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RESEARCH ARTICLE

Seedling Recruitment in the Long-Lived Perennial, *Echinacea angustifolia*: A 10-Year Experiment

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Abstract

Establishment of persistent plant populations may be restricted by limitations on the numbers of seeds, emergence of seedlings, or survival to reproductive maturity. The relative importance of these phases in establishment of new populations, particularly restorations, is poorly understood. In an experiment to quantify seedling emergence and juvenile survival of *Echinacea angustifolia* during its reintroduction to previously agricultural sites, we evaluated effects of two types of vegetation and prescribed burning at four times relative to sowing. We collected achenes from prairie remnants in western Minnesota, United States, and, each October 2000–2002, overseeded them into nearby study plots either in recently planted stands of native grasses or in oldfields abandoned 40 years earlier. For each cohort, we determined germinability of achenes in the laboratory and, in the field, monitored seedling emergence the following spring and subsequent survival in annual censuses through summer 2009. Germinability ranged from 20 to 37%, varying significantly among collection years. Seedlings emerged in every treatment combination, but emergence rarely exceeded 8% of achenes sown. Burns during the spring prior to sowing tended to enhance emergence, but to differing degrees depending on the year and vegetation. Burning in the spring after sowing reduced emergence. Burning enhanced juvenile survival in restored plots but not in oldfields. Strategies to reintroduce this species should include burning in the spring before sowing, sowing large quantities of seed, avoiding burning in the spring following sowing, and burning at least once within the first 6 years.

Key words: ***Echinacea angustifolia*, establishment limitation, fire, fragmentation, Minnesota, reintroduction, restoration, tallgrass prairie.**

Introduction

Seedling recruitment (including germination, emergence, and establishment) is critically important in dynamics of plant populations and in the establishment of new populations (Grubb 1977; Mulligan & Kirkman 2002). Recruitment may be limited by the number of seeds or by the availability of safe sites (reviewed in Turnbull et al. 2000). Survival of established seedlings to maturity is also important in population dynamics but is much less studied (Grubb 1977; Zobel & Kalamees 2005). It is unknown whether safe sites for seedling emergence also foster juvenile survival. Short-term seed introduction studies may identify regeneration niches that would not support establishment of a population that sustains itself.

The tallgrass prairie of North America, like many grasslands worldwide, is extremely fragmented; its preservation depends on managing nonpristine areas. Native prairie communities are being reconstructed on former crop fields, including land enrolled in the federal Conservation Reserve Program and public lands that have been planted with and are managed for native grasses but have few forbs. Reintroducing prairie plants to these sites poses a two-part challenge. First, even intensive intervention may not succeed in establishing populations (Turnbull et al. 2000). Though experiments documenting seed limitation suggest that seed addition alone might lead to establishment (reviewed in Turnbull et al. 1999; Foster & Tilman 2003), effects of habitat fragmentation on other organisms (Nickel et al. 2003; Perry & Galatowitsch 2004) and on fire regimes (Leach & Givnish 1996; Quintana-Ascencio et al. 2003) may have eliminated regeneration niches for some native plants. Second, the benefits of an intervention (e.g. drilling seeds or transplanting seedlings) in seedling establishment must be weighed against (1) its potential to harm existing vegetation and promote weeds (Lesica & Martin 2003) and (2) its costs compared with practices involving less intervention (e.g. broadcast seeding) but having lower success rates (Guerrant & Kaye 2007).

Extant vegetation may influence seedling recruitment through effects on resources and associated species (fungi and seed predators). Disturbances also affect regeneration. Prescribed fires are commonly used to reduce the abundance

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of invasive plants and promote growth and flowering of established, fire-adapted native plants (Bowles et al. 2003; Heisler et al. 2003). Fires reduce cover of live and dead vegetation and release nitrogen into the soil; both these effects can affect seedling recruitment (Suding & Goldberg 1999; Dzwonko & Gawronski 2002). For example, a prescribed spring burn may kill new seedlings but increase light availability for the next cohort. Fires were frequent in the tallgrass prairie of North America before European settlement (Trabaud et al. 1993). With the extreme fragmentation of the prairie ($<$1% remains in Minnesota) and active fire suppression, wildfires are now very rare (Collins & Wallace 1990). Leach and Givnish (1996) identified fire suppression as a major cause of species losses from remnant prairies in Wisconsin. The effect of fire on seedling recruitment is likely strong and probably depends on the type of vegetation and litter (Menges & Dolan 1998; Maret & Wilson 2005; Zimmerman et al. 2008) as well as timing.

Understanding the joint effects of prescribed burning and extant vegetation on recruitment, as well as its annual variation, can enhance the efficacy of overseeding as a component of management to increase the abundance of forbs in restored prairies. Here, we focus on *Echinacea angustifolia* (narrow-leaved purple coneflower; hereafter, *Echinacea*), a widespread plant characteristic of the tallgrass prairie and plains of North America (McGregor 1968). In western Minnesota, it persists in prairie remnants and is desired in restorations both for its conspicuous floral display and its use by threatened Lepidopterans as an oviposition site and nectar source (Dana 1991). We report on a 10-year experiment that quantifies seedling emergence of *Echinacea* in each of 3 years following overseeding into two vegetation types that are common targets for reintroduction. We tested the effect of year of prescribed burn relative to fall sowing. We assessed variability in recruitment among years with analyses that accounted for variation in germinability of seed lots, thus disentangling variation in viability of seed lots from annual variation in field conditions during germination. We also report juvenile survival over the 6 years following germination. Our experiment demonstrates that paucity of both seeds and safe sites poses important limitations on recruitment of *Echinacea* and informs management practice for restoration and reintroduction projects.

Methods

Study Species and Site

Echinacea angustifolia is an herbaceous long-lived perennial. In Kansas, its generation time has been estimated as 16–44 years (Hurlburt 1999). Plants in natural populations rarely flower before their seventh year and thereafter do not flower every year (personal observation). Spring burns promote summer flowering (Kuchenreuther 1996, personal observation). The plant is taprooted and does not spread vegetatively; thus, new plants arise solely from seeds, which are heavy (3–6 mg) and have no specialized mechanisms for dispersal. *Echinacea* seeds require specific conditions to break their over-winter dormancy. In particular, greenhouse studies indicate that light during germination and in pre-germination treatments helps break dormancy (Baskin et al. 1992; Macchia et al. 2001). *Echinacea* shares these characteristics with many other prairie plants used in restorations, and therefore serves as an appropriate model for detailed experimentation.

Echinacea's abundance and distribution are also typical of many prairie plants. Before European settlement, *Echinacea* was locally common with an extensive range from Canada to Texas and from the Mississippi River to the Rocky Mountains (Kindscher 1989). Today, natural populations are scattered and local populations often comprise fewer than 10 reproductive individuals (Wagenius 2006). This fragmentation of *Echinacea* habitat reduces its seed production, via both pollen limitation of seed set (Wagenius 2006; Wagenius et al. 2007; Wagenius & Lyon 2010) and severe inbreeding depression (Shaw et al. 2008; Wagenius et al. 2010).

The study sites in rural western Minnesota are centered near lat 45°49′N, long 95°42.5′W. The surrounding landscape was apparently suitable habitat for *Echinacea*, but the species now persists only in small prairie remnants. Most remnants are inaccessible to farm machinery or are otherwise unsuitable for agriculture. Near our sites, both prairie reconstructions and abandoned fields on public land far exceed the extent of remnant prairie. Many of these areas are targets for reintroduction of more native plant species to boost diversity and improve habitat for game and threatened species.

Experimental Design

The experiment occupied 10 study plots in sites that are candidates for reintroduction of native species. All tracts were agricultural fields, with production ceasing during 1960–1962. After release from agriculture, fields were planted with *Bromus inermis*, and thistles were controlled sporadically with herbicides. We positioned each experimental plot in a tract of land ($>$3 ha) that appeared to be potential habitat for *Echinacea* based on hydrology and plant species composition, yet had no *Echinacea* currently growing. The distance from each plot to the closest remnant *Echinacea* population ranged from 100 to 1000 m. Nine of the plots are on land owned by the State of Minnesota and managed by the state's Department of Natural Resources (DNR). Management on these areas promotes wildlife and suppresses weeds. The 10th plot, which is privately owned, adjoins state land. No pair of plots was separated by greater than 6 km.

The study plots were located in fields of two types, 'oldfields' as described above and oldfields into which native grasses were planted ('restored fields') in the following way. In the first year (1998 or 1999), a burn in May was followed by herbicide application. Then, seeds of native grasses (*Andropogon gerardii*, *Sorghastrum nutans*, *Stipa viridis*, *Elymus canadensis*, *Bouteloua curtipendula*, and *Panicum virgatum*) were drilled. The field was mowed each of the first 2 years, and the herbicide glyphosate was applied in October of the second year (when native grasses were dormant) to reduce

B. inermis. Prior to our experimental burning treatments, oldfields had more duff than restored fields. Total plant species richness per plot ranged from 18 to 59, with each plot containing at least a few desirable native species.

We established the 10 plots in fall 2000: 6 in oldfields and 4 in restored fields. Each plot consisted of six square units separated and surrounded by a 2-m buffer (Fig. 1). Each unit contained nine randomly placed, non-overlapping circular recruitment zones. In each unit, three zones were randomly assigned to each of 3 years of sowing: 2000, 2001, and 2002. In October, each of the appropriate recruitment zones received 100 achenes (fruits collected as described below) scattered evenly by hand on the surface of the litter. In late June of the following year, two observers searched each recruitment zone for seedlings and counted all present.

Two units in each plot were randomly assigned to each of the following burn treatments: burn in spring 2001, burn in spring 2002, or no burn. We mowed the appropriate buffer areas during the fall before a burn to establish fire breaks. Burns were conducted as scheduled with two accidental exceptions. First, in spring 2002, no burns occurred in one plot in a restored field. Second, in spring 2003, all units of two plots in restored fields were burned. As a result, 24 of the zones sown in 2002 were burned both before and after sowing; these 24 zones were excluded from the emergence analysis. Thus, the final design was not balanced. Additional spring burn treatments were applied in 2005, 2008, and 2009, such that experimental units experienced no burns, or were burned at least once on approximately 3- or 6-year intervals. All burn treatments, including inadvertent burns, occurred at a time consistent with the DNR manager's overall management plan (May 14–21). We were unable to count seedlings in the privately owned plot (an oldfield) after 2001 because the land was being prepared for development. This plot was excluded from the survival analysis. Thus, the unburned treatment in restored fields was restricted to 4 units in one experimental plot, compared with 20 units in four plots for burned restored fields, 12 units in three plots for unburned oldfields, and 18 units in five plots for burned oldfields.

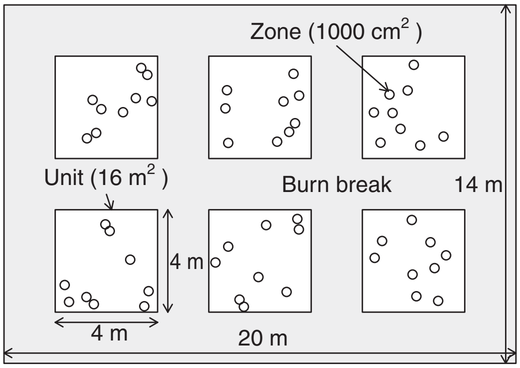

Figure 1. Layout of one of the 10 plots. Each plot contains six square units. Each unit contains nine circular zones in nonoverlapping random locations. A buffer (2-m wide) surrounds all units. Before conducting prescribed burns, we mowed parts of the buffer to serve as a burn break.

During September of each planting year when seedheads appeared ripe, we harvested seedheads from a local remnant *Echinacea* population, cleaned and mixed the achenes, and then filled envelopes, each with 100 achenes. A random sample of 15 envelopes was set aside for trials to assess germinability in the laboratory (see below). Remaining envelopes were randomly assigned to recruitment zones. In the first year of the study, we used two different seed lots, one collected in that year (2000) and the other in 1999. For each pair of units with the same treatment within the same plot, one unit was randomly assigned to be planted with the 1999 lot and the other with the 2000 lot. For the final 2 years of sowing, we sowed only achenes gathered that year.

Visual inspection usually cannot distinguish between a fertilized achene (filled with one seed) and an unfertilized (empty) achene. Previous studies on the reproductive biology of *Echinacea* in local remnants (Wagenius 2004, 2006) indicated that seed lots are likely to differ in their proportion of achenes having a seed. Laboratory trials to assess germinability of each seed lot were conducted synchronously with germination in the field. We spread seeds over moist filter paper in petri dishes and exposed them to defined light and temperature conditions according to a standard germination protocol (Feghahati & Reese 1994; as modified in Wagenius 2004) intended to maximize germination rates, rather than to mimic germination conditions in the field. We recorded the number of seeds germinating from each envelope.

Annual censuses of each recruitment zone continued each June through summer 2009. Teams of two researchers searched each zone and recorded the number of *Echinacea* plants found. No seedlings (plants bearing cotyledons) were found beyond the first spring after sowing. Three sources of observer errors affected counts. First, some counts were low because seedlings or juveniles were not seen. Juveniles with one leaf less than 2-cm tall and less than 5-mm wide were likely missed in some years, particularly in unburned oldfields where litter may be 10-cm deep. In 2009, searchers were provided with count data from 2008, and exceptional efforts were made to find all plants. Second, a *Solidago* or *Aster* seedling may occasionally have been misidentified as *Echinacea*. Third, juvenile *Echinacea* may have multiple rosettes of basal leaves. Distinguishing between these and multiple individuals occasionally proved difficult. In 2009, we performed molecular analyses on leaf tissue collected from adjacent rosettes for 8 of the 29 cases for which searchers noted uncertainty. DNA was extracted from dried leaf samples and nine microsatellite loci were amplified by PCR. In one case, this analysis suggested that a zone count should be decreased. In seven cases, the original count was supported. For the remaining 21 cases, the field searchers' count was retained.

Analysis

Emergence. We assessed the dependence of seedling emergence on burn treatment, vegetation type, and year of sowing. As is appropriate for a response variable recorded as counts, we assumed that emergence followed a Poisson distribution.

We used a Poisson family generalized linear model (GLM) with a logarithmic link function, implemented in the statistical package, R (R Development Core Team 2009). Our analysis accounted for the inherent germinability of each seed lot in quantifying emergence in the different environmental conditions in the field. We assigned a priori contrasts to the 'vegetation type' treatments such that the effects of the two field treatments on emergence ('oldfield' and 'restoration') were estimated as differences from germination in the laboratory, that is, treatment contrasts (R Development Core Team 2009). Burn treatments were named relative to sowing of seeds in fall, as follows: burned the spring (5 months) before seeds were sown ('before'), burned two springs (17 months) before sowing ('bef2'), burned the spring (7 months) following sowing ('after'), and no burn; only the latter treatment is represented in all years in both vegetation types. We excluded data from the plots that were inadvertently burned twice. The mean number of zones per combination of year sown, burn treatment, and vegetation type was 26 (range 6–60). The residual deviance of the full model greatly exceeded the residual degrees of freedom, indicating overdispersion; therefore, we used the quasipoisson family for the analysis. Estimates of seedling counts for each treatment combination were obtained from the simplest model found to adequately fit the data. In a supplementary analysis of the data from the single year of sowing, 2000, we evaluated effects of year of seed sampling (1999 vs. 2000) along with burn treatment and vegetation type. We also estimated the rate of emergence per germinable seed for each treatment combination using the delta method for ratios (Bolker 2008). As the numerator and denominator, we used model estimates of seedlings emerged in the field and of seedlings emerged in the laboratory, respectively.

Survival. We analyzed 6-year survival over all three seed cohorts combined. Owing to the challenges of finding and distinguishing *Echinacea* individuals (described above), plant counts in many zones showed an increase in one or more years as well as the expected decreases due to mortality. To accommodate these evident errors in the plant counts, we defined 'survival' as ending count subtracted from starting count, where starting count in each zone was the highest count recorded in the first 3 years of data collection in that zone. In analyses reported here, ending count was defined as the highest count 6 or more years after sowing. Thus, for the cohort sown in 2000, ending count was the highest observed in 2006, 2007, 2008, or 2009. For cohort 2001, ending count was the highest in 2007, 2008, or 2009. For cohort 2002, ending count was the highest in 2008 or 2009. Of 326 zones with a starting count ≥ 1, 10 zones had a higher ending count than starting count; these zones were excluded from the analysis. To evaluate the robustness of the inferences, we also performed analyses defining starting count as the highest count observed in any year and ending count as the highest count in year 6 or 7. Results from these analyses were very similar to the initial ones and are not included. In separate analyses, not shown, we investigated the distribution of erroneous plant counts and found no consistent association with treatments. Burn treatment levels, subsequent to the 2002 burn, were no burn, burned once, or burned at least twice. We used a binomial family GLM, implemented in R, with vegetation type, burn treatment, and year sown as predictors. As in the analysis of germination, the residuals indicated overdispersion; accordingly, the quasibinomial family was used for further analysis. As an alternative approach, we performed a mixed-effects GLM, using the R function glmer in package lme4, to account for the spatial clustering in the experimental design. Unit and plot were designated as random effects, whereas vegetation type, burn treatment, and year sown were fixed effects. Results from the mixed models were qualitatively similar to results from the GLM models; we here report only the results from the GLM using the quasibinomial.

Results

Emergence

Seedling emergence varied considerably among the sowing years, in part due to differences in germinability of achenes collected in different years. In the laboratory, a third or more of achenes collected in 1999, 2000, and 2001 germinated, whereas only about 20% of achenes collected in 2002 germinated (Fig. 2). In the field, emergence rarely exceeded 8% of achenes sown or 20% of germinable seeds sown (Table 2). The difference in germinability among seed lots was reflected in lower emergence for the cohort sown in 2002; the main effect of year sown was highly significant (Table 1). There was also a highly significant ($p < 0.0001$) interaction between year sown and vegetation type. Neither the three-way interaction nor the two-way interaction between year sown and burn treatment was significant ($p > 0.1$).

Germinability of the two seed lots sown in 2000 was directly compared in analysis of that subset of the data. Achenes collected in 2000 germinated with lower probability in the laboratory than those collected the previous year (Fig. 2). Correspondingly, emergence of achenes from 2000 was lower in the field plots; in that year, the main effect of collection year was highly significant ($p < 0.0003$), but its interactions with burn treatment and vegetation type were not.

The effect of the burn treatments depended on vegetation type, as reflected in a highly significant interaction between these two factors (Table 1). Seedling emergence was lower in plots burned after sowing compared with unburned plots (Fig. 2), especially in oldfields. Burning in spring before sowing enhanced emergence relative to the no-burn treatment. This was particularly true in 2001 for achenes sown into oldfields (Fig. 2b). In this instance, emergence exceeded 14% of achenes sown and 40% of germinable seeds sown (Table 2). The effect of this burn treatment was considerably more subtle in 2002, when overall germination percentage was lowest (Fig. 2c). The effect of burning two springs (17 months) before sowing was not distinguishable from burning one spring before sowing (Table 1).

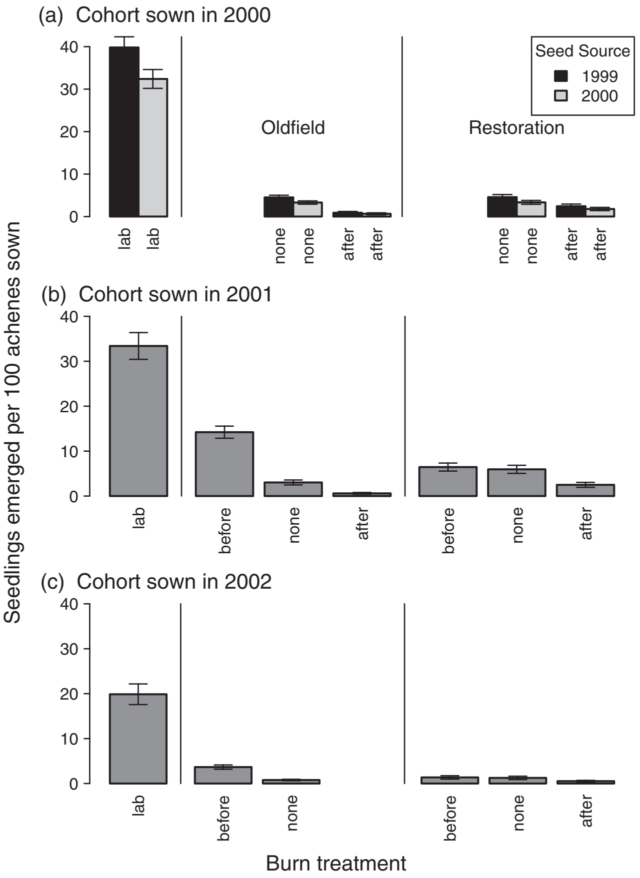

Figure 2. Estimated (±1 SE) number of seedling recruits from a sample of 100 *Echinacea angustifolia* achenes sown in 2000 (a), 2001 (b), and 2002 (c) into two vegetation types subjected to three burning treatments. Estimated counts were obtained from models containing main effects of year sown, vegetation type and burn treatment, and pairwise interactions between year sown and vegetation type, and between vegetation type and burn treatment. A separate analysis was performed on a subset of the data including only the seeds sown in 2000 (a). Estimated counts for the cohort sown in 2000 were obtained from a model containing main effects of seed source, vegetation type and burn treatment, as well as the two-way interaction between vegetation type and burn treatment. Burn timing is relative to sowing: 'before' = spring burn 5 or 17 months before sowing, 'none' = no burn, 'after' = spring burn 7 months after sowing. All achenes were sown 1 month after they were collected, except achenes collected in 1999 were sown 13 months after collection. The vegetation type labels refer to recent management ('oldfield' = no management or 'restoration' = planted with native grasses). Laboratory trials were conducted in the spring following autumn collection.

Table 1. Likelihood ratio tests for stepwise model simplification using backward elimination for the seedling emergence analysis.

Focal term (interaction or factor)	*df*	*Deviance*	*p*
Sown × vegm × burntrt	2	17.48	0.1076
Sown × burntrt	3	18.51	0.1979
Combine brntrt levels 'bef2' and 'bef'	2	0.55	0.9335
Sown × vegm	4	96.93	<0.0001
Vegm × brntrt	2	160.51	<0.0001
Vegm	1	11.96	0.1040
Sown	2	512.66	<0.0001
Burns	2	621.77	<0.0001

Deviance is the likelihood ratio test statistic. *p* values are for the *F* test of the null hypothesis that a model simplified by excluding the focal term is not significantly different from a model including the focal term. The full model included main effects of year sown (sown, three levels: 2000, 2001, and 2002), vegetation management (vegm, three levels: lab, oldfield, and restoration), burn treatment (brntrt, five levels: lab, bef2, before, none, and after), and all two- and three-way interactions. The burn treatment levels bef2 and before were combined, with no significant change in the model deviance. After model simplification, the minimal adequate model included main effects of sown, vegm, and brntrt (four levels) as well as the sown × vegm and vegm × brntrt interactions. Tests of main effects compared a model including main effects of all three factors and one pairwise interaction with a model excluding the main effect for the factor not involved in the interaction.

Table 2. Rate of seedling emergence for each germinable seed sown (±1 SE, delta method).

Vegetation	*Burn treatment*	*Cohort*	*Emergence per 100 germinable seeds (%)*
Oldfield	Before	2001	42.6 ± 5.5
Oldfield	Before	2002	18.4 ± 3.2
Oldfield	None	1999	11.3 ± 1.5
Oldfield	None	2000	10.2 ± 1.4
Oldfield	None	2001	9.1 ± 1.8
Oldfield	None	2002	3.9 ± 1.0
Oldfield	After	1999	2.2 ± 0.8
Oldfield	After	2000	2.0 ± 0.7
Oldfield	After	2001	1.8 ± 0.6
Restoration	Before	2001	19.3 ± 3.2
Restoration	Before	2002	6.8 ± 2.1
Restoration	None	1999	11.5 ± 1.7
Restoration	None	2000	10.3 ± 1.6
Restoration	None	2001	17.8 ± 3.1
Restoration	None	2002	6.3 ± 2.0
Restoration	After	1999	6.1 ± 1.3
Restoration	After	2000	5.5 ± 1.2
Restoration	After	2001	7.5 ± 1.8
Restoration	After	2002	2.6 ± 0.9

Estimates of germinable seeds with SEs for each cohort come from the laboratory germination trials. Estimates of seedlings emerged with SEs for each vegetation burn treatment combination are maximum likelihood estimates from the model described in Table 1.

Survival

Survival over 6 years was generally high, ranging from 47 to 66% except in unburned restored fields, where survival was less then 4%. Burn treatment and vegetation type interacted significantly (Table 3). Burning dramatically increased survival in restored fields; in contrast, survival decreased somewhat in burned relative to unburned oldfields (Fig. 3). Survival in fields burned once did not differ significantly from that in fields burned two or more times (Table 3). Experiment-wide, one plant flowered in 2007, three plants flowered in 2008, and 11 plants flowered in 2009 (two of these had also flowered in 2008). All 13 flowering plants were from cohort 2000 except for one of the plants that flowered in 2008. Two plants flowered for the first time in their seventh year, two in their eighth year, and nine in their ninth year. Thus, the study population had begun to contribute to its regeneration.

Table 3. Likelihood ratio tests for stepwise model simplification using backward elimination for the survival analysis.

Focal term (interaction or factor)	*df*	*Deviance*	*p*
Sown × vegm × burns	3	2.78	0.6962
Sown × vegm	2	0.69	0.8357
Sown × burns	4	3.62	0.7587
Sown	2	1.88	0.6142
Collapse burns to two levels	2	2.56	0.5122
Vegm × burns	1	75.1	<0.0001
Burns	1	3.15	0.2114
Vegm	1	9.77	0.0282

Deviance is the likelihood ratio test statistic. *p* values are for the *F* test of the null hypothesis that a model simplified by excluding the focal term is not significantly different from a model including the focal term. The full model included main effects of vegetation management (vegm), burn treatment (burns), and year sown (sown) as well as all two- and three-way interactions. The minimal adequate model included main effects of vegm and burns (collapsed from three to two burn levels) as well as the vegm × burns interaction.

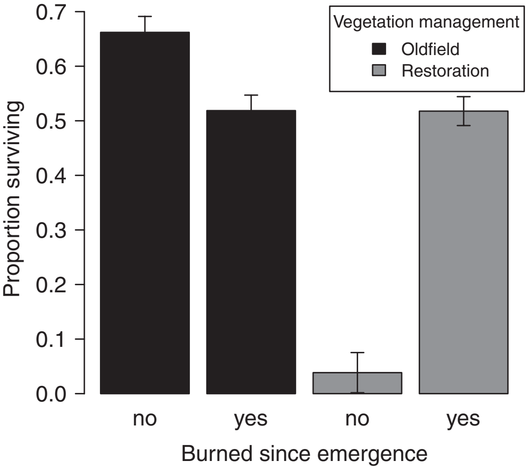

Figure 3. Estimated mean (±1 SE) proportion of *Echinacea angustifolia* survivors 5 years after initial seedling emergence in two vegetation types and exposed to two burn treatments. Proportions were predicted from a GLM including main effects of burn treatment and vegetation management and the interaction of burns and vegetation management. Seeds were sown into experimental plots in 2000, 2001, or 2002. 'Oldfields' were not otherwise managed; 'restored' fields were planted with native grass seeds in 1998 or 1999. Each experimental plot consisted of six units. During the 5 years following seedling emergence, units were not burned ('no') or burned one or more times ('yes').

Discussion

This experiment demonstrated the importance of both seed limitation and safe-site limitation in restricting recruitment of *Echinacea angustifolia* into suitable habitat in western Minnesota. At the outset, this species was absent from the experimental sites, though it grows in prairie remnants within 1 km. Addition of seeds resulted in seedling emergence both in sites recently planted with native tallgrass prairie grasses and in oldfields being considered for prairie restoration, demonstrating that these sites are typically subject to seed limitation. However, in all the cases, seed addition resulted in emergence of seedlings from only a small proportion of achenes sown, indicating that limitation on seedling establishment is also severe. Typically, five or fewer seedlings emerged per 100 achenes sown or less than 20% of germinable seeds. These findings reinforce the conclusions of Clark et al. (2007), Oster et al. (2009), and Uriarte et al. (2010) that limitations on both seed availability and opportunities for establishment of a seed once it arrives often play important roles in restricting recruitment.

Despite the strongly limited seedling emergence in this experiment, all the experimental factors (burn treatment, year of sowing, and vegetation type) demonstrated substantial effects on emergence. Burning in spring before seeds were sown in fall either enhanced recruitment or had little effect, depending on year and vegetation type. Additionally, in the absence of burning, emergence in oldfield vegetation was lower than that in areas recently planted to native grasses. A mechanism that could account for these findings is that burning of neighboring vegetation and litter increases light, soil temperature, and nutrient availability (Wan et al. 2001; Maret & Wilson 2005). This explanation seems likely because burning enhanced emergence especially in the oldfields that supported more standing pre-burn vegetation and litter (personal observation).

Burning in the spring following autumn sowing resulted in the lowest seedling emergence. This indicates that seeds or emerging seedlings did not tolerate burning. Similar results were obtained in a study on effects of burning on the grass *Aristida beyrichiana* in longleaf pine savanna of the southeastern United States. Mulligan and Kirkman (2002) found that burning during the growing season 1 year after germination dramatically reduced survival of the newest cohort of seedlings.

Sowing seeds in 3 different years from the collections of seeds in 4 years revealed considerable interannual variation in conditions that favor recruitment in the field, as predicted and regularly observed (e.g. Bowles et al. 2001; Morgan 2001; Dzwonko & Gawronski 2002; Dyer 2003; Forbis & Doak 2004). We emphasize the value of directly assessing the germinability of different seed lots and of accounting for differences in germinability directly in the analysis to distinguish the effects of differences among years in recruitment conditions from the effects of intrinsic differences among seed lots. We found considerable differences in germinability among the seed collections, with germination in laboratory trials highest in the 1999 seed lot and lowest in 2002. To a great extent, these differences likely results from variable pollination (Wagenius 2006).

Post-emergence survival was generally high and was enhanced by burn management. Survival of seedlings of six woodland species over 11 years in a study by Ehrlén et al. (2006) tended to be substantially lower. Our survival analysis was complicated by increases in counts of seedlings in many of the zones. Over all years of the study, we recorded slight increases in at least 40 of the 326 zones having at least one seedling. We attribute these anomalous increases primarily to errors in observation as described in the methods. We cannot absolutely rule out the possibility of some seeds sprouting the second or third year after sowing or dispersal of new seeds into the zones. However, while more than 1700 seedlings were identified in the first spring following sowing, we found zero new *Echinacea* seedlings during searches in years subsequent to initial emergence. *Echinacea* seedlings are readily distinguished from juveniles by their persistent cotyledons throughout the first several months following emergence.

We designed this experiment to mimic recruitment in natural populations except for two factors that represent real options for managers: timing of burns and vegetation types in available sites. Natural fires almost never occur in western Minnesota. Prescribed spring burns are increasingly commonly used, and their timing is constrained by weather. Summer and fall burns are less common. We chose vegetation types to represent the variety of local sites that are current and potential targets for reintroduction; these sites might also serve as sites for natural colonization from nearby remnants, but such events appear unlikely barring human intervention.

Our study system closely resembles Morgan's studies of *Rutidosis leptorrhynchoides* in its native Australia (Morgan 1995*b*, 1995*a*, 1997). As with *Echinacea*, this long-lived herbaceous member of the Asteraceae occupies grasslands that have largely been converted to agriculture and development. Thus, *Rutidosis* now occurs almost exclusively in lands not suited to these uses. Isolated plants tend to produce fewer seeds than those in extended populations (Morgan 1995*a*; cf. Wagenius 2004). Prescribed burning helps maintain this species. As in our study, Morgan (1995*a*) found that fewer than 10% of seeds produced emerge and establish. Our findings for *Echinacea* differ importantly from those for *R. leptorrhynchoides*, however. Whereas in our study, seedling survival over 6 years was typically about 50%, Morgan (1995*b*), following two cohorts of seedlings, found that only 13% survived for more than 14 months. Size of canopy gaps strongly influenced survival and growth of individuals (Morgan 1997). Similarly, burning promoted survival of *Echinacea*, provided it was delayed at least a year after emergence. Thus, gap-producing disturbances, such as fires, play an important role in facilitating establishment of individuals of *Echinacea* and *Rutidosis* and hence, their maintenance in native sites.

In a study of 17 grassland species in Sweden, Kiviniemi and Eriksson (1999) documented considerable variation in recruitment rates among species. Many rates fell within the range we found for *E. angustifolia* (see also Oster et al. 2009), but two hemiparasitic plants became established with considerably higher probability (i.e. 0.3). These authors also found that removal of vegetation significantly enhanced recruitment compared with undisturbed grasslands. In this study, we included only sites that were currently unoccupied by *Echinacea* populations and thus have no direct assessment of regeneration from seed within extant populations; studies on this process are under way. However, we have no reason to expect that rates of seedling recruitment are dramatically higher in remnant prairie sites than those documented here.

Implications for Practice

- Many *Echinacea* seeds must be sown to yield a few plants; seedling emergence varied around 5% of achenes sown and usually less than 10% of germinable seeds sown.
- The effect of burning on seedling recruitment depends on the extant vegetation type and promotes recruitment particularly in oldfields.
- Burning in spring, 5 or 17 months before autumn sowing can be used to enhance seedling recruitment, especially in oldfield vegetation.
- Burns should not be conducted in the spring after sowing in autumn to avoid damaging new seedlings.
- Sowing in two or more post-burn years can avoid recruitment failure due to a single unfavorable year.
- Once *Echinacea* seedlings emerge, their probability of survival is relatively high, estimated here as about 0.5 over 6 years. Burning enhances survival, particularly in restorations.

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