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## Using Endophytic Grasses to Reduce Small Mammal Populations

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**ABSTRACT:** Additional methods and integrated strategies to reduce damage by voles and other small rodents are needed, especially where rodenticides cannot be readily used. This field study examined if fields vegetated with endophytic grasses (i.e., grasses infected with the fungi, *Acremonium* spp. or *Neotyphodium* spp.) which produce alkaloids that impair herbivory, resulted in lower abundance or impaired reproduction of small mammals. We also determined if small mammals inhabiting fields with endophytic grasses had impaired capabilities (i.e., smaller body size and body condition). A lower abundance of small mammals in fields of endophytic grasses was evident. However, there appeared to be very little difference in the size, body condition, and pregnancy rates of small mammals from either field type. These results suggested that endophytic varieties of grasses could be used reduce population numbers of rodents, thereby reducing human-wildlife conflicts resulting from overabundance of rodents.

**KEY WORDS:** endophytic grasses, populations, rodent damage, small mammals, voles

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#### INTRODUCTION

Voles (*Microtus* spp.) and other small rodents cause severe damage to agricultural crops, orchards, and reforestation units (Lewis and O'Brien 1990, O'Brien 1994, Witmer et al. 1995, Witmer et al. 2007, Baldwin et al. 2013). Damage to agricultural fields is likely to be most significant when densities of voles reach ≥200 individuals/ha (Johnson 1958, Babinska-Werka 1979). Traditionally, broadcasting bait and using bait stations with toxic rodenticides (zinc phosphide or anticoagulants) in areas known to be infested with rodents have been used to reduce rodent densities (O'Brien 1994, Witmer et al. 2007, Witmer 2011). However, these treatments are not consistently successful or easy to implement because of the high reproductive potential and rapid reinvasion rates of rodents (Tobin and Richmond 1993, O'Brien 1994, Witmer et al. 2007). Therefore, managers have recognized a growing need for additional methods and integrated strategies to reduce damage by voles and other small mammals (Witmer et al. 1995, Witmer 2007, Witmer et al. 2007, Witmer 2018).

Grasses containing a symbiotic, endophytic fungi, Acremonium spp. (also referred to as Neotyphodium spp.) are known to reduce insect herbivory (Crawford et al. 2010, Uchitel et al. 2011). Livestock production can also be adversely affected by reducing feed intake, milk production, and weight gain; and affecting thermoregulation and reproduction (Ball et al. 1993, Hoveland 1993, Siegel 1993). In this way, the fungi protect grasses from herbivory and other environmental stresses by their production of alkaloids in the grasses (Bush et al. 1997). Some laboratory studies have also documented adverse effects on laboratory strains of mice and rats (Neal and Schmidt 1985, Zavos et al. 1990, Godfrey et al. 1994). Tannenbaum et al. (1998) noted suppression of reproduction in white-footed mice (Peromyscus leucopus). Uchitel et al. (2011) noted reduced seed removal my rodents in in the presence of endophytes. In contrast, Conover (1998)

did not find differences in reproduction and weights of captive voles fed diets of grasses that were infected and non-infected with the fungi, but did find higher mortality rates in voles fed the fungi-infected diet when temperatures were increased from 21°C to 31°C. Similarly, Washburn (2000) found no significant differences in the body condition or reproductive parameters of cottontail rabbits (Sylvilagus floridanus) from fields of endophytic and non-endophytic grasses, but he did not assess rabbit densities or mortality rates. Coley et al. (1995) reported lower abundance of small mammals on fields of endophytic grasses, but he only examined a few plots in one endophytic and one non-endophytic field and did not quantify reproductive or morphological parameters. Durham and Tannenbaum (1998) reported reduced growth and reproduction in captive prairie voles (Microtus ochrogaster) fed a combination of endophyte-containing rodent chow and endophytic grass seed. However, in fields with prairie voles, Fortier and others (2000), found little effect of endophytic grasses on reproduction, but suggested that the endophytic grasses may delay sexual maturation. Pelton and others (1991) reported a 4- to 5fold decrease in the number of rodents captured on fields of endophytic grasses than on non-endophytic grass fields. The results of these studies indicate that endophytic grasses could potentially be a biological control method used to maintain lower densities of small mammals, especially in agricultural areas. Endophytic grasses may also be useful at airports where rodents attract raptors and, hence, increase the risk of bird-aircraft collisions (Witmer 2011).

We evaluated the small mammal populations in fields of endophytic grasses (EG) and non-endophytic grasses (NG) in both perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*) in northeastern Oregon. We compared small mammal abundance, species richness, body size, body condition, and pregnancy rates between the fields to identify any differences potentially attributed

to the consumption of endophytic grasses. We hypothesized that EG fields supported fewer small mammals than NG fields and that the individuals from EG fields had reduced capabilities to survive and reproduce under ambient conditions than did individuals from NG fields.

## **METHODS Study Area**

This study area was located in the Columbia River Basin in Morrow and Umatilla Counties, northeastern Oregon, where the natural vegetation is shrub-steppe, comprised mainly of sagebrush (*Artemesia* spp.) and grasses (*Agropyron* spp. and *Poa* spp.; Franklin and Dyrness 1973). The study was conducted on irrigated agricultural grass fields maintained for the production and sale of grass seed. The fields, mostly flat, were comprised of well-drained loam or silt loam soils. Mean annual precipitation was about 20-25 cm and the summers were hot and dry (mean maximum July temperature is 33.5°C), while the winters were cold (mean minimum January temperature is -5°C). The mammalian fauna totaled about 61 species, as described by Verts and Carraway (1998).

## Assessing Populations and Condition of Small Mammals

Small mammals were collected between March 29-April 4, 1999, from six agricultural grass fields in northeastern Oregon: 1) two EG ryegrass fields, 2) two NG ryegrass fields, 3) one EG fescue field, and 4) one NG fescue field. Three 5×5 grids of snap traps with stations spaced at 10 m intervals (between and within rows) were established in each field and marked with wire flags. Each trapping grid encompassed about 0.13 ha. At each trapping station, we placed 2 Museum Special snap traps (Woodstream Corp., Lititz, PA). The traps were baited with peanut butter and rolled oats and were set in the late afternoon and checked early the next morning. Each trap grid was operated for 3 consecutive nights. All small mammal captures were collected to obtain morphological measurements and tissue samples and to evaluate reproductive status. All captures from each grid were stored in a sealed, plastic bag labeled with the date, field and grid number, and frozen for later examination. Grids were randomly located in each field. T-tests were used to compare relative abundance (captures per 100 trap nights) and morphological characteristics (body mass, total length, hind foot length, and an index of body condition [ratio of body mass to foot length]) between EG and NG fields (Proc T-Test, SAS Institute, Cary, NC). Species richness (i.e., total number of species of small mammals captured from a field type), was used as an index of biodiversity.

Additionally, saturation trapping [i.e., the placement of snap traps wherever rodent sign (burrow openings, runways) was observed] in the fields was conducted to increase the samples for physical examination and to obtain better estimates of relative abundance. The data was examined by individual trapping methodology and by combined trapping methodologies.

Pocket gophers (Thomomys talpoides) occupied the perimeters of some fields. This fossorial rodent rarely occurs in the interior of planted grass fields because farmers will not tolerate the damage they cause. A count of pocket gopher burrow systems (an aggregation of soil mounds resulting from the creation and maintenance of a gopher burrow system created by a single pocket gopher) around the periphery of the fields was used as an index of their relative abundance. Pocket gopher kill traps were placed in opened burrows to obtain specimens to be processed as described above for small mammals from snap traps. Note that pocket gopher capture rates per 100 trap-nights are not presented in the results because usually only one adult pocket gopher occupies a burrow system, but a variable number of traps may be placed in the burrow systems to expedite capture of that gopher.

## RESULTS Small Mammal Captures

Total small mammal captures with snap traps (from grids and saturation trapping) included 165 individuals of seven species (Table 1). The most commonly captured species, in decreasing order, were deer mice (*Peromyscus maniculatus*), montane voles (*Microtus montanus*), western harvest mice (*Reithrodontomys megalotis*), and house

Table 1 Cumma	m, of amall man	mal captures	with anan tra	na hv anasias	sex and field type
TADIE I SUITINA	IIV OI SIIIAII IIIAII	IIIIAI CADIIIIES	WIIII SHAD HA	DS DV SDECIES	Sex and neigrope

i icia i ype		eer Mouse Montane Vole			Western Harvest Mouse			House Mouse			Shrew*			Great Basin Pocket Mouse				
and ID	3	2	Total	3	♂ ♀ Total		3	♂ ♀ Total		♂ ♀ Total		♂ ♀ Total		♂ ♀ Total				
Endophytic Fields (EG)																		
2FI	0	3	3	0	2	2	0	0	0	5	2	7	0	0	0	0	0	0
5RI	1	2	3	8	5	13	1	0	1	0	0	0	0	0	0	0	0	0
6RI	11	6	17	2	2	4	3	6	9	0	1	1	0	0	0	1	0	1
Subtotal:	12	11	23	10	9	19	4	6	10	5	3	8	0	0	0	1	0	1
Non-endophytic Fields (NG)																		
1FU	7	6	13	2	5	7	8	3	11	0	1	1	0	0	0	0	0	0
3RU	4	2	6	9	5	14	0	0	0	5	4	9	2	0	2	0	0	0
4RU	7	11	18	5	5	10	5	5	10	0	0	0	3	0	3	0	0	0
Subtotal:	18	19	37	16	15	31	13	8	21	5	5	10	5	0	5	0	0	0
Total:	30	30	60	24	24	50	17	14	31	10	8	18	5	0	5	1	0	1

<sup>\*</sup>Both Meriam's and vagrant shrews may have been captured but positive species identification was not determined.

Table 2. Total small mammal captures and capture rate (animals per 100 trap-nights) with snap traps by trapping method (grids versus saturation trapping) and by field type.

Field Type and Trapping Strategy	Number of Total Captures	Trap- Nights	Capture Rate (per 100 trap-nights)
Endophytic Fields (EG):			
Snap Trap Grids	8	450	1.8
Saturation Trapping	51	650	7.8
Subtotal:	59	1,100	5.4
Non-endophytic Fields (NG):			
Snap Trap Grids	26	450	5.8
Saturation Trapping	76	435	17.5
Subtotal:	102	885	11.5
Overall Total:	161	1,985	8.1

mice (Mus musculus). These four species comprised >96% (159 of 165) of the total snap trap captures. Other species captured were five shrews (Sorex merriami and/ or S. vagrans) and one Great Basin pocket mouse (Perognathus parvus). Interestingly, it appeared that populations of the introduced, usually commensal, house mouse were able to persist away from human habitations where they usually occur in North America. Verts and Carraway (1998) had concluded that feral populations of house mice were rare in Oregon. The species richness of small mammals captured with snap traps was the same for both EG (5 species) and NG (5 species) fields. The low number of species was consistent with the statement by Verts and Carraway (1998) that the region is characterized by "depauperate mammalian fauna." While the data imply equal species richness on the two field types, interpretation of these data is not clear-cut. Although only five shrews were captured, all of them came from two NG fields. Perhaps the EG fields do not support adequate insect prey populations for insectivorous shrews, resulting in a lowered biodiversity in the small mammal community. In a study of small mammals in northeastern Oregon (in the general area of the current study), Moser and Witmer (2000) noted that no shrews were captured from sites with a history of heavy cattle grazing and speculated that there might be biodiversity implications for the small mammal community in such areas. Conversely, Pelton and others (1991) caught low numbers of shrews on both EG and NG fields in a field study in Tennessee.

Only 21% (34 of 161) of the small mammals captured with snap traps were collected from the snap trap grids; the capture rates from snap trap grids were 1.8-5.8 animals per 100 trap-nights (Table 2). Trapping success was much higher when saturation trapping was conducted; the captures rates using this method were 7.8-17.5 animals per 100 trap-nights (Table 2). It was not surprising that higher capture rates of small mammals would occur near activity centers (runways, burrow openings) where saturation trapping was focused. This is also why it is important to use an abundance index that is "standardized" to the number of captures per unit effort (i.e., animals per 100 trap-nights) versus reliance on only the total number of captures.

Fewer individuals were captured on EG fields (59) than

on NG fields (102; Table 2). This pattern held for both snap trap grid and saturation trapping results. However, the trap success rates on EG fields were not significantly lower on snap trap grids (t = 1.96, t = 6, t = 0.14) or with saturation trapping (t = 0.12) than the rates on NG fields. In summary, only about 36% of the total number of small mammals captured with snap traps came from EG fields. The overall capture rate per 100 trap-nights was much lower on EG fields (5.4) than on NG fields (11.5; Table 2). Conover (2003) reported only a slightly lower abundance of voles on EG fields than on NG fields.

Pocket gopher activity was considerable on the peripheries of four of the six fields (two EG and two NG; Table 3). With regard to the other two study fields, one EG field showed signs of much badger (Taxidea taxus) activity, indicating that this predatory species probably reduced the numbers of pocket gophers in that field. In the other case, one NG field was subject to trapping by the landowner to reduce crop damage by pocket gophers. While these latter two fields had fewer gophers and burrow systems than the other four fields, all data were included in the date summary (Table 3) because one field of each treatment (EG and NG) was involved. It should be noted that pocket gophers were trapped from other EG and NG fields than the six primary fields used in this study so that adequate numbers of carcasses would be available for body condition and reproduction analyses. More pocket gophers were trapped and more burrow systems found around EG fields than NG fields (Table 3). This is in contrast to the small mammal snap trap results and it could be that 1) pocket gophers are less sensitive to endophytic grasses than small mammals, 2) pocket gophers are foraging more on non-graminoid plants, or 3) pocket gophers are foraging more on plants outside the ÉG fields since farmers do not allow them to extend their burrow systems into the agricultural fields.

# Small Mammal Body Size, Body Condition, and Pregnancy Rates

Sample size limited analyses of body mass, body length, and body condition to the three most commonly captured species: deer mice, montane voles, and pocket gophers. There was little consistency found in measurements by species or sex between the two field types

Table 3. Number of pocket gophers captured by sex and field type and number of burrow systems observed

by field type.

Field Type and Field ID	Numb	er of Ca	ptures	Number of Burrow		
rieid Type and rieid ib	3	9	Total	Systems		
Endophytic Fields (EG):						
2FI	11	11	22	38		
5RI	7	12	19	65		
6RI	0	1	1	27		
Other EG	6	5	11	N/A*		
Subtotal:	24	29	53	110		
Non-endophytic Fields (NG):						
1FU	4	6	10	23		
3RU	2	1	3	8		
4RU	3	7	10	26		
Other NG	1	3	4	N/A*		
Subtotal:	10	17	27	57		
Total:	34	46	80	167		

\*N/A = not applicable; no burrow system data collected at these sites.

(Table 4). This inconsistency may have been an artifact of the relatively small sample sizes or to differences in microhabitat conditions of the various field types. Female deer mice from EG fields weighed more (t = 2.7, n = 11, P =0.04) and were in better body condition (t = 2.29, n = 11, P = 0.05) than females from NG fields; they also tended to be somewhat longer. In contrast, male deer mice were virtually the same from EG and NG fields (all  $P \ge 0.39$ ). Somewhat more than half (55.0%) of the 20 adult female deer mice examined were pregnant. Interestingly, the pregnancy rate was much higher for females (7 of 9, 77.8%) from EG fields than for females (4 of 11, 36.4%) from NG fields, but the sample sizes were relatively small.

The morphology and body condition of both female and male montane voles were virtually the same from EG and NG fields (all P  $\geq$  0.39). Conover (2003) reported reduced weight gain in male voles, but not females, when fed an EG diet. Almost all (16 of 17; 94.1%) adult females examined were pregnant regardless of field origin (EG, n = 5; NG, n = 12). Conover (2003) also found similar levels of reproduction in voles whether fed EG or NG diets.

Female pocket gophers captured near EG fields weighed more (t = 3.6,  $\hat{n}$  = 22,  $\hat{P}$  = 0.003), were longer (t = 2.33, n = 22, P = 0.03), and were in better body condition (t = 3.36, n = 22, P = 0.003) than females captured near NG fields. In contrast, male pocket gophers were virtually the same in morphology and body condition from EG and NG fields (all  $P \ge 0.38$ ). Over half (54.1%) of the 37 adult females examined were pregnant regardless of whether captured adjacent to EG ( $\hat{n} = 23, 52.2\%$ ) or NG (n = 14, 57.1%) fields.

Of the 18 house mice captured, only 8 were females and 5 of those were juveniles. Two of the adult females were pregnant and the third had previously reproduced. Based on those results, it appeared that a sustaining feral population may have established itself in the study area.

Table 4. Average adult body mass (g), total length (mm), body condition, and pregnancy rate (%) of three select small mammal species by species, sex, and field type.

Field Type	Deer	Mice	Montane	Voles	Pocket Gophers		
and Parameter	♂ (No.)	♀ (No.)	♂ (No.)	♀ (No.)	♂ (No.)	♀ (No.)	
Endophytic Fields (EG):							
Body Mass	18.8 (11)	20.7 (6)	33.8 (7)	32.1 (3)	118.9 (11)	99.2 (13)	
Total Length	149.6 (11)	151.5 (6)	145.7 (7)	138.7 (3)	201.9 (11)	194.8 (13)	
Body Condition <sup>a</sup>	0.93 (11)	1.06 (6)	1.73 (7)	1.76 (3)	4.46 (11)	3.77 (13)	
Pregnancy Rate	N/A <sup>b</sup>	77.8 (9)	N/A	100 (5)	N/A	71.4 (21)	
Non-endophytic Fields (NG):							
Body Mass	19.1 (10)	17.4 (5)	33.7 (11)	33.4 (7)	123.4 (5)	83.8 (9)	
Total Length	150.5 (10)	147.2 (5)	143.0 (11)	144.3 (7)	205.0 (5)	188.1 (9)	
Body Condition <sup>a</sup>	0.97 (10)	0.92 (5)	1.78 (11)	1.81 (7)	4.63 (5)	3.28 (9)	
Pregnancy Rate	N/A	26.7 (15)	N/A	100 (12)	N/A	78.6 (14)	

<sup>&</sup>lt;sup>a</sup> Index of body condition = body mass/hind foot length.

<sup>&</sup>lt;sup>b</sup> N/A = not applicable.

#### **DISCUSSION**

This study found a much lower abundance of small mammals on fields containing grasses with endophytic, alkaloid-producing fungi although differences in rodent body morphology, body condition and reproduction were not consistently apparent between the two field types. While the lower abundance reported in this study is not as dramatic as the abundance reduction reported by Pelton and others (1991), it nonetheless suggests that endophytic varieties of grass could potentially provide a management tool that could be used to reduce human-wildlife conflicts resulting from an overabundance of rodents (e.g., crop, cable/pipe, or structural damage from rodent foraging or gnawing; the attraction of predators and raptors that result in increased risk of wildlife-aircraft collisions; the role of rodents as important vectors or hosts of diseases of significance to humans or livestock). Endophytic grasses could also provide an important management tool for the grassy or fallow areas that border crop fields because these areas are known to provide "refugia" for rodent populations (Chambers et al. 1996, Martinelli and Neal 1995, Witmer et al. 2007). These areas provide the source populations of rodents that can quickly reinvade harvested crops fields once the next crop cycle begins. There is often a trade-off, however, when land managers use a combination of management tools (Witmer 2007). For example, Salminen and Grewal (2002) reported higher levels of alkaloids in endophytic grasses that are mowed less frequently, yet small mammal populations are lower at airports where the grasses are kept very short (Witmer 2011). The benefits of the endophytic grasses may be especially great where cyclic populations of small rodents, such as voles, occur that can achieve very high densities every few years, resulting in high levels of damage.

We note, however, that this study did not show consistent differences in rodent body morphology, body condition, and reproduction between endophytic and non-endophytic grass fields. Therefore, some species of small rodents (e.g., pocket gophers) may not be affected by endophytic grasses and some beneficial small mammals (e.g., insectivorous shrews) may be adversely affected.

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