Natural Selection for Survival Improves Freezing Tolerance, Forage Yield, and Persistence of Festulolium

M. D. Casler,* P. R. Peterson, L. D. Hoffman, N. J. Ehlke, E. C. Brummer, J. L. Hansen, M. J. Mlynarek, M. R. Sulc, J. C. Henning, D. J. Undersander, P. G. Pitts, P. C. Bilkey, and C. A. Rose-Fricker

ABSTRACT

Festulolium [*Festulolium loliaceum* (Hudson) P.V. Fournier] is a hybrid between meadow fescue (*Festuca pratensis* Huds.) and Italian ryegrass (*Lolium multiflorum* Lam.) or perennial ryegrass (*L. perenne* L.). The ryegrass parentage gives festulolium cultivars marginal winterhardiness in regions with severely cold winters. The objective of this research was to determine if natural selection for field survival resulted in a genetic improvement in freezing tolerance, forage yield, or persistence in northcentral and northeastern USA environments. The component strains of 'Spring Green' festulolium were compared with their unselected parents in a controlled-environment freezing test. The component strains of Spring Green had 186% greater plant survival and 34% greater tiller survival than their unselected parents. Spring Green was compared with its two commercially available parents in a 13-location field test. Spring Green averaged 5.0% higher in 2-yr forage yield than 'Kemal', but was similar in forage yield to 'Tandem' in a 13-location test ranging from Minnesota to Virginia. Spring Green averaged 30% more ground cover than its unselected parents at the six locations within USDA hardiness zones 2 through 4, but was generally similar to its parents outside of these severe hardiness zones. The increased freezing tolerance of Spring Green, obtained by phenotypic selection for field survival at several locations, appears to have resulted in increased adaptation to northern USA forage production environments.

Festulolium spp. represents a complex of interspecific hybrids between *Lolium* and *Festuca*. Meadow fescue (*Festuca pratensis* Huds.) is commonly used as one of the parents, crossed with either *Lolium perenne* L., resulting in hybrid progeny that are generally referred to as *Festulolium loliaceum* (Hudson) P.V. Fournier, or *L. multiflorum* Lam., resulting in hybrid progeny that are generally referred to as *Festulolium braunii* K.A. Hybrids between *Lolium* and *Festuca* are fairly common in nature, but did not receive a great deal of attention in agriculture until the 1950s (Wit, 1959).

Meadow fescue contributes midsummer growth, winter hardiness, and drought tolerance (Humphreys and Pasakinskiene, 1996; Thomas and Humphreys, 1991) while the ryegrass parent contributes rapid establishment and forage quality (Casler, 1990; Thomas and Humphreys, 1991) to the hybrids. Forage quality of festulolium cultivars is similar to that of ryegrass cultivars (Casler, 1990), which is higher than for any other agronomically important cool-season forage grass (Jung et al., 1996).

On the negative side, the ryegrass parent generally reduces winter survival of festulolium hybrids and their derivatives. The lack of adaptation of festulolium and other ryegrasses (*Lolium* spp.) to the northcentral and northeastern USA and southern Canada severely limits their use in pasture systems. Severe cold, dessicating winds, and lack of snow cover cause severe winter injury and mortality, limiting stand life under these extreme conditions (Casler and Walgenbach, 1990). Festulolium cultivars tend to have better winter survival than ryegrass in the northern USA, but not equal to that of meadow fescue. Three festulolium cultivars ranked 1, 2, and 3 out of 50 ryegrass and festulolium cultivars in a four-location evaluation of survival following two winters in Wisconsin (Casler and Walgenbach, 1990). The three festulolium cultivars averaged 56% ground cover, compared with 50% for the highest-ranked ryegrass cultivar and 38% for the mean of all 47 ryegrass cultivars.

Natural selection for survival was practiced in festulolium by digging and intercrossing survivors from swards that had been exposed to three winters (Novy et al., 1995). Selection increased progeny survival as measured by ground cover and botanical composition of mixed swards. Genetic variation for winterhardiness may be caused by genetic differences in fall dormancy, winter dormancy, soluble carbohydrate concentration, freezing tolerance, and possibly other factors. The objectives of this study were to (i) quantify freezing tolerance of four festulolium populations created by natural selection for field survival and their parental populations and (ii) determine if natural selection for survival can increase forage yield or persistence at multiple locations across the northern USA.

M.D. Casler and D.J. Undersander, Dep. of Agronomy, Univ of Wisconsin-Madison, Madison, WI 53706-1597; P.R. Peterson and N.J. Ehlke, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul, MN 55108 (P.R. Peterson formerly, Dep. of Crop, Soil, and Envt. Sci., Virginia Poly. Inst., Blacksburg, VA) 24061; L.D. Hoffman, Dep. of Agronomy, The Pennsylvania State Univ., University Park, PA 16802; E.C. Brummer, Dep. of Agronomy, Iowa State Univ., Ames, IA 50010; J.L. Hansen, Dep. of Plant Breeding and Biometry, Cornell Univ., Ithaca, NY 14853-1902; M.J. Mlynarek, Agric. Res. Stn., Univ. of Wisconsin-Madison, Ashland, WI 54806; M.R. Sulc, Dep. of Horticulture and Crop Sci., Ohio State Univ., Columbus, OH 43210; J.C. Henning, Dep. of Agronomy, Univ. of Kentucky, Lexington, KY 40506; P.G. Pitts, ITB Tech. Inc., Spring Green, WI 53588; P.C. Bilkey, AgResearch Intl. Inc., Madison, WI 53719; C.A. Rose-Fricker, Pure Seed Testing Inc., Hubbard, OR 97032. Received 27 Aug. 2001. *Corresponding author (mdcasler@facstaff.wisc.edu).

Published in Crop Sci. 42:1421–1426 (2002).

MATERIALS AND METHODS

Festulolium Germplasm

In April 1986, replicated 3-m row plots of 'Elmet', 'Prior', and Tandem festulolium were planted near Arlington, Ashland, Prairie du Sac, and Spooner, WI, in mixture with alfalfa, *Medicago sativa* L. (Casler and Walgenbach, 1990). Plots were harvested three or four times per year. Following three winters, mean ground cover of the festulolium row plots ranged from 10 to 85% among the five locations (Casler and Walgenbach, 1990). The most vigorous survivors were selected from the row plots, in approximately equal frequencies among cultivars and locations. All selections were transplanted to a common location and intercrossed to create the WFL-a89C population. Approximately 4000 plants of this population were transplanted near Hubbard, OR, in 1991. These plants were evaluated for stem rust (caused by *Puccinia graminis* Pers.) resistance in June 1992 and 100 of the most resistant plants were intercrossed to create the WFLr population.

Kemal festulolium was seeded to a 2-ha pasture near Spring Green, WI, in 1985. The pasture was set stocked for 5 yr, with 2 cows plus calves (*Bos taurus*) per ha. Following five winters, festulolium plant density in the Kemal pasture was less than 1 plant m^{-2}. A random sample of 160 plants were dug from the pasture, 80 representing heavily grazed plants in open areas and 80 representing plants ungrazed and relatively hidden from cattle by tall perennial broadleaf weeds. These plants were transplanted near Hubbard, OR, in August 1991. In 1992, 11 clones were selected for resistance to stem rust and intercrossed in two separate groups. The progeny populations were named W4KG (five clones from the grazed group) and W4KU (six clones from the ungrazed group).

Freezing Tolerance Test

Seed from the populations W4KG, W4KU, WFL-a89C, and WFLr and the cultivars Kemal, Elmet, Prior, and Tandem were planted in a glasshouse in June 1995. Each population was planted in 20 replicates of a completely randomized design. Each experimental unit was a rack of 67 30- by 130-mm cones, each with one seedling.

When seedlings reached a 56-d age, racks were placed in a controlled-environment chamber for 35 d of cold hardening at 2°C with an 8-h photoperiod. After hardening, four replicates of each population were placed in one of five controlled-environment chambers, maintained at one of the following five constant temperatures: −5, −7, −11, −14, or −17°C. Plants were maintained at that temperature for 3 d in the dark, after which they were removed and returned to the glasshouse, which was maintained at 18/13°C day/night.

After 30 d of recovery in the glasshouse, the number of living and dead tillers were counted on each plant. Tiller survival was computed on a plant basis and plant survival was computed on an experimental unit basis. Plants were harvested at a 0.5-cm cutting height, weighed, dried for 5 d at 60°C, and reweighed to determine dry matter concentration. Data for each freezing temperature were analyzed by analysis of variance. Means of each selected population were compared with means of their parent population(s) by contrasts.

Field Test

Three festulolium cultivars were seeded into 14 field trials in 1997. The cultivars were Spring Green (Casler et al., 2001), Kemal, and Tandem. The latter two cultivars comprise 67% of the parentage of Spring Green. Elmet and Prior, comprising the other 33% of Spring Green's pedigree, were unavailable. Previous field tests at four locations indicated that Elmet, Prior, and Tandem had similar survival over a 3-yr period (Casler and Walgenbach, 1990).

Each experiment was designed as a randomized complete block with three to 10 replicates (Table 1). In some cases, the festulolium cultivars were included in larger cultivar trials, but generally as a split-plot design in which species were whole plots. Plots were seeded with drill-type seeders, in rows spaced 15 cm apart, to simulate solid swards. Plot size varied among locations (Table 1). Plots were seeded at a rate of 18 kg ha^{-1} on a pure-live-seed basis. Germination was tested in January

Table 1. Site and management characteristics of 14 locations use to evaluate three festulolium cultivars.

Location	Soil type	r†	Plot size	Harvest schedule	Nitrogen applied‡
			m		kg ha^{-1}
Rosemount, MN	Waukegon silt loam (fine-silty over sandy-skeletal, mixed mesic Typic Hapludoll)	4	1.0 × 5.0	*Three harvests*: June, August, and September	50, 50, 50
Ames, IA	Nicollet loam (fine-loamy, mixed, super-active, mesic Aquic Hapludoll)	8	0.9 × 3.7	*Three harvests*: June, July, and September	112, 56, 56
Arlington, WI	Plano silt loam (fine-silty, mixed, mesic Typic Argiudoll)	8	0.9 × 6.1	*Three harvests*: June, August, and October	84, 84, 84
Ashland, WI	Portwing silt loam (fine, mixed, super-active, frigid Oxyaquic Glossudalf)	8	0.9 × 6.1	*Four harvests*: June, July, August, and October	84, 84, 84, 84
Lancaster, WI§	Fayette silt loam (fine-silty, mixed, mesic, Typic Hapludalf)	3	1.2 × 4.5	*Five grazings*: May, June, August, September, and October	40, 40, 40, 40, 40
Marshfield, WI	Withee silt loam (fine-loamy, mixed Aquic Glossoboralf)	8	0.9 × 6.1	*Three harvests*: June, August, and October	84, 84, 84
Lexington, KY	Maury well-drained silt loam (fine, mixed, mesic, semiactive Typic Paleudalf)	8	1.5 × 4.6	*Four harvests*: May, June, July, and October	67, 67, 67
Wooster, OH	Crosby silt loam (fine, mixed, mesic Aeric Ochraqualf)	8	0.9 × 6.1	*Three harvests*: May, June, and August	84, 78, 56
Rock Springs, PA	Hagerstown silt loam (fine, mixed, mesic, Typic Hapludolf)	8	0.9 × 6.1	*Three harvests*: May, July, and September	78, 56, 56
Rock Springs, PA¶	Hagerstown silt loam (fine, mixed, mesic, Typic Hapludolf)	6	0.9 × 6.1	*Three harvests*: May, July, and September	78, 56, 56
Ithaca, NY	Rhinebeck silt loam (fine-loamy, mixed Pachic Haploboroll)	10	1.1 × 4.0	*Four harvests*: May, July, August, and September	50, 50, 50, 50
Blacksburg, VA	Shottower cobbly loam (clayey, kaolinitic, mesic Typic Paleudult)	4	1.5 × 4.5	*Four harvests*: May, July, August, and November	80, 60, 40, 60
Blackstone, VA	Norfolk and Dothan sandy clay loams (fine-loamy, silceous, thermic, Typic Paleudult; fine-loamy, siliceous, thermic, Plinthic Paleudult)	4	1.5 × 4.5	*Three harvests*: May, June, and December	100, 100, 100
Orange, VA	Davidson clay loam (fine, kaolinitic, thermic Rhodic Kandiudult)	4	1.5 × 4.5	*Four harvests*: May, June, August, and December	100, 50, 0, 75

† Number of replicates.
‡ First number is kg N ha^{-1} applied in early spring, followed by applications made after the first and successive harvests.
§ Lancaster was grazed by beef cattle. All other locations were mechanically harvested.
¶ Seeded in late summer. All other locations were seeded in spring.

1997 according to AOSA standards (AOSA, 1998). Plots were clipped during the establishment year to control annual weeds.

Plots were harvested three or four times in 1998 and 1999 with mechanical harvesters at 13 of the 14 locations (excluding Lancaster, WI). Plots were cut to a stubble height of 7 to 9 cm. Nitrogen fertilizer was applied as indicated in Table 1. Phosphorus, potassium, and sulfur were added if soil tests indicated low levels of these elements. A 0.5- to 1-kg sample was collected from each plot for dry matter determination. Ground cover of living crown tissue was visually rated, using 5-percentage-point increments, immediately following the first harvest in spring 2000 at all locations except the three Virginia locations which showed little evidence of stand loss. Seeded rows had generally been obscured by this time because of tillering.

Plots at Lancaster were grazed with beef steers (*Bos taurus*) with a stocking rate sufficient to remove most standing forage within a 4- to 6-h period. Forage yield was determined with a capacitance meter calibrated to predict herbage mass from clipped samples (Murphy et al., 1995). Forage yield at all locations was computed as the sum over all cuttings or grazings.

Forage yield data and ground cover data were analyzed by mixed models analysis. For forage yield, cultivars, locations, and years were assumed to be fixed effects. Locations were assumed fixed because they belonged to different hardiness zones and we expected this to be an important factor influencing cultivar × location interaction. Cultivar × location interactions were partitioned into cultivar × region and cultivar × location(region), where two regions were defined based on locations in USDA hardiness zones 2 through 4 (Iowa, Minnesota, and Wisconsin) and zones 5 through 6 (all other locations).

Years were assumed to be fixed because of the potential importance of stand age for a species with marginal winter hardiness. All error terms involving replicates (R/L, Y × R, and Y × R/L, where R = replicates, Y = year, and L = location) were assumed to be random. Years were treated as repeated measures for which the optimal model was a heterogeneous-variance compound-symmetric covariance structure (Littel et al., 1996). Locations also had highly heterogeneous errors, so each location was modeled as a separate variance group within a single mixed model analysis, resulting in one residual variance estimate for each location–year combination (Littel et al., 1996). The resulting model was chosen as superior to simpler (homogeneous variance) models on the basis of both Akaike's Information Criterion and Schwartz'Bayesian Criterion (Littel et al., 1996). Ground cover was analyzed similarly, but without a source of variation for years. Means for Spring Green were compared with means of Kemal and Tandem by contrasts.

RESULTS AND DISCUSSION

Freezing Tolerance Test

There were no plants which survived the −14 or −17°C freezing treatments. Mean dry matter for these two temperatures was 904 g kg^{-1} and populations did not differ in dry matter at these two temperatures. The −11°C temperature allowed for considerable discrimination among populations, with mean plant survival of 32% and mean tiller survival of 39% (Table 2). The −7 and −5°C temperatures were relatively mild, with mean plant survival of 98% and mean tiller survival of 88%. The latter two temperatures only allowed a small amount of discrimination among populations. It appears that future studies of freezing tolerance in festulolium should utilize a narrower range of temperatures with smaller increments between consecutive temperature treatments.

Selection for field survival in the Kemal pasture increased plant survival of the progeny when measured at −11, −7, and −5°C (Table 2). Progress from selection was large when measured at the −11°C temperature and W4KG had greater plant survival than W4KU ($P < 0.01$). The difference between W4KG and W4KU suggests that plants growing in relatively open areas of the pasture, where cattle had ready access to their herbage, were under greater selection pressure for freezing tolerance compared with those protected by perennial weeds and ungrazed. These results underscore the importance of interacting environmental factors in determining the ability of marginally hardy herbage plants to survive harsh environmental conditions (Charles et al., 1975).

Selection for survival in the replicated row plots of Elmet, Prior, and Tandem was also successful at increasing plant survival, but only when measured at −11°C (Table 2). The progress made in this germplasm appeared to be considerably less than that observed in Kemal. This appeared to be due to the large differences in plant survival among the parental cultivars at −11°C (Elmet = 6%, Prior = 83%, and Tandem = 11%). The selected populations represented dramatic improvements in plant survival at −11°C over Elmet and Tandem, but not Prior. Differences in freezing tolerance among Elmet, Prior, and Tandem were unexpected, because field survival of these three cultivars, following two winters at four locations ranged from 52 to 60%

Table 2. Mean plant survival, tiller survival, and dry matter concentration for two festulolium populations (Kemal and EPT) and four populations selected for field survival and evaluated at three temperatures (−11, −7, and −5°C).

Population	Plant survival			Tiller survival			Dry matter		
	−11	−7	−5	−11	−7	−5	−11	−7	−5
	%			%			%		
Kemal (unselected)	3	96	94	25	70	82	915	440	342
W4KG	58**	98†	100†	45**	87**	92**	724**	375**	327
W4KU	34**	97	100†	35†	91**	96**	867	374**	308†
EPT (unselected)‡	33	98	97	41	81	94	841	412	328
WFLr	47**	99	97	43	87*	96	771*	367*	302
WFL-a89C	63**	99	100	55*	81	97	696**	421	283

* Mean significantly different from the respective parent (unselected) population at $P < 0.05$.
** Mean significantly different from the respective parent (unselected) population at $P < 0.01$.
† Mean significantly different from the respective parent (unselected) population at $P < 0.10$.
‡ Mean of Elmet, Prior, and Tandem, each comprising 33% of the parentage of WFL-a89C.

and were not significantly different (Casler and Walgenbach, 1990). Field survivors from Elmet and Tandem likely represented significant improvements in freezing tolerance, while survivors from Prior may have been more or less random plants with respect to freezing tolerance. Stand losses for Elmet and Tandem may have been due to mortality of freezing-intolerant plants, but stand losses for Prior were likely due to other factors. This is supported by results of Hides (1979), who found that selection for freezing tolerance of ryegrass seedlings did not confer increased adult-plant survival, suggesting the existence of alternative mechanisms of winter survival.

The difference between WFLr and WFL-a89C at −11°C was significant ($P < 0.05$) and suggested that selection for rust resistance in Oregon resulted in some loss of freezing tolerance that was accumulated among the initial selections. This may not signify a genetic correlation between rust resistance and freezing tolerance, but is more likely due to relaxation of selection pressure for survival during the cycle of selection for rust resistance in Oregon.

Differences in tiller survival were similar to those for plant survival (Table 2). Selection progress was evident in tiller survival at all three of the warmest freezing temperatures for the Kemal selections and at −11 and −7°C for the Elmet, Prior, and Tandem selections. These changes indicate that improved freezing tolerance can be manifested in two ways—reduced plant mortality and reduced injury (tiller mortality) of surviving plants. These two responses are positively correlated with each other, but not identical.

Dry matter concentration was determined as a measure of living tissue, on the assumption that the amount of living tissue was highly correlated with the concentration of moisture in the plants. Results for dry matter confirmed the combined results for plant and tiller survival, that selection for field survival increased freezing tolerance in both selection experiments, and that progress was greater for the grazed Kemal vs. the ungrazed Kemal (Table 2).

Genetic variation for freezing tolerance appears to be a mechanism by which adaptation of festulolium can be improved. Freezing tolerance can be increased in festulolium by field selection under either grazing or hay management. Although many interacting environmental factors combine to determine field survival of festulolium plants in harsh environments, selection of field survivors can be a useful means of increasing tolerance to freezing temperatures.

Field Test

Cultivar × location and cultivar × location × year interactions were significant ($P < 0.01$). Although there were some changes in ranking of the three cultivars across years and locations, these interactions were largely due to lack of significant differences at some locations (Table 3). On the basis of trials at Ames, IA, Ashland, WI, Lancaster, WI, Marshfield, WI, Lexington, KY, Blacksburg, VA, and Blackstone, VA, it was not possible to distinguish among festulolium cultivars over a 2-harvest-year period. Trials at Rosemount, MN, Arlington, WI, Wooster, OH, Rock Springs, PA, and Ithaca, NY, all showed some evidence that Spring Green had superior forage yield to either Kemal or Tandem. The cultivar × location × year interaction was manifested as inconsistency in these results across years; Rosemount, Arlington, and Wooster showed the largest differences among cultivars in 1998, while Rock Springs and Ithaca showed the largest differences in 1999. One location was an anomaly compared with the others; the trial at Orange, VA, showed superior forage yield for Tandem compared with Spring Green ($P < 0.05$). There was no pattern to soil types, N fertilization rate, latitude, longitude, or USDA hardiness zone of the locations that successfully discriminated among cultivars vs. those that did not discriminate.

Despite these interactions, cultivars differed in forage

Table 3. Forage yield of three festulolium cultivars evaluated at 13 locations in 1998 and 1999. Trials were seeded in 1997.

	1998			1999			Mean over years		
Location	Kemal	Tandem	Spring Green	Kemal	Tandem	Spring Green	Kemal	Tandem	Spring Green
	Mg ha^{-1}								
Rosemount, MN	4.62	4.24*	4.69	2.83	2.60	2.74	3.73	3.42	3.71
Ames, IA	2.76	2.24	2.49	3.78	3.87	3.53	3.27	3.05	3.01
Arlington, WI	3.73**	4.42*	4.12	2.00	1.66*	1.91	2.86*	3.04	3.02
Ashland, WI	3.72	4.05	3.81	5.65	6.02	5.87	4.68	5.03	4.84
Lancaster, WI†	2.67	3.05	2.73	1.56	1.53	1.84	2.12	2.29	2.28
Marshfield, WI	6.19	6.30	6.43	4.00	3.93	4.14	5.10	5.11	5.29
Lexington, KY	3.92	4.70	4.55	2.76	2.82	2.90	3.34	3.76	3.72
Wooster, OH	3.36**	3.45*	3.68	0.80	0.90	0.85	2.08*	2.17	2.27
Rock Springs, PA‡	3.86	4.01	3.76	–	–	–	–	–	–
Rock Springs, PA§	4.50	4.94	4.88	2.76**	3.54*	3.20	3.63**	4.24	4.04
Ithaca, NY	5.80	6.05	5.93	3.68**	4.36	4.19	4.74**	5.20	5.06
Blacksburg, VA	6.55	7.14	6.21	5.43	5.31	5.85	5.99	6.23	6.03
Blackstone, VA	4.55	4.82	5.20	3.80	4.85	4.31	4.17	4.83	4.75
Orange, VA	4.03	4.51*	4.13	2.34	2.81*	2.24	3.18	3.66*	3.19
Mean	4.30**	4.56	4.47	3.18**	3.40	3.35	3.74**	3.98	3.91

* Mean of Kemal or Tandem significantly different from mean of Spring Green at $P < 0.05$.
** Mean of Kemal or Tandem significantly different from mean of Spring Green at $P < 0.01$.
† Grazed site.
‡ Trial discontinued after the first year.
§ Late summer seeding.

yield across locations and years ($P < 0.01$) and the F-value for this test was over six times larger than the F-values for each of the cultivar × environment interactions. This is evident from the statistically significant differences between Kemal and Spring Green, averaged over years, at four of the six locations that had effective trials for forage yield discrimination (including Orange, VA).

Spring Green averaged 4.0 and 5.3% higher forage yield than Kemal in the first and second production years, respectively (Table 3). Spring Green ranked higher than Kemal for forage yield at 11 of 14 first-year locations and nine of 13 second-year locations. Averaged over years, Spring Green was 5.0% higher in forage yield than Kemal and ranked higher than Kemal at 11 of 13 locations. Differences in forage yield between Spring Green and Tandem were inconsistent and only occasionally significant. Spring Green ranked higher than Tandem at five of 14 first-year locations and six of 13 second-year locations. Averaged over locations within each year and over years, Tandem and Spring Green were not significantly different in forage yield.

Spring Green had significantly lower yield than Tandem at Orange, VA. Orange, VA, was one of only two locations in USDA hardiness zone 7 (along with Blackstone, VA); all other locations were in zones 2 through 6. The consistent superiority of Tandem to Spring Green at Orange may indicate that Spring Green is less adapted to warmer climates.

Mean ground cover in spring 2000 ranged from 27 to 91% at the 10 locations that experienced some loss of stand. The three Virginia locations were excluded from all ground cover analyses, because they suffered no stand losses. With the obvious exception of Rosemount, MN, mean ground cover was highly correlated with USDA hardiness zone classification (Table 4). Longer and more reliable snow cover may explain the higher ground cover at Rosemount.

Six of 10 locations had significant differences among cultivars for ground cover (Table 4). Spring Green ranked higher than Tandem in ground cover at nine of 10 locations, with five of these differences reaching statistical significance ($P < 0.01$). This difference was close to significance at Rosemount ($P = 0.08$). Spring Green ranked higher than Kemal at seven of 10 locations, with four of these differences reaching statistical significance ($P < 0.01$). Across all locations, Spring Green averaged 16% higher in ground cover than Tandem and 6% higher in ground cover than Kemal (both $P < 0.01$). Ithaca was the only anomaly for ground cover, where Kemal had significantly higher ground cover than Spring Green ($P < 0.01$).

Table 4. Mean ground cover of living crown tissue in spring 2000 for three festulolium cultivars evaluated at 10 locations, including LSD and P-value for differences among cultivar means.

Location	Zone†	Kemal	Tandem	Spring Green	Mean
		%			
Rosemount, MN	4	93	88	94	91
Ames, IA	4/5	61	51	58	57
Arlington, WI	4	25**	12**	43	27
Ashland, WI	2	23**	23**	36	28
Lancaster, WI‡	4	23**	20**	37	27
Marshfield, WI	3/4	32**	27**	46	35
Lexington, KY	6	81	81	84	82
Wooster, OH	5	84	83	85	84
Rock Springs, PA§	6	89	84**	88	87
Ithaca, NY	5	93**	85	84	87
Mean		61**	56**	65	61

* Mean of Kemal or Tandem significantly different from mean of Spring Green at $P < 0.05$.
** Mean of Kemal or Tandem significantly different from mean of Spring Green at $P < 0.01$.
† USDA Hardiness zone. Sites on the border between two zones are indicated.
‡ Grazed site.
§ Late summer seeding.

Differences in ground cover among cultivars were greatest for the six locations with the lowest USDA hardiness zone ratings (Table 4). For these six locations, Spring Green averaged 42% higher ground cover than Tandem (52 vs. 37%; $P < 0.01$) and 22% higher ground cover than Kemal (52 vs. 43%; $P < 0.01$). The cultivar × region interaction (2 df) accounted for 46% of the cultivar × location interaction (18 df) for ground cover. Both the cultivar × region and the cultivar × location(region) interactions were significant ($P < 0.01$). While there may be other climatic, soil, and/or management factors that might account for differences in cultivar performance across locations, USDA hardiness zone classification clearly accounts for approximately half of the cultivar × location interaction. It is unlikely that any single environmental factor could be found to account for a significant portion of the remainder of this interaction.

Because the five locations used to select field survivors (Arlington, Ashland, Prairie du Sac, Spring Green, and Spooner, WI) all fell within USDA hardiness zones 2 through 4, it is not surprising that persistence was improved in this region, but not outside this region. Freezing tolerance is important for survival in these hardiness zones, while it may be unimportant or less important at locations in hardiness zones 5 through 7. Thus, Spring Green festulolium appears to represent an adaptive improvement to a specific group of environments, defined by relatively severe winter conditions. Our ability to measure improvements in Spring Green across the range of soil types, N fertilization rates, and locations within hardiness zones 2 through 4 suggests that this adaptive improvement is quite broad within these hardiness zones.

REFERENCES

Association of Official Seed Analysts. 1998. Rules for seed testing. AOSA, Beltsville, MD.
Casler, M.D. 1990. Cultivar and cultivar × environment effects for relative feed value of temperate perennial grasses. Crop Sci. 30: 722–728.
Casler, M.D., P.G. Pitts, C. Rose-Fricker, P.C. Bilkey, and J.K. Wipff. 2001. Registration of 'Spring Green' festulolium. Crop Sci. 41: 1365–1366.
Casler, M.D., and R.P. Walgenbach. 1990. Ground cover potential of forage grass cultivars in binary mixtures with alfalfa at divergent locations. Crop Sci. 30:825–831.
Charles, A.H., F. England, and A.J. Thomson. 1975. The effect of nitrogen application and autumn management on winter hardiness

and spring growth of *Lolium perenne* L. (Part 1). J. Brit. Grassl. Soc. 30:315–325.

Hides, D.H. 1979. Winter hardiness in *Lolium multiflorum* Lam. III. Selection for improved cold tolerance and its effect on agronomic performance. Grass Forage Sci. 34:119–124.

Humphreys, M.W., and I. Pasakinskiene. 1996. Chromosome painting to locate genes for drought resistance transferred from *Festuca arundinacea* into *Lolium multiflorum*. Heredity 77:530–534.

Jung, G.A., A.J.P. van Wijk, W.F. Hunt, and C.E. Watson. 1996. Ryegrasses. p. 605–641. *In* L.E. Moser et al. (ed.) Cool-season forage grasses. ASA, CSSA, and SSSA, Madison, WI.

Littel, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS systems for mixed models. SAS Inst., Cary, NC.

Murphy, W.M., J.P. Silman, and A.D. Mena Barreto. 1995. A comparison of quadrat, capacitance meter, HFRO sward stick, and rising plate for estimating herbage mass in a smooth-stalked, meadowgrass-dominant white clover sward. Grass Forage Sci. 50:452–455.

Novy, E.M., M.D. Casler, and R.R. Hill, Jr. 1995. Selection for persistence of tetraploid ryegrasses and festulolium in mixture with perennial legumes. Crop Sci. 35:1046–1051.

Thomas, H., and M.O. Humphreys. 1991. Progress and potential of interspecific hybrids of *Lolium* and *Festuca*. J. Agric. Sci. (Cambridge) 117:1–8.

Wit, F. 1959. Hybrids of ryegrasses and meadow fescue and their value for grass breeding. Euphytica 8:1–12.