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RESEARCH ARTICLE

Does Seeding a Locally Adapted Native Mixture Inhibit Ingress by Exotic Plants?

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Abstract

Non-native plant species often colonize retired agricultural lands, creating monocultures with low species diversity that provide poor wildlife habitat. We assessed whether sowing a mix of 29 locally adapted native species reduced invasion of non-native plant species compared to allowing vegetation to colonize naturally following tillage. There was a sampling date × treatment interaction for canopy cover of perennial exotic plant species. Plots that were not sown to natives had two to six times greater canopy cover of exotic species than did plots with both preparation (woody vegetation removed, plowed, and disked) and control (no preparation or sowing) plots. Canopy cover of exotic plants was similar in prepared-only and control treatments from October 2008 to June 2010, ranging from 8 to 40%. Percent absolute canopy cover of native vegetation was 10–20 times greater on prepared and planted plots than on prepared-only plots during March 2009 to June 2010. Sowing a mix of locally adapted native species may inhibit encroachment by non-native species for up to two years after sowing on retired agricultural land in the Lower Rio Grande Valley of Texas.

Key words: bermudagrass, habitat, native plants, old world bluestems, post agricultural, sowing.

Introduction

Vegetation may become established through secondary succession on agricultural fields that have been cultivated and then abandoned (Cramer et al. 2008; Baeten et al. 2010). Exotic plants are often the primary species that colonize old agricultural fields that have been intensively cultivated. This is because the soil seed bank commonly contains ruderal agricultural weeds and few native species (Middleton 2003; Tognetti et al. 2010). Exotic plants may also invade from nearby habitats. Exotic plants may maintain dominance indefinitely in former agricultural fields they have invaded (Kulmatiski 2006; Baeten et al. 2010).

Presence of exotic grasses may make it difficult to restore native plant communities on retired agricultural land because they may invade and replace native plants that have been sown (D'Antonio & Meyerson 2002; Wilson & Pärtel 2003; Tjelmeland et al. 2008) and may hinder establishment of native plants by altering succession (Cramer et al. 2008; Tognetti et al. 2010). This is particularly true where subtropical C4 grasses such as buffelgrass (*Pennisetum ciliare* L.), are naturalized (Tjelmeland et al. 2008).

Restoration of native grasslands using native plants before exotic species become established may inhibit invasion by exotic plants (Bakker & Wilson 2004). Ingress of non-native species may also be reduced by establishing a diverse native plant community (Van der Putten et al. 2000; Kennedy et al. 2002). Sowing native plant seed mixtures more effectively inhibits non-native plant encroachment than sowing individual species (Blumenthal et al. 2003; Sheley & Half 2006).

Only about 5% of the original native plant communities in the Lower Rio Grande Valley of Texas are extant (Fulbright & Bryant 2002). Consequently, state and federal agencies including the Texas Parks and Wildlife Department and U.S. Fish and Wildlife service have purchased former cropland to restore native vegetation and to provide wildlife habitat (Jahrsdorfer & Leslie 1988). Restoration of native vegetation in these areas is difficult because of (1) the propensity of areas with fertile soils to be invaded by exotic plants (Stohlgren et al. 1999), (2) the presence of numerous exotic grass species in proximity to restoration sites (Wilson & Pärtel 2003), and (3) the lack of commercial seed of native ecotypes of plants adapted to the region (Lloyd-Reilley 2001).

Our objective was to test the hypothesis that a stand of vegetation produced by sowing a mixture of native plant seeds on former cropland would resist ingress of exotic plant species compared to areas allowed to revegetate naturally. Plots were sown with a mixture of native species representative of early,

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mid, and late stages of secondary succession that contained grasses, nonleguminous forbs, and legumes.

Methods

Experimental Design

Our study site was the Taormina unit of the Texas Parks and Wildlife Department Las Palomas Wildlife Management Area (26° 6′35.81″N, 98° 1′31.60″W). The Taormina unit is in the southern Rio Grande Valley of Texas, in an area typically described as a semi-arid to sub-humid ecosystem receiving an average of 71 cm of rain annually (1971–2000). Mean annual temperature is 23°C with few frosts (1971–2000) (U.S. Department of Commerce, National Oceanic & Atmospheric Administration 2004). During April 2009 through August 2009 this region of Texas was in a severe to extreme drought based on the Palmer *Z*-Index (Heim 2010) (Fig. 1). The study site is in the south Texas plains ecoregion and originally may have consisted of a mix of brushland and short grass prairie (Landers 1987). Soils at the study site were Harlingen Clay, which has a calcareous clayey alluvium parent material described as a Mollisol in the suborder ustolls (Soil Survey Staff 2008). Percent sand ranged from 15 to 29% across the study area, nitrate-N ranged from 4 to 16 µg/g, and pH was 8 (Falk 2010).

The study site was used for crop production for more than 50 years before our experiment. Since 1995, 33% of the area has been disked annually on a rotational basis to maintain early successional plants such as annual sunflower (*Helianthus annuus* L.) and *Croton* spp.

Site Preparation and Sowing

Treatments were arranged in a randomized, complete block design with four replications. Three treatments were randomly assigned to 2 ha experimental units within each 6 ha block. Treatments were: (1) control, (2) prepared-only, and (3) prepared and sown. The criterion for blocking was distance and direction from a stand of exotic plants that were potential sources of invasion by exotic plants. Blocks had an existing stand of exotic species located either on the upwind or downwind side of a prevailing south east wind. Treatments within blocks were located within 20 m of each other, and blocks were located an average of 20 m apart.

Plot preparation was conducted from 27 February to 8 March 2008. Woody plants on prepared-only and prepared and sown plots were shredded and removed from plots by grubbing and pushing with a bulldozer. After woody vegetation was removed, both the prepared-only and the prepared and sown plots were disked twice with an offset disk. Following disking, plots were moldboard plowed to a depth of 25 cm. After plowing, the plots were disked two more times and finished using a field float to level the seedbed.

Seeds of 29 plant species were sown within each 2 ha experimental unit assigned to the prepared and sown treatment. Seeds used in our study were harvested from regionally adapted ecotypes. Fourteen of these seed sources have been released by the South Texas Natives Project and the USDA NRCS Plant Materials Center in Kingsville, Texas (Smith et al. 2010). The remainder of the seed mixture was comprised of species being evaluated and increased by these two programs.

Sowing was accomplished during 19–20 March 2008. Plots were sown with a native seed drill and tub spreader (i.e. broadcast seeder) to sow the variety of seeds used. The drill was used to sow seeds that were heavier or coated and could easily pass through the drill, while the tub spreader was used to sow the lighter seeds that did not flow well. Two passes were necessary with the tub spreader to obtain the target seeding rate. Seeding rates were developed following USDA Natural Resources Conservation Service guidelines for sowing native vegetation with an overall seeding rate of 18 kg pure live seeds (PLS)/ha (Hidalgo County, Electronic Field Office Technical Guide) (Table 1). Seed mix composition was developed with an 8:2 ratio of grasses and forbs (Dillard 2004). Within this ratio, we attempted to have an equal proportion (on a PLS basis) of early, mid, and late successional species. A high proportion (50%) of the early successional grass species were made up of slender grama (*Bouteloua repens* Kunth.) and short-spike windmillgrass (*Chloris* × *subdolistachya* Müll.) which are aggressive native species that compete well with non-native grasses (Tjelmeland 2007). These species were sown to achieve rapid establishment. Following the final seed application, one pass with a roller packer was made to increase seed–soil contact, and to reduce depredation by birds and rodents.

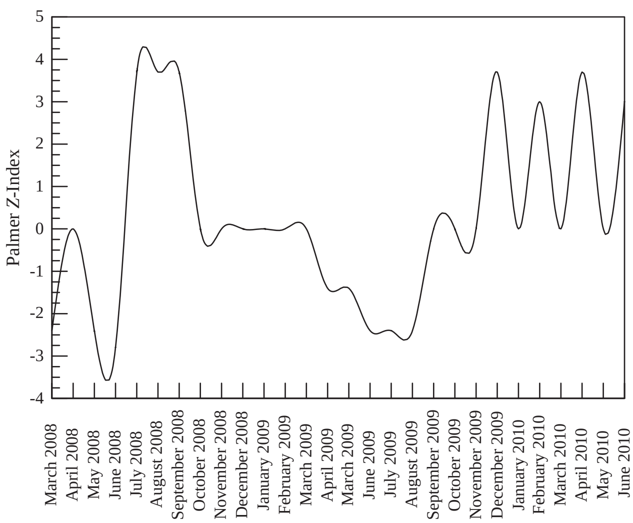

Figure 1. Palmer *Z*-Index of drought severity from March 2008 through June 2010 Hidalgo County, Texas (NOAA drought indices 2010).

Sampling

Vegetation sampling was conducted during October 2008, January 2009, March 2009, June 2009, August 2009, October 2009, March 2010, and June 2010. Percent canopy cover

Table 1. Percent of seed mix and sowing rate in grams pure live seed 19–20 March 2008, Hidalgo County, Texas, U.S.A., with percent of seed mix and sowing rate in grams pure live seed (PLS)/ha (NRCS, 2010).

Species	% of seed mix	Grams PLS/ha
Early successional grasses		
Slender grama (*Bouteloua repens*)	3.8	420
Shortspike windmillgrass (*Chloris* × *subdolistachya*)	11.4	78
Texas panicum (*Urochloa texana* Buckley)	8.6	591
Texas grama (Bouteloua rigidiseta Steud.)	1.2	123
Halls panicum (*Panicum hallii* Vasey)	0.2	3
Plains lovegrass (*Eragrostis intermedia* Hitchc.)	0.1	1
Mid-successional grasses		
Hairy grama (*Bouteloua hirsute* Lag.)	6.4	176
Tridens spp. (*Tridens* spp. Torr.)	3.8	52
Plains bristelgrass (*Setaria* spp.)	11.4	311
Arizona cottontop (*Digitaria californica* Benth.)	9.4	256
Late successional grass		
Multiflowered false Rhodes grass (*Tri chloris pluriflora* Fourn.)	3.5	58
False Rhodes grass (*Tri chloris crinite* Lag.)	6.0	98
Little bluestem (*Schizachyrium scoparium*)	2.4	112
Pink pappusgrass (*Pappophorum bicolor* Fourn.)	7.3	250
Longspike silver bluestem (*Bothriochloa longipaniculata* Gould.)	0.2	4
Sideoats grama (*Bouteloua curtipendula* Michx.)	3.1	232
Canada wildrye (*Elymus Canadensis* L.)	3.4	141
Annual forb		
Rio Grande clammyweed (*Polanisia dodecandra* L.)	2.2	30
Plantain blend (*Plantago* spp. L.)	2.3	128
Louisiana vetch (*Vicia ludoviciana var. texana* Nutt.)	0.7	39
Firewheel (*Gaillardia pulchella* Foug.)	0.8	86
Perennial forb		
Awnless bush sunflower (*Simsia calva* Engelm. & A. Gray)	0.3	10
Plains dozedaisy (*Aphanostephus ramosissimus* DC.)	2.1	28
Texas crownbeard (*Verbesina microptera* DC.)	0.2	6
Engelmann daisy (*Engelmannia pinnatifida* Raf.)	0.1	20
Orange zexmenia (*Wedelia hispida* A. Gray)	1.8	84
Mexican hat (*Ratibida columnifera* Nutt.)	3.4	93
Climax forb		
Low prairie clover (*Dalea scandens* Mill.)	0.1	2
Rio grande stickpea (*Calliandra conferta* Benth.)	0.1	1
Prairie acacia (*Acacia angustissima* var. *texensis* Mill.)	1.6	112

of vegetation was ocularly estimated with 40 20 × 50 cm quadrats within each treatment and block combination (Daubenmire 1959). All plants were identified to species, when possible. Sample points within each treatment and block combination were selected using a restricted random design in which four, 165 m transects were systematically placed within each experimental unit and 10 quadrats were randomly placed along each transect.

Statistical Analysis

Dependent variables in statistical analyses were percent bare ground and litter and percent canopy cover of perennial native plant species, perennial exotic species, and annual species. We analyzed data using repeated measures analyses with an arc sin transformation to normalize data (Littel et al. 1996). Independent variables were treatment and time. We selected the covariance structure for statistical models with the lowest AIC_c value. When there were competing structures, we chose the one that was different by ≤ 2 $AIC_{c.}$ When there was no difference in the values, we selected the simplest structure. We used a Kenward–Rogers degrees of freedom adjustment for a small sample size to avoid a Type I error associated with repeated measures analysis (Kowalchuk et al. 2004). We conducted a set of independent contrast comparisons comparing prepared and sown treatments against prepared-only, and prepared-only against control treatments for the six most recent sampling dates March 2009–June 2010 (Day & Quinn 1989). Species richness was calculated as number of species/4 m^2 by summing the number of different species found in each of the 40 20 × 50 cm frames. Evenness was calculated using the equation:

$$E = H/\log(S)$$

where H is the number derived from Shannon's index and S is the maximum value derived from Shannon's index (Chambers & Brown 1983).

Results

Native Plants

Lack of rainfall inhibited growth of vegetation on all treatments from the time that ground preparation was completed in March 2008 until July 2008, when 178 mm of rain fell in 2 days from Hurricane Dolly. The sampling date × treatment interaction was significant (Repeated measures ANOVA, $F_{[14,63]} = 2.37$, $p = 0.010$) for percent canopy cover of perennial native plants meaning that the percent cover of perennial native plants changed differently in each treatment over time (Table 2, Fig. 2). Percent canopy cover of perennial native plants was similar among treatments during the first growing season after sowing; however, cover of perennial natives ranged from 20 times greater at 15 months to 4 times greater at 27 months in prepared and sown plots than in prepared-only plots.

Percent canopy cover of native plant species in prepared and sown plots nearly quadrupled from 12 to 27 months after sowing, while percent canopy cover of native plant species changed minimally in control and prepared-only treatments during the same time period (Fig. 2). Canopy cover of native vegetation was similar between control plots and prepared plots for the first 19 months after sowing. Control treatments supported agricultural weeds such as Drummond's clematis (*Clematis drummondii* Torr. & A. Gray), *Baccharis* spp., silverleaf nightshade (*Solanum eleagnifolium* Cav.), tievine (*Ipomoea cordatotriloba* Dennst.), and *Sida* spp. remaining from past agricultural production.

Total percent canopy cover of sown native species ranged from $8 \pm 4\%$ (mean ± SE) to $67 \pm 5\%$ among replications of the prepared and sown treatment. In each replication, different combinations of sown species comprised this cover; consequently, percent canopy cover of individual plant species varied among replications. Plains bristlegrass (*Setaria leucopila* Scribn. & Merr.) canopy cover, for example, ranged from $5 \pm 1\%$ to $15 \pm 3\%$ among replications. Slender grama canopy cover varied from 0% to $24 \pm 4\%$ among replications. Sideoats grama (*Bouteloua curtipendula* Torr.) and firewheel (*Gaillardia pulchella* Foug.) were absent in one replication; greatest canopy cover of these two species in a replication was $11 \pm 2\%$ and $10 \pm 1\%$, respectively (Fig. 3). Total canopy cover of these four species comprised $64 \pm 15\%$ of the total native species canopy cover within each replication (Appendix 1).

Table 2. ANOVA table for repeated measures analysis of percent canopy cover of perennial native and exotic plant species; independent variables were sampling date (time) and treatment (control, prepared-only, and prepared and sown), October 2008–March 2010, Hidalgo County, Texas, U.S.A.

Source of variation	*Num DF*	*Den DF*	F	p
Perennial native				
Block	3	6	0.20	0.889
Sampling date	7	63	2.92	0.010
Treat	2	6	4.23	0.071
Sampling date × Treat	14	63	2.37	0.010
Perennial non-native				
Block	3	6	2.47	0.160
Sampling date	7	63	8.73	<0.001
Treat	2	6	6.72	0.029
Sampling date × Treat	14	63	2.44	0.008

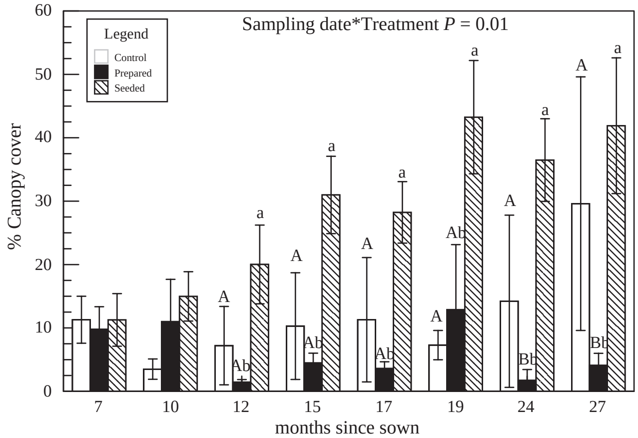

Figure 2. Absolute mean (± SE, $n = 12$) percent perennial herbaceous native canopy cover averaged across replications in control, prepared-only, and prepared and sown treatment for seven sampling dates, October 2008-June 2010, Hidalgo County, Texas, U.S.A. Statistical differences (0.05) within sample date from independent comparisons are indicated with different letters (A, B) for control versus prepared and (a, b) for prepared versus prepared and sown.

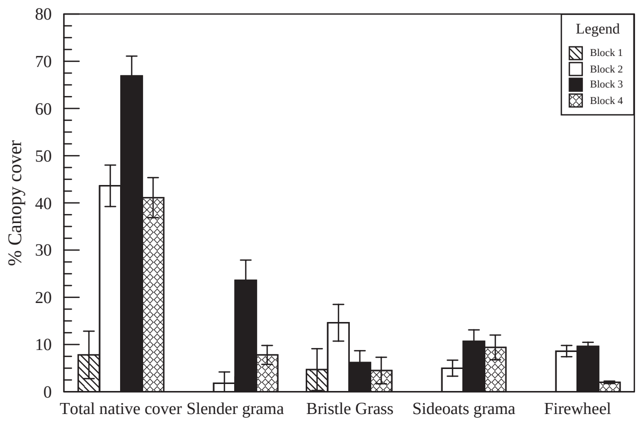

Figure 3. Absolute mean (± SE, $n = 4$) percent canopy of total native cover, three native grasses and one forb species in prepared and sown treatments for each replication, June 2010, Hidalgo County, Texas, U.S.A.

Perennial Exotic Plants

The sampling date × treatment interaction was also significant ($F_{[14,63]} = 2.44$, $p = 0.008$) for percent canopy cover of perennial exotic species which indicated that the percent canopy cover of perennial exotic species changed differently in each treatment over time (Table 2, Fig. 4). Percent canopy cover of exotic species increased eight fold from October 2008 to June

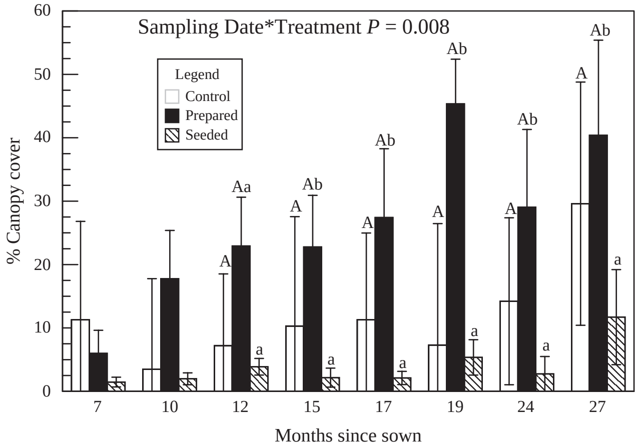

Figure 4. Absolute mean ($\pm$ SE, $n = 12$) percent perennial herbaceous non-native canopy cover averaged across replications in control, prepared and prepared and sown treatments for seven sampling dates, October 2008–June 2010, Hidalgo County, Texas, U.S.A. Statistical differences (0.05) within sample date from independent comparisons are indicated with different letters (A, B) for prepared versus prepared and sown and (a, b) for control versus prepared.

2010 in prepared only-plots; in prepared and sown and control treatment plots little change in percent canopy cover of exotic species occurred during this period. Percent canopy cover of perennial exotic plant species was three to nine fold greater in prepared-only than in prepared and sown plots during June 2009 to June 2010. Canopy cover of exotic grasses was similar in control and prepared-only plots on all sampling dates

Species Richness and Evenness

Species richness/4 m^2 varied among sampling dates ($F_{[7,77]} = 4.05$, $p < 0.001$). Highest species richness corresponded to the wettest 3 months during the study. Prepared and sown treatments had twice as many species/4 m^2 ($F_{[2,6]} = 18.61$, $p = 0.003$) than did control and prepared-only treatments. Evenness, on the other hand, was similar among sampling dates ($F_{[7,39.3]} = 0.66$, $p = 0.700$ and treatments ($F_{[2,4.692]} = 0.14$, $p = 0.871$).

Discussion

Sowing 29 different native plant species reduced ingress of exotic plants on former cropland in the lower Rio Grande Valley of Texas (for the 27 months of this experiment) compared to sites where vegetation established through secondary succession. Establishment of exotic plants was constrained in prepared and sown plots even though 14 species of exotic plants were present in the study area. Our results contrasted with previous research in southern Texas in which exotic grass canopy cover returned to pre-treatment conditions in less than 12 months following sowing of three native grass species (Tjelmeland et al. 2008).

Longer-term research is needed to determine how long native plants will dominate our study sites. Sowing a diverse mix of natives may simply postpone exotic grass encroachment. Native grasses dominated during the second year after restoration in California, for example, but by the fourth year few native perennial grasses remained (Rein et al. 2007). Harlingen Clay is a dominant soil series in the lower Rio Grande Valley of Texas (Kunze et al. 1955); however, our results could have varied if other soil series had been included in the study.

Old world bluestems (Kleberg's (*Dichanthium annulatum* (Forssk.) Stapf.), Angelton (*Dichanthium aristatum* (Poir.) C.E. Hubbard), and silky (*D. sericeum* (R. Br.) A. Camus) bluestems) were the major group of exotic plants in our study area (Appendix S1, supporting information). These grasses exhibit little evidence of habitat preference and commonly invade areas near seed sources (Gabbard & Fowler 2007). Prepared and sown plots and prepared-only plots were both surrounded by established stands of Kleberg's bluestem. Sown plots appear to have been more recalcitrant to invasion of the grass from the surrounding stands because there was little change in Kleberg's bluestem canopy cover in these plots during the study compared to an increase in canopy cover in prepared-only plots.

Bermudagrass was another common exotic grass on our sites. Once established, bermudagrass shows little evidence of habitat preferences in a manner similar to old world bluestems (Klementowski et al. 2005). In our study, bermudagrass invaded plots by producing stolons or by establishing from crowns that were not removed during land preparation. Small strips of bermudagrass that were missed during disking and tillage remained in both prepared-only plots and prepared and sown plots. As with the old world bluestems, prepared and sown treatments appeared to be more resistant than prepared-only treatments to the increase of this grass.

Native plant establishment in former cropland in the lower Rio Grande Valley with a long history of cultivation may be limited in part by low availability of native plant seeds and a high potential for dispersal of exotic plant seeds into the sites. Few native plants were present where we did not sow seeds of native plants, and those that were present consisted of species considered to be agricultural weeds by farmers (Everitt et al. 2007). Soil seed banks in agricultural fields that have been cultivated for decades are commonly deficient in plant species characteristic of stable plant communities (Cramer et al. 2008). Lack of native plants in the vegetation surrounding the study area was another probable cause for the deficiency of native plants in the study area (Jacquemyn et al. 2003). Scarcity of native plants to compete with exotic plants in old fields may be one of the explanations exotic plants are able to invade and become dominant (Seabloom et al. 2003).

Old World bluestems readily invade adjacent sites through seed dispersal (Gabbard & Fowler 2007; Ortega-S et al. 2007). One possible explanation for the lack of exotic plants in the prepared and sown treatments is that absence of gaps of unused resources reduced spaces available for exotic plants to establish (Dukes 2001; DiVittorio et al. 2007).

Establishment of individual native plant species in each replication varied greatly even though seeds were sown uniformly and soil physical and chemical properties were relatively uniform among replications. The large amount of unexplained variation in species establishment was similar to results of Ozinga et al. (2005), who found that they could only predict that a pool of species might become established in a given location, but could not predict which individual species would establish. They attributed this unpredictability to small scale (<1 cm) heterogeneity of resources and plasticity of individual plants. Unexplained variation in individual seedling establishment in an apparently homogenous environment is an important factor underlying the need to sow diverse native seed mixes.

The high amount of variation in species composition in sown treatments had little effect on the evenness or overall canopy cover of vegetation. If a particular microsite was unsuitable for germination and establishment of one species, there may have been a high likelihood that seeds of at least one of the 28 other species that were sown could establish in that microsite. Sowing a diverse mixture, consequently, probably resulted in higher and more uniform overall percent canopy cover, regardless of unpredicted sources of variation, than would have been achieved if only a few native species had been sown. Higher and more uniform vegetation cover reduced gaps that might be susceptible to ingress by exotic grasses.

Presence of several plant functional and successional groups possibly assisted in inhibiting invasion of exotic plants in prepared and sown plots. Several researchers have questioned whether species diversity or functional group diversity is more important in invasion resistance (Dukes 2001; Pokorny et al. 2005). One theory that explains invasion resistance of a diverse community is that different species have different competitive advantages, forming more complete resistance over time and space when coupled with the high resource utilization of diverse native communities (Theoharides & Dukes 2007).

Competition of native plants with exotic plants at the establishment stage is a key factor in restoring native grasslands (Yurkonis et al. 2005). The early cover provided from slender grama, for example, may have provided competition with exotics while later successional species were still in the seedling stage and would have been more vulnerable to being outcompeted by exotics. Evidence supporting this competition is the fact that the amount of canopy cover produced by native species in prepared and sown treatments was similar to the amount of canopy cover produced by exotics in prepared-only treatments, and the fact that slender grama provided a large contribution to this cover. The shift in species composition from slender grama, an early successional species, to bristlegrass and other later successional species near the end of our study demonstrates the need for different successional groups to provide cover at different times following sowing.

Climate of our study area was stochastic, with both drought and periods of high rainfall occurring during the study. Sowing a number of different species differing in moisture requirements increases the probability that some group of species will establish during drought or periods of excessive moisture (Seastedt et al. 2008).

Implications for Practice

- Sowing a diverse mix of 29 species of ecotypic native seeds in the lower Rio Grande Valley of Texas, helped restore native wildlife habitat and resist invasion from exotic species for up to 2 years after sowing.
- The addition of locally adapted native seed to former croplands increases species richness more than land preparation alone.
- If the objective of management is simply to have abandoned farmland covered by vegetation, sowing is an unnecessary expense because the sites will naturally revegetate from colonization by exotic grasses and agricultural weeds.
- In order to establish native vegetation on retired cropland in the lower Rio Grande Valley on Harlingen Clay soils, sowing seeds of adapted native plants is essential.
- Real estate value of native rangelands are higher than value of areas simply left fallow, largely because of demand for land to provide wildlife-related recreation (Krausman et al. 2009). With land prices in the Lower Rio Grande Valley nearing $5,000 USD/ha the benefits of spending approximately $1,235 USD/ha on native vegetation restoration may be economically viable.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Mean percent canopy cover ± SE of all species in each treatment averaged across replications and all sampling dates, Hidalgo County, Texas.