tolerance rating (C) in response to the treatment \times yrs-post-treatment interaction effect from Table 3 analysis (treatment yrs pooled). Vertical lines are standard error. Means with similar letters within each panel are not significantly different at $P \leq 0.05$. CA indicates clopyralid + aminopyralid; CA + Tr, CA + triclopyr; CA + Pc, CA + picloram; Cp + Tr, clopyralid + triclopyr.

tolerant (60–79.9) category (see Table 4, Fig. 4), with one exception. When averaged across all four treatments, lotebush declined from highly tolerant (> 80) to moderately tolerant (40–59.9) from 1 to 2 YPT after the 2014 treatment (see Fig. 4).

Including only the CA and CA + Pc treatments and 2013 and 2015 spray years, hackberry had significant main effects of treatment for the three response variables and a significant main effect of YPT for tolerance rating (see Table 3). Root-kill and canopy reduction were greater and tolerance rating was much lower (and in the susceptible category) in the CA + Pc than CA treatment (Table 5). Although there was no significant difference in root-kill and canopy reduction between 1 and 2 YPT, the combined effect of

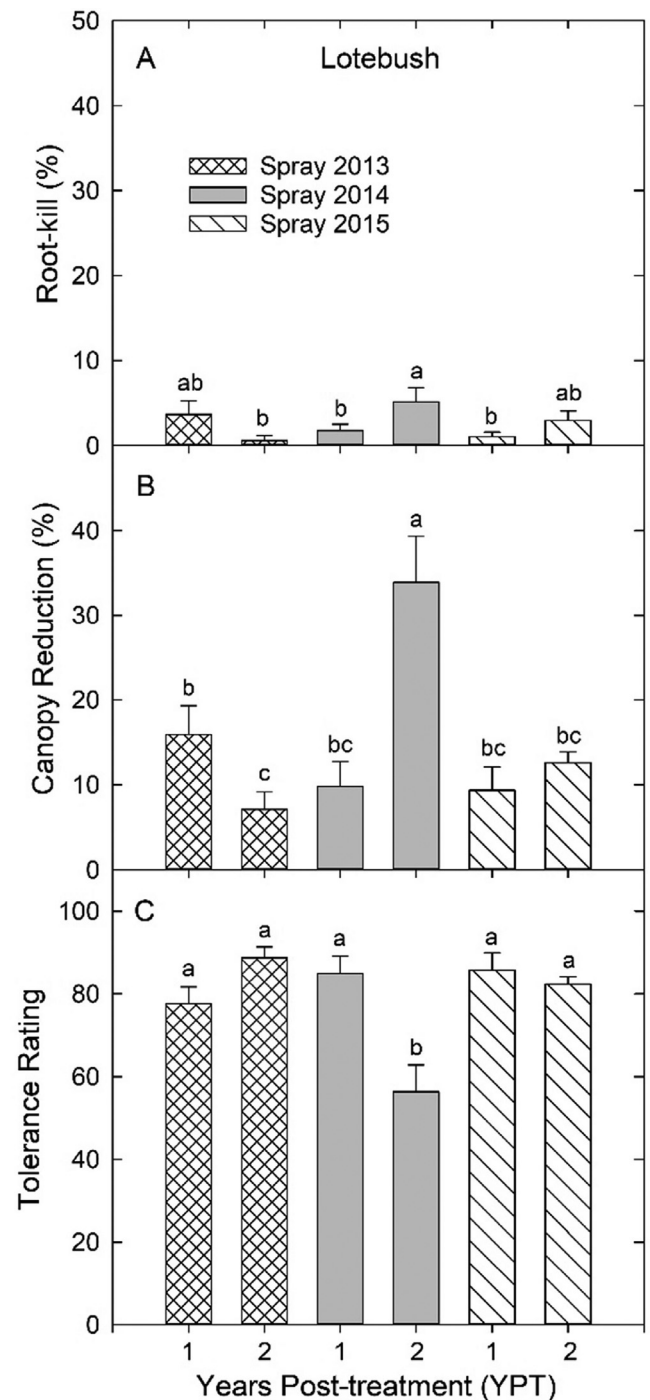


Figure 4. Lotebush root-kill (A), canopy reduction (B), and tolerance rating (C) in response to the spray yr \times yrs-post-treatment interaction effect from Table 3 analysis (herbicide treatments pooled). Vertical bars are standard error. Means with similar letters within each panel are not significantly different at $P \leq 0.05$.

these two variables resulted in an increased tolerance rating from 1 to 2 YPT when both treatments were combined.

There was sufficient replication of hackberry plants to include all four herbicide treatments in a two-way treatment \times spray year analysis at 2 YPT if only spray yr 2013 and 2015 were included. In this analysis, the main effect of treatment was significant for all three response variables (Table 6). Hackberry was highly tolerant or tolerant of all treatments except CA + Pc (Table 7). High root-kill and canopy reduction of surviving plants in this treatment yielded

Table 5

Hackberry percent root-kill (RK), percent canopy reduction (CR), and color-coded tolerance rating (TLR) as affected by herbicide treatment (pooled over 2013 and 2015 spray yrs and YPT) and YPT (pooled over herbicide treatment and 2013 and 2015 spray yrs) based on Table 3 analysis. Means (\pm least squares standard error) with similar letters within main effect and column are not significantly different at $P \leq 0.05$.

Effect	RK (%)	CR (%)	TLR ¹
CA	2.96 \pm 2.72 b	16.04 \pm 5.68 b	74.20 \pm 9.25 a
CA+Pc	58.71 \pm 5.79 a	81.37 \pm 5.81 a	10.95 \pm 5.26 b
1 YPT	29.95 \pm 17.16 x	55.64 \pm 19.55 x	33.04 \pm 17.15 y
2 YPT	31.73 \pm 16.25 x	41.77 \pm 19.05 x	52.12 \pm 20.62 x

¹Green indicates tolerant (60–79.9); yellow, moderately tolerant (40–59.9); orange, moderately susceptible (20–39.9); red, susceptible (0–19.9).

CA indicates clopyralid + aminopyralid; CA + Pc, CA + picloram.

Table 6

P values for PROC MIXED analysis of hackberry root-kill (RK), canopy reduction (CR), and tolerance rating (TLR) as affected by main effects of herbicide treatment (TRT) and spray yr (YEAR) for only yrs 2013 and 2015 at 2 YPT. *P* values < 0.05 are in bold. Analysis included three treatments (CA, CA + Pc, and Cp + Tr) but did not include the CA + Tr treatment due to insufficient replication at 2 YPT after the 2014 spray yr.

Effect	Df	RK	CR	TLR
TRT	3	0.0015	0.0005	0.0016
YEAR	1	0.1302	0.3329	0.4305
TRT · YEAR	3	0.7352	0.6364	0.5500

a tolerance rating of 18 that was in the susceptible category. This tolerance rating was higher than the 11 for the CA + Pc treatment shown in Table 5, but both ratings remained in the susceptible category. Tolerance rating was lower for this treatment in Table 5 because of the inclusion of 1 YPT in the analysis.

Regarding the effects of site, in the four-way analysis (Vernon vs. Hamlin in 2013 and 2014 spray years) there was a significant site effect on mesquite root-kill and a significant site \times YPT interaction effect on mesquite root-kill and canopy reduction (Table S3, available online at <https://doi.org/10.5061/dryad.tdz08kq65>). With all other sources of variation pooled, mesquite root-kill was slightly but significantly greater at Vernon (79.8%) than Hamlin (74.5%). The site \times YPT interaction occurred because there was no difference

in root-kill between 1 YPT (80.3%) and 2 YPT (79.4%) at Vernon, but at Hamlin, root-kill was significantly lower at 1 YPT (69.4%) than at 2 YPT (79.6%).

Regarding lotebush responses in the four-way analysis, there were significant site and site \times year effects on canopy reduction and tolerance rating (see Table S3). Canopy reduction was significantly greater at Hamlin (21.6%) than Vernon (12.9%), and tolerance rating was lower at Hamlin (70.0) than Vernon (80.1). The site \times year interaction occurred because canopy reduction at Vernon was significantly greater after the 2014 than the 2013 spray year (20.5 vs. 5.4%), but there was no difference in canopy reduction between spray years at Hamlin (20.1 vs. 23.2%).

In the three-way analyses (Vernon vs. Baird in the 2015 spray year), there were significant site and site \times treatment effects on mesquite root-kill, canopy reduction, and tolerance rating (Table S4, available online at <https://doi.org/10.5061/dryad.tdz08kq65>). Mesquite root-kill was significantly greater at Vernon (67.0%) compared with Baird (46.8%). The site \times treatment interaction occurred because root-kill and canopy reduction were significantly greater at Vernon than Baird in the CA (Vernon 66.2%; Baird 44.8%) and especially the Cp + Tr (Vernon 58.6%; Baird 14.9%) treatments, but there was no significant difference in root-kill in the CA + Pc (Vernon 77.5%; Baird 68.8%) or the CA + Tr (Vernon 65.8%; Baird 58.8%) treatments between sites. Regarding lotebush responses, there was a significant site effect on lotebush root-kill,

Table 7

Hackberry percent root-kill (RK), percent canopy reduction (CR), and color-coded tolerance rating (TLR) as affected by herbicide treatment (pooled over 2013 and 2015 spray yrs) at 2 YPT based on analysis from Table 6. Means (\pm standard error) with similar letters within each column are not significantly different at $P \leq 0.05$.

Effect	RK (%)	CR (%)	TLR ¹
CA	5.56 \pm 5.56 b	10.06 \pm 9.56 b	86.19 \pm 12.85 a
CA+Tr	12.95 \pm 5.80 b	27.24 \pm 0.36 b	63.11 \pm 2.52 a
CA+Pc	57.90 \pm 13.53 a	73.48 \pm 8.67 a	18.04 \pm 8.04 b
Cp+Tr	0.0 \pm 0.0 b	6.69 \pm 1.49 b	87.78 \pm 2.22 a

¹Blue indicates highly tolerant (80–100); green, tolerant (60–79.9); red, susceptible (0–19.9).

CA indicates clopyralid + aminopyralid; CA + Tr, CA + triclopyr; CA + Pc, CA + picloram; Cp + Tr, clopyralid + triclopyr.

canopy reduction, and tolerance rating (see Table S4). Lotebush root-kill (Vernon 3.5%; Baird 0.5%) and canopy reduction (Vernon 16.4%; Baird 5.5%) were significantly greater at Vernon than Baird.

Discussion

Treatment efficacy on target species mesquite

Mesquite root-kill and canopy reduction were more consistent with the CA and CA+Tr treatments than the Cp+Tr treatment across all spray years. The Cp+Tr treatment was included in this study because it had been the most common recommendation for mesquite control from about 1985 to 2010 (Hart et al. 2012). Most research found that this treatment achieved moderate to high (50–70%) mesquite root-kill without damaging associated herbaceous or woody species (Jacoby et al. 1981; Bovey and Whisenant 1991, 1992; Mitchell et al. 2004). However, anecdotal observations of some commercial applications of Cp+Tr on mesquite revealed more variable root-kill results. Clopyralid comprises 82% of the active ingredients in the CA treatment (acid equivalent: 0.276 kg.l⁻¹ for clopyralid; 0.06 kg.l⁻¹ for aminopyralid) (Trade Label 4, 2020). At the recommended rate of 2.046 l.ha⁻¹ (28 fl oz.ac⁻¹), the amount of clopyralid applied by weight is 0.56 kg.ha⁻¹ (2.046 l.ha⁻¹ × 0.276 kg.l⁻¹), which is twice the amount of clopyralid that is in the Cp+Tr treatment (Lyons et al. 2020). This may explain why root-kill is consistently higher in the CA treatments compared with Cp+Tr. Alternatively, it may be due to the inclusion of aminopyralid.

Lower mesquite root-kill in the CA, CA+Tr, and Cp+Tr treatments after the 2015 application compared with the 2013 application (see Fig. 2) may relate to the change in location of two of the four replicate plots in 2015 from Hamlin to the Baird site. However, this does not appear to be the reason because a similarly lower mesquite root-kill after the 2015 spray was observed among the two replicates at the Vernon location (CA = 82.4, 75.5, 65.4%; CA+Tr = 94.0, 71.4, 60.3%; Cp+Tr = 91.1%, 57.2%, 50.3% after 2013, 2014, and 2015 applications, respectively).

Precipitation patterns in each year may have affected susceptibility to these three herbicide treatments. Mesquite is drought tolerant partly because of leaf adaptations (Ansley et al. 1998; Qin et al. 2019) and utilization of different sections of an extensive root system as a facultative phreatophyte (Thomas and Sosebee 1978; Wan and Sosebee 1991). Mesquite distribution is typically limited to deeper soils (Eggemeyer and Schwinning 2009). Ansley et al. (2014) found that after 3 yr of artificial rain sheltering, mature mesquite trees on deep clay loam soils had redirected root growth to adjust to the extended soil drought and had healthy appearing foliage and predawn leaf water potentials that were similar or greater than (i.e., less water stress) non-rain-sheltered trees. Root system adjustment may allow mesquite to avoid the effects of extended drought for multiple years.

Because mesquite root-kill from the CA, CA+Tr, and Cp+Tr treatments was greater after the 2013 treatment yr than the other treatment years, we might assume that mesquite was in a weaker physiological state and more susceptible to herbicides in 2013 due to the 2011–2012 drought than in 2014 or 2015. However, we rated the appearance of mesquite foliage at the time of treatment applications in July 2013 as good to excellent. Foliage condition is a critical factor as the herbicide is best absorbed through physiologically active leaves (Meyer et al. 1983; Lyons et al. 2020). A visual observation of foliage condition provides only a general indication of leaf “receptiveness” to the herbicide, and we may have applied treatments in 2013 when mesquites maintained what appeared to be healthy foliage but remained under some level of moisture stress. That explanation contradicts the prevailing thought that so long as soil temperature is above a threshold level of 23.9°C

(Dahl et al. 1971), which it was, that moisture stress in mesquite either has minimal effect (Sosebee 1983) or decreases rather than increases foliar absorption of herbicides (Davis et al. 1968; Roche et al. 2002). We posit that mesquite adjusted to extended drought via root response as per Ansley et al. (2014) described earlier, and foliage was not water stressed at the time of herbicide treatments in 2013.

Different precipitation patterns in 2015 may have caused lower mesquite root-kill response to the CA, CA+Tr, and Cp+Tr treatments. Both the Vernon and Baird sites experienced well-above-normal precipitation in April and May 2015. This may have stimulated new foliage growth that reduced downward movement of carbohydrates (Fisher et al. 1956), which could have limited downward movement of the herbicide to the roots. The period of mesquite legume elongation may also limit downward translocation of carbohydrates and foliar-absorbed herbicides (Dahl and Sosebee 1984). Our treatments were applied in early to mid-July when mesquite legume elongation could occur. However, we did not observe many legumes on the trees at any site during herbicide application in any of the treatment years.

Mesquite response to the CA+Pc treatment did not follow the same pattern as with the three other treatments as root-kill was not different among spray years (hence, the significant treatment × spray year interaction in the Table 3 analysis). Sosebee et al. (1973) reported that large mesquite in dense stands were difficult to kill with aerial applications of picloram. Our CA+Pc treatment contained clopyralid and aminopyralid in addition to picloram, and this may explain the consistently high root-kill in all 3 spray yr.

Treatment efficacy on target species pricklypear

We did not expect the treatments without picloram to have much impact on pricklypear, and the results verified this. As expected, the CA+Pc treatment had a profound effect on reducing pricklypear presence. Root-kill and motte canopy reduction responses were consistent across treatment application years. Since we did not have a picloram-only treatment, we do not know if picloram alone would have been as effective as has been studied (Price et al. 1985; Peterson et al. 1988). An important finding was that the response to the CA+Pc treatment was delayed as root-kill and canopy reduction increased (and root-kill more than doubled) from 1 to 2 YPT (see Fig. 3). The opposite trend is typically found with mesquite; root-kill is often overestimated at 1 YPT compared with 2 YPT because any basal sprouting that occurs in top-killed mesquite may not yet have occurred or be easily visible under grass cover at 1 YPT. This did not happen in our study as main effects of YPT or any interactions with YPT were not significant in mesquite root-kill or canopy reduction. The low grass growth due to drought may have increased evaluation accuracy at 1 YPT.

Treatment effects on nontarget lotebush and hackberry

Lotebush, once perceived as a shrub species that needed to be controlled, is sensitive to picloram (Scifres and Kothmann 1976). Bovey et al. (1970) found that lotebush canopy reduction at 2 YPT was 20%, 39%, and 40% in response to 4.68, 9.35, and 14.03 l.ha⁻¹ (1.12, 2.24, and 3.36 kg.ha⁻¹) of picloram. Lotebush canopy reduction of 18.35% (see Table 4) in the CA+Pc treatment was similar to what Bovey et al. (1970) found with their lowest rate. The picloram rate in our CA+Pc treatment was 2.34 l.ha⁻¹ (0.56 kg.ha⁻¹), which was half of Bovey et al.’s (1970) lowest rate.

We do not have an explanation as to why lotebush tolerance rating declined from 1 to 2 YPT after the 2014 spray treatments (see Fig. 4). This was due more to changes in canopy reduction of

surviving plants than to root-kill. Typically, level of canopy reduction of surviving individuals is greatest the first year post treatment and decreases by the second year, as we observed with lotebush after the 2013 treatment yr (see Fig. 4B). After the 2014 treatments, canopy reduction increased to a greater degree from 1 to 2 YPT in the CA+Tr and CA+Pc treatments (average 10–43%) than in the CA and Cp+Tr treatments (10–25%). This reinforces results from Table 4 (pooled over all 3 spray yr) that the CA+Tr and CA+Pc treatments had a slightly more negative effect on lotebush than the other treatments.

Of greater concern in our study was the effect of the CA+Pc treatment on hackberry root-kill, which averaged 58.7% (averaged over both 1 and 2 YPT) and moved the tolerance rating to the susceptible category (see Table 5). When only 2 YPT was considered, hackberry percent canopy reduction was slightly less but the tolerance rating remained susceptible (see Table 7). Hackberry is less drought tolerant than mesquite (Qin et al. 2019) and grows in riparian areas or low-lying swales and small tributary channels on upland sites where soil moisture is more available (Van Auker et al. 1979; Everitt et al. 2006). In our study, many hackberry plants showed visible signs of leaf chlorosis likely due to drought stress just before our July 2013 treatment applications. Even if drought stress limits leaf absorption of foliar herbicides, as has been reported for mesquite (Davis et al. 1968), a different physiological process must have occurred with hackberry. This nontarget species may have been so physiologically weakened by drought stress that even if leaf activity was less than optimum for herbicide absorption, enough herbicide may have been absorbed to kill many of the plants.

Hackberry root-kill response to CA+Pc remained high after the 2015 application (44%), even though May that year had high rainfall. This suggests that by 2015, hackberry remained weakened by the severe drought in 2011–2013 and below average annual precipitation in 2014, or there were other factors independent of drought that made hackberry particularly susceptible to picloram. In contrast, nontarget lotebush had healthy-looking foliage at the time of spraying each year, like mesquite, and appeared to be drought tolerant. Foster et al. (1984) found that lotebush twig elongation occurred during a severe drought year. Several studies in Africa and central Asia have recognized other *Ziziphus* species as drought tolerant (Clifford et al. 1998; Arndt et al. 2001; Kalinganire et al. 2012).

Regarding response in other studies of nontarget woody plants near or beneath canopies of targeted woody plants, Whisenant (1987) found in Utah that clopyralid effectively controlled mountain big sagebrush (*Artemisia tridentata*) and killed only 5% of nontarget antelope bitterbrush (*Purshia tridentata*) and 6% of serviceberry (*Amelanchier alnifolia*). Bovey et al. (1970) found in south Texas that picloram intended to control huisache (*Acacia farnesiana*) also had a strong negative effect on spiny hackberry (aka granjeno) (*Celtis pallida*) and moderately damaged algerita (*Mahonia trifoliolata*; 15–63% canopy reduction) and Texas persimmon (*Diospyros texana*; 14–38%). While Bovey et al. (1970) did not refer to these species as nontarget, they are highly beneficial to wildlife (Hatch and Pluhar 1993; Linex 2014).

Several studies have quantified responses of nontarget forb species following broadcast treatment of picloram to control target species. Some found that that picloram reduced nontarget forb production and diversity (Arnold and Santelmann 1966; Meyer and Bovey 1985; Sheley and Denny 2006; Fuhlendorf et al. 2009). In contrast, two studies in the northern Great Plains found that picloram controlled target forbs spotted knapweed (*Centaurea maculosa*) and Canada thistle (*Cirsium arvense*) without damage to nontarget forbs (Rice et al. 1997; Travnicek et al. 2005). In mesquite control studies, McDaniel et al. (1982) found that 2,4,5-T+picloram temporarily reduced nontarget forb cover and production at 1 YPT,

but there was no difference compared with untreated by 2 YPT. Bedunah and Sosebee (1984) found that 2,4,5-T+picloram did not change forb production relative to untreated at 1 or 2 YPT.

Effect of site on mesquite and lotebush treatment responses

This study found, as have other studies (Dahl et al. 1971; Scifres and Kothmann 1976; Mitchell et al. 2004), that location or “site” may affect plant response to herbicide treatments. While differences among sites in this study were often statistically significant, they were mostly not numerically large, with one exception. After the 2015 treatments, root-kill of target mesquite was much lower at the Baird site than at Vernon when all treatments were pooled. This was especially true for the Cp+Tr treatment where mesquite root-kill was 59% at Vernon and 15% at Baird. The degree of difference in root-kill between sites was less with the CA treatment but still much greater at Vernon (66%) than Baird (45%). In addition, canopy reduction of nontarget lotebush was greater at Vernon (16%) than at Baird (6%) when all treatments were pooled.

While soils at Vernon and Hamlin were similar and mostly upland, those at Baird were different with one replicate predominantly loamy bottomland. This may explain why responses to treatments were more similar between Vernon and Hamlin after the 2013 and 2014 treatments than between Vernon and Baird after the 2015 treatments. Soil temperature at the time of treatment was cooler at Baird than any of the other sites and just above the threshold level needed for optimum response to foliar-applied herbicides (Dahl et al. 1971; Sosebee et al. 1973).

Management Implications

For woody species like mesquite that readily resprout following top-killing treatments such as prescribed fire, broadcast spraying of species-specific herbicides is one of the most effective means of reducing density and limiting woody plant encroachment. However, the effect of such treatments, especially new products, on nontarget woody plants needs to be determined. We found that the herbicide treatments evaluated in this study were mostly specific for target mesquite and did minor damage to nontarget lotebush and hackberry, with one exception. The treatment that contained picloram and was designed for simultaneous control of mesquite and pricklypear had a significantly negative effect on nontarget hackberry. We recommend using this treatment only if pricklypear dominates the understory and nontarget shrubs and forbs are not readily apparent. The study also revealed that drought is an important consideration when applying broadcast herbicides to mesquite as some nontarget species, such as hackberry, may be more vulnerable to drought than mesquite. Thus, we recommend a visual check of foliage condition of nontarget species before spraying for mesquite control.

The premix CA treatment was slightly more effective on mesquite than the commonly used Cp+Tr treatment that is currently available as generics (Lyons et al. 2020) because it provided more consistent root-kill and stand-level canopy reduction among different spray years. This was especially apparent in comparing responses at two different sites after the 2015 treatments. However, in years where environmental and mesquite physiological conditions yielded optimum treatment effectiveness (e.g., in this study the 2013 spray yr), there was no difference in root-kill and canopy reduction between CA and Cp+Tr. The addition of triclopyr to CA had no improved effect on mesquite over CA alone and no increased harm to nontarget lotebush. However, it did have a slightly more negative effect on hackberry after 1 of the 3 treatment yrs. The tolerance-rating model we developed was important because it integrated into a single numerical value (range: 0 for

least tolerant to 100 for most tolerant) the population-level root-kill and canopy reduction responses of a particular species. Our data revealed that statistically significant effects of treatments on the tolerance rating did not always match those found for percent root-kill or canopy reduction since the tolerance rating integrated those two variables.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Accessibility

Data can be found at Dryad; <https://doi.org/10.5061/dryad.tdz08kq65>.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.rama.2024.01.003](https://doi.org/10.1016/j.rama.2024.01.003).

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