

SNYDER. INTERSEEDING FORBS IN A TALLGRASS PRAIRIE REST.

**INTERSEEDING FORBS IN A TALLGRASS PRAIRIE
RESTORATION:
EFFECTS OF THREE DISTURBANCE REGIMES- FIRST
GROWING SEASON**

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ABSTRACT. Tallgrass prairie is one of the most endangered ecosystems in North America, but once covered vast areas of the Midwest, including regions of Indiana. Efforts to restore tallgrass prairie have not been able to replicate the species diversity of prairie remnants. Restoration seed mixes are often dominated with grasses, such as big bluestem (*Andropogon gerardii*), and typical management techniques can further increase their competitive advantage. Methods for increasing forb dominance and diversity are essential for diverse restoration. Interseeding (introducing seed into an already established community) is a restoration tool that has been growing in popularity but has been little studied. This experiment was conducted on the grass-dominated, 15-year-old Avis Prairie Restoration in Upland, IN. A nested factorial design compared three methods of disturbance – mowing, tilling, and herbicide treatment – on the success of interseeding seven species of forbs. Tilling produced the greatest numbers of seedlings by the end of the first growing season and all other treatments were not significantly different from one another for the sum of all sown species. Asteraceae and Fabaceae species reacted somewhat differently to landscape and treatment factors. Landscape position heavily determined interseeding success for all treatments and should be considered in management planning. This experiment suggests that patch tilling, followed by interseeding, could be a tool to increase heterogeneity and species diversity in grass-dominated tallgrass prairies.

Keywords: interseeding, species diversity, tallgrass prairie restoration, forb.

The few remnant tallgrass prairies and historical descriptions of the native tallgrass prairie that remain give restorationists glimpses of what restored prairie should resemble in both diversity and structure (Martin et al. 2005). However, when remnant tallgrass prairies are compared to prairies produced by reconstruction efforts, remnant prairies are more diverse than even long-term restorations (Howe 1994; McLachlan & Knispel 2005; Polley et al. 2005; Allison 2008), have different structure (Amman & Nyberg 2005), and different levels of habitat heterogeneity (Martin et al. 2005). Results from long-term sampling at Fermi National Accelerator Laboratory near Chicago, Illinois revealed stark species diversity differences between remnant and reconstructed prairies, with reconstructions always having lower diversity and failing to become more similar to remnants in composition over time (Sluis 2002). However, other studies show remnants and restorations becoming more similar over time, though there were strong differences in diversity between restorations and remnants (McLachlan & Knispel 2005). On remnant and restored prairies species diversity typically declines over time (Sluis 2002; McLachlan & Knispel 2005). The 16 ha Greene Prairie, located near the Curtis Prairie at the University of Wisconsin-Madison Arboretum, is one of the few restorations that has begun to closely resemble local remnants in plant species composition, at least in some regions, and is considered one of the highest quality restorations known (Howell & Jordan 1989). Aside from a few exceptional cases, restoration efforts do not duplicate the inherent species diversity found in remnant prairies.

Established restorations and degraded remnant prairies require management to maintain or increase species richness. A recent survey of six prairies (3 remnants and three >45-year-old restorations) in northern Illinois found that dominant grasses

accounted for 37-49% of the cover in remnants while restorations contained a much greater percentage (56-65%) of grasses (Allison 2002). Remnants vary broadly in their composition and forb or grass dominance, but restorations are almost always heavily grass dominated. In most degraded or reconstructed prairies, diversity is limited by low propagule availability and small species pools, not a lack of available microsites where seedlings can develop (Zobel et al. 2000). Increasing the available species pool by adding seed directly increases diversity, though to differing degrees depending on resource availability and competitive relationships among present vegetation (Foster 2001; Martin & Wilsey 2006; Dickson & Foster 2008). Interseeding and seedling transplanting have both been used to increase species pools and available propagules in remnant prairies and restorations (Packard 1997; Brown & Bugg 2001). However, according to Gibson (2006), very little research has been conducted on the process of interseeding, its viability, or success, even though it is gaining respect as a viable method for restoration.

Many early restorations focused on planting aggressive native grasses either as an initial step to restoration to out-compete weed species, because grass seed was all that was commercially available, or because forb seed was too expensive (Schramm 1990; Huber-Sannwald & Pyke 2005; Williams et al. 2007). There are also intentionally species-poor grasslands that were intended to combat erosion, such as the Conservation Reserve Program's (CRP) warm-season grass (WSG) plantings, developed by the Natural Resources Conservation Service (NRCS). Newer legislation has taken a broader view of these "functional grasslands" (Howell & Jordan 1989) and now requires mid-contract management of WSG plantings in order to open the grass sward for wildlife. Disking, herbicide treatment, prescribed burning, and mechanized interseeding are all approved

methods for mid-contract disturbances (USDA 2007). Dominant native grasses provide fuel for all-important prairie burns, but they also compete against the native forb component of the prairie mix, which contains the majority of the species richness in this community (Briggs & Knapp 2001). Unfortunately, initial seed mix largely controls long-term prairie structure and composition (Schramm 1990; Dickson & Busby *in press*).

In many restorations burning is conducted annually at the same time of year (typically early spring) (Schramm 1990). This can benefit C₄ grasses at the expense of the forb component of the community (Towne & Owensby 1984; Abrams et al. 1986; Howe 1994). Mosaic planting of grass-rich and forb-rich regions and differential seed conditioning (planting cold-damp stratified forb seed with unstratified grass seed) are ways to increase heterogeneity when building prairies (Schramm 1990). Dormant seeding during the fall or winter can favor forb establishment as well as stratify and incorporate the seed (Packard 1997). However, patch disturbance/interseeding techniques also could be used retroactively to increase heterogeneity and diversity, particularly in sites where managed grazing is not an option (Martin & Wilsey 2006). Increasing species diversity through the addition of seed is largely determined by the use of disturbance to reduce competition (Foster 2001; Dickson & Foster 2008). Disturbance is particularly important in productive grasslands, such as mesic tallgrass prairie, where species interactions determine the species richness of a site more than the available species pool (Dickson & Foster 2008).

Interseeding forb species, paired with various forms of disturbance, has been studied in German pastureland (harrowing, mowing, sward removal: Hofmann & Isselstein 2004) and Kansas C₃ grass-dominated field (raking and litter removal,

infrequent mowing: Foster et al. 2001; vegetation clipping: Dickson & Foster 2008). It has also been studied in Illinois old field (raking: Packard & Masters 2008), Illinois tallgrass prairie restoration (mowing, soil scarification, sod plug removal – all after burning: Warkins & Howell 1983), Iowa tallgrass prairie constructions (native ungulate grazing: Martin & Wilsey 2006), (timing and frequency of mowing: Williams et al. 2007), Iowa cool-season roadside vegetation (burning: Christiansen 1994), and Estonian alvar grasslands (plant removal from parts of plots: Zobel et al. 2000). However, there have been no comparisons of the effects of interseeding with herbicide treatment or superficial tilling disturbances, particularly in mesic prairie. This interseeding experiment compares superficial tilling, herbicide treatment, and mowing to determine which disturbance best promotes forb establishment over the first year in a well established and highly productive grass-dominated prairie. The disturbances were conducted in patches to increase heterogeneity and create species-rich “islands” that could then be seed sources for further restoration. The disturbance that best promotes seedling establishment could emerge as a practical tool for enhancing species-poor tallgrass prairies.

METHODS

Three disturbance methods and a control were compared during the summer of 2008 along an elevation gradient on the 10 ha Avis Prairie, owned by Avis Industrial Corp., in Upland, Indiana (N40°27.2', W85°0').

Study site.—The Avis Prairie was constructed in 1993 on an old agricultural field that had been used for both row crops and pasture (McIndoe et al. 2008). During the spring and summer of 1993, herbicide treatment and conventional tillage (plowing and

disking) were used before seeding. The original seed mix included approximately 50 hand-collected species (Rothrock & Squiers 2003) which were planted in a mosaic, with forb-rich regions in approximately 30 meter wide strips along the outside border and grass rich regions with <10% forbs on the rest of the site. The location of this experiment is within the grass-rich zone of the prairie on heavily eroded Morley Series clay subsoil (Jensen et al. 1983). Analysis of each block showed the relative coverage of grasses to be between 88.7% and 97.7% on July 2, 2008. Grasses were not flowering during sampling and were not distinguished by species, however, previous Floristic Quality Assessments of the grass-rich sections of Avis Prairie revealed Big Bluestem, *Andropogon gerardii* as the most dominant grass, though *Sorghastrum nutans* (ca. 5% cover) and *Panicum virgatum* (ca. <2% cover) also were present (Paul Rothrock, unpublished data). Results, reported by block, are shown in Table 1. The site is located on a south-facing slope. Blocks were placed on the slope, with the top block (block 1) occupying an upslope position, block 2 the steepest section, and block 3 the base of the hill (Figure 1).

The site has been burned each spring from 1994-2008, except for one controlled fall burn in 2004, and no subsequent spring burn in 2005. The Avis Prairie has developed a stable community structure that is now well developed in structure and species composition (Rothrock & Squiers 2003).

The mean annual precipitation for Marion, IN, <20 miles NE of the site is 991 mm and the mean annual temperature is 10.2 ° C. Weather for May and July, 2008, were similar to recorded normals. June, August, and September, each received approximately one-third the precipitation volume of recorded normals (Indiana State Climate Office 2008).

Design.—The experiment is a nested factorial design that utilizes three blocks aligned along the elevation gradient. Each block contains fifteen randomized swaths. Each swath consists of four randomized plots, each with one of four treatments: mowing, tilling, herbicide treatment, or control. Treatments were conducted in the center of an interseeded 2×2 m area at the center of a 4×4 m plot. This design provides an unseeded buffer surrounding each treatment isolating it from nearby treatments (Figure 2).

Paths were designated into the block design to avoid causing compaction or grass lodging that could affect the results. Block layout is permanent, with steel stakes marking the four corners of each block, and individual, brightly painted 1.5 m rebar stakes marking the NE corner of each of the 180 plots.

Mowing was conducted/simulated with a weedwacker and comprised the entire 2×2 m area. Vegetation was cut when it reached approximately 60 cm in height and reduced to approximately 15 cm in height four times during the growing season. Herbicide treatment was conducted with a 0.84% concentration of Glyphosate (Round-up®, Monsanto Co., recommended rate for general weed control) five days before planting and was intended to kill the vegetation. The herbicide was applied to a 1 m² square area at the center of randomly selected plots. Tilling was conducted with a Troy-Built® rototiller in 2×2 m patches less than 24 hours before seeding. Only the top few inches of soil were disturbed, and grass clumps were chopped, but not completely destroyed. Control treatments were seeded like all other treatments, but were left undisturbed.

Seed mix.—Seven regionally native forb species were selected. They had a variety of seed weights and represented a variety of families with different germination

requirements (Table 2). Seeds were purchased from JF New, Walkerton, IN. Seeding rates were applied according to Pure Live Seed (PLS) levels tested by an independent lab (AB Seed Laboratory LLC, Norcross, GA). The mix also included a species, *Sabatia angularis*, which has not been used in regional restorations because it is thought to have high conservatism, but is poorly understood and receives different treatments by regional FQA databases (Table 2). All seeds were cold-damp stratified in sand for 26 days and legumes were scarified, all according to their requirements (Nathan Simons pers. comm.). Each species was equally represented by seed number in the seed mix and seeding density was selected at 35 seeds/ft² (376.7/m²) (Diboll 1997; Williams et al. 2007). Initially seeds were carefully hand sorted to remove any weed seed, then mixed with sand in a ratio of one part seed to 1 part sand during stratification. After stratification, sand was added to increase the ratio to 1 part seed to 3 parts sand to aid in hand seeding (Packard 1997; Williams et al. 2007). The seed/sand mixture was thoroughly mixed, then individually measured and separated into 15 bags to avoid settling of small-seed. Seeding was conducted May 10. Each plot received an equal volume of the seed/sand mixture, sprinkled by hand over a 2×2 m area at the center of each plot. Seed was not incorporated into the soil but was left on the surface (Brown & Bugg 2001; Packard & Masters 2008).

Sampling methods.—In each of the 180 plots seedling counts of each of the seven seeded species, and the two most numerous weed seedlings per plot were taken monthly after seeding during the months of June, July, August, and September. Seedling counts were obtained using a 0.25 m² quadrat at the center of each plot. Seedlings were identified after they developed their first leaf and only were counted if they could be positively identified to species (Williams et al. 2007). However, if the species was

unknown, was clearly not one of the seven desired species, and was one of the two dominant weeds in that quadrat, it was marked as an unknown weed.

A random sample of 20 unseeded buffer areas per block was conducted using a 0.25 m² quadrat to quantify the natural levels of seedling germination and to identify germinating species.

Data analysis.—Treatment differences were analyzed with analysis of variance (ANOVA) General Linear Model (GLM) using Minitab 15 statistical software (Minitab Inc.). The data sets were tested for normality using Anderson-Darling tests. Datasets were considered normal if $p > 0.01$. Datasets that were not initially normal by the Anderson-Darling test were transformed using a square root transformation, then retested for normality. Back-transformed means are included in this paper for display, though all analyses were conducted with transformed data. The GLM model tested four sources of variation: block, treatment, swath, and block×treatment. All reported data utilized this model except for the unseeded buffer area sampling, which utilized one-way ANOVA. Tukey's HSD (Honestly Significant Difference) tests were conducted for all comparisons of means using $\alpha = 0.05$ to reduce family-wise Type 1 error.

RESULTS

Two of the seven species, *Sabatia angularis* (L.) Pursh and *Rudbeckia triloba* L, did not germinate during the 2008 growing season. Total weed species data did not meet assumptions of normality until the end of the growing season. Weed species composition (Appendix A) was very similar to the weeds that were present during the first few years of restoration (McIndoe et al. 2008). The two main plant families

represented in our seed mix, Asteraceae and Fabaceae, responded differently to treatments and their location on the hill slope. These two families have very different growth strategies and seed sizes. Patterns shown by these two families are presented in the August and September data sets.

Germination and initial response.—Data collected during June and July identifies the control treatment as the best facilitator of initial germination, with June averaging 3 times more seedlings compared to other treatments (Table 4). The high number of control treatment seedlings recorded in June was reduced by July, but was still significantly greater than mowing and herbicide treatments, though no longer from tilling (Table 4). Data for June sown species showed a minor interaction in the tilling treatment between blocks 2 and 3 (Table 3). Similar to the rest of the summer, the upland block (block 1) had the highest number of seedlings (figure 4). Mowing treatments showed relatively high levels of seedlings during the June sampling (the first mowing was conducted 5 days previous), but the seedlings were reduced to low levels by July (Figure 3).

Survival.—Between July and August the number of seedlings of sown species in the control treatments decreased by more than one half, while the numbers in the tilling treatment remained relatively constant (Figure 3). Seedling numbers in control treatments continued to decline throughout the rest of the summer. The seedling numbers in herbicide treatments decreased steadily after July and closely paralleled control treatments in both August and September (Figure 3). During August, mowing treatments remained significantly lower than herbicide and tilling treatments, but no longer from control treatments (Table 4). From August to September, mowing treatments maintained

their few seedlings while control and herbicide treatment numbers continued to decline (Figure 3). By mid September, tilling had statistically more seedlings than all other treatments and contained approximately six seedlings per 0.25 m² (Table 4). Control, herbicide, and mowing treatments were not statistically different and each sustained less than three individuals per 0.25 m² (Table 4). Seedling numbers by block decreased after July and developed a pattern of seedling numbers decreasing steadily from highest elevation block to lowest block (Figure 4). Analysis of unplanted weed species exhibited an interaction ($p = 0.019$) with the lowest block and the mowing treatment (Figure 5). Weed presence was greatest in till plots, while relatively minimal in other treatments.

Family group variation.—The two Fabaceae species responded to treatments and blocks differently than the two Asteraceae species throughout August and September. Asteraceae species showed a strong block preference by favoring higher elevations, which increased in September (Table 5). Legumes showed a similar, but weaker pattern, and the blocks were not significantly different for either month (Table 5). Asteraceae species showed similar seedling levels between control, herbicide, and till treatments, but mowing treatments produced significantly fewer seedlings than all other treatments (Table 4). By August, legumes were best represented in tilling treatments, followed by mowing treatments. By September, tilling produced the best result overall, with over twice the number of legume seedlings than mowing. Herbicide and control treatments were statistically lower than both mowing and tilling treatments (Table 4).

Natural seed bank germination.—Unseeded buffer zones were sampled during the middle of July and compared to the seeded plots. Naturally germinating seedling numbers were low and all species found were grouped together during analysis to meet

test assumptions. Block effect for natural germination closely resembled block variation in the seeded areas during July (Figure 6). The highest block (block 1) had significantly more seedlings for both the seeded and unseeded regions (Figure 6). The patterns for lower blocks within the seeded and unseeded regions were similar as well, neither having blocks different from each other. The similarities between seeded and unseeded regions suggest that seedling patterns along the elevation gradient were unrelated to seed availability and were instead a function of landscape position.

DISCUSSION

Tilling was more successful in promoting the first growing season establishment of our interseeded forbs than any other treatment. Control and herbicide treatments produced the highest germination rates, but seedlings were out-competed by established grasses as the summer progressed. End of season survivorship is the most important measure of treatment success, but germination patterns suggest that herbicide and mowing should be tested further using different application and timing.

Applying Glyphosate at recommended rates for general weed control (0.84% solution) did not accomplish a complete kill of the established perennial grasses. Grass clumps were heavily stunted and rarely reached a meter tall, but clumps grew denser for their height than untreated grasses. Higher Glyphosate concentrations might improve grass control and could change results for this treatment.

Mowing treatments performed poorly, producing low germination and survivorship of the interseeded forbs. It is possible that the first mowing was too late for ideal survival (June 9, vegetation <1 m tall) and the shade acclimated seedlings were

shocked by intense sun exposure. It is also possible that mowing sheltering grasses could have allowed seedling desiccation since June was an unusually dry month (Indiana State Climate Office 2008). One cannot rule out that the mowing thatch promoted fungal growth, reduced nutrient availability, or simply smothered many seedlings. Whatever the cause, the only species in our experiment to tolerate the mowing treatment was *Senna hebecarpa*. Haying, mowing and raking, time and frequency of mowing, and mowing height may change the results of this treatment (Williams et al. 2007).

Interseeding into the undisturbed grasses in control plots produced relatively high seedling germination in early summer, but seedling mortality was nearly complete after the grasses went to seed. It is likely that the sheltering effect of the undisturbed vegetation provided a microclimate favorable for seed germination. Since grasses shade the soil surface, preserving surface moisture and preventing desiccation.

Tilling treatments in this experiment utilized a walk-behind rotary tiller to create small disturbances. This equipment and patch size could be very useful for small restorations, but this method may not be practical for large sites. Larger equipment that could create similar results, such as large PTO-driven rotary tillers or disk harrows may be successful in disturbing patches for interseeding. Tilling produced the highest number of surviving seedlings, but the results from this experiment suggest that species or plant groups respond dissimilarly to various forms of disturbance. Incorporating diverse types of patch disturbance during different times of the year or during different years could enhance the heterogeneity of prairie restorations and make them more similar to historical or remnant prairies in diversity (Schramm 1990; Howe 1994).

The interseeded Asteraceae and Fabaceae species responded differently to both treatments and landscape location. The two Asteraceae species, *Ratibida pinnata* and *Rudbeckia hirta*, preferred higher elevations and tolerated shade in the control and herbicide treatments, but were unsuccessful in the mowing treatment. The two legumes, *Senna hebecarpa* and *Desmodium canadense*, were less affected by location, did not tolerate shade, and better tolerated mowing. Other recent studies suggest that individual species react to disturbances differently (Packard & Masters 2008). In a recent study that looked at interseeding prairie species into an old field in Illinois, incorporating seed by raking was compared to leaving seeds on the surface. After ten years two of the four successful species preferred incorporation (legumes), two did not (Packard & Masters 2008). Incorporation, either by fall seeding, or by raking, could have likely improved germination rates in this experiment, at least for some of the species, but incorporation is not always used in interseeding studies or practice (Warkins & Howell 1983; Brown & Bugg 2001; Martin & Wilsey 2006).

The declining survivorship of interseeded species down-slope, as well as unsown species outside of treatment areas, is similar to plant diversity gradients seen in other studies. Plant diversity gradients have been shown to parallel topographic gradients in sites similar to this experiment (Gibson & Hulbert 1987, Schimel et al. 1991). The causative factors for this relationship are thought to be soil depth and water availability, which directly influence competitive dynamics (Baer et al. 2005). There is evidence that as grassland productivity increases, seed additions become less successful, however, this relationship is weakened through the addition of disturbance (Foster 2001). In a study conducted on Konza Prairie (tallgrass) in NE Kansas during a drought year, forb

aboveground net primary productivity (ANPP) increased, while graminoid ANPP decreased (Briggs & Knapp 1995). In a later paper the authors suggest that competitive interactions with dominant grasses drive forb ANPP, while water relations and fire frequency determine grass ANPP (Briggs & Knapp 2001). Annual burning also has been determined to increase the variation of water availability between low regions and small ridges or slopes in tallgrass prairie (Knapp et al. 1993). Our data concur with these studies and suggest that the success of interseeding, even with patch disturbance, is adversely affected by the productivity of dominant grasses.

The complex interaction of factors that control competitive relationships, community structure, and function within tallgrass prairie ecosystems is not completely understood. However, patch application of varied disturbance regimes and timing of disturbance (Howe 1994), with seed addition, should increase diversity in grass-rich restorations (Dickson & Foster 2008). Both small and large scale restorations could benefit from patch tilling in conjunction with interseeding. Species groups also react somewhat differently to landscape position. *Ratibida pinnata* and *Rudbeckia hirta* seem to prefer drier upland sites, at least during the first growing season. *Senna hebecarpa* and *Desmodium canadense* may be able to recruit well regardless of landscape position, as long as there is an appropriate disturbance. Further study involving additional species could help restorationists optimize seed mixes for interseeding applications.

Many restorations in the Midwest are being conducted on small scales at parks, schools, homes, farms, and other similar locations. This study utilized equipment that is often accessible to restorationists working at these relatively small scales, but also simulates equipment that could be used for larger scales. As conservation and restoration

become more pervasive at the local level, the numbers of small-scale restorations are likely to increase. In small scale restorations, bison or even domestic cattle are not likely going to be available to disturb restorations, reduce dominance of C₄ grasses, and promote heterogeneity and diversity, as suggested for larger sites (Howe 1994; Knapp et al. 1999; Martin & Wilsey 2006). Other methods that provide similar ecological services, such as those tested in this experiment, could be particularly useful in improving diversity in small restorations.

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Table 1. Physiognomic relative coverage values by block. Sampling was conducted by randomly selecting 30 unseeded and untreated buffer zones (between treatments) per block. Sampling was conducted with ¼ m² quadrats on July 2, 2008.

<i>Physiognomy</i>	<i>Block 1</i>	<i>Block 2</i>	<i>Block 3</i>
Native Grass	88.7	97.7	96.8
Native Forb	10.7	2.2	3.2
Adventive Forb	0.6	0.1	0

Table 2. Interseeded species utilized in this experiment. C-value stands for Coefficient of Conservatism. These values are taken from the Indiana Floristic Quality Assessment (FQA) database (Rothrock 2004). Select C-values from the Chicago Region FQA database (Swink & Wilhelm 1994) are included within parentheses for comparison. PLS weights are included to show variation in seed weight. All species were equally represented at 4.8 individuals per ft².

<i>Species</i>	<i>C-value</i>	<i>Provenance</i>	<i>Family</i>	<i>PLS Quantity</i>
<i>Ratibida pinnata</i>	5 (4)	IN	Asteraceae	0.47 oz
<i>Rudbeckia hirta</i>	2	WS	Asteraceae	0.11 oz
<i>Desmodium canadense</i>	3	IN	Fabaceae	2.67 oz
<i>Senna hebecarpa</i>	4	IN	Fabaceae	8.58 oz
<i>Rudbeckia triloba</i>	3	IA	Asteraceae	0.36 oz
<i>Sabatia angularis</i>	4 (10)	IN	Gentianaceae	0.03 oz
<i>Penstemon digitalis</i>	3	IN	Schrophulariaceae	1.05 oz

Table 3. General Linear Model Results for summer 2008. Interseeded species are reported here. Tests were run on square-root transformed data.

<i>ANOVA General Linear Model (p values)</i>			
<i>Month</i>	<i>Block</i>	<i>Treatment</i>	<i>Block×Treatment</i>
Total sown species			
June	<0.001	<0.001	0.038
July	<0.001	<0.001	0.209
August	0.001	<0.001	0.329
September	0.001	<0.001	0.298
Asteraceae			
August	0.003	<0.001	0.461
September	0.001	<0.001	0.13
Fabaceae			
August	0.313	<0.001	0.705
September	0.19	<0.001	0.357

Table 4. Numbers of seedlings by treatment over the summer. Letters mark Tukey's HSD test results. Means with the same letters are not significantly different. Displayed data is back-transformed from their square-roots for presentation and SE's are presented as a range, ± 1 SE.

<i>Treatment</i>	<i>Mean Seedlings/0.25 m²</i>							
	<i>June</i>		<i>July</i>		<i>August</i>		<i>September</i>	
Total Sown Species								
Control	10.67 (12.6-8.9)	A	7.78 (8.9-6.7)	A	2.39 (2.9-1.9)	AB	1.08 (1.5-0.8)	A
Herbicide	3.65 (4.2-3.1)	B	5.27 (6.0-4.6)	B	3.13 (3.7-2.6)	A	1.62 (2.0-1.3)	A
Mow	3.88 (4.5-3.3)	B	1.75 (2.1-1.4)	C	1.37 (1.6-1.1)	B	1.35 (1.6-1.1)	A
Till	4.6 (5.1-4.1)	B	6.15 (6.7-5.7)	AB	5.28 (5.8-4.7)	C	5.92 (6.6-5.3)	B
Asteraceae								
Control					1.26 (1.6-0.9)	A	0.56 (0.8-0.4)	AB
Herbicide					1.69 (2.1-1.4)	A	0.99 (1.3-0.7)	AC
Mow					0.22 (0.3-0.1)	B	0.20 (0.3-0.1)	B
Till					1.68 (2.0-1.4)	A	1.79 (2.2-1.4)	C
Fabaceae								
Control					0.45 (0.6-0.3)	A	0.22 (0.3-0.1)	A
Herbicide					0.57 (0.7-0.4)	A	0.22 (0.3-0.1)	A
Mow					0.83 (1.0-0.7)	A	0.78 (1.0-0.6)	B
Till					2.52 (2.9-2.2)	B	2.92 (3.3-2.6)	C

Table 5. Plant families by block. Letters denote results of Tukey's HSD procedure. Means with the same letters are not significantly different. Displayed data is back-transformed from their square-roots and SE's are displayed as a range, ± 1 SE.

<i>Block</i>	<i>Mean Seedlings/0.25 m²</i>			
	<i>August</i>		<i>September</i>	
Asteraceae				
1 (High)	1.78 (2.1-1.5)	A	1.43 (1.8-1.1)	A
2 (Middle)	1.16 (1.4-0.9)	AB	0.90 (1.1-0.7)	A
3 (Low)	0.53 (0.7-0.4)	B	0.25 (0.4-0.2)	B
Fabaceae				
1 (High)	1.19 (1.5-1.0)	†	0.94 (1.2-0.7)	†
2 (Middle)	0.93 (1.1-0.7)	†	0.83 (1.0-0.7)	†
3 (Low)	0.78 (1.0-0.6)	†	0.59 (0.8-0.4)	†

† No significance

Figure 1. Map of the Avis Prairie showing block location. Taylor University is immediately West of SR 22.

Figure 2. Block design and layout. This example is the uppermost of three blocks, each aligned horizontally across the slope of a small hill in descending order.

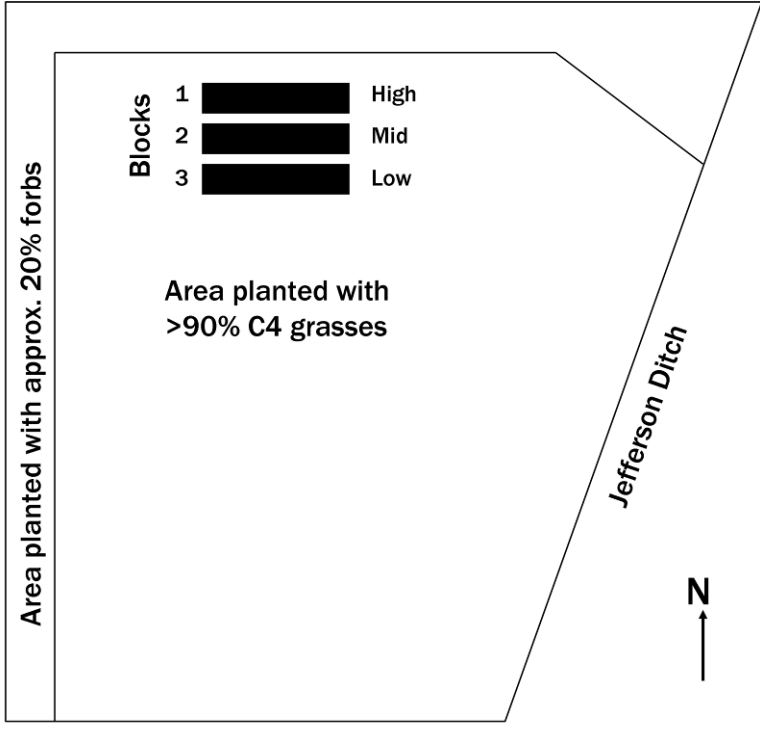
Figure 3. Mean forb seedlings of sown species and SEs by treatment. Seeding was conducted on May 10 and monthly samples were conducted the 12th -15th of each month. Displayed data is untransformed.

Figure 4. Mean forb seedlings of sown species and SEs by block. Means and SEs displayed are from back-transformed data.

Figure 5. Weed species per 0.25 m² within interseeded areas in September. There were some mow plots in the highest block that contained clonal patches of *Solidago altissima*. It was difficult to tell resprouts from new seedlings after repeated mowing within these patches. These factors likely account for some of the interaction revealed here.

Figure 6. Seedlings per 0.25 m² by topographic position of unseeded buffer zones and interseeded treatments. Bars are one SE and data is back-transformed for display. Interseeded includes sown species and dominant two weed species per plot. Both samples were taken within two days of July 15.

SR 22



Area planted with approx. 20% forbs

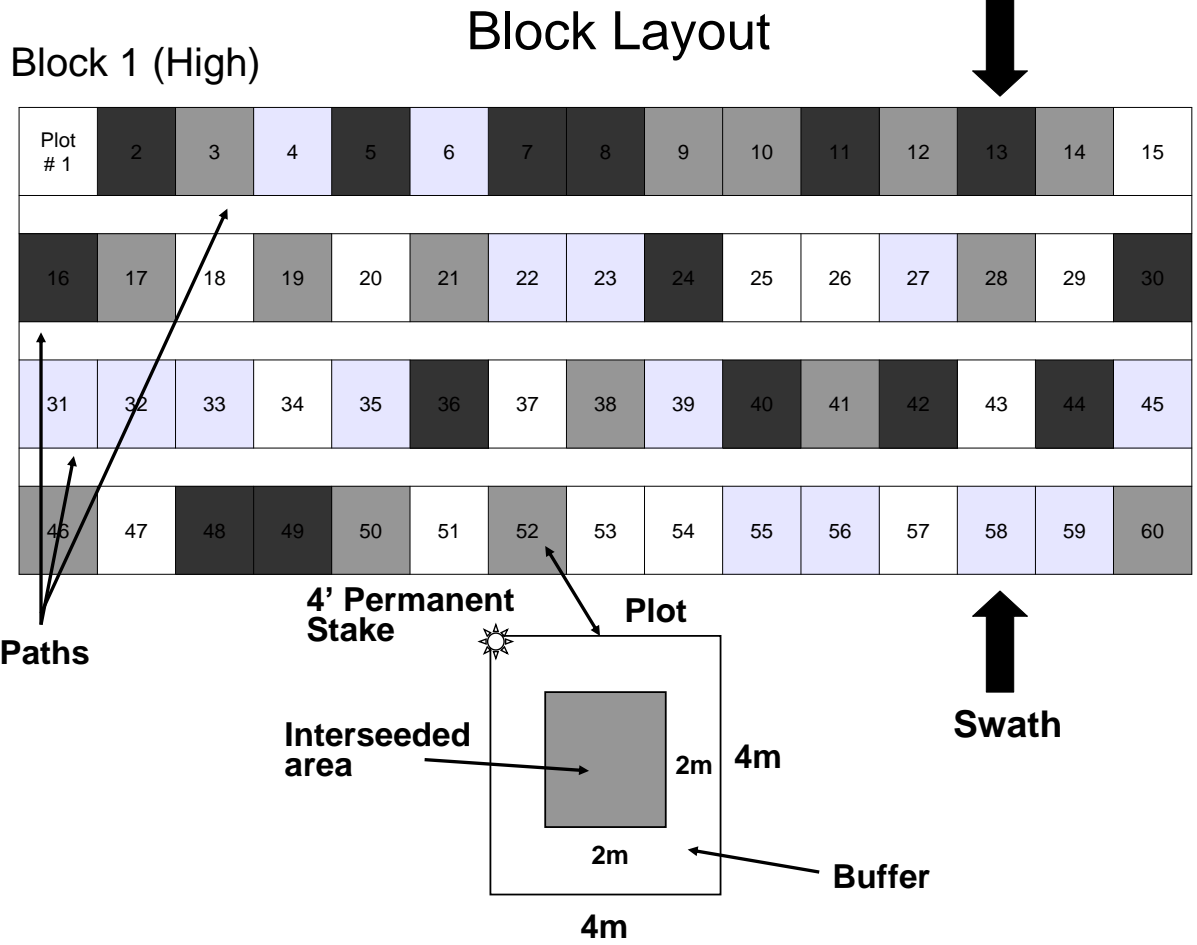
- Blocks
- 1 High
 - 2 Mid
 - 3 Low

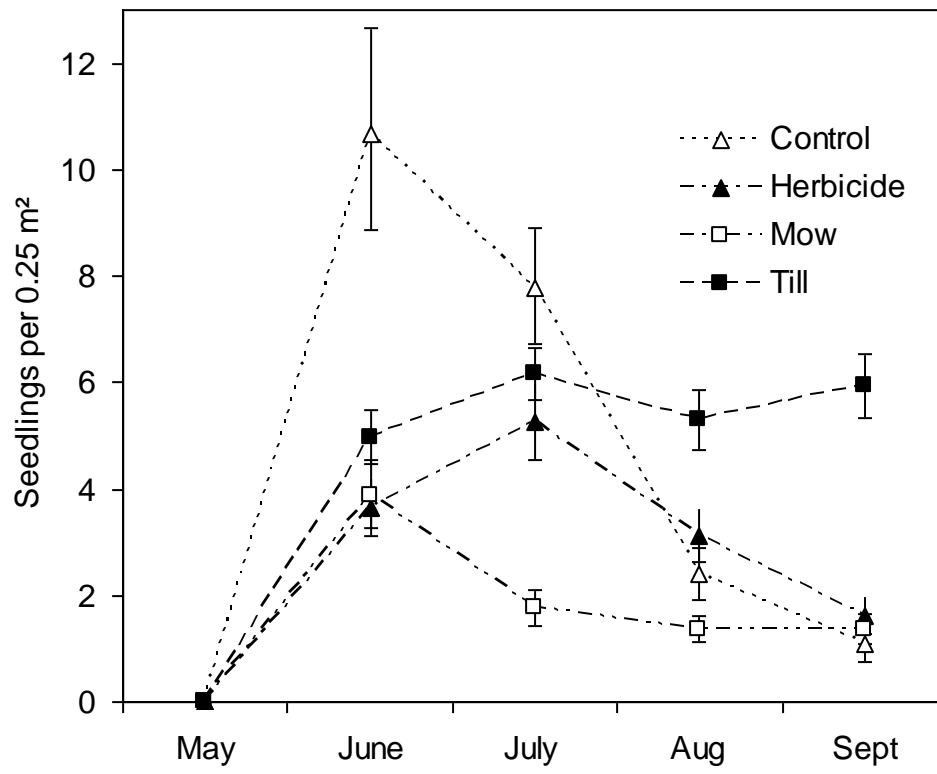
Area planted with >90% C4 grasses

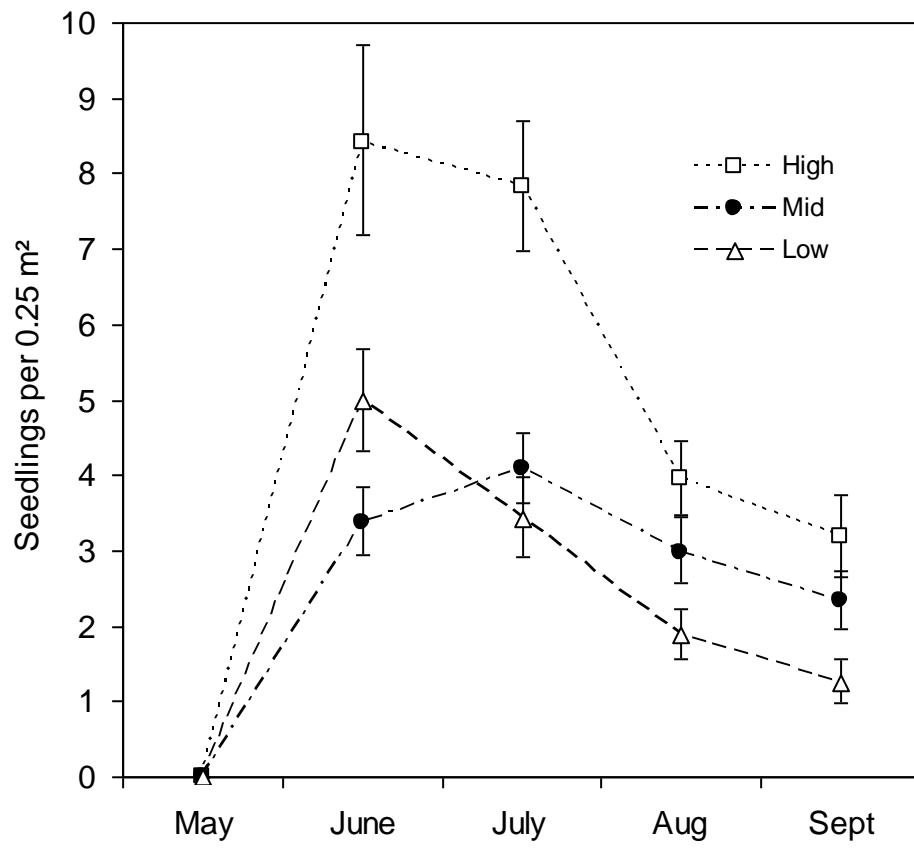
Jefferson Ditch



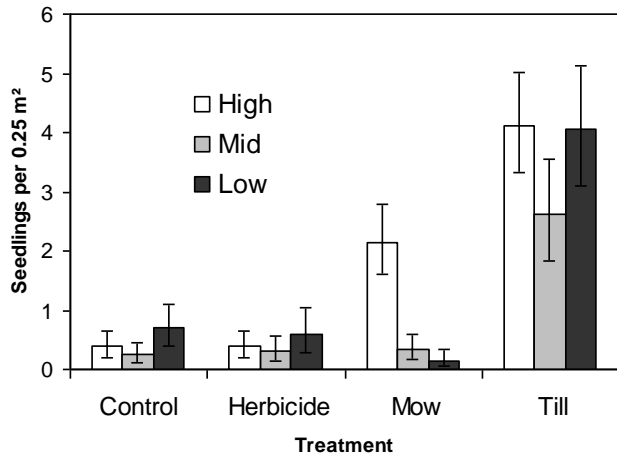
SR 26

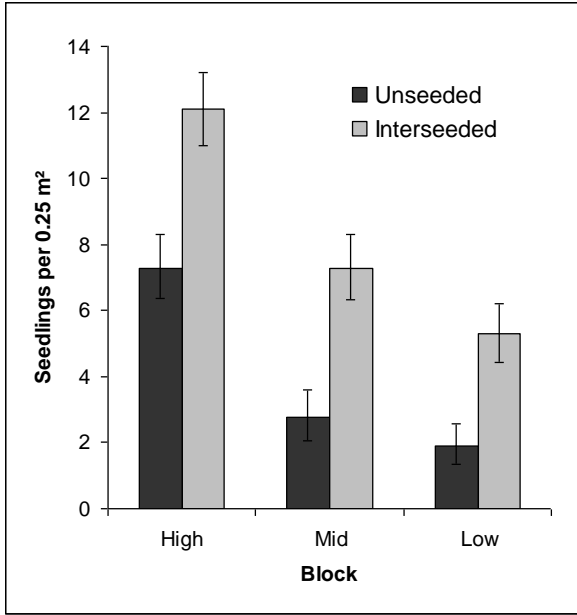






Interaction $p=0.019$





Appendix A. Weed species >8% of total individuals by month. All other weed species were present in very low numbers.

Species	June	July	August	September
<i>Hibiscus trionum</i>	62%	52%	37%	25%
<i>Convolvulus arvensis</i>	16%	14%	9%	9%
<i>Solidago altissima</i>	0%	8%	19%	19%

Appendix B. Tables and figures containing untransformed data.

Table 4. Numbers of seedlings \pm SE by treatment over the summer. Letters mark Tukey's HSD test results from square root transformed data (not displayed). Means with the same letters are not significantly different.

<i>Treatment</i>	<i>Mean Seedlings/0.25 m²</i>							
	<i>June</i>		<i>July</i>		<i>August</i>		<i>September</i>	
Total Sown Species								
Control	14.36 \pm 2.84	A	9.47 \pm 1.21	A	3.49 \pm 0.57	AB	2.31 \pm 0.58	A
Herbicide	4.56 \pm 0.55	B	6.38 \pm 0.81	B	4.04 \pm 0.54	A	2.51 \pm 0.39	A
Mow	5.04 \pm 1.09	B	2.42 \pm 0.37	C	1.87 \pm 0.24	B	2.0 \pm 0.29	A
Till	5.22 \pm 0.50	B	6.6 \pm 0.49	AB	5.91 \pm 0.60	C	6.62 \pm 0.73	B
Asteraceae								
Control					2.24 \pm 0.44	A	1.42 \pm 0.35	AB
Herbicide					2.44 \pm 0.38	A	1.78 \pm 0.33	AC
Mow					0.6 \pm 0.13	B	0.64 \pm 0.16	B
Till					2.33 \pm 0.28	A	2.64 \pm 0.41	C
Fabaceae								
Control					1.09 \pm 0.23	A	0.76 \pm 0.21	A
Herbicide					1.07 \pm 0.18	A	0.62 \pm 0.14	A
Mow					1.27 \pm 0.19	A	1.31 \pm 0.25	B
Till					3.16 \pm 0.37	B	3.4 \pm 0.32	C

Table 5. Untransformed means \pm SE for different plant families by block. Letters denote results of Tukey's HSD procedure, conducted on square-root transformed data (not displayed). Means with the same letters are not significantly different.

<i>Block</i>	<i>Mean Seedlings/0.25 m²</i>			
	<i>August</i>		<i>September</i>	
Asteraceae				
1 (High)	2.43 \pm 0.29	A	2.43 \pm 0.39	A
2 (Middle)	2.10 \pm 0.35	AB	1.68 \pm 0.25	AB
3 (Low)	1.18 \pm 0.22	B	0.75 \pm 0.16	C
Fabaceae				
1 (High)	1.93 \pm 0.26	†	1.70 \pm 0.26	†
2 (Middle)	1.57 \pm 0.24	†	1.48 \pm 0.22	†
3 (Low)	1.43 \pm 0.24	†	1.38 \pm 0.27	†

Figure 3. Untransformed

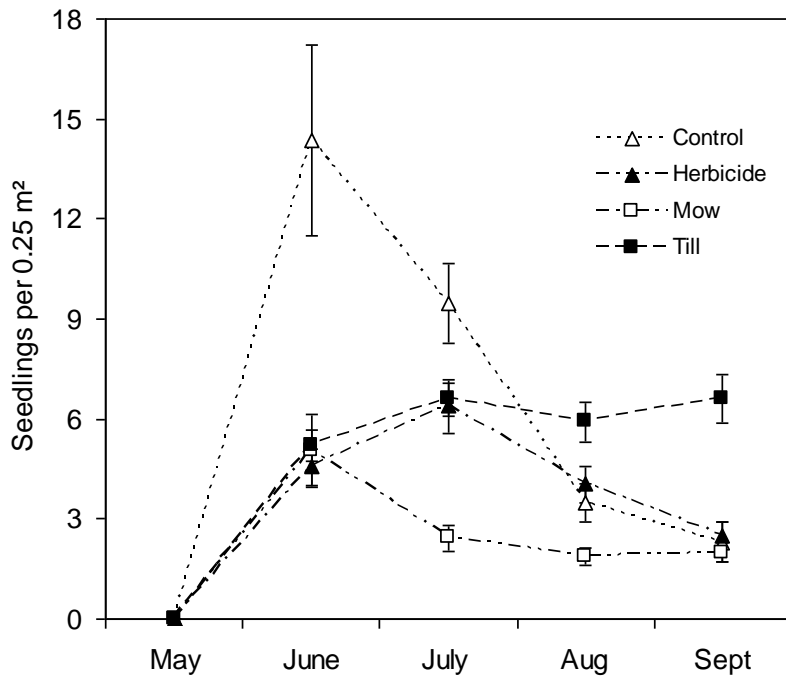


Figure 4. Untransformed

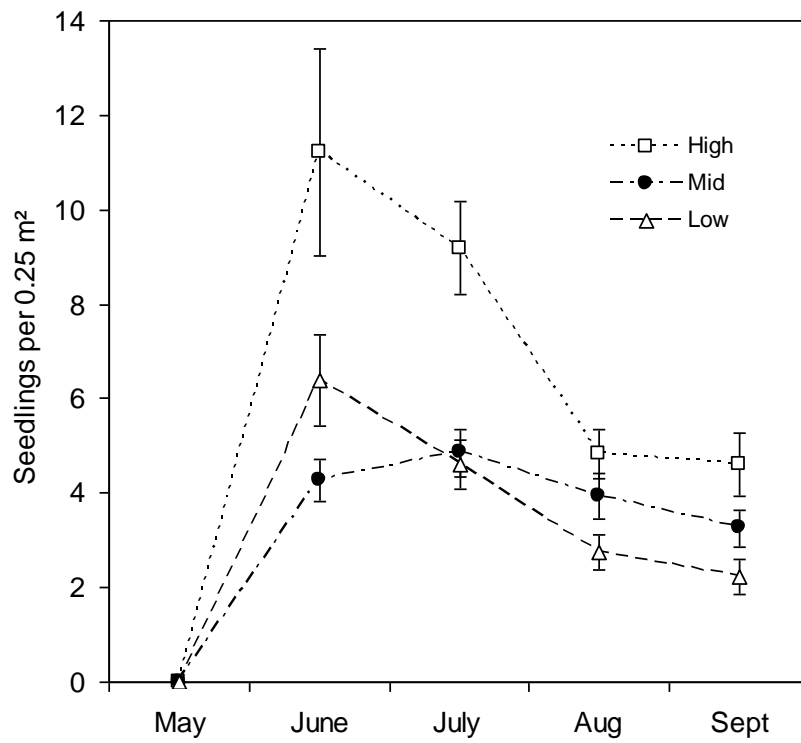


Figure 5. Untransformed

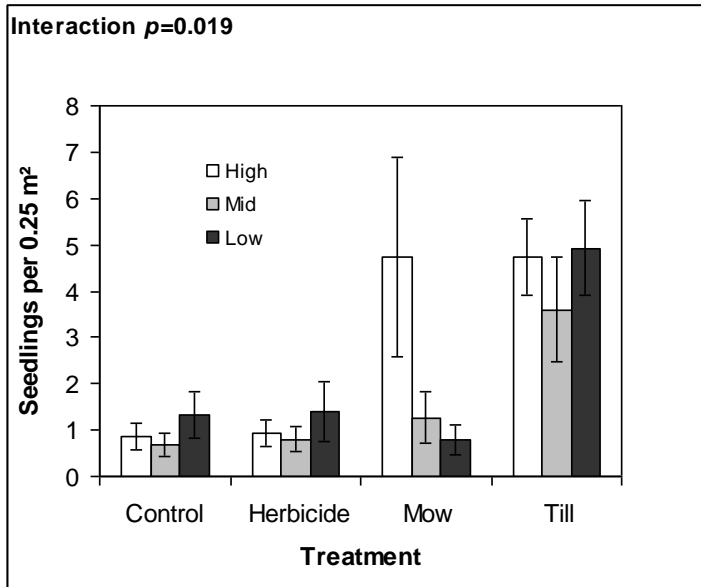
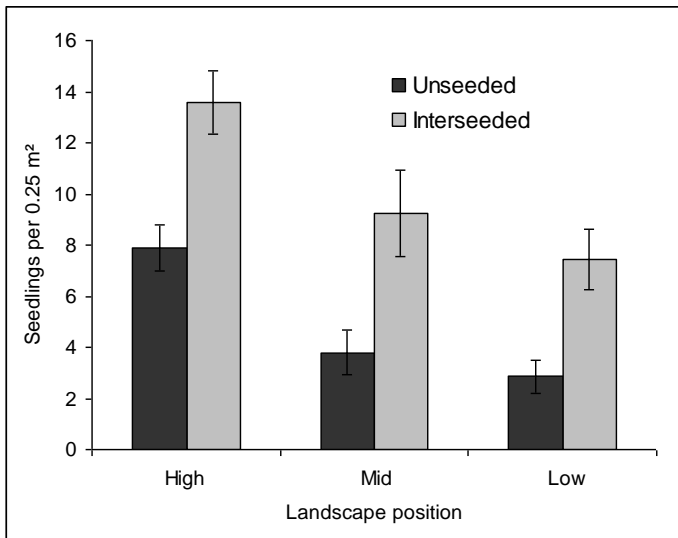


Figure 6. Untransformed



Appendix C. Explanation of data transformation/back-transformation procedure with example.

Issue: Transformed data is confusing when displayed. Transformed data must be returned to its original units to communicate effectively.

This transformation: On data that follow a Poisson distribution, such as count data, square-root transformation is typically the technique used. It is best for data that are either whole numbers over one (seedling counts) or else all decimals between 0 and 1 as taking the square-root effects these numbers differently. However, the principles discussed here also refer to other transformation methods, such as logarithmic transformation.

Back-transformation: Squaring the means derived from the test back to original units. Note that back-transformed means are similar to, but are not equal to untransformed means. These back-transformed means better represent the spread and character of the data that the statistical tests were run on and are therefore preferable to untransformed means.

Error bars: Error bars, whether using standard error, standard deviation, or confidence intervals require additional steps for back-transformation. Back-transforming SEs, SDs, or CIs that were generated on transformed data will not provide you the correct back-transformed error bars. Desired error bars must be calculated on transformed data, added to (for upper bar), and subtracted from (lower bar) the transformed means, then each column must be back-transformed (squared). Then the columns must be subtracted from the mean (lower), or the mean subtracted from the column (upper), giving you error bars. Note that the bars are not exactly even above and below the mean, this is to be expected. Example below using standard error:

Untransformed		Transformed				Back-transformed		
Mean	SE	Mean	SE	SE plus	SE min	Mean	SE plus	SE Min
11.22	2.18	2.90	0.22	3.12	2.68	8.40	9.71	7.18
4.28	0.45	1.84	0.12	1.96	1.71	3.38	3.85	2.94
6.38	0.96	2.23	0.15	2.39	2.08	4.98	5.69	4.31

(Mean subtracted from SE plus)

Upper Error Bar

1.31
0.47
0.71

(SE min subtracted from mean)

Lower Error Bar

1.22
0.44
0.66