Advantages of endophyte infection for irrigated pastures of semiarid, cold-desert environments

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Abstract

Little research has evaluated possible endophyte benefits to adaptation and production of grasses in the irrigated pastures of the semiarid, cold-desert environments of the western USA. Severe irrigation shortages are common; however, production demands are increasing, necessitating maximizing tall fescue's (*Festuca arundinacea* Schreb.) productivity when grown in sub-optimal conditions including drought, salinity, and cold temperatures. In a field study under irrigation in the Intermountain Western USA, the yield advantage to Kentucky 31 tall fescue infected with wild-type *Neotyphodium* over endophyte-free Kentucky 31 was greatest (over 15%) when irrigation was severely limited to natural precipitation. In an evaluation of salinity tolerance there were no significant differences in plant LD_{50} values due to endophyte infection. In a recent study Jesup MaxQ recovered better from winter injury than Jesup E– when grown in a high-elevation cold-desert, irrigated environment. These studies suggest the need for additional research to elucidate the potential advantages of wild-type and novel endophytes for tall fescue production in irrigated environments typical of the semiarid western USA.

Keywords: tall fescue, *Festuca arundinacea* Schreb., *Neotyphodium*, drought tolerance, salinity tolerance, winterhardiness

Introduction

Tall fescue (*Festuca arundinacea* Schreb.) is the predominant forage grass throughout the humid and subhumid eastern half of the United States, and is especially well adapted to the central transition zone (Moore 2003). In the western region of the USA, tall fescue's range of adaptation is limited by inadequate precipitation; even so, it is one of the most productive pasture grasses when grown with supplemental irrigation (Waldron *et al.* 2002). Unlike the eastern USA, endophyte-free rather than endophyte infected tall fescue is predominant in the western part of the country. However, increased grazing pressure on privately-owned, irrigated pastures, combined with severe water shortages, necessitates maximizing tall fescue's productivity when grown in sub-optimal conditions including drought, salinity, and cold temperatures.

The symbiotic relationship between *Neotyphodium* infection and tall fescue is well documented, showing increased insect, heat, and drought tolerance resulting in improved persistence and summer survival in the eastern USA (West *et al.* 1993). Malinowski and Belesky (2000) reviewed the mechanisms of endophyte-induced drought adaptation, including improved drought avoidance through expanded root growth and greater drought tolerance by better water use efficiency. West *et al.* (1993) reported that endophyte-infected tall fescue had enhanced tiller survival under zero irrigation as opposed to endophyte free stands, yet there was no consistent advantage to forage yield. Hesse *et al.* (2003) found that *Neotyphodium*-infected perennial ryegrass (*Lolium perenne* L.) collections that originated from dry sites in Germany had increased regrowth under drought than their uninfected counterparts. Conversely, it has also been reported that endophyte infection did not increase survival or growth of perennial ryegrass grown under low irrigation (Lewis 1992).

There has been less research to investigate the potential role of endophyte infection on increased grass tolerance to saline conditions and cold temperatures. One study found no benefit in growth between *F. rubra* plants infected or uninfected with *Epichloe* endophyte when watered with sea-water (Zabalgogeazcoa *et al.* 2006). Likewise, Simpson and Hume (2000) reported no relationship between salt tolerance and endophyte infection for tall fescue and perennial ryegrass. However, it was recently shown that barley (*Hordeum vulgare* L.) infected with the exotic endophyte *Piriformspora indica* was more tolerant to moderate salt stress than non-infected barley (Waller *et al.* 2005).

The relative effect of endophyte infection on tall fescue's winter survival and winter recovery has not been investigated. Likewise, there has been little research to evaluate possible endophyte benefits to adaptation and production of grasses in irrigated pastures of the semiarid, cold-desert environments typical of the western USA. We report here findings from a series of investigations conducted to elucidate the potential benefit of endophyte infection in tall fescue when grown in the western USA.

Materials and methods

Drought tolerance

Asay *et al.* (2001) evaluated the response of tall fescue to an irrigation gradient in the Intermountain West of the USA. Included in the study was a comparison of wild-type endophyte infected and endophyte free 'Kentucky 31'. Immunoblot tests confirmed the appropriate endophyte infection in the two entries. Complete results and details of methods can be reviewed in Asay *et al.* (2001). Here we review only the results at the lowest irrigation level. Briefly, yearly dry matter production (from five clippings) was compared for endophyte-status when tall fescue was grown without supplemental irrigation. The study was conducted in 1997 and 1998 near Logan, Utah, USA. Average precipitation that these plots received during the growing season was 361 mm.

Salt tolerance

Relative salt tolerance of tall fescue was evaluated in a greenhouse test in 2004. Entries included wild-type endophyte infected and endophyte free Kentucky 31, and novel-type endophyte infected (MaxQ) and endophyte free Jesup. Endophyte infection was confirmed in the Jesup lines, but not verified in the Kentucky 31 lines. The experiment was conducted by slightly modifying the procedures found in Peel *et al.* (2004). Briefly, the lethal dose to kill 50% of 20 plants per replicate for each cultivar (LD_{50}) was estimated using a replicated design and two separate runs. Six-week old plants, grown in silica sand, were dipped in a saline solution twice each week. Salt concentration started at an EC of 6 dS/m and increased in 6 dS/m increments every 1 week until an EC level of 42 dS/m was reached, where it remained for an additional 4 weeks of treatment. To account for both relative time and salt concentration, a cumulative linear value was calculated that accounted for salt concentration as measured by EC of the solution and the number of days at each EC concentration (EC_{days}). Probit analysis was used to estimate LD_{50} in terms of EC_{days} for each entry on individual replicates, and the results were subjected to mixed model analysis.

Winter hardiness and recovery

A space-planted trial of tall fescue was established in 2004 near Panguitch, Utah, USA. The area is a high elevation valley (1976 m above sea level) known for cold, open winters and late spring frosts. Kentucky 31 E+ and E–, and Jesup MaxQ and E– were again included in the test; however, immunoblot test of the material showed that 0% of the Kentucky 31 E+ plants were infected with the endophyte. Infection of Jesup MaxQ and Jesup E– was 100% and 0%, respectively. The experimental design was a randomised complete block with eight replicates of five-plant plots. Individual plants were visually evaluated for winter injury (1-9, 1 = winter killed and 9 = no winter injury) in early May 2005 and 2006. Plots were then harvested three times each year with a sickle-bar harvester to 8-cm stubble at the boot stage of plant development for the first harvest and when the height of regrowth was approximately 40 cm for subsequent harvests. Forage samples were taken from each plot and dried to a constant weight in a forced-air oven at 70°C to determine dry matter percentage. Forage yields were reported as yearly dry matter in kg/plot and were representative of tall fescue's ability to recover from winter injury. Analysis revealed a significant Entry × Year interaction, most likely due to 2004-05 winter being a mild with very little winter injury; therefore, only results from 2005-06 winter are reported.

Results and Discussion

Drought tolerance

Dry matter yield of Kentucky 31 E+ was significantly ($P<0.05$) higher (15%) than Kentucky 31 E– at the lowest irrigation level of 361 mm (natural precipitation only) (Table 1). Asay *et al.* (2001) reported that this difference was even more pronounced when yield was based only on late-season harvests after an obvious irrigation gradient existed due to loss/use of soil water reserves. West *et al.* (1993) could not associate a forage yield benefit to endophyte infection when tall fescue was grown under limited irrigation. However, their lowest irrigation (precipitation only) of approximately 540 mm is more comparable to the mid-irrigation levels reported in Asay *et al.* (2001) for which, in similar fashion, a trend for higher yield, although not significant, was found in favour of endophyte infection. Burns and Chamblee (1979) report that tall fescue is adapted to dryland conditions in the western USA when annual precipitation is approximately 500 mm; whereas, it lacks sufficient drought tolerance to persist when annual precipitation approaches 300 mm. It may be that endophyte enhancement of drought tolerance supercedes the plants own genetic expression somewhere between this 500 to 300 mm range. Based on these results, we would conclude that *Neotyphodium* may increase forage production of tall fescue when irrigation is limited in the western USA. However, additional studies are needed to further document the potential effect of wild-type and novel-type endophytes on persistence and forage yield of tall fescue grown under limited irrigation in the semiarid, cold-desert region of the USA.

Salt tolerance

We found no significant differences in LD_{50} values between Kentucky 31 E+ and E– lines, or between Jesup MaxQ and Jesup E– lines (Table 1). These results support Simpson and Hume (2000) findings that *Neotyphodium* confers little if any increased salt tolerance to tall fescue. However, in our overall study, we found very little genetic variation among tall fescue lines for salt tolerance as determined by plant death (data not shown). Because of this and absolute differences in favour of endophyte infected over endophyte free material, we feel additional research is warranted. To this end we are now investigating forage growth reduction (as opposed to death) at more moderate salinity levels.

Winter hardiness and recovery

There was no significant difference between Jesup MaxQ versus Jesup E– for winter injury (Table 1). Jesup is not adapted to the cold winter and spring temperatures typical of this environment, finishing near the bottom of the test. This suggests the possibility, that in this case, plant genetics made any endophyte enhancement ineffective. However, we did observe that approximately 20-30% of individual plants of Jesup MaxQ had little winter injury; whereas the remaining 70-80% of plants within the same plots were severely injured. We did not see this type of variable injury among plants of the other entries. The Jesup MaxQ plants with little winter injury contributed to, but were not solely responsible, for a significantly higher subsequent forage yield over the Jesup E– line (Table 1). We surmised that the uninjured Jesup MaxQ plants may have had a high level of endophyte expression, but this was not verified and our previous test had shown 100% infection in MaxQ plants. Overall, we concluded that Jesup MaxQ was better able to recover from winter injury as compared to Jesup E–. These results suggest that the novel endophyte may have a role in increasing recovery from winter injury. Additional research using adapted plant material is needed.

In conclusion, these studies suggest endophyte infection may improve abiotic stress tolerance in tall fescue grown in the

Table 1 Summary of performance data comparing endophyte status of tall fescue when evaluated under abiotic stress in Utah (USA).

Entry	Drought	Salt	Winter hardiness	Winter recovery
	Yield (Mg/ha)	LD_{50} (EC days)	Visual Injury 1-9 (1=dead)	Yield (kg/ plot)
Kentucky 31 E+	17.6	2746	---	---
Kentucky 31 E-	14.9	2672	---	---
Jesup MaxQ	---	2653	2.2	3.4
Jesup E-	---	2622	2.2	2.8
LSD (0.05)	1.9	158	0.7	0.4

irrigated pastures of the western USA. However, the studies were not conclusive and additional research is needed. Using adapted plant materials infected with both wild-type and novel endophytes will help elucidate the future role of endophytes in this area. Potential benefits will need to be carefully documented and shown to compensate for cattle performance issues of wild-type *Neotyphodium* and higher seed prices of novel-type endophyte/seed packages.

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