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Tall fescue is a potential spillover reservoir host for *Alternaria* species

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Abstract: The spread of invasive species is complicated and multifaceted. Enemy spillover (i.e. the transfer of a natural enemy from a reservoir host to a novel host) is one mechanism that facilitates the spread of non-native species. The reservoir host is a species that harbors high abundance of the enemy with little cost to fitness. We asked whether *Schedonorus arundinaceus* (tall fescue), a highly invasive grass species in North America, is a potential reservoir host for the ubiquitous genus of fungi, *Alternaria*. We also asked whether spillover of *Alternaria* is possible among grasses that commonly occur with *S. arundinaceus* in grassland ecosystems. We performed a greenhouse cross inoculation of three isolates of *Alternaria* and six grass species (three native, three invasive, including *S. arundinaceus*). We determined that spillover is possible because the fungal isolates infected and caused disease symptoms on all six grasses and decreased biomass in two of the grass species. We also determined that the invasive grass species appear to be more competent hosts than the native species and that *S. arundinaceus* could be a likely reservoir host for *Alternaria* spp. because it can harbor the pathogen with no apparent fitness cost.

Key words: invasive, native, pathogen, *Schedonorus arundinaceus*, spillback

Introduction

Pathogens can facilitate the invasion of non-native species through a process called spillover (Power and Mitchell 2004, Daszak et al. 2000), which occurs when an enemy, such as a pathogen or herbivore, is transferred or spills over from a reservoir host onto other, nearby hosts (Daszak et al. 2000, Power and Mitchell 2004). The reservoir host harbors high abundance of the enemy but suffers few consequences to its fitness and performance (Power and Mitchell 2004). Thus, the occurrence of pathogen spillover can increase the prevalence of the reservoir host through apparent competition (Holt et al. 1994, Beckstead et al. 2010), that is the reservoir host can more easily out-compete its neighbors if they are negatively affected by the higher abundance of the new enemy.

We specifically examined the potential of spillover among co-occurring grass species that are common in extant prairies and grassland ecosystems of the western USA. Since European settlement, prairies have sustained a larger percent decrease than any other ecosystem, including old growth forests, and account for less than 1% of their original area in some regions (Sampson and Knopf 1994). The competitive exclusion of native species by invasive species is a major obstacle for the success of restoration of native prairies (Ewing 2002, Pfeifer-Meister et al. 2008). Spillover may be one of the mechanisms that contribute to how invasive species gain this competitive advantage in prairie ecosystems (Malmstrom et al. 2005, 2007; Borer et al. 2007; Beckstead et al. 2010). Thus, it is important to identify reservoir species that harbor pathogens and to determine whether spillover can occur among cohabiting species within these plant communities.

We were particularly concerned about the non-native grass tall fescue, *Schedonorus arundinaceus* (Schreb.) Dumort. (synonyms = *Festuca arundinacea*, *Lolium arundinaceum*), as a potential reservoir host for fungal pathogens. Introduced from Europe in the late 19th century, *S. arundinaceus* is now one of the most prevalent perennial grasses across North America (Clay and Holah 1999). It is largely planted for forage, turf and soil conservation, but it has also become an invasive species and is a noxious weed in many ecosystems across North America (Clay and Holah 1999, Garrison and Stier 2010), including prairies of the western USA (Pfeifer-Meister et al. 2008, Severns and Warren 2008). Due to its prevalence across North America as both a crop and as a major invader in prairie ecosystems, it is important to determine whether *S. arundinaceus* could be a reservoir species for pathogens.

We surveyed fungal pathogens isolated from symptomatic leaf tissue samples of *S. arundinaceus,* which were collected from both its introduced range in North America and its native range in Europe. *Alternaria* species were the most common fungi isolated from leaf samples from both ranges. *Alternaria* species often are

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cited to be among the most common fungal species found on grasses and are known for their ubiquitous nature as saprotrophs and endophytes (Dugan and Lupien 2003, Gannibal 2004, Sánchez Márquez et al. 2011). However they also can be pathogenic, or latent pathogens—species that become pathogenic later in their life cycles (e.g. after sporulation) (Sánchez Márquez et al. 2011). *Alternaria* species are well known for producing a range of host-specific mycotoxins that can cause disease on a wide variety of economically important crop species (Thomma 2003, Kosiak et al. 2004). Despite their common occurrence on many species of grass, there is little information about the pathogenicity or differential infection ability of *Alternaria* species on prairie grasses.

To determine whether *S. arundinaceus* could act as a reservoir host, we chose three isolates of *Alternaria* from our survey that differed morphologically as the potential spillover enemies in this experiment. We performed a cross inoculation of the three *Alternaria* isolates onto *S. arundinaceus* and five other grass species that co-occur in grassland ecosystems of western USA. We were interested in determining whether the fungal isolates can infect and cause disease on all six grass species (i.e. we tested whether spillover of these *Alternaria* isolates is possible). Because of the cosmopolitan nature of *Alternaria*, we expected that all three fungal types would infect all six grass species, however we expected the degree of disease to differ among grass species and fungal isolates. If *S. arundinaceus* is a good reservoir species for these fungi, we hypothesized it would be easily infected and harbor the pathogen, with little loss of fitness relative to the other species.

MATERIALS AND METHODS

Grass species (potential hosts).—In addition to *Schedonorus arundinaceus*, we chose five other grass species that are abundant and commonly occur together in western prairie ecosystems: three native and two other introduced species. The three native species occur in most of the United States west of the Mississippi and include *Agrostis exarata* Trin. (spike bentgrass), *Danthonia californica* Bol. (California oat grass) and *Deschampsia cespitosa* (L.) P. Beauv. (tufted hair grass). *Deschampsia cespitosa* is the dominant grass in Pacific Northwest prairies and it is a circumboreal species that is native in both North America and Europe (Davy 1980, Clark and Wilson 2001). The two additional introduced species are *Anthoxanthum odoratum* L. (sweet vernalgrass) and *Holcus lanatus* L. (common velvetgrass). These two species also are common invaders worldwide (Cocks 1994, Buckland et al. 2001, Clark and Wilson 2001, Corbin and D'Antonio 2010, Pickering et al. 2011).

Fungal Isolates (potential enemies).—We isolated and identified fungal species from symptomatic leaf tissue samples collected from wild populations of *Schedonorus arundinaceus*. *Alternaria* species were among the most common fungi identified from these collections (25% of all samples, H. Wilson unpubl data). They were also the most common fungal species identified from a pathogen survey of the other two introduced grasses used (H. Wilson unpubl data) and have been identified from tape lifts taken from all six grass species in a related field study (Blaisdell 2011).

We identified the fungal isolates first morphologically and then by comparing the internal transcribed spacer (ITS) region rDNA sequences (with primers ITS1F and ITS4, which contain 18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, complete sequence and 28S ribosomal RNA gene, partial sequence) with data from GenBank BLAST queries. The first isolate used in this experiment was identified as *Alternaria infectoria* E.G. Simmons (GenBank accession number KC985250) and was isolated from a leaf sample of *S. arundinaceus* collected from grassland in Flaach, Switzerland (47.59°N, 8.61°E, 347 m). The second isolate was indentified as *Alternaria alternata* (Fr.) Keissl (GenBank accession number KC985248) and was isolated from a leaf sample of *S. arundinaceus* collected from a grassland in Crooked Creek, Wisconsin, USA (42.83°N, 88.477°W, 276 m). The third isolate also was identified as *A. alternata* (GenBank accession number KC985249) and was isolated from a leaf sample collected from the same *S. arundinaceus* population as the *A. infectoria* in Switzerland.

Although the two *A. alternata* isolates had identical ITS sequences, they had obvious morphological differences in culture; one isolate was a dark grayish brown and the other was pale gray. *Alternaria alternata* is a variable species complex and a number of *Alternaria alternata* species are distinct pathotypes producing different host-specific toxins (Nishimura and Kohmoto 1983). Thus, we treated these two *A. alternata* isolates as different potential pathogens, designated *A. alternata* 1 and *A. alternata* 2.

Experimental design.—To determine whether all six host species could be infected by the three *Alternaria* isolates, we used a randomized, fully crossed design in which each host species was inoculated with a spore suspension of each fungal isolate or a control solution containing no spores. Seeds were obtained from local seed companies of the southern Willamette Valley of Oregon, which obtain seed from local populations, or we used seed produced in nurseries that used the same local populations. The seeds were surface-sterilized with a 10% commercial bleach solution before being grown in flats 8 wk. The seedlings were transplanted to 13 × 13 cm, 500 mL pots containing one seedling per pot, with Black Gold® potting soil (Sun Gro Horticulture, Agawam, Massachusetts) until the plants were well established (150 d).

We used a total of 384 plants, 18 replicates of each plant species/treatment combination, except *Danthonia californica*, which had only six replicates of each treatment due to low germination. We attempted to ameliorate the poor germination of *D. californica* by treating the seeds with a 10% gibberellic acid in tap water. We did not treat seeds of

the other five grass species with gibberellic acid in that they had already germinated. After inoculation, we placed the plants in a predetermined, randomized order approximately 25 cm apart so that they did not touch one another in the greenhouse. The greenhouse was kept at approximately 25 C with 10 h/d natural light.

Inoculation.—To inoculate the plants with the *Alternaria* isolates, we prepared spore suspensions of each fungal isolate. To maximize sporulation, we plated the isolates on a starving medium of 2% agar containing 0.1% dextrose and 0.1% malt extract and let the fungi sporulate 1 wk under constant light in the greenhouse at 25 C. Twelve plates of dense spores from each fungal isolate were used to create the spore suspensions.

We washed the plates with 2–5 mL 0.05% Tween 80 (ICI Americas Inc., Wilmington, Delaware) in sterile deionized water. Because the spores are hydrophobic, the Tween 80 solution helped with separation of the spores from the agar plates (Sautour et al. 2003). We gently scraped the plates with a glass spreader and collected the spores in a 50 mL flask. For each fungal isolate we collected 40 mL of concentrated spore suspension containing 0.05% Tween 80 and then diluted the suspension 12.5× with deionized water to 500 mL final volume. Using a blood cell-counter slide (Counting Chamber No. 3500, Hausser Scientific, Northbrook, Illinois), we estimated that the spore concentration of the suspension was 10^6 spore cells/mL for each fungal isolate.

We used an airbrush to inoculate the plants with the spore suspensions. To ensure sufficient application of the suspension, each plant was sprayed with 3–6 mL (depending on plant size) spore suspension, until the leaves were saturated. We sprayed the control plants with sterile deionized water containing the same concentration of Tween 80 as the treatments (0.004%). The airbrush was thoroughly rinsed with sterile deionized water between applications of each treatment. To increase germination of the spores on the leaf tissue immediately after inoculation we placed the plants in large black plastic bags and incubated them at room temperature 48 h. Plants were grouped by treatment inside the plastic bags. After 48 h incubation, plants were transferred to the greenhouse and arranged in the predetermined randomized, blocked design. Post inoculation the plants were grown 41 d and watered as needed.

Data collection and analysis.—Height and width of all plants were measured 1 wk before applying the fungal isolates to obtain a measurement of pre-inoculation plant size. To verify germination of the fungal isolates on each host species, we collected tape lifts from the leaves of two individuals from each host species/treatment combination on the eighth day post inoculation; all plants had germinating fungal spores. On days 19 and 34 post inoculation, all individual plants were surveyed for total plant damage and overall senescence. Senescence was measured because *Alternaria* infection is known to cause senescence in leaves (Tu 1985, Jia et al. 2010), including those of grasses (Bertelsen et al. 2001). To measure total plant damage, we visually estimated the total area of leaf tissue with disease-like symptoms, which included various types of lesions, but mostly a tan and white lesion typical of *Alternaria* infections (FIG. 1a, b, d; Thomma 2003, van Kan 2006), recorded as a percentage of the entire plant (Nita et al. 2003). Similarly senescence was measured by visually estimating the proportion of the plant with senesced tissue. The same individual performed all observations, to maintain consistency in visual estimations.

On day 41 post inoculation, we harvested aboveground biomass and dried the plants for 24 h at 60 C before weighing. Because the plants had become pot-bound, we did not measure belowground biomass. We used biomass as an estimate of plant fitness because larger plants tend to reproduce more than smaller plants of the same species (Bazzaz et al. 1987). In addition, a related field study containing the same six grass species planted from the same seed sources found that vegetation biomass was significantly positively correlated with reproductive biomass for all six grass species (G.K. Blaisdell unpubl data). At the time of harvest we incubated leaf samples from all host species/fungal treatment combinations in humid chambers to verify that *Alternaria* spores germinated from the tissue. This was done to ensure that the damage surveyed was caused by *Alternaria* (FIG. 1c).

We collected data for plant damage and senescence twice: on day 19 and day 34 post inoculation. We therefore used repeated measures ANOVAs to compare the level of plant damage and of senescence among treatments, host species and their interaction at each collection time. We also included block as a random factor to determine whether placement in the greenhouse had an effect on total plant damage and/or senescence. A Tukey's HSD comparison was used to determine differences among host species in the overall ANOVAs of damage and senescence. We performed univariate ANOVAs of individual host species when we found a significant host by treatment interaction and used an a priori least squares contrasts to determine whether the fungal treatments significantly differed from the control treatments.

We ran a similar model to test the effects of the treatments on plant final biomass, with the addition of the covariate pre-inoculation plant width to correct for differences in initial plant size. We performed a logit transformation on total plant damage and senescence, which are proportion measures, to better fit a normal distribution of the residuals. Analyses were done with JMP® Pro 9.0.2 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Our tape mounts revealed germinating *Alternaria* spores on all host species from all fungal treatments. To determine whether the visible damage we recorded was from our *Alternaria* inoculations, we placed symptomatic leaf samples, at time of harvest, in humid chambers. Within these chambers, we observed *Alternaria* sporulating densely on symptomatic leaf tissue from all host/fungal treatment combinations (FIG. 1c). In contrast, there was little sporula-

FIG. 1. A. Total plant view of invasive *Anthoxanthum odoratum* infected by *Alternaria*. B. Close up of infected *A. odoratum* leaf demonstrating leaf lesions. C. Dense *Alternaria* (*A. alternata* 2) sporulation on symptomatic *Schedonorus arundinaceous* leaf sample taken post harvest and incubated in humid chambers. D. Symptomatic tissue of native *Agrostis exarata*.

tion from the controls. Although we did not re-isolate and re-sequence the *Alternaria* sporulating on the leaf samples, it is unlikely the visual damage we observed (FIG. 1a, b, d) was caused by an unknown fungal contamination. *Alternaria* made up nearly 100% of all fungal sporulation on the symptomatic leaf samples in the humid chambers (FIG. 1c), the type of visual leaf lesions were consistent across the six plant species and were consistent with what others have reported (Thomma 2003).

In our repeated measures analysis, total plant damage differed among the host species ($F_{5,342} = 77.48$, $P < 0.0001$), as well as fungal treatment ($F_{3,342} = 13.87$, $P < 0.0001$). Plants inoculated with any of the three *Alternaria* treatments had more damage than the control plants (least squares contrast: $F_{1,342} = 39.67$, $P < 0.0001$). The amount of damage from the fungal treatments depended on the host species (host species by fungal treatment interaction: $F_{15,342} = 1.67$, $P = 0.05$). Placement in the greenhouse also affected damage, as indicated by a significant block effect ($F_{17,342} = 2.30$, $P = 0.0026$), although we minimized the bench effects as much as possible with our randomized design, and there was not a significant interaction between treatment or host with placement in the greenhouse.

Time of measurement also mattered ($F_{1,342} = 81.74$, $P < 0.0001$), with less damage at the second census. In 20/24 fungal/host combinations (83.3%), there was less damage to the host at the second census

TABLE I. Percent change in plant damage and senescence between the two census dates: day 19 and day 34

	Percent change in plant damage				Percent change in plant senescence			
	Treatments							
Grass hosts	A. alt 1	A. alt 2	A. inf	Control	A. alt 1	A. alt 2	A. inf	Control
Agrostis exarata	−0.83	−0.97	−0.05	−0.06	−19.17	−6.67	−7.50	−13.94
Anthoxanthum odoratum	−0.45	−0.53	−1.00	0	−1.61	−2.00	−3.11	−3.22
Danthonia californica	0.16	−0.41	−0.70	−0.50	−2.17	−3.25	−1.60	−1.40
Deschampsia cespitosa	−0.41	−0.14	−0.03	−0.03	1.22	0.45	10.72	−0.16
Holcus lanatus	−4.25	−4.84	−5.83	−0.80	22.78	35.77	23.22	22.22
Schedonorus arundinaceus	−0.83	−0.24	0.41	−0.08	−3.45	−4.65	1.67	−8.90

(TABLE I). The treatments affected the hosts differently at the two census dates (time by treatment interaction: $F_{3,342} = 2.64$, $P = 0.0493$), although this was driven mainly by *Holcus lanatus* moving from most damaged at the first census to the least damaged at the second census, whereas the other species maintained their relative damage rankings. The effect of placement in the greenhouse on damage did not change over time (no block by time interaction: $F_{17,342} = 1.44$, $P = 0.1166$). There was a significant time by host by treatment interaction ($F_{15,342} = 1.44$, $P = 0.0012$). While damage was generally less in the second census, for *Danthonia californica* there was more damage by both *A. alternata 1* and *A. infectoria* at the second census (TABLE I). There was also more damage on *Schedonorus arundinaceus* by *A. infectoria* in the second census (TABLE I).

Because we found a significant interaction between host and treatment for total plant damage and we found the most damage during the first census, we focused on the first census. *Holcus lanatus* (invasive) and *Anthoxanthum odoratum* (invasive) had the highest damage; *Danthonia californica* (native) and *Schedonorus arundinaceus* (invasive) had intermediate damage; and *Agrostis exarata* (native) and *Deschampsia cespitosa* (native) had the least damage (Tukey's HSD $P < 0.05$, FIG. 2).

We performed individual univariate ANOVAs of each host to determine differences between the treatments for the first census (FIG. 2a). In these analyses, the a priori least squares contrasts revealed that the fungal treatments had more damage than the control treatment for all three introduced grasses (*Anthoxanthum odoratum* $F_{1,68} = 16.7$, $P < 0.0001$, *Schedonorus arundinaceus* $F_{1,68} = 11.7$, $P = 0.0011$, *Holcus lanatus* $F_{1,68} = 37.2$, $P < 0.0001$) and for the cosmopolitan *Deschampsia cespitosa* ($F_{1,68} = 4.2$, $P = 0.044$). For the native grass *Agrostis exarata*, we saw a trend that the fungal treatments had more damage than the controls ($F_{1,51} = 2.65$, $P = 0.108$), but for the native *Danthonia californica*, there was no difference between the fungal treatments and controls ($F_{1,13} = 0.31$, $P = 0.58$).

The repeated measures analysis of total plant senescence showed that senescence varied among host species ($F_{5,270} = 70.89$, $P < 0.0001$, TABLE I). However, there was no effect of fungal treatment ($F_{3,270} = 0.32$, $P = 0.8116$), treatment by host interaction ($F_{15,270} = 0.95$, $P = 0.5058$), nor did senescence depend on where the plants were located in the greenhouse (no block effect: $F_{17,270} = 0.66$, $P = 0.8438$). However, some results depended on time. In general there was less senescence at the second census than the first; it was lower in 16/24 (66.7%) host/treatment combinations (TABLE I, $F_{15,270} = 71.68$, $P < 0.0001$). The hosts differed in senescence at the two census dates, while most had less senescence at the second census, both *Deschampsia cespitosa* and *Holcus lanatus* had more (TABLE I). There were no effects of time (census date) on treatment ($F_{3,270} = 1.03$, $P = 0.3808$), nor was there a time by host by treatment interaction ($F_{15,270} = 0.56$, $P = 0.9067$).

We used final biomass to estimate the effects of each *Alternaria* isolate on host fitness (FIG. 2b). Not surprisingly, biomass differed among host species ($F_{5,313} = 29.83$, $P < 0.0001$), as did the covariate of pre-inoculation plant size ($F_{1,377} = 24.92$, $P < 0.0001$). There was no main effect of fungal treatment on biomass ($F_{3,313} = 0.82$, $P = 0.48$), however, there was a host species by treatment interaction, indicating that the treatments affected plant biomass differently depending on the host ($F_{15,313} = 1.96$, $P = 0.018$). Because of the significant interaction, we analyzed each host species individually. Because block was not significant and explained < 1% of the variance in the model, it was dropped from the tests of individual host species (Underwood 1981). The follow-up tests of individual host species showed reduced biomass in the fungal treatments relative to the controls for two of the six host species: the introduced *Anthoxanthum odoratum* (least squares contrast: $F_{1,64} = 4.04$,

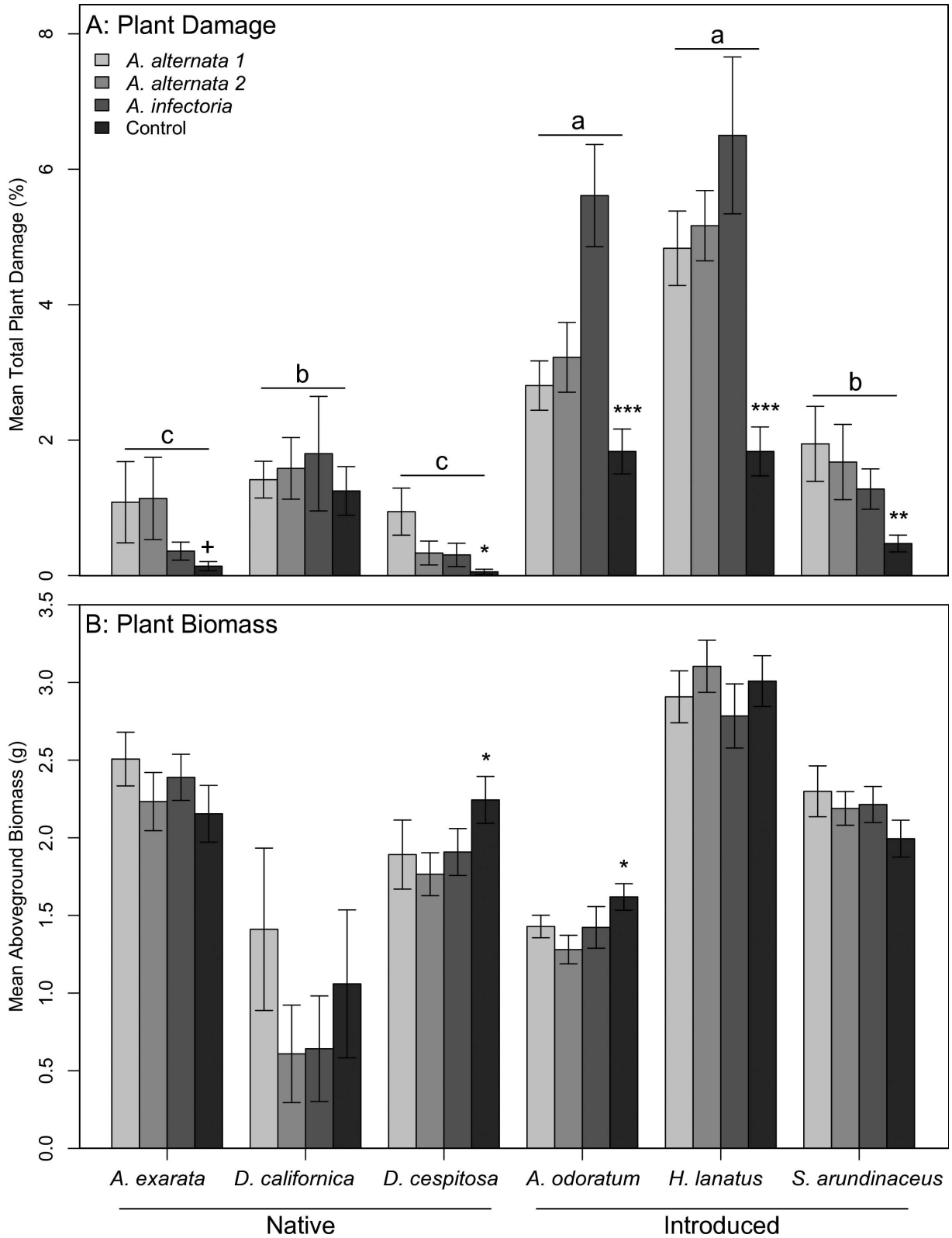

FIG. 2. A. Total plant damage of first census (day 19), measured as the percent of the plant with disease symptoms, caused by each treatment to each plant host. Letters indicate significant differences in damage among plant hosts while asterisks indicated significant differences among fungal treatments and controls within each plant host (*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$. + = $P < 0.15$). B. Final aboveground plant biomass (dry weight) at time of harvest for each host/treatment combination. Asterisks indicated significant differences among fungal treatments and controls within each plant host (* = $P < 0.05$). A, B. Bars are represented as one standard error.

$P = 0.049$) and the native *Deschampsia cespitosa* (least squares contrast: $F_{1,64} = 6.83$, $P = 0.010$).

DISCUSSION

Our results reveal that all three *Alternaria* isolates can infect and cause disease on all six grass species, and thus spillover of *Alternaria* has the potential to occur among these co-occurring grass species. In addition, the low level of damage on the control plants and low level of *Alternaria* sporulation found on the control tissue in the humid chambers post harvest indicates some airborne or water-splash transmission (Thomma 2003) in the greenhouse post inoculation. Although we did not originally intend this result, the ease of transfer of the *Alternaria* spores provides further evidence that spillover of *Alternaria* species among co-habitating grasses is probable within plant communities. The finding that all three *Alternaria* isolates could cause disease on the six grass species is novel because we establish that *Alternaria* can be pathogenic on these grass species given the right conditions, where previously *Alternaria* species were considered largely to be somewhat innocuous and saprotrophic endophytes on most grasses (Sánchez Márquez et al. 2011).

We did not see significant differences among the three fungal isolates in any of the grass species and conclude that these particular isolates have essentially the same pathogenicity on these grass species. It is also possible that, because there was some spore transfer within the greenhouse, differential effects between the fungal treatments might have been reduced. However, because there was relatively little spore transfer among plants within the greenhouse, compared to the initial inoculation, the conclusion that all isolates had essentially the same pathogenicity is more probable.

Our results also provide evidence that tall fescue, *Schedonorus arundinaceus*, could be a reservoir host for these *Alternaria* species. It had moderate visible damage, similar to or more damaged than the native species, but less damage than the other invasive species (FIG. 2a), and we did not see any differences in biomass between the fungal treatments and controls. Our results show that *S. arundinaceus* could be infected and harbor the fungi, with relatively small effects to its overall performance, and thus it has the characteristics of a reservoir species for *Alternaria*. Additional field studies are needed to test whether *S. arundinaceus* actually acts as a reservoir host for *Alternaria* and other potential pathogens in natural ecosystems and whether spillover of such pathogens may aid in its invasiveness.

Other highly invasive grass species have been shown to be good reservoir hosts for fungal and viral pathogens. Beckstead et al. (2010) showed that *Bromus tectorum*, the highly invasive cheatgrass, is a reservoir host for the fungal pathogen *Pyrenophora semeniperda*. Native species grown in areas planted with *B. tectorum* were more likely to die from *P. semeniperda* infection than native species planted in un-invaded areas (Beckstead et al. 2010). In addition, the invasive *Avena fatua* (wild oat) has been shown to be a reservoir host for barley yellow-dwarf virus. Its presence greatly increased the abundance of the virus on several other co-occurring species (Power and Mitchell 2004). It could be that invasive grass species have characteristics of being good reservoir hosts for fungal pathogens and other enemies, and this may be a causal factor in their invasiveness. However, further studies of spillover mechanisms and invasive grass species are needed to investigate this suggestion.

From our results *S. arundinaceus* appears to be a likely candidate as a reservoir host for *Alternaria* species in that it can harbor the pathogen without any cost to its performance. Similarly, the other invasive grasses, *Holcus lanatus* and *Anthoxanthum odoratum*, also appear to be easily infected (FIG. 2a). However, it is unlikely that *A. odoratum* would be a true reservoir host because it actually appears to be the most negatively affected by the *Alternaria* infection, sustaining the highest damage and a significant decrease in biomass (FIG. 2b). It is unclear whether *H. lanatus* could be a reservoir host because, while it had high damage with no effect on biomass, it also had a large percentage of senescence. This is not completely surprising, in that *H. lanatus* is more of a wetland species than the other grasses (Sánchez Márquez et al. 2010) and may have required more water than the other species in the greenhouse. Total senescence of the leaves significantly differed among grass species, but there was no treatment effect of senescence, indicating that senescence was largely an effect of the greenhouse environment combined with innate differences among hosts. Thus, it is hard to tease apart the question whether *H. lanatus* could be a reservoir species or whether the severity of disease was exacerbated by water stress in the greenhouse.

The spread of disease via *Alternaria* spillover is probable among the invasive hosts, and the high damage to *H. lanatus* and *A. odoratum* may serve to increase the inoculum density in that the incidence of disease (measured by number and size of leaf lesions) usually is positively correlated with inoculum density (El-Zik 1985, Chang and Hwang 2003, Carisse et al. 2008). However, it should be noted that the degree of damage we observed might be higher than what the plants would experience under natural conditions, due to our intentional inoculation using a concentrated spore suspension. On the other hand, our

plants were watered daily and protected in the greenhouse from other stresses (e.g. drought, freezing, predation) of their natural environment. Protection from environmental stresses could serve to increase the plants' ability to ward off infection (Schoeneweiss 1975). Because greenhouse experiments are innately limited in how much they can realistically extrapolate (Gibson et al. 1999), we stress the need for field experiments to determine how spillover of *Alternaria* may influence disease dynamics under natural conditions.

Working under the assumption that the more susceptible introduced hosts likely would increase the inoculum density within a plant community, cohabitation of these species could increase the chances of spillover onto neighboring native species. A variation of spillover, called spillback, happens when an introduced species serves as more competent host for native pathogens (Kelly et al. 2009). The higher than normal abundance of pathogens on the introduced species then can spill back onto native species at an increased rate compared to what the native species have been shown to do (Kelly et al. 2009). Because *Alternaria* species are ubiquitous across all types of grasses, we expect them to be present (whether native or not) in prairie ecosystems. The enhanced inoculum density among the invasive species could increase the potential of spillover or spillback onto neighboring native species.

Whether the native species are negatively affected by spillover is another question. The native *Agrostis exarata* appears to be the most resistant species to the *Alternaria* isolates. It had low overall percent visible damage, the controls only trended to have less damage than the fungal treatments ($P = 0.108$), and there was no significant difference in biomass among the treatments and controls at the final harvest. In addition, there was little to no damage on the *A. exarata* controls, suggesting that it is not easily infected via airborne or water-splash transfer. It also had the largest decrease in senesced material from the first to second censuses, indicating a large amount of new growth (TABLE I). This provides further evidence that *A. exarata* may be quite resilient to damage and stress from the initial fungal infection. This native species most likely would not be negatively affected by spillover of these fungi.

For the native *Danthonia californica*, we did not see any significant differences among any of the treatments. We suspect that the high variably in damage seen on *D. californica* (FIG. 2a) is likely due to the low sample size from poor initial plant germination rather than a trend that truly deviates from the other grasses. Of note, we did see relatively high overall damage compared to the other two native hosts (similar to that seen on *S. arundinaceus*), and the damage to controls also was quite high compared to the controls of the other native species (FIG. 2a). We also saw a slight increase in damage from the first to second census from both *A. alternata 1* and *A. infectoria* (two of three incidences of an increase in damage in TABLE I). The presence of accidental spore transfer onto the controls and the increase in damage from the first to second census dates suggests that *D. californica* may be particularly sensitive to *Alternaria* infection, however the low replicate number in our experiments of this species makes any definitive conclusion difficult.

In the native *Deschampsia cespitosa*, we saw a decrease in fitness, where biomass was significantly less for the fungal treatments compared to controls. Of interest however, we saw a relatively low amount of damage compared to other species. It could be that the pathogen was acting asymptomatically or that *D. cespitosa* is especially sensitive to even low levels of disease. This result provides evidence that *Alternaria* infection could be damaging to populations of *D. cespitosa*, a particularly important note in that *D. cespitosa* is widely used in restorations of prairie habitats (Clark and Wilson 2001, Paschke et al. 2005).

The consequences of spillover depend on the pathogen being transferred; thus it is important to know how common and how pathogenic *Alternaria* species are among grasses. We have observed *Alternaria* species in a related field experiment using the same six grass species, grown from the same seed sources as this study (Blaisdell 2011). The fact that we did not see differences in pathogenicity among the *Alternaria* isolates supports findings of their ubiquitous nature among grasses. It appears that *Alternaria* species (and other similar, ubiquitous endophytic fungi) in general have some mechanism that lets them bypass or override plant defenses long enough to ensure infection but not necessarily cause disease (Sánchez Márquez et al. 2011). Research also suggests that *Alternaria* species are able to live endophytically in nearly all grasses (Sánchez Márquez et al. 2011). Our results show that, depending on the grass host, they also can be potential grass pathogens that decrease host fitness. Generalist and common fungal endophytes and saprotrophs are important but often overlooked as potential enemies (Power and Mitchell 2004). Our results illuminate the importance of identifying such pathogens and their ability to spillover and cause disease in co-occurring species. Because most prairie grasses are perennial species, we stress the need for long term field experiments to help understand how spillover of fungal enemies among co-occurring species could influence invasion dynamics.

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