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Enhanced Survival and Animal Performance from Ecotype Derived White Clover Cultivars

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ABSTRACT

Most U.S. white clover (*Trifolium repens* L.) breeding projects have concentrated on developing ladino (*T. repens* var. *giganteum* Lagr-Foss) cultivars. Ecotypes collected from grazed, grass dominant pastures in Georgia are exclusively stolon dense, intermediate leaf types (*T. repens* f. *hollandicum* Erith ex Jav. & Soo) rather than ladinos. These ecotypes formed the parental base for the Durana (ecotypes only) and Patriot (an ecotype × ladino hybrid) cultivars. Our objective was to compare morphological characteristics and agronomic production and animal performance of Durana and Patriot against the ladino cultivar Regal and other appropriate checks. Durana and Patriot were distinct morphologically from Regal, but no differences were seen among these same entries for dry matter yield in small plots. Both cultivars possessed greater small plot stand survival than Regal under grazing with grass competition. In endophyte [*Neotyphodium coenophialum* (Morgan-Jones and Gams) Glenn, Bacon, and Hanlin] infected (E+) and endophyte-free (E−) tall fescue (*Festuca arundinacea* Schreb.) paddocks, Durana maintained a clover percentage of 43% by weight while Regal deteriorated to less than 5% by Year 2. Beef cattle (*Bos* spp.) gains on E+ paddocks with Durana, Regal, or grass alone check (fertilized with 65 kg N ha^{-1} grazing season^{-1}) treatments during Year 2 were 0.95, 0.49, and 0.43 kg head^{-1} d^{-1}, respectively, while gains on E− paddocks with Durana, Regal, and grass alone were 1.14, 0.75, and 0.99 kg head^{-1} d^{-1}, respectively. Similar trends were found for Patriot in a separate trial. The superior performance of these cultivars highlights the importance of f. *hollandicum* ecotypes to develop more persistent white clover cultivars.

WHITE CLOVER is the most widely used and persistent temperate pasture legume because of its aggressive stolons and reseeding ability. Among the cultivated types, there are three distinct, true breeding polymorphic forms: the small leaved, wild type (*T. repens* L. f. *repens* L.); the intermediate leaved, common type; and the large leaved, ladino type (Fick and Luckow, 1991; Pederson, 1995).

Most white clover breeding projects in the USA have centered on developing cultivars of ladino white clover. Ladino types are high yielding because of their large leaves and erect growth habit, but possess a low number of stolons compared to the stolon dense, intermediate types (Woodfield and Caradus, 1994). Low stolon density is a primary factor in the poor persistence of ladino cultivars, especially when used as a renovation legume for grass-based pastures (Bouton and Hoveland, 1996; Matches, 1989).

White clover ecotypes collected in southeastern USA pasture conditions (e.g., grass competition and heavy grazing pressure), even pastures planted previously with ladino cultivars, are exclusively the stolon dense, f. *hollandicum* type (Bouton et al., 1998; Widdup et al., 1996). Initial experiments demonstrated that this ecotype-based material, including hybrid population crosses of ecotypes with various cultivars, were more persistent than adapted ladino cultivars when established into tall fescue and grazed with beef cattle (Bouton et al., 1998). These data indicated that naturalized ecotypes should be used as the base germplasm to increase persistence and performance of white clover under grazing in the southeastern USA. The excellent performance of the ecotype hybrids also indicated the potential to capture simultaneously the persistence of the ecotypes along with the value added traits of the donor cultivars.

The Georgia ecotype collections formed the parental base for a breeding program that developed two commercial white clover cultivars, Durana and Patriot. More complete descriptions of the breeding history and morphology of Durana and Patriot white clover are found elsewhere (Bouton et al., 2005a, 2005b). Briefly, Durana is an 84-parent synthetic based strictly on ecotype material. Patriot is a population cross of an ecotype germplasm called GA-ETN with the ladino germplasm Southern Regional Virus Resistant (SRVR; Gibson et al., 1988).

In these experiments, we compare the morphological characteristics, dry matter yield, stand survival, and animal production performance of Durana and Patriot against the ladino cultivar Regal and other commercially available cultivars.

MATERIALS AND METHODS

Morphological Characteristics

Individual plants (120) from the cultivars Durana, Patriot, Louisiana S-1, Grasslands Huia, and Regal white clover were established at the Plant Sciences Farm near Athens, GA, on a Cecil sandy loam soil (clayey, kaolinitic, thermic, Typic Hapludult), and the Central Georgia Branch Station, near Eatonton, GA, on a Mecklenburg sandy loam soil (fine, mixed, thermic, Ultic Hapludalfs). Descriptions of Louisiana S-1, Grasslands Huia, and Regal are found elsewhere (Caradus, 1986; Caradus and Woodfield, 1997). Experimental design at each location was a randomized complete block with 10 blocks. Each plot consisted of a row of 12 plants spaced planted on

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Abbreviations: ADG, average daily gain; E−, endophyte-free; E+, endophyte-infected.

0.75-m centers. Each plant was then measured for the following characteristics: number stolon growing points, plant length, width, and height, leaflet length and width, petiole length, heading date, seedheads per plant, and cyanogenesis via the picric acid procedure of Corkill (1940).

Stand Survival

Stand survival was assessed in separate trials at two Georgia locations: two trials at the University of Georgia's College of Agricultural and Environmental Sciences (CAES) Northwest Georgia Branch Experiment Station near Calhoun and one trial at the CAES Central Georgia Branch Station near Eatonton. One of the Calhoun trials was established into an existing common bermudagrass [*Cynodon dactylon* (L.) Pers.] pasture and the other into an existing E− 'Jesup' (Bouton et al., 1997) tall fescue pasture. The soil type for both Calhoun trials was Pope fine sandy loam (fine-loamy, mixed, non-acid, thermic Fluventic Dystrochrepts). The Eatonton trials were established into E− Jesup tall fescue pastures with the soil being a Mecklenburg sandy loam.

In all trials, the experimental design was a randomized complete block with six blocks and white clover entries as treatments. The number of entries varied in each trial but Durana, Patriot, and Regal were present in all trials. The experimental area was clipped to a 7.5-cm height with a flail mower just before seeding the clovers. Each clover entry was sown with a Hege precision drill (Hege Equipment Inc., Colwich, KS) at a rate of 3 kg seed ha^{-1} on 10 Oct. 2000 for both Calhoun trials and 5 Nov. 1999 and 14 Nov. 2000 for the Eatonton trials. Plot size was 1.5 by 3.5 m. All experimental areas were fertilized uniformly with 30 kg N, 15 kg P, and 28 kg K ha^{-1} as a complete fertilizer at establishment. No additional fertilizer was applied for the duration of the study. The experimental areas were continuously grazed with beef cattle from April until November of each year. Animals were placed into or removed from the paddock area to maintain a grazing height of approximately 5.0 to 7.5 cm.

Final percent basal area estimates were determined on each plot approximately 26 mo after establishment using a 0.5-m^2 point-quadrat grid that contained 100 intersecting points formed on a 750-cm^2 frame via wires placed at 5-cm intervals. Basal area was therefore defined as any portion of the white clover plant (leaves, stolons, etc.) that came into direct contact with the intersecting points.

Dry Matter Yield

Dry matter yield was assessed in replicated small plots at two locations in Georgia; these were the Plant Sciences Farm near Athens and the Northwest Georgia Branch Experiment Station near Calhoun. The soil at Athens site was a Cecil sandy loam, and at Calhoun was a Pope fine sandy loam. The experimental design was a randomized complete block with six blocks and white clover entries as treatments. Entries were Regal, Durana, Patriot, and 'Grasslands Pitau' white clover. Plots (1.5 by 3.5 m) of each entry were established with a Hege precision drill in cultivated soil on 1 Oct. 1999 at the Calhoun location and 29 Oct. 1999 at the Athens location. Plots were fertilized uniformly with 30 kg N, 15 kg P, and 28 kg K ha^{-1} as a complete fertilizer at establishment. No additional fertilizer was applied for the duration of the study. All plots were harvested with a flail mower three to five times during each subsequent year at both test locations. At each harvest, subsamples were taken to determine dry matter percentage and all plot yields were calculated on a dry matter basis.

Animal Performance—Durana

Twelve 0.81-ha paddocks were established at Eatonton in November 1998 with Jesup tall fescue in a Mecklenburg sandy loam soil. Six of these paddocks contained E+ Jesup and the remaining six paddocks contained E− Jesup. In November 1999, the paddocks were assigned the following clover treatments: no clover (fertilized annually with 65 kg ha^{-1} N), sprayed with paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) herbicide to reduce grass competition and seeded with Durana white clover at 3 kg ha^{-1}, and sprayed with paraquat herbicide and seeded with Regal white clover at 3 kg ha^{-1}. Treatments were therefore a factorialized combination of E+ or E− tall fescue with the clover treatments (no clover, Durana, or Regal). There were two replications of each treatment.

Paddocks were fertilized uniformly with 30 kg N, 15 kg P, and 28 kg K ha^{-1} as a complete fertilizer at establishment. In February and September of each of each subsequent year of the trial, 65 kg N ha^{-1} was applied to each grass only paddock as liquid N. No lime or additional fertilizer nutrients were applied for the duration of the trial.

Poor rainfall for the winter and spring establishment period delayed stocking the paddocks until October 2000 when stands were judged adequate to begin grazing. The grazing periods for the 2000–2001 growing season were therefore 10 October 2000 until 20 November 2000 (autumn) and 13 March 2001 until 3 Aug. 2001 (spring). The grazing periods for the 2001–2002 growing season were 2 Oct. 2001 until 13 Nov. 2001 (autumn) and 28 March 2002 until 14 June 2002 (spring). The initial stocking rate was four to six beef cattle per paddock with each weighing from 250 to 350 kg depending on the year and grazing season. In this put and take system, two animals were initially designated as testers and the others as grazers. On the basis of forage availability, stocking rate was adjusted during each grazing period by removal of one or more grazer animals. Target available forage was 1200 kg ha^{-1} throughout the grazing season. The testers stayed on the paddock for the duration of the grazing season. The liveweight of each animal was measured before allocation of animals to treatments and approximately every 4 wk during each grazing period. Forage available yield was determined initially and every 4 wk by sampling 10 0.09-m^2 quadrate areas throughout each paddock. The botanical composition by weight of these samples was determined by hand separation into their component parts (e.g., grass, clover, other).

Animal Performance—Patriot

These trials were established at the Calhoun location in the same soil as for the yield trials and with many of the same methods described above for the Durana performance trials. Before planting, the experimental area contained a mixture of common bermudagrass and E− tall fescue. The entire area was sprayed with Roundup herbicide and then divided into 1-ha paddocks. In October 1999, the following treatments were assigned as separate paddocks: wild-type E+ Georgia 5 (Bouton et al., 1993) tall fescue (no clover, N Fertilizer control), Durana white clover planted together with E+ Georgia 5 tall fescue, and Regal white clover planted together with E+ Georgia 5 tall fescue. Seeding rate for the clover was 3 kg ha^{-1} and for the tall fescue was 20 kg ha^{-1} when planted together, and 25 kg ha^{-1} for the fescue when planted alone. There were two replications of each treatment.

Paddocks were fertilized uniformly with 30 kg N, 15 kg P, and 28 kg K ha^{-1} as a complete fertilizer at establishment. In March and September of each of subsequent year of the trial,

Table 1. Characteristics of white clover cultivars tested at two locations in Georgia (Watkinsville and Eatonton).

Cultivar	Stolon growing points	Plant			Leaflet		Leaf	Heading date	Seedheads	Cyanomorphic plants‡
		Length	Width	Height	Length	Width	Petiole length			
	no./m^2	cm			mm			DOY†	no./plant	%
Durana	368	13.1	12.8	3.3	12.6	9.6	29.1	106.2	42	79.3
Patriot	336	13.8	13.5	4.8	14.4	10.9	39.1	109.1	31	44.1
Louisiana S-1	242	10.6	9.9	4.7	14.3	11.3	37.6	106.6	23	83.6
Grasslands Huia	234	10.7	10.7	3.6	13.1	10.9	41.9	115.1	3	62.5
Regal (ladino)	190	12.2	12.4	8.9	18.9	14.2	62.0	112.9	23	7.5
LSD ($P < 0.05$)	33	1.7	1.6	0.7	0.9	1.0	6.1	2.2	8	12.2

† DOY = Day of year or average number of days from 1 January.
‡ Analyzed via picric acid test by procedures from Corkill, L. (1940).

65 kg N ha^{-1} was applied to each grass alone paddock as liquid N. No lime or additional fertilizer nutrients were applied for the duration of the trial.

Stands were grazed the initial spring (25 April until 19 July 2000) and during the autumn and spring of each subsequent year. The remaining grazing periods were 14 Sept. 2000 until 2 Nov. 2000 (autumn); 22 March 2001 until 19 June 2001 (spring); 18 Sept. 2001 until 12 Nov. 2001 (autumn); 19 March 2002 until 9 July 2002 (spring); 1 Oct. 2002 until 26 Nov. 2002 (autumn); 25 March 2003 until 16 July 2003 (spring). Stocking rates, animal data, and forage sampling were as described previously for the Durana animal performance trials.

Statistical Analysis and Animal Care

All data were analyzed with analysis of variance procedures and means comparisons made with least significant differences (LSD at $P < 0.05$) via SAS (SAS Inst., 1982). All grazing studies were conducted under University of Georgia animal use regulations in compliance with the Animal Welfare Act of 1966 & 9 CFR Subchapter A.

RESULTS

Morphological Characteristics

Durana differed from Louisiana S-1 and Regal in having more stolon growing points per unit area, a shorter plant height, a smaller leaflet, shorter petioles, an earlier heading date, and a greater number of seedheads per plant (Table 1). It also showed a higher frequency of cyanogenic plants than Regal. Durana differed from Grasslands Huia in having more stolon growing points per unit area, shorter petioles, an earlier heading date, a greater number of seedheads per plant, and a higher frequency of cyanogenic plants.

Patriot differed from Louisiana S-1 in having more stolon growing points per unit area, a greater plant width and length, more seedheads per plant, and a lower frequency of cyanogenic plants (Table 1). Patriot differed from Durana in having taller individual plants, larger leaflets, longer petioles, a later heading date, and lower frequency of cyanogenic plants. Patriot differed from Regal in having more stolon growing points per unit area, a shorter plant height, a smaller leaflet, shorter petioles, an earlier heading date, a greater number of seedheads per plant, and a higher frequency of cyanogenic plants. Patriot differed from Grasslands Huia in having more stolon growing points per unit area, greater plant spread (both width and length), taller plant height, larger leaflet length, an earlier flowering date, a greater number of seedheads per plant and a lower frequency of cyanogenic plants (Table 1).

Stand Survival

When tested in continuously grazed tall fescue pastures, Durana and Patriot showed significantly ($P < 0.05$) higher final basal area than the Regal check in three separate trials at the Calhoun and Eatonton locations (Table 2). When interseeded into bermudagrass and continuously grazed, Durana and Patriot also showed significantly ($p < 0.05$) higher final plant basal areas than the Regal check at the Calhoun location.

Dry Matter Yield

In the yield trials, there was a significant ($P < 0.05$) year × location by cultivar interaction mainly because of the cultivar × year effect being very significant ($p < 0.05$) at Athens (Table 3). This was probably due to the magnitude of the overall yield being much higher in 2001 versus 2000 and 2002 as no real changes in ranking took place. At Calhoun, no year × cultivar interaction was found so data are combined over years (Table 3). The yield data, and especially the overall averages across both years and locations, demonstrated that the dry matter yield potential of Durana, Patriot, and Regal are similar in the absence of grazing (Table 3). However, the cultivar Pitau was the lowest yielding at both locations.

Animal Performance—Durana

At Eatonton during the 2000 autumn grazing period, Durana showed greater clover content in the available forage than Regal. Animal liveweight gains were variable across all treatments and did not necessarily match clover content but did show a general positive effect of E− over E+ tall fescue especially when grown without clover (Table 4). However, by the 2001 spring grazing

Table 2. Final percentage of basal areas of white clover cultivars when interplanted into tall fescue or bermudagrass sods and continuously grazed by beef cattle at two locations in Georgia (Calhoun and Eatonton) for either two or three growing seasons.

Cultivar	Calhoun (2000–2002)		Eatonton (fescue)	
	Bermuda	Fescue	1999–2002	2000–02
	%			
Durana	73	90	30	44
Patriot	63	64	28	40
Regal	31	17	4	2
LSD ($P < 0.05$)	14	21	23	22

Table 3. Annual dry matter yield of different white clover cultivars at two Georgia locations (Athens and Calhoun) for 3 yr.

	Athens				Calhoun	Athens/Calhoun
Cultivar	2000	2001	2002	3-yr average	3-yr average	3-yr average
	kg ha^{-1}					
Regal	4672	5232	3153	4351	2579	3466
Patriot	4273	5966	3804	4680	2479	3581
Durana	3604	6397	2880	4293	2331	3313
Pitau	3465	5354	647	3155	836	1996
LSD ($P < 0.05$)	1169	NS	893	606	556	437

period, each clover treatment was found to improve liveweight gains per hectare over both E+ and E− tall fescue alone with N fertilizer treatments (Table 4). Total gain and average daily gain (ADG) on E− tall fescue alone were superior to gains on E+ tall fescue alone in 2001. However, no differences in total gain and ADG were observed among any of the fescue plus clover treatments. This particular spring grazing period was also noted for its long duration grazing period (13 March until 3 August) because of excellent forage growing conditions and grazing animals that continued to gain.

In the 2001 autumn grazing period at Eatonton, no differences were seen among treatments for both total gain and ADG (Table 5). Durana demonstrated better clover content than Regal for which near base line amounts of clover were recorded. During the 2002 spring grazing period, Durana maintained a high average clover content in the available forage while Regal had deteriorated to less than 5% of the available forage (Table 5). Therefore, Durana was the sole clover treatment able to provide improved animal gains when compared to E+ tall fescue (no clover) with N fertilizer (Table 5). This is reflected in the higher ADG in higher gains per ha in the E+ Jesup.

Animal Performance—Patriot

There were no year interactions with season or cultivar, but there was a season × cultivar interaction. Therefore, the data are presented to show this main interaction of season × cultivar (Table 6). There were no significant differences in forage production among all treatments in either autumn or spring.

During the autumn grazing period at Calhoun, there was no significant difference in ADG across treatments when averaged for 3 yr. However, the two clover treatments had higher liveweight gains per hectare than the grass alone treatment (Table 6). The substantially higher clover content for Patriot ($P < 0.05$) did not result in higher animal performance during the autumn when compared with Regal.

During the spring grazing period, Patriot gains were higher than E+ fescue with N fertilizer (no clover). The higher clover content for Patriot resulted in better total gain and ADG than Regal when averaged across the four spring grazing seasons (Table 6).

DISCUSSION

Ladinos are predominant white clover cultivars used commercially throughout the USA since their introduction from Italy in the early 1900s (Gibson and Cope 1985). The first USA-bred ladino cultivar, Pilgrim, was released in 1953, and ladinos have remained the main focus of white clover breeding programs over the past 50 yr (Woodfield and Caradus 1994; Brink et al., 1999). The poor persistence of ladino cultivars in grazed pastures, which has been reconfirmed in the studies reported here, has led to their suitability being increasingly questioned (Widdup et al., 1996; Bouton et al., 1998; Brink et al., 1998). Reasons suggested for this failure to persist under grazing include poor drought and heat tolerance and susceptibility to a number of pests and diseases such as clover root curculio [*Sitona hispidula* (Fabricus)], *Peanut stunt virus*, and *Alfalfa mosaic virus* (Barnett and Gibson 1975; Pederson et al., 1991).

The improved persistence of naturalized white clover ecotypes was reported by Bouton and Hoveland (1996) and Widdup et al. (1996). Both groups identified that ecotype populations collected from grazed pastures generally had smaller leaf size and a higher frequency of cyanogenic plants than the commonly sown ladino white clover cultivars. Only Durana and Patriot consistently maintained a high clover content in the current trials.

Table 4. Average total forage yield, clover percentage of the total forage yield, and beef steer average daily gain (ADG) and total gain on Jesup tall fescue (E+ or E−) paddocks either interseeded with different white clover cultivars (Durana and Regal) or fertilized with 65 kg N ha^{-1} grazing season^{-1} at Eatonton, GA, during the 2000–2001 autumn and spring growing seasons.

	Autumn 2000				Spring 2001			
Paddock	Forage	Clover	ADG	Gain	Forage	Clover	ADG	Gain
	kg ha^{-1}	%	kg head^{-1} d^{-1}	kg ha^{-1}		%	kg head^{-1} d^{-1}	kg ha^{-1}
E+/N Fert	2440	<1	0.35	67	2292	<1	0.84	291
E+/Regal	1606	14	0.57	119	1958	39	1.45	538
E+/Durana	1592	39	0.54	94	2054	63	1.35	523
E−/N Fert	2381	<1	0.99	193	1932	<1	1.26	411
E−/Regal	1966	4	0.89	159	1754	17	1.54	547
E−/Durana	1691	26	0.65	131	2312	55	1.42	511
LSD ($P < 0.05$)	582	13	0.19	43	934	21	0.30	68
LSD ($P < 0.10$)	456	10	0.15	34	732	16	0.23	54

Table 5. Average total forage yield, clover percentage of the total forage yield, and beef steer average daily gain (ADG) and total gain on Jesup tall fescue (E+ or E−) paddocks either interseeded with different white clover cultivars (Durana and Regal) or fertilized with 65 kg N ha^{-1} grazing season^{-1} (N Fert) at Eatonton, GA, during 2001–2002 autumn and spring growing seasons.

	Autumn 2001				Spring 2002			
Paddock	Forage	Clover	ADG	Gain	Forage	Clover	ADG	Gain
	kg ha^{-1}	%	kg head^{-1} d^{-1}	kg ha^{-1}		%	kg head^{-1} d^{-1}	kg ha^{-1}
E+/N Fert	2124	<1	0.91	72	2465	<1	0.43	105
E+/Regal	1878	<1	0.65	38	1626	3	0.49	77
E+/Durana	1596	12	0.85	60	1719	46	0.95	166
E−/N Fert	1697	<1	0.84	68	2066	<1	0.99	244
E−/Regal	1940	<1	0.89	77	894	<1	0.75	114
E−/Durana	1881	13	0.80	50	1641	39	1.14	245
LSD ($P < 0.05$)	645	6	0.44	35	564	18	0.37	36
LSD ($P < 0.10$)	505	5	0.35	28	442	14	0.29	28

These results validate the use of naturalized ecotypes as suggested by Widdup et al. (1996), Bouton and Hoveland (1996) and Brink et al. (1999). The increases in persistence of Durana and Patriot resulted in higher clover content beyond Year 2 and animal performance compared with the Regal ladino type. Durana and Patriot have a high percentage of cyanomorphic plants compared with ladinos (Table 2), but there were no apparent negative effects on animal performance.

Several mechanisms have been suggested to explain the improved white clover survival. Caradus and Williams (1989) reported that the most persistent white clovers in New Zealand were collected from New Zealand pastures and possessed moderate to high levels of cyanogenesis and high stolon densities. Results from the current studies also indicate that both of these traits are important for improved white clover persistence in southeastern U.S. pastures. The absence of both these traits in *T. repens* f. *ladino* germplasm partially explains its poor adaptation in grazed pastures with grass competition, whereas both of these traits are highly associated with the f. *hollandicum* ecotypes. Brink et al. (1999) clearly demonstrated that southeastern ecotypes had a greater proportion of stolon nodes that produced secondary stolon branches and nodal roots. They suggested that this was a mechanism that improves persistence in heat- and drought-stressed environments. However, it is clear that other traits are also required since introduced cultivars such as Prestige and Tahora, which have high stolon densities and are cyanogenic, fail to persist in southeastern U.S. pastures. Similarly, Pitau, which is highly cyanogenic (Caradus and Woodfield 1997), failed to persist at Athens and Calhoun. Bouton and Hoveland (1996) suggested the naturalized ecotypes also had improved adaptation to pests and diseases found in southeastern pastures, while Pederson and Brink (1997) reported that the ecotypes produced consistently higher numbers of inflorescenes and seed even under continuous grazing.

The introduction of ladinos to North America is relatively recent when compared with the initial introduction of European white clover populations including Dutch wild white in the 17th Century (Leffel and Gibson 1973). Although the ancestry of the ecotypes that have formed the basis of Durana and Patriot is unknown, the morphological results confirm that neither Durana nor Patriot could be classified as f. *ladino* cultivars. Durana is a f. *hollandicum* type cultivar, while Patriot is a hybrid between the f. *hollandicum* and f. *ladino* with mostly intermediate values for stolon density, leaf size, petiole length, and frequency of cyanogenic plants. Dutch wild white is viewed as the protypical f. *hollandicum* type and was widely used before the introduction of ladino cultivars. Similarly Louisiana S-1, which has been extensively used through southern and eastern USA, is also a f. *hollandicum* type (Caradus and Woodfield 1997). Genetic diversity analysis of 32 white clover cultivars including Patriot, Durana, Lousiana S-1, Regal, and SRVR, using microsatellite markers indicated that Durana was not derived from Lousiana S-1 and was also dissimilar to the ladino cultivars (Jahufer et al., 2003). Patriot and Durana clustered with Colt, a cultivar derived from North Carolina ecotypes and was genetically similar to two cultivars (Nomad and Triffid) with a strong New Zealand parentage.

The general absence of yield differences among the Regal, Durana, and Patriot cultivars was surprising. However, the higher than expected yield of Durana may have been due to emphasis placed on plant vigor during

Table 6. Average total forage yield, clover percentage of the total forage yield, and beef steer average daily gain (ADG) and total gain on Georgia 5 (E+) tall fesuce paddocks either simultaneously seeded with different white clover cultivars (Patriot and Regal) or seeded and then fertilized with 65 kg N ha^{-1} grazing season^{-1} at Calhoun, GA, for three autumn (2000–2002) or four spring grazing seasons (2000–2003).

	Autumn				Spring			
Treatment	Forage	Clover	ADG	Gain	Forage	Clover	ADG	Gain
	kg ha^{-1}	%	kg head^{-1} d^{-1}	kg ha^{-1}		%	kg head^{-1} d^{-1}	kg ha^{-1}
N Fert	1926	<1	0.58	190	1594	<1	0.85	307
Regal	1780	15	0.70	224	1698	19	0.86	354
Patriot	1808	29	0.71	222	1478	31	1.02	435
LSD ($P < 0.05$)	347	2	0.19	28	473	7	0.08	52
LSD ($P < 0.10$)	276	1	0.15	22	381	6	0.06	43

its development (Bouton et al., 2005a) or simply its excellent adaptation to the region.

As expected from previous work with the Jesup cultivar at the same location (Bouton et al., 2002; Hoveland et al., 1997), animal performance on E− Jesup tall fescue was greatly superior to that on E+ Jesup. When white clover made up 20 to 40% of the available forage supply, improvements in animal performance were found even for nontoxic, E− tall fescue. This was especially true for the spring through early summer grazing period. However, when forage yields and clover content were very high, as seen at Eatonton in the spring grazing period of 2001 (Table 4), animal performance increases because of white clover were extremely high and economically very viable.

The E+ Georgia 5 used at the Calhoun location was only 60% endophyte infected while the E+ Jesup used at the Eatonton location was >90% infected. This may explain the better animal gains for the E+, N fertilizer control (no clover) treatment at Calhoun (Table 6) relative to the same treatment at Eatonton (Tables 4 and 5). However, we cannot rule out the possibility that cultivar or even location differences could explain this variation in the performance of this particular treatment.

The autumn results from Eatonton were inconsistent in demonstrating the positive effects of clover addition to the grass sward due first to the invasion of annual summer grasses [especially crabgrass, *Digitaria sanguinalis* (L.) Scop., and signalgrass, *Setaria* spp.] into the paddocks at Eatonton during the autumn. These are high quality grasses that were variable in their presence across the paddocks and may have confounded the autumn results. Second, the amount of clover found in all clover paddocks during the autumn, on average, tended to be lower than that found in the spring. Importantly, the overall autumn results from Calhoun did show increased animal performance with addition of clover and support the speculation that the invasion of warm-season grasses was probably confounding the Eatonton autumn results.

There are several important conclusions from these trials. First, the clear benefit in animal performance of increased white clover content was shown in the southeastern USA, an area known for inconsistent legume performance. Second, the poor survival of ladino cultivars when used in perennial, grass-based grazing systems was reconfirmed. Third, the ability of the stolon dense, ecotype-based white clover cultivars, specifically Durana and Patriot, to provide better animal performance than ladinos because of their increased persistence and ability to sustain a higher clover content in the total available dry matter was demonstrated. The combined effect of these data endorse the use of naturalized ecotypes to develop more persistent white clover cultivars that are suitable for use as a renovation legume in grazed, grass pastures of southeastern USA. Finally, Durana and Patriot should be valuable additions for livestock producers in the Southeast by improving long-term animal performance, even on nontoxic tall fescue forage, and at the same time, reducing the need for expensive nitrogen fertilizer.

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