

1 **Field-based effects of allelopathy in invaded tallgrass prairie**

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5
6 *Abstract* Allelopathic phytochemicals have been linked to invasion success, but their role in the
7 invasion process remains unclear. Toxicity effects demonstrated with lab bioassays may be neutralized in
8 soils, and their role in population expansion can be intertwined with non-allelopathic processes that also
9 influence dispersal and establishment. Here, we use greenhouse experiments to test the soil-based impacts
10 of invasive fine fescue (*Festuca rubra*) on recruitment in tallgrass prairie. Fescue roots release the growth
11 inhibitor *m-tyrosine*. Using root washes and fescue-conditioned soils to mimic field potency, we determine
12 allelopathic impacts on recruitment including intraspecific limitation. We also test if non-allelopathic
13 factors (propagule pressure, disturbance, fertility) influence invasion into constructed fescue and prairie
14 mesocosms, and whether root washes inhibit arbuscular mycorrhizal (AM) fungi. We observed significant
15 negative effects of fescue soils and root washes on germination and seedling survival, including on fescue
16 itself. Mesocosm invasion, however, was determined more by non-allelopathic mechanisms (propagule
17 pressure, rapid growth). Fescue invasion was higher in prairie mesocosms than its own understory, with no
18 effects of disturbance or fertility. Tallgrass species had difficulty establishing in all environments
19 regardless of propagule pressure. Impacts on AM fungal hyphae length and spore production were
20 insignificant. Our results suggest that non-allelopathic traits may be sufficient to explain fescue invasion,
21 with allelopathy likely emerging as a final *coup-de-grâce* for recruiting native grasses once dominance has
22 been attained. Allelopathic species may thus not necessarily be invasive unless non-allelopathic traits can
23 facilitate establishment prior to the accumulation of soil-based toxins.

24

25 *Keywords:* Allelopathy, *Festuca rubra*, novel weapons, plant invasion, *m-tyrosine*, tallgrass prairie

26

1 **Introduction**

2 Allelopathic ‘novel weapons’ have emerged as a prominent mechanism to explain why some
3 plant invaders can rapidly expand in habitats where they lack evolutionary familiarity (Bais et al. 2003;
4 Interjit et al. 2006). These novel weapons are powerful phytochemicals to which native competitors or
5 herbivores are assumed to have no evolved tolerance. They can create advantages for invaders within
6 trophic levels, by directly inhibiting competitors, or indirectly by interfering with beneficial symbiotic
7 interactions between native plants and soil organisms (Vivanco et al. 2004; Stinson et al. 2006; Hawkes
8 et al. 2006; Bertin et al. 2007; He et al. 2010). They can also work across trophic levels, where untasty
9 chemicals in foliage or roots result in less consumption or disease infestation compared to co-occurring
10 native species, thereby favoring invader expansion (Mitchell et al. 2006).

11 It remains unclear, however, the exact role by role allelopathic novel weapons contribute to
12 invader dominance (Levine et al. 2006; Kaur et al. 2009). One uncertainty is the potency of novel
13 weapons in natural settings. Many studies of phytotoxic root exudates have used lab-based *in vitro*
14 bioassays, but soil microbial processes may weaken or neutralize their toxicity in the field (Interjit et al.
15 2008; Kaur et al. 2009; Bertin et al. 2009). Another uncertainty is determining the explicit role that soil
16 effects have in population expansion, given that invader spread occurs into soils that have yet to be
17 modified (Levine et al. 2006). If positive soil feedbacks drive increased demographic performance in
18 high-density invader populations, then these populations could push invasion forward by showering the
19 invasion front with seed. As such, soil effects have a direct role in population spread. Alternatively,
20 allelopathic species can possess traits that influence expansion independently of soil processes (e.g.,
21 seed mass, growth rates, emergence time), such that allelopathy plays little or no role in expansion.
22 These traits may be especially critical if there are negative intraspecific soil effects, where soil toxicity
23 reduces invader fitness such that propagule pressure is constrained (Dwyer and Morris 2006, Hansen
24 and Wilson 2006). In this latter case, allelopathic effects will not drive expansion, but could contribute

1 to invader persistence and impact by preventing recruitment of other species once dominance has been
2 attained.

3 We examine these issues with invasive fine fescue grass (*Festuca rubra*) [hereafter ‘fescue’] in
4 remnant tallgrass prairie. A perennial species, fescue produces one of the stronger novel weapons that
5 has been described, the non-protein amino acid *m-tyrosine* which acts as a powerful growth inhibitor on
6 many plant species in lab-based bioassays (Aronson and Wermus 1965; Bertin et al. 2007). There are
7 red fescues that are native to North America but these have not been associated with the release of *m-*
8 *tyrosine*. Assemblages of invasive fine fescue occur widely across temperate North America, often in
9 mixtures of different species and cultivars, with *m-tyrosine* implicated as mechanism explaining its
10 success. The degree to which this is true, however, is unclear. Fescue also possesses numerous non-
11 allelopathic traits that favor invasion, including high seed production, drought tolerance, rapid
12 colonization rates, and clonal spread which leads to the formation of dense sod. It is also widely
13 introduced via cultivation and planting, such that human activity may explain dominance more than life
14 history traits. In addition, field studies have not been able to validate high *m-tyrosine* potency
15 demonstrated by lab bioassays, possibly because the chemical is quickly neutralized by microbes or soil
16 chemical processes (Bertin et al. 2009; Kaur et al. 2009). The influence of allelopathic effects on fescue
17 dominance, therefore, may be minimal.

18 Using experimental greenhouse work, we test the relative importance of allelopathic versus non-
19 allelopathic mechanisms on recruitment dynamics of fescue and native grasses from tallgrass prairie
20 where fescue is invading. To conduct this work, we use fescue-conditioned soils and root washes rather
21 than commercially purchased synthetic *m-tyrosine* as is typically used (e.g., Bertin et al 2007, 2009),
22 with the assumption that the former more closely resembles field concentrations. We examine the
23 separate effects of field-collected soils and root washes on germination, growth rates, and seedling
24 survival of fescue and native grasses. Using mesocosms of fescue and tallgrass communities, we test the
25 effects of three non-allelopathic mechanisms on recruitment - propagule pressure, disturbance

1 (clipping), and fertility - and whether these factors interact with community type. We also examine the
2 impact of root washes on arbuscular mycorrhizas (AM), given their established importance for fescue
3 (Skalova and Vosatka 1998) and for native C₄ grasses in tallgrass prairie (Jastrow and Miller 1993;
4 Vogelsang et al. 2006; Johnson et al. 2010).

5

6 **Materials**

7 *Study area*

8 Field soils and native seed were obtained in September 2008 from a ~1 ha remnant tallgrass prairie near
9 Cambridge, Ontario, Canada (43°23' N, 80°19' W). Tallgrass prairie occurs at the northeastern edge of
10 its North American distribution in Ontario (Packard and Mutel 2005). It formerly covered ~100,000 ha
11 of southwestern Ontario, of which < 1% remains (Roberts et al. 1977; Bakowsky and Riley 1993). The
12 site occurs on the southwest-facing slope of a glacial moraine, composed of rapidly drained fine gravels,
13 sand, and silt till (Fig. 1A). It is also bisected by an abandoned railway corridor from the 1800s, which
14 may have contributed to the persistence of its prairie species by occasionally introducing fire (Howe
15 1994). The site is dominated by the native C₄ grass *Andropogon gerardii*, with the C₄ grasses
16 *Sorghastrum nutans*, *Schizachyrium scoparium*, and the C₃ grass *Elymus canadensis* at lower
17 abundances. The top of the moraine is old-field, with high cover of fine fescue grass that was likely
18 planted at least several decades earlier. Fescue now occurs in high abundance throughout the prairie
19 understory up to 20 m from the edge (Fig. 1A). It is unknown whether the native overstory grasses can
20 recruit from seed, once fescue dominates the prairie understory (Fig. 1B).

21 *Fescue seed*

22 Our study objective was to test for allelopathic effects of fine fescue grass. Allelopathic potency in fine
23 fescues, however, can vary widely with some studies reporting that 90% of cultivars lack potency in lab
24 trials (Bertin et al. 2007). To reduce the possibility that field collected seed contain low-potency cultivars
25 by chance, we substituted field-collected fescue seed with commercially available seed known to emit *m*-

1 *tyrosine* (“Ecolawn”, Wildflower Farm Inc., Coldwater, Ontario). This allowed us to contrast the
2 potential allelopathic effects of two sources: the fescue-conditioned soils collected from the prairie site,
3 and the mesocosms and root washes prepared using the commercially purchased seed. The disadvantage
4 of this approach is that soil dynamics of these two fescue types may differ widely, and our tests of
5 intraspecific effects in particular are interpreted in this context (e.g., in some cases we added commercial
6 seed to field-conditioned soils). Our germination tests, however, showed that the suppressive effects of
7 the soils and root washes on recruitment were statistically indistinguishable (see experiments 1 and 3
8 below), suggesting that potency levels are similar.

9 *Experiments*

10 The first experiment tested the effects of field-collected soils on germination, seedling growth rate, and
11 seedling survival of fescue and the native C₄ grass *A. gerardii* over nine weeks. We used a fully factorial
12 design including reciprocal intraspecific soil effects to test whether fescue soils inhibit fescue
13 recruitment. We also used a greenhouse soil treatment as a control (Sunshine mix: sphagnum peat moss,
14 horticultural grade perlite, dolomite lime and a wetting agent; Sun-gro Horticulture, Vancouver, BC,
15 Canada). A 10 cm layer of field-collected soil was added to sand-filled 1 litre containers (Streuss and
16 Sons, Eugene OR), so that the seedlings grew down through the undiluted soil before contacting the
17 sand (Klironomos 2002). Sand was selected to resemble the substrate conditions at the field site. There
18 were 40 replicates of each plant species in either native or exotic soil respectively, and 20 replicates per
19 plant species for each control treatment, for a total of 200 pots. Planting occurred in October 2008. Prior
20 to planting, *A. gerardii* seeds were moistened and cold-stratified at 4 °C for one month (Beckman et al.
21 1993), after which germination was >90%. Germination trials of unstratified *A. gerardii* seed had
22 germination success <1%, as is typical for this species. No stratification treatment was required for
23 fescue. Seeds were first overplanted, with emergence time and total seedling establishment recorded as
24 they were thinned down to one plant per pot. Final height and leaf production were recorded after three
25 months, comparing performance among three soil types.

1 The second experiment tested the relative influences of propagule pressure, disturbance, and
2 nutrients on invasion into mesocosms of fescue and native-grass. We constructed these mesocosms in
3 five litre pots in October 2008, planting either fescue or a mixture of the four dominant prairie grasses of
4 the study site. The mesocosms were allowed to establish for 3 months, with assumption that *m-tyrosine*
5 levels would accumulate in the fescue pots over this period. A confounding challenge of greenhouse-
6 based feedback experiments is that field soils may contain both negative and positive soil partners.
7 Strictly using greenhouse soils, for example, allows the quantification of negative soil conditioning by
8 phytotoxic plants but also excludes the potentially beneficial effects of symbiotic soil partners found in
9 field soils (e.g., Callaway and Aschehoug 2000). Plants grown in conditioned greenhouse soils may thus
10 suffer the effects of negative feedbacks, of an absence of beneficial partners, or both. To account for
11 this, we established our fescue and prairie mesocosms on three different soil types: 100% greenhouse
12 soil, or greenhouse soil mixed with either field-collected fescue soils or prairie soils (both at 9:1 ratios).
13 The field soils served as an inoculant for potentially beneficial microbes, but were diluted to 9:1 ratios to
14 reduce the potential concentrations of *m-tyrosine* introduced in the field soils. The net result is that the
15 primary *m-tyrosine* effects should derive from the mesocosm fescue sod, and not the added field soil.

16 After 12 weeks, seed was added at high (32 seeds per replicate) or low densities (8 seeds per
17 replicate). There were four treatments, testing the interacting effects of cover type, resource pulses,
18 disturbance, and soil type on invasion into the mesocosms. The resource pulses were N fertilization,
19 given its importance as a limiting resource in tallgrass prairie (Milchunas and Lauenroth 1995, Maron
20 and Jeffries 2001; Gross et al. 2005). The disturbance was clipping, which reduced canopy height to five
21 cm. Fertilization began immediately at planting of the mesocosms in October. Clipping occurred three
22 months after mesocosm construction, just prior to seed addition. The experiment was a full factorial
23 design. For each cover type, there were three replicates of fertilization and clipping, and two control
24 replicates, on the three soils. We measured establishment, growth rate, and survival of seedlings at three
25 intervals over three months.

1 The third experiment tested the effects of fescue and prairie root washes on germination, with
2 the assumption that these washes capture root exudates including *m-tyrosine*. We did not test this
3 directly, but given the documented strength of this chemical (e.g., Bertin et al 2007) we assumed that
4 toxicity effects would be detectable (which they were) even if our dosage levels may have been low
5 relative to field concentrations. Roots were harvested from the unfertilized and unclipped mesocosms of
6 the second experiment. We manually removed soil particles, but did not wash the roots. Roots were then
7 placed in 400 ml of double-deionized water until the contents totalled one litre. Roots were soaked for
8 three days at 4 °C, after which the samples were passed through cheesecloth to remove soil debris.
9 Given that the root washes could potentially contain fescue- or prairie species microbes that would
10 affect germination, the solutions were next passed through a .22 µm syringe filter to greatly reduce the
11 microbial fraction. Finally, we added the solutions to petri dishes containing 35 seeds of either fescue or
12 cold and moisture-stratified *A. gerardii*. The timing and percentage of seeds that germinated were
13 recorded until no new germinants emerged, which occurred after six weeks. Each seedling was removed
14 after it was counted.

15 In the fourth experiment, we examined the effect of the fescue and prairie root washes on
16 mycorrhizal colonization, using the micro-filtered root washes described above. We conducted this
17 experiment on the mycorrhizal fungus *Glomus intraradices* (Schenck & Smith) growing on sterile root
18 organ culture of carrot (*Daucus carota* L.) (Antunes et al. 2007). Fungal propagules of *G. intraradices*,
19 isolated from a carrot root organ culture (Premier Tech, IRBV'95) that was established in 1995, were
20 transferred to a non-mycorrhizal carrot root organ culture that was established in 1996 (Antunes et al.
21 2007). Both the mycorrhizal and non-mycorrhizal root organ cultures were further propagated by the
22 placement of plugs of mature root organ cultures (6–8 weeks) on new medium (Becard and Fortin
23 1988). We then added 100 ml of each root wash preparation (fescue or prairie), as well as a control
24 treatment using only double-deionized water, to the M-Medium after autoclaving and cooling to ~60
25 °C. Plates with each extract addition were randomized and then 4 cm lengths of the mature root organ

1 cultures were added to the center of plates. New root organ cultures received plugs either with or
2 without *G. intraradices*. Each of the three extract additions were replicated 10 times on root organ
3 cultures either with or without *G. intraradices*, for a total of 60 experimental units (30 with AM fungi,
4 30 without). All root organ cultures were then randomly placed in an unlit growth chamber set at a
5 constant temperature of 26 °C. After 13 weeks, we quantified root length, hyphal length and estimated
6 spore abundance using the grid-line intersect method (Tennant 1975). For root intersections we counted
7 six intersections per plate. For hyphae and spores we counted four intersections per plate.

8 *Statistical analysis*

9 All experiments were analysed using ANOVA, using either JMP 8.0 for experiments 1-3 (SAS) or R for
10 experiment 4 (R Development Core Team 2008). All data were log + 1 transformed to normalize their
11 distribution. Post-hoc comparisons followed Fisher's protected LSD procedure, restricting post-hoc
12 Tukey's test comparisons to significant higher order interactions.

13

14 **Results**

15 *Experiment 1*

16 The field-collected fescue soils significantly inhibited the germination, rate of growth, and
17 seedling survival of the native C₄ grass *A. gerardii* ($F_{2,163} = 11.7$, $p < 0.0001$; Tukey's test), with an
18 average of only 2.1 (SE 0.04) seedlings emerging per pot over three weeks (Table 1). Of the 31/200 pots
19 that failed to produce any seedlings after three months, 27 were for *A. gerardii* planted in fescue soils.
20 Germination and survival of fescue showed no effects of soil type (fescue or *A. gerardii* field soils)
21 (Table 1). Growth rates of fescue, however, were significantly higher on the native soils during the first
22 week ($F_{2,293} = 6.1$; $p = 0.003$). Growth of fescue was 0.45 cm per day at this time (SE = 0.01), compared
23 to less than half that rate in its own soil and in the greenhouse soils (Table 1). There was no significant
24 difference in emergence time between the fescue seeds and the native seeds, averaging 8.13 (0.7 SE)
25 and 9.10 (0.5) days respectively ($F_{2,163} = 0.15$, $p = 0.84$).

1 For both grasses, the numbers of surviving seedlings and the speed of seedling emergence were
2 significantly higher on the greenhouse soils than the field soils (seedlings: $F_{2,163} = 24.7$, $p < 0.0001$,
3 Tukey's test; emergence: $F_{2,163} = 3.1$, $p = 0.04$, Tukey's test) (Table 1). This effect was likely explained
4 by substrate, as the field soils had sands and fine gravel that appeared to become moisture limited even
5 with daily watering (personal observation). This suggests that field-based recruitment in this system may
6 be challenged by abiotic conditions, in addition to any biotic influences including allelopathy.

7 *Experiment 2*

8 Using vegetation height as a surrogate for community structure in general (Grime 2001), the
9 fertilization, clipping, and soil treatments had strong influences on mesocosm development. The
10 development of the unclipped prairie mesocosms were influenced by two factors: soil type and
11 fertilization ($F_{2,35} = 3.60$; $p = 0.043$). Average grass height was lowest in the fertilized control soils
12 (31.7 cm [SE 1.75]; Tukey's test), which was associated with dense crowding. Native grasses grew
13 significantly taller in the fescue soils compared to native or greenhouse soils, regardless of fertilization
14 (unfertilized 50.4 cm [5.7]; unfertilized 48.7 [1.9]; Tukey's test). We could not differentiate if this
15 effect was a result of fescue soils stimulating growth directly or indirectly by reducing crowding by
16 suppression (i.e., there was a phytotoxic effect of the fescue field soil, even though it was diluted to
17 10%). These differences, however, had no significant influence on recruitment success.

18 Recruitment success in the mesocosms was determined by interactions between seed type, seed
19 density, and cover type, ($F_{1,35} = 14.1$, $p = 0.0002$) with high additions of fescue seed resulting in more
20 establishment in the native mesocosms compared to fescue mesocosms (Tukey's test). This result
21 occurred independently of disturbance, fertility, or soil type. Native grass, in contrast, was a relatively
22 poor invader, establishing in small numbers regardless of cover type, soil type, or propagule pressure
23 (Tukey's test).

24 *Experiment 3*

1 Fescue root wash significantly suppressed germination of both species, compared to the A.
2 *gerardii* root wash and distilled water alone ($F_{1,48} = 4.48$; $p=0.017$). Seeds of *A. gerardii* were the most
3 suppressed, with an average of 43% failure (SE 5%) after 30 days (Tukey's test). Failure percentages
4 for *A. gerardii* in distilled water averaged 12% (SE 3%) and 26% (SE 6%) in its own root wash; these
5 two values were not statistically different (Tukey's test). Germination success of fescue in the root
6 wash of *A. gerardii* averaged 97% (SE 9%), which was significantly greater than germination in its
7 own root wash (71% [SE 3%]).

8 Unlike experiment one, *A. gerardii* was significantly slower to emerge than fescue, with this
9 effect exacerbated by the fescue root wash ($F_{2,157} = 3.43$; $p=0.035$). There was no difference in *A.*
10 *gerardii* emergence in distilled water vs. *A. gerardii* extract (Tukey's). Fescue emergence times did not
11 differ by extract type (Tukey's test).

12 *Experiment 4*

13 Density of AM fungal hyphae did not differ among the control treatment (double-deionized
14 water) and root washes from *A. gerardii* and fescue ($F_{2,27}=1.41$, $p=0.26$). While root washes did
15 increase sporulation of AM fungi ($F_{2,27}=3.63$, $p<0.05$), these increases were similar between *A. gerardii*
16 and fescue ($p>0.5$). As expected, AM fungi reduced overall root density of in-vitro carrot roots
17 ($F_{1,54}=7.46$, $p<0.01$), but fescue root extracts did not significantly alter carrot root density alone
18 ($F_{2,54}=2.22$, $p=0.12$) or through interactions with AM fungi ($F_{2,54}=0.62$, $p>0.5$).

19

20 **Discussion**

21 Determining how soil feedbacks influence population expansion can be challenged by potential
22 interactions with non-allopathic factors, including the possibility that the latter can be more prevalent if
23 toxicity effects are slow to unfold (Levine et al. 2006). Our work supports this scenario. We found
24 significant negative effects of field-conditioned fescue soils and root washes on germination,
25 supporting the results of lab-based bioassays (Bertin et al. 2007). Recruitment by fescue within the

1 mesocosms, however, was associated mostly with non-allelopathic factors including seed density,
2 rapid and high seed emergence, and fast growth rates determining invasion. We did not directly test for
3 positive soil feedbacks in fescue, where its soil effects favour its own growth and fecundity. However,
4 our observations of negative intraspecific impacts on germination and mesocosm invasion suggest that
5 fescue restricts its own performance when at higher abundance, at least in terms of recruitment from
6 seed. Allelopathy, therefore, would function more for persistence, by preventing recruitment by other
7 species once dominance has been attained.

8 The initial stages of mesocosm invasion by fescue were explained by two factors: seed density
9 and established cover type, with native grassland being more susceptible to invasion. These results
10 suggest the presence of favourable microsites within the mesocosm, with higher propagule pressure
11 increasing the odds of hitting these locations. This is consistent with observations of invasion by
12 propagule pressure in general (von Holle and Simberloff 2003; Lockwood et al. 2005, Colautti et al.
13 2006; MacDougall and Wilson 2007). It is also consistent with observations of microsite availability in
14 xeric prairies such as ours, where sandy substrates result in a structurally more open canopy that
15 facilitates invasion (White and Glenn-Lewin 1984). The disturbance and fertility treatments did not
16 significantly change this result, although recruitment success by fescue was slightly reduced with
17 fertilization presumably due to increased stand density and lower microsite availability (MacDougall
18 and Wilson 2007).

19 Propagule pressure can operate in a strictly probabilistic sense, where more seeds mean a higher
20 likelihood of winning the establishment lottery (e.g., Sale 1977). If this was the only factor determining
21 success, however, we would expect similar levels of recruitment by the native species. The native grass
22 was added at exact densities and showed similar success rates in the germination trials (experiment 1).
23 This did not translate into invasion success in the mesocosms, however, suggesting that factors in
24 addition to seed density favoured fescue invasion. These factors appear to be a range of traits including
25 high germination success, rapid emergence times, and fast rates of seedling growth. For the subset of

1 seeds that hit favourable microsites by probability, these traits appear to magnify establishment
2 success. This is consistent with previous observations of establishment by fescue in undisturbed
3 perennial grass monocultures, where establishment rates of ~40% and emergence times averaging eight
4 days (Milbau et al. 2003). In that study, seedlings surviving the initial establishment stage subsequently
5 had high levels of survival (Milbau et al. 2003), with growth rates thus determining long-term success,
6 instead of factors such as seed mass which is typically described as more important (Ross and Harper
7 1972; Thompson et al. 2001).

8 Native grass, in contrast, struggled to recruit in all mesocosm environments. These results are
9 consistent with the life history of prairie grasses, where seed bank dormancy, high stratification
10 requirements, and larger seed mass (but slower seedling growth rates) are important recruitment
11 mechanisms in highly variable prairie environments. What has become unclear is whether those
12 recruitment strategies remain relevant given the habitat changes that are affecting tallgrass remnants in
13 general, and whether fine fescue grasses are better matched to these changes (e.g., MacDougall and
14 Turkington 2005). Recruitment dynamics in tallgrass prairie are typically associated with fire and
15 grazing, neither of which occurs in fragmented prairie sites including our study area. In the absence of
16 disturbance, recruitment from seed may constitute as little as 2% of total native grass recruitment per
17 year (Knapp et al. 1998). Fescue, however, appears capable of recruiting at least occasionally from
18 seed under these conditions, as described above. Fescue seed has no dormancy capabilities but also
19 needs no stratification (Williams 1983), which may allow it to rapidly respond to microsite favorability
20 in time and space.

21 Our results suggest that allelopathic effects are not necessarily needed for the expansion of
22 fescue into native tallgrass prairie. We also observed no impact on mycorrhizal hyphae and spore
23 production, presumably because *m-tyrosine* functions as a growth inhibitor of vascular plants, to which
24 fungi show no sensitivity. Its soil impacts, however, may play a substantial role in invader persistence
25 and impact on dominance has been attained. These results are consistent with the long-term persistence

1 of fescue dominated old fields in central North America, where diversity levels remain low and with
2 little tree invasion despite the absence of fire and grazing. It remains untested whether there are
3 intraspecific soil effects that constrain fescue performance at high density. In soil-based positive
4 feedback models of invasion, the soil effects increase invader fitness thereby accelerating invader
5 expansion. Our results, however, showed that fescue germination and seedling growth rates were
6 significantly higher in tallgrass soils, root washes, and mesocosms, with lower germination in its own
7 soils compared to ambient control treatments. We did not determine fescue soil effects on adult plants.
8 By implication, however, our data tentatively suggest that performance of individuals at the invasion
9 front could exceed those in the interior, as has been reported elsewhere (Dwyer and Morris 2006,
10 Hansen and Wilson 2006). Higher fitness by these plants could be largely responsible for population
11 expansion by seed output, lateral spread, or both, with allelopathic effects being relatively unimportant.

12 Tests of allelopathy are best conducted by matching potency levels in the field (Callaway et al.
13 2008), which we did not explicitly do. There may be other methodological limitations. Phytotoxin
14 production by fescue may increase when experiencing water stress (Duke 2007), but we watered daily.
15 We also did not test for endophytes, although it has been previously shown that an endophyte produced
16 by tall fescue (*F. arundinacea*) had low allelopathic effects on the germination and emergence of
17 tallgrass species (Renne et al. 2004). Despite these issues, we still observed significant negative soil
18 effects on recruitment. They were not as strong as those observed in lab bioassays for synthetic *m*-
19 *tyrosine* (Bertin et al 2007), which may be expected given the likelihood of microbial breakdown or
20 immobilization by binding to soil organic matter (Weston and Duke 2003; Bertin et al. 2009). However,
21 they suggest that the soil-based effects of fescue are relatively robust and contribute to fescue
22 persistence at some level.

23 In conclusion, two factors have limited the testing of how soil feedbacks influence population
24 expansion, both of which we accounted for. We saw suppressive effects despite the likelihood of
25 dilution compared to lab bioassays. We also saw that spread can occur independently of allelopathic

1 effects. Past studies of feedbacks have measured effects using soils already conditioned by the invader
2 (Bever 1994, Klironomos 2002), leaving unanswered the question of how spread occurs in unmodified
3 soils beyond the invasion front (Levine et al. 2006). Our work shows that this can occur exclusively by
4 non-allelopathic mechanisms, with toxicity effects potentially emerging later. These results are
5 consistent with the prediction that soil effects can unfold in a range of ways depending on the traits of
6 the invader, and the rate at which toxicity levels build in the soil. Our results also imply that exotic
7 allelopathic species need not also be highly invasive species, unless there are mechanisms that also
8 drive its population expansion.

9

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14

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1 Table 1. Mean growth rates (cm per day), emergence times (days), and the total number of emerged
 2 seedlings (from 30 planted seeds) for the invasive grass *Festuca rubra* and the native prairie species
 3 *Andropogon gerardii* [1 SE]. The data derive from three soil types: field-collected *Festuca* soil, field-
 4 collected prairie soil, and greenhouse soil. The two growth rates measures per soil type are after one
 5 week and three weeks respectively. Letters refer to Tukey's test comparisons, and are specific to each
 6 row.

7

Species	Festuca soil		Prairie soil		Greenhouse soil	
growth rates						
<i>Festuca rubra</i>	0.20 ^c [0.01]	0.27 ^c [0.02]	0.45 ^a [0.02]	0.20 ^c [0.01]	0.25 ^c [0.01]	0.34 ^b [0.02]
<i>Andropogon gerardii</i>	0.03 ^a [0.006]	0.13 ^b [0.01]	0.29 ^c [0.01]	0.18 ^b [0.01]	0.22 ^b [0.01]	0.23 ^b [0.01]
emergence time						
<i>Festuca rubra</i>	8.13 ^a [0.05]	-	8.15 ^a [0.05]	-	6.15 ^b [0.03]	-
<i>Andropogon gerardii</i>	10.2 ^a [0.07]	-	9.1 ^a [0.04]	-	7.3 ^b [0.04]	-
total emerged seedlings						

<i>Festuca</i>	16.7 ^a [2.1]	-	15.1 ^a [1.1]	-	25.4 ^b [1.3]	-
<i>rubra</i>						
<i>Andropogon</i>	2.1 ^a [0.04]	-	5.5 ^b [0.05]	-	10.7 ^c [1.3]	-
<i>gerardii</i>						

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1 Table 2. Summary of factors that significantly influenced invasion success in the constructed
 2 mesocosms over three months. Seed type: *Festuca rubra*, *Andropogon gerardii*; seed density (high [32
 3 seeds per pot]; low [8 seeds per pot]); Treat: all possible combinations of fertilization and clipping;
 4 Soil: fescue soil mixture, native soil mixture, 100% greenhouse soil; Date: monthly sampling intervals
 5 (n = 3); Cover: fescue, native prairie. Non-significant values are not shown.

6

Source	DF	F	p
Seed Type	1	207.8	<0.0001
Seed Type*Seed Density	1	46.7	<0.0001
Seed Density	1	46.2	<0.0001
Date	4	21.8	<0.0001
Date*Seed type	4	10.6	<0.0001
Treat*Soil*Seed Type	4	4.3	0.001
Date*Seed Density	4	4.3	0.001
Treat*Soil	4	4.2	0.001
Cover*Date	4	4.1	0.002
Cover*Date*Seed Type	4	3.8	0.004

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