AN ABSTRACT OF THE THESIS OF

Clarence Ellis Watson, Jr. for the degree of Doctor of Philosophy in Agronomic Crop Science presented on June 28, 1976

Title: HERITABILITY FOR PERLOLINE, NITROGEN, AND DIGESTIBILITY CHARACTERISTICS IN TALL FESCUE (FESTUCA ARUNDINACEA, SCHREB.) SINGLE-CROSSES GROWN IN TWO LOCATIONS

Abstract approved: Redacted for Privacy
Dr. R. V. Frakes

Tall fescue often results in poor liveweight gains. This may be accounted for by variations in perloline content, nitrogen content, or digestibility of the plant. Two groups of tall fescue plants were examined for each of these traits. These groups consisted of (a) fifteen single-crosses of a six parent diallel and (b) seven cultivars of tall fescue.

The diallel cross was planted at Corvallis, Oregon and Columbia, Missouri. Plots were harvested and sampled on April 8 and September 22, 1975 at Corvallis, Oregon and on October 16, 1975 at Columbia, Missouri. The two cuttings at Corvallis, Oregon and the fall cuttings at Corvallis and Columbia were analyzed as multiple environments. The cultivars were planted at Corvallis, Oregon and harvested April 10, July 11, and September 22, 1975. All samples were analyzed for perloline and nitrogen content. Samples from the fall harvests at Corvallis, Oregon and Columbia, Missouri were analyzed for *in vitro* digestibility.

There were significant differences among crosses for perloline content. Significant general and specific combining ability was

found for perloline content. Broad-sense heritabilities ($H_{B.\ S.}$ = .21–.94) tended to be larger than narrow-sense heritabilities ($H_{N.\ S.}$ = .15–.53). There appeared to be a high degree of dominance for low perloline. Significant differences existed among the cultivars for perloline content. Dates of harvest were a significant source of variation for perloline content, with perloline increasing steadily over the season. Significant genotype x environment interactions were found, suggesting that lines should be tested over locations and years. It should be possible to select for low perloline lines with a program of recurrent selection using a high perloline tester.

Significant differences were found among crosses for nitrogen content. General combining ability was significant in all cases. Narrow-sense heritabilities ($H_{N.\ S.}$ = .50–.72) were almost as large as the broad-sense heritabilities ($H_{B.\ S.}$ = .55–.89), suggesting primarily additive gene action for nitrogen content. No differences were found among the cultivars for nitrogen content, but cuttings were a significant source of variation. Differences among cuttings as well as the cutting x cultivar interaction appeared to be due to soil fertility levels and disease. Selection for nitrogen content should be possible with recurrent selection. Lines should be tested over environments.

There were differences in <u>in vitro</u> digestibility among crosses for the September harvest at Corvallis, Oregon, but not for the October harvest at Columbia, Missouri. General combining ability was a significant source of variation. The broad-sense heritability

($H_{B.\ S.}$ = .74) was high. The narrow-sense heritability ($H_{N.\ S.}$ = .18) was very low, however, indicating that in vitro digestibility was controlled primarily by non-additive gene action. Digestibilities were significantly higher at Corvallis, Oregon than at Columbia, Missouri. No genotype x environment interaction was found. Selection for high in vitro digestibility should be possible with a program of recurrent selection for specific combining ability.

# Heritability for Perloline, Nitrogen, and Digestibility Characteristics in Tall Fescue (*Festuca arundinacea*, Schreb.) Single-Crosses grown in two locations

by

Clarence Ellis Watson, Jr.

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1977

APPROVED:

Redacted for Privacy

Professor of Plant Breeding
in charge of major

Redacted for Privacy

Head of Department, Agronomic Crop Science

Redacted for Privacy

Dean of Graduate School

Date thesis is presented June 28, 1976

Typed by Alice C. Watson for Clarence Ellis Watson, Jr.

ACKNOWLEDGEMENTS

The author wishes to thank his major professor, Dr. R. V. Frakes, for his guidance during the course of study and preparation of the manuscript. Thanks are extended to Dr. D. O. Chilcote for making laboratory space available to the author and for serving as a member of the committee. The author wishes to express his gratitude to Dr. H. J. Jenson, Dr. W. W. Chilcote, and Mr. J. W. Johnson for their interest in the project and for serving as members of the graduate committee.

The author would like to express his appreciation to Mr. W. T. W. Woodward, Mr. J. K. Beeson, and Dr. M. Hile who aided in the collection and analysis of samples.

Sincerest appreciation is extended to Dr. D. A. Sleper, University of Missouri, Columbia, Missouri, for the collection of samples at Columbia, and to Dr. A. G. Matches, ARS-USDA, University of Missouri, Columbia, Missouri, for providing the digestibility data.

Thanks are extended to Dr. S. G. Yates, ARS-USDA, Northern Regional Research Laboratory, Peoria, Illinois, for providing the perloline monohydrochloride used in the preparation of standards.

The author wishes to extend his deepest appreciation to his wife, Alice, for her encouragement and patience during the course of study and for typing the manuscript.

TABLE OF CONTENTS

LIST OF TABLES

Appendix Table

# HERITABILITY FOR PERLOLINE, NITROGEN, AND DIGESTIBILITY CHARACTERISTICS IN TALL FESCUE (*FESTUCA ARUNDINACEA*, SCHREB.) SINGLE-CROSSES GROWN IN TWO LOCATIONS

## INTRODUCTION

Tall fescue is a vigorous, high yielding, cool season grass, widely used as a forage crop. Poor animal performance is not uncommon in animals fed tall fescue as hay or pasture. Vigorous, high yielding tall fescues have, on occasion, shown significantly lower liveweight gains than other grasses with similar nutrient content.

Tall fescue contains alkaloids among which is perloline, which has been shown to be physiologically active in ruminants. Perloline is reported to have considerable genetic and environmental variation. Variation in perloline content may account for the unpredictable occurences of poor animal performance on tall fescue.

Nitrogen content is a measure of the amount of crude protein in tall fescue. Variation in the protein content could also be an explanation for poor animal performance. The nitrogen content of tall fescue is reported to be positively correlated with the perloline content.

Digestibility is reported to be related to liveweight gain and dry matter intake. Digestibility shows both genetic and environmental variation in some cool season grasses. This variation could account for the occurence of poor animal performance in tall fescue.

The objectives of this study were:

1. To determine if there were significant differences among genotypes and cultivars of tall fescue for perloline content,

nitrogen content, and *in vitro* digestibility.

2. To determine the type of gene action controlling each of the traits.
3. To estimate the broad-sense and narrow-sense heritabilities of each trait.
4. To determine if environmental variation, represented by cuttings and locations, influenced the perloline content, nitrogen content, and digestibility.
5. To determine if there was a significant genotype x environment interaction for each of the three characteristics.

# LITERATURE REVIEW

Perloline is a major alkaloid of tall fescue (Festuca arundinacea, Schreb.) and ryegrass (Lolium spp.)(16, 29, 32). It has also been detected in high concentration in Setaria lutescens and meadow fescue (Festuca elatior) (12, 16). It occurs in trace amounts in timothy (Phleum pratense) and orchardgrass (Dactylis glomerata)(16, 35).

Perloline has been shown to be physiologically active in animals and may account for poor animal performance on tall fescue (3, 11, 13, 15). Perloline has been demonstrated to inhibit ruminal cellulose digestion and volatile fatty acid production (11, 13, 15). High concentrations of perloline can kill the rumen microflora (11, 19). Bush and Buckner (13) found that the seasonal distribution of perloline in tall fescue corresponded to the time of poor animal performance. Boling et al. (15) compared the effects of two diets, varying only in perloline content, on lambs. One diet contained no perloline and the other contained 0.5% added perloline monohydrochloride. The lambs fed the diet with perloline showed lower crude protein and cellulose digestibility than those on the control diet. The digestibility of crude fiber, nitrogen-free extract, ether extract, and ash also tended to be lower in lambs on the perloline diet, although not significantly lower. Lambs on the perloline diet had a larger urine volume and more urinary nitrogen than lambs on the control diet. Lambs fed added perloline retained 0.44 g of nitrogen per day while the lambs fed the control retained 0.94 g. Urea nitrogen in the blood plasma tended to be lower with the perloline group. The concentrations of acetic,

isovaleric, and valeric acids were reduced and the concentration of propionic acid was higher with the perloline diet. The body temperature of the lambs on the perloline diet was higher than that of those on the control diet during the last two days of the trial. The hypothesis was that this added heat load caused reduced intake and lower weight gains. Bush and Buckner (13) have estimated that a large lactating cow could consume up to 50-100 g of perloline per day during periods of high perloline accumulation in the plant.

Yates (41) reported that perloline might be a factor in the development of fescue foot in cattle. It was noted that the worst incidence of fescue foot occurred at the time of highest perloline accumulation in the plant. Poor animal performance is reported to be accompanied by symptoms similar to fescue foot, such as diarrhea, rapid respiration rate, rough hair coat, and high rectal temperatures (13). Jacobson et al. (30) have stated that poor animal performance may be due to a subclinical toxicity. Fescue foot produces symptoms similar to ergot poisoning (13, 39, 41). Wilson (39) reported that fescue and ergot extracts were physiologically active in mice, while ryegrass extracts were not active. This would seem to be contradictory if perloline is an agent in the development of fescue foot, since the major alkaloids of ryegrass and tall fescue are identical (42). Yates (42) further noted that the alkaloids of toxic fescue hay, fresh toxic fescue, and non-toxic fescue hay were identical.

Tall fescue contains at least eleven alkaloids, including perloline, perlolidine, festucine, and several pyrrolizidine alkaloids (13,

26, 42, 43). Bush and Buckner (13) reported that several of these alkaloids are physiologically active and they suggested that alkaloids other than perloline should be considered in assessing tall fescue toxicity.

Perloline has been shown to have an inhibitory effect on plant growth (22, 23). Fejer (22) found that perloline and indole acetic acid inhibited top growth of ryegrass in a similar manner. Perloline was also found to inhibit root growth of perennial ryegrass (23). Butler (16) found that high perloline clones of perennial ryegrass tended to be high yielding and vigorous which disagrees with the inhibition theory.

Bush et al. (14) reported that perloline accumulations were greatest in the leaves and lowest in the seed of mature tall fescue plants. In seedlings, more perloline accumulated in the roots than the leaves. Gentry et al. (26) found that the concentration of perloline was greater in stems than leaves of mature tall fescue plants. They also reported that the concentration of perloline decreased as the plant aged.

There is seasonal variation in the perloline content of tall fescue and ryegrass (12, 13, 16, 26, 29, 32, 35). The perloline content is usually highest during periods of rapid growth (29, 32, 35). Bush and Buckner (12) noted that the perloline content of tall fescue was low and showed little variation in the spring, high in July and August, and declined during the fall. Secondary patterns within this were attributed to variation in rainfall. Reifer and Bathurst (35)

found that rapid changes in the weather resulted in drastic changes in the perloline content of ryegrass. They stated that no single environmental factor could account for the variation in the perloline content.

Perloline content is influenced by the fertility status of the soil (2, 12, 13, 14, 26, 35). The concentration of perloline in the plant increases with increasing nitrogen fertilization. Bush and Buckner (12) found that nitrates resulted in a greater increase in perloline than did urea. Bennett (2) reported that the effect of added nitrate on the perloline content of ryegrass was of comparable magnitude to genetic effect. It was further noted that added phosphorous had no effect on the perloline content. Gentry et al. (26) found that both phosphorous and potassium lowered the perloline content of tall fescue.

Variation in perloline content among genotypes of ryegrass has been reported (16, 32). Gentry et al. (26) found differences in perloline content among and within varieties of tall fescue. Cornelius et al. (18) found differences among $F_1$ progenies of Lolium x Festuca hybrids. Perloline content in these hybrids was controlled mainly by non-additive gene action although there was significant general combining ability. Perloline content in Lolium x Festuca hybrids is reported to be controlled by relatively few genes, showing a high degree of dominance for low perloline (8, 9, 10, 18). Butler (16) noted that tetraploid ryegrass generally had more perloline than diploid ryegrass. Broad-sense heritability estimates for perloline content in Lolium x Festuca hybrids range from 0.57 to 0.98 (9, 12,

15). Parent-progeny correlations are large and positive (9, 15). It is suggested that selection for low perloline lines should be possible (16, 18). Cornelius et al. (18) suggest that a high perloline tester be used for selection since high perloline appears to be recessive.

Bush et al. (15) reported a significant progenies x years interaction for perloline content in Lolium x Festuca hybrids. They stated that although the interaction was significant, it should not interfere with selection for high or low perloline content.

A second factor to be considered in assessing poor animal performance is the nitrogen content. Percent nitrogen is used as a measure of the crude protein content of forages. Tall fescue is reported to be low in crude protein among the forage grasses (13, 24, 33). Crude protein or percent nitrogen is negatively correlated to percent soluble carbohydrates (7, 17, 28, 31). Soluble carbohydrates account for the major differences in digestibility among forage species (24). Jones (31) found that selection of lines high in crude protein resulted in lines low in soluble carbohydrates. Grimes et al. (28) reported that crude protein was negatively correlated with digestibility, soluble carbohydrate content, and dry matter intake of forage grasses. There was no correlation between crude protein and liveweight gain. Bennett (2) found that the nitrogen content of perennial ryegrass was positively correlated with the perloline content.

Environment can affect the nitrogen content of tall fescue. Miles et al. (33) found that the nitrogen content of tall fescue differed among harvest dates. The lowest nitrogen levels were found

during the middle of the growing season. Differences in nitrogen content of tall fescue over the growing season were not due to temperature changes (21). Nitrogen levels in tall fescue have been shown to decrease with aging of the plant (6).

The nitrogen content of tall fescue increases with increasing nitrogen fertilization (21, 31). Duncan et al. (21) was unable to find significant differences in the nitrate content of tall fescue plants before the application of nitrogen fertilizer.

Significant differences in nitrogen content among cultivars of tall fescue have been reported (17, 33). Cooper (17) found that nitrogen content in ryegrass and orchardgrass was controlled by additive gene action. Heritabilities for nitrogen content ranged from 0.20 to 0.75 for ryegrass and from 0.53 to 0.69 for orchardgrass.

Miles et al. (33) found a significant genotype x environment interaction for nitrogen content in tall fescue. There was no consistency in the ranking of cultivars from cut to cut.

Differences in digestibility may also account for differences in animal performance on tall fescue. Tall fescue has been reported to have digestibilities comparable to other forage grasses (13, 30). Digestibility is important since it is reported to be positively correlated with dry matter intake and liveweight gain (28). Digestibility of forage grasses is positively associated with the soluble carbohydrate content (24, 28, 30). It is thought that the variation in soluble carbohydrate content accounts for the major differences in digestibility among forage grasses (24). The digestibility of tall

fescue is influenced by the perloline content (3, 11, 13, 15). Grimes et al. (28) reported that digestibility was negatively correlated with nitrogen content.

Jones (31) reported that most of the variation in digestibility among cultivars of forage grasses could be accounted for by growth stage and morphology. Brown et al. (6) noted that the digestibility of tall fescue did not decrease with age of the plant.

Digestibility is greatly influenced by the environment in which the plant is growing (1, 20, 33, 40). Digestibility of tall fescue declines as temperature increases (1, 20, 40). Allinson (1) reported that long days resulted in lower digestibility in tall fescue. Miles et al. (33) found differences among cutting dates for digestibility of tall fescue. Lowest digestibilities were found in the middle of the season.

Miles et al. (33) found significant differences in digestibility among six cultivars of tall fescue. Cooper (17) reported differences in digestibility among genotypes of both ryegrass and orchardgrass. Heritability estimates for digestibility in orchardgrass ranged from 0.52 to 0.53, while estimates for ryegrass were low and non-significant. Breese and Davis (4) have successfully selected for higher digestibility in orchardgrass.

A genotype x environment interaction has been reported for digestibility in forage grasses (33). Cultivars showed no consistency in ranking from cutting to cutting.

# MATERIALS AND METHODS

The experimental material consisted of two groups of tall fescue plants. One group included fifteen single-crosses of a six-parent diallel and the other group included seven cultivars of commercially available cultivars and experimental lines from Oregon, Kentucky, and Missouri.

Single-cross seed was obtained by bagging panicles of the parental genotypes together in all possible combinations of two, prior to anthesis. The fifteen single-crosses were planted in a randomized complete block design with four replications. Each plot consisted of a single row of fifteen plants. Identical material was planted at Corvallis, Oregon and Columbia, Missouri.

The seven cultivars were planted in a randomized complete block design with four replications. Each plot consisted of five rows, fourteen feet long and six inches apart.

All fields at Corvallis, Oregon were fertilized with 112.5 kg/ha of nitrogen on March 31, 1975. Fields were fertilized again with 70.6, 89.6, and 90.0 kg/ha on July 16, September 24, and October 20, 1975, respectively. Fields were irrigated following each harvest and fertilization.

Plots from two replications of each set of plant material were harvested and a 50.0 g (dry weight) sample was taken. The single-crosses were sampled on April 8 and September 22, 1975 at Corvallis, Oregon and on October 16, 1975 at Columbia, Missouri. The cultivars were sampled on April 10, July 11, and September 22, 1975. All

samples were dried in a force air oven and ground in a Wiley mill to pass through a 1.0 mm screen. All samples were analyzed for perloline and nitrogen content. Only the single-crosses from the September harvest at Corvallis, Oregon and the October harvest at Columbia, Missouri were analyzed for *in vitro* digestibility.

Perloline was measured using a technique described by Shaffer et al. (37). Perloline was extracted from the tall fescue samples with 50% ethanol and isolated on a cation exchange resin. Perloline content of the samples was determined with a spectrofluorometer and compared to a standard curve. Purified perloline used in the preparation of standards was provided by S. G. Yates, ARS-USDA, Northern Regional Research Laboratory, Peoria, Illinois.

Nitrogen content was determined by microkjeldahl analysis. Samples were digested as described by Nelson and Sommers (34). The digested samples were distilled by the procedure of Brimner and Edwards (5). Ammoniacal nitrogen was determined by titration.

The procedure of Tilley and Terry (38) was used to determine *in vitro* digestibility. Digestibility data were provided by A. G. Matches, ARS-USDA, University of Missouri, Columbia, Missouri.

The single-crosses were analyzed as a randomized complete block for each sampling date and location. The two cuttings at Corvallis, Oregon were considered as a multiple environment with cuttings as environments. The September cutting at Corvallis, Oregon and the October cutting at Columbia, Missouri were also considered as a multiple environment with locations as environments. The multiple environments were analyzed as split-plot designs with environments as

main plots. The cultivars were analyzed as a split-plot design with cuttings as main plots.

Diallel analysis was performed on both single and multiple environment data according to method four, model two, of Griffing (27). For multiple environment data, the model of Rojas and Sprague (36) was used for the estimation of combining ability x environment interactions. Broad-sense and narrow-sense heritabilities were estimated from the variance components of general and specific combining ability according to Gardner (25).

# RESULTS AND DISCUSSION

The analysis of variance revealed significant differences among crosses for perloline content, for the April and September harvests at Corvallis, Oregon, but not for the October harvest at Columbia, Missouri (Table 1). There was significant general combining ability for both harvest dates in Oregon as well as significant specific combining ability for the September harvest. Means for general combining ability (mean of all single-crosses involving a single line) ranged from 6.3 µg/g for line A59-90 to 33.1 µg/g for line V8-696 in April at Corvallis, Oregon (Table 2). In September general combining ability means ranged from 219.9 µg/g to 498.1 µg/g with A59-90 being the low line and V8-696 the high line. All lines demonstrated specific combining ability. Lines V2-248 and V8-696 demonstrated the widest ranges in perloline content among their single-cross progenies. In September any cross involving A59-90 tended to be low in perloline, indicating dominance for low perloline. This agrees with published reports that there is a high degree of dominance for low perloline (8, 9, 10, 18). Since low perloline appears to be dominant it has been suggested that a high perloline tester should be used to select for low perloline lines. Parent V8-696 appears to be such a line. It had the highest perloline content in April and September. Single-crosses involving V8-696 tended to reflect the genotype of the other parent.

The analysis of variance for multiple environments revealed significant differences between environments in both cases (Table 3).

Table 1. Analysis of variance and combining ability analysis for perloline content of fifteen single-crosses in each of three environments in 1975.

| Source | df | Mean Square | | |
|---|---|---|---|---|
| | | Oregon, Apr. 8 | Oregon, Sept. 22 | Missouri, Oct. 16 |
| Replications | 1 | 158.88 | 5728.11 | 1530.82 |
| Crosses | 14 | 703.82* | 81166.00** | 1849.71 |
| GCA | 5 | 1348.67** | 149943.54** | —— |
| SCA | 9 | 345.61 | 42956.28** | —— |
| Error | 14 | 251.41 | 8367.11 | 858.44 † |

*, ** Significant at the 5 and 1% levels of probability, respectively.

† Error degrees of freedom = 13.

Table 2. Perloline content (μg/g) of fifteen single-crosses grown at Corvallis, Oregon and harvested April 8 (upper diagonal) and September 22, 1975 (lower diagonal).

| | Parent | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Mean |
| A59-90 | ——— | 3.0 | 3.0 | 12.5 | 3.0 | 10.2 | 6.3 |
| B5-62 | 221.9 | ——— | 2.5 | 15.6 | 10.0 | 33.3 | 12.9 |
| B17-9 | 240.1 | 497.7 | ——— | 8.1 | 7.0 | 20.9 | 8.3 |
| V2-248 | 170.8 | 460.9 | 348.2 | ——— | 5.0 | 74.8 | 23.2 |
| V2-448 | 133.3 | 490.6 | 236.7 | 275.0 | ——— | 26.3 | 10.3 |
| V8-696 | 333.6 | 664.1 | 257.8 | 886.2 | 349.0 | ——— | 33.1 |
| Mean | 219.9 | 467.0 | 316.1 | 428.2 | 296.9 | 498.1 | |

Table 3. Analysis of variance and combining ability analysis for perloline content for two multiple environments.

| Source | df | Mean Square | |
|---|---|---|---|
| | | Oregon (2 cuttings) | Oregon-Missouri |
| Replications | 1 | 3898 | 6591 |
| Environments (E) | 1 | 1894450* | 1495020* |
| Error (A) | 1 | 1989 | 668 |
| Crosses (C) | 14 | 46969** | 45163** |
| GCA | 5 | 87140** | 87085** |
| SCA | 9 | 24651** | 21873** |
| E x C | 14 | 34900** | 37853** |
| E x GCA | 5 | 64152** | 66503** |
| E x SCA | 9 | 18651** | 21936** |
| Error (B) | 28 | 4309 | 4752 † |

*, ** Significant at the 5 and 1% levels of probability, respectively.

† Error (B) degrees of freedom = 27.

In the first multiple environment with cuttings as environments, the mean perloline content was 15.7 µg/g in April and 371.1 µg/g in September (Table 2). In the second multiple environment with locations as environments, the mean perloline content was 371.1 µg/g for Corvallis, Oregon and 53.4 µg/g for Columbia, Missouri (Table 4). Environmental variation was large in both instances.

Significant differences were found among crosses for each multiple environment experiment (Table 3). General and specific combining ability were significant sources of variation in both cases. In the experiment using cuttings as environments, general combining ability means ranged from 113.1 µg/g for line A59-90 to 265.6 µg/g for line V8-696 (Table 5). All lines demonstrated some specific combining ability with lines V2-248 and V8-696 showing the widest ranges in perloline content among their progenies. In the study with locations as environments, general combining ability means ranged from 129.7 µg/g for A59-90 to 355.5 µg/g for V8-696 (Table 6). All lines exhibited some specific combining ability with lines V2-248 and V8-696 showing the greatest amount of specific combining ability. In both experiments the performance of line A59-90 indicated a high degree of dominance for low perloline. Lines V2-248 and V8-696 were both high perloline lines and their single-cross progenies tended to reflect the genotype of the lines they were crossed to in both experiments. Each of these lines appeared to carry many recessive genes for high perloline with V8-696 having more than V2-248.

The environments x crosses interaction was significant for both

Table 4. Peroline content (μg/g) of fifteen single-crosses harvested September 22, 1975 at Corvallis, Oregon (upper diagonal) and October 16, 1975 at Columbia, Missouri (lower diagonal).

| | Parent | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Mean |
| A59-90 | ——— | 221.9 | 240.1 | 170.8 | 133.3 | 333.6 | 219.9 |
| B5-62 | 34.4 | ——— | 497.7 | 460.9 | 490.6 | 664.1 | 467.0 |
| B17-9 | 81.3 | 50.0 | ——— | 348.2 | 236.7 | 257.8 | 316.1 |
| V2-248 | 29.7 | 54.7 | 68.8 | ——— | 275.0 | 886.2 | 428.2 |
| V2-448 | 14.1 | 52.2 | 70.3 | 21.9 | ——— | 349.0 | 296.9 |
| V8-696 | 37.5 | 90.6 | 128.1 | 70.3 | 26.6 | ——— | 498.1 |
| Mean | 39.4 | 56.4 | 79.7 | 49.1 | 37.0 | 70.6 | |

Table 5. Perloline content (µg/g) of the fifteen single-crosses averaged over the two cuttings at Corvallis, Oregon.

| | Parent | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Mean |
| A59-90 | ——— | 112.4 | 121.5 | 91.7 | 68.2 | 171.9 | 113.1 |
| B5-62 | | ——— | 250.1 | 238.3 | 250.3 | 348.7 | 240.0 |
| B17-9 | | | ——— | 178.2 | 121.9 | 139.4 | 162.1 |
| V2-248 | | | | ——— | 140.0 | 480.5 | 225.7 |
| V2-448 | | | | | ——— | 187.6 | 153.6 |
| V8-696 | | | | | | ——— | 265.6 |

Table 6. Perloline content (μg/g) of the fifteen single-crosses for the fall cutting, averaged over the two locations.

| | Parent | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Mean |
| A59-90 | ——— | 128.1 | 160.7 | 100.3 | 73.7 | 185.5 | 129.7 |
| B5-62 | | ——— | 273.8 | 257.8 | 271.4 | 377.3 | 261.7 |
| B17-9 | | | ——— | 208.5 | 153.5 | 193.0 | 197.9 |
| V2-248 | | | | ——— | 148.4 | 478.3 | 238.7 |
| V2-448 | | | | | ——— | 187.8 | 167.0 |
| V8-696 | | | | | | ——— | 355.5 |

multiple environment studies. Both general combining ability x environments and specific combining ability x environments interactions were significant in each case. In the experiment with cuttings as environments, the general combining ability x environments interaction is due mainly to the performance of line B5-62. This line moved from third to second in ranking from April to September at Corvallis, Oregon (Table 2). Although the change in ranking is not great, the magnitude of change in perloline content needed to cause the change in ranking is large. The specific combining ability x environments interaction cannot be attributed to the performance of any one line. Most lines exhibited some change in specific combining ability across cuttings. The most dramatic change was that of cross B5-62 x B17-9. This cross changed from the lowest ranking cross in April to the third highest cross in September. In the study with locations as environments the general combining ability x environments interaction is due primarily to the performance of line B17-9. This line ranked fourth in perloline content at Corvallis, Oregon and ranked first at Columbia, Missouri (Table 4). Again, the specific combining ability x environments interaction cannot be attributed to the performance of any single line. One of the more notable changes was that of cross V2-248 x V8-696. This was the highest ranking cross at Corvallis, Oregon, but was only intermediate at Columbia, Missouri.

Broad-sense heritabilities for perloline content in the two single environments were high with narrow-sense heritabilities only half as large (Table 7). The broad-sense estimates for single

Table 7. Broad-sense ($H_{B.S.}$) and narrow-sense heritabilities ($H_{N.S.}$) for perloline and nitrogen content for three single environments and two multiple environments.

| Environment | Perloline | | Nitrogen | |
|---|---|---|---|---|
| | $H_{B.S.}$ | $H_{N.S.}$ | $H_{B.S.}$ | $H_{N.S.}$ |
| Oregon, Apr. 8 | .73 | .53 | .79 | .62 |
| Oregon, Sept. 22 | .94 | .41 | .79 | .68 |
| Missouri, Oct. 16 | ns† | ns | .89 | .50 |
| Oregon, Apr. 8 and Sept. 22 | .37 | .15 | .76 | .43 |
| Oregon-Missouri, Sept. 22 and Oct. 16 | .21 | .21 | .55 | .72 |

† ns = non-significant.

environments are comparable to those reported in the literature (9, 12, 15). The broad-sense heritabilities for the multiple environments were low. The narrow-sense heritability for the multiple environment with cuttings as environments was only half as large as the broad-sense estimate. In the multiple environment with locations as environments, the narrow-sense heritability was equal to the broad-sense estimate. This was the only case where it appeared that perloline content was controlled by additive gene action, which does not agree with the other estimates. Data from the single environments and the multiple environment with cuttings as environments indicated a large amount of non-additive gene action for perloline content, but there was significant additivity present. This additivity may be due to dominant genes which act in an additive fashion. The more loci with a dominant allele present, the greater the perloline content. Selection for low perloline lines should be possible by using a program of recurrent selection with a high perloline tester.

Analysis of the cultivars revealed differences among cultivars and cuttings, as well as a significant cutting x cultivar interaction (Table 8). Cultivars ranged from a low of 34.5 µg/g for Missouri experimental, Hunch I, to a high of 108.0 µg/g for Alta (Table 9). Fawn and Alta, cultivars originating from Oregon, were the highest in perloline. Kenhy, a Lolium x Festuca hybrid, was intermediate in perloline content.

There was a significant increase in the perloline content each cutting (Table 9). This pattern does not agree with that reported by Bush and Buckner (13). They reported that perloline reached its

Table 8. Analysis of variance for nitrogen and perloline content of seven cultivars of tall fescue, harvested April 10, July 11, and September 22, 1975.

| Source | df | Mean Square | |
|---|---|---|---|
| | | Nitrogen | Perloline |
| Replications | 1 | 0.0309 | 628.7 |
| Cuts | 2 | 15.3372** | 39866.8** |
| Error (A) | 2 | 0.0672 | 92.5 |
| Cultivars | 6 | 0.0447 | 4055.6* |
| Cuts x Cultivars | 12 | 0.0721* | 3172.7* |
| Error (B) | 18 | 0.0254 | 1199.0 |

*, ** Significant at the 5 and 1% levels of probability, respectively.

Table 9. Perloline content (μg/g) of seven cultivars for the harvests of April 10, July 11, and September 22, 1975.

| Cultivar | Harvests | | | Mean |
|---|---|---|---|---|
| | April 10 | July 11 | September 22 | |
| Alta | 12.4 | 187.5 | 124.2 | 108.0 |
| TFM | 1.6 | 106.3 | 82.8 | 63.6 |
| Ky 37G1-309 | 9.2 | 46.1 | 135.2 | 63.5 |
| Kenmont | 2.7 | 16.4 | 104.7 | 41.3 |
| Kenhy | 16.4 | 133.6 | 84.4 | 78.1 |
| Hunch I | 6.6 | 42.2 | 54.7 | 34.5 |
| Fawn | 6.7 | 85.9 | 176.6 | 89.7 |
| Mean | 7.9 | 88.3 | 108.9 | 68.4 |

highest levels in mid-summer and declined in the fall in Kentucky. In this experiment perloline content was highest in the fall. There are several possible explanations for this discrepancy. Nitrogen fertilizer was not applied after the spring harvest. The nitrogen contents of the cultivars dropped in July to levels about one-third of those in April. Nitrogen was applied following the July harvest, but the nitrogen contents rose only slightly by September. Low nitrogen may have greatly reduced the perloline content since nitrogen is a necessary substrate for perloline formation. The failure of the nitrogen contents for September to equal those of April may have been due to low soil nitrogen levels or due to an epidemic of crown rust which occurred in September. Free water on the leaves coupled with cool temperatures resulted in the rust epidemic which affected some cultivars worse than others. Temperature patterns may also account for the discrepancy between these data and those reported for Kentucky. Perloline was highest in Kentucky during the period of highest temperatures. In Oregon, in 1975, the warmest period was approximately early September. If perloline content is influenced by temperature, then the two studies may not be in conflict.

The cutting x cultivar interaction can be seen in the performance of the cultivars, TFM, Kenhy, and Fawn (Table 9). Oregon experimental, TFM, which had the lowest perloline content of any cultivar in April, was the third highest cultivar in July and the sixth ranking cultivar in September. This cultivar was a winter-active line. TFM had its lowest perloline content during April when it was growing most active-

ly. It became very dormant following the April cutting and the perloline content rose during this period. This disagrees with reports that perloline content is highest during periods of rapid growth (29, 32, 35). It would appear that temperature or photoperiod were more important than growth rate in determining the perloline content. The cultivar, Kenhy, showed a steady decline in ranking over the season. It was the highest cultivar in April, second in July, and fourth in September. Fawn, which ranked fourth in both April and July, was the highest entry in September. The rust epidemic may have influenced the perloline content in September, resulting in a significant interaction. Fawn and Alta, which were most susceptible to rust, ranked first and third, respectively, in September. Hunch I and TFM, which had the highest levels of resistance to rust, were the lowest ranking cultivars in September. Possibly infection by Puccinia coronata may have resulted in higher levels of perloline.

The analysis of variance revealed significant differences among crosses for nitrogen content in each of the three environments (Table 10). There was significant general combining ability for nitrogen content in all cases. Means for general combining ability ranged from 2.80% to 3.09% for the April cutting at Oregon and from 3.45% to 3.68% for the September cutting (Table 11). General combining ability means varied from a low of 2.09% to a high of 2.40% for nitrogen content at Columbia, Missouri in October 1975 (Table 12). Line B5-62 had the lowest general combining ability in each environment.

Table 10. Analysis of variance and combining ability analysis for nitrogen content of fifteen single-crosses in each of three environments in 1975.

| Source | df | Mean Square | | |
|---|---|---|---|---|
| | | Oregon, Apr. 8 | Oregon, Sept. 22 | Missouri, Oct. 16 |
| Replications | 1 | 0.0282 | 0.1281** | 0.1968** |
| Crosses | 14 | 0.0569* | 0.0402* | 0.0764** |
| GCA | 5 | 0.1183** | 0.0922** | 0.1509** |
| SCA | 9 | 0.0226 | 0.0149 | 0.0350 |
| Error | 14 | 0.0160 | 0.0118 | 0.0131† |

*, ** Significant at the 5 and 1% levels of probability, respectively.

† Error degrees of freedom = 13.

Table 11. Nitrogen content (%) of fifteen single-crosses grown at Corvallis, Oregon and harvested April 8 (upper diagonal) and September 22, 1975 (lower diagonal).

| | Parent | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Mean |
| A59-90 | ——— | 2.84 | 3.11 | 2.96 | 3.20 | 3.00 | 3.02 |
| B5-62 | 3.33 | ——— | 2.94 | 2.70 | 2.77 | 2.77 | 2.80 |
| B17-9 | 3.51 | 3.46 | ——— | 3.15 | 3.02 | 3.26 | 3.09 |
| V2-248 | 3.48 | 3.37 | 3.70 | ——— | 3.13 | 3.06 | 3.00 |
| V2-448 | 3.69 | 3.65 | 3.73 | 3.78 | ——— | 2.90 | 3.00 |
| V8-696 | 3.42 | 3.43 | 3.67 | 3.52 | 3.55 | ——— | 3.00 |
| Mean | 3.48 | 3.45 | 3.61 | 3.57 | 3.68 | 3.52 | |

Table 12. Nitrogen content (%) of fifteen single-crosses harvested September 22, 1975 at Corvallis, Oregon (upper diagonal) and October 16, 1975 at Columbia, Missouri (lower diagonal).

| | Parent | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Mean |
| A59-90 | ——— | 3.33 | 3.51 | 3.48 | 3.69 | 3.42 | 3.48 |
| B5-62 | 1.83 | ——— | 3.46 | 3.37 | 3.65 | 3.43 | 3.45 |
| B17-9 | 2.27 | 2.32 | ——— | 3.70 | 3.73 | 3.67 | 3.61 |
| V2-248 | 2.15 | 2.10 | 2.32 | ——— | 3.78 | 3.52 | 3.57 |
| V2-448 | 2.40 | 2.15 | 2.29 | 2.38 | ——— | 3.55 | 3.68 |
| V8-696 | 2.53 | 2.08 | 2.46 | 2.31 | 2.60 | ——— | 3.52 |
| Mean | 2.23 | 2.09 | 2.33 | 2.25 | 2.36 | 2.40 | |

Analysis of the multiple environments revealed significant differences between environments and among crosses in both cases (Table 13). The interaction was not significant for either experiment as the ranking of the crosses relative to each other did not change drastically over environments (Tables 11 and 12).

In the experiment using cuttings as environments, the mean nitrogen content was significantly higher in September than April at Corvallis, Oregon (Table 11). This may have been due to a reduced rate of growth and lower forage production in September. In the second experiment with locations as environments, crosses at Oregon had a mean nitrogen content of 3.55% while crosses at Columbia, Missouri had a mean nitrogen content of 2.28% (Table 12).

Crosses were a significant source of variation and there was significant general combining ability in both multiple environments (Table 13). In the study with cuttings as environments, general combining ability means varied from a low of 3.12% for B5-62 to a high of 3.36% for line B17-9 (Table 14). In the study with locations as environments, general combining ability means varied from 2.77% for line B5-62 to 3.02% for line V2-448 (Table 15).

Broad-sense heritabilities for nitrogen content were high for both single and multiple environments (Table 7). Narrow-sense heritabilities were only slightly lower than the broad-sense estimates, indicating that nitrogen content was controlled mainly by additive gene action. Selection for nitrogen content should be easily accomplished with a program of recurrent selection.

Table 13. Analysis of variance and combining ability analysis for nitrogen content for two multiple environments.

| Source | df | Mean Square | |
|---|---|---|---|
| | | Oregon (2 cuttings) | Oregon-Missouri |
| Replications | 1 | 0.1382 | 0.3212 |
| Environments (E) | 1 | 4.8053* | 24.3334** |
| Error (A) | 1 | 0.0180 | 0.0036 |
| Crosses (C) | 14 | 0.0788** | 0.0849** |
| GCA | 5 | 0.1700** | 0.2013** |
| SCA | 9 | 0.0294 | 0.0202 |
| E x C | 14 | 0.0183 | 0.0318 |
| Error (B) | 28 | 0.0139 | 0.0203† |

*, ** Significant at the 5 and 1% levels of probability, respectively.

† Error (B) degrees of freedom = 27.

Table 14. Nitrogen content (%) of the fifteen single-crosses averaged over the two cuttings at Corvallis, Oregon.

| | Parent | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Mean |
| A59-90 | ——— | 3.08 | 3.31 | 3.22 | 3.44 | 3.21 | 3.25 |
| B5-62 | | ——— | 3.20 | 3.03 | 3.21 | 3.10 | 3.12 |
| B17-9 | | | ——— | 3.43 | 3.37 | 3.47 | 3.36 |
| V2-248 | | | | ——— | 3.45 | 3.29 | 3.28 |
| V2-448 | | | | | ——— | 3.22 | 3.34 |
| V8-696 | | | | | | ——— | 3.26 |

Table 15. Nitrogen content (%) of the fifteen single-crosses for the fall cutting, averaged over the two locations.

| | Parent | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Mean |
| A59-90 | ——— | 2.58 | 2.89 | 2.81 | 3.04 | 2.98 | 2.86 |
| B5-62 | | ——— | 2.89 | 2.73 | 2.90 | 2.76 | 2.77 |
| B17-9 | | | ——— | 3.01 | 3.01 | 3.07 | 2.97 |
| V2-248 | | | | ——— | 3.08 | 2.92 | 2.91 |
| V2-448 | | | | | ——— | 3.08 | 3.02 |
| V8-696 | | | | | | ——— | 2.96 |

The analysis of variance for nitrogen content of the cultivars disclosed differences among cuttings and a significant cutting x cultivar interaction (Table 8). There were no differences among cultivars.

Nitrogen content changed drastically with cuttings (Table 16). Nitrogen contents in April were approximately three times as high as in July. This was probably due to lack of fertilization following the April harvest. Nitrogen was applied following the July harvest. Nitrogen content rose in September, but was only half as great as that in April. It was thought that this was the result of the rust epidemic in September.

The cutting x cultivar interaction is most noticeable with the cultivars, Alta, TFM, Hunch I, and Fawn (Table 16). It was felt that the rust epidemic was primarily responsible for the interaction. Missouri experimental, Hunch I, and Oregon experimental, TFM, had the highest levels of resistance to rust, while Alta and Fawn were completely susceptible. Hunch I and TFM had the lowest nitrogen contents among the cultivars in April, but both were high in nitrogen in September when rust was most severe. Fawn and Alta were both high in nitrogen in April, but were the lowest ranking varieties in September. It appeared that rust greatly reduced the nitrogen content. Oregon experimental, TFM, which had the lowest nitrogen content in April, was the top cultivar in July and September. This may have been a result of its winter-active growth habit. It made its greatest production in April after which it became very dormant. It accumulated

Table 16. Nitrogen content (%) of seven cultivars for the harvests of April 10, July 11, and September 22, 1975.

| Cultivar | Harvests | | | Mean |
|---|---|---|---|---|
| | April 10 | July 11 | September 22 | |
| Alta | 3.05 | 1.13 | 1.49 | 1.89 |
| TFM | 2.75 | 1.33 | 1.82 | 1.97 |
| Ky 37G1-309 | 3.26 | 1.00 | 1.76 | 2.01 |
| Kenmont | 3.21 | 1.00 | 1.76 | 1.99 |
| Kenhy | 3.43 | 1.14 | 1.67 | 2.08 |
| Hunch I | 2.86 | 0.83 | 1.74 | 1.81 |
| Fawn | 3.20 | 1.02 | 1.59 | 1.93 |
| Mean | 3.11 | 1.06 | 1.69 | 1.95 |

nitrogen in the summer, but was not using much for growth at that time.

Significant differences for *in vitro* digestibility were found for the September cutting of the diallel at Corvallis, Oregon, but not for the October cutting at Columbia, Missouri (Table 17). There were significant differences among crosses with crosses ranging from 66.36% to 74.00% dry matter digestibility (Table 18). General combining ability was a significant source of variation. General combining ability means ranged from a low of 69.42% for line V8-696 to a high of 71.69% for line A59-90. It was interesting to note that line V8-696 had the highest perloline content and the lowest digestibility, while line A59-90 had the lowest perloline content and the highest digestibility. The cross with the lowest digestibility, V2-248 x V8-696, was the cross with the highest perloline content. The correlation coefficient for perloline content and *in vitro* digestibility was only -0.36, however.

The broad-sense heritability for *in vitro* digestibility was 0.74 and the narrow-sense heritability was 0.18. This indicated that *in vitro* digestibility was controlled mainly by non-additive gene action. Selection for improved digestibility may be possible with a program of recurrent selection for specific combining ability.

Analysis of the multiple environment with locations as environments, revealed a significant difference between locations. The mean *in vitro* digestibility was 70.79% at Corvallis, Oregon and 61.39% at Columbia, Missouri. There were no differences among crosses and no

Table 17. Analysis of variance and combining ability analysis for digestibility of fifteen single-crosses in two environments.

| Source | df | Mean Square | |
|---|---|---|---|
| | | Oregon, Sept. 22 | Missouri, Oct. 16 |
| Replications | 1 | 16.75* | 18.47 |
| Crosses | 14 | 7.92* | 9.71 |
| GCA | 5 | 10.67* | ——— |
| SCA | 9 | 6.39 | ——— |
| Error | 14 | 3.05 | 13.68† |

* Significant at the 5% level of probability.

† Error degrees of freedom = 13.

Table 18. Digestibility (%) of fifteen single-crosses harvested September 22, 1975 at Corvallis, Oregon (upper diagonal) and October 16, 1975 at Columbia, Missouri (lower diagonal).

| | Parent | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Mean |
| A59-90 | ——— | 72.03 | 70.78 | 72.69 | 70.70 | 72.27 | 71.69 |
| B5-62 | 62.25 | ——— | 70.81 | 74.00 | 71.54 | 68.95 | 71.47 |
| B17-9 | 61.56 | 62.38 | ——— | 69.02 | 71.30 | 67.86 | 69.95 |
| V2-248 | 60.79 | 61.89 | 58.74 | ——— | 71.98 | 66.36 | 70.81 |
| V2-448 | 63.14 | 64.40 | 61.33 | 64.15 | ——— | 71.66 | 71.43 |
| V8-696 | 62.34 | 56.38 | 61.05 | 62.63 | 58.00 | ——— | 69.42 |
| Mean | 62.01 | 61.46 | 61.01 | 61.62 | 62.20 | 60.06 | |

crosses x environments interaction. Until further information is available, lines should be tested over locations and years in the event that there may be a genotype x environment interaction.

# SUMMARY AND CONCLUSIONS

There was significant genetic variation for perloline content to allow for selection of low perloline lines. Perloline content was controlled mainly by non-additive gene action with dominance for low perloline, although there was significant additive gene action present. Broad-sense heritabilities were generally high with narrow-sense heritabilities only half as large. Selection for low perloline lines should be possible using a program of recurrent selection with a high perloline tester. Perloline content varied with environment, as represented by cuttings and locations. Further work is needed to determine the effects of specific environmental factors, such as temperature, soil fertility, moisture, disease, and photoperiod, on the perloline content of tall fescue. There were significant genotype x environment interactions. As a result of these interactions, it is recommended that selections for perloline content be tested over locations and years.

Significant genetic variation was found for nitrogen content. Nitrogen was controlled primarily by additive gene action. Narrow-sense heritabilities were high and only slightly lower than the broad-sense estimates. Selection for nitrogen content should be readily accomplished with a program of recurrent selection. There was significant variation due to environment. Soil fertility and disease appeared to be the major factors involved in this variation. The cutting x cultivar interaction was significant indicating that lines should be tested over locations and years.

There was significant genetic variation for *in vitro* digestibility in one environment. Digestibility was controlled mainly by non-additive gene action. The narrow-sense heritability was very low. There was a significant difference between the environments tested. No significant genotype x environment interaction was found. Until more information is available, lines should be tested over locations and years in the event that there is a genotype x environment interaction for *in vitro* digestibility in tall fescue.

# BIBLIOGRAPHY


1. Allinson, D. W. 1971. Influence of photoperiod and thermoperiod on the IVDMD and cell wall components of tall fescue. Crop Sci. 11:456-459.

2. Bennett, W. D. 1963. A note on the effect of nitrate and phosphate on the perloline content of perennial ryegrass (Lolium perenne L.). N. Z. J. Agric. Res. 6:310-313.

3. Boling, J. A., L. P. Bush, R. C. Buckner, L. C. Pendlum, P. B. Burrus, S. G. Yates, S. P. Rogovin, and H. L. Tookey. 1975. Nutrient digestibility and metabolism in lambs fed added perloline. J. Anim. Sci. 40:972-976.

4. Breese, E. L. and W. E. Davis. 1970. Herbage plant breeding. Jubilee Rep. Welsh Plant Breed. Sta. p. 11-47.

5. Brimner, J. M. and A. P. Edwards. 1965. Determination and isotope-ratio of analysis of different forms of nitrogen in soils: I. Apparatus and procedure for distillation and determination of ammonium. Soil Sci. Soc. Am. Proc. 29:504-507.

6. Brown, R. H., R. E. Blaser, and J. P. Fontenot. 1966. Digestibility of fall grown Kentucky 31 fescue. Agron. J. 55: 321-324.

7. Buckner, R. C., P. B. Burrus, and G. T. Webster. 1968. Breeding, hybridization, and cytogenetic studies of cool-season grass species. Kentucky Agric. Exp. Sta. Ann. Rep. 81:30-31.

8. Buckner, R. C., P. B. Burrus, G. T. Webster, and L. P. Bush. 1974. Breeding pasture, hay, and turf grasses. Kentucky Agric. Exp. Sta. Ann. Rep. 87:14-15.

9. Buckner, R. C., L. P. Bush, and P. B. Burrus. 1973. Variability and heritability of perloline in Festuca sp., Lolium sp., and Lolium-Festuca hybrids. Crop Sci. 13:666-669.

10. Buckner, R. C., L. P. Bush, P. B. Burrus, and G. T. Webster. 1972. Breeding pasture, hay, and turf grasses. Kentucky Agric. Exp. Sta. Ann. Rep. 85:15-17.

11. Bush, L. P., J. A. Boling, G. Allen, and R. C. Buckner. 1972. Inhibitory effects of perloline to rumen fermentation in vitro. Crop Sci. 12:277-279.

12. Bush, L. P. and R. C. Buckner. 1969. Physiologic and genetic studies of alkaloids in Festuca, spp., Lolium, spp., and Lolium-Festuca hybrids. Kentucky Agric. Exp. Sta. Ann. Rep. 82:24-25.

13. Bush, L. P. and R. C. Buckner. 1973. Tall fescue toxity. p. 99-112. In A. G. Matches (ed.) Anti-quality components of forages. Am. Soc. of Agron., Madison, Wis.

14. Bush, L. P., R. C. Buckner, and P. B. Burrus. 1970. Physiological studies of fescue and ryegrass genotypes. Kentucky Agric. Exp. Sta. Ann. Rep. 83:22-23.

15. Bush, L. P., R. C. Buckner, and P. B. Burrus. 1973. Tall fescue x ryegrass forage quality. Kentucky Agric. Exp. Sta. Ann. Rep. 86:20.

16. Butler, G. W. 1962. Genetic differences in the perloline content of ryegrass (Lolium) herbage. N. Z. J. Agric. Res. 5:158-162.

17. Cooper, J. P. 1961. Selection for nutritive value. Welsh Plant Breed. Sta. Rep. p. 145-146.

18. Cornelius, P. L., R. C. Buckner, L. P. Bush, P. B. Burrus, and J. Byars. 1974. Inheritance of perloline content in annual ryegrass x tall fescue hybrids. Crop Sci. 14:896-898.

19. Cunningham, I. J. and E. M. Clare. 1943. A fluorescent alkaloid in ryegrass (Lolium perenne L.). V. Toxicity, photodynamic action, and metabolism of perloline. N. Z. J. Sci. Tech. 24 (B):167-178.

20. Deinum, B. and J. G. P. Dirven. 1971. Climate, nitrogen, and grass. IV. The influence of age on chemical composition and in vitro digestibility of maize (Zea mays L.) and tall fescue (Festuca arundinacea, Schreb.). Neth. J. Agric. Sci. 19: 264-272.

21. Duncan, C. C., M. Schupp, and C. M. McKell. 1969. Nitrogen concentration of grasses in relation to temperature. J. Range Management 22:430-432.

22. Fejer, S. O. 1960. Effects of gibberellic acid, indole-acetic acid, coumarin, and perloline on perennial ryegrass (Lolium perenne L.). N. Z. J. Agric. Res. 3:734-743.

23. Fejer, S. O. 1962. The activity of perloline and of root exudates and shoot extracts of Lolium spp. in the Cress test. Die Naturwissenschaften 49:354.

24. Fonnesbeck, P. V. 1968. Digestion of soluble and fibrous carbohydrate of forage by horses. J. Anim. Sci. 27:1336-1344.

25. Gardner, C. O. 1963. Estimates of genetic parameters in cross-fertilizing plants and their implications in plant breeding. p. 225-252. In W. D. Hanson and H. F. Robinson (eds.) Statistical genetics and plant breeding. Natl. Acad. Sci., Natl. Res. Coun. Pub. 982, Washington, D. C.

26. Gentry, C. E., R. A. Chapman, L. Henson, and R. C. Buckner. 1969. Factors affecting the alkaloid content of tall fescue (Festuca arundinacea, Schreb.). Agron. J. 61:313-316.

27. Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci. 9:463-493.

28. Grimes, R. C., B. R. Watkin, and J. R. Gallagher. 1967. The growth of lambs grazing on perennial ryegrass, tall fescue, and cocksfoot, with and without white clover, as related to the botanical and chemical composition of the pasture and pattern of fermentation in the rumen. J. Agric. Sci. 68: 11-21.

29. Grimmett, R. E. R. and D. F. Waters. 1943. A fluorescent alkaloid in ryegrass (Lolium perenne L.). II. Extraction from fresh ryegrass and separation from other bases. N. Z. J. Sci. Tech. 24(B):151-155.

30. Jacobson, D. R., S. B. Carr, R. H. Hatton, R. C. Buckner, A. P. Graden, D. R. Dowden, and W. M. Miller. 1970. Growth, physiological responses, and evidence of toxicity in yearling dairy cattle grazing different grasses. J. Dairy Sci. 53:575-587.

31. Jones, D. I. H. 1970. Chemistry. Jubilee Rep. Welsh Plant Breed. Sta. p. 183-194.

32. Melville, J. and R. E. R. Grimmett. 1941. Isolation of a new alkaloid from perennial ryegrass. Nature 148:782.

33. Miles, D. G., G. Griffith, and R. J. K. Walters. 1963. Variation in the chemical composition of four grasses. Welsh Plant Breed. Sta. Rep. p. 110-114.

34. Nelson, D. W. and L. E. Sommers. 1973. Determination of total nitrogen in plant material. Agron. J. 65:109-112.

35. Reifer, I. and N. O. Bathurst. 1943. A fluorescent alkaloid in ryegrass (Lolium perenne L.). III. Extraction and properties. N. Z. J. Sci. Tech. 24(B):155-159.

36. Rojas, B. A. and G. F. Sprague. 1952. A comparison of variance components in corn yield trials: III. General and specific combining ability and their interaction with locations and years. Agron. J. 44:462-466.

37. Shaffer, S. R., M. Williams, B. J. Harmon, E. E. Pickett, and G. B. Garner. 1975. Determination of perloline by a fluorometric method. J. Agric. Food Chem. 23:346-348.

38. Tilley, J. A. and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grasslands Soc. 18:104-111.

39. Wilson, G. F. 1964. Identifying the toxic factor in tall fescue grass. N. Z. J. Agric. Res. 7:766-769.

40. Wilson, J. R. and C. W. Ford. 1973. Temperature influences on the in vitro digestibility and soluble carbohydrate accumulation of tropical and temperate grasses. Aust. J. Agric. Res. 24:187-198.

41. Yates, S. G. 1962. Toxicity of tall fescue forage: A review. Econ. Bot. 16:295-303.

42. Yates, S. G. 1963. Paper chromatography of alkaloids of tall fescue hay. J. Chromatography 12:423-426.

43. Yates, S. G. and H. L. Tookey. 1965. Festucine, an alkaloid from tall fescue (Festuca arundinacea, Schreb.): Chemistry of the functional groups. Aust. J. Chem. 18:53-60.

# APPENDIX

Appendix Table 1. Analysis of variance for digestibility for a multiple environment with locations (Corvallis, Oregon and Columbia, Missouri) as environments.

| Source | df | Mean Square |
|---|---|---|
| Replications | 1 | 34.9301* |
| Environments (E) | 1 | 1327.4700** |
| Error (A) | 1 | 0.0147 |
| Crosses (C) | 14 | 10.9387 |
| E x C | 14 | 6.6734 |
| Error (B) | 27 | 8.2179 |

*,** Significant at the 5 and 1% levels of probability, respectively.

Appendix Table 2. Variances of GCA ($S_g^2$) and SCA ($S_s^2$) effects for perloline content for harvests of April 8 and September 22, 1975 at Corvallis, Oregon.

| Parent | April 8 | | September 22 | |
|---|---|---|---|---|
| | $S_g^2$ | $S_s^2$ | $S_g^2$ | $S_s^2$ |
| A59-90 | 84 | -101 | 33936 | 1553 |
| B5-62 | -40 | -173 | 12648 | 1511 |
| B17-9 | 32 | -130 | 2978 | 9841 |
| V2-248 | 37 | 76 | 3364 | 19868 |
| V2-448 | -6 | -112 | 6844 | -135 |
| V8-696 | 422 | 86 | 23485 | 26363 |

Appendix Table 3. Variances of GCA ($S_g^2$) and SCA effects ($S_s^2$) for nitrogen content for harvests of April 8 and September 22, 1975 at Corvallis, Oregon and October 16, 1975 at Columbia, Missouri.

| Parent | Oregon, April 8 | | Oregon, September 22 | | Missouri, October 16 | |
|---|---|---|---|---|---|---|
| | $S_g^2$ | $S_s^2$ | $S_g^2$ | $S_s^2$ | $S_g^2$ | $S_s^2$ |
| A59-90 | -.0015 | -.0029 | .0047 | -.0076 | .0003 | .0050 |
| B5-62 | .0494 | -.0091 | .0149 | .0127 | .0496 | .0122 |
| B17-9 | .0153 | -.0031 | .0031 | -.0148 | .0015 | .0070 |
| V2-248 | -.0030 | -.0057 | -.0019 | -.0061 | -.0014 | -.0057 |
| V2-448 | -.0030 | .0035 | .0229 | -.0017 | .0086 | .0011 |
| V8-696 | -.0032 | -.0033 | .0008 | -.0027 | .0194 | .0029 |

Appendix Table 4. Variances of GCA ($S_g^2$) and SCA effects ($S_s^2$) for digestibility for the harvest of September 22, 1975 at Corvallis, Oregon.

| Parent | $S_g^2$ | $S_s^2$ |
|---|---|---|
| A59-90 | 0.6252 | 0.0851 |
| B5-62 | 0.0687 | -0.1788 |
| B17-9 | 0.4729 | -1.9814 |
| V2-248 | -0.6346 | 1.2782 |
| V2-448 | 0.0012 | 0.0563 |
| V8-696 | 2.3279 | 1.6920 |

Appendix Table 5. Analysis of variance for nitrogen and perloline content of the parental genotypes of the diallel for the harvests of September 22, 1975 at Corvallis, Oregon and October 16, 1975 at Columbia, Missouri.

| Source | df | Mean Square | |
|---|---|---|---|
| | | Nitrogen | Perloline |
| Replications | 1 | 0.0067 | 1666.7 |
| Locations (L) | 1 | 3.0104 | 786170.0 |
| Error (A) | 1 | 0.1601 | 5104.1 |
| Genotypes (G) | 5 | 0.1525* | 240390.0** |
| G x L | 5 | 0.0179 | 117690.0** |
| Error (B) | 10 | 0.0346 | 18017.3 |

*, ** Significant at the 5 and 1% levels of probability, respectively.

Appendix Table 6. Nitrogen content (%) of the parental genotypes of the diallel for the harvests of September 22, 1975 at Corvallis, Oregon and October 16, 1975 at Columbia, Missouri.

| | Location | | |
|---|---|---|---|
| Genotype | Oregon | Missouri | Mean |
| A59-90 | 2.77 | 1.99 | 2.38 |
| B5-62 | 2.89 | 2.36 | 2.63 |
| B17-9 | 3.23 | 2.45 | 2.84 |
| V2-248 | 3.20 | 2.54 | 2.87 |
| V2-448 | 2.83 | 2.22 | 2.53 |
| V8-696 | 3.23 | 2.34 | 2.78 |
| Mean | 3.02 | 2.32 | 2.67 |

Appendix Table 7. Perloline content (μg/g) of the parental genotypes of the diallel for the harvests of September 22, 1975 at Corvallis, Oregon and October 16, 1975 at Columbia, Missouri.

| | Location | | |
|---|---|---|---|
| Genotype | Oregon | Missouri | Mean |
| A59-90 | 112.50 | 18.75 | 65.63 |
| B5-62 | 193.75 | 25.00 | 109.38 |
| B17-9 | 256.25 | 17.19 | 136.72 |
| V2-248 | 1225.00 | 206.25 | 715.63 |
| V2-448 | 212.50 | 9.38 | 110.94 |
| V8-696 | 468.75 | 20.32 | 244.53 |
| Mean | 411.46 | 49.48 | 230.47 |

Appendix Table 8. Analysis of variance for digestibility of the parental genotypes of the diallel for the harvest of October 16, 1975 at Columbia, Missouri.

| Source | df | Mean Square |
|---|---|---|
| Replications | 1 | 0.9185 |
| Genotypes | 5 | 22.9156 |
| Error | 5 | 10.0252 |

Appendix Table 9. Digestibility (%) of the parental genotypes of the diallel for the harvest of October 16, 1975 at Columbia, Missouri.

| Genotype | Digestibility |
|---|---|
| A59-90 | 65.62 |
| B5-62 | 62.93 |
| B17-9 | 62.84 |
| V2-248 | 55.47 |
| V2-448 | 62.02 |
| V8-696 | 61.24 |
| Mean | 61.69 |

Appendix Table 10. Analysis of variance for nitrogen and perloline content of the eight parental genotypes of the cultivar, Fawn.

| Source | df | Mean Square | |
|---|---|---|---|
| | | Nitrogen | Perloline |
| Replications | 1 | 0.1058 | 32395.3 |
| Cuts | 1 | 5.7291* | 1087360.0 |
| Error (A) | 1 | 0.0153 | 39924.3 |
| Genotypes | 7 | 0.1509** | 90203.0** |
| Cuts x Genotypes | 7 | 0.0598** | 48936.3** |
| Error (B) | 14 | 0.0113 | 5479.4† |

*, ** Significant at the 5 and 1% levels of probability, respectively.

† Error (B) degrees of freedom = 13.

Appendix Table 11. Nitrogen content (%) of the parental genotypes of the cultivar, Fawn, for the harvests of April 10 and September 8, 1975.

| Genotype | Harvests | | Mean |
|---|---|---|---|
| | April 10 | Sept. 8 | |
| 304 | 3.77 | 2.57 | 3.17 |
| 314 | 3.53 | 2.56 | 3.05 |
| 315 | 3.09 | 2.33 | 2.71 |
| 339 | 3.32 | 2.13 | 2.72 |
| 340 | 3.04 | 2.34 | 2.69 |
| 342 | 3.21 | 2.53 | 2.87 |
| 351 | 2.99 | 2.27 | 2.63 |
| 352 | 2.97 | 2.42 | 2.70 |
| Mean | 3.24 | 2.39 | 2.82 |

Appendix Table 12. Perloline content (μg/g) of the parental genotypes of the cultivar, Fawn, for the harvests of April 10 and September 8, 1975.

| | Harvests | | |
|---|---|---|---|
| Genotype | April 10 | Sept. 8 | Mean |
| 304 | 6.41 | 242.19 | 124.30 |
| 314 | 37.82 | 359.38 | 198.60 |
| 315 | 5.94 | 285.16 | 145.55 |
| 339 | 33.28 | 324.22 | 178.75 |
| 340 | 17.19 | 76.18 | 46.68 |
| 342 | 23.13 | 738.29 | 380.71 |
| 351 | 174.63 | 847.66 | 511.14 |
| 352 | 66.72 | 441.41 | 254.06 |
| Mean | 45.64 | 414.31 | 229.97 |

Appendix Table 13. Numerical example for the combining ability analysis and derivation of broad and narrow-sense heritability estimates from the expected mean squares for perloline content in a multiple environment with locations as environments, utilizing the analysis presented by Griffing (27) and Rojas and Sprague (36).

| | Expected Mean Square Components for Method 4, Model II | |
|---|---|---|
| Source | df | Expected Mean Squares |
| Crosses | p(p-1)/2 | |
| GCA | p-1 | Ve + rVsl + rlVs + (p-2)rVgl + (p-2)rlVg |
| SCA | p(p-3)/2 | Ve + rVsl + rlVs |
| Crosses x Environment | [p(p-1)/2](l) | |
| GCA x Environment | (p-1)(l-1) | Ve + rVsl + (p-2)rVgl |
| SCA x Environment | [p(p-3)/2](l-1) | Ve + rVsl |
| Error | m | Ve |

m = degrees of freedom for experimental error

p = number of parents

l = number of locations

Appendix Table 13. (continued)

r = number of replications

Vg = variance for general combining ability (GCA)

Vs = variance for specific combining ability (SCA)

Vgl = variance for GCA x location interaction

Vsl = variance for SCA x location interaction

Single-Cross Means Across Locations and Replications

| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | Total |
|---|---|---|---|---|---|---|---|
| A59-90 | ——— | 128.1 | 160.7 | 100.3 | 73.7 | 185.5 | 648.3 |
| B5-62 | | ——— | 273.8 | 257.8 | 271.4 | 377.3 | 1308.4 |
| B17-9 | | | ——— | 208.5 | 153.5 | 193.0 | 989.5 |
| V2-248 | | | | ——— | 148.4 | 478.3 | 1193.3 |
| V2-448 | | | | | ——— | 187.8 | 834.8 |
| V8-696 | | | | | | ——— | 1421.9 |
| Total | | | | | | | 6396.2 |
| Total/2 | | | | | | | 3198.1 |

$\sum_i x_{i\cdot}^2 = (648.3)^2 + (1308.4)^2 + \ldots + (1421.9)^2 = 7253969.2$

$x_{\cdot\cdot}^2 = (3198.1)^2 = 10227843.6$

Appendix Table 13. (continued)

$$\sum_{i<j}\sum x_{ij}^2 = (128.1)^2 + (160.7)^2 + \ldots + (187.8)^2 = 839922.5$$

$$\text{GCA SS} = \left[\frac{1}{p-2}\left(\sum_i x_{i\cdot}^2\right) - \frac{4}{p(p-2)}\, x_{\cdot\cdot}^2\right] \times (r)(l)$$

$$\text{SCA SS} = \left(\sum_{i<j}\sum x_{ij}^2 - \frac{1}{p-2}\sum x_{i\cdot}^2 + \frac{2}{(p-1)(p-2)}\, x_{\cdot\cdot}^2\right) \times (r)(l)$$

Where SS = sum of squares

$$\text{GCA SS} = \left[\frac{1}{4}(7253969.2) - \frac{1}{6}(10227843.6)\right] \times (2)(2) = 435426.7$$

$$\text{SCA SS} = \left[839922.5 - \frac{1}{4}(7253969.2) + \frac{1}{10}(10227843.6)\right] \times (2)(2) = 196858.4$$

Analysis of Variance Table

| | | SS | | |
|---|---|---|---|---|
| Source | df | Oregon–Missouri | Oregon | Missouri |
| Crosses | 14 | 632283.4 | 1136324.0 | 25895.9 |
| GCA | 5 | 435426.7 | 749717.7 | 18221.7 |
| SCA | 9 | 196858.2 | 386606.5 | 7674.2 |

$$\text{GCA x Environment SS} = (\text{GCA SS}_{\text{Environment 1}} - \text{GCA SS}_{\text{Environment 2}}) - \text{GCA SS}_{\text{Multiple Environment}}$$

Appendix Table 13. (continued)

SCA x Environment SS = (SCA $SS_{\text{Environment 1}}$ + SCA $SS_{\text{Environment 2}}$) − SCA $SS_{\text{Multiple Environment}}$

GCA x Environment SS = (749717.7 + 18221.7) − 435426.7 = 332512.7

SCA x Environment SS = (386606.5 + 7674.2) − 196858.2 = 197424.5

Analysis of Variance Table

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Crosses | 14 | 632283.4 | 45163.1 | 9.50 |
| GCA | 5 | 435426.7 | 87085.3 | 18.33 |
| SCA | 9 | 196858.2 | 21872.9 | 4.60 |
| Crosses x Environment | 14 | 529937.8 | 37852.7 | 7.97 |
| GCA x Environment | 5 | 332512.7 | 66502.5 | 14.00 |
| SCA x Environment | 9 | 197424.5 | 21936.1 | 4.62 |
| Error | 27 | 128299.4 | 4751.8 | |

$$\hat{g}_i = \frac{1}{p(p-2)} \left[p \sum x_{i\cdot} - 2(x_{\cdot\cdot}^2)\right]$$

$$\hat{s}_{ij} = x_{ij} - \frac{1}{p-2}(x_{i\cdot} + x_{\cdot j}) - \frac{2}{(p-1)(p-2)}(x_{\cdot\cdot})$$

Where $\hat{g}_i$ = GCA effect for the $i^{th}$ parent

$\hat{s}_{ij}$ = SCA effect for the $ij^{th}$ cross

$$g_1 = \frac{1}{24}[6(648.3) - 2(3198.1)] = -104.43$$

Appendix Table 13. (continued)

$\hat{s}_{1,2} = 128.1 - \frac{1}{4}(648.3 + 1308.4) - \frac{1}{10}\ (3198.1) = -41.27$

| | SCA Effects | | | | | | |
|---|---|---|---|---|---|---|---|
| Parent | A59-90 | B5-62 | B17-9 | V2-248 | V2-448 | V8-696 | GCA Effects |
| A59-90 | ——— | -41.27 | 71.05 | -40.31 | 22.72 | -12.19 | -104.43 |
| B5-62 | | ——— | 19.14 | -47.82 | 55.40 | 14.55 | 60.62 |
| B17-9 | | | ——— | -17.40 | 17.26 | -90.05 | -19.15 |
| V2-248 | | | | ——— | -38.77 | 144.30 | 31.80 |
| V2-448 | | | | | ——— | -56.61 | -57.80 |
| V8-696 | | | | | | ——— | 88.96 |

$S_{g_i}^2 = (\hat{g}_i)^2 - \frac{p-1}{p(p-2)}$ (error mean square)

$S_{s_i}^2 = \frac{1}{(p-2)} \sum_i \hat{s}_{ij}^2 - \frac{(p-3)}{(p-2)}$ (error mean square)

Where $S_{g_i}^2$ = variance of GCA effects for the $i^{th}$ parent

$S_{s_i}^2$ = variance of SCA effects for the $i^{th}$ parent

Appendix Table 13. (continued)

$$S_{g_1}^2 = (-104.43)^2 - \frac{5}{24}(4751.8) = 9916.44$$

$$S_{s_1}^2 = \frac{1}{4}(-41.27^2 + 71.05^2 + \ldots + -12.19^2) - \frac{3}{4}(4751.8) = -1303.47$$

| Parent | $S_g^2$ | $S_s^2$ |
|---|---|---|
| A59-90 | 9916.44 | -1303.47 |
| B5-62 | 2685.35 | -1654.63 |
| B17-9 | -623.29 | -32.72 |
| V2-248 | 21.20 | 3070.87 |
| V2-448 | 2350.86 | -1416.32 |
| V8-696 | 6923.81 | 4560.08 |

Heritability estimates (Gardner (25)):

$Ve = MS_{error} = 4751.8$

$Vsl = (MS_{SCA \times environment} - MS_{error})/r = (21936.1 - 4751.8)/2 = 8592.2$

$Vgl = (MS_{GCA \times environment} - MS_{SCA \times environment})/r\,(p-2) = (66502.5 - 21936.1)/2(4) = 5570.8$

$Vs = (MS_{SCA} - MS_{SCA \times environment})/r(1) = (21872.9 - 21936.1)/2(2) = -15.8$

Appendix Table 13. (continued)

---

$$Vg = [(MS_{GCA} + MS_{SCA \times environment}) - (MS_{SCA} + MS_{GCA \times environment})]/r(l)(p-2)$$

$$= [(87085.3 + 21936.1) - (21872.9 + 66502.5)]/2(2)(4) = 1290.4$$

$$\text{Total variance} = 4Vg + 4Vs + Vgl + Vsl + Ve$$

$$\text{Total genetic variance} = 4Vg + 4Vs$$

$$\text{Additive genetic variance} = 4Vg$$

$$\text{Broad-sense heritability} = \frac{4Vg + 4Vs}{4Vg + 4Vs + Vgl + Vsl + Ve} = \frac{5098.4}{24013.2} = 0.21$$

$$\text{Narrow-sense heritability} = \frac{4Vg}{4Vg + 4Vs + Vgl + Vsl + Ve} = \frac{5161.6}{24013.2} = 0.21$$

---