# Grass Growth Promotion by Dark Septate Endophytic Fungi is Host Specific

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**Abstract**

Isolates of dark septate endophytic fungi (DSE) were obtained from healthy looking roots of two early season grasses [crested wheatgrass (*Agropyron cristatum* L), Russian wildrye (*Elymus junceus* Fisch)] and one late season grass [blue gramma (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths] growing in southwest Saskatchewan. The capacity of some fungal isolates to colonize the roots or to promote the growth of these grasses was tested under controlled conditions. A first study revealed that DSE isolates AC 1 and EJ 5 colonize with more intensity the roots of the grass species from which they were isolated. A second study showed that the ability of each fungal isolate to promote plant growth depended on the plant species inoculated. Inoculation with four out of five isolates reduced *B. gracilis* growth, but increased the growth of one of the early season grasses. The results indicate that DSE fungal isolates are not species specific colonizers but have a strong preference for certain plant species, and some isolates can promote or depress plant growth depending on specific DSE isolate-grass combinations.

## Introduction

Plant roots are naturally colonized by many microorganisms that can develop symbiotic associations. Dark septate endophytes (DSE) are fungi that colonize the roots of plants without causing any apparent damage. Research results indicate that some species of DSE can mediate important functions related to water and nutrient absorption by plants, and provide plant tolerance to stress under extreme environmental conditions. For instance, it has been suggested that the colonization by fungal endophytes in grasses growing in arid zones, can help to maintain the hydraulic continuity between roots and dry soil (Barrow, 2003). In an alpine environment, the direct involvement of a DSE in the early accumulation of nitrogen was reported (Mullen et al., 1998) and a DSE isolate was also involved in plant survival at extreme soil temperature (Redman et al., 2002). A wealth of research on the role of fungal endophytes in plant tolerance to stress, suggests that plant-DSE symbiosis can help to mitigate the impact of climate change on plant communities (Rodriguez et al., 2008).

The ecological role of DSE colonizing the roots of prairie grasses is unclear. In particular their level of host specificity is not known. Defining levels of host preference and plant growth promotion capacity of DSE will help to understand the involvement of these fungi in plant performance and community dynamics. The purpose of this research was to test the specificity and growth promotion capacity of DSE previously isolated in southwest Saskatchewan from roots of crested wheatgrass (*Agropyron cristatum* L), Russian wildrye (*Elymus junceus* Fisch) and blue gramma (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths.

## Materials and methods

Two independent experiments were conducted under controlled conditions in order to test the capacity of DSE isolates to colonize plant roots or to promote plant growth. These DSE isolates are referred to, in the following sections, according to the plant from which they were isolated (e.g. EJ = *E. junceus,* AC = *A. cristatum,* BG = *Bouteloua gracilis*) followed by a number assigned to the isolate in a microbial collection at the Soil Microbiology laboratory in SPARC.

**Experiment 1 Host preference by dark septate endophytic fungi:** Isolates of 2 DSE isolates and 2 grass species were combined in a factorial experiment to evaluate the capacity of DSE to colonize a host different from the plant species from which the fungi was originally isolated. Treatments consisted in individual plants of each grass species (*A. cristatum* or *E. junceus*) inoculated with one of two DSE isolates. Non inoculated plants of each grass served as controls. The plants were grown in sterile microcosms, prepared according to a method presented before (Scher et al., 1984). Briefly, glass tubes 15 cm long and 2 cm in diameter were filled with a 8 cm layer of quartz sand (bottom) and 2.5 cm layer of soil (top). Five mL of a 1:1000 diluted 20-20-20 fertilizer were added to each microcosm and then sterilized during 1 hour at 115°C, 20 PSI. Sterile PDA plugs or plugs from 3 week old cultures of isolates AC 1 or EJ 5 were used to inoculate germinated surface sterile seeds of each grass species. Seeds were sterilized by successive immersion in 95% ethanol for 10 sec, sterile water for 10 sec, 2.5% Javex® for 2 min, and in sterile water for 2 min. The inoculated seeds were kept overnight onto wet sterile filter paper inside Petri dishes and then, axenically transplanted in each microcosm. The lower portion of the tube containing substrate and the opening were externally wrapped with aluminum foil and placed with a 45° inclination in the growth cabinet. The microcosms were kept in controlled

conditions at 23:19°C and 16:8 hours (day:night), relative humidity of 60% and lights adjusted to provide 445 μmol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation (PAR). Microcosms in each treatment were replicated 3 times. Water content was adjusted as needed and the plants were harvested after 75 days. The roots were stained using Schaeffer black ink (Vierheilig et al., 1998) and colonization assessed at 400X using a Olympus compound microscope and the line intercept method (Giovannetti and Mosse, 1980).

**Experiment 2 Grass growth promotion by DSE:** Five DSE isolates and 3 grass species were combined in a factorial experiment to identify the grass growth promotion capacity of each fungal isolate. Sterile seeds of two cool season grasses (*A. cristatum* and *B. gracilis* ) and a warm season grass (*B. gracilis*) were inoculated with sterile PDA plugs or plugs colonized by DSE isolates AC 1, EJ 2, AC 4, EJ 5 or BG 17 and grown during 44 days in mesocosms. Seed sterilization, plant inoculation and growth chamber conditions were the same as described above. Mesocosms were prepared in plastic pots containing 450 g of pasteurized soil. The soil water content was adjusted to 15% and 5 seedlings were transplanted to each pot. Each plant received 2 mL of water daily during the first week. After a week, plants were thinned to two per pot and 25 mL of water were added daily until the end of the experiment. This quantity of water equals 5.5% of soil water content, a suboptimal level of soil moisture. Aerial parts were harvested after 44 days and dried at 55°C to obtain dry mass produced in each pot. Mesocosm in each treatment were replicated 4 times.

**Statistical analysis:** Data from root colonization in experiment 1, and dry mass in experiment 2 were analyzed by two-way ANOVA. In experiment 2, inoculation effects are presented as relative growth, and were calculated as follows:

$$\text{Relative growth} = \frac{\text{DMa DSEx}}{\text{DMa control}} \text{ X } 100$$

Where **DMa DSEx** is the dry mass of shots in a pot containing grass specie **a** inoculated with **DSE x**, and **DMa control** is the average shoot dry mass measured in pots of grass species **a** treated with sterile PDA.

## Results

**Experiment 1 Host preference by DSE:** A significant interaction between plant species and fungi ($p < 0.05$) showed that colonization by isolate AC 1 and EJ 5 was higher in the plant species from which they were isolated (Figure 1). The level of root colonization of *A. cristatum* with isolate AC1, which was isolated from the same plant species, was 28 times higher than the colonization in *E. junceus*. Inoculation of *E. junceus* by isolate EJ 5 (isolated from *E. junceus*), resulted in three times higher colonization in *E. junceus* than in *A. cristatum*.

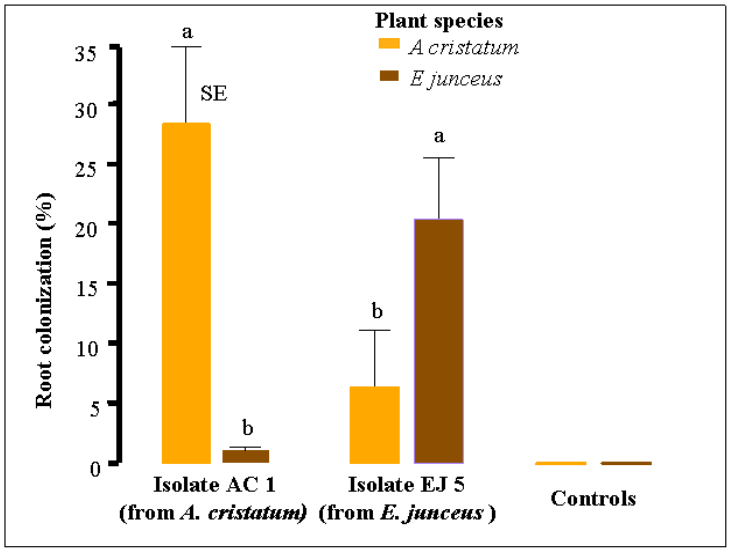


**Figure 1. Extent of root colonization by two fungal isolates in the grass species from which they were obtained, as compared with the colonization in a different host. Bars are means of three replicates. Means with different letters are different ($p$<0.05, protected LSD test).**

**Experiment 2 Effect of DSE on plant growth:** The effect of the DSE isolates were plant specific ($p<0.05$). For example, inoculation with fungal isolates AC 1 and BG 17 increased the growth of *A. cristatum* and *E. junceus*, but depressed the growth of *B. gracilis* (Figure 2). Similarly, inoculation with isolates EJ 2 and AC 4 increased the growth of *E. junceus* as compared to *B. gracilis.* In general, the inoculation with DSE resulted in growth depression of the late season grass, but not in the early season grasses.

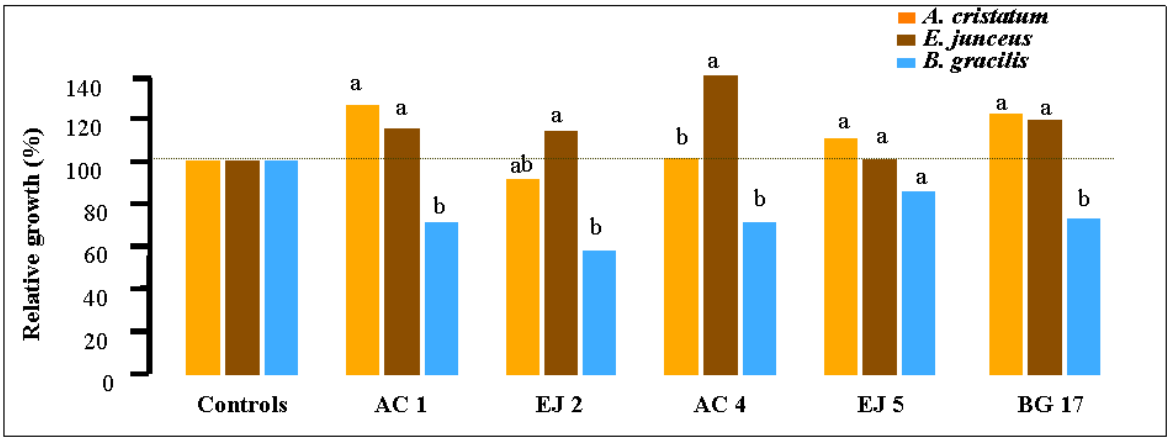


**Figure 2 Plant dependent growth promotion or depression by dark sepate endophytic fungi. Bars are means of four replicates. For each isolate, means with different letters are different ($p$<0.05, protected LSD test). Values above or below the dotted line indicate relative increase or decrease in dry weight as compared with control plants.**

## Discussion

We found that root colonization by DSE can be largely host specific. Furthermore, the effect of DSE on plant growth is also host specific. The results suggest that DSE play a role in plant community dynamics. Biomass of both early season grasses, *A. cristatum* and *E. junceus*, was increased by inoculation with DSE isolates AC 1 and BG 17. However, the productivity of the late season grass, *B. gracilis*, was about 25% lower when inoculated with any of these two DSE isolates. The fact that BG 17, which was isolated from *B. gracilis*, provided no benefit to this plant species under the suboptimal watering conditions of this experiment, but increased the growth of *A. cristatum* and *E. junceus*, suggests that DSE do not always promote plant growth, and that the growth response under water stress is plant specific. The role of endophytic fungi in alleviating plant stress under extreme saline or soil temperature, had been shown before to depend on specific plant isolate combinations, but the effect of fungal endophytes in providing drought tolerance was reported to be less dependent on the plant host (Rodriguez et al., 2008).

The result suggests that *B.gracilis* would be out competed by the other two grass species and would disappear if these particular isolates immigrate in mixed plant stands including the three grasses. It is also possible that BG 17 improves the fitness of *B. gracilis* by relieving the effect of a growth limiting factor that was not present in our experiment; for example, BG 17 may confer disease resistance, winter hardiness, or induce the production of grazer-deterring molecules in plant tissues. But we did not test for these stresses. It is also possible that BG 17 simply helps *B. gracilis* survive long drought periods by slowing-down the growth of the plant, reducing plant's water requirement.

DSE would offer an interesting option in the design and management of sustainable forage production systems. Perhaps the most important challenge at this time of escalating input costs is to improve the efficiency of crop production. We believe that managing plant symbiosis is an avenue to reach this goal.

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