Susceptibility of a tallgrass prairie to invasion by the herbicidal producing species, *Festuca rubra*

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Disturbance, nutrients, propagule pressure, and the presence of arbuscular mycorrhizal fungi (AMF) have been shown to influence invasion in plant communities, although the relative importance of each remains unclear. A remnant of tallgrass prairie outside Cambridge, ON appears to be resilient to invasion by *Festuca rubra*, despite its herbicidal properties. A greenhouse experiment was conducted to determine what factors are responsible for the resilience of the native grassland, using synthesized fescue and native sod. The effects of disturbance, nutrient enrichment, propagule pressure, and the presence of AMF and phytotoxin on the establishment ability of *F. rubra* and the native species *Sorghastrum nutans* were examined. Propagule pressure was the only factor shown to influence fescue invasion and establishment of *S. nutans* was unaffected by all factors. These results suggest that the resilience of the native grassland may be due to decreased seed production or viability of *F. rubra* and not any characteristics of the grassland. Also, the restoration of native grasslands will not be limited by remnants of phytotoxin in the soil, following removal of exotic species.

Invasive species are considered a major threat to ecosystems worldwide, but the factors that contribute to a community's susceptibility to invasion are, for the most part, still under debate. Disturbance, increased nutrients, and propagule pressure are widely accepted as facilitators of invasion.^{5, 7, 8} The presence of mycorrhizal fungal communities has also been shown to influence a plant community's resistance to invasion.^{9, 14} However, the relative importance of each factor on the establishment of exotic and native species is not clear, as their effects are difficult to separate.

Disturbance can significantly impact invasion by reducing adult cover, allowing for increased seedling density and richness of non-native species.⁸ This positive effect on seedling addition could result from disturbance decreasing the amount of resources sequestered by resident vegetation and, as a consequence, the amount of nutrients available to invasive species increases.⁵ Phosphorus and nitrogen are often limiting resources⁵ and nitrogen addition in a Californian grassland enhanced the invasive ability of exotic species.⁸

Previous studies have shown that propagule pressure is an important determinant of seedling growth and survival. Some studies suggest that propagule pressure is the most important determinant of establishment success for non-native species, as it is capable of overcoming native grassland resilience.^{8, 17} Tall grass prairies historically covered a substantial amount of Southern Ontario, but less than 5% remain and many contain exotic species.¹⁵ Once

established, exotic species can impact ecosystem functioning by altering the interactions between plant and microbial communities,¹⁹ or in some grassland communities, changing the composition of the soil microbial community.⁹ Soil organisms, such as arbuscular mycorrhizal fungi (AMF), play an important role in the health, productivity and overall structure of plant communities. AMF have been shown to affect the susceptibility of a plant community to invasion, by increasing or decreasing the ability of exotic species to invade, depending on what soil fungal communities are present.^{14, 16}

Red fescue (*Festuca rubra*) is a creeping invasive grass that has established in grasslands across North America and has an increased tolerance of disease and stress.² Red fescue has an increased competitive ability through production of the highly potent phytotoxin *m*-tyrosine. This toxin is produced quickly following germination in the roots of the plant and is released into the surrounding soil. After release, the toxin comes into contact with other grasses and inhibits their root growth, effectively decreasing the fitness of the plant. However, there is no evidence that the phytotoxin affects AMF present in native soil.² Alternatively, a previous study determined that the fitness of red fescue is limited by AMF, with a decreased biomass, a decrease in the number of tillers, and decreased maximum height of tillers when AMF is present in the soil.¹³ Thus, AMF may limit the invasibility of a native community by red fescue.

The purpose of this study is to determine the factors that influence the ability of exotic species to invade natural

grasslands, by using soil and native seed from an existing resistant grassland. It is unclear if the resilience of the grassland is a result of soil processes preventing exotic species from further colonizing, or if red fescue establishment depends upon disturbance or increased nutrients. The presence of red fescue in the native grassland appears to have no effect on the adult natives due to their deep rooting system, but could impact native seedling establishment. In order to determine the ability of the grassland to persist in the presence of a highly invasive species the following question will be addressed: What factors determine the resilience of native grasslands to the highly competitive and toxic invasive species, red fescue?

If soil conditions are responsible for the resilience of the native grassland by AMF decreasing the fitness, and consequently toxin production of red fescue, then red fescue will show a decreased establishment in native sod. If resilience of the grassland is due to a lack of fescue phytotoxins, then invasion resistance and resilience by native grasses will decrease in fescue conditioned soil. If the resilience of the native grassland is due to a lack of disturbance limiting the establishment of red fescue, then red fescue will be able to establish in native sod following disturbance and native grass will be unable to establish in exotic sod. If invasion success is dependent upon an influx of nutrients increasing resource availability, then red fescue will be capable of establishment in native sod with increased nitrogen and native grass will be unable to establish in exotic sod. If propagule pressure has an effect on invasion by increasing the ability of exotic species to overcome native resilience, then red fescue will show higher invasion success with a higher number of seeds.

METHODS

Determining invasion success

Invasion success in this experiment was defined as the number of seeds that germinate and will be determined for each treatment and propagule pressures as follows:

Establishment success = # of seeds emerged (1)

Study site and species

The experiment was carried out in the University of Guelph, ON (43°33'N, 80°15'W) greenhouse. All soil samples and native seed used for the construction of sod were collected from the field site located in Cambridge, ON (43°26'N, 80°19'W) which contains the native species big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), Indian grass (*Sorghastrum nutans*), and Canadian wild rye (*Elymus Canadensis*), as well as the exotic grass red fescue. Soil was collected in both native and exotic grassland areas at random in early fall of 2008. Plant

roots were gathered with the soil to ensure the presence of biologically active materials (fungi and toxins) in the samples. The collected soil samples were kept refrigerated at 4°C until construction of the sod. A commercially available potting soil (Sunshine mix) was used as a control soil, and was obtained from the greenhouse. Native seeds of all four species were refrigerated at 4°C and soaked in water until needed, either for sod construction or addition to established sod. Exotic seed was "Eco-lawn" and was obtained commercially.

Experimental set-up

In late October of 2008, 5 litre planters were filled with either control soil (Sunshine mix #4), exotic soil or native soil. Both exotic and native soil were mixed with control soil, with the mixtures approximately 1:9 (field soil : control soil), as the amount of soil that could be collected from the field was limited. Either exotic seed or a mixture of native grasses were over sown to ensure germination and a healthy sod. A total of 48 planters were constructed, with 24 containing exotic sod and 24 containing native sod. All planters were watered daily as necessary. Three treatments (disturbance, nitrogen or control) were applied to each soil type, for a total of 9 treatments. For every treatment, there were 3 replicates for each exotic and native soil planters and 2 replicates for control soil.

Determining the effect of propagule pressure

13 weeks following initial sod set-up, each planter was split into halves and either red fescue or Indian grass seed was added to each half. The seeds were added in groups of approximately 8 and 32, which acted as low and high propagule pressure.

Determining the effect of disturbance

16 planters had the grass cut to approximately 10cm in height just prior to seed addition, to act as a surrogate for disturbance. This height represents a disturbance event typical of mowing.

Determining the effect of increased nutrients

16 planters were watered with 20-20-20 fertilizer immediately after seed addition, and again 1 week later, to increase nitrogen availability.

Determining the effect of soil processes

16 planters acted as controls for disturbance and nutrient levels and were used to measure soil effects.

Statistical analysis

All data was analyzed using a multi-factor ANOVA. To determine the effect of disturbance, nutrient availability, soil

Table 1: ANOVA model of red fescue and Indian grass establishment success in native and exotic sod following three treatments (disturbance, increased nutrients and control) at high and low propagule pressures

Source	DF	F ratio	Prob>F
Cover	1	2.952	0.086
Treat	2	2.412	0.091
Soil	2	0.288	0.750
Treat*Soil	4	4.299	0.002
Date	4	21.898	<0.0001
Cover*Date	4	4.182	0.002
Seed Type	1	207.861	<0.0001
Treat*Soil*Seed type	4	4.384	0.002
Date*Seed type	4	10.662	<0.0001
Cover*Date*Seed type	4	3.877	0.004
Seed Density	1	46.211	<0.0001
Date*Seed density	4	4.303	0.002

processes and propagule pressure, the establishment success of Indian grass and red fescue were compared across all treatments and time periods. Significant interactions were analyzed using Tukey's HSD test.

RESULTS

When the germination success of red fescue and Indian grass were compared between treatments, the model was found to be highly significant ($R^2=0.88$; $F_{359,958}=12.76$; $p<0.0001$). Seed type and date had significant effects on fescue invasion, with higher establishment success occurring at an increased rate compared to Indian grass. Seed density was also significant, as red fescue establishment increased with higher seed availability (**Table 1**). These results occurred regardless of soil type and treatment, with native soil conditions having no detectable effects on the ability of fescue to establish. However, cover type had a significant influence on fescue invasion, with higher establishment in native sod at later time periods. Native grass, in contrast, was a relatively poor invader, as Indian grass was only capable of low establishment. These results occurred regardless of cover type, treatment or soil type, with fescue soil conditions

having no detectable effects on the ability of Indian grass to establish. Neither propagule pressure nor date had significant effects on Indian grass, as establishment success did not increase with time or seed density (**Tables 2-8**).

DISCUSSION

The ANOVA model provided strong evidence that propagule pressure is the most important factor determining fescue invasion in resilient grassland. Despite hypothesized effects, there was no evidence that soil processes, disturbance or nutrient availability influence the establishment success, and consequently, invasive ability of red fescue. These results support previous studies, which have found that the effects of other environmental factors, such as disturbance and nutrient enrichment, are negligible compared to propagule pressure.^{3, 4, 8, 10, 17} The increase in establishment with increased seed availability indicates that propagule pressure acts as a "lottery" effect for invasive species, but not for native species, as these results were only shown for fescue establishment. This suggests that the resilience of the native grassland is not due to a high seed production by tallgrass species maximizing its establishment and sustainability.

Table 2: Tukey's HSD test for the effects of treatment*soil on establishment success of red fescue and Indian grass

Level (Treatment[D:disturbance, N:nutrients, C:control], Soil [E:exotic, N:native, C:control])	Significant relativity (levels not connected by same letter are significantly different)	Least Square Means
C, C	A B	4.313
D, E	A	4.292
N, C	A B	4.063
C, N	A B	3.792
D, N	A B	3.500
C, E	A B	3.083
N, E	A B	2.167
D, C	A B	1.563
N, N	B	1.458

Table 3: Tukey's HSD test for the effects of cover*date on establishment success of red fescue and Indian grass

Level (Cover [E:Exotic, N:Native], Date [1-5 consecutive sampling periods])	Significant relativity (levels not connected by same letter are significantly different)	Least Square Means
N, 5	A	6.426
N, 4	A B	5.944
N, 3	A B	5.894
N, 2	A B C	5.213
E, 5	B C	4.912
E, 4	B C D	4.833
E, 3	B C D	4.741
E, 2	C D	4.218
E, 1	D E	3.509
N, 1	E	2.764

Table 4: Tukey's HSD test for the effects of treatment*soil*seed type on establishment success of red fescue and Indian grass

Level (Treatment[D:disturbance, N:nutrients, C:control], Soil [E:exotic, N:native, C:control], Seed type [E:exotic, N:native])	Significant relativity (levels not connected by same letter are significantly different)	Least Squares Mean
C, C, E	A B	8.625
D, E, E	A	8.583
N, C, E	A B C	8.125
C, N, E	A B C	7.583
D, N, E	A B C D	7.000
C, E, E	A B C D	6.167
N, E, E	B C D E	4.333
D, C, E	C D E F	3.125
N, N, E	D E F	2.833
N, N, N	F	0.083
D, C, N	E F	7.238×10^{-15}
C, E, N	F	3.775×10^{-15}
N, E, N	F	2.776×10^{-15}
C, N, N	F	1.887×10^{-15}
D, N, N	F	0.000
N, C, N	E F	0.000
D, E, N	F	-8.882×10^{-16}
C, C, N	E F	-1.499×10^{-15}

Table 5: Tukey's HSD test for the effects of date*seed type on establishment success of red fescue and Indian grass

Level (Date [1-5 consecutive sampling periods], Seed type [E:exotic, N:native])	Significant relativity (levels not connected by same letter are significantly different)	Least Square Means
5, E	A	10.380
3, E	A	10.148
4, E	A	10.107
2, E	A	9.208
1, E	B	6.264
5, N	C	0.958
4, N	C	0.671
3, N	C	0.486
2, N	C	0.222
1, N	C	0.009

Table 6: Tukey's HSD test for the effects of cover*date*seed type on establishment success of red fescue and Indian grass

Level (Cover [E:exotic, N:native], Date [1-5 consecutive sampling periods], Seed type [E:exotic, N:native])	Significant relativity (levels not connected by same letter are significantly different)	Least Square Means
N, 5, E	A	11.769
N, 3, E	A	11.306
N, 4, E	A	11.222
N, 2, E	A	10.204
E, 4, E	B	8.991
E, 5, E	B	8.991
E, 3, E	B	8.991
E, 2, E	B	8.213
E, 1, E	C	7.019
N, 1, E	C	5.509
N, 5, N	D	1.083
E, 5, N	E	0.833
E, 4, N	E	0.676
N, 4, N	E	0.667
E, 3, N	E	0.491
N, 3, N	E	0.481
N, 2, N	E	0.222
E, 2, N	E	0.222
N, 1, N	E	0.019
E, 1, N	E	2.332x10 ⁻¹⁵

Instead, it may be a result of fescue experiencing limitations in its seed set or viability, decreasing its establishment success. Although in this study, red fescue did not show any reductions in seed viability or germination limitations, fescue seeds are not capable of surviving enforced dormancy periods and persisting in the soil.¹⁸ Red fescue has been shown to have a high seedling emergence at a high germination rate,^{11, 18} but invasive ability has been shown to be dependent upon both propagule pressure and the number of seedlings. Therefore, the resilience of the grassland may be a result of fescue suffering high seed mortality in the field and not a result of native species characteristics.

The lack of an effect of soil processes on fescue invasion, evident by the high establishment success of fescue in all soil types, suggests that the AMF symbionts of native tallgrass species are not responsible for the resilience of the native grassland. Red fescue showed the highest establishment success in native sod, suggesting that the AMF communities may actually facilitate fescue invasion into tallgrass prairies by enabling increased establishment. This increase in establishment success indicates the existence of positive interactions between invaders and native communities, supporting results previously shown by von Holle and Simberloff.¹⁷ Therefore, instead of acting as a barrier, AMF may permit positive interactions by providing a pathway for exotic species to negatively impact the performance of native species.⁹ This supports the findings of Stampe and Daehler,¹⁴ which showed that the presence of AMF provided

invading species with strong competitive advantages. Alternatively, the increased establishment of fescue in native sod could be due to the decreased density of native sod compared to fescue sod, as germination of invasive species has been shown to increase with gaps in vegetation.⁸

Similarly, there was no evidence that the phytotoxin, *m*-tyrosine, produced by fescue roots negatively impacts, or that AMF facilitates, the health or establishment of native species. The ability of Indian grass to establish in fescue monoculture at the same capacity as native sod indicates that *m*-tyrosine is not as toxic to native tallgrass species as previously believed.² A previous study by Renne et al. showed that extract of the toxic endophyte produced by tall fescue (*F. arundinacea*) had low allelopathic potential on the germination and emergence of tallgrass species,¹² and that fescue monocultures have shown a comparatively high susceptibility to invasion.¹¹ These results indicate that the resilience of the grassland may be due to a lack of allelopathic effects of the fescue phytotoxin.

However, the lack of toxic effects may have been due to the level of *m*-tyrosine in the sod being lower than the concentration typically found in the field. The production of phytotoxin by red fescue has been shown to increase when the species is experiencing water stress,⁶ and as the sod was regularly watered, toxin production may have been minimal. Although the sod had a dense rooting system, which may have helped minimize the loss of phytotoxin produced by the fescue roots, the sod was constructed in sterilized soil. The

Table 7: Tukey's HSD test for the effects of date*seed density on establishment success of red fescue and Indian grass

Level (Date [1-5 consecutive sampling periods], Seed Density [H:High or L:Low])	Significant relativity (levels not connected by same letter are significantly different)	Least Square Means
5, H	A	8.222
4, H	A	7.903
3, H	A	7.787
2, H	A	6.954
1, H	B	4.611
5, L	C	3.116
4, L	C D	2.875
3, L	C D	2.847
2, L	C D	2.477
1, L	D	1.662

use of sterilized soil in sod construction has been shown to alter allelopathic effects, through changes in the movement and degradation of allelochemicals compared to field soil.¹² Therefore, the lack of a toxic effect of *m*-tyrosine on Indian grass could be unrepresentative of the interactions under natural conditions. Alternatively, the lack of an effect of soil processes on the establishment of native grasses could be due to the age of the sod, as young monocultures are not fully functioning ecosystems that accurately encompass phytotoxic effects.¹¹ Thus, soil conditioning may have effects on the resilience of the native grassland, but experimental conditions were too dilute to detect them.

There was no evidence that disturbance or nutrient enrichment facilitated invasion, suggesting that the prairie is not vulnerable to nutrient changes, nor is the resilience due to a lack of disturbance. However, the lack of an effect of increased nutrient availability, either through nutrient addition or disturbance decreasing nutrient acquisition, could be due to the use of sunshine mix, as it contains gypsum which provides an initial nutrient charge. The sod may not have completely depleted the nutrients in the soil and any increase in nutrients as a result of the clipping or fertilization may have been in excess to the nutrients already available in the soil. Alternatively, the sod may have sequestered the additional soil nutrients, resulting in a decrease in the light available for seed germination. Sod in the fertilization treatment was noticeably taller, more dense and had increased green coloration compared to unfertilized sod, which could have resulted in a decreased light availability,

similar to conditions found by Gross et al.⁸

The low establishment of Indian grass regardless of disturbance, nutrient enrichment, soil processes and propagule pressure indicates that it faces severe limitations similar to other tallgrass species.¹ These germination limitations represent a trade-off between dormancy periods and seedling survival, which prevents germination during harsh environmental conditions and thereby decreases seedling mortality. However, these germination limitations make the grassland susceptible to invasion, even during favorable environmental conditions, due to the high establishment success of fescue, even in native sod. Although there was no evidence that the establishment of Indian grass is inhibited by *m*-tyrosine, the differences in germination limitations of the native species and red fescue indicates that despite its persistence, the grassland is extremely susceptible to invasion and its persistence may be dependent upon the presence of adult bunchgrasses. However, the ability of Indian grass to establish in fescue sod suggests that restoration of tallgrass prairies will not be inhibited by the persistence of fescue toxin remnants after removal of fescue sod.

The conclusions drawn by this study are limited as it only examined the overall establishment success of exotic and native species in diluted soil conditions. Further studies should examine the invasibility of tallgrass prairies and the ability of native species to establish in fescue sod with undiluted soil conditions, to gain a clearer understanding of the effects of soil processes on invasion success.

Table 8: Tukey's HSD test for the effects of seed type*seed density on establishment success of red fescue and Indian grass

Level (Seed Type [E:exotic, N:native], Seed Density [H:high, L:low])	Significant relativity (levels not connected by the same letter are significantly different)	Least Square Means	Prob>F
E, H	A	9.222	0.086
E, L	B	3.306	0.091
N, L	C	0.019	0.750
N, H	C	5.773x10 ⁻¹⁵	0.002

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