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RESEARCH ARTICLE

# Use of shelter tubes, grass-specific herbicide, and herbivore exclosures to reduce stressors and improve restoration of semiarid thornscrub forests

Krysten Dick[1], Heather D. Alexander[2,3], Jonathan D. Moczygemba[1]

**In semiarid south Texas, land conversion has reduced thornscrub forests by greater than 95%, and stressors, including competition with invasive grasses, mammalian herbivory, and drought, threaten the success of restoration efforts. This study assessed the effectiveness of multiple restoration treatments aimed at improving survival and growth of thornscrub forest seedlings planted in old agricultural fields. In January 2013, we treated greater than 1,100 seedlings with grass-specific herbicide, herbivore exclosures, and shelter tubes, used separately or combined. We further evaluated the effects of shelter tube duration (0, 6, 12, and 18 months). For each seedling, we quantified surrounding invasive grass cover, browse intensity, height, and basal diameter every 4 months until September 2014. Herbicide application decreased invasive grass cover approximately 5-fold and increased seedling survival (23%) and basal diameter (26%). Shelter tube application for 12 and 18 months increased seedling survival (10%) and height (43 and 74%, respectively), whereas seedlings treated with tubes for only 6 months performed similar to those left untreated. Exclosures had no impact on seedling survival but increased seedling height (23%) and basal diameter (26%). We found no significant interactive effects of treatments. Overall, herbicide most effectively increased seedling survival and basal diameter growth, whereas shelter tubes proved most useful for promoting height growth. Combined, these treatments increased implementation and maintenance costs 2-fold, but minimized seedling mortality and maximized restoration potential. These findings highlight the necessity of post-planting seedling management to reduce stress from invasive grasses, mammalian herbivory, and drought and improve restoration potential in semiarid thornscrub forests.**

**Key words:** herbicide, revegetation, seedling, semiarid, shelter tube, thornscrub forest

### Implications for Practice

- Simply planting thornscrub forest seedlings in old agricultural fields is insufficient to restore semiarid thornscrub forests because stressors, including competition with invasive grasses, mammalian herbivory, and drought, limit survival and growth of revegetated plants.
- Competition with invasive grasses is a major barrier to survival and basal diameter growth of thornscrub forest seedlings but can be partially overcome through the control of invasive grasses using a grass-specific herbicide.
- Shelter tubes help planted seedlings to grow taller faster, allowing them to overcome height competition with invasive grasses; however, basal diameter growth is most effectively increased by herbicide or exclosures.
- Post-planting management of revegetated seedlings to reduce stress from invasive grasses, mammalian herbivory, and drought is critical for successful restoration of semiarid thornscrub forests.

## Introduction

Tamaulipan thornscrub forests of south Texas and northeastern Mexico represent a diverse assemblage of approximately 50 woody plant species (Shindle & Tewes 1998) that have experienced substantial loss and fragmentation since the early to mid-1900s due to land conversion to pasture, agricultural development, and urbanization (Reid et al. 1990; Návar 2008). These land use changes have played a major role in wildlife population losses (Jahrsdoerfer & Leslie 1988; Foroughbakhch et al. 2006). Approximately 145 animal species use thornscrub forest habitat, and many of these are listed by the United States Fish and Wildlife Service (USFWS) as target species that require immediate protection (Jahrsdoerfer & Leslie 1988). Most notably, the Federally endangered ocelot (*Leopardus pardalis*), with less than 80 individuals remaining in the United States (H. Swarts, USFWS, personal communication, 2016), relies on dense thornscrub forests of south Texas as their only suitable habitat. In addition, thornscrub forests influence many other

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[1]U.S. Fish and Wildlife Service, Laguna Atascosa National Wildlife Refuge, Los Fresnos, TX 78566, U.S.A.
[2]Department of Forestry, Forest and Wildlife Research Center, Mississippi State University, MS 39762, U.S.A.
[3]Address correspondence to H. D. Alexander, email heather.alexander@msstate.edu


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ecosystem processes, including carbon sequestration (Návar et al. 2008), soil water availability as a consequence of plant uptake and (Rodrıguez et al. 2004) rainfall redistribution (Návar et al. 2009), and soil stability (Návar et al. 2000). In response to thornscrub forest loss and implications for wildlife habitat and ecosystem function, USFWS has dedicated management efforts throughout south Texas to restore these forests.

One restoration approach underway is the planting of nursery-grown thornscrub forest seedlings in old agricultural fields. Over the last 5 years, approximately 120,000 seedlings have been hand-planted across 36 ha at Laguna Atascosa National Wildlife Refuge (LANWR), location of one of two remaining breeding populations of ocelots in the United States (Haines et al. 2005). Many hundreds of thousands more seedlings have been planted across the Lower Rio Grande Valley NWR since the 1980s as part of a large-scale effort to reclaim farmland and build a wildlife corridor along the Rio Grande River (Ewing & Best 2004). Despite extensive research leading to the successful cultivation of thornscrub forest seedlings in a nursery environment and ongoing revegetation efforts, studies have rarely investigated the fate of seedlings once transplanted into agricultural fields (Ewing & Best 2004), and only recently have we begun to explore various post-management strategies to improve restoration potential (Alexander et al. 2016).

Understanding seedling success post-planting is critically important because old agricultural fields commonly used for revegetation are inhospitable environments with a suite of abiotic and biotic stressors that challenge thornscrub forest seedling survival and growth. South Texas climate is semiarid, with low annual precipitation and high evaporation rates that are predicted to become even more extreme with climate warming (Norwine & John 2007). Prior to land conversion, thornscrub seedlings likely overcame water stress by growing in the shaded canopy of nurse plants (Jurado et al. 1998, 2006). In reclaimed fields, however, nurse plants are uncommon, and if present, invasive grasses proliferate in their understories. Brought to the southwest in the mid-twentieth century for cattle forage and erosion control, these grasses aggressively spread throughout the region after their introduction (Ruffner et al. 2012) and are now major competitors with woody plants (Burquez-Montijo et al. 2002; Arriaga et al. 2004), especially in disturbed areas like old fields. In addition to growing exposed and among highly competitive invasive grasses, seedlings are often heavily browsed by mammalian herbivores, including white-tailed deer (*Odocoileus virginianus*) and eastern cottontail rabbits (*Sylvilagus floridanus*) (Alexander et al. 2016), who rely on browse for a portion of their diet (Davis 1952; Davis & Winkler 1968; Varner et al. 1987). Ultimately, restoration is incomplete unless thornscrub seedlings survive and grow into mature forests, and the factors above appear to hinder this process. Thus, there is a pressing need to understand restoration strategies that alleviate these common stressors and promote seedling success.

Common strategies used in dryland ecosystems to help revegetated woody seedlings cope with these stressors include shelter tubes, herbicides, and herbivore exclosures. Across arid regions of the Mediterranean, shelter tubes have improved survival and growth of planted woody vegetation (Bellot et al. 2002; Jiménez et al. 2005; Oliet et al. 2005; Oliet & Jacobs 2007; Padilla et al. 2011) by (1) condensing dew and funneling water to a narrow region near the stem's base (Del Campo et al. 2006), (2) shading soils, reducing evaporative water losses (Puértolas et al. 2010), and (3) preventing herbivory (Tuley 1985; Jiménez et al. 2005). In semiarid shrublands of California, grass-specific herbicide targeted at monocotyledons has reduced competition with invasive grasses (Cione et al. 2002), although continual application is required for grass management and successful restoration (Cox & Allen 2008). Herbivore exclosures have proven useful in semiarid rangelands of Africa to limit browse and restore native woody vegetation (Yayneshet et al. 2009). Dryland ecosystems targeted for restoration are often highly degraded and experience multiple interacting stressors (Call & Roundy 1991; Aronson et al. 1993; Allen 1995; Padilla et al. 2009); however, few studies in these ecosystems have investigated the simultaneous implementation of various restoration strategies to overcome co-occurring stressors (Young & Tewes 1994; Alexander et al. 2016).

We evaluated thornscrub seedling survival and growth within the LANWR of south Texas over an 18-month period in response to three post-planting treatments, used singly and combined: grass-specific herbicide, shelter tubes, and herbivore exclosures. We experimented with duration of shelter tube application to determine how this treatment impacted plant success over time. In addition, we estimated costs associated with implementing and maintaining each treatment to help managers evaluate cost-benefit scenarios. Understanding which restoration strategies most effectively alleviate stressors and promote survival and growth of revegetated thornscrub forest seedlings is imperative for implementing successful restoration practices.

## Methods

### Study Area

This research was conducted at the 39,257-ha LANWR, located in Cameron County, Texas, approximately 20 km west of the Gulf of Mexico and 40 km north of the city of Brownsville. LANWR contains the largest remaining tracts of thornscrub forest in south Texas and is comprised of other ecosystems such as coastal prairies, salt marshes, and salt flats. Nearly every plant species (approximately 50) of Tamaulipan thornscrub forest is found at LANWR (Shindle & Tewes 1998), but composition is dominated by Texas ebony (*Ebenopsis ebano* [Berl.] Barneby & Grimes), spiny hackberry (*Celtis ehrenbergiana* [Klotzsch] Liebm.), brasil (*Condalia hookeri* M.C. Johnst.), coyotillo (*Karwinskia humboldtiana* [Schult.] Zucc.), narrow-leaf forestiera (also known as elbow bush; *Forestiera angustifolia* Torr.), Texas persimmon (*Diospyros texana* Scheele), and lotebush (*Ziziphus obtusifolia* Hook. ex Torr. & A. Gray) A. Gray).

The region is semiarid and subtropical, with sporadic rainfall events that usually occur in September and October (Norwine et al. 1995). During this study, LANWR received 48 cm of rainfall in 2013, and 56 cm from January to September 2014 (NOAA National Climatic Data Center, Stations USR0000TLAG and USW00012957; Fig. S1, Supporting Information). However, in

**Table 1.** Percent (%) of seedlings of each species that were planted within each treatment in the thornscrub forest study at Laguna Atascosa National Wildlife Refuge in south Texas. Numerical values in column headings refer to length of shelter tube application (0, 6, 12, or 18 months). "Other" category includes all species planted at less than 5%. Nomenclature follows Lady Bird Johnson Wildflower Center Database (www.wildflower.org).

| | | | *No Exclosure* | | | | | | | | *Exclosure* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *No Herbicide* | | | | *Herbicide* | | | | *No Herbicide* | | | | *Herbicide* | | | |
| *Genus* | *Species* | *Common Name* | *0* | *6* | *12* | *18* | *0* | *6* | *12* | *18* | *0* | *6* | *12* | *18* | *0* | *6* | *12* | *18* |
| *Forestiera* | *angustifolia* | elbow bush | 13 | 19 | 13 | 15 | 18 | 17 | 18 | 31 | 15 | 11 | 28 | 18 | 14 | 19 | 19 | 21 |
| *Ebenopsis* | *ebano* | Texas ebony | 18 | 13 | 15 | 17 | 10 | 14 | 11 | 15 | 15 | 18 | 15 | 19 | 14 | 15 | 13 | 14 |
| *Karwinskia* | *humboldtiana* | coyotillo | 10 | 15 | 15 | 14 | 15 | 13 | 13 | 13 | 15 | 11 | 18 | 17 | 8 | 14 | 17 | 15 |
| *Castela* | *erecta* | goatbush | 11 | 10 | 10 | 6 | 11 | 13 | 11 | 8 | 13 | 6 | 8 | 8 | 7 | 11 | 10 | 10 |
| *Celtis* | *ehrenbergiana* | spiny hackberry | 6 | 13 | 1 | 10 | 8 | 4 | 7 | 7 | 14 | 8 | 6 | 7 | 11 | 7 | 6 | 8 |
| *Havardia* | *pallens* | tenaza | 10 | 4 | 8 | 7 | 3 | 6 | 6 | 3 | 6 | 10 | 6 | 6 | 15 | 8 | 4 | 13 |
| *Ziziphus* | *obtusifolia* | lotebush | 7 | 8 | 6 | 1 | 4 | 8 | 3 | 7 | 6 | 8 | 8 | 1 | 1 | 6 | 3 | 1 |
| *Acacia* | *farnesiana* | huisache | 7 | 1 | 6 | 6 | 11 | 4 | 11 | 0 | 1 | 3 | 1 | 6 | 1 | 3 | 4 | 8 |
| *Lycium* | *berlandieri* | Berlandier's wolfberry | 6 | 4 | 4 | 6 | 1 | 6 | 10 | 3 | 6 | 8 | 0 | 3 | 3 | 1 | 6 | 0 |
| *Prosopis* | *glandulosa* | mesquite | 1 | 1 | 7 | 7 | 7 | 7 | 1 | 1 | 4 | 4 | 4 | 1 | 1 | 4 | 3 | 1 |
| *Citharexylum* | *berlandieri* | Berlandier's fiddlewood | 6 | 0 | 3 | 6 | 4 | 3 | 1 | 4 | 1 | 6 | 1 | 4 | 6 | 3 | 1 | 1 |
| Other | Other | Other | 3 | 4 | 6 | 3 | 3 | 1 | 3 | 3 | 0 | 3 | 1 | 1 | 4 | 4 | 1 | 4 |
| *Phaulothamnus* | *spinescens* | snake-eyes | 1 | 1 | 3 | 3 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 6 | 4 | 3 | 10 | 1 |
| *Condalia* | *hookeri* | brasil | 3 | 3 | 0 | 0 | 1 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 |
| *Amyris* | *texana* | Texas torchwood | 0 | 1 | 0 | 0 | 3 | 1 | 3 | 3 | 0 | 1 | 0 | 1 | 4 | 0 | 3 | 0 |
| *Chromolaena* | *odorata* | blue mistflower | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 | 1 | 0 | 0 |
| *Malpighia* | *glabra* | Barbados cherry | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

2011 and 2012, annual rainfall only amounted to 25 and 42 cm. Strong southeasterly winds from the Gulf of Mexico are predominant from March to October, and mean monthly air temperatures range from 15.7 in January to 28.9°C in August (Norwine et al. 1995). Terrain at LANWR is generally flat with an average slope of 0.29 to 0.38 m/km.

### Experimental Design

In October 2012, we established a seedling population study within a 21-ha old agricultural field (County Road, Unit 8; 26°15′23″ N, 97°21′53″ W) that had been allowed to fallow for approximately 25 years and was dominated by exotic grasses, including buffelgrass (*Cenchrus ciliaris* L.) and Kleberg bluestem (*Dicanthium annulatum* [Forssk.] Stapf), and an occasional "native-invasive" shrub of honey mesquite (*Prosopis glandulosa* Torr.) and huisache (*Acacia farnesiana* (L.) Willd.). The site was flat, with an elevation of approximately 5 m above MSL; soils were 90% clay and loam (Chargo and Laredo silty-clay) (Williams et al. 1977). Prior to revegetation, the site was bulldozed to simplify the planting process. Bulldozed slash and grass were burned in piles away from the study site. Rows were then ripped 20–25 cm (8–10 in) deep into the soil with the back of a motor grader. One- to two-year-old old thornscrub seedlings, grown in local soils within a nursery and established from locally harvested seed (Ewing & Best 2004), were hand-planted inside the rip at approximately 20 cm depth and at 1-m intervals within rows and 2-m between rows. Seedlings were planted in fall when rainfall events are more common (Norwine et al. 1995), and soils are more malleable.

Within the study site, a split–split plot, randomized complete block (RCB) design (Fig. S2) was used to experiment with post-planting treatments (grass-specific herbicide, shelter tubes, and herbivore exclosures). Each of three blocks of 0.13 ha were separated by 25 m. Areas were randomly split into "exclosure" and "no exclosure" treatments, and split further into "herbicide" and "no herbicide" treatments. Within the exclosure and herbicide splits, further subdivisions into 8-m$^2$ plots were randomly assigned to shelter tube treatment durations (0, 6, 12, and 18 months). This design created 16 treatment combinations, each replicated three times (i.e. plot = experimental unit). Each plot contained 24 seedlings, and the study evaluated a total of 1,152 seedlings. In each plot, there were at least three seedlings each of Texas ebony, coyotillo, and elbow bush. The remaining seedlings were a random assortment of thornscrub forest species commonly used in the revegetation program (Table 1). Because this study was set-up post-planting, we were unable to control the relative distribution of species planted in each plot. Seedlings within each 8-m$^2$ plot were numbered (1–24) on a 0.61-m flag adjacent to the seedling for relocation purposes, and each plot was marked at all four corners with PVC tubing and flagging.

Treatments were initiated in winter/spring 2013. Herbivore exclosures were installed in January 2013 and constructed from 2.3-m tall heavy-duty deer fencing (TENAX, Baltimore, MD, U.S.A.) attached to 2.4-m tall T-posts. Shelter tubes (Tubex U.S.A., Oak Hickory, TN, U.S.A.) made of translucent plastic (0.6-m tall, 6–10 cm diameter) were carefully placed over seedlings and secured to a bamboo stake (1-m tall; 12–14 mm diameter) using two cable ties, one at the top of the tube and one at the bottom in March 2013. Tubes were secured by pushing the bottom approximately 3 cm into the soft soil after rainfall. Shelter tubes remained on seedlings for 6, 12, or 18 months. A grass-specific herbicide, Clethodim 2E (Albaugh, Inc., Ankeny, IA, U.S.A.; 3% solution of Clethodim), was initially applied using a backpack sprayer in May 2013, when grasses first appeared “green” and receptive to chemicals. Following this initial treatment, herbicide was applied on two other occasions, when grasses initiated a new flush of green growth following a period of dormancy (September 2013 and April 2014).

Invasive grass cover, browse intensity, height, basal diameter, and survival were measured for each seedling at 4-month intervals from January 2013 to September 2014. Percent cover of invasive grass species was visually estimated within a 0.25-$m^2$ area around each seedling. Browse intensity was determined by counting the number of browsed stems on each seedling. Seedling herbivory was mammalian (mainly eastern cottontail rabbits and white-tailed deer), and very little insect herbivory was noted in this study. Seedling height was measured from the root collar to the tallest terminal bud or green leaf (for seedlings that had experienced die-back) using a ruler to measure the nearest 0.1 cm. Basal diameter was measured to the nearest 0.01 cm using calipers perpendicular to the base of the main stem at 0.5 cm above the root collar. For seedlings with multiple stems, only the largest stem was measured. We measured basal diameter because this parameter often correlates with root biomass in other systems (Burger et al. 1997). To confirm this correlation in thornscrub forest seedlings, we excavated 30 total seedlings of Texas ebony (24), elbow bush (2), and spiny hackberry (4) in areas adjacent to the sampling plots in fall 2014 (2 years post-planting) and created an allometric equation relating basal diameter (BD) with root biomass (root biomass = BD × 25.5 − 9.3; $r^2 = 0.68$, $p < 0.01$). Seedling survival was quantified by noting if the seedling was dead or alive at each time period. Because thornscrub seedlings are drought-adapted, some seedlings were counted as dead because they had no foliage (drought dormancy). Occasionally, when we returned at the next sampling period, some seedlings had resprouted, and in these instances, we reclassified the seedling as alive.

We estimated costs associated with implementing and maintaining each treatment. Costs included labor and supplies associated with growing ($1.70 per seedling; L & L Growers, San Benito, TX, U.S.A.) and planting seedlings ($0.10 per seedling without tube and $0.20 per seedling with tube), installing exclosures ($1.45 per seedling) and/or seedling tubes ($1.12 per seedling), and spraying herbicide two times per year for the first five years post-planting using a Utility Task Vehicle (UTV; $0.60 per seedling). Herbicide application for this length of time should be more than sufficient to allow seedlings to reach a height above that of the invasive grasses (1.5 m), which we estimated to be approximately 3.5 years based on growth rates during the study period. Herbicide application costs would decrease substantially (to only $0.16 per seedling) if a crop-duster was used for application, but this would approach would only be suitable across large planting areas.

### Statistical Analyses

A four-way, repeated measures analysis of variance (ANOVA) was conducted using SAS v 9.2 to compare invasive grass cover, browse intensity, seedling survival, height, and basal diameter (repeated measure = seedling; covariance structure = autoregressive) among the four factors (date, herbicide, tube, and exclosure) and their interactions (Table S1). Plots were blocked by sampling area (random factor) to account for natural variation seen across the site such as grass composition and potential soil differences. Transformations (height = square root, basal diameter = log, invasive grass cover and browse intensity = arcsin) were required to better meet assumptions of normality (Kolmogorov–Smirnov–Lilliefors test) and homogeneity of variances (Levine’s test). Seedlings showed no significant difference in initial values of height and basal diameter in January 2013. When higher-order significance was found ($p < 0.05$) between fixed factors, a post-hoc Fisher’s LSD test was performed to further examine multiple treatment comparisons. We did not assess species-specific differences because revegetated sites are planted with a random mixture of species to increase diversity. The percent contribution of each species to each treatment was relatively even across the study site (Table 1); thus, variations in composition across plots likely had little effect on the pronounced results of seedling survival, height, and basal diameter (very small standard error of the mean) seen in this study.

## Results

### Invasive Grass Cover and Browse Intensity

Grass-specific herbicide reduced invasive grass cover (Fig. 1A), but the magnitude of this effect varied with sampling date (date × herb, $p < 0.0001$). At the beginning of the study, invasive grass cover was relatively low (<20%), reflecting the recent bulldozing of the site. By study cessation, however, grass cover had increased approximately 5-fold on plots without herbicide application (approximately 90%) and remained unchanged with herbicide application. Exclosures and tubes interacted to affect grass cover (excl × tubes, $p = 0.04$), but there was no consistent pattern to these effects.

Exclosures decreased seedling browse, but only after the third sampling date (date × exclosure, $p < 0.0001$; Fig. 1B). At this time, browse intensity outside of exclosures spiked to 1.5 stems browsed per seedling, and remained relatively high for the remainder of the study.

### Seedling Survival, Height, and Basal Diameter

Over the study, herbicide (Fig. 2A; date × herb, $p < 0.001$) and tubes (Fig. 2B; date × tube, $p < 0.001$) increased seedling survival, but there was no effect of exclosures (Fig. 2C). For the first half of the study, survival gradually decreased, with no significant differences ($p > 0.05$ for all comparisons) between seedlings treated with herbicide or tubes and those left untreated. After a year of treatment, however, survival of seedlings treated with herbicide or tubes leveled off and remained above 80% for the remainder of the study. In contrast, seedlings without herbicide or tube treatment or with tube treatment of only 6 months exhibited a decline in survival after the

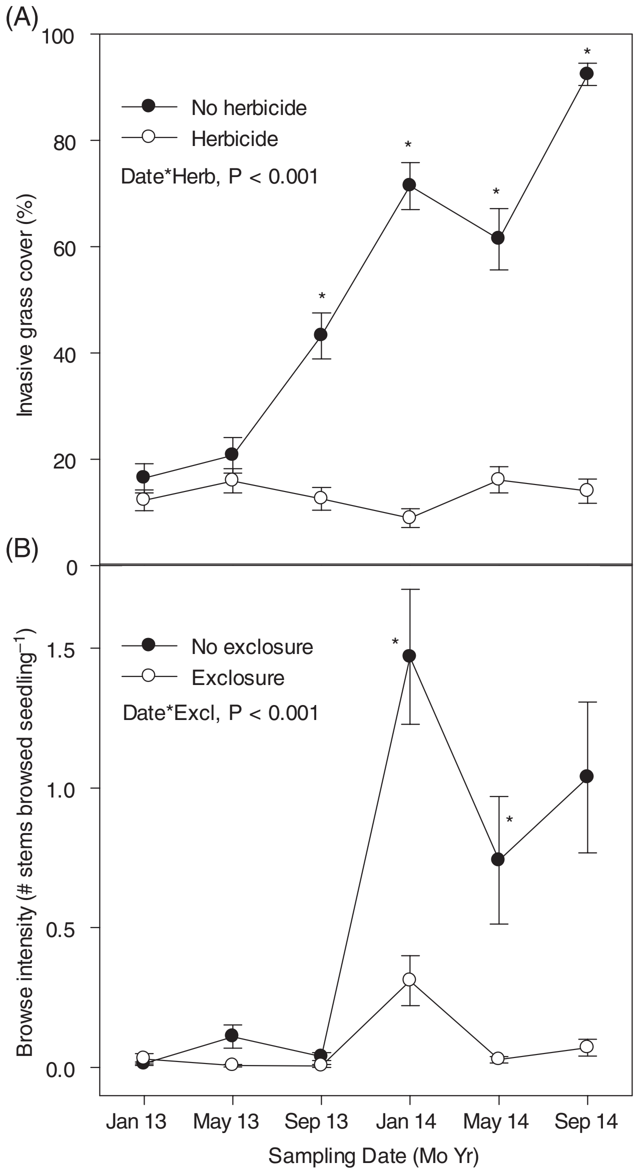


Figure 1. (A) Percent invasive grass cover (mean ± 1 SE) around planted thornscrub forest seedlings in response to grass-specific herbicide applied in May 2013, September 2013, and April 2014, and (B) browse intensity (mean ± 1 SE) on planted thornscrub forest seedlings in response to exclosure treatment. Seedlings were planted in old agricultural fields within the Laguna Atascosa National Wildlife Refuge in south Texas in October 2012. Asterisks (*) denote significant differences between treatments at $p < 0.05$ on a particular sampling date.

third sampling date, reaching approximately 70% by the end of the study.

Tubes and exclosures increased seedling height, but this effect varied with sampling date (date × excl, $p < 0.01$; date × tubes, $p < 0.001$; Fig. 2E and 2F, respectively); herbicide had no significant effect on height (Fig. 2D). Applying shelter tubes for 18 months had the greatest impact on seedling height, with seedlings reaching greater than 61 cm (74% increase) by the end of the study. A 12-month tube application also increased seedling height to approximately 50 cm (43% increase), but height growth stopped as soon as the tubes were removed. Similarly, a 6-month application increased height until the tubes were removed, but by the end of the study, these seedlings had a height similar to those left untreated ($p = 0.09$). Within exclosures, seedling height increased from approximately 30 to 52 cm over the study. Outside of exclosures, seedlings grew to only 41 cm.

Herbicide and exclosure application led to a gradual increase in seedling basal diameter over the study (date × herb, $p < 0.001$; date × excl, $p < 0.001$; Fig. 2G and 2I, respectively), but tubes had no effect (Fig. 2H). Basal diameter of untreated seedlings increased approximately 1.5-fold during the study, from approximately 0.4 to 0.6 cm, whereas that of those treated with herbicide or exclosures increased approximately 2-fold.

### Treatment Costs and Benefits

Although untreated seedlings cost less ($1.80 per seedling), they were the least successful; while more expensive, the addition of at least one treatment substantially improved seedling success (Table 2). Herbicide application improved seedling survival and basal diameter growth, and increased costs to only $2.41 per seedling (34% increase). Shelter tubes improved seedling survival and height growth, and increased costs to $3.02 per seedling (68% increase). Although exclosures protected seedlings from browse and improved seedling height and basal diameter, they cost $4.62 per seedling, double the cost of herbicide and 1.5 times the cost of shelter tubes.

## Discussion

Reclaimed agricultural fields used as thornscrub forest restoration sites are highly degraded, devoid of potential nurse trees, and experience multiple stressors, including invasion by exotic grasses, intense mammalian herbivory, and high exposure to environmental extremes, especially drought and wind. Our findings show that seedlings planted in these conditions experience relatively high mortality and slow growth. This translates into low potential for eventual restoration and high costs associated with losing greenhouse-grown seedlings.

The periodic use of grass-specific herbicide substantially reduced invasive grass cover and improved seedling survival and basal diameter growth during this 18-month study. These findings likely reflect reduced competition for resources. Buffelgrass, the dominant invasive on our site, develops a thick, fibrous root system with creeping rhizomes and is known for its ability to aggressively compete for water in semiarid regions (Tix 2000). The lack of an herbicide effect on seedling height and the finding that basal diameter growth responded to herbicide application only after grass cover exceeded 50% (fall 2013) suggest that most of the competition was belowground.

These seedling responses to herbicide differ from those of a pilot study on a nearby site (Alexander et al. 2016) where seedlings exhibited high survival and growth on plots with the greatest invasive grass cover. However, grass cover in the pilot

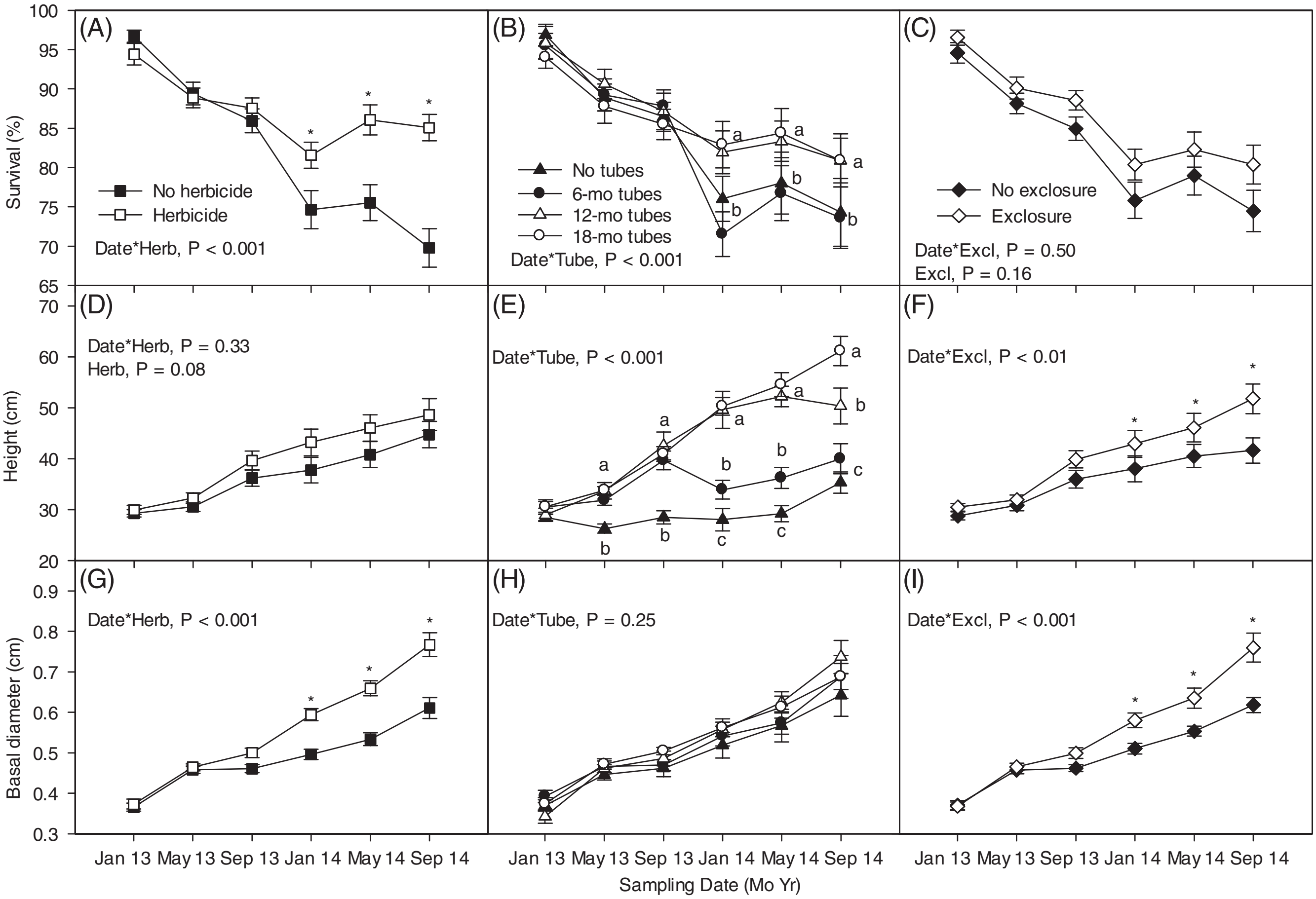


Figure 2. Planted thornscrub forest seedling survival, height, and basal diameter (mean ± 1 SE) in response to grass-specific herbicide (A, D, G), shelter tube application for different durations (B, E, H), and herbivore exclosures (C, F, I). Seedlings were planted in old agricultural fields within the Laguna Atascosa National Wildlife Refuge in south Texas in October 2012. Asterisks (*) (for herbicide or exclosures treatments) or different letters (tube duration treatments) indicate significant ($p < 0.05$) differences between treatments on a particular sampling date.

study increased slowly, varied seasonally, and rarely exceeded 70%, which may reflect drought conditions during the first two years of that study (only 25 cm of rainfall in 2011 and 42 cm in 2012). In the current study, invasive grasses maintained a steady increase in cover in the absence of herbicide application, reaching near 100% by the end of the study. Thus, the interactions between invasive grasses and thornscrub forest seedlings may vary depending on the level of grass cover. At relatively low cover, grasses may actually facilitate seedlings by hiding them from browsers and shading soils, but as grass cover increases, competition is likely the overriding interaction (MacDougall & Turkington 2005; Wolkovich et al. 2009).

We based herbicide application costs on two treatments per year for a five-year period because we estimated that seedling height would exceed that of grasses during this timeframe. If this assumption holds true, herbicide would be a cost-effective treatment application, increasing costs by only 33% compared to untreated seedlings. However, invasive grasses in south Texas are persistent, with continual seed inputs from nearby untreated areas and prolific belowground root systems (Jahrsdoerfer & Leslie 1988; Smith et al. 2010). Thus, the continued application of herbicide may remain a necessary, yet intensive, treatment for curbing any competitive consequences. Consequently, associated costs would increase, along with any other negative effects of adding herbicide to the system.

**Table 2.** Final seedling survival, height, and basal diameter and costs associated with implementing and maintaining each treatment during the thornscrub forest study at Laguna Atascosa National Wildlife Refuge in south Texas. Herbicide costs are based on application using a UTV.

| *Treatment* | *Survival (%)* | *Height (cm)* | *Basal Diameter (cm)* | *Cost Per Seedling* |
|---|---|---|---|---|
| *Herbicide* | | | | |
| No | 69 | 45 | 0.61 | $1.80 |
| Yes | 85 | 49 | 0.77 | $2.41 |
| *Shelter tubes* | | | | |
| 0 months | 74 | 35 | 0.64 | $1.80 |
| 6 months | 74 | 40 | 0.69 | $3.02 |
| 12 months | 81 | 50 | 0.74 | $3.02 |
| 18 months | 81 | 61 | 0.69 | $3.02 |
| *Exclosures* | | | | |
| No | 75 | 42 | 0.61 | $1.80 |
| Yes | 80 | 52 | 0.76 | $4.62 |

Shelter tube application for 12 and 18 months increased seedling survival and promoted height growth. These findings support those of two previous studies in thornscrub forests (Young & Tewes 1994; Alexander et al. 2016) and several others in semiarid Mediterranean climates (e.g. West et al. 1999; Sharrow 2001; Oliet & Jacobs 2007). Tubes increased soil moisture and moderated air and soil temperatures around the seedlings (Dick 2015), likely because they condense dew (del Campo et al. 2006) and reduce light intensity (Bergez & Dupraz 2009). These conditions have been shown to create a favorable microclimate for seedling survival and promote seedling height growth in dryland ecosystems (Padilla et al. 2011; Defaa et al. 2015). In addition, tubes protect against wind damage (Bainbridge 1994), restrict browse until seedlings exceed the height of the tubes (Dubois et al. 2000; Sharrow 2001), and physically restrict grasses from growing on top of the seedlings (Alexander 2015, Mississippi State University, personal observation).

The length of shelter tube application was important for determining seedling success. When tubes were removed after only 6 months, seedlings often died or experienced die-back, likely due to re-exposure to stressors. By the end of the study, survival and height of these seedlings were similar to those left untreated. Seedlings treated with tubes for 12 months grew approximately 20 cm taller than the 6-months application and experienced little mortality or die-back after tube removal, probably because they were big enough to tolerate the increased stress. However, seedling height growth stopped when tubes were removed. When treated for 18 months, seedlings continued to increase in height and began to branch out once they exceeded the height of the tube, but survival was similar to seedlings treated for only 12 months. Although tube treatment increased costs by 66% compared to untreated seedlings, tubes require little maintenance once installed, and if removed prior to plants reaching above them and branching out (approximately 18 months), they can be reused, thereby lowering overall costs.

In contrast to some studies in dryland ecosystems (Jiménez et al. 2005; del Campo et al. 2006), tubes had no effect on basal diameter. A major concern in systems where water is limiting and development of a belowground root system is essential for survival is that tubes cause plants to reallocate resources to aboveground tissues at the expense of roots. Although not significant, we found that seedlings growing in tubes for 18 months had slightly smaller basal diameters (0.69 cm) than those growing in tubes for 12 months (0.74 cm). Thus, basal diameter growth should be monitored if applying tubes for longer intervals, as there is potential for tubes to hinder basal diameter growth.

Exclosures reduced herbivore browse intensity and improved seedling height and basal diameter, but had no significant impact on survival. Repetitive browsing not only restricted height growth but also appeared to hinder belowground growth, likely through repetitive reallocation of resources to new tissues. Exclosure impacts on browse were only evident after the third sampling date, which is when height of seedlings growing in tubes tended to exceed the height of the tube. Once above the tubes, seedlings began to branch out horizontally, creating an obvious bunch of foliar growth perched at the perfect height for deer browse. Limiting browse through exclosures, however, was less effective for promoting height than seedling tubes and substantially more expensive to implement, and the effects on basal diameter were equivalent to using herbicide, which was also considerably cheaper than exclosures. Thus, while exclosures may facilitate thornscrub restoration, this treatment is expensive to install, difficult to implement across large areas, and does not improve seedling success more than the other less expensive treatments.

A primary finding of this research was that abiotic and biotic stressors play a critical role in the survival and growth of planted thornscrub forest seedlings, and that post-planting treatments are necessary to help seedlings overcome these stressors. Without post-planting treatments, thornscrub seedlings have poor growth and survival rates (Vela 2015; Alexander et al. 2016) and are unlikely to develop into restored thornscrub forests with characteristic structure and form of remnant old-growth patches. Degraded drylands of south Texas are a hotspot for conservation priority because of high biodiversity coincident with high human land use, and several threatened or endangered fauna depend on thornscrub habitat. Thus, the importance of effective reforestation cannot be understated. This study promotes the evaluation and improvement of restoration projects in dryland ecosystems, especially those used to create habitat for endangered species.

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## Supporting Information

The following information may be found in the online version of this article:

**Figure S1.** Monthly average high and low temperature and precipitation data from January 2013 to September 2014 collected at the National Oceanographic. Atmospheric Administration (NOAA) National Climatic Data Center (Stations USR0000TLAG and USW00012957)

**Figure S2.** The complete randomized block, split–split plot design used in this study created 16 different treatment combinations using herbivore exclosures, herbicide, and shelter tubes applied for 0, 6, 12, and 18 months.

**Table S1.** Statistical results for planted thornscrub forest seedlings at Laguna Atascosa National Wildlife Refuge in south Texas, from January 2013 to September 2014, displaying the effects of restoration treatments and their degrees of freedom (df; between group, within group), *F*-value (*F*), and *p*-value (*p*) for each response variable.

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