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The XXII International Grassland Congress (Revitalising Grasslands to Sustain Our Communities) took place in Sydney, Australia from September 15 through September 19, 2013.

Proceedings Editors: David L. Michalk, Geoffrey D. Millar, Warwick B. Badgery, and Kim M.

Broadfoot

Publisher: New South Wales Department of Primary Industry, Kite St., Orange New South Wales, Australia

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Endophyte status in summer-dormant tall fescue in the southern Great Plains of USA

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Keywords: Endophytes, summer-dormant tall fescue.

Introduction

Non-toxic fungal endophytes provide persistence-related benefits to summer-active, continental-type tall fescue (*Festuca arundinacea* Schreb.), as well as reduced animal toxicosis compared to toxic endophytes. However, the benefits of fungal endophytes to summer-dormant, Mediterranean-type tall fescue persistence or production are unclear. Summer-dormant tall fescue has potential to replace traditional, annual small grain graze-out systems in the Southern Great Plains region of the USA. This region is characterized by severe water deficits accompanied by extreme heat in summer, and by relatively mild, rainy winters (Malinowski *et al.* 2009). Summer-active tall fescues are better suited to high rainfall areas (>900 mm annual average rainfall (AAR)) east of the 97° longitudinal meridian, while summer-dormant tall fescues are best adapted to lower rainfall areas (600 to 900 mm AAR) between the 97° and 99° longitudinal meridian (Butler *et al.* 2011).

The objective of this field study was to determine the effects of the 'novel' endophyte strain AR542 on persistence of summer-dormant tall fescue 'Flecha' in the Southern Great Plains of the USA. Two field experiments were conducted to evaluate the effect of the novel endophyte AR542 on the survival of Flecha.

Methods

An on-farm demonstration of commercially available Flecha AR542 was planted at 16 kg/ha during autumn each year from 2004 to 2009 on 2-10 ha paddocks of a producer's farm at Vashti, TX (33°34'N, 98°1'W). The soil was an Anacon (fine, mixed thermic Udic) loam. Tall fescue was planted according to guidelines outlined by Butler *et al.* (2008), whereby annual grass weeds were sprayed with glyphosate in the spring, to prevent seed production, followed by a second application of glyphosate after rainfall to control emerged grass weeds, prior to planting in the autumn. Paddocks were moderately grazed with cattle (to 15-cm stubble height) during April-May of the establishment year. In the years after establishment, paddocks were grazed when sufficient forage (1.7 to 2.2 t dry matter (DM)/ha) was present (October through May), and grazing was terminated when forage availability was less than 1.1 t

DM/ha. Tillers (24) were randomly collected by sampling pseudostems (approximately 3-4 mm in length), as close to the soil as possible, from each paddock during spring 2007, 2010, 2011 and 2012 for each existing stand. These were analyzed by polymerase chain reaction (PCR), using endophyte-specific primers, to estimate the percent infection. Tall fescue stand percent observations were recorded in spring each year to estimate plant persistence.

In a separate experiment, Flecha AR542 and Flecha Nil (endophyte-free) lines were planted at Burneyville, OK (33°53'N; 97°15'W) in 2005 and at Vernon, TX (34°03'N, 99°16'W) and Burneyville, OK in 2008, using a randomized complete block design with 4-10 replications per location. Soil at the Burneyville location was a Minco fine sandy loam (coarse, silty, thermic udic Haplustoll; pH 6.1, 26 mg P/kg, 67 mg K/kg), while soil at Vernon was a Miles fine sandy loam (fine-loamy, mixed, superactive, thermic typic Paleustalf; pH 6.6, 20 mg P/kg, 300 mg K/kg). Entries were planted with a small plot drill in 1.5 m by 1.5 m sward plots at all locations, at a rate of 20 kg of pure live seed (PLS)/ha. Once established, plots at Burneyville were grazed with 3-8 head of beef cattle (*Bos* sp.) in spring and/or summer, in order to maintain stubble height at about 5 cm. Plots at Vernon were clipped when necessary to prevent seed head production. Tall fescue stand % observations were recorded in spring each year to estimate plant persistence.

Prior to planting from 2007 onwards, each seed batch was tested for total endophyte infection levels using PCR with endophyte-specific primers. DNA from individual seeds was extracted using the MagAttract 96 plant DNA Core Kit (Qiagen) and PCR performed as described in Charlton *et al.* (2012).

The growing season from October through April was extremely dry during the 2010-11 season, being 82% below the 30-yr normal average (342 mm) at Vashti, TX, 85% below average (296 mm) at Vernon, TX, and 36% below average (342 mm) at Burneyville, OK. In addition to the dry growing season, the 2011 summer (June-August) was extremely hot (100 d exceeding 38°C) with negligible rainfall (96, 94, and 72% below normal for Vashti, Vernon, and Burneyville, respectively), resulting in the hottest, driest year on record.

Results

In the on-farm demonstration, initial endophyte infection was approximately 59% and generally increased with increasing stand life, ranging from 67-92%, until the severe heat and drought of 2011. This suggests the endophyte may be beneficial during the first (establishment) season. After the 2011 drought, endophyte infection varied with the number of years since establishment. The older, more mature plots from establishment years 2004, 2005, and 2006 had greater endophyte infection levels of 67-79%, compared to the later establishment years of 2007, 2008, and 2009, which had infection levels of 14-46%. This suggests the endophyte was lost in the plant and that plant age may influence endophyte survival.

In the second experiment, initial tall fescue stand persistence was excellent (100%) and decreased after the 2011 season. However, persistence did not differ between endophyte-free and novel endophyte (AR542)-infected tall fescues. The beneficial effect of this combination of novel endophyte-infected summer-dormant tall fescue was considerably less than that observed with endophyte-infected summer-active tall fescue.

Conclusion

Data comparing endophyte-infected with endophyte-free Flecha tall fescue indicates that stand persistence was not improved with the inclusion of the 'novel' endophyte, AR542. In continental tall fescue, increased stress tolerance and persistence, due to endophyte infection, has been demonstrated (Hopkins *et al.* 2010) and the benefit of 'novel' endophytes in alleviating animal health concerns and improving performance has been well

documented (Hopkins *et al.* 2010). Similar studies are yet to be performed with endophyte-infected summer-dormant tall fescue, but based on the findings described here, 'novel' endophyte AR542 did not provide a distinct benefit to the summer-dormant variety Flecha in the southern Great Plains. However, we cannot discount the possibility that endophyte-infection does provide a benefit that has not been explored as part of this study. A more thorough examination of different endophyte-infected and endophyte-free summer-dormant lines is currently underway, to determine factors that influence summer-dormant tall fescue survival in the southern Great Plains.

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