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Divergent Selection for Anthesis Date in Annual Ryegrass

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ABSTRACT

Anthesis date is the major factor controlling distribution of forage yield and quality of annual ryegrass (*Lolium multiflorum* Lam.) in the spring. This study was initiated to evaluate variation and selection response for anthesis date and to measure correlated responses in annual ryegrass. Four annual ryegrass cultivars were evaluated for heading date, anthesis date, spikelets per spike, and florets per spikelet over 3 yr in the field at Mississippi State, MS. Only spikelets per spike and florets per spikelet, showed significant cultivar × year interactions. Differences among cultivars were observed for all four characteristics. Two cycles of phenotypic recurrent selection for earlier and later anthesis date were performed on each cultivar. Response after two cycles of selection for late anthesis in the early cultivar, Florida 80, was greater (11.1 d) than selection for early anthesis (6.0 d); however, response to selection for early anthesis in the late cultivar, Marshall, was greater (4.8 d) than selection for late anthesis (2.7 d). The tetraploid, 'Multimo', exhibited similar responses to selection for both early and late anthesis date. Late selections within each cultivar showed increased numbers of spikelets per spike compared with the respective unselected parent population. Number of florets per spikelet decreased in most selected populations compared with the respective unselected parent populations. There appeared to be sufficient genetic variation after two cycles of selection to allow for additional gain from selection for anthesis date in most populations.

ANNUAL RYEGRASS is the major winter annual forage in the southeastern USA. Attributes, such as rapid establishment, high yield, and excellent forage quality, make ryegrass a highly desirable forage species. Annual ryegrass is an open-pollinated species that has a determinate flowering habit, with anthesis date being the major factor controlling the distribution of forage yield and quality in the spring. Extending the growing season of annual ryegrass by delaying the flowering date could maintain a high proportion of leaves to stems for a longer time period, thus increasing the nutritive value, digestibility, and palatability of the forage.

Maturity date is influenced by both environmental and genetic factors, and interactions of these factors. Wilson (1959) and Evans (1960a) noted the effects of soil fertility, juvenile requirement, and vernalization criteria on maturity in ryegrass. Annual ryegrass is considered nitropositive in that it flowers earlier with increased nitrogen (Wilson, 1959). He also reported minimum leaf numbers as low as four for the minimum vegetative requirement in annual ryegrass before floral initiation. The vernalization criteria for annual ryegrass are insignificant; however, exposure to low temperatures may make the plant more responsive to photoperiod. Major (1980), Evans (1960b), and Aitken (1966) have shown that photoperiod is one of the main environmental factors that influences flowering in annual ryegrass. Breese (1961) observed in annual or short-lived perennial ryegrass, that growth and maturity traits exhibited a high degree of dominance and epistasis. Thomas (1967) reported that in the adult stage of annual ryegrass, flowering time was genetically controlled and there were no maternal effects.

The potential genetic variation for maturity in *Lolium* sp. was demonstrated by experiments of Cooper (1954, 1959a, b, and c). Cooper (1959c) practiced divergent selection for early and late ear emergence date in two perennial ryegrass (*L. perenne* L.) populations over several generations. Response was rapid in both directions in both populations. The response to selection was asymmetrical in both populations, the advancement toward lateness being greater than that toward earliness, even though the selection differential was the same in both directions. Cooper (1960) observed few significant correlations between date of ear emergence and other ear measurements. Spikelet number was highly heritable in both populations, while floret number showed little genetic variation.

Our objectives were to (i) evaluate the variation for heading date, anthesis date, number of spikelets per spike, and number of florets per spikelet in four diverse annual ryegrass cultivars used for forage production in the USA, (ii) evaluate the selection response for both early and late anthesis date in these cultivars, and (iii) measure correlated responses of these characteristics to selection for either early or late anthesis date within each cultivar.

MATERIALS AND METHODS

The four cultivars of annual ryegrass evaluated in these experiments consisted of three diploids, Florida 80, 'Gulf,' and Marshall, and one tetraploid, Multimo. Florida 80 is earlier in maturity than Gulf and much earlier than common ryegrass (Prine et al., 1982). Gulf is intermediate in maturity and is ≈10 d earlier than common ryegrass (Weihing, 1963). Marshall evolved as the result of 29 yr of natural selection from common ryegrass and matures ≈2 wk later than Gulf in Mississippi (Arnold et al., 1980). Multimo is an artificially induced autotetraploid that is similar to or slightly later in maturity than Marshall.

In the parental evaluations, the four cultivars were planted in the greenhouse in late summer of 1985, 1986 and 1987. Seed were planted in 6 by 6 by 8 cm peat pots in a mixture of 1:1 sand and peat moss. Plants were thinned to one plant per pot 1 wk after emergence. Six-week-old seedlings of the four cultivars were transplanted to the field on 1-m centers in early fall each year at Mississippi State, MS, on a Leeper silty clay loam soil (fine, montmorillonitic, nonacid, thermic Vertic Haplaquept) with pH of 6.5. The experimental design for each year was a randomized complete block with six replicates and eight spaced plants per plot.

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Abbreviations: Y × C, year × cultivar.

Table 1. Analyses of variance for heading date, anthesis date, spikelets per spike, and florets per spikelet for four annual ryegrass parental cultivars, 1986 to 1988.

Source	df	Mean square			
		Heading date	Anthesis date	Spikelets spike^{-1}	Florets spikelet^{-1}
Years (Y)	2	1 429.4**	2 172.2**	0.7	401.0*
Reps/Y	15	53.4	36.2	23.0	5.2
Cultivars (C)	3	19 746.5**	15 549.9**	1612.7**	263.2**
Y × C	6	65.4	39.8	65.1**	59.1*
C × Reps/Y	45	40.6**	44.6**	19.3	3.7*
Plants/Plots	504	25.0	18.1	17.5	2.3

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

Two applications of fertilizer were applied to the field each growing season. The first application consisted of 430 kg ha^{-1} of 13-6-11 (N P K) 1 wk after transplanting. The second application of fertilizer consisted of 170 kg ha^{-1} of NH_4NO_3 in early spring.

In the selection phase, two sets of 64 plants each from each of the four cultivars were planted as spaced plants in 8 by 8 grids on 1-m centers in isolation blocks in the fall of 1985 on the Leveck Animal Research center at Mississippi State University on the same soil type as the parental evaluations. Within each cultivar, one block was selected for early anthesis date and the other selected for late anthesis date. A 2-m border of rye (*Secale cereale* L.) was planted around each block to serve as a means of isolation. Fertilizer applications and amounts were the same as in the parental evaluations. Approximately 20% of the ryegrass plants within each block was retained and allowed to intercross. The other 80% was removed from the block and discarded. Seed from each selected plant within each block was individually harvested in the spring of 1986. Equal quantities of seed from each plant within each block were bulked to constitute the seed of the first selection cycle for anthesis date for the respective populations. These first selection cycle populations for the four cultivars were included with the parental evaluations in the 1986–1987 growing season. First cycle populations were also subjected to a second cycle of selection for either early or late anthesis date in the 1986–1987 growing season. Procedures for the second cycle were the same as for the first cycle. The second cycle, first cycle, and unselected parent population were included in the parental evaluations in the 1987–1988 growing season.

Mature spaced plants of the four cultivars of annual ryegrass were evaluated for heading date, anthesis date, number of spikelets per spike, and number of florets per spikelet. The field plots were evaluated everyday between 1400 and 1800 h from 5 March until the last plant had achieved anthesis each year. Both heading and anthesis date were noted and recorded as days since the beginning of the year. Heading date was recorded when the first ear emerged from the boot. Anthesis date was recorded when the first flower opened and anthers appeared. Five randomly chosen spikes were taken from each plant after anthesis had occurred and the number of spikelets on each spike were counted. Four spikelets were randomly chosen on each spike and the number of florets per spikelet was recorded. Data for florets and spikelets were summed over all five spikes and means for each plant were obtained.

All data were subjected to analysis of variance. Means for the experiments were separated using Fisher's protected least significant difference. Observed variances of parental cultivars and their respective selected populations were tested using Cochran's (1941) C test for homogeneity of variances as

$$C = \frac{\sigma^2_{\text{largest variance}}}{\Sigma\, \sigma^2_{\text{sum of all variances}}}$$

with $(n - 1)$ degrees of freedom, where n = number of observations per population.

Genotypic and phenotypic correlation coefficients among characteristics were calculated for the parental cultivars over the 3-yr period. The genotypic correlations were obtained by using the following formula from Falconer (1989):

$$r_G = \frac{\text{Cov}_{xy}}{[(\text{Var}_x)(\text{Var}_y)]^{1/2}}$$

where

r_G = genotypic correlation of X and Y
Cov_{xy} = genetic covariance of characters X and Y
Var_x = genetic variance of character X
Var_y = genetic variance of character Y.

Realized heritabilities (Falconer, 1989) for anthesis date were calculated using the following formula:

$$h^2 = (\bar{X}_{px} - \bar{X}_{\text{cultivar}})/(\bar{X}_{\text{selected}} - \bar{X}_{\text{cultivar}})$$

where

Table 2. Means and variances for heading date, anthesis date, spikelets per spike, and florets per spikelet of four annual ryegrass cultivars, 1986 to 1988.

Cultivar	Heading date 3 yr	Anthesis date 3 yr	Spikelets spike^{-1}			Florets spikelet^{-1}		
			1986	1987	1988	1986	1987	1988
	d	d	no.			no.		
			Means					
Florida 80	91.8	105.7	25.9	26.5	27.8	12.8	12.8	14.8
Gulf	99.2	114.1	29.5	31.1	30.5	13.1	13.5	17.3
Marshall	115.5	127.3	35.1	32.9	33.8	13.0	16.1	14.9
Multimo	114.7	126.4	34.4	34.0	32.3	9.9	12.0	13.4
LSD (0.05)	1.5	1.6	2.0	1.8	1.9	1.0	0.7	0.8
			Variances					
Florida 80	28.2	28.8	13.8	18.1	10.2	3.3	2.9	1.4
Gulf	30.7	29.5	18.6	18.3	24.0	3.3	1.3	2.9
Marshall	36.6	30.2	16.7	16.4	31.6	3.1	3.2	2.6
Multimo	33.9	25.2	18.5	16.8	10.8	1.3	2.6	2.1
Heterogeneity of variances	NS	NS	NS	NS	**	NS	NS	NS

** Significant at the 0.01 level of probability, according to Cochran's (1941) test.

Table 3. Means of four characteristics in selected and unselected populations of four annual ryegrass cultivars, 1987 to 1988.

Selection cycle	Florida 80		Gulf		Marshall		Multimo	
	1987	1988	1987	1988	1987	1988	1987	1988
				Heading date, d of year				
2nd cycle early	—	89.3	—	95.5	—	107.6	—	108.5
1st cycle early	86.2	90.3	95.8	100.4	106.9	112.1	109.4	110.8
Unselected	87.9	94.4	97.5	102.4	112.2	118.5	112.3	116.5
1st cycle late	92.0	99.1	103.6	107.5	116.2	123.6	117.3	122.3
2nd cycle late	—	105.9	—	110.5	—	122.7	—	125.1
LSD (0.05)	1.2	2.5	1.7	1.9	2.5	2.4	2.0	2.5
				Anthesis date, d of year				
2nd cycle early	—	102.4	—	110.9	—	121.9	—	122.1
1st cycle early	100.6	103.5	110.0	116.3	118.0	126.4	120.6	124.2
Unselected	102.6	108.4	111.2	118.5	123.3	131.2	123.6	129.5
1st cycle late	105.3	112.9	116.0	122.7	127.1	134.1	128.8	133.7
2nd cycle late	—	119.5	—	125.4	—	133.9	—	135.6
LSD (0.05)	1.1	2.7	0.8	1.9	2.4	2.0	1.2	2.1
				Spikelets spike^{-1}, no.				
2nd cycle early	—	26.4	—	27.1	—	30.9	—	27.9
1st cycle early	26.6	27.6	29.3	28.6	32.6	32.0	32.8	30.7
Unselected	26.5	27.8	31.1	30.5	32.9	33.8	34.0	32.3
1st cycle late	29.1	27.9	33.1	33.2	35.2	35.9	38.7	35.7
2nd cycle late	—	30.0	—	32.9	—	36.2	—	36.8
LSD (0.05)	2.1	1.6	2.0	2.0	1.7	2.3	2.3	1.2
				Florets spikelet^{-1}, no.				
2nd cycle early	—	14.8	—	15.6	—	15.8	—	13.4
1st cycle early	14.0	14.7	15.7	15.6	15.6	15.8	12.6	12.9
Unselected	12.8	14.8	13.5	17.3	16.1	14.9	12.0	13.4
1st cycle late	13.5	13.9	15.0	16.9	14.5	14.4	10.9	12.1
2nd cycle late	—	13.5	—	15.9	—	15.9	—	12.0
LSD (0.05)	0.7	0.8	0.8	0.7	1.0	0.7	0.8	0.7

$\bar{X}_{px}$ = mean of the polycross progeny
$\bar{X}_{cultivar}$ = mean of parent cultivar
$\bar{X}_{selected}$ = mean of the individuals selected from the parent cultivar.

Standard errors for the estimates of realized heritabilities were calculated according to Prout's (1962) formula for variance and taking the square root.

$$SE(h^2) = \left\{\frac{1}{D^2}\left[\frac{h^2(1 - h^2)S_p^2}{N_p} + \frac{S_o^2}{N_o}\right]\right\}^{1/2}$$

where

$D^2 = (\bar{X}_{selected} - \bar{X}_{cultivar})^2$
N_p = number of selected parents
N_o = number of offspring measured
S_p^2 = phenotypic variance of population from which parents were drawn
S_o^2 = phenotypic variance of offspring population
h^2 = realized heritability.

RESULTS AND DISCUSSION

The maturity characteristics (heading and anthesis date) did not show a Y × C interaction, while the seed yield characteristics (spikelets per spike and florets per spikelet) exhibited significant Y × C interactions in the four parental cultivars over three growing seasons (Table 1). Differences in the main effects of cultivars and years were significant for heading and anthesis date.

Heading and anthesis date followed similar patterns among the four ryegrass cultivars (Table 2). Number of spikelets per spike tended to increase with later maturity in the cultivars, which is similar to what Cooper (1960) found in perennial ryegrass. Number of florets per spikelet in the intermediate and late maturing diploid cultivars was greater than or equal to the number of florets per spikelet in the early diploid cultivar.

Table 4. Realized heritabilities (h^2) and associated standard errors (SE) based on selections for anthesis date in four annual ryegrass cultivars, 1987 to 1988.

Selection cycle	Florida 80				Gulf				Marshall				Multimo			
	1987		1988		1987		1988		1987		1988		1987		1988	
	h^2	SE	h^2	SE	h^2	SE	h^2	SE	h^2	SE	h^2	SE	h^2	SE	h^2	SE
2nd cycle early	—		0.26	± 0.16	—		1.10	± 0.14	—		0.76	± 0.15	—		0.41	± 0.18
1st cycle early	0.41	± 0.14	0.69	± 0.14	0.92	± 0.14	0.40	± 0.16	0.99	± 0.10	0.70	± 0.14	0.51	± 0.15	0.83	± 0.13
1st cycle late	0.51	± 0.15	0.60	± 0.15	0.35	± 0.14	0.73	± 0.13	0.76	± 0.20	0.39	± 0.13	0.79	± 0.13	0.85	± 0.14
2nd cycle late	—		1.01	± 0.11	—		0.61	± 0.16	—		0.00	± 0.14	—		0.33	± 0.13

Variances within the cultivars only differed significantly for spikelets per spike in 1988. Variances within cultivars were similar for maturity characteristics and number of florets per spikelet.

Large phenotypic (r_p) and genotypic (r_G) correlations were observed between heading date and anthesis date over the three growing seasons. The high genotypic correlation ($r_G = 1.00$) between heading date and anthesis date suggests that either characteristic may be used for evaluating maturity in annual ryegrass. Genotypic correlations for number of spikelets per spike with heading ($r_G = 1.01$) and anthesis date ($r_G = 1.02$) were also high, while their corresponding phenotypic correlations ($r_p = 0.60$ and 0.61, respectively) were approximately half that of the genotypic correlations. There were no significant phenotypic or genotypic correlations with other traits found for the number of florets per spikelet.

Genetic variation for heading and anthesis dates was observed in all four cultivars and variances within each of the parental cultivars were approximately equal. Heading and anthesis dates showed no significant Y × C interactions. Genetic variation was also observed for the seed yield characteristics in each of the four parental cultivars, but not to the extent found in the maturity characteristics. Variances were similar for number of spikelets per spike and number of florets per spikelet in the parental cultivars. The seed yield characteristics were found to be more susceptible to environmental variation than the maturity characteristics, which is in agreement with Cooper (1960), who suggested that heritabilities for the seed yield characteristics would be lower and consequently the response to selection would be slower. Because of the significant Y × C interaction, selection for spikelets per spike and florets per spikelet would benefit from multiple environment evaluations.

Significant differences among cycles of selection were observed for all four characteristics within each of the four parental cultivar sources in both 1987 and 1988 (Table 3). In 1987, responses to selection were not of the same magnitude for early compared with late anthesis date. Realized heritabilities were generally high with the exception of selection for early anthesis in Florida 80 and selection for late anthesis in Gulf (Table 4). Correlated response for heading date followed a similar pattern to anthesis date (Table 3). Selection for later anthesis date resulted in an increased number of spikelets per spike in all cultivars. Florets per spikelet showed no pattern relative to selection for early or late anthesis date.

In 1988, responses to selection for anthesis date were asymmetric in early and late maturing diploid cultivars (Tables 3 and 4). Responses to selection for late anthesis were greater than for early anthesis in Florida 80, an early-maturing diploid cultivar; while responses to selection for early anthesis were greater than for late anthesis in Marshall, a late-maturing diploid cultivar. Gulf, an intermediate-maturing diploid cultivar, exhibited similar selection responses for both early and late anthesis dates. In the tetraploid cultivar, Multimo, responses for anthesis dates were also similar for both early and late selections. Indirect selection responses for heading date were similar to those for anthesis date in all cultivars. Late selections within each of the four cultivars showed increased numbers of spikelets per spike when compared with their respective parent cultivar, while number of spikelets per spike decreased in the early selections. In general, number of florets per spikelet decreased with selection for either early or late anthesis compared with the parent cultivar. The exception was Marshall, where the number of florets per spikelet increased with selection.

Variances for the four parental cultivars and their respective selected populations were generally homogeneous for the four characteristics in 1987 and 1988 (data not shown) with the exception of spikelets per spike in Marshall and Gulf populations. Marshall populations, as a whole, tended to produce more abnormal inflorescences than the other three cultivars. A common abnormality was production of inflorescences with two or three branches off the rachis that caused the inflorescence to resemble a panicle. These abnormal inflorescence types also occurred in the late selections of Gulf populations, but not to the extent observed in Marshall populations. Variances for spikelets per spike in Marshall populations and in the late selections for Gulf were substantially higher than for the other two cultivars. These abnormal inflorescences apparently had no effect on mean or variance for florets per spikelet in either Marshall or Gulf populations.

In the Florida 80 and Marshall cultivars, environmental barriers may have restricted selection for early and late anthesis, respectively. It may be difficult to select for early anthesis in Florida 80 in that winter temperatures generally restrict plant growth during January and February in Mississippi, thus restricting the degree of earliness that can be obtained. On the other hand, selection for late anthesis date in the Marshall populations may be difficult, because later flowering would be subjected to higher temperatures and drought, which can reduce both pollination and fertilization. Another factor which could interfere with selection in these two cultivars is length of photoperiod. With long photoperiod at northern latitudes, selection for lateness is more effective than selection for earliness; while at southern latitudes, selection for earliness is more effective than selection for lateness, in the northern hemisphere (Cooper, 1950). For example, extremely late genotypes of Marshall will not flower in extreme South Mississippi. To obtain extremes for earliness in the early-maturing cultivar, Florida 80, selections should be made in the lower south; while extremes for lateness in the late-maturing cultivar, Marshall, should be made in the mid to upper south.

In most cases there appeared to be sufficient genetic variance and the realized heritabilities were high enough to suggest that further gains from selection are possible. Heading date and anthesis date were highly correlated ($r_G = 1.00$) and either characteristic could be used as a measure of maturity. The association of increased number of spikelets per spike with later maturity in annual ryegrass could have implications for seed yield; however, further research is needed to determine if this would result in increased seed yield or simply in the production of more small seed without any increase in seed yield.

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