

Prairie forb response to timing of vole herbivory

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Abstract. The timing of herbivory can be an important factor in the strength and direction of plant response to herbivore damage. To determine the effect of vole herbivory timing within a growing season on tallgrass prairie forbs, we used individual plant enclosures to limit vole access to three species, *Desmanthus illinoensis*, *Echinacea purpurea*, and *Heliopsis helianthoides*, in an experimental restoration in northern Illinois, USA. As part of a long-term experiment, we implemented five vole access treatments in 2003: (1) vole access for the entire growing season, (2) early-season access, (3) mid-season access, (4) late-season access, and (5) no vole access. We protected all plants from herbivory in the following growing season (2004) to test whether the effects of herbivory in one growing season carried over to the next. We also tested how restoration planting design, including seeding time (June or December) and density (35 or 350 seeds/m² of each species) affected patterns of herbivory and plant recovery.

Vole access for the entire growing season was most detrimental for the growth and reproduction of all three species. In contrast, vole access for a portion of the growing season had different effects on the three species: *Desmanthus* growth and reproduction was negatively affected by early-season access, *Echinacea* reproductive output was reduced by late-season access, and *Heliopsis* was not affected by early-, mid-, or late-season vole access. Negative effects of continual vole access carried over to the following growing season for *Desmanthus* and *Heliopsis*, but not for *Echinacea*. Effects of herbivory did not carry over to the next season for *Echinacea* and *Heliopsis* when plants were accessible to voles for only part of the growing season. In contrast, *Desmanthus* plants exposed to early-season herbivory in one year continued to produce fewer seeds per plant after being protected from vole herbivory for a growing season.

Planting density and planting season had mixed effects. *Echinacea* and *Desmanthus* were larger in plots planted in June, showing that restoration design continued to affect plant population dynamics seven years after seeding. However, there was no interaction between plant response to vole herbivory and restoration design.

Key words: *Desmanthus illinoensis*; *Echinacea purpurea*; experimental restoration; *Heliopsis helianthoides*; herbivory; Illinois, USA; life history; *Microtus ochrogaster*; *Microtus pennsylvanicus*; tallgrass prairie; vole.

INTRODUCTION

Vertebrate herbivory shapes grassland plant communities (McNaughton 1985, Huntly 1991, Frank et al. 1998, Olf and Ritchie 1998). In North America, bison and probably elk herbivory increases plant diversity by reducing dominance of C₄ grasses in tall and mixed-grass prairies (Collins 1987, Howe 1999, Knapp et al. 1999, Towne et al. 2005). Extirpated in all but the largest prairie remnants, large vertebrate herbivores are no longer members of the naturally occurring complement of tallgrass herbivores (Noss et al. 1995). However, most remnant and restored prairies do host much smaller plant-eating vertebrates, including prairie and meadow voles (*Microtus ochrogaster* and *M. pennsylvanicus*), that often behave as potent “cryptic consumers” (sensu Keesing 2000) that can be major forces in grassland

systems. Voles feed on forbs and grasses in tallgrass prairie habitats, having important potential effects on vegetation when they reach population highs of 60–300+ individuals per hectare (Taitt and Krebs 1985, Howe and Lane 2004, Getz et al. 2006, Howe et al. 2006, Howe 2008). At high population densities, these small rodents sometimes have ungulate-level effects on aboveground biomass and species composition by cutting, if not consuming, tallgrass vegetation (Howe et al. 2002). In other cases they strongly influence species composition without obviously altering community productivity (Batzli and Pitelka 1970, Howe and Brown 2001, Howe and Lane 2004). An inescapable message is that 30–50 g rodents in large numbers can be a major force shaping grassland remnants and restorations.

A variety of general effects of vole herbivory in tallgrass vegetation are known. Voles have short- and long-term effects on community composition (Howe and Brown 1999, Howe and Lane 2004, Clay et al. 2005, Howe et al. 2006, Howe 2008), reduce biomass (Howe et al. 2002), alter intensity of competition between plants

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(Olofsson et al. 2002; see also Martínez-Garza et al. 2004), impact forb growth and reproduction (Zorn-Arnold et al. 2006), and alter the outcome of competition between plants (Clay and Holah 1999, Howe and Lane 2004, Olofsson et al. 2004; also Ostfeld et al. 1997). General effects are most apparent in restorations and other artificial grassland assemblages (Howe et al. 2002, Howe and Lane 2004) and have clearly directed the trajectory of these communities.

It matters when during a growing season vertebrates eat plants (Lortie and Aarssen 2000, García and Ehrlén 2002, Marshall et al. 2005, McKague and Cappuccino 2005). Late-season herbivory is most detrimental for survival, growth, and reproduction of some plant species (Michaud 1991, Obeso and Grubb 1994, Escarré et al. 1996), while for others early-season herbivory has the greatest effect (Knight 2003, Gustafsson 2004). For some species, timing can even change the sign of effects on plants: simulated herbivory on an annual legume (*Sesbania macrocarpa*, Fabaceae) late in the season results in overcompensation in components of reproduction, while early damage reduces them (Marshall et al. 2005). Differential effects of herbivory timing are likely related to resources available to plants when they are damaged and time remaining in the growing season; plants with access to necessary resources and ample time remaining before the end of the growing season are likely to compensate for and regrow lost tissue, while those that have little time and few resources do not (Augustine and McNaughton 1998, Anderson and Frank 2003). To date, plant response to temporal patterns of small mammal herbivory in grasslands is virtually unexplored.

Restoration practices and site characteristics can lead to different plant communities, even when planted species are the same (Hobbs and Norton 2004). Seeding time, seeding density, soil preparation, and soil type all play a role in creating the plant community that ultimately occupies an area. The plant communities created by different restoration practices result in different neighborhoods that present a well-recognized set of contingencies for both the herbivore and its forage. Neighborhoods represent the options that an herbivore has and often affect which plants it selects (Baraza et al. 2006). Such neighborhood effects are the basis of associational susceptibility, where a non-preferred plant near favored forage may be consumed by herbivores, and associational refuge, where palatable plants mixed among non-preferred plants are avoided by herbivores (Hambäck et al. 2000, White and Whitham 2000). In addition, the matrix in which a plant grows has the potential to affect its response to herbivory. Plants in a more competitive matrix are less likely to recover from herbivory than are those in a less competitive matrix (Machinski and Whitham 1989). It follows that restoration design and implementation could create neighborhoods that affect patterns of herbivory and plant

recovery, leading to interaction of neighborhood and timing effects.

To understand the role of timing in tallgrass forb response to herbivory, we used individual plant enclosures to test how vole herbivory at different times of the growing season affects three prominent species from two of the largest prairie forb families: from Fabaceae, *Desmanthus illinoensis*, and from Asteraceae, *Echinacea purpurea* and *Heliopsis helianthoides*. Here we ask whether (1) focal plant species experience different patterns of vole herbivory, (2) timing of vole herbivory affects forb reproductive and vegetative attributes, and (3) the plant communities resulting from restoration design (seeding time and density) affect patterns of vole herbivory and plant recovery from herbivory.

METHODS

Study site

This study was conducted within a larger tallgrass prairie experimental restoration established in 1997 at the Morton Arboretum in Lisle, Illinois, USA. The restoration consisted of 96 7×7 m plots that were seeded with 18 prairie species. Half of the plots were seeded at low (35 seeds of each species/m²) and half at high (350 seeds of each species/m²) density. Half of the plots were seeded in June and half in December, fully crossed with seeding density. Plots were delineated by hardware cloth fences 1.23 m high and 0.6 m deep, and small mammals were allowed access to half of the plots through gates cut in the fences at ground level. Only plots with small-mammal access were used for the current study.

Overall plant density and biomass measurements within these restoration plots illustrate community-level differences resulting from the planting design and give context for the current experiment. When the treatments in this study were applied in 2003, total plant density within the restoration was higher in plots seeded in June than in December (94.5 ± 7.3 and 78.7 ± 6.2 plants/m², respectively; all data are mean \pm SE), though total biomass was lower in the June than the December-seeded plots (77.3 ± 4.9 and 113.8 ± 7.0 g/m², respectively; H. F. Howe and J. S. Brown, *unpublished data*). Plant density was higher in plots seeded at a low density (85.5 ± 5.4 plants/m²) and lower in plots seeded at high density (65.9 ± 4.2 plants/m²). Total biomass was similar for plots seeded at low and high density (122.4 ± 8.0 and 118.2 ± 6.5 g/m², respectively).

Focal forbs

We chose focal species that were common in the restoration experiment and widespread in the tallgrass prairie region. The three species selected are from two of the most common tallgrass prairie forb groups: legumes and composites (Great Plains Flora Association 1986). We knew from prior studies in an experimental tallgrass prairie restoration that vole herbivory reduced *Desmanthus* and increased *Echinacea* size and numbers but had

TABLE 1. Number of plots containing at least five individuals for the three forb species according to restoration seeding time (December and June) and seedling density (high and low).

Species	December		June		Total plots
	High	Low	High	Low	
<i>Desmanthus</i>	4	4	7	8	23
<i>Echinacea</i>	12	12	10	12	46
<i>Heliopsis</i>	11	12	11	12	46

Notes: Total possible plots are 48 for each species. High density was 350 seeds/m², and low density was 35 seeds/m².

little effect on *Heliopsis* (Howe et al. 2002). The wide range of response of these three species to herbivory makes them a good starting point for mapping forb response to vole herbivory timing.

Desmanthus illinoensis (Michx.) MacM. (Mimosaceae) is a long-lived perennial, nitrogen-fixing legume found throughout the tallgrass prairie region of North America. *Desmanthus* is cultivated in some areas as forage for livestock. *Echinacea purpurea* L. Moench. (Asteraceae) is a long-lived perennial that occurs widely throughout the tallgrass prairie region and is often used as an ornamental. *Echinacea* is the only one of the three focal species that has a rosette of basal leaves. *Heliopsis helianthoides* (L.) Sweet (Asteraceae) is a widespread perennial that is often grown as an ornamental (Great Plains Flora Association 1986). Voles generally prefer *Echinacea* achenes and avoid *Desmanthus* seeds and *Heliopsis* achenes (Turner 2005).

Herbivore species

Meadow voles (*Microtus pennsylvanicus*) and prairie voles (*Microtus ochrogaster*) are both present at the study site. They clip stalks of plants at ~10 cm height and often clip basal leaves as well. Clipped stems may or may not be eaten. Whether or not voles consume clipped stems, these stems represent lost biomass, photosynthetic area, and reproductive potential of the affected shoot. The vole population during access treatments in 2003 was about 140 known individuals per hectare (128 in a June census, 157 in September), 47% of which were prairie voles (Howe et al. 2006).

Individual exclosures

Vole access to individuals of the three focal forb species was controlled during the growing season. Hardware cloth cylinders (60 cm high, 10 cm deep, 30 cm diameter) were placed around five individuals of each forb species in each small-mammal-access plot in summer 2002. Plots containing fewer than five individuals of a species were not used for that species. The number of plots with at least five individuals of each species by seeding time and by planting density differs among the three focal species (Table 1).

Within each plot, the five cylinders for each species were randomly assigned to one of five treatments: (1) all-

season vole access, (2) early-season vole access (first seven weeks of the growing season), (3) mid-season vole access (second seven weeks of the growing season), (4) late-season vole access (third seven weeks of the growing season), and (5) no vole access. Access was controlled by opening or blocking 5 × 5 cm gates at the base of the cylinders in 2003. Seven-week intervals for treatments corresponded roughly to phenologic stages of the species. Early-season access targeted emergence of the plant and flower-bud formation. Flowering occurred in the mid-season access treatment; fruits ripened during the late-season access treatment. Each of the three species emerges and flowers at different times, and treatments commenced for each species when emergence began. Accordingly, treatments for *Echinacea* were started two weeks before treatments for *Heliopsis*, which were started two weeks before treatments for *Desmanthus*. Cylinders were weeded periodically to prevent weedy species such as clover (*Trifolium* spp.), which are aggressive in the absence of voles, from taking over the cylinders.

At the end of each treatment, total number of stems and number of clipped stems were counted. At the end of the growing season as plants were senescing, all plants were clipped at the base and dried to constant mass. Plants were separated into stems, leaves, and fruits/seed heads and weighed. All fruits/seed heads on each plant were counted. To estimate seed production per plant, the fruits/seed heads were subsampled. For *Desmanthus*, five infructescences were haphazardly chosen and the number of pods determined. Then seeds in five pods from each infructescence were counted. For *Echinacea* and *Heliopsis*, three seed heads were haphazardly chosen and achenes were counted. The mean number of achenes per seed head was multiplied by the number of seedheads per plant to estimate achenes per plant. A sample of ~250 seeds/achenes for each individual plant of the three species were counted using a seed counter (Pfeuffer Contador) and weighed to the nearest milligram. Individual seed/achene mass was determined by dividing achene mass by number.

Analysis

To determine the effect of restoration factors and forb species on vole herbivory patterns, we used a repeated-measures ANOVA with forb species, seeding time, and seeding density as independent variables and stem herbivory intensity (number of stems clipped/total number of stems per plant) in early, mid, and late season as repeated variables. We used another repeated-measures ANOVA to determine whether there are differences in vole clipping of *Echinacea* basal leaves and stems during the growing season. Post hoc Tukey pairwise comparisons were used to determine differences in vole use of the species in each portion of the growing season, as well as changes in vole use of a single species over the course of the growing season.

We tested whether seeding density, seeding time, or forb species affected mortality using counts of present and absent individuals and chi-square tests. *Heliopsis* experienced high mortality overall, and we used a chi-square to test whether mortality was affected by vole access treatment.

Plant response to herbivory was assessed with a variety of potentially interacting variables. Above-ground biomass at the end of the growing season (hereafter biomass) estimates relative resources accumulated and general vigor within a population. The ratio of reproductive to vegetative biomass (hereafter reproductive:vegetative biomass) reflects allocation to reproduction in seeds and fruits as contrasted with production of shoots and stems. Number of seeds per plant estimates individual fecundity as the most commonly used surrogate for potential fitness. Mean mass per seed is a potential fitness measure if seed size influences seedling size and viability (Leishman et al. 2000). The response variables were transformed to meet the assumptions of normality: masses were log transformed, seed numbers were square-root transformed, and reproductive:vegetative biomass was arcsine square-root transformed.

We used repeated-measures ANOVAs to determine the effect of vole access treatment and restoration seeding time and density on focal plant growth, both in the growing season in which treatments were applied and in the following growing season when plants were protected from vole access. Because the design of this experiment is partially hierarchical, the mean sum of squares for plot nested within density and season was used as the error for density, season, and density by season interaction effects. Initial analyses showed, as expected, that the three species were affected differently by vole herbivory. These species were therefore analyzed separately. Plants that survived through 2004 were included in the analysis. A separate repeated-measures ANOVA was performed for each response variable, for a total of four for each species. Bonferroni adjustments minimize experiment-wise error rate for each forb species and provide conservative estimates of statistical significance for the four repeated-measures analyses. Reported significance values are Bonferroni adjusted; an uncorrected value of $P < 0.0125$ is reported as a corrected experiment-wise error rate of $P < 0.05$ and $P < 0.0025$ corrected as $P < 0.01$. Post hoc tests (Hotelling's T^2) tested for differences between treatment means when univariate ANOVAs showed that treatment had a significant impact on the response variables. Data are presented with untransformed means and standard errors for visual clarity. All analyses were performed using SYSTAT 12 (Systat Software, Chicago, Illinois, USA).

RESULTS

Effects of herbivory on focal forbs

Vole herbivory patterns varied between the three plant species; *Desmanthus* experienced the highest total

clipping intensity (number of clipped stems/total number of stems; 0.63 ± 0.08 ; all data are mean \pm SE), followed by *Echinacea* (0.37 ± 0.04), and *Heliopsis* (0.24 ± 0.05) (repeated-measures ANOVA $F_{2,413} = 11.016$, $P < 0.001$; P values are Bonferroni corrected throughout). Vole use of plant species changed over the growing season (repeated-measures ANOVA $F_{4,826} = 6.951$, $P < 0.001$). *Desmanthus* experienced higher clipping intensity in the early and mid growing season relative to the other species (post hoc Tukey tests, $P < 0.001$) and was clipped most early in the growing season, though not significantly so (Fig. 1a). Clipping intensity of *Heliopsis* stems was relatively constant through the growing season (Fig. 1b). Voles used *Echinacea* stems and basal leaves differently over the course of the growing season (repeated-measures ANOVA $F_{2,836} = 34.498$, $P < 0.001$; Fig. 1c, d). Basal leaves were harvested at a relatively constant rate through the growing season, while *Echinacea* stems experienced a dramatic increase in clipping intensity late in the growing season (post hoc Tukey test, $P < 0.001$). Late season *Echinacea* clipping intensity was significantly greater than that of late-season *Heliopsis* clipping (post hoc Tukey test, $P < 0.001$), though not different from late-season *Desmanthus* clipping.

Restoration seeding time also affected the pattern of herbivory over the course of the growing season (repeated-measures ANOVA $F_{2,826} = 5.746$, $P < 0.01$). Plants growing in plots seeded in June experienced relatively constant clipping intensity (0.15 ± 0.01 early, 0.10 ± 0.02 mid, and 0.16 ± 0.02 late) while clipping intensity was more than three times as high in the late than early or mid growing season in December-seeded plots (0.07 ± 0.02 early, 0.08 ± 0.02 mid, and 0.25 ± 0.03 late).

Heliopsis experienced unexpectedly high mortality over the course of this study. While $<2\%$ of *Desmanthus* (1 of 115 plants) and *Echinacea* plants (4 of 230 plants) died, $\sim 50\%$ of all *Heliopsis* (119 of 230 plants) in the study died ($\chi^2 = 206.371$, $P < 0.001$). The highest mortality was in the all-season access treatment where almost three times as many *Heliopsis* individuals died than survived. *Heliopsis* survival and mortality in other access treatments was roughly equal ($\chi^2 = 13.329$, $P < 0.05$; Table 2). *Heliopsis* mortality was unaffected by planting time or planting density.

All-season and early-season access resulted in significantly lower *Desmanthus* seed numbers, both in the growing season in which voles had access and in the subsequent season when they were protected (Fig. 2; $F_{4,76} = 13.2$, $P < 0.001$). All-season and early-season access also resulted in lower biomass, reproductive:vegetative biomass, and seed mass in 2003 (treatment by year interaction, repeated-measures ANOVA $F_{4,76} = 17.9$, $F_{4,76} = 13.5$, and $F_{4,76} = 18.5$, respectively, all $P < 0.001$; Fig. 3, Table 3). The effects of early-season access did not carry over to 2004, but all-season access treatments did.

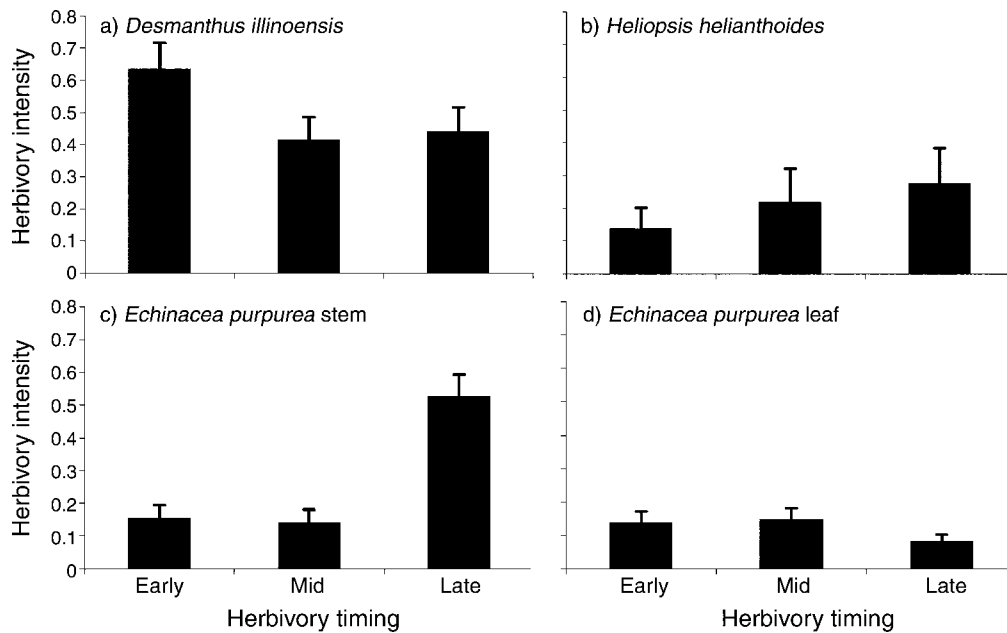


FIG. 1. Vole herbivory patterns for (a) *Desmanthus*, (b) *Heliopsis*, (c) *Echinacea* stems, and (d) *Echinacea* leaves. Herbivory intensity (mean + SE) is the proportion of stems clipped to total stems. Post hoc Tukey pairwise comparisons tested for differences between all species and herbivory timing. *Desmanthus* was clipped more intensively than the other two species in the early and mid portions of the growing season (Tukey test, $P < 0.001$). *Echinacea* stems were clipped significantly more than *Echinacea* basal leaves and *Heliopsis* stems late in the growing season (Tukey test, $P < 0.001$).

Vole access affected the ratio of reproductive:vegetative biomass and the number of achenes per plant for *Echinacea* in 2003, with no carryover to 2004 (treatment by year interaction, repeated-measures ANOVA $F_{4,156} = 5.2$ and $F_{4,156} = 5.3$, respectively, both $P < 0.01$; Fig. 4, Table 4). *Heliopsis* biomass was significantly lower where voles had access for the entire growing season in both 2003 and 2004 (0.9 ± 0.6 g dry mass for all-season access, 12.2 ± 2.7 g mean for the other treatments; repeated-measures ANOVA $F_{4,67} = 3.48$, $P < 0.05$).

Effects of restoration design

Seeding time affected all *Echinacea* response variables. *Echinacea* plants in June-seeded plots were larger (40.3 ± 4.0 g dry mass in June vs. 14.3 ± 1 g dry mass in December; repeated-measures ANOVA $F_{1,156} = 18.6$, $P < 0.001$), had higher reproductive:vegetative biomass (0.15 ± 0.01 g dry mass in June vs. 0.11 ± 0.01 g dry mass in December; $F_{1,156} = 17.0$, $P < 0.001$), produced

more achenes per plant (1151 ± 129 in June vs. 325 ± 43 in December; $F_{1,156} = 26.9$, $P < 0.001$), and had heavier individual achenes (1.3 ± 0.08 mg in June vs. 0.83 ± 0.07 mg in December; $F_{1,156} = 10.0$, $P < 0.01$; Table 4) than plots seeded in December. *Desmanthus* biomass was also higher in June- than December-seeded plots (130 ± 13.7 g and 114.8 ± 20.3 g, respectively; $F_{1,76} = 7.5$, $P < 0.05$; Table 3). Seeding density had no effect on the size and reproductive output of the three forb species.

TABLE 2. Number of *Heliopsis* individuals present and absent in each vole access treatment after treatments in 2003.

Treatment	Present	Absent
All-season access	12	34
Early-season access	27	19
Mid-season access	22	24
Late-season access	27	19
No access	23	23

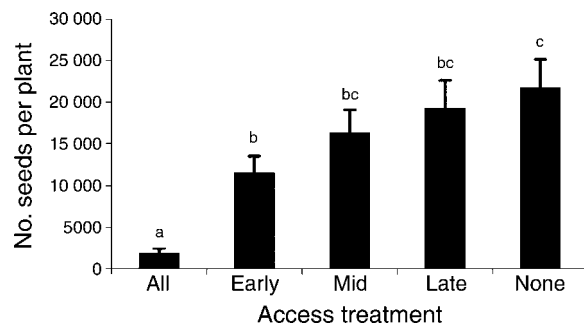


FIG. 2. *Desmanthus* seed production in relation to timing of vole access. Seed production continued to be lower where voles had continual and early-season access in 2003 even after being protected from vole herbivory in 2004. The number of seeds shown for each treatment is the mean of 2003 and 2004; bars represent standard error. Different letters indicate significant differences in access treatment means from repeated-measures ANOVA (Hotelling's T^2 , $P < 0.05$).

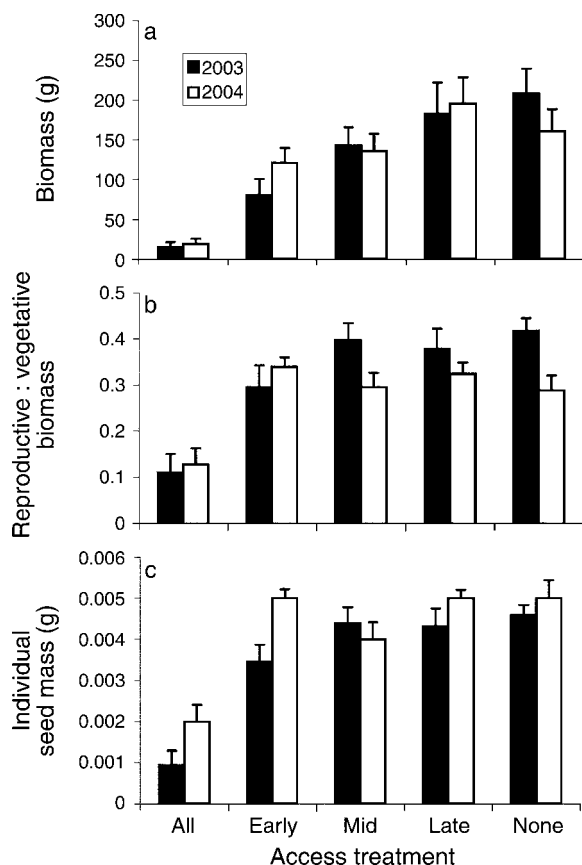


FIG. 3. Effects of continual vole access through the growing season on *Desmanthus* (a) biomass, (b) reproductive : vegetative biomass, and (c) individual seed mass in 2003, carried to 2004 when plants were protected from voles. Bars represent standard error.

DISCUSSION

Vole herbivory patterns

Patterns of vertebrate herbivory are affected by nutritive and defensive components of available plant species, plant phenology, herbivore density, and the choices of forage species that vary greatly in relative value (e.g., Lindroth and Batzli 1984, Marquis and Batzli 1989, Olofsson et al. 2004, Howe et al. 2006). Voles favored *Desmanthus*, a nutritious legume commonly planted as a forage crop (Springer et al. 2001), more than other focal plant species for most of the growing season. The decline in clipping of *Desmanthus* stalks as the growing season progressed probably reflected structural changes in the stems; as *Desmanthus* plants matured, the stems became thicker and woody. Young growth is less lignified and silicified and therefore less tough than older growth (Van Soest 1995), a trait that generally affects herbivore foraging (e.g., Lucas et al. 2000, Teaford et al. 2005) and may be particularly important for small vertebrates like voles (Massey and Hartley 2006). The increase in clipped *Echinacea* stems late in the growing season likely reflects the availability of a new food resource. Voles consume large amounts of seeds in the late summer and early fall (Lindroth and Batzli 1984) and *Echinacea* achenes are a favorite vole food (Turner 2005). These achenes seem to be the primary goal of *Echinacea*-stem clipping by voles late in the growing season because the clipped stalks are usually left uneaten and the seedhead is removed. In contrast, vole clipping of *Heliopsis* stems remains low and constant through the growing season. In feeding trials, *Heliopsis* achenes and stems have low palatability (A. T. Sullivan, unpublished data).

Effects of vole herbivory timing

Reductions in plant biomass and reproductive output experienced by plants following herbivory play major

TABLE 3. Repeated-measures ANOVA results for *Desmanthus* with vole access treatment and restoration seeding time and density as independent variables.

Source of variation	df	F value			
		Biomass	Reproductive : vegetative	Seeds per plant	Seed mass
Treatment	4, 76	17.91***	13.45***	13.20***	18.54***
Seeding	1, 76	7.46*	1.75	1.30	4.20
Density	1, 76	3.62	3.079	0.85	3.39
Treatment × seeding	4, 76	0.49	0.92	0.60	0.83
Treatment × density	4, 76	0.91	0.16	0.65	1.56
Seeding × density	1, 76	3.53	1.33	2.90	1.80
Plot (seeding × density)	19, 76	4.877	1.48	0.99	1.257
Treatment × time	4, 76	3.82*	4.29*	3.01	3.90*
Seeding × time	1, 76	0.16	0.18	0.35	0.19
Density × time	1, 76	0.34	0.88	0.34	1.15
Treatment × seeding × time	4, 76	0.50	0.60	0.25	0.31
Treatment × density × time	4, 76	0.28	0.33	1.81	0.24
Seeding × density × time	1, 76	0.02	0.46	1.69	0.02
Plot (seeding × density) × time	19, 76	1.68	1.65	1.32	1.73

Notes: Response variables were measured in 2003, when access treatments were applied, and again following the 2004 growing season, when plants were protected from voles. P values reflect Bonferroni adjustment to correct the experiment-wise error rate. * P < 0.05; *** P < 0.001.

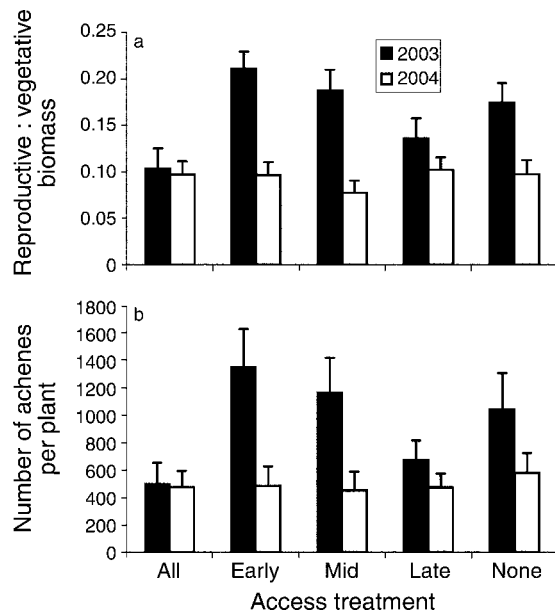


FIG. 4. Effects of vole access to *Echinacea* on (a) reproductive:vegetative biomass and (b) achenes per plant. There was no carryover effect of vole access treatments in 2003 to 2004 when plants were protected from voles. Error bars represent standard error.

roles in plant community dynamics (Huntly 1991, Bowers 1993, Olf and Ritchie 1998). The extent of these “cryptic effects” (sensu Zorn-Arnold et al. 2006) can be related to herbivory timing and may continue to affect plant populations beyond the growing season in which the herbivory occurred. For example, García and Ehrlén (2002) found that early-season defoliation of *Primula veris* reduced future growth, while leaf removal later in the growing season reduced both future flowering probability and growth. Knight (2007) found that early-season defoliation of *Trillium grandiflorum* by

deer reduced the probability that those plants would be reproductive the following growing season, reducing the population growth rate. These cryptic effects can clearly have lasting effects.

In the present study, some cryptic effects lasted and some did not. *Desmanthus* plants affected by early-season herbivory in 2003 continued to produce fewer seeds in 2004 compared with protected plants, though seed mass was similar to that of seeds from protected plants. It appears that these *Desmanthus* plants that were affected by early-season herbivory invested their resources in fewer larger seeds rather than more smaller seeds, a strategy that may increase seedling vigor and growth (Bonfil 1998, Leishman et al. 2000). In contrast, while late-season herbivory in 2003 affected *Echinacea* reproductive:vegetative biomass and achenes per plant, these effects did not last into the next growing season. Differences in continued effects of herbivory may be amplified by herbivory in consecutive growing seasons. Gustafsson (2004) found that herbivory in a second consecutive growing season reduced plant survival, growth, and reproduction, probably because resources become exhausted. It is likely that herbivory in consecutive growing seasons would further limit *Desmanthus* seed production. In contrast, the lack of continued effects for *Echinacea* indicates that established plants should be fairly resilient to late-season herbivory in consecutive growing seasons. It should be noted that repeated removal of seed heads, though it does not affect established plants, may have a demographic effect if large quantities of removed achenes are consumed and if the *Echinacea* population is seed limited (Eriksson and Ehrlén 1992). If it is seed limited, then the population will be negatively affected, but if it is safe-site (a microsite that is suitable for seed germination and seedling survival) limited, the loss of achenes to vole consumption will have less effect.

TABLE 4. Repeated-measures ANOVA results for *Echinacea* with vole access treatment and restoration seeding time and density as independent variables.

Source of variation	df	F value			
		Biomass	Reproductive:vegetative	Achenes per plant	Achene mass
Treatment	4, 156	3.82	0.13	2.37	2.10
Seeding	1, 156	18.56***	17.00***	26.86***	10.00**
Density	1, 156	4.80	2.50	2.97	0.75
Treatment × seeding	4, 156	0.43	1.65	0.86	1.13
Treatment × density	4, 156	0.56	0.10	1.16	0.95
Seeding × density	1, 156	0.98	0.67	3.07	0.05
Plot (seeding × density)	4, 156	2.39**	1.19	2.24**	1.64
Treatment × time	4, 156	2.65	5.16**	5.30**	2.94
Seeding × time	1, 156	1.23	0.17	0.58	0.64
Density × time	1, 156	1.78	3.00	2.56	0.27
Treatment × seeding × time	4, 156	0.37	1.61	0.55	1.05
Treatment × density × time	4, 156	1.01	2.01	2.07	2.27
Seeding × density × time	1, 156	1.13	0.33	0.22	0.09
Plot (seeding × density) × time	38, 156	1.69	1.84*	1.27	2.08**

Notes: Response variables were measured in 2003, when access treatments were applied, and again following the 2004 growing season, when plants were protected from voles. *P* values reflect Bonferroni adjustment to correct the experiment-wise error rate.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

While the three focal species responded differently to the temporally isolated vole access treatments, all three species were most detrimentally affected by continual vole access. The difference between all-season and temporally isolated access was likely the result of repeated clipping during the growing season, which can limit the available time and resources for plants to recover from herbivory (Reece et al. 1996), leading to smaller plants with lower reproductive output.

Plant response to herbivory timing is likely to be affected by resource availability and time available to recover from herbivory before the end of the growing season or subsequent herbivory event (Anderson and Frank 2003). For species affected by early-season herbivory, resource availability may limit regrowth and reproduction (Knight 2003). In our experiment, *Desmanthus* plants that experienced early-season herbivory were not able to grow as big or produce as many seeds as those that had been protected, while plants that experienced mid- and late-season herbivory compensated for lost vegetative and reproductive mass. This suggested that *Desmanthus* plants that experienced herbivory early in the growing season are unable to assimilate enough resources to compensate for removed biomass. For species affected by late-season herbivory, the remaining growing season may have been too short for affected plants to regrow and regenerate reproductive structures (Machinski and Whitham 1989, Obeso and Grubb 1994, Gustafsson 2004, McKague and Cappuccino 2005). *Echinacea* reproductive: vegetative biomass and achenes per plant were reduced by late-season herbivory, but stem clipping took place as the plant senesced and the stem was turning brown. This indicated that although there was no opportunity to regrow following late-season herbivory in 2003, *Echinacea* did not expend extra resources that reduced its growth and reproductive output in the following growing season.

Plant structure can also influence the outcome of herbivory (e.g., Marquis 1996). For forbs and grasses, basal meristems constitute an important adaptation for herbivory compensation. For example, the ability of *Gentianella campestris* to compensate for high levels of herbivory (75% of the stalk removed) is related to the induction of growth from basally located meristems (Huhta et al. 2000). Similarly, in order for stems clipped by voles to regrow, their lowest meristem had to be below the point of vole clipping, typically about 10 cm high. Basal meristem location likely influenced the relative mortality of our three species. *Desmanthus* and *Echinacea* typically regrew from meristems either at the base of the stalk or in leaf axils below where voles clipped and experienced little mortality. *Heliopsis* stalks regrew from meristems in the leaf axils. Because voles usually clipped *Heliopsis* stalks below the lowest leaves, clipped stalks typically did not regrow. If the clipped stalk was the only stalk of a *Heliopsis* individual, it died. *Heliopsis* probably persisted because voles normally avoided it.

Effects of herbivory timing are also affected by vole population density. Differences in consumption within species results from differential palatability from chemical or mechanical defenses (Dirzo and Harper 1982, Marquis 1992), or detectability (Baraza et al. 2006). The most palatable, least defended, and most detectable individuals will be consumed first (see Augustine and McNaughton 1998). When vole populations are high, more plant individuals will be found and less palatable individuals will be consumed or cut. The effects presented here are based on a vole density of ~140 known individuals per hectare. If more voles are present, more individual plants are likely to be clipped; with fewer voles, there is likely to be less effect.

Effects of restoration design

Restoration creates distinct plant communities that result in neighborhoods that differ in available forage and competitive interactions (see Howe et al. 2006, Parsons et al. 2007). Neighborhoods, here reflected by planting design, can affect the growth and reproduction of resident plants, as well as the occurrence and outcome of herbivory (Goldberg 1987, Machinski and Whitham 1989, Palmer et al. 2003, Baraza et al. 2006). In this restoration, the ecological effects of season and density of seeding continued to affect the focal plant species seven years after seeding. June-seeded plots had bigger and more productive *Echinacea* plants and more, larger *Desmanthus* plants. The difference in plant size and reproductive output probably reflected competitive environment. Overall plant density was higher and biomass lower in June-seeded plots, but much of the extra plant density in June-seeded plots was due to short species, such as invasive Kentucky bluegrass (*Poa pratensis*), that did not compete effectively with larger *Echinacea* and *Desmanthus*. More intensive clipping in December- compared to June-seeded plots may have also affected *Echinacea* size and reproductive output. However, this difference in clipping was probably less important than competitive environment since *Desmanthus* plants were also larger in June-seeded plots but experienced similar levels of clipping in December- and June-seeded plots.

While restoration design had some effect on *Echinacea* and *Desmanthus* growth and reproduction, *Heliopsis* experienced high mortality everywhere in the restoration regardless of seeding time or density, even when it was protected from herbivory. *Heliopsis* is often associated with weedy and disturbed areas and has a more ruderal lifestyle (Great Plains Flora Association 1986, Swink and Wilhelm 1994). It decreased rapidly in the restoration in 2002 and 2003 regardless of the seeding time or density, or vole access treatment of the neighborhood in which it was growing (Howe et al. 2006). High mortality observed in this study reflects that trend. Overall, effects of planting design on plant response to vole herbivory appears minimal.

CONCLUSIONS

To our knowledge, this is the first study to investigate plant response to the timing of small mammal herbivory within a growing season. The three focal plant species in this study showed a wide range of responses. Of the three species, one was most negatively affected by early-season herbivory, one by late-season herbivory, and one was unaffected by a season of herbivory, indicating that a wide variety of responses to herbivory timing among plant species is likely.

All three species were most negatively affected by continual vole access. This implies multiple bouts of herbivory that drained resources and produced the more dramatic effects of continual vole access when compared to single-season vole access. Carryover of effects of herbivory into the next growing season differed for the three species, allowing a glimpse into mechanisms behind vole suppression of certain plant species and their overall effects on plant communities. One expects that species that carryover herbivory effects to a subsequent growing season will ultimately decrease in a plant community relative to species that do not, particularly if herbivory continues in consecutive growing seasons.

Finally, although restoration seeding time influenced herbivory patterns and plant size seven years after planting, it did not influence plant response to timing of vole herbivory. Vole effects on individual species were surprisingly similar across treatments, despite differences in restoration design that resulted in differences in density and biomass of the plant communities. In this study, plant response to vole clipping was affected more by the loss of resources and limited time for regrowth than by differences in the competitive environment created by restoration design.

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