Effect of Seed Multiplication Regimes on Genetic Stability of Kenstar Red Clover

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ABSTRACT

Genetic shifts frequently occur in red clover (*Trifolium pratense* L.) cultivars when seed is increased outside the area of development. Continual monitoring of possible genetic changes ensures stability of cultivars. These investigations examined genetic shifts (changes in longevity and forage yield) in the Kenstar cultivar of red clover during seed increases in Kentucky, California, and Washington. Management variables were imposed so that the effect of class of seed, location of seed production, clipping the first growth, lineage (descent from different breeder seed lots), and year of seed production could be examined. Seed lots thus produced were grown and evaluated at Lexington, Kentucky, and Arlington, Wisconsin. Persistence and yield generally declined from breeder to recertified, particularly for seed lots produced at the California location. Lineage and clipping treatments had no effect on stand or yield, but stand and yield were lower from stands established with seed produced in the third year than from those established with second-year seed. Percent bloom was negatively correlated with persistence and yield suggesting, as has been shown in previous studies, that blooming types produce the most seed and are less persistent than later blooming types. Regrowth height at Wisconsin substantiated the Kentucky data and confirmed our earlier conclusion that the ability to regrow rapidly is associated with decline in performance. Washington is the recommended location for seed increase of Kenstar because less genetic shift occurred at the site compared with California and Kentucky.

Additional index words: **genetic shift, plant longevity, forage yield, persistence, seed certification.**

INTRODUCTION

Red clover is a cross-pollinated, heterozygous and heterogenous species. It is normally spring sown, and seed crops may be produced during the first, second, and third years of the stand. After the third year, stands are usually so thin that seed production is not economical. Cultivars are subject to genetic change when seed is multiplied outside the area of development (Taylor, May, Decker, Rincker and Garrison, 1979). A genetic shift towards lower yield and less persistence generally occurs with advancing generation and is more pronounced in seed lots produced in southern locations (California) than in northern locations (Washington). A study of genetic shift in red clover cv Kenland established that earliness of bloom was negatively correlated with forage yield, suggesting that in southern locations, short day lengths allowed more unequal blooming and seed production among genotypes than at northern locations (Taylor *et al.*, 1979). No advantage of third-year over second-year breeder seed (from Kentucky) was shown; however, third-year certified seed from southern latitudes generally was less productive than second-year seed, possibly because of more opportunity for differential selection. Differences in performance of foundation and certified seed lots could not be associated with their parental seed lots.

The previous study with Kenland red clover was conducted with seed collected from increase fields of commercial growers, and the effects examined in performance trials were those that resulted from variable commercial practices. Kenland is a broad-based cultivar resulting from recurrent selection in Kentucky (Hollowell, 1951). When the cultivar Kenstar, a 10-clone synthetic, was released (Taylor and Anderson, 1973), an opportunity was available to impose management variables during seed multiplication regimes and to study the factors involved in genetic shifts of a cultivar with a narrow genetic base. Variables considered to be potentially important were generation of seed increase (comparable to breeder, foundation, registered, and certified classes); location of increase (California, Washington, and Kentucky); effect of lineage (different parental seed lots); effect of removal or non-removal of first growth for hay prior to seed production (clipping vs. no clipping); and effect of year of seed production (first, second, or third year after

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sowing). Evaluation locations chosen were Kentucky, where Kenstar originated, and Wisconsin. The evaluation methods used were those shown to be effective in a previous study of genetic shifts (Taylor *et al.*, 1979). Obviously, the location where changes in longevity and yield can be evaluated most optimally is where the cultivar was bred, i.e., Kentucky. Evaluation at other locations may impose a set of environmental conditions that may not measure true genetic shifts. Nevertheless, if data from the Wisconsin location were correlated with those from Kentucky, it would indicate that the genetic shift phenomena was widespread. Therefore, the objectives of the present study were to examine the effect of the variables outlined above on genetic shift of Kenstar red clover during seed multiplication.

MATERIALS AND METHODS

Seed Increases

In 1970, Kenstar breeder seed (Descent 1) that had been produced in Kentucky was established at Lexington, Kentucky, Prosser, Washington, and Shafter, California (Fig. 1). These are locations where red clover seed is produced for commerce. In addition, the 10 clones of Kenstar were polycrossed in Washington and California to form Descent 2 and 3, the seeds of which were increased at these two locations, respectively. These increases provided 12 more lots at each location similar to the increases shown in Fig. 1 except for the seeding-year crop at Shafter. In summary, Descent 1 included 6 lots increased at Kentucky, 12 lots increased in Washington, and 16 lots increased in California; Descent 2 included 12 lots increased in Washington; and Descent 3 included 12 lots increased in California, making a total of 60 lots. Four breeder seed lots from Kentucky were added to bring the final total to 64. After stands were established, management variables were imposed as follows:

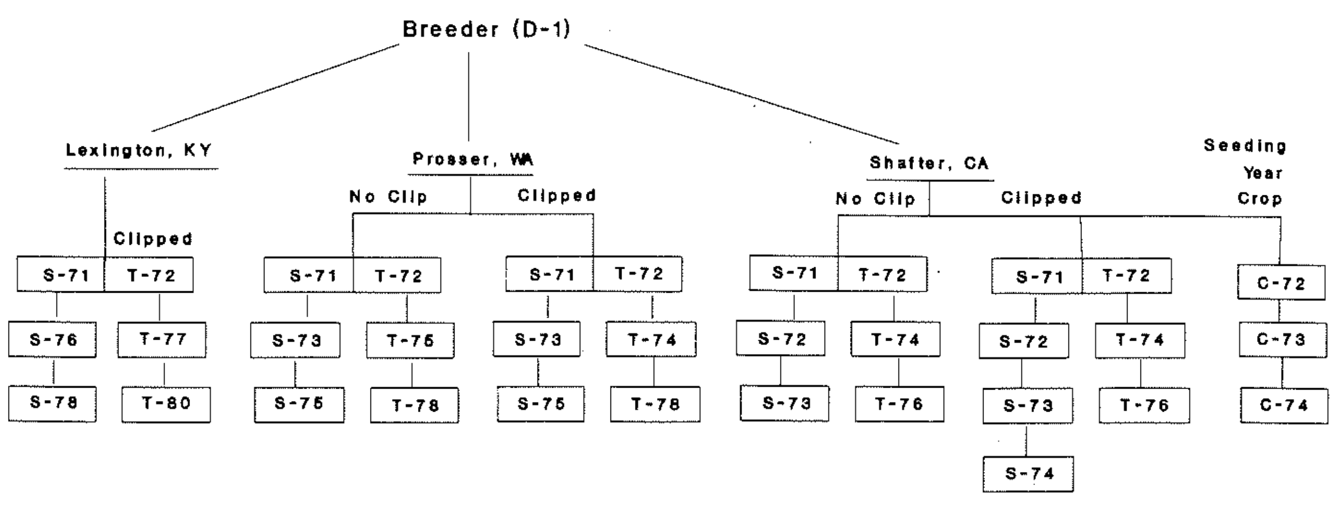
1. Clipping vs. no clipping: In Washington and California, first-growth foliage was removed (clipping) or left standing to produce seed (no clipping). First growth in Kentucky was clipped as is the prevailing management practice (Taylor, Anderson and TeKrony, 1972).

2. Year of harvest: Seed was harvested with conventional harvesting equipment in the first year (year of sowing) only in California, and in the second and third years at all three locations.

3. Class of seed: Harvested seed was resown in the same locations so that five classes of seed were produced. These classes were equivalent to breeder (only in Kentucky), foundation, registered, and certified (at all three locations), and one extra generation at California, termed recertified, for seed crops harvested in the second year only.

Seed increases were completed in 1976 in California, in 1978 in Washington, and in 1980 in Kentucky (Fig. 1). Remnant seeds of the 64 lots were stored in Washington and in Kentucky at -5 to -15°C until evaluation. Seed stored at these temperatures has been shown to maintain viability almost indefinitely (Rincker, 1974; 1980).

Figure 1.
Diagram of seed increases of Kenstar red clover from Descent 1 at Kentucky, Washington, and California. Number in each block indicates the year of seed increase. S = seed crop in first year after sowing, T = seed crop in second year after sowing, C = seed crop in year of sowing. Descent 2 and 3 not shown for brevity.



Evaluation

Samples of all seed lots were shipped to Lexington and Arlington for evaluation. At Lexington, seeds of all entries were sown on Spindletop Farm in drilled plots on 26 March 1981 in wheat (*Triticum aestivum* L.) that had been established the previous autumn. Plot size was 1.52 by 4.87 m. Seeding rate was 11 kg ha^{-1} and original stands were nearly 100%. Harvested plot size was 0.91 by 3.0 m. Entries (seed lots) were arranged in an 8 x 8 repeated triple lattice design and were coded as to class of seed, clipping vs. no clipping, year of seed production, location of seed increase, and lineage (descent from original breeder-seed lot). Soil type was a Maury silt loam (fine, mixed, mesic Typic Paleudalph). Stand percentage (percent ground cover) was determined on 10 June 1983; percent bloom on 23 June 1982; and dry matter yields on 20 May, 24 June, 2 August, and 16 September 1982, and on 26 May and 30 June in 1983. The percentage stand data in 1983 near the end of the experiment represent survival or persistence of original plants. To determine dry matter yields, plots were mowed at approximately 10 days after first bloom at a 7.6 cm height. Forage was bagged and dried at 70°C to a constant dry weight.

At Arlington the seeds of the 64 entries were sown in 1981 in 6.1 m long drilled rows and arranged in an 8 x 8 quadruple lattice. Rows were spaced 1 m apart and were thinned to 20 evenly spaced plants (about 30.5 cm apart). Soil type was a Plano silt loam (fine, silty, mixed, mesic Typic Arginoll). Data collected in 1981 on each plant included plant type (Bird, 1948), scored on a scale of 1 to 5 with 5 = most erect; leaf mark (presence or absence); and resistance to powdery mildew caused by *Erysiphe polygoni* DC em Salm., scored on a scale of 1 to 5 with 1 = most resistant. In 1982, maturity was scored on a scale of 1 to 5 with 5 = most mature or most flowering. Resistance to northern anthracnose caused by *Kabatiella caulivora* (Kirchn.) Karak was scored on a scale of 1 to 5 with 1 = most resistant. Inoculum of these pathogens are universally present under Wisconsin conditions (R.R. Smith, unpublished). Plots were mowed on 15 June and regrowth height was measured on 6 July 1982. Methods used were similar to that of a previous study with Kenland and were shown to be adequate for evaluating genetic shifts (Taylor *et al.*, 1979).

All data were subjected to analyses of variance. To reduce the volume of data from Kentucky, only percentage stand on 10 June 1983, total dry matter yields for 1982 and 1983, and percentage bloom on 23 June 1982 are presented in tabular form. In Wisconsin, only plant type and regrowth height data had significant differences (P = 0.05) in any of the analyses, and are the only ones presented.

Methods of analysis

Adjusted entry means were obtained by estimated generalised least squares (EGLS) methods (Cornelius, 1985; 1986) using a model in which lattice blocks, plots within blocks, and for variables for which individual plants were evaluated, plants within plots were considered random. For variables that were recorded as one observation per plot, restricted maximum likelihood (REML) estimates of variance components (Corbeil and Searle, 1976) for use in EGLS estimation were computed as described by Cornelius (1985). For variables on which there were individual plant data, REML variance estimates could not be computed due to the magnitude of the computational problem; thus, analysis of variance ('fitting of constants' method) estimates were used instead.

The effective variance of an entry mean was calculated as average variance minus average covariance, this quantity being algebraically identical to half the average variance of the pairwise difference. The effective variance of a mean then was used as an estimate of error for approximate inferences concerning differences among entry means. The degrees of freedom of this error estimate were taken as 273 and 161 in Kentucky and Wisconsin evaluations respectively, these being the df for intrablock error (i.e., plots within blocks, eliminating treatments) in the two experiments.

Entries consisted of an incomplete five-factor factorial arrangement of treatments with class of seed, location of seed production, lineage, clipping, and year of seed production as treatment factors. Sums of squares among adjusted entry means in subsets of entries that would allow effects of one or more factors to be evaluated free of differences owing to other factors were computed and subpartitioned by conventional analysis of variance techniques. These were tested by approximate F-tests using the effective variance of an entry mean as the denominator of F.

To achieve a balanced set of location comparisons, 6 lots from Kentucky and 12 lots from each of the Washington and California locations consisting of foundation, registered, and certified seed lots were compared. Data on breeder seed from Kentucky and recertified from California were omitted from this analysis to achieve the balanced set.

The only unconfounded comparison of lineages was between the Washington and California produced seed lots of Descent 1 vs. the average of the Descent 2 seed lots produced in Washington and the Descent 3 seed lots produced in California. Therefore, data from seed lots of Descent 1 produced in Kentucky were omitted from the analysis.

TABLE 1.
Stand and bloom percentages, type, regrowth scores, and total dry matter forage yields of Kenstar classes of seed produced in Kentucky, California and Washington and evaluated in Kentucky and Wisconsin.

		Kentucky				Wisconsin	
		Stand	Bloom	Total yield		Type[2]	Regrowth height
Seed class	Lots	June 1983	June 1982	1982	1983	1981	1982
	no.	---------- % ----------		---- Mg ha^{-1} ----			cm
Breeder[3]	3	64.6 a[1]	26.2 c	10.9 a	4.7 ab	2.3 a	41.6 ab
Foundation[4]	19	61.9 ab	31.0 b	10.7 ab	4.6 b	2.5 a	42.9 b
Registered[4]	19	61.6 b	29.8 b	10.7 ab	4.6 b	2.4 a	42.1 b
Certified[4]	19	57.8 c	31.3 a	10.5 c	4.5 bc	2.4 a	42.1 b
Recertified[5]	4	53.5 d	34.0 a	10.4 c	4.4 c	2.5 a	40.6 a

[1]Means with same latter not significantly different according to LSD (P = 0.05); [2]Type scored on a scale of 1 to 5 with 5 = most erect; [3]KY only; [4]Produced in CA and WA; [5]CA only.

Correlation coefficients on entry means were calculated among variables of the two experiments, but only those involving the variables indicated above are presented.

RESULTS

Class of seed

Performance of five classes (breeder, foundation, registered, certified and recertified) of Kenstar seed produced in three locations is presented in Table 1. Stands, forage yields, and regrowth heights of seed lots generally declined and percentage bloom increased from breeder to recertified, although not all differences were significant, particularly those of the middle classes. Some differences were confounded with location inasmuch as breeder seed was produced only at Kentucky and recertified grown only in California. Type scores in Wisconsin were not significantly different.

Location

Performance of seed lots from California was inferior compared with the other two locations as shown by stand (persistence) and dry matter yields in 1983 (Table 1). A shift towards greater blooming of the California lots was also indicated. At Wisconsin, the California seed lots produced more erect and taller plants than did those from Washington.

The location x class interaction was significant for stand and dry matter yield (1982) (Table 2). Performance of the registered class produced in Kentucky was greater than the foundation class, in contrast to a decline for seed produced either in California or Washington.

Lineage

When the Washington and California produced seed lots were evaluated in Kentucky, no significant differences in lineage were detected (Table 3). One lineage-class interaction occurred in the Wisconsin evaluation. Although the difference between Descent 1 and 2 was not significant, a significant interaction occurred when mildew infection was lower in the certified seed lots from Descent 2 than from Descent 1.

Clipping treatment

No significant differences due to clipping before seed production were found during the Kentucky evaluations of Washington and California-produced seed (Table 3). In Wisconsin, the only difference was found in maturity (data not presented). Clipping resulted in slightly later maturity than not clipping. A significant interaction of clipping treatment with seed class occurred in the registered and certified seed classes. Foundation seed lots exposed to clipping variables were not significantly different.

TABLE 2.

Stand and bloom percentages, type scores, regrowth heights, and dry matter forage yields of Kenstar seed lots from Kentucky breeder seed (Descent 1) produced in Kentucky, California and Washington and evaluated in Kentucky and Wisconsin.

			Kentucky				Wisconsin	
			Stand	Bloom	Yield		Type[1]	Regrowth height
Location	Class	Lots			1982	1983	1981	1982
		No.	------- % -------		---- Mg ha^{-1} ----			cm
KY	Found.	2	61.4	30.8	10.5	4.7	2.5	43.4
KY	Regr.	2	65.9	28.5	10.9	5.0	2.3	43.9
KY	Cert.	2	64.3	34.2	10.3	4.6	2.3	43.1
WA	Found.	4	63.7	31.6	10.8	4.7	2.4	42.4
WA	Regr.	4	62.3	27.7	10.6	4.6	2.4	41.9
WA	Cert.	4	58.4	27.7	10.7	4.7	2.4	41.9
CA	Found.	4	59.9	33.3	10.7	4.7	2.8	43.8
CA	Regr.	4	59.4	35.5	10.6	4.5	2.3	42.8
CA	Cert.	4	54.6	34.1	10.5	4.9	2.4	43.2

[1]Scored on a scale of 1 to 5 with 5 = most erect.

TABLE 3.

Stand and bloom percentages, type scores, regrowth heights, and total forage dry matter yields evaluated in Kentucky and Wisconsin of Kenstar seed lots produced in Washington and California averaged by lineage, clipping treatments, and year of seed production.[1]

		Kentucky				Wisconsin	
		Stand	Bloom	Total yield		Type[2]	Regrowth
Seed class	Lots	10 June 1983	23 June 1982	1982	1983	1981	1982
	no.	----------- % -----------		---- Mg ha^{-1} ----			cm
Lineage							
Descent 1	24	59.6 a[3]	31.6 a	10.6 a	4.6 a	2.4 a	42.7 a
Descent 2,3[4]	24	60.1 a	30.1 a	10.8 a	4.8 a	2.4 a	42.0 a
Clip							
No clip	24	60.0 a	31.1 a	10.7 a	4.6 a	2.4 a	41.8 a
Clipped	24	60.0 a	31.6 a	10.7 a	4.8 a	2.4 a	42.8 a
Year							
2nd	24	60.9 a	28.9 b	10.8 a	4.8 a	2.4 a	41.8 b
3rd	24	58.8 b	32.8 a	10.6 b	4.5 b	2.7 a	42.8 a

[1]Comparisons within lineage, clipping treatments, and years of seed production.
[2]Scored on a scale of 1 to 5 with 5 = most erect.
[3]Means within a comparison with the same letter are not significantly different according to LSD (P = 0.05).
[4]Mean of Descent 2 and 3.

TABLE 4.
Simple correlation coefficients among characters measured or scored on Kenstar seed lots evaluated in Kentucky and Wisconsin.

Character	Stand[1] 10 June 1983 (KY)	Bloom[1] 23 June 1982 (KY)	Total yield 1982 (KY)	Total yield 1983 (KY)	Type[2] 1981 (WI)
Bloom June 1983 (KY)	-0.46**				
Total yield 1982 (KY)	0.45**	-0.54**			
Total yield 1983 (KY)	0.60**	-0.50**	0.51**		
Type 1981 (WI)	-0.12	0.22	-0.11	-0.10	
Regrowth height 1982 (WI)	0.08	0.25*	-0.01	0.00	0.34**

*,** Significant at P + 0.05 and 0.01 levels, respectively.
[1]Percent stand and bloom.
[2]Scored on a scale of 1 to 5 with 5 = most erect.

Year of seed production

Seed lots from third-year stands produced lower stands, yields, and more bloom than lots from second-year stands in the Kentucky evaluations (Table 3), indicating the general inferiority of the third-year seed production. Regrowth height was the only significant variable in the Wisconsin evaluation. A significant location by year interaction for regrowth height occurred. The Washington third-year seed lots produced taller plants than did the corresponding California seed lots, as contrasted to the second-year seed lots, which did not differ from the California seed lots.

Correlations

Except for percent bloom with regrowth height, only correlations within locations were significant (Table 4). In Kentucky, stands and dry matter yields were positively correlated, and both were negatively correlated with bloom. In Wisconsin, type and regrowth height data were correlated. Other significant correlations at Wisconsin (not shown) were maturity vs. percentage of plants with leaf mark (r = 0.329) and anthracnose resistance with regrowth height (r = 0.293).

Other characters

Powdery mildew and northern anthracnose resistances, percentage of plants with leafmark, and maturity, all scored in Wisconsin, could not be related to class of seed, location of seed increase, lineage, clipping treatment, or year of seed production.

DISCUSSION

The results of these experiments on Kenstar generally agree with the earlier experiment on Kenland red clover (Taylor *et al.*, 1979). Although Kenstar is more narrowly bred then Kenland (Taylor and Anderson, 1973), the same conclusions may be formulated. Yield and stand generally declined from breeder to certified seed and even further to the recertified seed class. This corroborates the earlier decision to eliminate the registered seed class and to prohibit recertification from certified seed. The smallest decline in performance outside the area of forage use occurred in Washington, which also justifies the decision to limit seed increases to areas north of approximately 40°N latitude (Taylor *et al.*, 1979). No differences in lineage effects were associated with any of the variables of the study. Likewise, clipping the first forage growth had no effect on longevity or forage yields of progeny from any of the seed-increase locations. However, the detrimental effect of third-year seed production as compared with second-year seed production was demonstrated in progeny from seed produced in Washington and California. This effect probably is a result of genetic shift, and the same factors that cause reduction of performance in advanced generations are probably involved (Taylor *et al.*, 1979).

The negative correlation of bloom with survival and forage yield substantiates earlier evidence (Taylor, Dade and Garrison, 1966; Taylor, Kendall and Stroube, 1968) that the decline in performance is associated with shifts in flowering response. The earlier types produce the most seed but are less persistent. These shifts are smaller if seed lots are produced in the northern location. The clipping practice apparently did not overcome this problem in California. The data on regrowth height at Wisconsin substantiated the Kentucky data on genetic shifts. Regrowth height at Wisconsin was correlated with percent bloom at Kentucky which suggests that ability to regrow rapidly is also associated with decline in performance.

In conclusion, the Washington location is recommended for seed increase because less genetic shift occurs than at the California and Kentucky locations. Clipping and lineage treatments did not affect stand or yield of progeny. These data indicate the present programme (Taylor *et al.*, 1979) is adequate in all respects except that further consideration should be given to the decline in performance of progeny of third-year seed compared with second-year increases.

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