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## Correlated responses in annual ryegrass (*Lolium multiflorum* Lam.) selected for improved winter growth

Amandeep K. Dhaliwal, Kamal Chugh, Edzard van Santen\*

Department of Agronomy and Soils, Auburn University, AL 36849-5412. USA.

\* Corresponding author: Edzard van Santen, E-mail: vanedza@auburn.edu

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### ABSTRACT

Annual ryegrass is a major annual cool season crop in the southeastern USA, available from mid autumn to late spring. It has high palatability, forage quality, seedling vigor and dry matter (DM) yield. Due to low availability of cool season annuals and perennials in early autumn and winter, animals are fed on stored forages. Annual ryegrass, along with perennial grasses, has high spring production but is lacking in late autumn and winter production. The primary objective of the breeding project was to shift the yield distribution of annual ryegrass towards the winter to make it available for grazing and to reduce the dependence of livestock on stored forages and grain. The base population was created from six top performing cultivars in Alabama. Plants were open-pollinated and bulk harvested for two seasons to create a random mating base population ( $C_0$ ). Three cycles of phenotypic recurrent selection along with  $C_0$  were used to study correlated responses such as heading date, plant type and seed yield. Each population was increased in replicated seed increase nurseries using a randomized complete block (RCB) design with four (2007/08) or two (2008/09) individual isolation nurseries per population. Each plot consisted of 200 plants and heading and plant type data were collected on each plant during the reproductive stage. For all correlated responses,  $C_1$  showed a significant change from  $C_0$  with exception of seed yield in 2008. There was no further change during successive cycles ( $C_2$ ,  $C_3$ ). With three cycles of selection, selected populations shifted their heading date 8 d earlier, had a more erect growth habit and increased seed yield than  $C_0$ . Of the three correlated responses, seed yield has shown a population x evaluation year interaction.

**Key Words:** *recurrent phenotypic selection; dry matter yield; heading date; seed yield; plant erectness.*

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## INTRODUCTION

Annual ryegrass (*Lolium multiflorum* Lam.), also known as Italian ryegrass, is grown on 9.3 million ha in the USA, of which 90% of the area is in the southeast states (Balasko et al., 1995) where it is utilized as forage crop. Seed production occurs in Oregon and Washington (Youngberg and Wheaton, 1979). Selection of annual ryegrass outside its agronomic utilization area has resulted in no genetic improvement during the last 20 years. Therefore forage traits should be selected in their respective agronomic areas to increase forage production (Casler et al., 2003).

Seeding dates for annual ryegrass in southeastern USA range from late summer to late autumn due to the mild temperate to subtropical climate. In this region autumn grown ryegrass attains 40% of its growth during the winter (December-February) and the remaining 60% late in the season (March-May). In a 12-year Louisiana study, approximately 30% of total production occurred in April alone (Redfearn et al., 2002). Annual ryegrass attains a high growth rate in the spring months, which is lower during cooler months. Due to the limited availability of standing forages during the cooler months, cattle feed on stored forages and grain to meet their nutritional requirements, which increases management costs (Ball et al., 2002).

Winter production can be improved by agronomic means, such as early seeding, with or without supplementary irrigation. A long-term approach would be to shift dry matter yield distribution through plant breeding. A phenotypic recurrent selection program, initiated in 2005 at Auburn University has resulted in improved winter performance (Dhaliwal, 2009). When evaluated in solid seeded plots at the selection location first-cut yield increased linearly at 477 kg ha<sup>-1</sup> cycle<sup>-1</sup> ( $P < 0.001$ ). This selection method has increased forage yield in Pensacola bahiagrass (*Paspalum notatum* Flüggé) (Burton, 1974; Burton, 1982), rye (*Secale cereale* L.) (Bruckner et al., 1991), maize (*Zea mays* L.) (Dudley and Lambert, 2004), and seed yield in perennial ryegrass (*Lolium perenne* L.) (Marshall and Wilkins, 2003).

A correlated response is defined as a change in a non-selected trait while selecting for the trait of interest and may respond either positively, or negatively, to selection for the trait of interest. A recent study with meadow brome grass (*Bromus riparius* Rehm) (de Araujo and Coulman, 2002) reported a positive albeit low correlation between DM yield and seed yield ( $r = 0.36$ ). The underlying genetic principle is the same as for indirect selection, i.e., a change in the non-selected trait will only happen if there is heritability for the trait of interest, a genotypic correlation between the two traits, and additive genetic variance for the trait not directly selected.

The objective of this research was to assess the changes in heading date, plant type, and seed yield in spaced-plant nurseries of populations of annual ryegrass selected for improved winter growth.

## MATERIALS AND METHODS

### SELECTION PROCEDURES

Details of creation of the base population and selection procedures can be found in Dhaliwal (2009). Briefly, a random mating base population was created from six high performing cultivars through two cycles of random mating (2002/3 and 2003/4). Selection for improved winter productivity was carried out in spaced plant nurseries for three cycles by transplanting in early November, followed by selection of a single plant per 25-plant block in mid January to early February, 750 growing degrees days (Celsius scale, Base temperature 3°C) post transplanting. The selection criterion used was individual-plant dry matter yield. Selected plants were recombined in isolation. Bulk-harvested seed was used as the base to advance to the next cycle.

### SEED YIELD NURSERIES

For seed production, the  $C_1$  and  $C_2$  populations along with the base population ( $C_0$ ) were seeded into "Ray Leach Cone-tainers" (Stuewe & Sons, Inc., Tangent, Oregon, USA) in a greenhouse in late August 2007. Replicated seed increase nurseries (Randomized Complete Block Design,  $r = 4$  in 2007/8 and  $r = 2$  in 2008/9) were established at the Plant Breeding Unit of the E.V. Smith Research Center of the Alabama Agricultural Experiment Station, Tallassee, Alabama (32.49 N, 85.89 W) in early November 2007 from the greenhouse grown material. Each plot consisted of approximately 200 plants on 90 cm centers arranged in an equal number of rows and columns to minimize the average distance between plants and promote random mating. Plots were surrounded on all sides by a 6 m border of cereal rye (*Secale cereale* L.) cv. Wren's Abruzzi to prevent pollen flow. This rye cultivar is at least 60 cm taller than the annual ryegrass at the time of anthesis and is very effective in preventing pollen flow. Similar procedures were used for the 2008 seed yield nurseries where  $C_3$  was included along with  $C_0$ ,  $C_1$  and  $C_2$ , except that only two seed increase blocks per population were established due to space constraints.

Individual plant notes were taken during reproductive development by visiting the plots every three days. The heading date for a given plant was the day when at least five spikes had emerged 75% from the boot. Plant type was scored on a scale of 1-5, where one represented a prostrate plant with a tiller angle of less than 30° and five an erect plant with a tiller angle greater than 75°. At maturity, when the rachis below panicle turned brown, seed from each plot was bulk-harvested, dried in a shed and threshed in a stationary thresher. Seed was then cleaned with an Airblast Cleaner (ALMACO, Nevada, IA) with a common airflow setting for all plots

### STATISTICAL ANALYSIS

Data for heading date and plant erectness were taken for each individual plant but analyzed on a plot mean basis. Mixed models methodology as implemented in SAS® PROC GLIMMIX was used to analyze the response variables. Population, year, and their interaction were considered as fixed effects, whereas block within year was the sole random effect. R-side modeling using the GROUP option of the RANDOM statement was used to account for heterogeneous variances; the smallest number of variance groups was selected based on the AICC criterion. Least squares population x year means were calculated using the SLICEDIFF option to compare populations within a year. Dunnett's test was performed to compare the selected populations ( $C_1$ ,  $C_2$ ,  $C_3$ ) to the base population ( $C_0$ )

## RESULTS AND DISCUSSION

### HEADING DATE

Selection for improved winter productivity gave populations ( $C_1$ ,  $C_2$ ,  $C_3$ ) that had an 8 d earlier heading date than the base population ( $P < 0.001$ ). The selected populations did not differ from one another ( $P > 0.26$ ). There was no population x evaluation year interaction (Figure 1). The change in heading date occurred during the first selection cycle. These changes are not trivial as they were based on the heading date of 800 genotypes in 2008 and 400 in 2009.

In Figure 2, plants falling under the range of the 5<sup>th</sup> to 95<sup>th</sup> percentile showed less difference among populations compared to the large differences among populations due to inclusion of plants in the range of the 1<sup>st</sup> to 99<sup>th</sup> percentile. Therefore plants under the extreme percentiles gave the most difference among base and selected populations. It indicates that the variation is explained by small number of plants that were eliminated in selected populations because of late maturity. The non-synchronized maturity was observed during harvesting and therefore late maturing plants might have been excluded from contributing pollen.

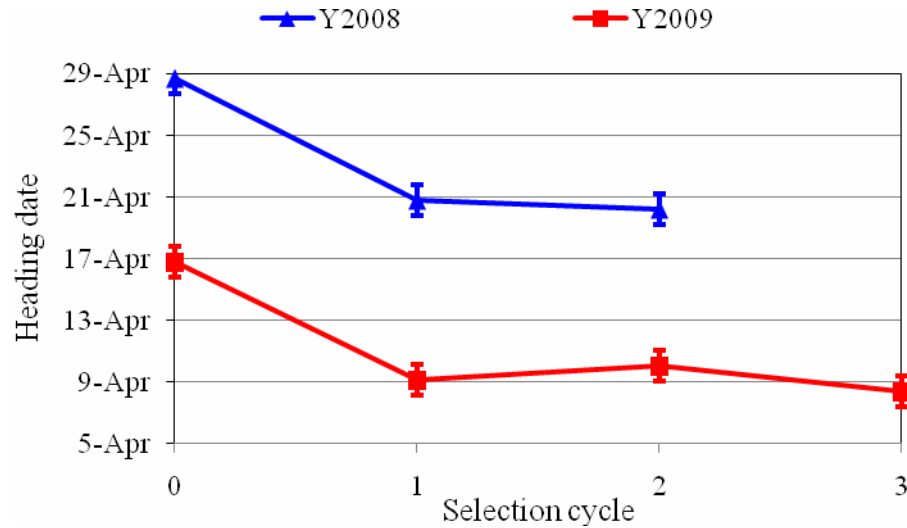


Figure 1. Effect of three cycles of phenotypic recurrent selection for winter productivity on heading date of annual ryegrass. Heading was defined as the day that a given plant had at least five panicles 75% emerged from the boot.

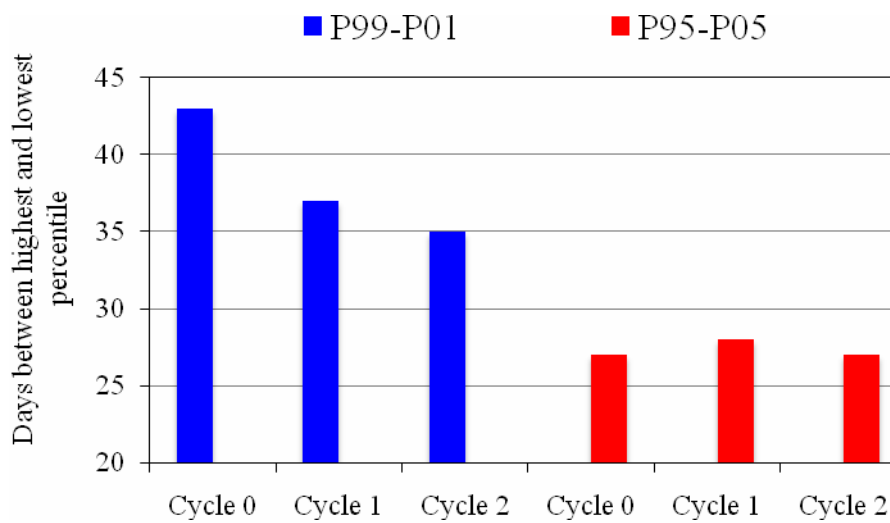


Figure 2. Intra-population variation for heading date within three cycles (Cycle 0, Cycle 1, Cycle 2 selected for dry matter yield) at different percentile ranges. (P99 - P01 includes plants for heading date falling in the range of the 1<sup>st</sup> to 99<sup>th</sup> percentile; P95 - P05 includes plants in the range of the 5<sup>th</sup> to 95<sup>th</sup> percentile).

It has to be remembered that selection occurred in late January to early February, at least two months prior to stem elongation. Selected genotypes, with an extreme late heading date, may have simply been eliminated from contributing to the next generation. It is known that reproductive maturity is highly heritable and thus eliminating certain late-maturing genotypes might have induced this change. Similar results were observed in another study with annual ryegrass, where dry matter yield was genetically correlated (0.99) with heading date (Fujimoto and Suzuki, 1975).

### PLANT TYPE

Plant type data were collected in mid March to obtain an accurate estimate of tiller angle because with time (age), zenith angle (angle with respect to vertical) increases resulting in plants with wide angles. All selected populations were significantly ( $P < 0.0001$ ) more erect than the base population (Figure 3). As with heading date, these changes occurred during the first selection cycle and no significant ( $P > 0.44$ ) differences among selected populations ( $C_1$ ,  $C_2$ ) were observed. During the evaluation phase of the cycle, all plants were harvested uniformly at 5 cm above ground level because the main criterion was biomass production of individual plants without considering plant habit (erect or prostrate). The association of tiller angle with DM yield may explain the change in growth habit. Plants with narrow tiller angle have more leaf area exposed to sunlight resulting in high DM accumulation. Divergent selection for reproductive maturity in spaced orchard grass plants (*Dactylis glomerata* L.) also showed a significant correlation between maturity and growth habit. Populations selected for early maturity became more erect in growth habit and had a high total DM yield. Selection for late maturity resulted in a significant change towards prostrate growth (Short and Carlson, 1989). Similarly, there was a high correlation between early maturity and erect growth habit in *Poa annua* L., where the same results were found in both individual plant and family trials (Warwick and Briggs, 1978).

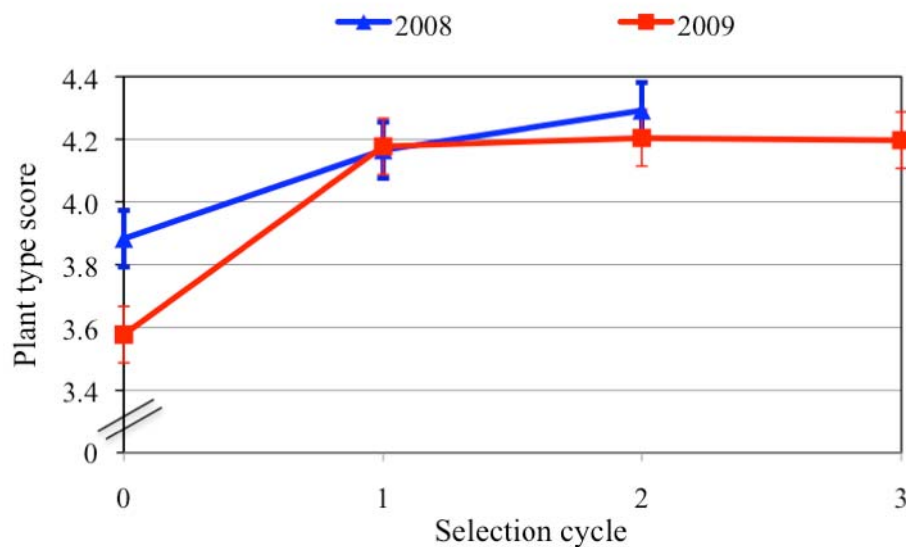


Figure 3. Effect of three cycles of phenotypic recurrent selection for winter productivity on plant type score of annual ryegrass. (Plant type scored on a scale of 1 = tiller angle  $< 30^\circ$  to 5 = tiller angle  $> 75^\circ$ ).

### SEED YIELD

In 2008, seed yield increased during the first two cycles of phenotypic recurrent selection (Table 1). Similarly, seed yield showed a significant correlation with DM yield in meadow brome grass (*Bromus riparius* Rehm.) (de Araujo and Coulman, 2002; de Araujo and Coulman, 2004). Increased seed yield can be explained by genotypic and phenotypic correlation of dry weight with tiller number (Fujimoto and Suzuki, 1975), which is a major component of seed yield. The population selected for one cycle, based on forage DM yield, exceeded the base population by 31% ( $P < 0.001$ ) and the population selected for two cycles exceeded it by 20% ( $P = 0.006$ ).

In 2009,  $C_1$  gave a 51% ( $P = 0.195$ ) higher yield than the  $C_0$  but with insignificant differences (Table 1), whereas in 2008, this increase was significant ( $P < 0.001$ ). After three years of selection,  $C_2$  and  $C_3$  yielded 95% ( $P < 0.001$ ) and 52% ( $P = 0.019$ ) more seed than the

C<sub>0</sub> population respectively. The significant year × population interaction was expected given the variable spring weather generally experienced in southeastern United States. Ultimately, seed yield will have to be measured in the target environment (Willamette Valley, Oregon, USA) to get a true measure under actual seed production conditions of the effect of selection for improved winter growth on seed yield.

Table 1 Effect of three cycles of phenotypic recurrent selection for winter dry matter production on seed yield.

Selection cycle	2008		2009	
	Seed yield (kg ha <sup>-1</sup> )	Dunnett's <i>P</i> vs C <sub>0</sub>	Seed yield (kg ha <sup>-1</sup> )	Dunnett's <i>P</i> vs C <sub>0</sub>
C <sub>0</sub>	452		214	
C <sub>1</sub>	592	<0.001	323	0.195
C <sub>2</sub>	544	0.006	418	<0.001
C <sub>3</sub>			326	0.019
SE	72		61	

## SUMMARY

All indirect responses showed drastic modification in cycle 1 and did not change any further in cycles 2 and 3. This is in contrast to the direct selection response evaluated at the selection location, where forage yield increased linearly over two cycles. A possible explanation for the drastic changes in heading date from C<sub>0</sub> to C<sub>1</sub> might be the inclusion of late maturing plants in cycle 1 that were not included in the next generations because of non synchronous flowering. The association between high DM and erect plant type might be explained by a narrow plant tiller angle that resulted in more photosynthate accumulation. Increased seed yield may be due to tightening of maturity in advanced selection cycles making pollination more effective.

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