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Evaluation of Turf-Type Intergeneric Hybrids of *Lolium perenne* with *Festuca pratensis* for Improved Stress Tolerance

A Thesis submitted in partial satisfaction of the requirements for the degree of

Master of Science

in

Plant Biology

by

Brent D. Barnes

March 2012

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This Thesis of Brent D. Barnes is approved:

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Committee Chairperson

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General Introduction

Turfgrass is the largest irrigated crop in the United States, covering nearly three times the surface area of agricultural crops (Milesi et al. 2005). While turfgrass offers many benefits including: aesthetics, fire protection, dust prevention, carbon sequestration, and environmental cooling (Beard and Green 1994), all are more difficult to quantify than crop yield. Given the current and anticipated water shortages, if turfgrasses are to remain a viable part of the landscape in arid climates, such as Southern California, they must be managed wisely and accurately when it comes to water-use.

With increasing air temperatures and lower water availability, warm-season (C4) grasses are of interest because they possess metabolic and anatomical features that enhance photosynthetic efficiency while minimizing water loss. Overall, these grasses are more drought and heat tolerant than cool-season (C3) grasses. However, winter dormancy is the weak point of warm-season turfgrass use in Southern California and other areas because most people demand that grass be green year-round. In contrast to warm-season turfgrasses, cool-season grasses, with ample irrigation or precipitation, can provide year round color, but they are not well adapted to high temperatures and drought. So, whereas one approach to water conservation would be to improve winter color retention of the warm-season turfgrasses, another could be to improve drought and heat tolerance of cool-season grasses.

Classical breeding has created many drought tolerant grasses by selection of survivors from populations subjected to prolonged drought, but greater improvements are

warranted given declining water resources and greater water-use restrictions. Usually, breeding for stress tolerance comes at the expense of turf quality characteristics (Alderson and Sharp 1994, Read et al. 1999, Abraham et al. 2004). Some grasses may possess sufficient drought tolerance, but lack desirable visual quality, and vice versa. Hybridization and selection may combine positive traits of both parents. So, interspecific and intergeneric hybridization may generate progeny that have better visual quality of one grass, and the better drought tolerance of another.

In the general concept of combining contrasting characteristics of selected parents, many turfgrass species and genera have been intercrossed to create better performing hybrids. Crosses have been made to improve seed yield (Bradshaw 1958), sterility (Alderson and Sharp 1994), finer leaf texture (Gibeault et al. 1997), and abiotic stress tolerance (Marcum 1998, Humphreys et al. 1997). Many of these hybrids have shown differences in rooting length (Lehman and Engelke 1991, Salaiz et al. 1991, Burt and Christians 1990, Marcum et al. 1995, Hays et al. 1991). Deeper rooting has been associated with drought avoidance and tolerance in many species of turf (Burton et al. 1954, Carrow 1996, Huang et al. 1997, Sheffer et al. 1987, White et al. 1993) and other crops. Selection for deeper rooting characteristics has been used to produce more drought tolerant turf in tall fescue and perennial ryegrass (Bonos et al. 2004), and deeper and infrequent irrigation practices have been used to promote deeper rooting in turfgrasses (Fu et al. 2007).

Deficit irrigation is a common practice in Southern California and other areas during periods of drought and water-use restrictions. In practice it involves applying water amounts below that of reference evapotranspiration (ET_o) as measured from a well-watered turf crop (Feldahake et al. 1984, Fry and Butler 1989, Qian and Engelke 1999). So, a breeding program focused on selecting plants for deeper root production and greater turf quality under deficit irrigation might identify superior new plants that could be a replacement for currently used species.

The *Festuca-Lolium* complex offers turf-type plants that combine deep rooting characteristics of the genus *Festuca* with the superior visual quality of the genus *Lolium*. *Festuca-Lolium* hybrids have been used extensively as forage grasses in Europe to create more stress tolerant and more productive stands. Hybrids have been made from *F. pratensis, F. arundinacea,F. mairei,* and *F. glaucescens* on one hand and two common *Lolium* species, *L. perenne* and *L. multiflorum* the other (Kopecký et al. 2008). Using several approaches, from cytological to marker-assisted selection, tolerances to abiotic and biotic stress, including drought, low and freezing temperatures, and crown rust, have been directly associated with specific regions of the parental genomes (Humphreys and Pasakinskiene 1996, Lesniewska et al. 2001, Kosmala et al. 2006, and Roderick et al. 2003). In addition to improved stress tolerance found among some of these hybrids, deeper rooting traits were associated with a specific region of chromosome 3 from *F. pratensis* in *L. multiflorum* backgrounds that significantly enhanced drought tolerance of *Festuca-Lolium* hybrids (Turner et al. 2010). However, many of these hybrids were

forage types, selected and bred for large biomass production, and biomass production is not a highly desirable characteristic in turfgrass.

The goal of this program is to create drought tolerant, turf-type hybrids of fescues (*Festuca spp.*) with ryegrasses (*Lolium spp.*), commonly referred to as Festulolium. Original forage-type tetraploid hybrids were subjected to androgenesis to reduce their ploidy level to diploid thereby reducing their vigor, and selected plants were crossed and backcrossed to a diploid, turf-type *L. perenne* (Kopecký et al. 2005). The resulting new Festulolium hybrids have the visual appearance of *L. perenne* with but still carry substantial chromatin introgressions from *F. pratensis*. With stringent selection, the less vigorous hybrids may achieve stress tolerance and posses deep root growth characteristics like the forage-type Festuloliums created in the United Kingdom. In this study, new Festulolium hybrids were eveluated for the traits associated with drought tolerance in turf-type phenotypes. The focus was on root growth characteristics of the Festulolium relative to: (i) the backcross parent *L. perenne*, (ii) a representative meadow fescue, and (iii) drought tolerance industry standard lines of both *F. arundinacea* and *L. perenne* in experimental conditions in the greenhouse, with ample irrigation. The second study focused on drought tolerance and root growth characteristics of similar experimental populations and local drought tolerant populations under deficit irrigation in field conditions.

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Root Characteristics of Fescues, Ryegrasses, and Their Hybrids Selected for Improved Stress Tolerance

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Abbreviations: FISH, Florescent *in*-situ hybridization; FL, Festulolium; MAP, Months After Planting; UCR, University of California, Riverside.

ABSTRACT

Recurrent selection for drought and heat tolerance among hybrids of perennial ryegrass (*Lolium perenne* L.) with meadow fescue (*Festuca pratensis* Huds.), was used to develop turf-type populations with a marked increase in stress tolerance. Increased tolerance was associated with the presence of an introgression of *F. pratensis* chromatin on chromosome 3 of *L. perenne*. To determine if root characteristics were responsible for the improved stress tolerance, a greenhouse study was conducted to compare sister lines of Festulolium both with or without the introgression; the recurrent backcross parent; a representative *F.* pratensis; and turf-type tall fescue (*Festuca arundinacea* Schreb.) grown under well-watered conditions in 5.1-cm diam. x 160-cm long tubes containing sand. Two separate 120-day experiments revealed that *F. pratensis* produced deeper roots, more root biomass, and a higher root:shoot, while the industry standard, *F. arundinacea,* ranked at or near the lowest value. For the hybrids and ryegrass, the root parameters were intermediate between the 2 fescues, with no statistically significant difference among the 3 lines tested. The results indicate that in the tested Festulolium turf, drought and heat tolerance were not a consequence of increased root depth or root biomass as reported in previous reports for forage-type intergeneric hybrids of ryegrass and fescue.

Cool-season turfgrasses dominate the landscape throughout California and most of the United States because they retain green color year-round better than warm season turfgrasses, but often only with supplemental irrigation. However, diminishing potable water supplies coupled with increasing drought and water-use restrictions are making it more difficult and costly to maintain cool-season turf during hot, dry summers. While developing warm-season turfgrasses that retain green color during winter would be an ideal solution, an easier approach at the present time is to increase drought and heat tolerance in cool-season species so that they can survive hot summers with less water.

Hybridization, both interspecific and intergeneric, is used by turfgrass and forage breeders to improve various traits, including stress tolerance (Brilman 2001). Hybridization has the potential to combine and exploit positive complementary traits from both parents, thus creating more desirable genotypes. Breeders have used interspecific hybridization in warm-season turf species such as bermudagrass (*Cynodon spp.*) to create sterility, improve turf quality, and increase stress tolerance (Alderson and Sharp 1994, Marcum 1998). It has also been used to improve winter color retention and leaf texture in *Zoysia* spp. (Gibeault et al. 1997). In cool-season grasses, wide hybridization has been used to improve bluegrasses (*Poa* spp.), fescues (*Festuca spp.*), and bentgrasses (*Agrostis spp.*) (Alderson and Sharp 1994, Humphreys and Thomas 1993, Bradshaw 1958). Hybrid bluegrass, a cross between Texas bluegrass (*Poa arachnifera* Torr.) and Kentucky bluegrass (*Poa pratensis*), exhibits both the drought and heat stress tolerance seen in Texas bluegrass while having a visual quality similar to Kentucky bluegrass (Read et al. 1999, Abraham et al. 2004). Most species that have been

used for turf have, at one time or another, been hybridized to enhance stress tolerance (Brilman 2001). However, wide hybridization has had a broader impact in forage-type grasses both in the USA and in Europe.

Hybrids of fescues (*Festuca spp.*) and ryegrasses (*Lolium spp.*), commonly referred to as Festulolium, have been exploited by forage breeders for decades because of complimentary genetic and agronomic profiles of the species (Lewis et al. 1973, King et al. 2007). Hybrids of *Lolium* and *Festuca* species were first made by Jenkin (1933). Soon thereafter, it was found that chromatin of these 2 genera readily recombined with high frequencies of homeologous pairing (Humphreys 1989, Humphreys and Ghesquiere 1994, Kopecky et al. 2008). This has made *Festuca* and *Lolium* desirable genera for introgression breeding (Jauhar 1975, King et al. 1999, Zwierzykowski et al. 1998, Zwierzykowski et al. 1999, Zwierzykowski et al. 2008). Researchers began to use the high rate of genetic recombination to select for optimal agronomic traits of the hybrids. *Lolium* provided rapid germination and establishment, wear tolerance, palatability, and nutrition; *Festuca* offered greater stress tolerance and persistence. Most of these hybrids were bred specifically for forage, yet both fescues and ryegrass offer many positive characters for turfgrass use.

Tetraploid hybrids were obtained from a forage breeding and genetic program of Z. Zwierzykowski, Institute of Plant Genetics. Poznan, Poland. Among others, some of these hybrids were created from forage-type perennial ryegrass (*L. perenne* L.) and meadow fescue (*F. pratensis* Huds.). Androgenesis was used to reduce their ploidy level

to diploid (Kopecký et al. 2005), and perennial ryegrass 'SR 4220' was used as the backcross parent to produce turf-type populations. These populations were subjected to extreme selection pressure for drought and heat tolerance in the field; all irrigation was withheld from a plot of these hybrids between the months of July and October with daytime temperatures regularly reaching 40 ºC. The few surviving plants were potted, moved to the greenhouse and backcrossed and intercrossed to generate the next generation. Cytological screening suggested that this extreme selection pressure favored retention of a specific introgression of *F. pratensis* chromatin into *L. perenne* on the distal end on the short arm of chromosome 3 and, to a lesser extent, on the long arm of chromosome 2. Similar introgressions had been associated with drought and freezing tolerance, and increased rooting in forage-type Festulolium (Humphreys et al. 1997, Humphreys et al. 2003, Turner et al. 2010). Furthermore, chromosomes 3 of *Festuca* and *Lolium*, and chromosome 1 of rice (*Oryza spp.*) are syntenic and known to carry certain genes for improved stress tolerance (King et al. 2007). Major genes for drought (Zhi-Kang et al. 2005) and salinity (Malhotra and Blake 2005) have been mapped on rice chromosome 1, the potential also exists for greater salinity and drought tolerance among the turf-type Festulolium accessions. The greater rooting depth of fescues and other cool-season grasses contributes to drought avoidance (Weaver and Himmel 1930, Olmsted 1941, Bennett and Doss 1960, Doss et al. 1960, Cheesman et al. 1965). These grasses also undergo morphological changes in response to drought that increase the root:shoot ratio (Weaver and Himmel 1930), decrease tillering, reduce leaf number (Olmsted 1941) and contribute to drought tolerance. Ryegrasses do not share the same characteristics,

but the turf-type Festulolium hybrid selections from UCR may have had these characteristics integrated into their genome and they may be expressed under well-watered conditions.

As increased drought tolerance in response to selection under drought affects root characteristics in many crops, the objective of this study was to compare rooting characteristics among the Festulolium selections and related species under well-watered conditions in the greenhouse to determine if greater root growth characteristics were inherited and responsible for increased stress tolerance observed during selection in the field.

MATERIALS AND METHODS

General Study Conditions and Duration

Greenhouse studies were conducted at University of California, Riverside during the growing season of 2010. The experiment was started on April 5 and repeated on April 21 in a different greenhouse. Each experiment lasted 3 months. Average day/night temperatures, relative humidity ranges, and maximum photon flux density were 27/12 ºC, 40% to 90%, and 1200 μmol m^{-2} s^{-1}, respectively, in both greenhouses. Day length was set to 16-hr day/8-hr night using high-pressure sodium light.

Genotypes and Cultivars

The following genotypes or cultivars were evaluated: (i) turf-type *Festuca arundinacea* cv. 'Tulsa Time' (Seed Research of Oregon, Corvallis, OR); (ii) turf-type

Lolium perenne cv. 'SR 4220' (Seed Research of Oregon, Corvallis, OR) used as the backcross parent for the Festulolium populations; (iii) *Festuca pratensis* cv. 'Pasja' (provided by Dr.Virginia Lehman, Blue Moon Farms, Lebanon, OR); and (iv) two populations of Festulolium cytologically verified by *in-situ* DNA probing to carry ('FL3S') or not to carry ('FL0') the *F. pratensis* introgression on chromosome 3. Cv. 'Pasja' was a representative forage-type *F. pratensis* used here in lieu of the unavailable parent of the Festulolium populations originated from material generated by Kopecký et al. (2008) and selected after being subjected to extreme drought and heat, as explained above. The florescent *in-situ* hybridization (FISH) protocol used to verify the status of 3S introgressions was that of Masoudi-Nejad et al. (2002). Plants with this introgression were backcrossed to *L. perenne* 'SR4220' and intercrossed among themselves in isolation from other plants, to create a verified population containing the 3S introgression. The population used in the study was verified to have at least 95% of the plants carrying this segment by Dr. Adam Lukaszewski at the University of California, Riverside. Sister plants without the introgression were labeled FL0 and used as the negative control.

Preparation and Maintenance of Turfgrasses in Slant Tubes

Seeds of the five genotypes were germinated in the dark at 23 ºC in a temperature-controlled incubator and transplanted into 72-cell plastic flats filled with dry #30 silica sand (Carmeuse Industrial Sands, San Juan Capistrano, CA). Individual seedlings were grown to ca. the 3-tiller stage and transplanted into 165-cm long polyethylene tubing bags (Bradley's Plastic Bag Co., Downey, CA) filled with 4.6 kg of dry #30 silica sand with a

water-holding capacity of 24% (w/w). Bags were supported outside by 5.1-cm diam. x 160-cm long polyvinyl chloride (PVC) tubes with a PVC cap at the bottom and a 1.25-cm hole drilled in the middle for drainage. After transplanting seedlings, the tubes were irrigated daily to field capacity using 125 ml of a nutrient solution containing 240 mg/L nitrogen, 120 mg/L phosphorus, 240 mg/L potassium (Peters Excel 20N-10P_2O_5-20K_2O; Milpitas, CA), 3.15 mg/L sulfur, and 1.69 mg/L iron (Peters STEM; Milpitas, CA). Turf was cut weekly to maintain a height of 5.1-cm.

The experiment was in a completely randomized block design with five replicates of each population. Sub-plots consisted of sampling date (4, 8, and 12 weeks after planting) where the five replicates of each genotype were randomly chosen for measurement of rooting and other parameters described below. This totaled 75 rooting columns per experiment, and with 25 harvested every 4 weeks.

Turfgrass Quality, Clippings, Above Ground Biomass, and Tiller Number

Turfgrass was evaluated every 4 weeks for color, texture, density, and uniformity (Emmons 2000) and rated on a scale from 1 to 9 (1 = dead turf, 6 = minimally acceptable, coarse, light green, thin, and 9 = best, fine, dark green, dense). Clipping yield was taken every 2 weeks by cutting the turf at a height of 5.1 cm. Clipping fresh weights were recorded and dry weights determined after drying in an 80 ºC forced-air oven for 72 hr. At the time of root harvest, all green tissue was cut above the soil surface and washed free of sand. Tillers were counted and the sample was weighed, dried, and re-weighed to determine relative water content and above ground biomass fresh and dry weight.

Root Biomass, Longest Root, and Number of Roots at a Soil Depth of 60-160 cm

At all three root harvest dates of 4, 8, and 12 weeks after planting, the plastic sleeve was removed from the PVC root tube and placed in a cold room at 4 ^{o}C until roots could be washed. For washing, tubes were placed on a table and divided into three sections, 0-30 cm, 30-60 cm, and 60-160 cm from the soil surface. Each section was then placed on a 6-mm mesh screen and the plastic sleeve was cut and removed. Sand was washed away gently using a hose with a water break nozzle attached. Visual length and number measurements were then gathered by straightening and counting the roots in the two deepest sections. Lastly, roots were placed in an oven, allowed to dry for 72 hr, and weighed.

Root Scanning

At the three-month harvest date, roots at a soil depth of 30-60 cm and 60-160 cm were washed free of all sand, and stained with a neutral red dye for 4 hr to enhance scanning accuracy. For scanning, roots were placed on a clear scanning tray, water was added to cover the roots to an even depth, and the roots were spread to minimize overlap of tissue. The roots were placed on an Epson 1680 scanner (Epson America Inc., Long Beach, CA) set at 1000 dpi resolution and scanned using WinRHIZO software (Regent Instruments, Inc. Quebec, Canada). Root diameter was set to intervals of 0.1 mm and measurements of length, surface area and volume were determined for roots in each section (LeCain et al. 2006).

Data were subjected to ANOVA (Statistix 8.0, Tallahassee, FL). Differences in means were separated using Fisher's protected LSD analysis ($\alpha = 0.05$). Root:shoot ratio was calculated by taking total root dry mass and dividing it by the total above ground biomass dry weight.

RESULTS AND DISCUSSION

With the exception of the root:shoot ratio (Table 1.1), there were no significant differences among treatments in the two experiments. Therefore, all data were combined for presentation.

Turfgrass Quality, Clippings, Above Ground Biomass, and Tiller Number

The turf was maintained under well-watered conditions throughout the experiment. Under these conditions *L. perenne* 'SR4220', FL0, and FL3S exhibited the greatest turfgrass quality throughout the experiment due to their finer leaf texture and darker green color (Fig. 1.1). The meadow fescue *F. pratensis* 'Pasja' had the greatest clipping biomass dry weight (data not shown). No significant differences were found among the other genotypes. On all harvest dates, *F. pratensis* 'Pasja' ranked highest in the above ground biomass (Fig. 1.2). There were no differences among the parent *L. perenne* 'SR4220' and the two Festulolium lines.

Tiller numbers were variable during the study, but in general were related to leaf texture, with tiller number decreasing with coarser leaf texture. At all harvest dates of 4, 8, and 12 weeks, *F. pratensis* 'Pasja' had the lowest number of tillers (Fig. 1.3). At 3

months after planting, FL0 exhibited the most tillers with all other genotypes having an intermediate number of tillers. In general, the two Festulolium populations tested shared similar aboveground morphological characteristics, including tiller number and turf quality ratings, with *L. perenne* 'SR4220' and were significantly different from *F. arundinacea* 'Tulsa Time' or *F. pratensis* 'Pasja'.

Longest Root and Number of Roots at a Soil Depth of 60-160 cm

At 60-160 cm root depth, there were no differences among tested accessions in the number of roots or in the length of the longest root (data not shown). At all three harvest dates, the order from highest to lowest for these root characteristics was random.

Total Root Biomass

At each sampling time, *F. pratensis* 'Pasja' produced the largest total root biomass dry weight, and the greatest root dry weight in each of the three sections of the rooting columns when growth had reached those depths (Figs. 1.4 – 1.7). At the 2nd and 3rd sampling, tall fescue had the lowest root biomass at a depth of 0-30 cm (Fig. 1.5). During most of the experiment, *L. perenne*, FL0, FL3S, and *F. arundinacea* did not significantly differ in root biomass at the 30-60 cm or 60-160 cm depths (Figs. 1.6 and 1.7).

Root:Shoot Ratio

At 1, 2, and 3 months after planting, the root:shoot ratio of *Fp* was the greatest among the genotypes tested (Fig. 1.8). Also, for the first two months, the root:shoot ratio

of FL3S was significantly greater than *F. arundinacea*. In experiment 2, at 3 months, FL0, FL3S, and *L. perenne* 'SR4220' all had root:shoot ratios significantly greater than tall fescue (Fig. 1.8c).

Root Scanning Data

WinRHIZO root scanning data produced variable data. In the 30-60-cm and the 60-160-cm ranges, trends among genotypes were similar for root biomass dry weight (Figs. 1.9 and 1.10). Thus, *F. pratensis* 'Pasja' had significantly greater average root diameter, root length, surface area, and volume at the 30-60-cm depth than all other genotypes. At the 60-160-cm depth, *F. pratensis* 'Pasja' had a significantly greater root diameter and root surface area than FL0 and *F. arundinacea* 'Tulsa Time', significantly greater root length and root diameter than FL0 and FL3S, and significantly greater root volume than FL0, FL3S, and *F. arundinacea* 'Tulsa Time'. At a soil depth of 60-160 cm, root diameter of FL3S and *L. perenne* was similar to *F. pratensis* (Fig. 1.10a).

The root biomass results were unexpected. Tall fescue, a common cool-season grass throughout California, was expected to produce a large and deep root system. In both experiments, *F. arundinacea* 'Tulsa Time' displayed root characteristics similar to *L. perenne* 'SR4220', FL0, and FL3S. Perhaps this was a consequence of the fact that the plants were grown under well-watered conditions in this study. Turner et al. (2010) reported that hybrids with introgressions on the short arm of chromosome 3 had deep rooting characters with much variability, but regardless of the conditions the recombinant lines produced the deepest roots, which correlated with increased leaf extension. Greater

total root biomass dry weight was observed in the ryegrass, FL0, and FL3S populations compared to tall fescue under well-watered conditions at 3 months after planting (Fig. 1.4). If the Festulolium populations were to show increased root plasticity in times of stress, they would have a greater ability to uptake water, and possibly be more drought tolerant. In this study, the root systems of the Festulolium hybrids did not differ in any character measured from the root system of the recurrent ryegrass parent.

After three months of growth in the second experiment, Festulolium populations showed a larger root:shoot ratio when compared to tall fescue, but not different from the recurrent parent ryegrass. Drought tolerance in similar species improved with selection for increased root:shoot ratios (Bonos et al. 2004). Plasticity in root systems sometimes requires a trigger event to stimulate the growth of roots that reaches valuable water resources in a dry environment (Fu et al. 2007). A plant that is more adaptable in its ability to grow a larger root system, could possibly be more drought tolerant than a plant without the same plasticity. Tall fescue is known for large deep rooting systems that aids in drought avoidance (Carrow 1996). This study shows that some turf-type *L. perenne* have even larger root systems than *F. arundinacea*, and they can survive extreme drought and heat pressure in Southern California summers. Therefore they could easily replace tall fescue as the major cool-season turfgrass, offering not only better performance under drought, but also superior turf characteristics under optimal conditions. So, while this study failed in the assumed goal of associating root characteristics with enhanced drought and heat tolerance, it might have inadvertently

indentified a new cool-season grass more suitable for Southern California conditions than the widely accepted *F. arundinacea*.

Among this study and others including Festulolium, there were few characteristics of root growth between *L. perenne* 'SR4220' and the Festulolium hybrids (FL3S and FL0) that gave a clear explanation as to why the Festulolium hybrids had increased drought tolerance (Crush et al. 2007, Turner et al. 2008, Turner et al. 2010). Our data show a large amount of genetic variation in these populations of grasses with regard to root responses under well-watered conditions. This agrees with other research into introgression lines of Festulolim hybrids of *L. perenne* and *F. pratensis*, for which large amounts of variation were found in Festulolium lines with chromosome 3 introgressions (Turner et al. 2010). With no significant differences between the rooting characters of *L. perenne* 'SR4220' and the Festulolium hybrids, it is possible that the recurrent parent *L. perenne* 'SR4220' confers a physiological process that is giving these plants greater drought and heat tolerance. While we cannot entirely exclude the possibility there are other *F. pratensis* introgressions in the studied material that are below the resolution limit of the FISH technique used, absence of apparent difference between the recurrent parent (SR4220) and the Festulolium hybrids, more or less eliminates such interpretation. Further research is planned to also examine the factors quantified under well-watered conditions under controlled deficit irrigation in the field to test these hypothesis.

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Table 1. Overall ANOVA table for the effects of experiments 1 and 2 on above and below ground ratings for all genotypes.

a. 1 Month

	AGB‡	Longest	Number	Root DW	Root DW	Root DW	Total	Root:Shoot
DW root		of roots	0-30 cm	30-60 cm	60-160 cm	root DW		
Genotype (G)	***	ns	ns	***	ns	ns	***	***
Experiment (E)	ns	ns	ns	ns	ns	ns	ns	ns
G X E	ns	ns	ns	ns	ns	ns	ns	ns

b. 2 Months

	AGB	Longest	Number	Root DW	Root DW	Root DW	Total	Root:Shoot
DW root		of roots	0-30 cm	30-60 cm	60-160 cm	root DW		
Genotype (G)	**	ns	ns	***	***	ns	***	***
Experiment (E)	***	*	*	*	ns	ns	*	**
G X E	ns	ns	ns	ns	ns	ns	ns	__

c. 3 Months

	AGB	Longest	Number	Root DW	Root DW	Root DW	Total	Root:Shoot
DW root		of roots	0-30 cm	30-60 cm	60-160 cm	root DW		
Genotype (G)	***	ns	ns	***	***	**	***	***
Experiment (E)	***	ns	*	**	*	ns	**	ns
G X E	ns	ns	ns	ns	ns	ns	ns	*

*, **, ***, ns, Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.
‡ Above ground biomass.

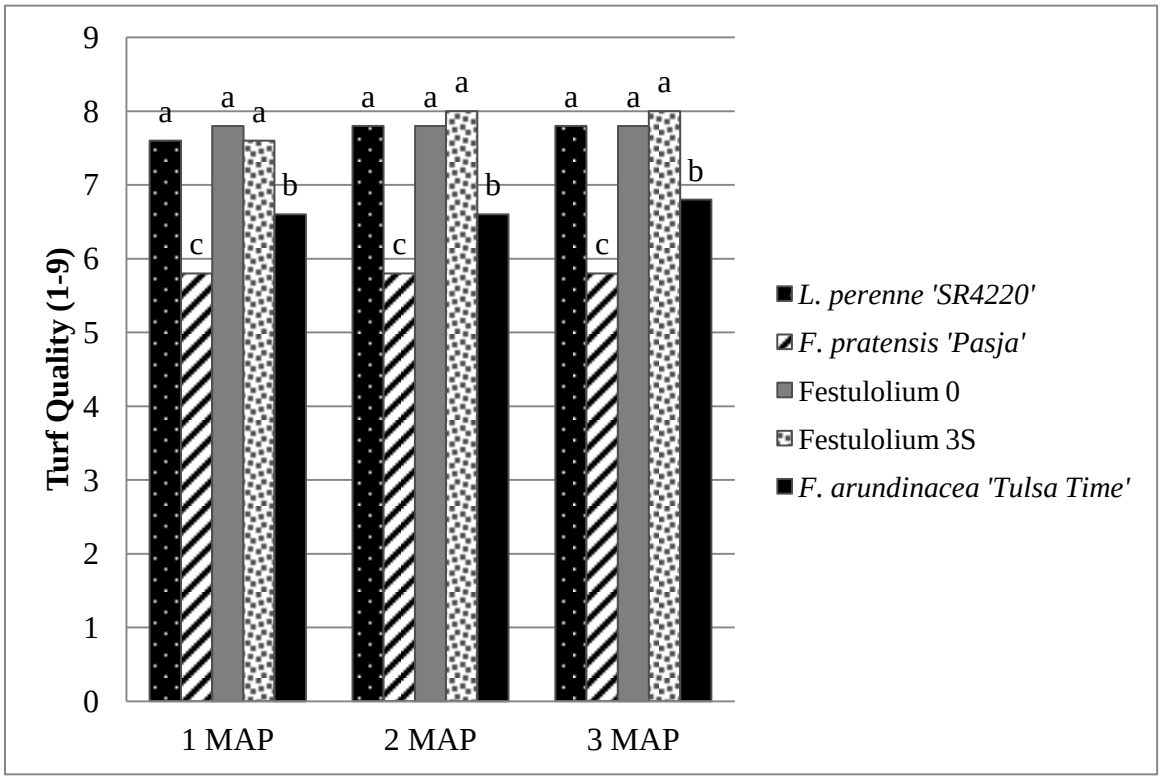

Figure 1.1 Turf quality (color, texture, density, and uniformity) rated 1-9 (1 = dead turf, 6 = minimally acceptable, coarse, light green, thin, and 9 = best, fine, dark green, dense) under well-watered conditions. Riverside, CA. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA (α = 0.05).

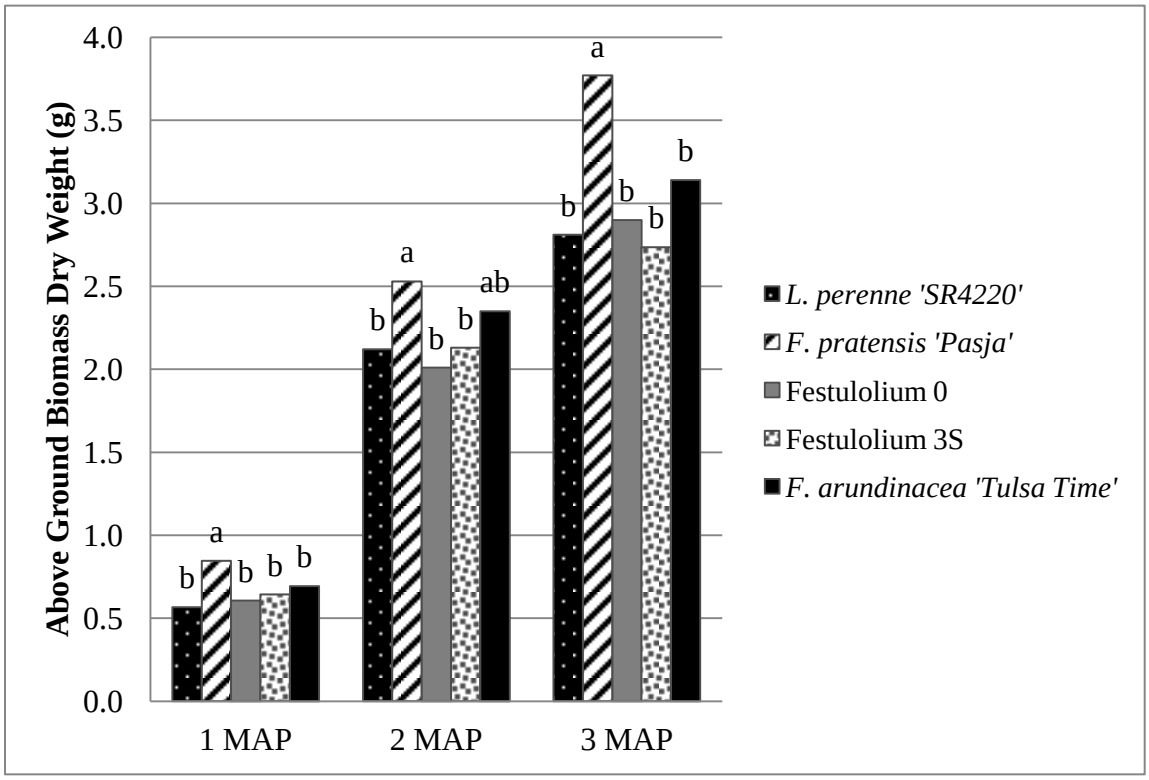

Figure 1.2. Above ground biomass dry weight under well-watered conditions. Riverside, CA. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA ($\alpha = 0.05$).

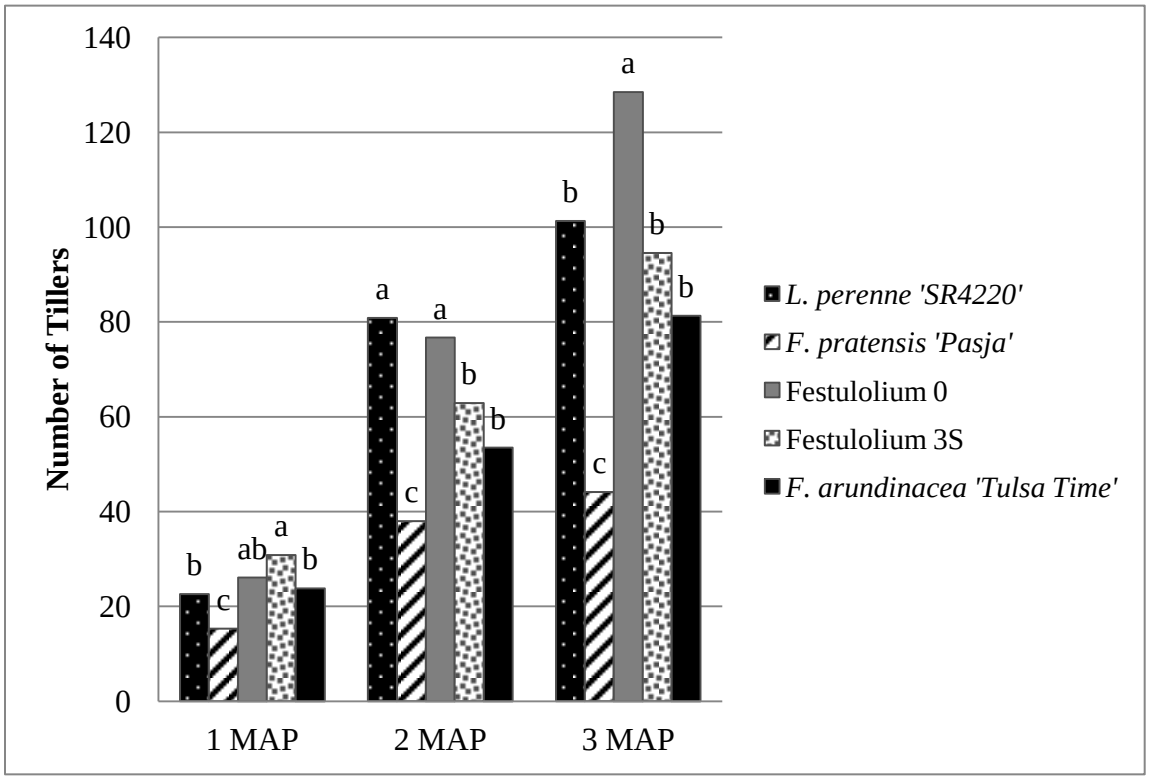

Figure 1.3. Number of tillers under well-watered conditions. Riverside, CA. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA ($\alpha = 0.05$).

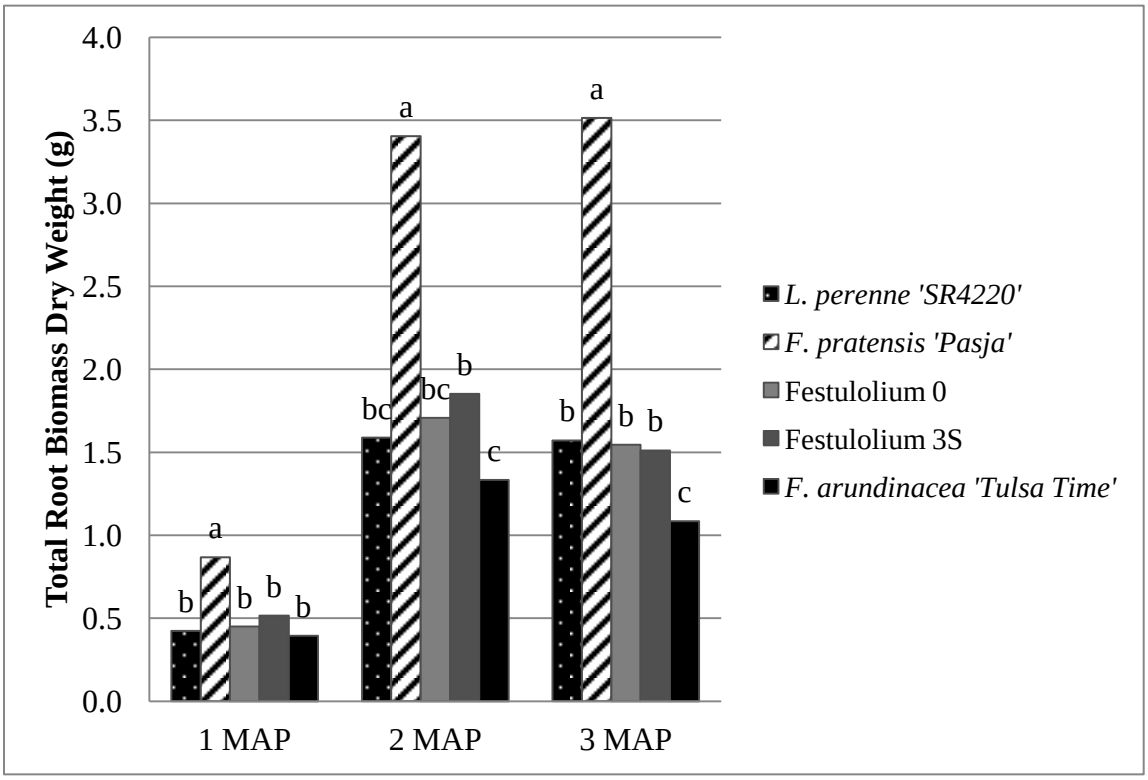

Figure 1.4. Total root biomass dry weight under well-watered conditions. Riverside, CA. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA ($\alpha = 0.05$).

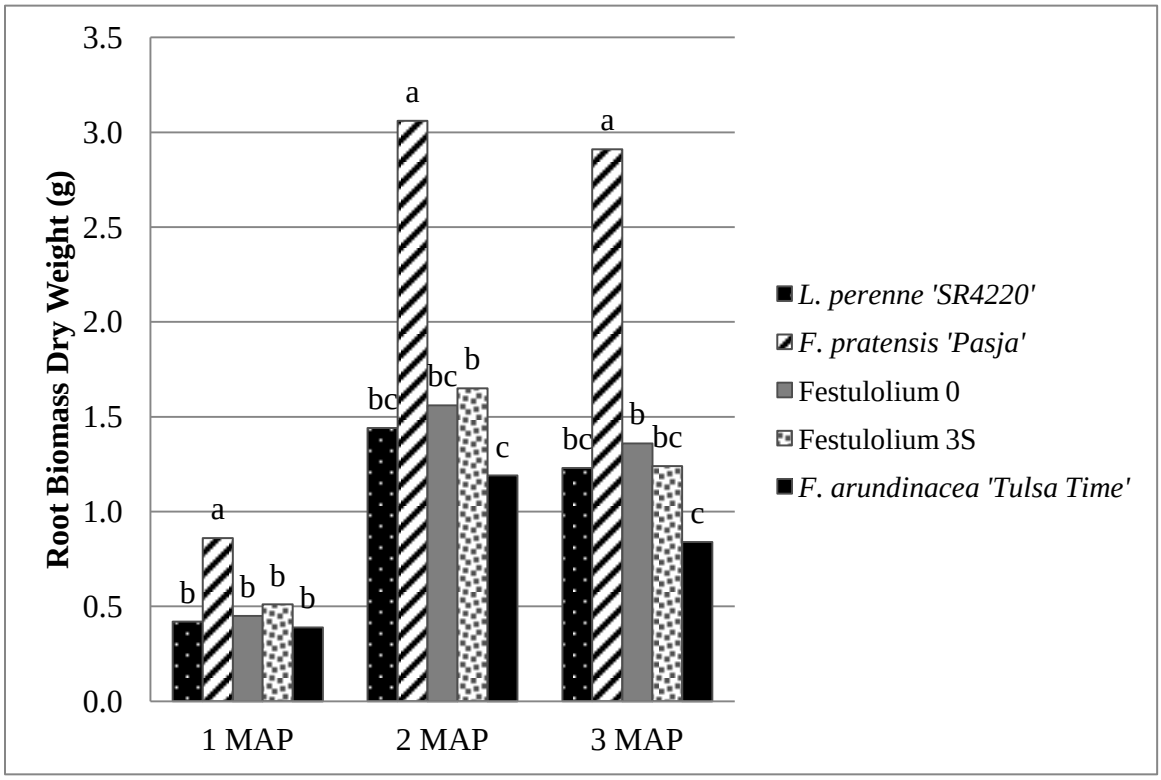

Figure 1.5. Root biomass at a soil depth of 0-30 cm under well-watered conditions. Riverside, CA. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA ($\alpha = 0.05$).

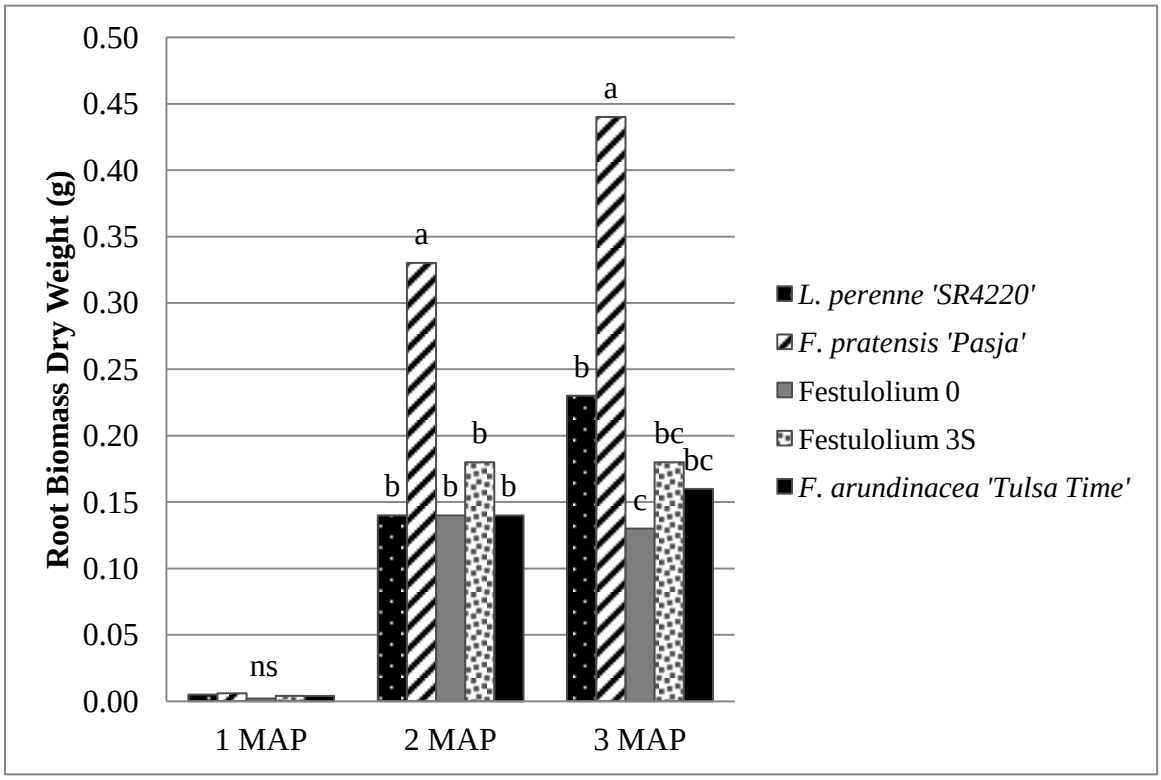

Figure 1.6. Root biomass at a soil depth of 30-60 cm under well-watered conditions. Riverside, CA. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA ($\alpha = 0.05$).

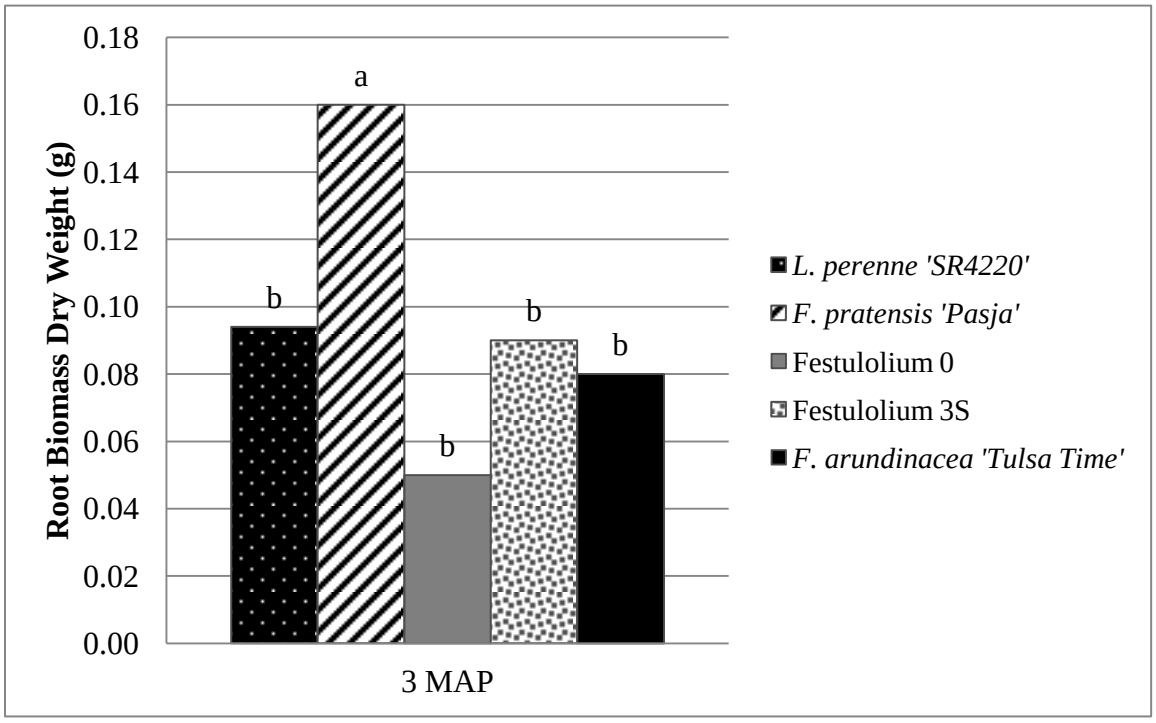

Figure 1.7. Root biomass at a soil depth of 60-160 cm under well-watered conditions. Riverside, CA. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA ($\alpha = 0.05$).

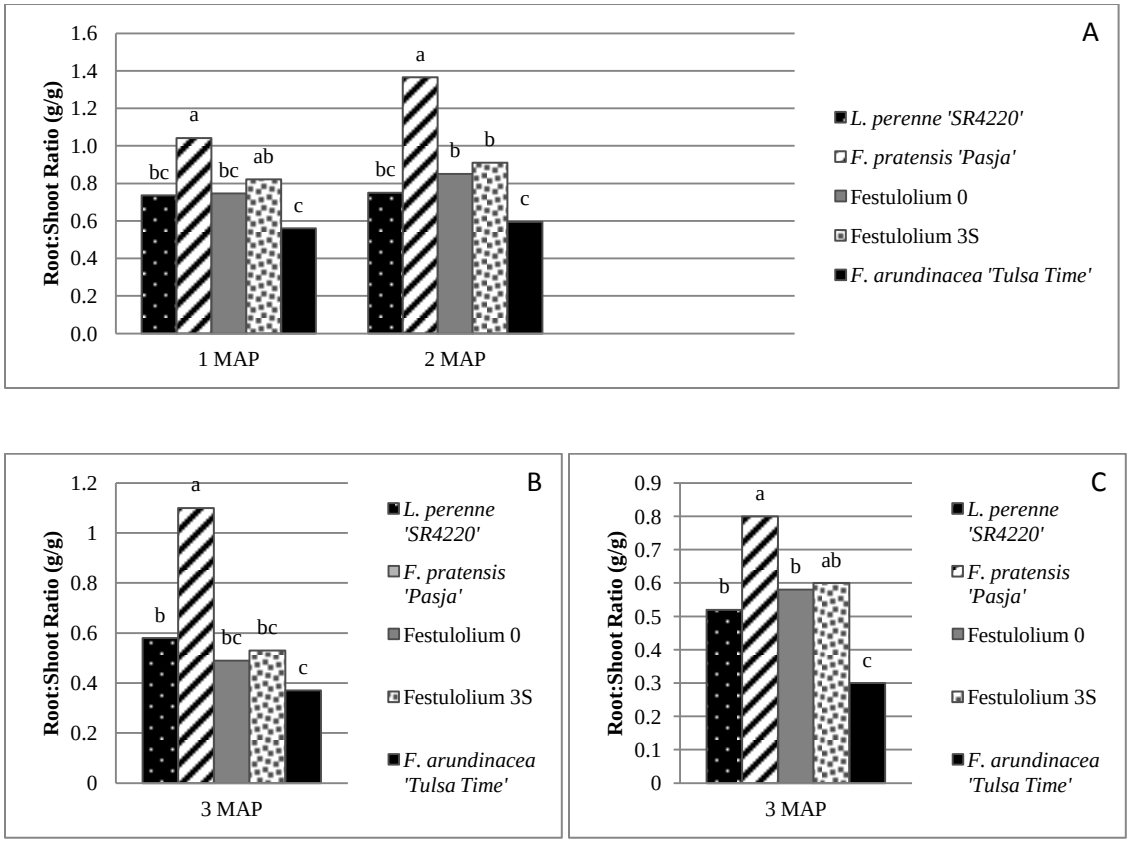

Figure 1.8. Root:Shoot ratio under well-watered conditions. Riverside, CA. A = Mean Root:Shoot ratio during the first two months of growth in experiments 1 and 2. B = Root:Shoot ratio three months after planting of experiment 1. C = Root:Shoot ratio three months after planting of experiment 2. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA ($\alpha = 0.05$).

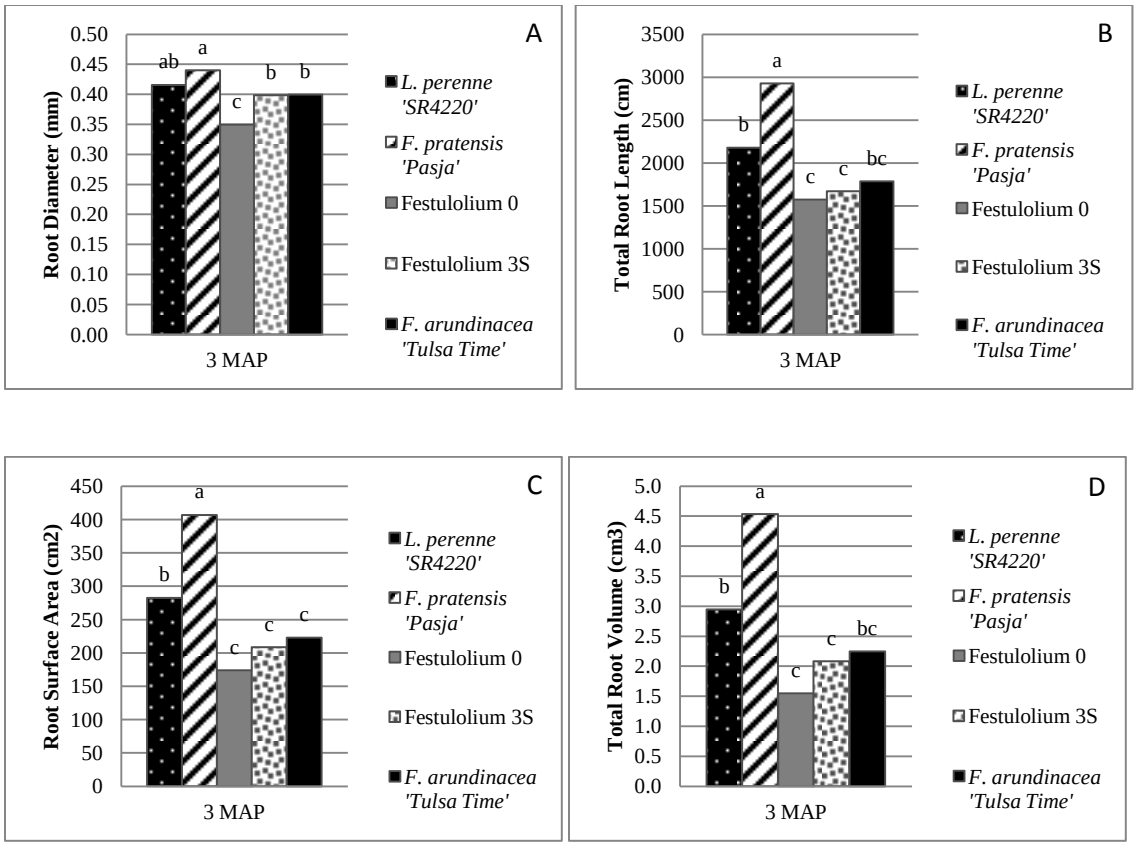

Figure 1.9. WinRHIZO analysis of roots at a soil depth of 30-60 cm under well-watered conditions. Riverside, CA. A = average root diameter, B = total root length, C = total root surface area, and D = total root volume. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA (α = 0.05).

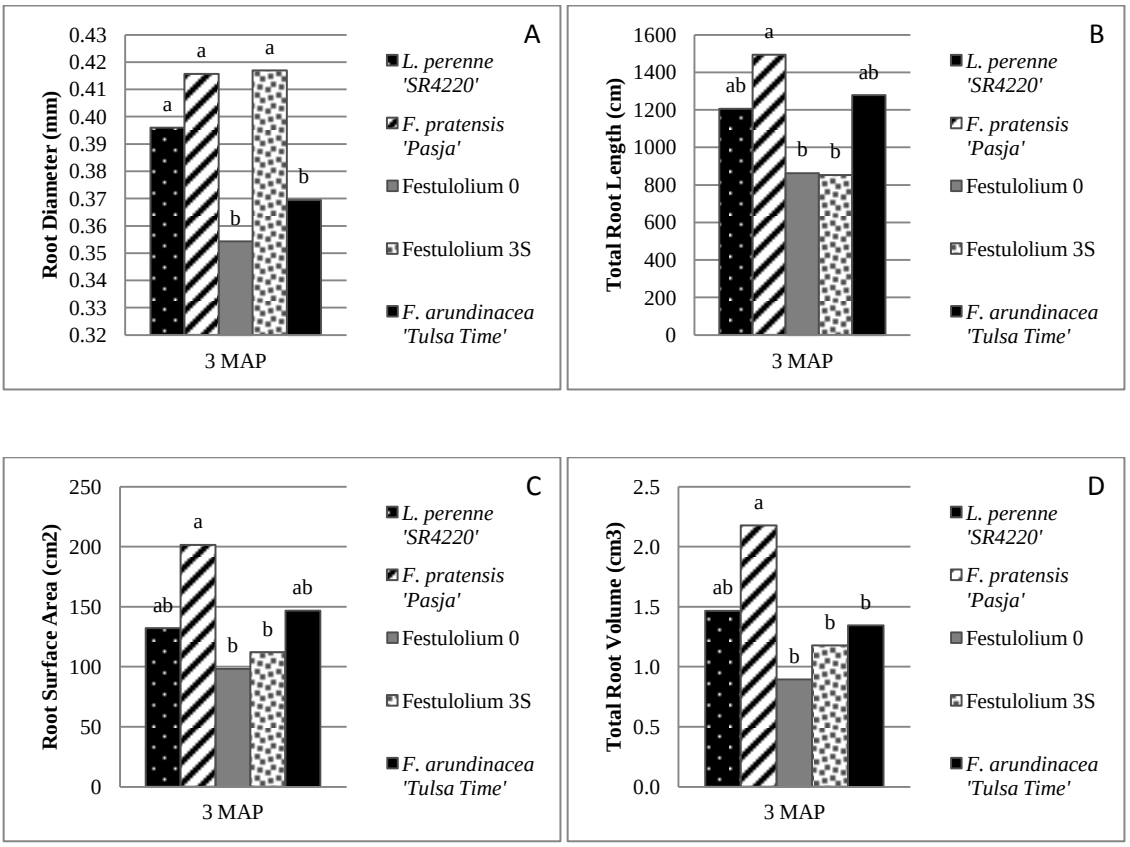

Figure 1.10. WinRHIZO analysis of roots at a soil depth of 60-160 cm under well-watered conditions. Riverside, CA. A = average root diameter, B = total root length, C = total root surface area, and D = total root volume. MAP = Months After Planting. Means with the same letter at each rating date were not significantly different using ANOVA ($\alpha = 0.05$).

Performance of Fescues, Ryegrasses, and Their Hybrids under Field Deficit Irrigation

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Abbreviations: CIMIS, California Irrigation Management Information System; DAT, Days After Treatment; ET_o, reference evapotranspiration; FL, Festulolium; NDVI, Normalized Difference Vegetation Index; TDR, Time-Domain Reflectometry.

ABSTRACT

Fescues (*Festuca spp.*) are known for providing increased abiotic stress tolerance in hybrids with ryegrasses (*Lolium spp.*). Introgressions of *Festuca* chromatin on chromosome 3 of *Lolium* have already been shown to contribute to drought tolerance and deeper root growth. A field study was conducted in 2010 and 2011 in Riverside, CA, to evaluate relative drought tolerance in response to deficit irrigation (50-70% ET_o) among four populations of turf-type hybrids with verified introgressions of meadow fescue [*F. pratensis* (Huds.) P. Beauv.] chromatin on chromosome 3 into *L. perenne* (UCRFL); the recurrent ryegrass backcross parent (*L. perenne* L.); a drought tolerant perennial ryegrass cultivar; a representative meadow fescue; and 3 turf-type tall fescue cultivars (*Festuca arundinacea* Schreb.). During most rating dates, turf quality was significantly greater among the populations of Festulolium and the ryegrasses than fescues. At a soil depth of 30-60 cm, tall fescues 'Grande II' and 'Speedway' showed greater root biomass when compared to all other accessions except UCRFL001. The Festulolium populations provided greater turf quality during drought conditions than fescues, but did not differ significantly from their parent line, *L. perenne* 'SR 4220' or the drought tolerant ryegrass 'Zoom'. The experiments suggest that at least some *L. perenne* turfgrasses could be better adapted to limited irrigation under Southern California conditions.

With predicted global climate change, some believe that the 21st century can expect more years of drought than the previous centuries (Hoerling and Eischeid 2007). Drought is an increasing problem for Californian agriculture, especially when water deliveries are restricted during times of drought. One of the first crops to always face water restrictions during drought is turfgrass. Idealy, new turfgrass species or hybrids can be discovered that require less irrigation while maintaining high turf quality.

Festulolium hybrids were first created by Jenkin (1933) by controlled crosses of *L. perenne* and *F. pratensis* Huds., but such hybrids are known to occur naturally in European grasslands (Brilman 2001). These two genera have many complimentary agronomic traits, and it was only natural that grass breeders looked to combine these profiles into superior hybrids, originally bred for forage use. Some of the original Festulolium cultivars were created to emphasize the abiotic stress tolerance of the *Festuca* genus while having the optimal growing capabilities, palatability, and digestability of nutritious forage of ryegrass (Lewis et al. 1973, Kopecký et al. 2008).

UCR's turf-type Festulolium hybrids originated from a research program geared toward forage grasses. Since our goals focused on turf-type hybrids, we selected for different traits and characteristics than it would be expected in a forage oriented program. In the turf industry, perennial ryegrass contributes a high turf quality that is a combination of dark green color, fine leaf texture, and rapid rate of establishment. Fescues contribute superior abiotic stress tolerance to its hybrids. The sets of characteristics have been successfully combined in hybrids, or have been introgressed

from the *F. pratensis* genome into *L. perenne*-like stocks (Lesniewska et al. 2001, Skibinski et al. 2002, Kosmala et al. 2003). Other abiotic and biotic stress tolerance traits have also been found in hybrids from the *Festuca-Lolium* complex including delayed senescence (Thomas et al. 1994, Thomas et al. 1997), cold tolerance (Lesniewska et al. 2001, Skibinski et al. 2002, Kosmala et al. 2003), freezing tolerance (Kosmala et al. 2006, Grønnerød et al. 2004, Guo et al. 2005), and crown rust (*Puccinia coronata*) resistance (Roderick et al. 2003, Armstead et al. 2006). With the use of genomic and florescent *in-situ* hybridization (GISH and FISH), selection for introgressions can be cytological, or replaced with suitable DNA markers.

Increased drought tolerance in Festulolium has been associated with introgression of chromatin from *F. pratensis* on chromosomes 2 and 3 (Humphreys and Pasakinskiene 1996, Humphreys et al. 1997, Humphreys et al. 2005, Turner et al. 2010). The results of these studies provided evidence that specific introgressions into the long arm of chromosome 2 and the short arm of chromosome 3 gave the Festulolium hybrids greater tolerance to drought and increased rooting depths (Humphreys and Pasakinskiene 1996, Humphreys et al. 1997, Humphreys et al. 2005, Turner et al. 2010). Previous studies at UCR showed Festulolium lines with increased root:shoot ratios compared to tall fescue (Barnes et al. 2012). The use of high root:shoot ratios for successful selection of drought tolerant turf has been demonstrated within both parental genera of the *Festuca-Lolium* complex (Karcher et al. 2008, Bonos et al. 2004). Greater root densities at lower soil depths have been related to less tissue firing (chlorosis of leaf tissue), and improved turf quality (Carrow 1996). Greater turf quality has also been correlated to deeper rooting in

many other grasses like *Zoysia spp.* (Marcum et al. 1995), *Cynodon spp.* (Hays et al. 1991), and *Festuca spp.* (Qian et al. 1997).

Tall fescue is one of the most commonly used cool-season turfgrasses in the Mediterranean transition climate of Southern California for home lawn and landscaping use (Baird et al. 2009). Its popularity for turf use stems from its ability to avoid drought, and this avoidance is believed to be associated with roots penetrating deeper than most other cool season turf species. Wilman et al. (1998) found that, when looking at deep root production at soil depths between 50-100 cm for fescues, ryegrasses, and their hybrids under drought conditions, ranked as follows: *F. arundinacea* > *L. perenne* > *L. perenne* X *F. pratensis*, *F. pratensis*, *L. multiflorum* X *F. pratensis*, *L. perenne* X *L. multiflorum* > *L. multiflorum* > Westerwolds ryegrass. Newer Festulolium lines may be a potential replacement for tall fescue lawns in this climate under deficit irrigation due to increased potential for deeper root growth and higher turf quality.

Southern California turf managers began to realize that turfgrass stands can be maintained at an acceptable turf quality with reduced or deficit irrigation in the 1970's and 1980's. Irrigation maintained as a percentage of ET_o divided by the distribution uniformity of the irrigation system could be used to maintain acceptable turf quality (Doorenbos and Pruitt 1984). Later, it was shown that tall fescue irrigated at 80% of ET_o divided by distribution uniformity, watered deep but infrequently, retained acceptable quality (Richie et al. 2002). Thus, a suitable replacement for tall fescue would have to maintain acceptable turf quality with less irrigation.

Current Festulolium hybrids were bred for their vigor and high biomass production in forage, and would not be a good replacement for a turf use. Our program started from tetraploid hybrids, and used androgenesis to reduce their ploidy to diploid (Kopecký et al. 2005). Lower ploidy levels reduced biomass production and improved turf quality characteristics. Additionally, the hybrids were crossed and backcrossed to diploid, turf-type *L. perenne,* 'SR4220', a selection with optimal drought tolerance and turf quality. Direct and extreme selection pressure for drought tolerance in the field appeared to have favored a region on the short arm of chromosome 3 and to a lesser extent on chromosome 2.

A study was conducted at the University of California, Riverside, to determine root characteristics of these Festulolium hybrids under well-watered conditions in a greenhouse (Barnes et al. 2012). No significant differences were found among the accessions of *L. perenne*, including the introgression line on chromosome 3S and its sister line with normal karyotype. Among lines tested, the accession of *F. pratensis* used as a control, had the greatest root biomass overall, and the 3S introgression line had a higher root:shoot ratio than the tall fescue check. This led to the development of a field study investigating drought tolerance and root biomass production of these turf-type Festulolium hybrids under deficit irrigation. Deficit irrigation is known to trigger a response in some accessions that leads to different root growth characteristics different from growth under well-watered conditions (Fu et al. 2007). This is known as root plasticity.

The objectives of this study were to compare root depth and drought tolerance of the Festulolium hybrids to drought tolerant industry standard turfgrasses for the Southern California climate under field conditions in accessions with verified presence of *F. pratensis* chromatin on the short arm of chromosome 3.

MATERIALS AND METHODS

General Conditions

Plant material evaluated in this study were: (i) three cultivars of *F. arundinacea* 'Tulsa Time', 'Speedway', and 'Grande II' (Seed Research of Oregon, Corvallis, OR); (ii) two cultivars of *L. perenne* 'Zoom' and 'SR 4220' (Seed Research of Oregon); (iii) a representative *F. pratensis* cv. 'Pasja' (Blue Moon Farms, LLC; Lebanon, OR) used here as a control in lieu of the Festulolium parents lost after hybrids were made; and (iv) four populations of Festulolium: 'UCRFL001', 'UCRFL002', 'UCRFL003', and 'UCRFL004'. These populations were developed from material subjected to severe drought and heat pressure under field conditions in Riverside, CA (Barnes et al. 2012), cytologically verified to contain introgression from *F. pratensis* on the short arm of chromosome 3, and intermated in isolation under greenhouse and field conditions.

Experiments ran from January 2010 through November 2012, and deficit irrigation was induced from 19 August to 2 December 2010 and repeated from 27 July to 9 November 2011 at the University of California, Riverside Turfgrass Research Facility. The cultivars and genotypes were established from seed on 14 January 2010 at a rate of 4.5 kg ha^{-1} in 1.5-m^2 plots on a Hanford fine sandy loam (course-loamy, mixed,

superactive, nonacid, thermic Typic Xerothents). Turf was maintained at a 5-cm height of cut, fertilized annually at 293 kg N ha^{-1} in 24.4 kg N ha^{-1} monthly increments (15N-$5P_2O_5$-$8K_2O$; Simplot, Boise, ID), and kept well-watered during establishment and following deficit irrigation treatments.

Deficit Irrigation

At the time of drought initiation, plots were watered by hand using a water break nozzle. The plots were separated by 15-cm tall strips of sheet metal inserted to the depth of 14-cm to prevent belowground movement of water to adjacent plots. Irrigation was based on the previous 7-d cumulative ET_o obtained from an on-site California Irrigation Management Information System (CIMIS) weather station that was based on a modified Penman equation with a wind function. The CIMIS reference crop was a well-watered, 11.9-cm tall cool-season grass. The weekly irrigation amount was equally divided into three irrigation events per week. Irrigation events were cycled to prevent runoff and maximize infiltration. Plots were irrigated at 70% ET_o replacement for the first month, 60% ET_o replacement for the second month, and 50% ET_o replacement for the third month before irrigation was returned to 140% ET_o during a 2-week recovery period.

Data Collection

Bi-weekly measurements included: (i) turfgrass quality (1 = dead turf, 6 = minimally acceptable, coarse, light green, thin, and 9 = best, fine, dark green, dense) (Emmons, 2000); (ii) volumetric soil water content 7.5 cm below the soil surface using time-domain reflectometry that converts multiple electrical signals into a percent soil

moisture level (TDR 300, Spectrum Tech. Inc., Plainfield, IL); and (iii) normalized difference vegetation index (NDVI), which senses light at 660 nm and 840 nm to estimate plant health by taking a ratio of absorbed light and reflected light (CM-1000, Spectrum Tech. Inc., Plainfield, IL). Clippings were collected every month and dried for 72 hr in an 80 ^{o}C oven before weighing. After stress recovery, root data were collected by taking 3 samples per plot using a 2.5-cm hammer drive corer to a soil depth of 60 cm, and dividing each core into two sections of 0-30 cm and 30-60 cm. The cores were placed in water, soaked for 20 min to remove soil, and roots were gently washed of soil to collect root biomass for each depth before drying in a forced air oven at 80 ^{o}C for 3 days prior to weighing.

The experimental design was a randomized complete block with 3 replicates of cultivars or genotypes. Data were subjected to ANOVA using Statistix 8.0 (Statistix Analytical Software, Tallahassee, FL). Means were separated using a Fisher's Protected LSD test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Volumetric soil water content ranged from 3.6% to 60.1% during the experiments and there were no significant differences among treatments or years, indicating uniform soil wetting (data not shown). Average monthly temperatures and ET_o were higher in 2011 than 2010, especially during the initial months of deficit irrigation (Table 2.1). Weather and turf maturity were likely responsible for significant treatment by year

interactions. Consequently, all data, except root analyses, are presented separately by year.

Turfgrass Quality

In 2010, the *L. perenne* cultivars 'Zoom' and 'SR 4220' showed high turfgrass quality before, during, and after recovery from deficit irrigation (Table 2.2). During the same period, UCRFL001 was similar to *L. perenne* 'SR4220'. As drought progressed, the ryegrasses and most of the Festulolium populations retained higher quality than the fescues. It appeared that for the fescues irrigation at 60% ET_o or higher was needed to maintain acceptable turf quality. Few significant differences in quality were observed among the tall fescue cultivars, and collectively they possessed better quality than the *F. pratensis* until 56 DAT.

Higher temperatures in 2011 adversely affected turf quality of the fescues early in the experiment with at least 70% ET_o irrigation required to maintain minimally acceptable quality (Table 2.3). However, from 75-91 DAT, the ryegrasses and FL showed higher quality than the fecues. By the end of the irrigation recovery period, tall fescue quality was similar to that of ryegrass and Festulolium.

Overall, the Festulolium populations were among the top performers for turf quality never dropping below minimally acceptable turf quality until 77 DAT in 2010 and 31 DAT in 2011; however, they were not significantly different from the ryegrasses including 'SR 4220', their backcross parent. All tall fescue cultivars dropped below

minimally acceptable turf quality by 56 DAT in 2010, and all but *F. arundinacea* 'Tulsa Time' dropped below minimal quality by 31 DAT in 2011.

Normalized Difference Vegetation Index (NDVI)

NDVI provides an objective indication of turf health and color, these data corresponded to visual observations indicating that *L. perenne* 'Zoom', *L. perenne* 'SR4220', UCRFL002-004 hybrids, and, in some instances, *F. arundinacea* 'Speedway' held the highest turf quality and health in 2010 (Table 2.4). In 2011, data were more variable in comparison. *L. perenne* 'Zoom' usually displayed the greenest color of all cultivars and genotypes before, during, or after deficit irrigation and, among the fescues, *F. arundinacea* 'Speedway' possessed slightly higher NDVI values throughout the study (Table 2.5).

Dry Clipping Yield

'Pasja' meadow fescue, a forage-type grass, produced the greatest biomass in both years (Tables 2.6 and 2.7). Like turf quality and NDVI, warmer weather in 2011 also reduced dry clipping yields compared to 2010, with exception of 91 DAT. When expressed as percent change from initial clipping yields, the FL populations were similar to the ryegrasses and fescues in terms of dry matter losses during drought stress (data not shown).

Dry Root Biomass

F. arundinacea ‘Grande II’ root biomass dry weight from the soil depth of 30-60 cm was significantly greater than the other cultivars and genotypes with the exception of *F. arundinacea* ‘Speedway’ and ‘UCRFL001’ (Table 2.8). The same general trend was observed in the upper region of the root zone although the data were not significant.

Discussion

Ryegrass cultivars and Festulolium hybrids in this study produced greater turf quality compared to the fescues. A majority of lawns in Southern California consist of tall fescue. Under deficit irrigation, our results suggest ranking grasses, by performance, to *L. perenne* > *L. perenne* X *F. pratensis* (diploid) > *F. arundiacea*> *F. pratensis* (diploid). This is in contrast to the results of Wilman et al. (1998) who demonstrated that *F. arundinacea* was best suited for drought conditions due to its deep root production. Moreover, the Festulolium hybrids and *L. perenne* selections maintained acceptable turf quality above 60% of ET_o for 2010, and at 70% of ET_o for 2011, a much lower irrigation level than previously reported for tall fescue (Richie et al. 2002). Also, recovery ratings of the *L. perenne* cultivars and the Festulolium accessions recovered in less days than the fescues in 2010 and recovered in less days than only ‘UCRFL001’ and *F. pratensis* in 2011 (Table 2.2 and 2.3).

Clipping yield differences among these species and genotypes could be explained in part by their morphological characteristics. For example, fescues have coarser leaf texture than ryegrasses and this may account for a good part of the increased clipping weight. The Festulolium hybrids were identical in texture to their parent line *L. perenne*

'SR4220', a somewhat surprising result given that these Festulolium populations were generated after only two backcrosses to the recurrent parent.

Previous research demonstrated that drought avoidance or tolerance of tall fescue is aided by its deeper root system (Qian et al. 1997). The greenhouse study by this author did show that the accessions of *L. perenne* and Festulolium tested here did not have deeper roots than the *F. arundinacea* check under normal irrigations, and in this study, under serious deficit irrigation all Festulolium and *L. perenne* populations exceeded *F. arundinacea* in turf quality. We did not observe an association between deeper rooting and decreased leaf firing as reported by Carrow (1996) for tall fescue cultivars. However, *F. arundinacea* 'Grande II' and 'Speedway' produced larger root biomass than all other turfgrasses except 'UCRFL001'. And, 'UCRFL001' was the only Festulolium to be significantly greater in root biomass from *L. perenne* 'SR4220'. Previous studies with our Festulolium hybrids showed Festulolium to have increased root:shoot ratios when compared to tall fescue and this could be the reason for increased turf quality of our Festulolium populations in the field (Barnes et al. 2012). Increased root:shoot ratios have been shown to decrease leaf firing in tall fescue and perennial ryegrass cultivars under drought (Bonos et al. 2004).

All Festulolium populations used for this study were BC_2 progenies with only two cycles of drought selection. They were young introgression lines possibly with insufficient backcrosses to create stable and uniform populations homozygous for the introgression on the short arm of chromosome 3. There are indications that gametic

competition may, in fact, select against the introgression. Therefore, the frequency of the introgression does not remain contstant from generation to generation of propagation: an intercross of cytologically verified introgression heterozygotes does not guarantee 50% introgression frequency among progeny. This may explain, to some extent, absence of significant differences between the Festulolium accessions and the recurrent *L. perenne* parent in the majority of test performed here and the greenhouse study.

All tests performed so far do not indicate that deeper rooting is responsible for increased drought and heat tolerance of the tested Festulolium accessions, and we cannot state with confidence that the high level of tolerance is, in fact, associated with the *F. pratensis* intorgressions. In favor of this explanation is a dramatic increase in the frequency of the introgression among the survivor of the extreme test, from several precent to over 60%. On the other hand, we did not study the root systems in detail under deficit irrigation so we cannot exclude extensive root plasticity in response to drought among accessions under study. And, lastly, we cannot exclude the possibility that by, a lucky coincidence, an accession of *L. perenne* was used in this study that has a innately high drought tolerance, significantly above that of industry standard *F. arundinacea*. Overall, this study shows that *L. perenne* and Festulolium hybrids can serve as a replacement for *F. arundinacea* in the Southern California climate.

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Table 2.1. Environmental data during the deficit irrigation and recovery phases of the studies in 2010 and 2011 in Riverside, CA.

Year	Month	Natural Precipitation (mm)	Average Daily ET_o (mm)	Average Daily Temperature (ºC)		
				Minimum	Maximum	Average
2010	July	0.0	166.8	16.1	30.7	22.5
	August	0.0	177.5	16.0	32.6	23.7
	September	0.5	138.4	15.2	32.0	22.9
	October	9.5	53.3	13.2	24.3	18.2
	November	25.3	81.8	8.2	21.5	14.7
	December	218.1	45.3	7.8	18.7	12.6
2011	July	7.3	197.2	17.0	31.9	23.7
	August	0.0	194.3	16.9	33.7	24.4
	September	0.0	139.1	16.0	31.3	22.6
	October	10.8	102.3	12.5	27.6	19.2
	November	39.4	62.12	7.9	20.4	13.6
	December §	0.10	26.15	4.1	18.0	10.8

§ Weather data averaged from 1 Dec 2010 through 9 Dec 2011

Table 2.2. Turf quality[z] during the deficit irrigation and following recovery phase in 2010 in Riverside, CA. Deficit irrigation consisted of 70%, 60%, and 50% ET_o from 0-30, 30-60, and 60-90 DAT[‡], respectively. Recovery was 140% ET_o for 2 weeks.

Treatment	5 DAT	29 DAT	56 DAT	77 DAT	91 DAT	Recovery
L. perenne 'SR4220'	8.7 ab	7.7 b	6.3 b	5.0 a	3.0 a	6.0 a
L. perenne 'Zoom'	9.0 a	8.3 a	7.3 a	5.0 a	3.0 a	6.3 a
UCRFL001	8.3 bc	7.7 b	6.3 b	5.0 a	3.0 a	6.0 a
UCRFL002	8.0 cd	7.3 bc	6.3 b	5.0 a	3.0 a	6.0 a
UCRFL003	8.0 cd	7.0 c	6.0 bc	5.0 a	3.0 a	6.0 a
UCRFL004	8.0 cd	7.0 c	5.7 bcd	4.3 b	3.0 a	5.3 b
F. pratensis 'Pasja'	6.0 f	5.0 e	5.0 d	4.0 c	2.3 b	4.0 c
F. arundinacea 'Grande II'	7.5 e	6.0 d	5.0 d	3.0 d	2.0 b	4.0 c
F. arundinacea 'Tulsa Time'	7.7 de	6.0 d	5.3 cd	3.0 d	2.0 b	4.0 c
F. arundinacea 'Speedway'	7.5 e	6.0 d	5.7 bcd	3.0 d	2.3 b	4.0 c
LSD (α = 0.05)	**0.5**	**0.6**	**0.9**	**0.3**	**0.5**	**0.5**

Mean separation within columns by Fisher's protected LSD test (α = 0.05).

[‡]Days after treatment (DAT)

[z] Turfgrass quality (1 = dead turf, 6 = minimally acceptable, coarse, light green, thin, and 9 = best, fine, dark green, dense)

Table 2.3. Turf quality[z] during the deficit irrigation and following recovery phase in 2011 in Riverside, CA. Deficit irrigation consisted of 70%, 60%, and 50% ET_o from 0-30, 30-60, and 60-90 DAT[§], respectively. Recovery was 140% ET_o for 2 weeks.

Treatment	0 DAT	31 DAT	61 DAT	75 DAT	91 DAT	Recovery
L. perenne 'SR4220'	8.0 a †	5.7 ab †	4.0 a ‡	4.0 a †	3.0 a †	4.3 ab †
L. perenne 'Zoom'	8.0 a	5.7 ab	3.7 a	3.7 a	2.3 bc	4.0 abc
UCRFL001	8.0 a	3.7 c	3.0 b	3.7 a	2.7 ab	3.0 c
UCRFL002	8.0 a	4.3 bc	3.7 a	4.0 a	2.7 ab	4.3 ab
UCRFL003	8.0 a	5.7 ab	3.0 b	4.0 a	2.7 ab	5.0 a
UCRFL004	7.3 b	5.7 ab	3.7 a	4.0 b	3.0 a	5.0 a
F. pratensis 'Pasja'	6.0 d	4.3 bc	3.0 b	3.0 b	2.0 c	3.7 bc
F. arundinacea 'Grande II'	6.7 c	5.3 ab	2.0 c	3.0 b	2.0 c	4.0 abc
F. arundinacea 'Tulsa Time'	7.0 bc	6.3 a	2.7 b	2.7 b	2.0 c	4.0 abc
F. arundinacea 'Speedway'	7.0 bc	5.3 ab	3.0 b	3.0 b	2.0 c	5.0 a
LSD (α = 0.05)	**0.4**	**1.5**	**0.7**	**0.6**	**0.6**	**1.0**

† Mean separation within columns by Fisher's protected LSD test (α = 0.05).
‡ Mean separation within columns by Fisher's protected LSD test (α = 0.10).
[§]Days after treatment (DAT)
[z]Turfgrass quality (1 = dead turf, 6 = minimally acceptable, coarse, light green, thin, and 9 = best, fine, dark green, dense)

Table 2.4. Normalized difference vegetation index (NDVI) during the deficit irrigation and following recovery phase in 2010 in Riverside, CA. Deficit irrigation consisted of 70%, 60%, and 50% ET_o from 0-30, 30-60, and 60-90 DAT‡, respectively. Recovery was 140% ET_o for 2 weeks.

Treatment	5 DAT	29 DAT	56 DAT	77 DAT	91 DAT	Recovery
L. perenne 'SR4220'	0.76 d †	0.76 bc	0.80 b	0.58 b	0.50 ab	0.68 bc
L. perenne 'Zoom'	0.85 a	0.85 a	0.86 a	0.65 a	0.53 a	0.76 a
UCRFL001	0.75 d	0.76 bc	0.83 ab	0.59 b	0.52 a	0.72 ab
UCRFL002	0.75 d	0.76 bc	0.82 b	0.57 b	0.49 ab	0.72 ab
UCRFL003	0.75 d	0.77 bc	0.81 b	0.57 b	0.49 ab	0.72 ab
UCRFL004	0.76 d	0.76 bc	0.79 bc	0.56 b	0.47 b	0.71 ab
F. pratensis 'Pasja'	0.67 e	0.67 d	0.73 d	0.50 c	0.42 cd	0.59 de
F. arundinacea 'Grande II'	0.77 cd	0.74 c	0.74 d	0.49 c	0.42 d	0.58 e
F. arundinacea 'Tulsa Time'	0.79 bc	0.76 bc	0.76 cd	0.49 c	0.41 d	0.59 de
F. arundinacea 'Speedway'	0.80 b	0.79 b	0.79 bc	0.56 b	0.46 bc	0.64 cd
LSD (α = 0.05)	**0.026**	**0.038**	**0.041**	**0.055**	**0.045**	**0.055**

† Mean separation within columns by Fisher's protected LSD test (α = 0.05).
‡Days after treatment (DAT)

Table 2.5. Normalized difference vegetation index (NDVI) during the deficit irrigation and following recovery phase in 2011 in Riverside, CA. Deficit irrigation consisted of 70%, 60%, and 50% ET_o from 0-30, 30-60, and 60-90 DAT‡, respectively. Recovery was 140% ET_o for 2 weeks.

Treatment	0 DAT	31 DAT	61 DAT	75 DAT	91 DAT	Recovery
L. perenne 'SR4220'	0.82	0.74 ab †	0.73	0.67 a	0.60 a	0.77
L. perenne 'Zoom'	0.88	0.76 ab	0.74	0.63 ab	0.58 abc	0.71
UCRFL001	0.82	0.61 d	0.69	0.58 bcd	0.50 de	0.69
UCRFL002	0.82	0.70 bc	0.73	0.63 abc	0.58 abc	0.77
UCRFL003	0.82	0.75 ab	0.72	0.63 abc	0.53 bcde	0.79
UCRFL004	0.81	0.73 ab	0.74	0.67 a	0.60 a	0.81
F. pratensis 'Pasja'	0.81	0.64 cd	0.70	0.57 bcd	0.56 abcd	0.73
F. arundinacea 'Grande II'	0.82	0.72 bc	0.67	0.55 d	0.52 cde	0.74
F. arundinacea 'Tulsa Time'	0.86	0.81 a	0.71	0.56 cd	0.49 e	0.72
F. arundinacea 'Speedway'	0.85	0.77 ab	0.73	0.61 abcd	0.58 abc	0.76
LSD (α = 0.05)	**ns**	**0.084**	**ns**	**0.071**	**0.068**	**ns**

† Mean separation within columns by Fisher's protected LSD test (α = 0.05).
‡Days after treatment (DAT)

Table 2.6. Dry clipping yield of turfgrasses during the deficit irrigation and following recovery phase in 2010 in Riverside, CA. Deficit irrigation consisted of 70%, 60%, and 50% ET_o from 0-30, 30-60, and 60-90 DAT‡, respectively. Recovery was 140% ET_o for 2 weeks.

Treatment	29 DAT	56 DAT	91 DAT	Recovery
	Grams			
L. perenne 'SR4220'	3.83 cd †	1.69 d	2.12 cd	4.48 b
L. perenne 'Zoom'	3.76 cd	2.35 d	1.69 d	4.17 b
UCRFL001	4.72 bcd	2.98 cd	2.67 bcd	4.82 b
UCRFL002	3.52 d	3.35 cd	2.09 cd	5.58 b
UCRFL003	4.62 bcd	1.72 d	2.07 cd	4.78 b
UCRFL004	4.58 bcd	2.26 d	2.06 cd	4.66 b
F. pratensis 'Pasja'	19.84 a	11.72 a	6.51 a	20.79 a
F. arundinacea 'Grande II'	7.96 bc†	5.85 b	3.89 b	6.94 b
F. arundinacea 'Tulsa Time'	7.44 bcd	4.41 bc	3.33 bc	6.32 b
F. arundinacea 'Speedway'	8.81 b	2.66 cd	2.76 bcd	6.73 b
LSD (α = 0.05)	**4.24**	**1.78**	**1.47**	**3.15**

† Mean separation within columns by Fisher's protected LSD test (α = 0.05).
‡Days after treatment (DAT)

Table 2.7. Dry clipping yield of turfgrasses during the deficit irrigation and following recovery phase in 2011 in Riverside, CA. Deficit irrigation consisted of 70%, 60%, and 50% ET_o from 0-30, 30-60, and 60-90 DAT‡, respectively. Recovery was 140% ET_o for 2 weeks.

Treatment	0 DAT	31 DAT	61 DAT	91 DAT	Recovery
			Grams		
L. perenne 'SR4220'	17.16 cd	2.04 c	3.83	3.16 d	1.47 d
L. perenne 'Zoom'	18.83 cd	1.77 c	2.37	3.53 d	1.21 d
UCRFL001	13.88 d	2.42 c	5.70	6.61 b	1.27 d
UCRFL002	16.60 cd	2.01 c	3.20	4.41 bcd	1.88 cd
UCRFL003	20.28 bcd	1.90 c	3.53	3.50 d	2.47 bcd
UCRFL004	17.53 cd	2.19 c	4.90	3.80 cd	1.97 cd
F.pratensis 'Pasja'	41.48 a	7.65 a	5.67	9.86 a	6.20 a
F. arundinacea 'Grande II'	27.66 bc	6.11 ab	6.17	6.33 bc	5.55 a
F. arundinacea 'Tulsa Time'	27.00 bc	6.69 ab	6.57	5.32 bcd	3.29 bc
F. arundinacea 'Speedway'	32.61 ab	5.75 b	7.60	5.25 bcd	3.69 b
LSD (α = 0.05)	**12.58**	**1.66**	**ns**	**2.76**	**1.67**

† Mean separation within columns by Fisher's protected LSD test (α = 0.05).

‡Days after treatment (DAT)

Table 2.8. Pooled data for dry root biomass at a soil depth of 0-30 cm and 30-60 cm at the conclusion of the experiments in 2010 and 2011. Riverside, CA.

	Root biomass dry weight 0-30 cm core (g)	Root biomass dry weight 30-60 cm core (g)
L. perenne 'SR4220'	0.19	0.019 d †
L. perenne 'Zoom'	0.20	0.024 cd
UCRFL001	0.24	0.039 abc
UCRFL002	0.20	0.033 bcd
UCRFL003	0.22	0.022 cd
UCRFL004	0.19	0.029 cd
F. pratensis 'Pasja'	0.14	0.021 cd
F. arundinacea 'Grande II'	0.28	0.053 a
F. arundinacea 'Tulsa Time'	0.22	0.033 bcd
F. arundinacea 'Speedway'	0.27	0.048 ab
LSD (α = 0.05)	**ns**	**0.019**

† Mean separation within columns by Fisher's protected LSD test (α = 0.05)