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Title: CER AND LEAF CHARACTERISTICS OF FOUR TALL FESCUE (FESTUCA ARUNDINACEA SCHREB.) SELECTIONS DIFFERING IN FORAGE YIELD

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Gas exchange, leaf area development, leaf anatomy and stomatal characteristics of four tall fescue selections differing in forage yield were examined to provide further insight into characteristics associated with yield differences of these selections. Winter-growing TFM 26 and TFM 16, the highest and lowest-yielding selections, respectively, and summer-growing selections TFK 4 and TFK 12, high and low yielding, respectively, were studied.

Carbon dioxide-exchange rate (CER) of ten genotypes per selection was measured in December, 1978, and April, 1979, using infrared gas analysis. In April, 1979, dark respiration (Rd) and transpiration (Tn) rates were also evaluated and resistances to gas diffusion were calculated. Specific leaf weight (SLW) of leaves used for gas exchange measurements was determined. Leaf area development, as described by leaf elongation (Le) rate, leaf width (w) and leaf area expansion (La) rate, was assessed during three measurement periods.

Photomicrography of leaf cross-sections and microscopic observation of leaf impressions were used to study leaf anatomy and stomatal characteristics, respectively.

The lowest-yielding selection, TFM 16, had significantly greater CER (December), narrower leaves and lower boundary layer resistance (r_a). While differences among selections were not significant in April, TFM 16 tended to have higher mean CER and Tn and lower mean resistance components - total resistances to water vapor and CO_2 diffusion (Σr_{H_2O} and Σr_{CO_2}, respectively) and stomatal resistance (r_s). The highest-yielding selection, TFM 26, tended to have lower CER and Tn and higher Σr_{H_2O}, Σr_{CO_2}, r_s and r_a. Differences among selections for mesophyll resistance (r_m) were not significant. Nonsignificant differences among selections for SLW and Rd were found. CER and SLW did not appear to be closely related in this study since highly significant differences in CER were found (December) while none were found for SLW.

High-yielding TFM 26 had significantly greater Le and La rates in one experiment, but differences among selections were not significant in two other experiments. Leaf width was a relatively more stable morphological character than Le or La rate and appeared to have an important influence on La rate. Leaf width, therefore, merits further consideration in forage physiology and breeding studies.

A generalized description of leaf anatomy of these selections includes several noteworthy characteristics. Large contributions

by bulliform cells and leaf ridging to adaxial epidermal tissue area are expected to be important in the control of leaf roll and boundary layer depth, respectively. The presence of lignified vascular bundle fibers and fiber caps may have negative effects on forage quality. Approximately half of the veins are major veins, important in long-distance transport. While mesophyll cell area comprises the major fraction of leaf cross-sectional area, large air spaces are also found within the leaf. Study of stomatal characteristics revealed that adaxial stomatal frequency exceeded that of the abaxial leaf surface by more than 3.5 times but adaxial stomatal size was only somewhat smaller than abaxial stomatal size. Consequently, adaxial stomata may be of primary importance to gas exchange when stomata are open.

The lowest-yielding selection, TFM 16, had a significantly larger percentage of cross-sectional area invested in epidermal cell layers and in bulliform cell area. This latter feature, in concert with TFM 16's generally smaller abaxial stomata and lower abaxial stomatal frequency, may provide an advantage in the restriction of water loss during water-stress periods compared to the other selections. Furthermore, TFM 16's generally greater adaxial stomatal frequency may be relatively more advantageous for gas exchange when water is not limiting and may be related to its higher CER. However, TFM 16's greater investment in fiber cap tissue may represent a negative forage quality factor.

Selections TFM 26 and TFK 12 had more vascular bundles than TFM 16. While TFM 26 had more minor veins, TFK 12 had more major veins and, consequently, greater phloem tissue height and width than TFM 16. However, TFM 16's significantly shorter interveinal distance may facilitate transport of photosynthate and may, therefore, be related to TFM 16's higher CER.

Results of this study indicate that differences in physiological, morphological, anatomical and stomatal characteristics exist among these four tall fescue selections. Further study of the relationships among these characteristics and of their importance to forage yield and quality may suggest characteristics which can be profitably manipulated by plant breeders to bring improvement in tall fescue forage.

CER and Leaf Characteristics of Four Tall Fescue (*Festuca arundinacea* Schreb.) Selections Differing in Forage Yield

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Gas Exchange and Leaf Area Characteristics of Four Tall Fescue Selections Differing in Forage Yield

ABSTRACT

Gas exchange and leaf area characteristics of four tall fescue selections differing in forage yield were examined to provide further insight into characteristics associated with yield differences of these selections. Carbon dioxide-exchange rate (CER) of ten genotypes per selection was measured in December, 1978, and April, 1979, using infrared gas analysis. In April, 1979, dark respiration (Rd) and transpiration (Tn) rates were also evaluated and resistances to gas diffusion were calculated. Specific leaf weight (SLW) of leaves used for gas exchange measurements was determined. Leaf area development, as described by leaf elongation (Le) rate, leaf width (W) and leaf area expansion (La) rate was assessed during three measurement periods.

The lowest-yielding selection, TFM 16, had significantly greater CER (December), narrower leaves and lower boundary layer resistance (r_a). While differences among selections were not significant in April, TFM 16 tended to have higher mean CER and Tn and lower mean resistant components-total resistances to water vapor and CO_2 diffusion (Σr_{H_2O} and Σr_{CO_2}, respectively) and stomatal resistance (r_s). The highest-yielding selection, TFM 26, tended to have lower

CER and Tn and higher Σ_{H_2O}, Σr_{CO_2}, r_s and r_a. Differences among selections for mesophyll resistance (r_m) were not significant, but the negative r_m values obtained indicate that extreme care in the determination and interpretation of components of resistance to gas diffusion is needed.

Nonsignificant differences among selections for SLW and Rd were found. CER and SLW did not appear to be closely related in this study since highly significant differences in CER were found (December) while none were found for SLW.

High-yielding TFM 26 had significantly greater Le and La rates in one experiment, but differences among selections were not significant in two other experiments. Leaf width was a relatively more stable morphological character than Le or La rate and appeared to have an important influence on La rate. Leaf width, therefore, merits further consideration in forage physiology and breeding studies.

INTRODUCTION

Physiological and morphological characteristics of tall fescue genotypes have been the subject of several studies attempting to explain contrasting forage yield. Gas exchange and leaf area characteristics are undoubtedly important yield criteria. However, the extent to which these components influence forage production is still not well understood.

Genetic variation in CO_2-exchange rate (CER) of tall fescue has been reported (Asay et al., 1974; Jewiss and Woledge, 1967), but an inconsistent relationship between tall fescue CER and forage yield was also observed (Nelson et al., 1975).

Leaf area development has been suggested as an important component determining forage yield. Horst et al. (1978) reported that leaf elongation (Le) rate was positively correlated with forage yield of tall fescue genotypes grown in a competitive field environment. Tall fescue genotypes differing in tillering capacity were studied by Nelson et al. (1977) under conditions of low competition, and they found the forage yield component dry matter per tiller positively correlated with leaf area expansion (La) rate, which is a function of Le rate and leaf width. Wilhelm and Nelson (1978) studied four tall fescue genotypes differing in CER-yield relationships and observed that high-yielding genotypes had considerably greater rates of both Le and La than did low-yielding genotypes regardless of CER

level. They concluded that low-yielding genotypes do not have the rapid leaf growth characteristics to exploit a high-CER trait.

Dark respiration (Rd) and its maintenance and growth components have recently received increased attention due to their importance in providing energy and carbon skeletons for synthesis of new plant material (Thornley, 1971; Penning de Vries, 1974 and 1975; Wilson, 1974, 1975 and 1976). For instance, Jones and Nelson (1979) recently reported that genetic variation for Rd existed among tall fescue genotypes differing in growth habit and CER-yield relationships.

Lhamby (1978) studied four tall fescue selections differing in yield under competitive field conditions and reported that leaf width, tiller number and tiller dry weight contributed most to forage dry matter yield. The present study was undertaken to provide further insight into characteristics associated with yield differences of these selections. Experimental objectives were to evaluate these four different-yielding tall fescue selections for variation in CER, Rd and components of resistance to gas diffusion. In addition, specific leaf weight (SLW) and leaf area development were assessed.

MATERIALS AND METHODS

Gas exchange characteristics, specific leaf weight and leaf area development of four tall fescue selections differing in forage yield were examined. Two winter-growing types, TFM 26 and TFM 16, and two summer-growing types, TFK 4 and TFK 12, were used in this study. TFM 26 and TFM 16 are high and low yielding fall-winter growing selections, respectively, while TFK 4 and TFK 12 are high and low yielding selections, respectively, which produce their most vigorous growth in spring and early summer months. Lhamby (1978) found that TFM 26 produced the highest forage yield and TFM 16 the lowest, averaged over three years; the TFK selections were intermediate in forage yield.

Twenty genotypes of each selection were obtained from replicated field plots in September, 1978, by removing widely separated single tillers. These tillers were established in 10 cm pots in sandy loam soil in the greenhouse and were tagged to maintain the identity of original tillers. Pots were arranged in a completely randomized fashion on the greenhouse bench, were watered daily and a complete nutrient solution was applied each week. The natural daylength period was extended to a 15-hour day to maintain plants in the vegetative state. Greenhouse temperature was maintained at 18±3 C.

Ten genotypes per selection were used for CER measurement in

December, 1978, and again in April, 1979. In December we selected plants having leaves of comparable tiller position on the original tiller and comparable degree of leaf expansion. One leaf per genotype was to be used for CER measurement. Selected plants were moved from the greenhouse to the laboratory for an acclimatory period one week before CER measurements were to begin. The plants were placed on a bench under cool white fluorescent tubes which provided 145±27 μ E m^{-2} s^{-1} irradiance (photosynthetic photon flux density, PPFD) at the leaf surface. Daytime laboratory temperature averaged 25.5 C which was approximately 7 C higher than greenhouse temperature. Because light energy and temperature varied along the bench, plants were rotated daily. A fan was used to provide mixing of air layers under the lights but did not blow directly on the plants. Although plants were watered daily, moisture stress symptoms, such as leaf roll and leaf-tip dieback, became apparent for plants of all selections during the course of this experiment. Also, not all leaves were fully expanded by the time CER measurements began.

When CER was measured in April, plants were moved back and forth daily between the greenhouse and the laboratory. Two fully-expanded leaves per genotype were used in April's CER experiment. Recent collar formation was the criterion used to select leaves; consequently, leaves chosen were not necessarily on the original

tiller and their position on a tiller was not considered.

Assimilation Chambers

The use of two acrylic plastic (plexiglass) assimilation chambers allowed leaves in one chamber to equilibrate while measurements of leaves in the second chamber were being made. The chambers were ten-sided and measured 58 cm across and 3 cm deep (see Figure 1). Thus, leaves of all ten genotypes per selection were placed in a chamber through slots in its sides so that a mean value of CER was obtained across genotypes for each selection. An air seal was provided by pumping air into a chamber at a rate of 2.5 l min^{-1}, for example, and by withdrawing an air sample at a rate of 0.5 l min^{-1}; the excess air flow of 2.0 l min^{-1} was exhausted out of the slots where leaves had been inserted. A reference air stream was pumped into the chamber through an inlet located in the center of the chamber. This inlet consisted of a plexiglass cylinder through which 2 mm diameter holes had been drilled. Ten groups of three holes were spaced 1 cm apart around the inlet; the two outer holes of a group faced downward and upward, respectively, at an angle of 45° while the center hole faced directly outward so that an equal air flow rate across each side of a leaf was provided. This was checked visually by watching the pattern formed when smoke was pumped through the air inlet. A sample air stream was withdrawn from the chamber

through 0.4 mm holes spaced 1 cm apart in 10 mm O.D. plastic tubing positioned approximately 4 cm from the outer edge of the chamber along its periphery. A 3-cm deep water bath was placed on top of the assimilation chamber to provide chamber temperature control.

Gas Exchange Measurements and Calculations

CER measurements were made using a LIRA 300 infrared gas analyzer calibrated in the differential mode and an open system as described by Stanwood (1974). Atmospheric air was used as the reference air stream and was pumped into the system through tubing that extended from above the roof of the building; the inlet was well removed from any exhaust ducts. The reference air stream was humidified before it was introduced into the plant chamber during December's measurements but it was not humidified during April's measurements. Relative humidity of the reference air stream averaged 72±2% in December and 43±4% in April. Leaf temperature was estimated by placing two thermocouples in the assimilation chamber at the same height at which leaves were positioned. Incident radiation on leaves averaged 792±83 μ E m^{-2} s^{-1} (PPFD) and was provided by a GE 1000-watt Lucalox high pressure sodium lamp. Five CER measurement runs (each run included CER measurement of all four selections) were made in December and three were made

in April. The order in which selections were measured was randomized for each run. CER on a per unit leaf area basis was calculated using the following equations:

$$CER = \frac{\Delta CO_2 \text{ (mg } CO_2\ l^{-1}) \text{ X air flow rate (l min}^{-1})}{\text{total chamber leaf area}}$$

$$\text{mg } CO_2\ l^{-1} = \frac{\mu l CO_2}{\text{liter}} \text{ X} \frac{273K}{\text{leaf T(K)}} \text{ X} \frac{\text{barometric pressure}}{1013 \text{ mb}}$$

$$\text{X} \frac{44,000 \text{ mg}}{\text{mole } CO_2} \text{ X } \frac{\text{mole } CO_2}{22.4 \text{ l } CO_2} \text{ X } 10^{-6}$$

In April transpiration data were also collected using a Cambridge dew point hygrometer. Transpiration (Tn) on a leaf area basis was calculated as:

$$Tn = \frac{[H_2O]_{out} - [H_2O]_{in} \text{ X } 2.5 \text{ l min}^{-1} \text{ air flow rate}}{\text{total chamber leaf area}}$$

Water vapor concentrations were determined from the dew points of the sample (out) and reference (in) air streams. Carbon dioxide diffusion resistances were calculated from April's CER and Tn data. Total resistance to CO_2 diffusion (Σr_{CO_2}) was partitioned into boundary layer resistance (r_a), stomatal resistance (r_s) and residual or mesophyll (r_m) resistance.

Total resistance to CO_2 diffusion in s cm^{-1} was calculated as:

$$\Sigma r_{CO_2} = \frac{[CO_2]_a - [CO_2]_{chl}}{CER}$$

Atmospheric CO_2 concentration ($[CO_2]_a$) was determined by comparing the reference air stream's CO_2 concentration with that of a known standard gas. Chloroplast CO_2 concentration ($[CO_2]_{chl}$ was assumed to equal compensation point CO_2 concentration, 45 ppm (Chen et al., 1970).

Total leaf resistance to water vapor diffusion (ΣrH_2O) in s cm^{-1} was calculated as:

$$\Sigma r_{H_2O} \quad \frac{[H_2O]_c - [H_2O]_a}{Tn}$$

Water vapor concentration inside the leaf ($[H_2O]_c$) was assumed to equal the water vapor concentration of saturated air at the estimated leaf temperature, while $[H_2O]_a$ is the water vapor concentration of the reference air stream entering the leaf chamber.

Boundary layer resistance (r_a) in s cm^{-1} was estimated using the relationship:

$$r_a = k\sqrt{\frac{\text{leaf width, cm}}{\text{wind speed, cm s}^{-1}}} \quad \text{where } k = 1.3\ s^{1/2}\ cm^{-1}$$

Wind speed was set at 100 cm s^{-1}. Since tall fescue leaves have prominent ridges on the adaxial leaf surface which are expected to influence the depth of the still air boundary layer and, therefore, r_a, we adjusted mean leaf width to take into consideration the extent of leaf ridging. We had determined the mean number of ridges and

mean ridge height for leaves of each selection used in December's CER measurements.[1] We, therefore, added the following factor to the mean width of leaves used in April's measurements for each selection:

(mean no. ridges) (2 sides of ridge) (mean ridge height, cm)

Stomatal resistance to CO_2 diffusion (r_s) in s cm^{-1} was calculated as:

$$r_s = \Sigma r_{H_2O}\left(\frac{D_{H_2O}}{D_{CO_2}}\right) - r_a \quad \text{where} \left(\frac{D_{H_2O}}{D_{CO_2}}\right) = 1.56$$

The factor 1.56 was used to reflect the lower diffusivity of CO_2 through air compared to that of water vapor.

Mesophyll resistance to CO_2 diffusion (r_m) is the residual resistance:

$$r_m = \Sigma r_{CO_2} - (r_a + r_s)$$

(The previous equations are taken from Zelitch, 1971, and Sestak et al., 1971).

Dark respiration (Rd) of the leaves used in the April CER study was estimated by determining the rate of oxygen uptake at 29 C using

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[1]Cohen, C. J., Chilcote, D. O. and R. V. Frakes. 1979. Leaf anatomy and stomatal characteristics of four tall fescue selections differing in forage yield. In: CER and leaf characteristics of four tall fescue (Festuca arundinacea Schreb.) selections differing in forage yield. M.S. Thesis. Oregon State University.

a Gilson differential respirometer. The two leaves of each genotype used in the CER study were treated as a pair and randomly-selected genotypes of all four selections were included in each respirometer run. Each leaf was excised just above the collar and was cut in half. A 5 cm section above the midpoint for one leaf and below the midpoint for the other leaf of the pair was used. Areas of the sections were measured using a Licor LI 3000 leaf-area meter. The two sections per genotype were placed in the outer well of a clean, dry 15 ml reaction flask. Two ml of 4 N KOH were added to the center well to absorb evolved CO_2. The respirometer was shielded from light and flasks were equilibrated in a 29 C water bath for 30 min before the rate of O_2 uptake was measured manometrically by taking readings at 30-min intervals over a 2-hr period; measurement periods began approximately four hours after sunrise. Two reference flasks without leaf sections were included with each run to compensate for slight variations in temperature and for possible diffusion of oxygen through the tygon tubing used to connect flasks to the manometers. After O_2 uptake measurements were complete, leaf sections were microwaved for 30 sec, were oven dried at 70 C and were then weighed.

Leaf Area Characteristics

Specific leaf weight of the leaves used for gas exchange

measurements was determined. In December, five leaf discs of known area were removed from the mid-leaf position of each leaf using a leather hole punch; leaf discs were placed in pre-weighed, aluminum sample cans and were dried in a 70 C oven until they reached constant weight. In April entire leaves, rather than leaf discs, were used to calculate specific leaf weight. Each leaf was cut in half at the midpoint and a 5-cm long section to one side of the midpoint was used to measure Rd while the rest of the leaf was microwaved for 30 sec and was then placed in a 70 C oven to dry. Leaf areas were determined using a Licor LI 3000 leaf area meter prior to oven drying or Rd measurement.

Leaf area development was assessed by measuring leaf elongation (Le) rate and leaf width (W) and by calculating leaf area expansion (La) rate as the product of Le X W X 0.905 (Kemp, 1960). Leaf elongation rates were determined by measuring the length of an expanding leaf, from its tip to the collar of the previously expanded leaf, on an every-other-day basis for a period of eight days. Leaf width at the midpoint was measured on the eighth day. Leaf area development was evaluated in this manner in November and December, 1978, and in May, 1979, using leaves of 8, 10 and 10 genotypes, respectively, for each selection.

RESULTS AND DISCUSSION

CER and Rd

Highly significant differences ($P \leq 0.01$) in CER were found among selections in December's study; though differences were not statistically significant in April, the same relative ranking of selection means was observed (Table I). The lowest-yielding selection, TFM 16, had significantly greater CER than all other selections in December ($P \leq 0.05$). The lower CER rates found in December, compared with April's values, may reflect the water stress conditions (e.g. leaf roll) that were observed during December's measurements.

While the existence of genetic variation for single-leaf CER in tall fescue has been reported (Asay et al., 1974), a consistent relationship between CER and forage yield has not been found (Nelson et al., 1975). In fact, it has been possible to select individual tall fescue genotypes having high or low yield in combination with high or low CER per unit leaf area (Wilhelm and Nelson, 1978). In the present study, the two lower-yielding selections had significantly higher CER (December). While CER on a single-leaf basis is a necessary component of forage yield, it apparently is not a sufficient component for yield determination. Canopy characteristics, leaf area development, dry matter distribution and response to

environmental stress are examples of other factors that are likely to influence forage yield production and the relationship between CER and yield.

Differences among selections in Rd rates were not significant. Since all leaves were fully-expanded, the rates observed should represent the maintenance respiration component of dark respiration (Wilson, 1976). Slow rates of maintenance Rd in mature plant tissue may benefit crop growth by reducing respiratory losses (Wilson, 1976). However, Jones and Nelson (1979) recently reported that Rd of mature leaf blades of tall fescue genotypes having low, medium or high dry matter yield per tiller corresponded to their yield/tiller classification. But, they also found that a low CER-low yield/tiller genotype had higher Rd of mature leaf blades compared to other genotypes having contrasting CER-yield relationships. In our study, selections differing in yield did not differ significantly in Rd. Further study of Rd and of its growth and maintenance respiration components is needed before conclusions regarding relationships between Rd and tall fescue forage yield can be drawn.

Tn and Resistance Components

Nonsignificant differences among selections were also found for Tn rate and the components of resistance to gas diffusion--Σr_{H_2O}, Σr_{CO_2}, r_s and r_m; however, highly significant differences among

selections for r_a were found (Table II). TFM 16 had significantly lower r_a than all other selections ($P \leq 0.01$) while TFM 26 had significantly higher r_a than TFK 4 ($P \leq 0.05$) but was comparable in r_a to TFK 12. The differences found among selections for r_a are parallel to those found for leaf width adjusted to take into consideration the extent of leaf ridging (Table II). This result would be expected since the only variable in the equation used to calculate r_a was adjusted leaf width. However, since r_a is a relatively minor component of resistance to gas diffusion (Zelitch, 1971), the differences in r_a among selections were not expected to be reflected in differences in total resistance to diffusion of water vapor or CO_2 or in rates of Tn or CER. In calculating r_a, leaf widths were adjusted in a simple linear fashion to take into consideration extent of ridging, but this correction may still not adequately reflect the increased depth of the boundary layer due to ridging and resultant increased r_a.

Although significant differences among selections for Tn and the other resistance components were not found, several relationships are noteworthy. TFM 26 had the lowest mean Tn rate and the highest calculated mean r_s. Conversely, TFM 16 had the highest mean Tn rate and the lowest calculated mean r_s. This inverse relationship between Tn and r_s is expected since r_s is the major component of resistance to diffusion of water vapor when stomata are open. Also, the relatively lower mean r_s of TFM 16 and its somewhat higher

mean Tn rate may be related to the significantly higher adaxial stomatal frequency that was found for TFM 16.[2] However, the values reported for r_s and Tn of these selections are relatively high and low, respectively, and may, therefore, reflect a certain amount of stomatal closure. For instance, Nobel (1974) reports r_s values ranging from 0.4 to 2 s cm^{-1} for open stomates of dicotyledonous crop plants and Tn values of 12 to 60 mg H_2O dm^{-2} min^{-1} when leaf temperature was 25 C, air temperature was 20 C and ambient relative humidity was 50%.

Examination of Table II shows that negative values for mean r_m were obtained. Such values are difficult to interpret and suggest that the assumptions made in the calculation of the resistance components should be examined. The major assumption that was made in the calculation of Σr_{CO_2} was that $[CO_2]_{chl}$ is equal to the compensation point $[CO_2]$, Γ. We did not measure Γ but used the value reported by Chen et al. (1970) for tall fescue, 45 ppm. This value was obtained for an undesignated variety of tall fescue and under experimental conditions of saturating light intensity and 25 C leaf temperature (T). Since Γ was not measured in the present experiment, we do not know the actual Γ's of the selections under the experimental conditions used or whether Γ varied for the selections. Also, though

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[2] Ibid.

saturating irradiance was provided, the estimated leaf T was higher than that in the work of Chen et al. and averaged 29.8 ± 4.0 C across selections and measurement runs. Increasing leaf T under light-saturating conditions has been found to increase Γ through increased Rd and photorespiratory rates (Troughton and Slatyer, 1969). If Γ had been greater than 45 ppm in our experiment, Σr_{CO_2} would have been lower than calculated and r_m values would have been more negative than shown in Table II.

In this experiment, leaf T was estimated by measuring assimilation chamber temperature at approximately leaf level during CER runs. This may have been an inadequate approximation of leaf T, and as such, could have had a particularly marked effect on the calculation of Σr_{H_2O} and, consequently, r_s and r_m, since the saturated water vapor concentration at leaf T was used to calculate Σr_{H_2O}. For instance, if leaf T were considerably lower than estimated, then a considerably lower water vapor gradient could have existed between the leaf interior and the ambient atmosphere. This would result in lower calculated values for Σr_{H_2O} and r_s. If r_s values were considerably lower than those shown in Table II, then a positive r_m value might have been obtained after subtraction of r_s and r_a from Σr_{CO_2}.

O'Toole et al. (1976) point out that the simple model generally used for the calculation of r_m (i.e., the equations list in Materials

and Methods) can provide nothing more than an approximation which may not actually reflect leaf physiological processes. They also note Jarvis and coworkers' (see Sestak et al., 1971) comment that r_m is frequently defined by the methods used for its determination and, thus, may reflect methodology rather than accurately provide information on the nonstomatal phase of the CO_2 fixation process. It is suggested that interpretation of r_m be verified using an indicator of biochemical response, such as ribulose-1,5-bisphosphate carboxylase activity.

Leaf Area Characteristics

No differences in SLW were found among selections in either experiment (Table I). The higher April values may be more representative of these selections, since water stress symptoms were observed in December. Though a positive relationship between CER and SLW has previously been reported (Delaney and Dobrenz, 1974; Carlson et al., 1970), our data do not suggest a close relationship--though highly significant differences in CER were found in December, none were found for SLW. Wilhelm and Nelson (1978) also found no significant differences in SLW among genotypes having contrasting levels of CER and yield.

As suggested previously, leaf area development is expected to be an important factor influencing forage yield. Of the three

components used in this study to describe leaf area development, only leaf width was found to differ clearly and consistently among selections (Table III). The lowest-yielding selection, TFM 16, had significantly narrower leaves at all three measurement dates ($P \leq 0.10$, $P \leq 0.01$ and $P \leq 0.01$, respectively), while the other three selections had similar leaf width.

Differences in Le and La rates were only evident in November when TFM 26, the highest-yielding selection, had a greater Le rate than both TFK selections and was comparable in Le rate to TFM 16. In addition, TFM 26 had a greater La rate than TFM 16 and TFK 12 but was comparable in La rate to TFK 4. Though narrow-leafed TFM 16 had a Le rate which was similar to that of TFM 26, it had a lower La rate. And, while TFK 4 had a lower Le rate than did TFM 26, its rate of La was similar. Thus, leaf width effects on La rate appeared to be quite important in this study.

Rates of Le and La were lower in December and May compared to November. However, values for leaf width appeared to remain relatively more stable. Leaf elongation rate--and through it La rate--is expected to be quite sensitive to environmental variability due to the requirements of adequate turgor and energy supply for continued cell expansion. Lhamby (1978) also found that leaf width was a more stable morphological characteristic for these selections than either Le or La rate and he reported that leaf width was

consistently related to the forage yield of these selections. However, Asay et al. (1977), who studied tall fescue genotypes selected to represent a range in leaf widths, found that leaf width was not significantly correlated with dry matter yield even though they also found that forage yield increased significantly as leaf width increased. While the possible value of leaf width as a selection criterion for forage yield (either used singly or in combination with other traits) remains uncertain, leaf width as a relatively stable component of leaf area development appears to be important and should be considered further in forage breeding and physiology studies.

SUMMARY AND CONCLUSIONS

1. The lowest-yielding selection, TFM 16, had significantly greater CER (December), narrower leaves and lower r_a.
2. While differences among selections were not significant in April, TFM 16 tended to have higher mean CER, higher mean Tn, lower mean Σr_{H_2O}, Σr_{CO_2}, and r_s.
3. The highest-yielding selection, TFM 26, tended to have lower CER and Tn and higher Σr_{H_2O}, Σr_{CO_2}, r_s and r_a.
4. TFM 26 had significantly higher Le and La rates in one experiment but differences among selections were not significant in two other experiments.
5. Leaf width was a relatively more stable morphological character than Le or La rate and appeared to have an important influence on La rate. Leaf width, as a major determinant of forage yield for these selections (Lhamby, 1978) and as an important and consistent component of leaf area development, merits further consideration in forage physiology and breeding studies.
6. Nonsignificant differences among selections were found for SLW and Rd. Further investigation of Rd and of its growth and maintenance components, is needed before conclusions regarding the relationships between Rd and forage yield can be drawn.
7. Extreme care in the determination and interpretation of

components of resistance to gas diffusion is recommended and it may be more useful to accompany resistance component determination, e.g. r_m, with study of biochemical processes involved in the CO_2 fixation process.

Figure 1. Top view of assimilation chamber with water-bath cover removed

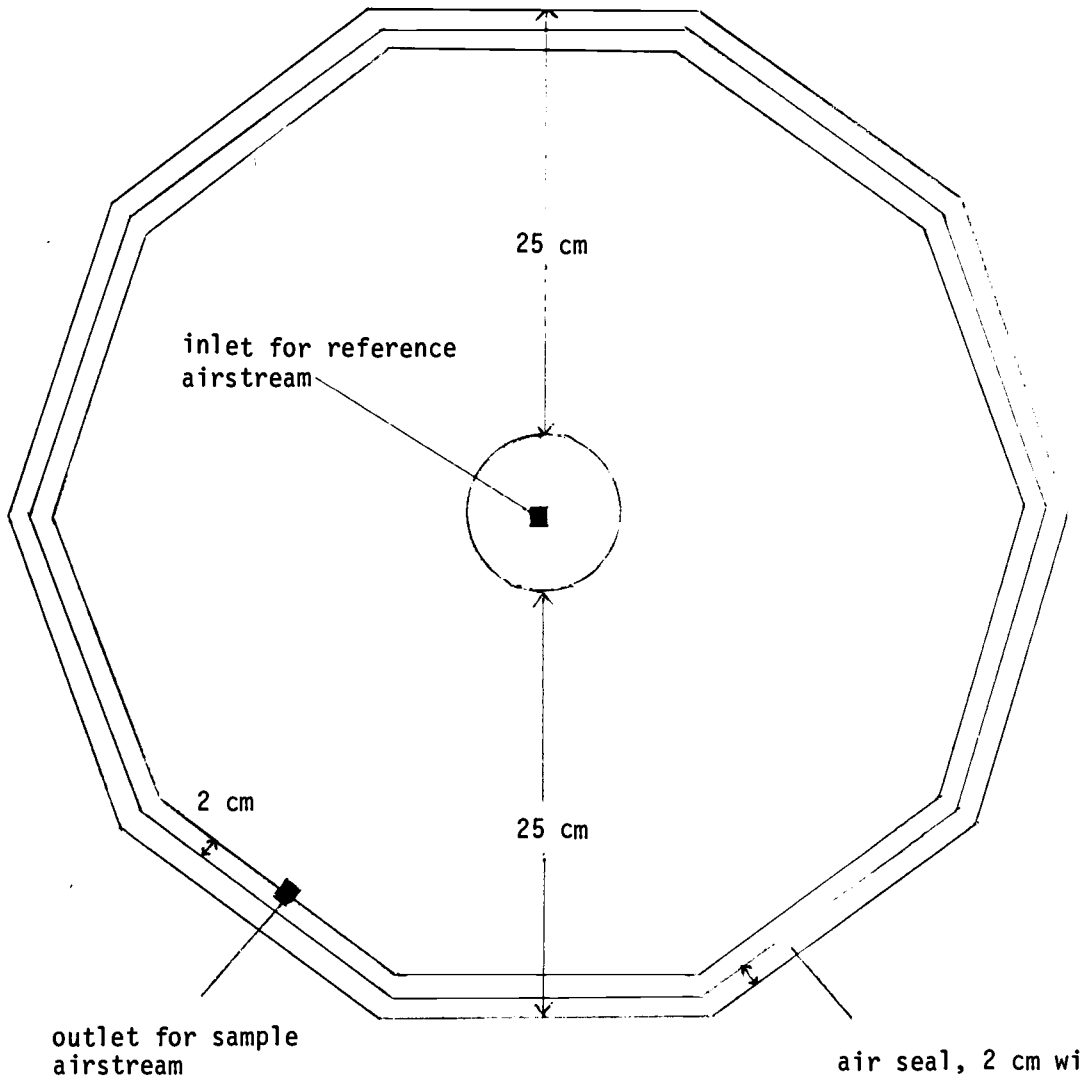

Table I. Net CO_2-exchange rate (CER), dark respiration (Rd) and specific leaf weight (SLW) of four tall fescue selections differing in yield.

Selection-Yield	CER mg CO_2 $dm^{-2}hr^{-1}$ December	CER mg CO_2 $dm^{-2}hr^{-1}$ April	Rd - April $\mu l\ O_2 g^{-1}hr^{-1}$	Rd - April $\mu l\ O_2\ dm^{-2}hr^{-1}$	SLW mg cm^{-2} December	SLW mg cm^{-2} April
TFM 26 - H	8.57 a*	12.25	714	374	3.06	4.98
TFM 16 - L	10.26 c	13.41	608	326	3.04	5.58
TFK 12 - L	9.63 b	13.04	897	437	2.72	5.08
TFK 4 - H	8.69 a	12.71	796	353	2.66	4.68
L. S. D., 0.05	0.59	n. s.	n. s.	n. s.	n. s.	n. s.
$S_{\bar{x}}$	0.19	0.81	90	45	0.21	0.25

*Values not followed by the same letter are significantly different at the 5% level of probability.

Table II. Transpiration (Tn), components of resistance to gas diffusion and adjusted leaf width of four tall fescue selections differing in yield.

Selection - Yield	Tn $mg.H_2O\ dm^{-2}\ min^{-1}$	Σr_{H_2O}	Σr_{CO_2}	r_s	r_a	r_m	Adjusted leaf width, cm
				s cm^{-1}			
TFM 26 - H	10.18	14.18	15.91	21.99	0.134 c*	-6.20	1.078 c
TFM 16 - L	13.16	10.30	14.17	15.95	0.122 a	-1.90	0.885 a
TFK 12 - L	12.03	12.33	14.91	19.10	0.133 bc	-4.33	1.051 bc
TFK 4 - H	12.87	11.29	15.06	17.48	0.130 b	-2.55	1.004 b
L. S. D., 0.05	n. s.	n. s.	n. s.	n. s.	0.004	n. s.	0.073
$S_{\bar{x}}$	1.05	1.08	1.00	1.69	0.002	-1.44	0.026

*Values not followed by the same letter are significantly different at the 5% level of probability.

Table III. Leaf area development of four tall fescue selections differing in yield as described by leaf elongation rate (Le), leaf width (W) and leaf area expansion rate (La).

Selection - Yield	November, 1978			December, 1978			May, 1979		
	Le ($mm\ day^{-1}$)	W (mm)	La ($mm^2\ day^{-1}$)	Le ($mm\ day^{-1}$)	W (mm)	La ($mm^2\ day^{-1}$)	Le ($mm\ day^{-1}$)	W (mm)	La ($mm^2\ day^{-1}$)
TFM 26 - H	19.73 b*	6.28 b+	109.44 b	9.70	6.05 b	54.20	8.25	5.37 b	41.90
TFM 16 - L	14.11 ab	5.22 a	67.60 a	12.31	4.95 a	57.52	9.47	4.27 a	41.51
TFK 12 - L	10.22 a	6.53 b	62.33 a	9.13	6.70 b	54.80	9.25	5.98 b	51.75
TFK 4 - H	13.98 a	6.53 b	83.05 ab	9.01	5.95 b	48.98	9.52	6.22 b	54.35
L. S. D., 0.10		0.90							
L. S. D., 0.05	5.65	n.s.	33.93	n.s.	0.90	n.s.	n.s.	0.95	n.s.
$S_{\bar{x}}$	1.95	0.38	11.71	1.57	0.31	9.29	1.42	0.33	9.11

*Values not followed by the same letter are significantly different at the 5% level of probability.

+Values not followed by the same letter are significantly different at the 10% level of probability.

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Leaf Anatomy and Stomatal Characteristics of Four Tall Fescue Selections Differing in Forage Yield

ABSTRACT

Leaf anatomy and stomatal characteristics of four tall fescue selections differing in forage yield were examined through photomicrography of leaf cross-sections and microscopic observation of leaf impressions, respectively. Winter-growing TFM 26 and TFM 16, the highest- and lowest-yielding selections, respectively, and summer-growing selections TFK 4 and 12, high and low yielding, respectively, were studied.

A generalized description of leaf anatomy of these selections includes several noteworthy characteristics. Large contributions by bulliform cells and leaf ridging to adaxial epidermal tissue area are expected to be important in the control of leaf roll and boundary layer depth, respectively. The presence of lignified vascular bundle fibers and fiber caps may have negative effects on forage quality. Approximately half of the veins are major veins, important in long-distance transport. While mesophyll cell area comprises the major fraction of leaf cross-sectional area, large air spaces are also found within the leaf. Study of stomatal characteristics revealed that stomatal frequency of the adaxial leaf surface was more than 3.5 times greater than abaxial stomatal frequency, while adaxial stomatal

size was only somewhat smaller than abaxial stomatal size. Consequently, adaxial stomata may be of primary importance to gas exchange when stomata are open.

The lowest-yielding selection, TFM 16, had a significantly larger percentage of cross-sectional area invested in epidermal cell layers and in bulliform cell area within the adaxial epidermis. This latter feature, in concert with TFM 16's generally smaller abaxial stomata and lower abaxial stomatal frequency, may provide an advantage in the restriction of water loss during water-stress periods compared to the other selections. Furthermore, TFM 16's generally greater adaxial stomatal frequency may be relatively more advantageous for gas exchange when water is not limiting and may be related to the significantly higher CO_2-exchange rate (CER) found previously for this selection. However, TFM 16's greater investment in fiber cap tissue may represent a negative forage quality factor.

Selections TFM 26 and TFK 12 had more vascular bundles than TFM 16. While TFM 26 had more minor veins, TFK 12 had more major veins and, consequently, greater phloem tissue height and width than TFM 16. However, the significantly lower interveinal distance found for TFM 16 may facilitate transport of photosynthate and may, therefore, be related to TFM 16's higher CER.

Nonsignificant differences in percent cross-sectional areas of vascular bundle, phloem, metaxylem and mesophyll tissues and in

number of ridges, ridge height, leaf thickness and metaxylem and mesophyll cell diameters were found. Mesophyll cell diameter did not appear to be related to CER in this study since significant differences for CER were found previously but none were found for mesophyll cell diameter of the same leaves.

INTRODUCTION

Examination of leaf anatomy is important in studies which attempt to relate plant structure to function. Interspecific differences in leaf anatomy are well documented. For example, plants possessing the photosynthetically-efficient C_4 pathway also have the distinctive Kranz leaf anatomy (Downton and Tregunna, 1968; El-Sharkawy and Hesketh, 1965). Variation in leaf anatomy among cultivars or genotypes within a species has also been found, e. g. for alfalfa (Delaney and Dobrenz, 1974; Dobrenz et al., 1971), soybean (Dornhoff and Shibles, 1976) and ryegrass (Wilson and Cooper, 1969).

Several investigators have studied the relationship between leaf anatomy and CO_2-exchange rate (CER). Wilson and Cooper (1970, 1969a, 1969b, 1969c, 1969d, 1967) found a strong negative relationship between light-saturated CER and mesophyll cell size in ryegrass. Specific leaf weight (SLW) of soybean cultivars appeared to be a function of leaf thickness and both features were consistently, positively correlated with soybean CER (Dornhoff and Shibles, 1976). Takeda and Fukuyama (1971) studied 60 grass species and reported that species which have short distances between adjacent vascular bundles had high CER while greater distances were correlated with lower CER. Carlson et al. (1970) found that CER was positively correlated with

thickness of palisade and mesophyll cell layers and with amount of intercellular air space in alfalfa.

Stomatal characteristics have also been studied by many workers because stomates provide the major pathway for both photosynthetic CO_2 uptake and transpirational water loss. Genetic variation in stomatal frequency and size has been reported for bromegrass (Tan and Dunn, 1976), soybean (Ciha and Brun, 1975) and triticale (Sapra et al., 1975), and genetic variation for stomatal frequency has also been reported for barley (Miskin et al., 1972), bentgrass (Shearman and Beard, 1972), corn (Heichel, 1971), ryegrass (Wilson, 1969b) and sorghum (Liang et al., 1975). Stomatal frequency has been shown to vary with leaf surface or position within leaves (Ciha and Brun, 1975; Liang et al., 1975; Shearman and Beard, 1972; Tan and Dunn, 1976). In a number of monocot species, e.g., oats, barley, wheat and corn, stomatal frequency is fairly similar for both leaf surfaces (Nobel, 1974).

The relationships between stomatal characteristics and CER or crop yield remain controversial. Heichel (1971) found that a corn inbred which had higher CER had lower stomatal frequency than an inbred with lower CER. Miskin et al. (1972) found no correlation between stomatal frequency and CER of five barley populations; but since lines having low stomatal frequency transpired less water, they concluded that it might be possible to reduce transpirational water loss

without effecting CER by selecting varieties with fewer stomata. Soybean adaxial stomatal aperture was strongly correlated with CER in one year but showed no relationship the next (Dornhoff and Shibles, 1976). Finally, Walton (1974) reported a positive correlation between stomatal frequency and bromegrass forage yield.

Objectives of the present study were to describe leaf anatomy and stomatal characteristics of four tall fescue selections differing in forage yield and CER and to provide additional insight into characteristics associated with these yield and CER differences.[1]

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[1] Cohen, C. J., Chilcote, D. O. and R. V. Frakes. 1979. Gas exchange and leaf area characteristics of four tall fescue selections differing in forage yield. In: CER and leaf characteristics of four tall fescue (Festuca arundinacea Schreb.) selections differing in forage yield. M.S. Thesis. Oregon State University.

MATERIALS AND METHODS

Plant material and culture have been described previously.[2] Briefly, winter-growing selections TFM 26 and TFM 16, high and low yielding, respectively, and summer-growing selections TFK 4 and TFK 12, high and low yielding, respectively, were studied. Each selection was represented by 10 genotypes which were grown in a greenhouse during fall, 1978. Greenhouse temperature was maintained at 18 ± 3 C. The natural daylength period was extended to a 15-hr day to maintain plants in a vegetative state.

In December, 1978, a series of CO_2-exchange rate (CER) measurements was made using one leaf per genotype and 10 genotypes per selection to describe CER of selections. When this study was complete, each leaf used was excised, then cut in half at the midpoint and several sections approximately 2 mm wide were cut near the midpoint and were placed in a cold 10% (v/v) acrolein solution for tissue fixation. Vials containing the leaf pieces were placed under vacuum (-25 lbs Hg) for 4 hr and were then refrigerated (ca. 4C). Following dehydration and embedding of leaf pieces in glycol methacrylate plastic, 5 μ-thick cross sections were cut using a rotary microtome and were mounted on microscope slides. Slides were stained with toluidine blue, pH 4.4. Two cross sections per leaf

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[2] Ibid.

(i.e. 20 cross sections per selection) were photographed at 100 X magnification with Kodak High Contrast Copy Film.[3] Negatives were mounted in slide holders. Counts, measurements and tracings of anatomical features were made on projected images at 700X magnification. Counts and measurements of the numbers of ridges, vascular bundles, major veins and minor veins, and ridge height, leaf thickness, mesophyll and metaxylem cell diameters, phloem tissue height and width, and interveinal distance were made across an entire cross section. Cross-sectional percentages of epidermal layer, bulliform cell, fiber cap, vascular bundle, vascular bundle fiber, phloem, metaxylem, mesophyll and air space areas were based on tracings of the midridge area of each cross section. Diameters of three mesophyll cells per ridge area and two metaxylem vessels per major vein were measured. Interveinal distance was measured as the linear distance separating outer bundle sheath cells of adjacent vascular bundles. Values for counts and measurements were averaged across each leaf cross section so that 20 values per selection were compared. Components of the leaf tracings (e.g. mesophyll cell area) were cut out and their areas were measured using a Licor LI 3000 leaf area meter. Component areas are expressed as percentages of traced cross-sectional areas so that relative tissue make-up can be

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[3]Trade names are included for the information of the reader and do not imply endorsement by the Oregon Agricultural Experiment Station.

compared; selections were also compared on an absolute-area basis.

Stomatal characteristics of the four selections were also examined. Leaf impressions of fully-expanded leaves of comparable tiller position of the genotypes used in the previous study were made. Clear nail polish was applied to both leaf surfaces and was allowed to dry for approximately 5 min. Double-sided Scotchtape was used to peel dried impressions from the leaves. Five cm-long sections of the impressions at the tip, center and base leaf-blade positions of each leaf surface were mounted on microscope slides and cover slips were applied. Stomatal frequency was evaluated by counting stomata in five 1.85 mm^2 microscopic fields of view (100X magnification) for each surface and leaf-blade position. Stomatal size was estimated by measuring exterior guard cell length of five stomata per surface and leaf-blade position using 430X magnification. A micrometer scale was used to calibrate measurements.

RESULTS AND DISCUSSION

Leaf Anatomical Characteristics

Total cross-sectional area did not differ significantly among selections. However, the relative distribution or partitioning of tissue components, expressed as the percentage of total cross-sectional area, did vary. Before discussing the specific differences that were found, it is appropriate to note several general trends.

General Description of Leaf Anatomy

The two epidermal layers comprised a large fraction of the total cross-sectional area, from approximately 17 to 22% (Table I). The adaxial epidermis made a larger contribution than did the abaxial epidermis to the total epidermal cross-sectional area. This is partly due to the presence of large, specialized bulliform cells--which contribute to the control of leaf roll during water stress as they respond to turgor changes (Esau, 1977)--and partly to the presence of ridges on the adaxial surface.

On the average, ridge height contributed over 44% (Table II) of leaf thickness and an average of 17 ridges were found across the width of leaves. Consequently, this feature is likely to have a strong influence on the thickness of the still air boundary layer and on the associated boundary layer resistance to gas diffusion. Adaxial

stomata are generally located near the bottom of the ridges as well as in the grooves between ridges and they are recessed beneath the outer surface of the epidermis.

Most vascular bundles are located directly below the peaks of ridges. On the basis of tracings to the outside of the outer bundle sheath, vascular bundle tissue comprised about 10% of total cross-sectional area. Sclerenchymatic cells frequently form fiber plates or caps which can extend from the outer bundle sheath of major veins all the way to both epidermal layers in some cases.

Nearly half of all veins across the width of the leaf were major veins (i.e. 47.17 and 51.91% of the veins were major veins for selections TFM 26 and TFK 12, respectively). This contrasts with Hanson and Rasmusson's (1975) finding that all of 210 barley varieties examined had at least twice as many minor as major veins. The arrangement of phloem cells within the vascular bundles is different for major and minor veins with respect to the relationship between phloem height and phloem width of the phloem cell area. While in minor veins, phloem width is generally only slightly greater than phloem height, in major veins phloem width is often two times as great as phloem height (data not shown). The data collected do not indicate whether the greater phloem width of major veins is due to greater cell size, greater cell number or both.

Thick-walled, lignified cells lie between the xylem and phloem

of major veins (Akin and Burdick, 1973) and are referred to as vascular bundle fibers. Cell walls of the inner bundle sheath are thickened radially and toward the inside of the vascular bundle while the outer bundle sheath has larger, thin-walled cells.

Mesophyll cell area comprised the major fraction of the cross-sectional area and averaged 42% of cross-sectional area across selections. Large air spaces are found within the leaf. The traced air space area contributed from 20.29 to 27.19% of the cross-sectional area for selections TFM 16 and TFK 12, respectively.

Selection Differences in Tissue Distribution

While the four tall fescue selections examined have a number of anatomical features in common, they do differ in a number of respects. TFM 16 had a greater percentage of cross-sectional area invested in epidermal layers than did some of the other selections (Table I). The generally greater percentage of adaxial epidermis of TFM 16 can be attributed to its generally greater percentage of bulliform cell area. This latter feature may have noteworthy consequences in terms of control of leaf roll and control of water loss during water-stress periods.

While TFM 16 generally had a lower percentage of vascular bundle fiber area, it generally had a greater percentage of fiber cap area. Since fiber caps are made up of lignified cells and since lignin

is generally recognized as one of the factors limiting forage digestibility (Mowat et al., 1969; Waite et al., 1964), the relatively greater investment by TFM 16 in fiber cap material may have a negative impact on its digestibility and, therefore, its quality as a forage grass.

Selections also differed in percent air space. Both TFM selections had significantly lower percent air space than did the TFK selections. The amount of intercellular air space within a leaf is thought to effect gas diffusion and, therefore, CER (and transpiration). For instance, Dornhoff and Shibles (1976) found significant, positive correlations between the volume of intercellular air space and CER of soybean cultivars in a two-year study ($r=0.64^{**}$ and $r=0.45^{**}$); and, they found significant, negative correlations between volume of intercellular air space and residual resistance to CO_2 diffusion ($r=-0.69^{**}$ and -0.49^{*}). However, in our study, percent cross-sectional air space does not appear to be related to CER since both a high- and a low-CER selection (TFM 16 and TFM 26, respectively) had lower percent air space than did the high- and low-CER selections TFK 12 and TFK 4, respectively. However, the tracing procedure used in this study was not sensitive enough to include all intercellular air space--while large air spaces were easy to see and to trace, the fine intercellular air spaces between more compactly-arranged mesophyll cells were not. The latter portion of the intercellular

air space may be considerably more important to the facilitation of gas diffusion and may have been missed to a large extent in this study.

While the TFM selections had significantly lower percent air space, they did not--as a result--have significantly more mesophyll cell area. Part of this difference among selections in percent air space may have been made up by somewhat greater percent mesophyll and fiber cap area for TFM 26 and by greater percent fiber cap and epidermis in TFM 16. Selections did not differ with respect to percent mesophyll, vascular bundle, phloem or metaxylem tissue areas.

On an absolute-area basis, rather than percent cross-sectional-area basis, significant differences among selections were found for areas of adaxial epidermis, abaxial epidermis, bulliform cell, vascular bundle, vascular bundle fiber, phloem and air space (data not shown).

Differences in areas of adaxial epidermis, abaxial epidermis, bulliform cell, vascular bundle fiber and air space tended to be comparable to those found on a percent cross-sectional area basis. For example, TFM 16 had the greatest mean areas of adaxial epidermis ($P \leq 0.10$), abaxial epidermis ($P \leq 0.01$) and bulliform cells ($P \leq 0.01$) and the lowest mean areas of vascular bundle fiber ($P \leq 0.10$) and air space ($P \leq 0.01$). However, while differences among selections on a percent cross-sectional-area basis were found for fiber cap, nonsignificant differences in fiber cap area on an

absolute-area basis were found. This indicates relatively greater variation within selections, compared to that among selections, for fiber cap area on an absolute basis than for fiber cap area as a percentage of cross-sectional area. Thus, while the absolute tissue area invested in fiber cap material may vary considerably from leaf to leaf within a selection, the percentage contribution of fiber cap to total cross-sectional area may be relatively more stable for these selections.

While nonsignificant differences for percent phloem were found, the two selections having lowest mean percent phloem, TFM 16 and TFK 4, had significantly less phloem area on an absolute basis ($P \leq 0.05$). And, while nonsignificant differences were found for percent vascular bundle tissue, TFM 16 had a lower mean percent vascular bundle tissue and significantly less vascular bundle area on an absolute basis ($P \leq 0.10$).

Selection Differences in Tissue Measurements

Significant differences among selections were found for numbers of vascular bundles, major veins and minor veins, for height and width of phloem tissue and interveinal distance (Table II). TFK 12 and TFM 26 had more vascular bundles per leaf cross-section than did TFM 16. This would not seem to be due simply to differences in leaf width (TFK 12 and TFM 26 having wider leaves than TFM 16)

since the wide-leafed selection TFK 4 had a similar number of vascular bundles compared with either TFM 16 or TFK 12.

TFK 12 had a greater number of major veins than did the other three selections which had a similar number of major veins. Differences in the number of minor veins were significant at the 10% level of probability with TFM 26 having more minor veins than TFM 16. Thus, while both TFK 12 and TFM 26 had more vascular bundles than did TFM 16, TFK 12 had a greater number of major veins but TFM 26 had a greater number of minor veins compared to TFM 16. Major veins have a greater area of phloem tissue and larger metaxylem vessels through which transport can occur, while minor veins, through lateral connections to major veins, may be quite important in the transfer of material (e.g. assimilate, amino acids and other metabolites) to major veins for long-distance transport. TFM 26, having more minor veins than TFM 16, may be better able to move material to major veins for long-distance transport.

It can be seen in Table II that there is a discrepancy between the number of ridges and the number of vascular bundles, especially for TFK 12. While there was usually only one vascular bundle beneath each ridge, this was not always the case. The midridge of some leaves had three vascular bundles, rather than one, and this occurred for more leaves of TFK 12 than it did for other selections.

Although the percent phloem area of traced midridge areas of

leaves did not differ significantly among selections, phloem tissue height and width--averaged across each entire leaf cross section--did vary significantly. Phloem tissue height and width were greatest for TFK 12 which probably reflects the fact that TFK 12 had more major veins than did the other selections.

The average distance between veins, i.e. interveinal distance, was shorter for TFM 16 than it was for other selections. Thus, TFM 16 had fewer vascular bundles than did other selections (i.e. TFM 26 and TFK 12) and they were closer together. While reduced interveinal distance is thought to enhance translocation of assimilate (Crookston and Moss, 1974; Takeda and Fukuyama, 1971), it may not be appropriate to suggest that TFM 16's reduced interveinal distance, relative to other selections, may enhance its translocation of photosynthetic products. (This suggestion would be attractive in light of the hypothesized feedback-inhibition of photosynthesis and the fact that TFM 16 had greater CER per unit leaf area than did the other selections.) A great deal of error is likely to have been associated with measurement of interveinal distance in this study. Differences in the degree of leaf roll among selections could have contributed to this error and an objective test of the degree of leaf roll was not made. Since TFM 16 had a greater percent bulliform cell area, it may have had a greater degree of leaf roll which would have led to reduced interveinal distance measurements in this study.

Leaf thickness, ridge height and number and both mesophyll and metaxylem cell diameters did not differ significantly among selections.

Stomatal Characteristics

Differences Among Selections

A highly significant difference ($P \leq 0.01$) was found across all selections and leaf-blade positions between stomatal frequency of the adaxial and abaxial leaf surfaces. The adaxial surface had more than 3.5 times as many stomata per mm^2, on the average, than did the abaxial surface.

Significant differences among selections for stomatal frequency were also found (Table III). TFM 16 had significantly greater stomatal frequency at the tip and base leaf-blade positions ($P \leq 0.01$ and $P \leq 0.05$, respectively) of the adaxial surface, but no significant differences in stomatal frequency were found among selections at the adaxial center leaf-blade position. TFM 16 had significantly lower stomatal frequency ($P \leq 0.01$) at the abaxial tip and center leaf-blade positions compared to other selections. While nonsignificant differences were found at the abaxial base leaf-blade position, TFM 16 again tended to have lower stomatal frequency at this position.

Significant differences in stomatal length, as estimated by

measurement of exterior guard cell length, between leaf surfaces and among selections were also found. Though occurring with much greater frequency on the adaxial leaf surface, stomata were only somewhat smaller on this surface compared with stomata of the abaxial surface. That is, while stomatal frequency was more than 3.5 times greater on the adaxial surface (averaged across selections and leaf-blade positions), stomata were only about 10% smaller on the adaxial surface compared with those of the abaxial surface (averaged across selections and leaf-blade positions).

Stomata of TFM 16 were smaller ($P \leq 0.05$) at the adaxial tip leaf-blade position than were those of all other selections. At the center leaf-blade position, stomata of TFM 16 and TFM 26 were smaller than were those of TFK 4, while stomatal length was comparable for TFK 12 and TFK 4. Nonsignificant differences in stomatal length were found at the base leaf-blade position.

On the abaxial surface, TFM 16's stomata again were smaller at the tip leaf-blade position and they were also smaller than those of all other selections at the center leaf-blade position. Again, nonsignificant differences in stomatal length occurred at the base leaf-blade position.

Interestingly, TFM 16 generally had greater adaxial stomatal frequency and smaller adaxial stomatal size compared with other selections but the stomata on the abaxial surface--though occurring

with lower frequency--were also smaller. Also, since TFM 16 had greater percent bulliform cell area, it may be able to respond to water stress with greater leaf roll than the other selections and may further restrict water loss to a greater extent since it has fewer and smaller stomata on the abaxial leaf surface (the exposed leaf surface when significant leaf roll has occurred).

The rather lop-sided distribution of stomata between leaf surfaces of these selections suggests that the adaxial stomata may be of primary importance to gas exchange at times of adequate water availability (i.e. when significant leaf roll does not occur). The generally greater adaxial stomatal frequency of TFM 16 might then enhance gas exchange of TFM 16, compared to the other selections, and may be related to its greater CER.

Differences Within Selections

Stomatal size and frequency within selections were also examined for possible differences between leaf surfaces and among positions within each surface (data not shown). Adaxial stomatal frequency was significantly greater than abaxial stomatal frequency within all selections ($P \leq 0.01$), while abaxial stomatal length exceeded that of adaxial stomata within all selections ($P \leq 0.01$).

Three selections, TFM 26, TFM 16 and TFK 12, exhibited similar trends for stomatal frequency and length among positions

within each leaf surface. On their adaxial surfaces, stomatal frequency at the tip leaf-blade position exceeded that at the other two positions ($P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.01$ for TFM 26, TFM 16 and TFK 12, respectively). Nonsignificant differences in stomatal frequency among abaxial leaf-blade positions were found within each of these selections. Also, stomatal length did not vary significantly among leaf-blade positions of either leaf surface within each of these selections.

However, somewhat different results were found for selection TFK 4. On its adaxial surface, while stomatal frequency at the tip leaf-blade position was greater than at the base ($P \leq 0.05$), it was similar to that at the center leaf-blade position. On its abaxial surface, stomatal frequency decreased significantly from the tip to the center to the base leaf-blade position ($P \leq 0.05$). Stomata were significantly longer at the adaxial center position than at the tip or base positions ($P \leq 0.05$) where stomatal size was similar. The same pattern for abaxial stomatal length of TFK 4 was found ($P \leq 0.05$).

For all four selections, stomatal frequency was greatest at the tip, or oldest leaf-blade position, when significant differences were found. This may indicate a similar response among selections to environmental conditions during earlier leaf growth. For instance, development under higher light intensity generally results in increased stomatal frequency (Ciha and Brun, 1975; Cooper and Qualls, 1967;

Hanson, 1917; Penfound, 1931). Water-stress periods during leaf development can also lead to increased stomatal frequency through reduced cell enlargement (Ciha and Brun, 1975).

Though differences for stomatal length among leaf-blade positions were only significant for TFK 4, similar trends were observed for the other selections, i. e. stomatal length tended to be greater at the center leaf-blade position of both leaf surfaces. This also suggests possible similar response to environmental factors. However, similar patterns of genetic variation in stomatal frequency and length could also be responsible for the similarities that were observed.

SUMMARY AND CONCLUSIONS

1. The generalized description of leaf anatomy of these selections included the following noteworthy characteristics--large contributions by bulliform cells and leaf ridging to adaxial epidermal tissue area are expected to be important to the control of leaf roll and boundary layer depth, respectively;

   the presence of fiber caps and vascular bundle fibers as supporting tissues may have negative effects on forage quality;

   about half of the veins are major veins, important in long-distance transport; and,

   while mesophyll cell area comprises the major fraction of leaf cross-sectional area, large air spaces are also found within the leaf and comprised approximately 20 to 27% of cross-sectional area.

2. TFM 16 had a significantly larger percentage of cross-sectional area invested in epidermal cell layers and in bulliform cell area within the adaxial epidermis. This latter feature, in concert with TFM 16's generally smaller abaxial stomata and lower abaxial stomatal frequency, may provide an advantage in the restriction of water loss during water-stress periods compared to the other selections.

3. TFM 16's greater investment in fiber cap tissue, compared to other selections, may represent a negative forage quality factor; furthermore, this difference in dry matter partitioning may reflect a greater investment of energy into formation of support tissue rather than of photosynthetically active leaf tissue.

4. Selections TFM 26 and TFK 12 had more vascular bundles than TFM 16; while TFM 26 had more minor veins, TFK 12 had more major veins and, consequently, greater mean phloem tissue height and width than TFM 16.

5. TFM 16's generally greater adaxial stomatal frequency may be relatively more advantageous for gas exchange when water is not limiting and may be related to its generally higher mean CER and Tn and generally lower mean r_s[4]; also, if the significant differences found for interveinal distance are real, TFM 16's reduced interveinal distance may facilitate movement of assimilate from mesophyll cells to veins and may, therefore, be related to its generally greater CER.

6. The nonsignificant differences found in SLW[5] may reflect nonsignificant differences in leaf thickness since the two features

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[4] Op. cit.

[5] Op. cit.

have been strongly associated in several previous studies (e.g. Dornhoff and Shibles, 1976; Delaney and Dobrenz, 1974.

7. Nonsignificant differences in percent cross-sectional area of vascular bundle, phloem, metaxylem and mesophyll tissue areas and in number of ridges, ridge height, metaxylem cell diameter and mesophyll cell diameter were found. Mesophyll cell diameter did not appear to be related to CER in this study since significant differences for CER were found but none were found for mesophyll cell diameter of the same leaves.

8. Results of this and a previous study[6] indicate that differences in physiological, morphological, anatomical and stomatal characteristics exist among these four tall fescue selections. Further study of the relationships among these characteristics and of their importance to forage yield and quality may suggest characteristics which can be profitably manipulated by plant breeders to bring improvement in tall fescue forage.

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[6] Op. cit.

Table I. Component tissue areas of four tall fescue selections differing in forage yield as a percentage of leaf cross-sectional area.

Selection-Yield	Adaxial Epidermis	Abaxial Epidermis	Bulliform	Fiber Cap	Vascular Bundle	Vascular Bundle Fiber	Phloem	Metaxylem	Mesophyll	Air Space
					%					
TFM 26 - H	12.28 ab*	6.74 ab	6.95 ab	4.99 ab	10.01	0.75 b	1.16	0.64	44.28	21.70 a
TFM 16 - L	14.53 c	7.56 b	9.06 c	5.71 b	9.93	0.54 a	1.07	0.72	41.97	20.29 a
TFK 12 - L	10.77 a	5.91 a	5.57 a	4.13 a	10.11	0.76 b	1.20	0.66	41.89	27.19 b
TFK 4 - H	12.85 bc	6.91 b	7.94 bc	4.22 a	10.17	0.67 ab	1.07	0.63	40.21	25.62 b
L.S.D., 0.05	1.95	0.93	1.46	0.99	n.s.	0.17	n.s.	n.s.	n.s.	3.45
$S_{\bar{x}}$	0.69	0.33	0.52	0.35	0.34	0.06	0.05	0.04	1.16	1.22

*Values not followed by the same letter are significantly different at the 5% level of probability.

Table II. Leaf anatomical characteristics of four tall fescue selections differing in yield.

Selection-Yield	No. Ridges	Ridge Height (μ)	No. Vascular Bundles	Leaf Thickness (μ)	Mesophyll Cell Diameter (μ)	Metaxylem Cell Diameter (μ)	Phloem Height (μ)	Phloem Width (μ)	Interveinal Distance (μ)	No. Major Veins	No. Minor Veins
TFM 26 - H	17.6	126.7	17.7 b*	275	25.6	21.5	29.1 bc	44.7 ab	242 bc	8.3 a	9.3 b+
TFM 16 - L	16.2	120.1	16.3 a	271	24.6	22.3	26.6 a	43.5 a	218 a	8.1 a	8.2 a
TFK 12 - L	17.7	127.1	18.3 b	286	26.0	20.7	30.8 c	46.8 b	254 c	9.5 b	8.8 ab
TFK 4 - H	17.1	117.7	17.1 ab	269	25.3	21.2	27.9 ab	42.4 a	237 b	8.3 a	8.7 ab
L.S.D., 0.10											0.7
L.S.D., 0.05	n.s.	n.s.	1.4	n.s.	n.s.	n.s.	2.0	3.0	15	1.0	
$S_{\bar{x}}$	0.5	3.9	0.5	6	0.5	0.7	0.7	1.0	5	0.3	0.3

*Values not followed by the same letter are significantly different at the 5% level of probability.

+Values not followed by the same letter are significantly different at the 10% level of probability.

Table III. Stomatal frequency and length at three leaf-blade positions for adaxial and abaxial leaf surfaces of four tall fescue selections differing in yield.

Selection - Yield	Leaf-blade position: Tip	Center	Base	Leaf-blade position: Tip	Center	Base
	stomatal frequency/mm²+			stomatal length, u++		
			Adaxial			
TFM 26 - H	89.0[1] a*	76.4[2]	79.3[3] ab	38.6 b	40.8[6] a	37.7
TFM 16 - L	102.5 b	79.9[4]	86.1[5] b	34.4 a	39.0[7] a	38.3
TFK 12 - L	88.4 a	75.5	74.8 a	39.7 b	41.6 ab	40.0
TFK 4 - H	89.8 a	80.0	74 4 a	40.4 b	44.5 b	39.7
L. S. D., 0.05	8.3	n.s.	9.3	3.8	3.6	n.s.
$S_{\bar{x}}$	2.9	2.8	3.2	1.3	1.2	1.4
			Abaxial			
TFM 26 - H	24.5 b	19.4 a	24.0	43.2 b	46.6 b	41.6
TFM 16 - L	10.3 a	15.5 a	17.9	38.9 a	41.8 a	41.8
TFK 12 - L	27.9 b	28.4 b	28.4	45.9 b	46.0 b	45.6
TFK 4 - H	29.6 b	28.9 b	23.5	44.8 b	48.5 b	42.7
L. S. D., 0.05	9.5	8.1	n.s.	4.2	3.7	n.s.
$S_{\bar{x}}$	3.3	2.8	2.9	1.5	1.3	1.2

* Values not followed by the same letter are significantly different at the 5% level of probability.

\+ Each value is the mean no. of stomata from 50 microscopic fields except as noted below.

++ Each value is the mean of 50 stomata except as noted below.

[1] Mean no. of stomata from 45 microscopic fields.

[2] Mean no. of stomata from 35 microscopic fields.

[3] Mean no. of stomata from 45 microscopic fields.

[4] Mean no. of stomata from 20 microscopic fields.

[5] Mean of stomata from 40 microscopic fields.

[6] Mean of 45 stomata.

[7] Mean of 40 stomata.

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APPENDIX I - LITERATURE REVIEW

LEAF ANATOMY

Genetic Variation

Examination of leaf anatomy is important in studies which attempt to relate plant structure with function. Interspecific differences in leaf anatomy are well documented. For example, plants possessing the photosynthetically efficient C_4 pathway also have the distinctive Kranz leaf anatomy (23, 24, 75). El-Sharkawy and Hesketh (24) examined 15 species--both dicots and monocots--which represent a range of photosynthetic capacity. Their data show an apparent negative relationship between photosynthetic rate and the average diameter of palisade cells; the tropical grasses they examined had a larger ratio of internal cell surface area exposed to air:cell volume. This higher surface area-to-volume ratio is thought to facilitate diffusion of CO_2 into cells (24, 52, 93, 95, 96, 97, 98). El-Sharkawy and Hesketh also found a significant negative correlation between photosynthetic rate and leaf thickness which they thought might reflect the effect of an increased path length for CO_2 diffusion on photosynthetic rate (24).

Variation in leaf anatomy among cultivars or genotypes within a species has also been found (19, 20, 21, 93, 94, 95, 96, 97, 98). Delaney and Dobrenz (19) found that alfalfa genotypes which differed in photosynthetic rate also had differences in internal leaf anatomy. Clones which had the largest area per leaflet had the lowest photosynthetic rates (leaf area basis), thinner leaves and lower specific leaf weight. Specific leaf weight was largely dependent on leaf thickness while leaf thickness and palisade tissue thickness were closely associated. Leaves having thick palisade tissue appeared to have long cells and two layers of palisade cells were found in the thickest leaves. Net photosynthetic rate was also significantly associated with thickness of palisade tissue.

Dornhoff and Shibles (21) found that specific leaf weight and leaf thickness of four soybean cultivars were consistently, positively correlated with net CO_2 exchange rate (CER); specific leaf weight seemed to be a function of leaf thickness rather than density. Also, both CER and the combined mesophyll-carboxylation resistance (r_r) were related to the thickness and cellular volume of the several leaf tissue

layers; the association of these characters with CER and r_r was positive and negative, respectively. Watanabe and Tabuchi (96), however, found no correlation between leaf photosynthetic rate and mesophyll volume per unit leaf area for 15 soybean varieties when they were extremely careful to use leaves of comparable age and stem position. Dornhoff and Shibles may not have used comparable leaves since they simply examined the most recent fully-expanded leaf of the main axis for each cultivar each day. The characters cell diameter, exposed cell surface area and cell surface-to-volume ratio appeared poorly related to CER in Dornhoff and Shibles' study and they concluded that smaller cells having relatively more surface area provide no advantage in soybean.

This contrasts with the findings of El-Sharkawy and Hesketh reported above and with the reports of Wilson and Cooper who have worked with different Lolium species and with various Lolium perenne L. populations and have found a strong negative relationship between light-saturated photosynthetic rate and mesophyll cell size (93, 94, 95, 96, 97, 98). (Note: Dornhoff and Shibles also measured CER under saturating irradiance.) However, when light intensity was limiting, apparent photosynthesis was not related to any anatomical feature (95). Wilson and Cooper's 1970 paper reports work designed to test the efficacy of using mesophyll cell size (i.e. mean cross-sectional area) as a selection criterion for light-saturated, single leaf photosynthetic rate. Selection for small mesophyll cells within a population of "Veya" perennial ryegrass generally resulted in smaller mesophyll cells, heavier seed and greater yield of shoot dry matter than did selection for large cells; families from small-celled parents had greater NAR (and lower LAR and RLGR since these were negatively correlated with NAR) than those from large-celled parents between the third and fifth leaf stage; and, light-saturated photosynthetic rate of young, fully-expanded leaves was negatively related to cell size in the F_1 generation. Thus, Wilson and Cooper concluded that mesophyll cell size is a useful criterion for selecting for light-saturated photosynthesis in Lolium perenne.

Environmental Variation

The environmental conditions under which plants are grown and leaves develop are also known to effect leaf anatomy. The most commonly examined environmental variable appears to be light intensity, though temperature and moisture status effects have also been examined. The effects of temperature and light intensity may be difficult to separate under field conditions since solar radiation

and ambient temperature generally vary together. Brown (6) studied leaf anatomy of several grass types (e.g. festucoid, panicoid, chloridoid and aristidoid) and suggested that the various types of leaf anatomy found may be correlated with the light intensity and/or temperature conditions that are typical of the habitats generally occupied by the various grass groups.

Numerous reports compare leaf anatomical features of sun and shade leaves (13, 29, 57, 82). Sun leaves generally are thicker than shade leaves and often have more cells and more highly organized and differentiated cells (56). For example, Cooper and Qualls (13) found that the greater leaf thickness of sun leaves of alfalfa and birdsfoot trefoil plants appeared to be related to both larger number and greater size of palisade and mesophyll cells; the palisade layer also appeared more clearly differentiated in sun leaves. However, shaded leaves of both species appeared to have more intercellular air space than sun leaves. Hanson (29) had reported previously that sun leaves of various tree species had smaller air spaces. And, Turrell (82) found that the ratio of internal-to-external surface area of leaves is low for shade leaves and high for sun leaves of the same plant.

In a controlled environment study, Nobel et al. (52) wished to determine if the increased photosynthetic rates of Plectranthus parviflorus Henckel, which occur for plants grown under higher illumination, could be accounted for by changes in leaf anatomy. Plants were grown at 20 C, 55% relative humidity and light levels of 1000 to 40,000 lux. Illumination received during development was found to have a major effect on internal leaf anatomy and all of the increases in photosynthetic rate at higher light levels were accounted for by the increases in internal leaf area which occurred (expressed as mesophyll cell surface area per unit leaf area, A^{mes}/A). The increase in A^{mes}/A resulted from lengthening of cells in the upper two palisade layers, development of more palisade layers and increased size and frequency (cell number per unit leaf area) of spongy mesophyll cells. From this report it appears that increased photosynthetic rates can result from development under high light of thick leaves which have both increased cell size and increased cell surface area exposed to intercellular air spaces (per unit leaf area).

Wilson and Cooper (97) reported that temperature during growth had a significant effect on cell size, cell number and mesophyll thickness of Lolium genotypes--all genotypes had thinner leaves with smaller mesophyll cells when grown at 21 C compared to growth at 15 or 9 C and they again found a negative association between

light-saturated net photosynthetic rate and mean mesophyll cross-sectional area. However, the reports of Bula (7) and Carlson et al. (8) suggest a positive relationship of cell size and photosynthetic capability in alfalfa. Bula found that alfalfa plants grown under higher temperatures developed smaller leaves and lower leaf area per plant and these leaves had smaller, more densely-packed cells; he suggested that this arrangement could impose a much greater barrier to CO_2 diffusion from the stomatal cavity to palisade cells compared to leaves having large cells and more intercellular spaces. Carlson et al. found that net photosynthesis was positively correlated with thickness of palisade and mesophyll cell layers and with amount of intercellular space but was negatively correlated with cell surface-to-cell volume ratio--which increases as cell size decreases.

Bula found that the larger cell size which resulted from cooler growth temperature mainly reflected increased xylem vessel and bundle sheath parenchyma cell size. He also provides a description of his notion of an ideal leaf anatomy: "The optimum leaf anatomy may be one of small palisade cells to facilitate movement of CO_2 to chloroplasts, large intercellular spaces in the parenchyma region to facilitate CO_2 diffusion from the stomata to the palisade cells, and large vascular cells to facilitate translocation of water and photosynthates."[1]

Vascular System Variability

As Bula suggests, vascular cell development is an important component of leaf anatomy. Numerous workers suggest that photosynthetic rate might be influenced by the degree and/or rate of translocation of photosynthate from leaves. Neales and Incoll (48) provided a comprehensive review of the hypothesis of feedback inhibition of photosynthesis and concluded that although a negative correlation exists between assimilate accumulation and photosynthesis, the two processes have not been proved to be causally related. Nevertheless, it is clear that inter- and intraspecific differences in the amount and rate of translocation exist which are correlated with differences in both photosynthetic rates and in leaf anatomy.

For instance, Moss and Rasmussen (46) found that translocation of $^{14}CO_2$-labeled assimilate was three times as fast from maize leaves as from sugar beet leaves. The tropical, C_4 grasses sorghum

[1] Bula, R. J. 1972. Morphological characteristics of alfalfa plants grown at several temperatures. Crop Sci. 12:686.

and millet have been shown to export 70% or more of ^{14}C-labeled assimilate during the first six hours after assimilation while the C_3 species tomato, castor bean, soybean and Nicotiana affinis exported only 45 to 50% during the same time period (33). Hofstra and Nelson (33) report a correlation (r) value of 0.967 between maximum photosynthetic rates and percent of ^{14}C-labeled assimilates rapidly translocated from the leaves of different species--tomato, nicotiana, castor bean, soybean, sunflower, sorghum, millet, sugarcane and corn.

Gallaher et al. (26) studied the relationship between ^{14}C-photosynthate translocation and phloem cross-sectional area for several C_4 and C_3 species (both monocots and dicots were compared) and found that percent translocation from and cross-sectional area of phloem were higher in the photosynthetically more efficient C_4 species. For example, Panicum maxicum had 100% greater translocation out of its 96% greater phloem area (in labeled spots) compared to P. melioides. However, though photosynthetic rates were higher for the C_4 species, the differences in photosynthetic rate were not as great as those found in translocation rate--so, more favorable translocation alone cannot account for the higher photosynthetic rates of the C_4 species examined.

C_4 grasses have been shown to have fewer cells between leaf vascular bundles than C_3 species (15, 75). Crookston and Moss (15) found that the C_3 species they examined had an average of 12 rows of mesophyll cells between vascular bundles and the distance between vascular bundles averaged 0.27 mm while only 2 rows of mesophyll cells and 0.11 mm, on the average, separated vascular bundles of C_4 species. They also found that C_4 leaves had about three times as many vascular bundles per unit leaf width as leaves of C_3 species. The greater vein frequency in C_4 species permits direct access to a vascular bundle sheath cell for each C_4 mesophyll cell and is thought to be an extremely efficient arrangement for rapid translocation of photosynthetic products (15, 75). Takeda andFukuyama (75) studied 60 grass species and found that species which have short distances between adjacent vascular bundles had high photosynthetic rates while greater distances were correlated with lower photosynthetic rate. Hanson and Rasmusson (28) examined 210 barley varieties and found two-fold differences in leaf vein frequencies among varieties. Varieties also differed significantly with respect to numbers of major and minor veins and ratio of minor to major veins; all varieties had at least twice as many minor veins as major veins.

Stanwood (73) examined winter wheat varieties and found

significant cultivar differences in total vascular area though none occurred for the number of vascular bundles per unit leaf cross-sectional area. Correlations between vascular area and net carbon exchange rates were also generally lacking.

Segovia and Brown (66) provided information on the relationship of phloem size to leaf size and position in tall fescue, sugarcane, peanut and soybean. In all four species, leaf area increased with leaf position from the stem base upward. However in tall fescue, soybean and peanut, the last leaf or two to appear were smaller than the one previous. Phloem area in leaf bases of tall fescue and sugarcane and in petioles of peanut and soybean was closely related to leaf area and followed the same trend as leaf area with leaf position. This relationship was linear in tall fescue and the correlation coefficient (r) was 0.96. Phloem area was also a linear function of bundle number in tall fescue leaves; as bundle number increased from 9 to 15 in the smallest and largest tall fescue leaves, respectively, phloem area per bundle increased only from 500 to 593 $\mu 2$. Therefore, increased phloem area with leaf position resulted primarily from increased bundle number rather than increased phloem area per bundle.

Segovia and Brown also found that shading treatments reduced phloem area in soybean and peanut both in absolute amount and when expressed per unit leaf area since leaf area increased with shading. Shading also reduced specific leaf weight and, therefore, phloem area per unit leaf weight increased with shading. Since shading increased leaf area but decreased phloem area, the two characteristics do not seem to be as closely related as the correlation data initially suggested.

These workers caution that phloem cross-sectional area provides at best only an approximation of conductive tissue for several reasons: what tissue is included in phloem measurements is quite subjective, especially in monocots; sieve tube cross-sectional area may be more relevant to photosynthate translocation but is hard to measure; and, not all phloem tissue or sieve tube elements measured may be functional.

Segovia and Brown's data point out the importance of careful leaf selection in sampling; and, Metcalfe (44) indicates that for comparative purposes it is bewt to take thin sections from the same part along leaf blades, since structural variations along the leaf blade occur. Metcalfe also provides a generalized description of tall fescue leaf anatomy and notes that variation occurs to some extent, particularly with respect to sclerenchyma distribution.

SPECIFIC LEAF WEIGHT

The relationship between photosynthetic rate and specific leaf weight (i. e. leaf dry weight per unit leaf area) is reported by some as positive (8, 19, 36, 55, 103) but others report a negative association (10, 32, 34) or no association (86) between these parameters. The positive relationship found by some workers may be explained if, in these instances, SLW reflects photosynthetically advantageous leaf anatomical characteristics. This appears to be the case in Delaney and Dobrenz's study of alfalfa genotypes which exhibited a wide range of leaflet sizes (19) and Carlson et al.'s study of alfalfa, orchardgrass and birdsfoot trefoil plants (8). In these reports, SLW reflected leaf thickness; photosynthetic rate was associated with thickness of palisade and/or mesophyll layers as well as SLW.

Specific leaf weight, however, can also reflect the relative balance between photosynthate production and translocation (11) and Chatterton (10) interpreted the close, negative relationship he found between NCE and SLW of alfalfa leaves as evidence supporting the hypothesis of feedback inhibition of photosynthesis. The accumulation of soluble sugars is proposed to exert this feedback control (47), but starch accumulation, rather than soluble sugar accumulation, has also been associated with reduced photosynthetic rate and a concomitant increase in mesophyll resistance (r_m) (32, 47). Hofstra and Hesketh (32) were among those who reported a negative association between photosynthetic rate and SLW, and they concluded that the suppression of photosynthetic rate they observed was primarily due to increased r_m which they attributed to leaf starch content.

Different interpretations of the usefulness of SLW as a selection criterion are also possible. Both Barnes et al. (2) and Song and Walton (71) have found significant variation for SLW among alfalfa varieties and genotypes, respectively. They have also both concluded that SLW and leaf area are independent traits and have suggested that it might, therefore, be possible to enhance light interception, via larger leaflet size, and photosynthetic capability, via high SLW, simultaneously in a selection program. Inherent in this proposal is the assumption that selection for higher SLW will bring higher photosynthetic rate. On the other hand, Chatterton--who also worked with alfalfa--suggests that translocation characteristics of genotypes (e.g. low SLW) must be evaluated as well as NCE rate if selection for high NCE rate is to bring significant progress toward increased productivity.

Specific leaf weight has also been shown to vary with environment. Several reports indicate that SLW increases as light intensity increases (3, 11, 56, 101, 102) and as temperature increases (7, 103). Chatterton et al. (11) found diurnal variation in SLW in alfalfa and corn--SLW increased with increasing sun light intensity from a morning low to an afternoon high and then decreased as light intensity decreased during the afternoon. However, minor light intensity fluctuations were not accompanied by significant changes in SLW. Total nonstructural carbohydrate (TNC) level displayed a similar diurnal pattern and a highly significant correlation between SLW and TNC was found for both alfalfa and corn. Chatterton et al. suggest that if feedback inhibition of photosynthesis does occur, photorespiration may be seen as a regulatory mechanism which compensates for slower translocation.

Specific leaf weight, as well as photosynthetic rate, light saturation intensity and RuBP carboxylase activity, has been shown to increase as light intensity during growth increases. Also, Pearce and Lee (56) demonstrated that SLW and net photosynthesis adapt to changes in light intensity. In their experiment, alfalfa plants were grown under high (32-43 klux) or low (13-14 klux) light intensity; one week after leaf unfolding, half of the plants in each regime were transferred to the other, and two weeks later, plants were again moved so that all combinations of light intensity and leaf age were obtained in fourteen treatments (H, L, HH, HL, LH, LL, HHH, HHL, HLH, HLL, LHH, LHL, LLH, LLL). Generally, SLW and net photosynthesis of alfalfa leaves changed when light intensity was changed and the observed change was almost enough to bring the values to levels similar to those of leaves which had continuously been at that light intensity. But, carry-over effects were seen since SLW and net photosynthesis of leaves developed under high light, for example, did not decrease to the same level of those developed and kept under low light intensity; a similar discrepancy occurred when plants which developed under low light were moved to high light intensity. This apparent pre-conditioning may reflect morphological differences due to light intensity received during leaf development, while the apparent adaptability may have been due to changes in light absorption (e. g. pigment characteristics) and biochemical activity in response to changed light intensity (56).

Since they had previously found that alfalfa SLW and NCE were influenced by leaf position in the canopy (102), Wolf and Blaser designed an experiment which separated the effects of leaf aging and low light intensity that basal leaves normally experience in dense alfalfa canopies (101). They concluded that the observed decline of

photosynthetic efficiency and SLW was due to low light intensity. When light intensity was maintained at 100% normal daylight by plant thinning, SLW and NCE values remained high; these values dropped sharply in normal dense canopies as light decreased with increasing canopy depth. The large magnitude of this effect is considered quite important since "even in young developing alfalfa canopies, 20 cm in height, there may be less than 15% of incident light penetration to bottom leaves."[2] Wolf and Blaser also suggested that a moderate negative relationship between yield and SLW might be expected. They pointed out that SLW and NCE will decline due to reduced light intensity in canopies that develop large leaf areas quickly and that large and fast-developing canopies are associated with high yields.

STOMATAL CHARACTERISTICS

Control Theory

The following summary of information on control of stomatal behavior is primarily taken from Meidner and Mansfield's 1968 book, Physiology of stomata (42), and Raschke's 1975 review article, "Stomatal action" (61).

Ambient external factors do not always control stomatal aperture since endogenous rhythms also appear to be important--stomata tend to open during the day and close at night. However, rhythms apart, light usually produces stomatal opening; the maximum speed of this opening response varies with species.

As early as 1932, Scarth proposed that the light-dark responses of stomata might be due to photosynthetic removal and respiratory production of CO_2 inside the leaf. Freudenberger (1940) tested the effects of CO_2 concentrations over a range within physiological levels and found that increased CO_2 concentration produced closure while decreased concentration produced opening in illuminated or darkened leaves and in etiolated or normal leaves. Heath and Heath and Russell (1948-1954) further established the close relationship between CO_2 concentration and stomatal aperture and found that both light intensity and CO_2 concentration determine extent of opening--the higher the light intensity, the higher the CO_2 concentration required

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[2]Wolf, D. D. and R. E. Blaser. 1972. Growth rate and physiology of alfalfa as influenced by canopy and light. Crop Sci. 12:24.

to produce a given degree of stomatal closure. Since greater CO_2 conconsumption in photosynthesis occurs at higher light intensity a greater external CO_2 concentration was required to maintain a given internal concentration.

Evidence also exists for light effects independent of CO_2 concentration. Blue light has been found to be much more efficient than red light in producing stomatal opening and blue light at light intensities below the light compensation point can produce opening. Meidner and Mansfield suggested that shorter wavelengths can maintain some degree of stomatal opening under low light conditions. Thus, when light level falls below the compensation point, stomatal opening might still allow CO_2 influx. Also, they suggest that large fluctuations in aperture might be somewhat avoided in cloudy weather with rapidly changing light intensity.

Temperature can effect stomatal aperture through CO_2 concentration as well as through its effects on plant water status, and, therefore, cell turgidity. For example, the midday closure frequently seen in plants growing in hot climates can be explained by the relatively greater temperature stimulation of respiratory CO_2 output than photosynthetic CO_2 utilization. Heath and Orchard (1957) studied the effect of temperature on CO_2 compensation point of onion and coffee plants and found that the CO_2 concentration at compensation rose with temperature over the range 10-35 C. They found they could prevent the stomatal closure that normally occurred in response to a temperature increase from 25 to 35 C by flushing the central leaf cavity of onion with CO_2-free air. Also, stomatal opening increased progressively with temperature if the onion leaf was swept with air of normal CO_2 concentration, but when sweeping was stopped at higher temperature, closure occurred.

Plant water status also effects stomatal behavior and both passive and active movement have been distinguished. Stalfelt (1955) referred to passive movements as those determined by forces outside the guard cells while active movements are brought about by participation of the guard cells. For example, passive opening occurs when a subsidiary cell is punctured, relieving the pressure normally exerted by the turgid subsidiary cell on the guard cell (Heath, 1938); passive closing occurs to some extent when adjoining epidermal cells are relatively more turgid than the guard cells--thus, maximum stomatal opening can occur when plants are slightly water stressed since this supraturgid state is relieved.

Active stomatal behavior is accompanied by changes in osmotic pressure, then water content of guard cells as potassium ions move

into and out of guard cells. Potassium ion influx accompanies opening movements and operates against a concentration or free energy gradient, and thus requires energy input.

It has also been found that stomatal sensitivity to CO_2 concentration increases progressively with increasing water deficit (Heath and Mansfield, 1962). This provides an enhanced ability to retard transpirational water loss. Though both water loss and CO_2 uptake are reduced by stomatal closure, the proportional drop in photosynthesis is probably less than that for transpiration due to the importance internal resistances play in determining photosynthetic rate.

The above summary of stomatal behavior is greatly simplified. The importance of interactions between environmental factors in control of stomatal behavior needs to be stressed since a complex of factors, often mutually reinforcing, occurs in the field. Also, hormonal influences appear to be involved as well. Increased leaf abscisic acid concentrations are associated with stomatal closure (27, 41, 58, 60, 61, 62, 63) while kinetin treatment is associated with an opening response (43).

Environmental and Genetic Variation

Details of stomatal development in corn and oat leaves have been provided by ultrastructural studies (37, 72). According to Esau (25a), stomatal development begins just before the main period of epidermal meristematic activity is completed and continues through cell enlargement during much of the leaf extension period; in parallel-veined leaves, in which stomata are arranged in longitudinal rows, stomatal formation begins at leaf apices and progresses downward. Brown and Rosenberg (4) found that stomatal size increased while density (i.e. number per unit area, also referred to as frequency) decreased during sugar beet leaf development so that the two factors tended to compensate for one another in their contributions to leaf resistance. A negative, or inverse, relationship between stomatal size and frequency is widely reported (12, 40, 57, 65, 76, 77, 91).

Supraoptimum stomatal density results in interstomatal interference; however, Parlange and Waggoner (53) state that if interstomatal spacing is at least three times stomatal length, interstomatal interference is negligible. Based on Salisbury's early work with English woodland flora, Teare et al. (79) inferred that under given

environmental conditions, a species tends to develop a definite proportion of stomata compared to epidermal cell number. Using Salisbury's stomatal index: $I = [S/(E+S)]\ 100$, where S is stomatal frequency and E is epidermal cell frequency, Teare et al. confirmed their inference based on a two-year study of 41 Triticum selections.

Davis et al. (18) noted that a generally reported trend during plant development is of an increase in stomatal density in later-produced leaves; this trend was not supported by the results of their study of leaf aging effects on stomatal resistance. They did find, however, that beginning at leaf maturity, diffusive resistance of a whole, fully-expanded bean leaf, exposed to constant light intensity, increased continuously with age until leaf abscission. Furthermore, this leaf age effect on leaf resistance was found to be uniform for different leaves of the same plant when they were compared on a relative developmental age basis (i.e. leaf resistance plotted as function of percent time from leaf maturity to death) and it operated independently of both immediate and long-term shading effects, which also produce increased leaf resistance (14). Perhaps this increase in stomatal resistance with leaf aging contributes to the decline in photosynthetic capacity of leaves as they age.

As was just mentioned, light intensity effects stomatal behavior. Development is also effected. Development under higher light intensity generally results in increased stomatal frequency (9, 12, 13, 29, 57) while shading reduces stomatal frequency (9, 13, 29, 57). However, the effect of light intensity on stomatal characteristics may not be the same for both leaf surfaces. For instance, Penfound (57) reported that stomatal frequency was 85% greater for the upper epidermis and 75% greater for the lower epidermis of sunflower leaves grown in full sun. Charles-Edwards and Ludwig (9) also reported a similar unsymmetrical increase in upper and lower stomatal frequencies of tomato leaves with increasing light intensity during growth. Also, as Sharpe (67) found with field-grown cotton, stomata on one leaf surface can respond differently to light, temperature, and moisture conditions than do stomata on the other leaf surface.

Environmental factors other than light intensity, e.g. temperature and moisture status, are also likely to effect stomatal development since they effect cell and leaf expansion. For instance, one might expect to find relatively low stomatal frequency for leaves grown under optimum moisture conditions, since cell enlargement would be favored and relatively large epidermal cells might separate stomata. Teare et al. (79) report that this was found to be the case

by Van de Roovaart and Fuller (1935) who examined stomatal frequency in cereals. More recently, Ciha and Brun (12) found that water-stressed soybean plants had significantly greater stomatal frequency, smaller leaf area and fewer stomata per leaflet surface than did nonstressed plants.

Genetic variation, as well as environmental variation, effects stomatal characteristics. Variation in stomatal frequency has been reported for barley (45), bentgrass (68), bromegrass (76, 77, 85), corn (30, 31), ryegrass (95, 97), sorghum (40), soybean (12) and wheat (65). Stomatal frequency can also vary with leaf position on the plant (30, 31, 40, 65, 68, 77, 79) and with leaf surface or position within leaves (12, 16, 30, 31, 40, 65, 68, 77, 79). Genetic variation in stomatal size has also been reported (12, 65, 77, 91).

The relationships between stomatal characteristics and photosynthetic rate and crop yield remain controversial. Heichel (31) found that a corn inbred which had higher net photosynthetic rate had lower stomatal frequency than an inbred with lower net photosynthesis; he interpreted this finding as a demonstration of the importance of mesophyll resistance. Miskin et al. (45) found no correlation between stomatal frequency and photosynthetic rate of five barley populations; but, since lines having low stomatal frequency transpired less water, they concluded that it might be possible to reduce transpirational water loss without effecting photosynthesis by selecting varieties with fewer stomata. Soybean adaxial stomatal aperture was strongly correlated with CER in one year but showed no relationship the next (21). Leaf permeability, measured by use of a viscous-flow porometer, was significantly rank-correlated with short-term photosynthesis and grain yield of wheat cultivars (70) but stomatal frequency was negatively correlated with sorghum grain yield (40). Walton (85) reported a positive correlation between stomatal frequency and bromegrass forage yield. In fact, factor analysis showed that stomatal length and frequency accounted for more of yield variation than did factors such as plant height and days to heading (20% versus 15%).

Walton also found indications of the role of additive genetic variances in genetic control of stomatal length and frequency. This contrasts with Heichel's report (30) that stomatal frequency of maize depended on a single gene (perhaps conditioned by modifiers). Walton found this report surprising since he believes such a character, i.e. one which influences photosynthesis, respiration and water use,

"must be the outcome of complex, evolutionary pressures and would be expected to have a system of genetic control that seeks to balance a diversity of genetic pathways."[3]

Other genetic studies have examined the heritability of and the effect of selection for stomatal characteristics. High heritability for stomatal frequency was reported in sorghum (40) and smooth bromegrass (76). Wilson (91) reported that heritability of frequency was twice that of length in a ryegrass F_1 population but response was much reduced in the F_2. He also found that direct selection for stomatal resistance was largely ineffective and heritability was low; a genetic shift in stomatal length was found to be compensated for by an opposite shift in frequency. However, selection for long and frequent stomata was more successful than for short or infrequent stomata.

TALL FESCUE INFORMATION

Genetic variability for net photosynthetic rate in tall fescue has been reported (1, 35, 49, 89). For instance, Asay et al. (1) found significant differences in NCE among clonal lines of tall fescue and among their polycross progenies in the field. Variation in dark respiration rate and photorespiration have also been reported (50, 87). Nelson et al. (50) found no significant relationship between dark respiration rate and NCE in Alta tall fescue; a positive relationship between these characters was found in a Kentucky tall fescue base population but none was found after selection. Nelson et al. concluded that independent selection should be possible for these characters. However, photorespiratory rate was positively correlated with NCE; Nelson et al., therefore, concluded that selection for increased NCE would probably also increase photorespiration unless genetic control is independent and selection pressure is exerted for both characters during a breeding program. Nelson et al. (51) also found genetic variation among tall fescue genotypes of diverse origin for activities of several important enzymes: ribulose bisphosphate carboxylase, phosphoenol pyruvate carboxylase, alanine aminotransferase, phosphoglyceryl kinase, glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase and glucose-6-phosphate dehydrogenase.

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[3]Walton, P. D. 1974. The genetics of stomatal length and frequency in clones of Bromus inermis and the relationships between these traits and yield. Can. J Plant Sci. 54:753.

The 1975 Welsh Plant Breeding Station Report (87) included information on individual leaf photosynthesis of tall fescue, though details of experimental conditions were not provided. It was found that maximum photosynthetic rate increased with increasing growth temperature (15, 20, or 25 C) and the greatest rate occurred at about the growth temperature. Leaf size and rates of leaf extension were maximal at 20 C and declined at 25 C. Mesophyll cell area, vascular area and percentage of mesophyll all decreased as growth temperature increased. And, photosynthetic rate per unit mesophyll volume was negatively correlated with mesophyll cell cross-sectional area and also with area of vascular tissue.

Leaf position has also been found to influence photsynthetic rate in tall fescue (88). Seedlings of two tall fescue varieties were grown at 20 C, 16 hr daylength at 70 Wm^{-2} and photosynthetic rates of the third through seventh leaves on the main tiller were measured at light saturation (180 Wm^{-2}) over a temperature range from 13 to 35 C. The temperature response curves were of similar shape for both varieties and all leaf positions with a broad-topped optimum occurring between 20 C and 25 C. But, the maximum photosynthetic rate per unit leaf area declined pretty steadily in both varieties from leaf three to leaf six before reaching a steady value. If leaf area increased with stem position as Segovia and Brown found (66), this might explain this declining trend of photosynthetic rate expressed on a leaf area basis.

Temperature effects on stomatal development in tall fescue have also been found (88). Stomatal frequency greatly increased in plants grown at 25 C versus 10 C largely due to an increase on the abaxial surface. However, stomatal conductance on the abaxial, but not the adaxial, leaf surface also increased significantly for plants grown at 10 C. But, groups of plants showed a similar steady increase in stomatal conductance up to 25-30 C but rapid closure above 30 C.

Treharne and Nelson (80) concluded that leaf growth of temperate grasses is more affected by growth temperature than are physiological parameters such as net assimilation rate. Tall fescue plants grown at 10 C had lower RGR, lower shoot: root ratio, and lower LAR. Leaf growth (area per day) was about 25% of that at 25 C. The much reduced rate of leaf expansion resulted in leaves of 10 C-grown plants having higher SLW and 18% higher net assimilation rate; however, LAR was 42% higher for plants grown at 25 C.

When NCE of plants grown at 10 versus 25 C was measured at different temperatures from 10 to 35 C, a sharp drop in NCE above

30 C occurred for both 10 and 25 C plants and in both 21% and 2% O_2. Porometer measurements of leaf diffusive resistances revealed that stomatal closure occurred at temperatures above 30 C and this was thought to explain the sharp decline in NCE at temperatures above 30 C.

As mentioned previously, variation for dark respiration rate (Rd) among tall fescue genotypes has been reported. Wilson (87) evaluated selected slow- and fast-Rd tall fescue and perennial ryegrass genotypes under field conditions and found that dry matter production of the slow-Rd genotypes exceeded that of fast-Rd genotypes in both species and at all harvests over two years. Dark respiration has been divided into maintenance and growth components. The former is thought to be associated with protein turnover and the repair and maintenance of unstable cell structure of mature plant tissue (57a), while the latter supports synthesis of new plant tissue. Slow rates of maintenance Rd in mature plant tissue may benefit crop growth by reducing respiratory losses (87). However, Jones and Nelson (35a) recently reported that Rd of mature leaf blades (presumably maintenance Rd) of tall fescue genotypes having low, medium or high dry matter yield/tiller corresponded to their yield/tiller classification. But, they also found that a low CER -low yield/tiller genotype had higher Rd of mature blades compared to other genotypes having contrasting CER-yield relationships.

Leaf area development as well as gas exchange characteristics, must be important to forage yield production since the harvested plant material is primarily leaf material. Leaf elongation rate (Le) and leaf width determine the rate of leaf area expansion (La). All three leaf area characteristics have been associated with tall fescue forage yield. Horst et al. (33a) suggested that Le may be an acceptable selection criterion for forage regrowth and yield potential because it was positively related to tall fescue forage yield. Leaf width, along with tiller number and tiller dry weight, contributed most to forage dry matter yield of different-yielding tall fescue selections (39). However, though Asay et al. (1) found that forage yield increased significantly as leaf width of diverse tall fescue genotypes increased, they reported that leaf width was not significantly correlated with yield. Finally, La was positively correlated with dry matter yield per tiller, a major forage yield component, in a study with low competition conditions (48a); and, under field sward conditions, Lhamby (39) found that higher-yielding, wider-leafed selections generally had greater La than a lower-yielding, narrow-leafed selection.

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Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance

Character	SV	df	Mean Square	$S_{\bar{x}}$	C.V. %
CER - Dec, 1978	Among Sel.	3	3.55**	0.19	4.70
	Within Sel.	16	0.19		
CER - Apr, 1979	Among Sel.	3	0.73 n.s.	0.81	10.93
	Within Sel.	8	1.97		
R_d - g^{-1} basis	Among Sel.	3	150,250.13 n.s.	90.47	37.94
	Within Sel.	36	81,850.30		
R_d - cm^{-2} basis	Among Sel.	3	2.23 n.s.	0.45	38.42
	Within Sel.	36	2.02		
Transpiration	Among Sel.	3	5.39 n.s.	1.05	15.12
	Within Sel.	8	3.32		
Σr_{H_2O}	Among Sel.	3	8.24 n.s.	1.08	15.58
	Within Sel.	8	3.51		
Σr_{CO_2}	Among Sel.	3	1.53 n.s.	1.00	11.53
	Within Sel.	8	3.00		
r_s	Among Sel.	3	20.00 n.s.	1.69	15.69
	Within Sel.	8	8.54		
r_a	Among Sel.	3	0.0006**	0.0016	5.44
	Within Sel.	76	0.00005		
r_m	Among Sel.	3	11.23 n.s.	1.44	(-)66.50
	Within Sel.	8	6.18		
Unadjusted leaf width	Among Sel.	3	0.067**	0.026	19.70
	Within Sel.	76	0.013		
Adjusted leaf width	Among Sel.	3	0.145**	0.026	11.49
	Within Sel.	76	0.013		

+, *, ** Mean squares are significant at 0.10, 0.05 and 0.01 levels of probability, respectively.

Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance

Character	SV	df	Mean Square	$S_{\bar{x}}$	C.V %
SLW - Dec, 1978	Among Sel.	3	0.43 n.s.	0.21	23.38
	Within Sel.	36	0.45		
SLW - Apr, 1978	Among Sel.	3	0.62 n.s.	0.25	15.90
	Within Sel.	36	0.65		
Le - Nov, 1978	Among Sel.	3	123.05*	1.95	38.00
	Within Sel.	28	30.41		
Leaf W - Nov, 1978	Among Sel.	3	3.13+	0.38	17.33
	Within Sel.	28	1.13		
La - Nov, 1978	Among Sel.	3	3,574.97*	11.71	41.11
	Within Sel.	28	1,097.90		
Le - Dec, 1978	Among Sel.	3	23.85 n.s.	1.57	49.52
	Within Sel.	36	24.72		
Leaf W - Dec, 1978	Among Sel.	3	5.22**	0.31	16.75
	Within Sel.	36	0.98		
La - Dec, 1978	Among Sel.	3	127.39 n.s.	9.29	54.54
	Within Sel.	36	863.13		
Le - May, 1979	Among Sel.	3	3.51 n.s.	1.42	49.26
	Within Sel.	36	20.18		
Leaf W - May, 1979	Among Sel.	3	7.57**	0.33	19.08
	Within Sel.	36	1.08		
La - May, 1979	Among Sel.	3	440.49 n.s.	9.11	60.80
	Within Sel.	36	829.84		

Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance

Character	SV	df	Mean Square	$S_{\bar{x}}$	C. V. %
% adaxial epidermis (including bulliform)	Among Sel.	3	48.30**	0.69	24.48
	Within Sel.	76	9.53		
% abaxial epidermis	Among Sel.	3	9.26**	0.33	21.65
	Within Sel.	76	2.15		
% bulliform	Among Sel.	3	43.93**	0.52	31.38
	Within Sel.	76	5.36		
% fiber cap	Among Sel.	3	10.95**	0.35	33.07
	Within Sel.	76	2.48		
% vascular bundle	Among Sel.	3	0.24 n.s.	0.34	15.10
	Within Sel.	76	2.30		
% vascular bundle fiber	Among Sel.	3	0.20*	0.06	38.74
	Within Sel.	76	0.07		
% phloem	Among Sel.	3	0.09 n.s.	0.05	21.03
	Within Sel.	76	0.05		
% metaxylem	Among Sel.	3	0.03 n.s.	0.04	28.51
	Within Sel.	76	0.03		
% mesophyll	Among Sel.	3	56.07 n.s.	1.16	12.38
	Within Sel.	76	27.17		
% air space	Among Sel.	3	210.10**	1.22	23.11
	Within Sel.	76	30.01		

Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance++

Character	SV	df	Mean Square	$S_{\bar{x}}$	C.V. %
Adaxial epidermis area (including bulliform)	Among Sel.	3	1,549.01+	5.37	26.33
	Within Sel.	76	577.85		
Abaxial epidermis area	Among Sel.	3	389.15**	2.04	18.71
	Within Sel.	76	83.33		
Bulliform cell area	Among Sel.	3	1,618.74**	4.22	35.38
	Within Sel.	76	357.03		
Fiber cap area	Among Sel.	3	546.87 n.s.	3.69	46.21
	Within Sel.	76	272.96		
Vascular bundle area	Among Sel.	3	398.02+	2.99	18.20
	Within Sel.	76	178.97		
Vascular bundle fiber area	Among Sel.	3	22.37**	0.49	43.35
	Within Sel.	76	4.87		
Phloem area	Among Sel.	3	21.04*	0.54	28.95
	Within Sel.	76	5.84		
Metaxylem area	Among Sel.	3	1.26 n.s.	0.26	25.00
	Within Sel.	76	1.40		
Mesophyll area	Among Sel.	3	13,092.28 n.s.	19.05	27.05
	Within Sel.	76	7,258.29		
Air space area	Among Sel.	3	15,745.49**	13.10	33.03
	Within Sel.	76	3,435.09		
Total cross-sectional area	Among Sel.	3	30,386.92 n.s.	33.99	20.49
	Within Sel.	76	23,105.73		

++ Analysis performed on raw data (not converted to actual tissue areas)

Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance++

Character	SV	df	Mean Square	$S_{\bar{x}}$	C. V. %
No. ridges	Among Sel.	3	8.77 n. s.	0.46	12.00
	Within Sel.	76	4.24		
Ridge height	Among Sel.	3	217.99 n. s.	2.70	14.05
	Within Sel.	76	146.05		
No. vascular bundles	Among Sel.	3	14.60*	0.48	12.43
	Within Sel.	76	4.64		
Leaf thickness	Among Sel.	3	565.85 n. s.	3.89	9.02
	Within Sel.	76	302.98		
Mesophyll cell diameter	Among Sel.	3	3.36 n. s.	0.33	8.22
	Within Sel.	76	2.13		
Metaxylem cell diameter	Among Sel.	3	3.94 n. s.	0.47	13.89
	Within Sel.	76	4.34		
Phloem height	Among Sel.	3	30.71**	0.49	10.91
	Within Sel.	76	4.77		
Phloem width	Among Sel.	3	35.46*	0.74	10.64
	Within Sel.	76	10.91		
Interveinal distance	Among Sel.	3	2,184.80**	3.69	9.90
	Within Sel.	76	271.86		
No. major veins	Among Sel.	3	7.88*	0.35	18.25
	Within Sel.	76	2.45		
No. minor veins	Among Sel.	3	4.42+	0.29	14.60
	Within Sel.	76	1.64		

++ Analysis performed on raw data (not converted to actual tissue measurements)

Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance++

Character	SV	df	Mean Square	$S_{\bar{x}}$	C.V. %
Adaxial stomatal frequency	Among Sel.	3	2,735.17**	3.97	13.45
	Within Sel.	103	426.31		
Abaxial stomatal frequency	Among Sel.	3	3,999.30**	3.22	41.09
	Within Sel.	116	311.05		
Adaxial tip stomatal frequency	Among Sel.	3	1,534.68**	5.37	9.93
	Within Sel.	35	288.06		
Adaxial center stomatal frequency	Among Sel.	3	154.15 n.s.	5.13	9.92
	Within Sel.	27	204.25		
Adaxial base stomatal frequency	Among Sel.	3	878.91*	5.99	12.52
	Within Sel.	33	331.64		
Abaxial tip stomatal frequency	Among Sel.	3	2,631.09**	6.12	45.28
	Within Sel.	36	374.30		
Abaxial center stomatal frequency	Among Sel.	3	1,506.86**	5.24	38.94
	Within Sel.	36	274.59		
Abaxial base stomatal frequency	Among Sel.	3	633.15 n.s.	5.37	39.24
	Within Sel.	36	288.63		

++ Analysis performed on raw data (not converted to actual stomatal frequency)

Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance[++]

Character	SV	df	Mean Square	$S_{\bar{x}}$	C.V.%	L.S.D.
TFM 26: Adaxial stomatal frequency	Among positions	2	1,243.05*	5.77	11.05	9.16 (0.05)
	Within positions	22	277.83			
TFM 26: Abaxial stomatal frequency	Among positions	2	261.42 n.s.	5.35	40.44	
	Within positions	27	286.65			
TFM 16: Adaxial stomatal frequency	Among positions	2	3,352.45**	4.87	7.96	7.79 (0.05)
	Within positions	19	173.78			10.65 (0.01)
TFM 16: Abaxial stomatal frequency	Among positions	2	523.00 n.s.	7.47	87.68	
	Within positions	27	558.31			
TFK 12: Adaxial stomatal frequency	Among positions	2	2,024.58**	4.56	9.79	7.15 (0.05)
	Within positions	27	207.70			9.65 (0.01)
TFK 12: Abaxial stomatal frequency	Among positions	2	3.26 n.s.	5.53	33.50	
	Within positions	27	306.11			
TFK 4: Adaxial stomatal frequency	Among positions	2	2,064.53*	6.53	13.72	10.25 (0.05)
	Within positions	27	426.61			
TFK 4: Abaxial stomatal frequency	Among positions	2	377.80*	3.15	19.67	4.93 (0.05)
	Within positions	27	98.95			

++ Analysis performed on raw data, except L.S.D. values which are based on stomatal frequency mm^{-2}

Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance ++

Character	SV	df	Mean Square	$S_{\bar{x}}$	C. V %
Adaxial guard cell length	Among Sel.	3	0.117**	0.027	10.90
	Within Sel.	113	0.021		
Abaxial guard cell length	Among Sel.	3	0.168**	0.027	10.18
	Within Sel.	116	0.022		
Adaxial tip guard cell length	Among Sel.	3	0.080*	0.044	10.80
	Within Sel.	36	0.019		
Adaxial center guard cell length	Among Sel.	3	0.052*	0.041	9.05
	Within Sel.	33	0.016		
Adaxial base guard cell length	Among Sel.	3	0.013 n.s.	0.047	11.41
	Within Sel.	36	0.022		
Abaxial tip guard cell length	Among Sel.	3	0.106*	0.049	10.82
	Within Sel.	36	0.024		
Abaxial center guard cell length	Among Sel.	3	0.087**	0.043	8.97
	Within Sel.	36	0.019		
Abaxial base guard cell length	Among Sel.	3	0.038 n.s.	0.042	9.20
	Within Sel.	36	0.017		

++ Analysis performed on raw data (not converted to guard cell length in microns)

Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance++

Characters	SV	df	Mean Square	$S_{\bar{x}}$	C.V. %	L.S.D.
TFM 26: Adaxial guard cell length	Among positions	2	0.026 n.s.	0.047	11.17	
	Within positions	26	0.021			
TFM 26: Abaxial guard cell length	Among positions	2	0.072 n.s.	0.055	11.86	
	Within positions	27	0.030			
TFM 16: Adaxial guard cell length	Among positions	2	0.063 n.s.	0.059	12.90	
	Within positions	25	0.026			
TFM 16: Abaxial guard cell length	Among positions	2	0.032 n.s.	0.039	9.09	
	Within positions	27	0.015			
TFK 12: Adaxial guard cell length	Among positions	2	0.011 n.s.	0.040	9.31	
	Within positions	27	0.016			
TFK 12: Abaxial guard cell length	Among positions	2	0.0004 n.s.	0.034	7.13	
	Within positions	27	0.0119			
TFK 4: Adaxial guard cell length	Among positions	2	0.072*	0.037	8.60	3.27 (0.05)
	Within positions	27	0.014			
TFK 4: Abaxial guard cell length	Among positions	2	0.094*	0.048	10.06	4.19 (0.05)
	Within positions	27	0.023			

++ Analysis performed on raw data, except L.S.D. values which are based on guard cell length in microns

Appendix II. Analysis of variance mean squares, degrees of freedom and levels of significance

Character	SV	df	Mean Square	$S_{\bar{x}}$	C. V. %
No. tillers/pot - Dec, 1978	Among Sel.	3	6.42*	0.47	35.22
	Within Sel.	36	2.21		
No. leaves/tiller - Dec, 1978	Among Sel.	3	0.96^{+}	0.19	19.00
	Within Sel.	36	0.36		
No. leaves/pot - Dec, 1978	Among Sel.	3	173.49**	1.68	39.53
	Within Sel.	36	28.37		
No. tillers/pot - Jan, 1979	Among Sel.	3	4.76 n.s.	0.60	40.73
	Within Sel.	36	3.62		
No. leaves/tiller - Jan, 1979	Among Sel.	3	0.50^{+}	0.14	13.87
	Within Sel.	36	0.20		
No. leaves/pot - Jan, 1979	Among Sel.	3	136.30*	1.78	37.85
	Within Sel.	36	31.70		
No. tillers/pot - May, 1979	Among Sel.	3	19.47 n.s.	1.12	32.27
	Within Sel.	36	12.60		
No. leaves/tiller - May, 1979	Among Sel.	3	0.86*	0.14	14.43
	Within Sel.	36	0.21		
No. leaves/pot - May, 1979	Among Sel.	3	332.89^{+}	3.74	33.75
	Within Sel.	36	140.11		
Photosynthetic leaf no. on tiller - Dec, 1978	Among Sel.	3	0.97*	0.16	12.66
	Within Sel.	36	0.25		

Appendix III. Supplementary data

Table I. Tillering data

Mean:	December, 1978			January, 1979			May, 1979		
	tillers/pot	leaves/tiller	leaves/ pot	tillers/pot	leaves/tiller	leaves/pot	tillers/pot	leaves/tiller	leaves/pot
TFM 26	3.9a*	2.9 a+	11.2 a*	4.2	3.1 a+	12.9 a*	9.0	3.0 ab*	27.0 a+
TFM 16	5.4 b	3.6 b	19.7 b	5.7	3.6 b	20.4 b	11.6	3.5 c	40.7 b
TFK 12	4.0 a	3.0 a	11.9 a	4.4	3.1 a	13.4 a	12.2	2.9 a	36.4 b
TFK 4	3.6 a	3.1 a	11.1 a	4.4	3.1 a	12.8 a	11.2	3.3 bc	36.2 b
L.S.D., 0.10		0.4			0.3				8.9
L.S.D., 0.05	1.3		4.8	n.s.		5.1	n.s.	0.4	
L.S.D., 0.01			6.5						
$S_{\bar{x}}$	0.5	0.2	1.7	0.6	0.1	1.8	1.1	0.1	3.7
C.V. %	35.2	19.0	39.5	40.7	13.9	37.8	32.3	14.4	33.7

* Means not followed by the same letter are significantly different at the 5% level of probability

+ Means not followed by the same letter are significantly different at the 10% level of probability

Appendix III. Supplementary data

Table II. Photosynthetic leaf number on tiller used in Dec. 1978 CER measurements and regrowth

Selection	Photosynthetic leaf number	Total photosynthetic leaf regrowth (cm)	No. of leaves showing regrowth
TFM 26	4.1 bc*	31.3	7
TFM 16	4.3 c	26.7	6
TFK 12	3.8 ab	15.8	7
TFK 4	3.6 a	37.7	9
L.S.D., 0.05	0.4		
$S_{\bar{x}}$	0.2		
C.V. %	12.7		

* Means not followed by the same letter are significantly different at the 5% level of probability

Appendix IV. Gas exchange and resistance component example calculations

Example CER Calculation:

Given: $44.37 \frac{\mu l CO_2}{l}$ (i.e. 44.37 ppm CO_2 from IRGA deflection)

26.25°C leaf T = 299.25°K

1020.32 mb atmospheric pressure

116.4 cm^2 leaf area

2.8 l min^{-1} air flow rate

$$mg\ CO_2\ l^{-1} = \frac{44.37\ \mu l\ CO_2}{l} \times \frac{273°K}{299.25°K} \times \frac{1020.32\ mb}{1013\ \ mb} \times \frac{44,000\ mg}{mole\ CO_2} \times \frac{mole\ CO_2}{22.4\ l\ CO_2} \times 10^{-6}$$

$$= 0.08008469\ mg\ CO_2\ l^{-1}$$

$$CER = 0.08008469 \frac{mg\ CO_2}{l} \times \frac{2.8\ l}{min} \times \frac{60\ min}{hr} \times \frac{1}{116.4\ cm^2} \times \frac{100\ cm^2}{dm^2}$$

$$= 11.56\ mg\ CO_2\ dm^{-2}\ hr^{-1}$$

Example Tn Calculation:

Given that the reference air stream dew point is 9.50 C and its temperature is 24.17 C, then its water vapor content is 11.2 mg H_2O l^{-1}. And, given that the sample air stream dew point is 18.00 C and its temperature is 30.00 C, then its water vapor content is 19.1 mg

$H_2O\ l^{-1}$.

$$Tn = [H_2O]_{out} = [H_2O]_{in} \times \text{air flow rate} \div \text{leaf area}$$

$$= (19.1 - 11.2)\ \frac{\text{mg } H_2O}{l} \times 2.8\ \frac{l}{\text{min}} \times \frac{1}{116.4\ \text{cm}^2} \times \frac{100\ \text{cm}^2}{\text{dm}^2}$$

$$= 19.00\ \text{mg } H_2O\ \text{dm}^{-2}\ \text{min}^{-1}$$

Example Resistance Component Calculations

r_a: Given that mean leaf width is 0.623 cm, that the leaf has 17.6 ridges of 0.012 cm height and that wind speed is 100 cm s^{-1}, then adjusted mean leaf width =

$$0.623 + (17.6\ \text{ridges} \times \frac{2\ \text{sides}}{\text{ridge}} \times 0.012\ \text{cm})$$

$$= 1.045\ \text{cm}$$

$$r_a = 1.3\ s^{1/2}\ \text{cm}^{-1} \sqrt{\frac{1.045\ \text{cm}}{100\ \text{cm}\ s^{-1}}} = 0.13\ s\ \text{cm}^{-1}$$

Σr_{H_2O}: Given that the water vapor concentration inside the leaf is the saturated water vapor concentration at leaf T, i.e. 27.7 mg $H_2O\ l^{-1}$ at T = 26.25 C, or 0.0277 mg $H_2O\ \text{cm}^{-3}$, that the water vapor concentration of the air stream entering the leaf chamber is 11.2 mg $H_2O\ l^{-1}$, or 0.0112 mg $H_2O\ \text{cm}^{-3}$, and that the Tn rate is 19.00 mg $H_2O\ \text{min}^{-1}$, or 0.00317 mg $H_2O\ \text{cm}^{-2}\ s^{-1}$, then

$$\Sigma r_{H_2O} = \frac{[H_2O]_c - [H_2O]_a}{Tn}$$

$$= \frac{0.0277 - 0.0112}{0.00317} \text{ s cm}^{-1}$$

$$= 5.20 \text{ s cm}^{-1}$$

Σr_{CO_2} : Given that the atmospheric CO_2 concentration is 355.1 ppm, or 0.6356 μg cm^{-3}, that the CO_2 concentration at the chloroplast is the compensation point CO_2 concentration, 0.0805 μg cm^{-3} and that CER is 11.56 mg CO_2 dm^{-2} hr^{-1}, or 0.0321 μg CO_2 cm^{-2} s^{-1}, then

$$\Sigma r_{CO_2} = \frac{[CO_2]_a - [CO_2] \text{ Chl}}{CER}$$

$$= \frac{0.6356 - 0.0805}{0.0321} \text{ s cm}^{-1}$$

$$= 17.29 \text{ s cm}^{-1}$$

r_s : Given the information calculated previously and using 1.56 as $\frac{D_{H_2O}}{D_{CO_2}}$, then

$$r_s = \Sigma r_{H_2O} \left(\frac{D_{H_2O}}{D_{CO_2}} \right) - r_a$$

$$= (5.20 \times 1.56) - 0.13 \text{ s cm}^{-1}$$

$$= 7.98 \text{ s cm}^{-1}$$

r_m : The mesophyll resistance to CO_2 diffusion is the residual resistance,

$$r_m = \Sigma r_{CO_2} - (r_a + r_s)$$
$$= 17.29 - 8.11 \text{ s cm}^{-1}$$
$$= 9.18 \text{ s cm}^{-1}$$

Appendix V. Microtechnique Procedures

Embedding in Glycol Methacrylate for Light Microscopy

References: Feder, N. and T. P. O'Brien, 1968. Plant Microtechnique: Some Principles and new methods. Amer. Journal of Botany. 55(1):123-142.

and

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and

Dr. Fred Rickson, Botany, Oregon State Univ., Corvallis, Oregon.

Fixation of Tissues:

1) Acrolein -

Small pieces of tissue should be fixed in a 10% solution of acrolein in distilled water cooled to about 0°C in ice. This must be done in the fume hood since acrolein is a potent tear gas. Specimens should be fixed 12-24 hours.

2) Glutaraldehyde -

Specimens less than a few mm in diameter should be used since glutaraldehyde penetrates slowly. A 3% solution of glutaraldehyde in 0.025 M phosphate buffer at pH 6.8 should be cooled to 0°C. Material should be left in fixative overnight.

Dehydration and Embedding:

All steps should be carried out at 0°C-4°C.

1) Make two changes in 8 hrs of ethylene glycol monomethyl ether

(synonyms: methyl cellosolve or 2-methoxyethanol).

2) Make two changes in 8 hrs of absolute ethyl alcohol.

3) Change twice in 8 hours with n-propanol.

4) Change twice in 8 hours with n-butanol. Tissue can be stored at this stage for several months (best at 0°C).

5) 1 part n-butanol: 2 parts plastic mixture* for 8 hours in vacuum (make sure vials are in ice).

6) Pure plastic for 8 hours in vacuum (again at 0°C).

7) 8 hours in vacuum at room temperature.

8) Embed tissue in Beem capsules making sure that the tissue is oriented. Cover Beem capsule with a piece of Parafilm and place into oven at 60°C-70°C for 2 hours (or 40°C for 3 days).

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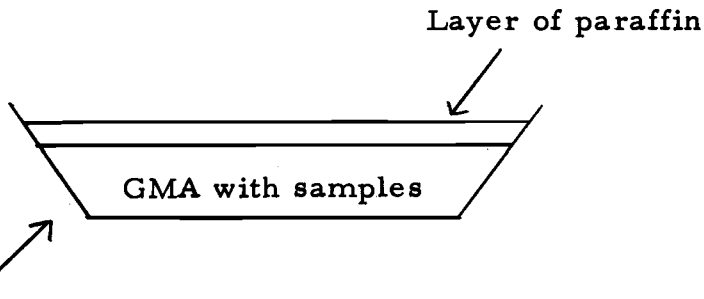
*Plastic mixture: The glycol methacrylate must be purified 5 times as follows: Mix 2 g charcoal with 100 ml glycol methacrylate and stir with a magnetic stirrer. Filter through Whatman #1 filter paper. Repeat this 4 more times. Use two sheets of paper for the final filter. The purified glycol methacrylate is then mixed with 2, 2' Azobis (2-methylpropionitrile) (0.3 g/100 ml methacrylate-polethylene glycol 400 mixture) and polyethylene glycol 400 (10% by volume). This mixture should be stirred for 3 hours, filtered and stored in the freezer.

A low acid glycol methacrylate can be purchased from:

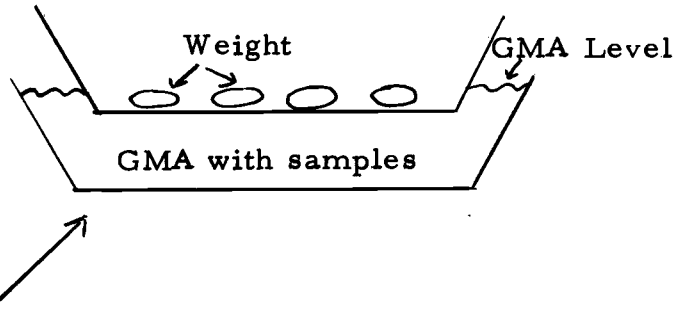
Hartung Associates
33rd Street and Cleveland Avenue
Camden, NJ 08105

The price is $10.00/pound (pound = 500 ml) and you will lose about 200 ml in the filtering process.

Two methods of flat embedding are shown below. The important point of the embedding process is to exclude oxygen as much as possible.



Aluminum weighing pan
Use only at 40°C or the paraffin will melt



Two aluminum weighing pans
Use enough weight to "squeeze" the GMA to the top of the bottom pan. Three-four days at 40°C.