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The University of Arizona PH.D. 1982

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SELECTION OF SPECIFIC STEM WEIGHT AND ASSOCIATION WITH AGRONOMIC AND QUALITY CHARACTERISTICS IN ALFALFA

by

Jerry Gerdine Legg

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Jerry Gerdine Legg

entitled Selection for specific stem weight and association with agronomic and quality characteristics in alfalfa

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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Dissertation Director	Date

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ABSTRACT

The study of readily measurable agronomic traits which may estimate total dry matter production and the determination of their relationships with quality characteristics needs more emphasis in alfalfa breeding. The main objective of this study was the selection of specific stem weight (SSW) and its relationship to other agronomic and nutritive traits. A secondary study was on agronomic and nutritive traits and their relationship with one another. Means, standard deviations, analyses of variance, and correlations were obtained on 98 Mesa-Sirsa alfalfa clones for SSW, plant height, yield, leaf-stem ratio, stem acid detergent fiber (ADF), leaf ADF, stem acid detergent lignin (ADL), and leaf ADL.

The means of SSW (70-47 mg/5 cm), plant height (58-48 cm), yield (52-23 g), leaf-stem ratio (2.1-1.0), stem ADF (45-42%), and stem ADL (10.1-9.1%) varied from one harvest to another depending on the temperature. Significant differences were observed among alfalfa clones on June 1980 for SSW, plant height, yield, leaf-stem ratio, stem ADF, stem ADL, and leaf ADL. Range values were as follows: SSW (92-53 mg/5 cm), plant height (70-41 cm), yield (82-22 g), leaf-stem ratio (1.6-0.6), stem ADF (50-36%) stem ADL (13-8%), leaf ADF (18-13%), and leaf ADL (5-3%). A low correlation was reported between stem ADF and SSW (0.34) and also was observed between stem ADF and leaf ADF (0.46).

The SSW probably would be more effective in increasing total dry matter production in dense stands. The coarseness of the stem was not related with high fiber. High leaf ADF could be useful in selecting for leaf cells which are more resistant to rupture and would gradually release soluble protein in the rumen; thus, the incidence of bloat probably would be reduced. A low stem ADF would make the stem more digestible; therefore, intake of the stem fraction may be greater. With more coarse material in the rumen, salivary secretion might be stimulated. The SSW and stem ADF could be used to select individual genotypes which could increase growth and development and at the same time improve forage quality, since SSW and stem ADF are not related.

INTRODUCTION

Alfalfa (Medicago sativa L.) is the most important cultivated forage crop in Arizona. Its popularity is due primarily to its importance in the dairy ration. Dairy animals consume the greatest quantity of the crop. Dairymen generally are more discriminating about the quality of the feed fed to their animals than people with other classes of livestock. Dairymen have complained about a problem with cattle leaving the coarser, less digestible stem material in the feed bunks. As a result of these complaints, many alfalfa producers are becoming more interested in maintaining or improving on the nutritive value of their hay.

From the animal nutrition standpoint, it would be advantageous to lower the fiber content in the stem. This would increase the digestibility of the stems. Since there is very little information on the relationship between agronomic traits and forage yield in alfalfa, a need exists for research in this area. Specific stem weight could be used as an estimator of growth and development. Some researchers (Dobrenz, Schonhorst, and Thompson, 1969) have noted that increased stem production was associated with high yielding cultivars. With increasing stem production it would be advantageous to select for stems with lower fiber.

The lower fiber in the stem would probably increase forage intake; thus, more nutrients would be available for animal performance.

With more coarse stem material in the rumen, salivary glands would be stimulated and increase saliva flow; thus, bloat incidence may be reduced. Saliva contains antifoaming agents (Mishra et al., 1967; Mishra et al., 1969).

The rupture of cells in the leaves of non-bloating legumes is slower than that of bloating legumes. If there is variation in the fiber content in leaves of bloating legumes, the rupture of leaf cells could be slowed by increasing wall strength of leaf cells. A slower rupture of leaf cells would release soluble protein in the rumen more gradually.

A rapid method of prediction of acid detergent fiber (ADF) and acid detergent lignin (ADL) is needed to study quality traits of alfalfa. The near infrared (NIR) reflectance method might be a technique of predicting these chemical values. Since a large number of individuals can be studied by this method, it is ideal for genetic studies where a large population is needed to find a certain trait.

The main objectives of this study were: (a) to determine the genetic variation of specific stem weight and its relationship with other agronomic traits in a population of alfalfa plants, (b) to determine whether a genotype with low acid detergent fiber of the stem could be found in this population, (c) to determine variation in the acid detergent fiber in the leaf of a bloating legume such as alfalfa, and (d) to determine whether NIR reflectance could be used as a predictor of acid detergent fiber and acid detergent lignin.

LITERATURE REVIEW

Growth and Development

Stems of the alfalfa plant (Medicago sativa L.) are morphological structures which have been studied very little. Increasing specific stem weight could be a useful agronomic trait for increasing forage yield. An abstract by Schonhorst et al. (1977) was the only paper found on specific stem weight (SSW). They reported that an increase in specific stem weight resulted in an increase in dry matter production. The SSW was greatest for the early spring harvest. When a large-leaflet clone was compared with a small-leaflet clone, no difference in specific stem weight was observed; thus, leaflet size appears uncorrelated with SSW (Marcarian, 1980).

As the temperature rises above the optimum level for plant growth, specific stem weight is expected to decrease as the rate of cell elongation decreases. Synthesis of cell wall constituents is involved in cell enlargement; thus, some cell-wall components are not laid down as the temperature increases (Sutcliffe, 1978). Delaney, Dobrenz, and Poole (1974) reported that an increase in leaf-stem ratio resulted as a response to high temperatures. This response indicates that a decrease in stem weight contributes more to yield reduction than does a decrease in leaf weight.

Leavitt, Dobrenz, and Stone (1979) reported that total leaf area per plant also appears to be correlated with forage yield. Dry matter production was positively and significantly associated with total

CO_2 uptake per plant and per leaflet of primary leaves. Large-leaflet clones had higher forage yield, larger leaflet size, and lower SLW than small-leaflet clones when data were compared over the entire growing season. Delaney (1972) and Delaney and Dobrenz (1974a) observed a positive correlation between forage yield and total CO_2 uptake per plant. Foutz, Wilhelm, and Dobrenz (1976) reported that high-yielding alfalfa clones incorporated more CO_2 per plant than low-yielding clones. Forage yield appears more intensely associated with total leaf area per plant because of an increase in leaf area available for photosynthesis. Total leaf area per plant and total CO_2 uptake appears associated positively with total dry matter production, because more photosynthates were available for stem elongation and growth.

Liang and Riedl (1964) found high-yielding alfalfa plants were taller, more upright, and had thicker and more woody stems than low-yielding plants. High-yielding plants usually had a greater number of stems. The forage-yielding advantage of taller plants was probably due to having a greater leaf area resulting in greater photosynthetic activity.

Temperature is important in regulating plant growth. Stem and leaf weights and total leaf area per plant were highest for alfalfa grown at 25 C as reported by Bula (1972) and lowest at 35 C. Leaf-to-stem ratios were the lowest for plants at 25 C. Stem weight was highest for the bottom 10-cm segment of the plants and gradually decreased in succeeding ascending 10-cm segments. Plant response to high temperature was shorter growth and earlier flowering. Both stem and leaf weights

were reduced. Smith (1970) also found that weight of stems of alfalfa decreased from the bottom 10-cm segment to the top, while leaf yields were higher at the center of the plant. Crude fiber also decreased from the bottom to the top of the plant.

Leaf-stem ratio varies among alfalfa cultivars (Dobrenz, Schonhorst, and Thompson, 1969). Leaf-stem ratio also varies among harvest dates. They found harvests made during June and July had the lowest leaf-stem ratio when the dry matter production was the highest, that is, more stem production occurred during June and July.

Smith (1969) found that first-flower stage of growth of 'Vernal' alfalfa was reached in 21 days in a warm regime (32C day/24C night), but not until 37 days when grown in a cool regime (18C/10C). Forage produced during the cool regime contained more in vitro digestible dry matter. Crude fiber was highest in leaflets during the warm temperature period, but was highest in stems during cool temperatures. Shoot height and yields of leaflets, stems, and total herbage were greater under cool temperatures.

The effect of temperature on acid detergent fiber (ADF) and forage yield was studied by Jensen, Massengale, and Chilcote (1967). Forage yield was reduced under the hot environment. The leaf ADF remained relatively constant but stem ADF was higher when plants were grown under high temperatures. Acid detergent lignin (ADL) in the stem was also higher under high temperature (81 F). Total plant ADF and ADL were similar. When alfalfa was cut at 1/10th bloom, ADF and ADL were similar. Feltner and Massengale (1965) found that growth of alfalfa was retarded during August at Tucson, Arizona.

Marten (1970) found at a specific bloom stage that ADF and ADL were similar under warm and cool temperatures, but were slightly lower under warm regimes. Finer stems and shorter internodes of warm-regime alfalfa contributed to the higher leaf percentage. The primary effect of increased temperature on quality of alfalfa was hastened maturity and an accompanied decline in quality.

Luckett and Klopfenstein (1970) and Luckett et al. (1967) found that changes in the cell-wall components of stems and changes in leaf-stem ratio were responsible for variation in digestibility. The amount of ADF, ADL, or cell wall constituents (CWC) in stems was negatively correlated with digestibility of stems and also of the whole plant. Lignin in the leaves was of little importance because of the small amount of lignification of the cell wall.

When Cole, Dobrenz and Massengale (1971, 1972) treated alfalfa with gibberellic acid (GA), there was a significant increase in total dry matter production primarily by increasing stem weight. The GA significantly increased stem-petiole weight and stem length, but decreased leaflet to stem-petiole ratios. Alfalfa responded to GA by producing longer stems but GA had no apparent effect on the amount of leaf tissue.

When indole-3-acetic acid (IAA) was added to woody shoots, cambial division was stimulated and the cambium differentiated into xylem tissue. When GA was applied, cambial derivatives on the xylem side of cambium did not differentiate. A high IAA/low GA concentration ratio favors xylem formation, whereas a low IAA/high GA concentration ratio

favors phloem production. The IAA was important in elongation of cambial initials because it stimulated production of xylem vessels and fibre elements. Application of GA causes further elongation of xylem fibres. The IAA was also an important factor in determining vessel diameter (Digby and Wareing, 1966).

The enzyme, peroxidase, was important in the deposition of lignin on the cell wall and governs the oxidation of phenols to form lignins. The enzyme must be bound to the cell wall before deposition will occur (Brown, 1966; Wardrop, 1971; Freudenberg and Neish, 1968).

Shenk and Elliott (1971) found that progeny from plants high in 6-hr in vitro dry matter disappearance (IVDMD) and low in neutral detergent fiber (NDF) contained an average of 24 vascular bundles per stem. Stems which were low in 6-hr IVDMD and high in NDF contained 31 vascular bundles. Stems also contained a greater number of xylem cells in plants high in NDF and low in 6-hr IVDMD. Stems high in NDF contained 17,217 xylem cells as compared to 13,335 in stems low in NDF. This was a difference of 22.9%. This difference was due to different rates of cambial differentiation.

Location of Digestible and Undigestible Portions of Plants Using Histological Examination

Akin, Burdick, and Amos (1974), using scanning electron microscopy (SEM), found that mesophyll, small vascular bundles, and the outer bundle sheaths of the large vascular bundles were more digestible in leaves of 'Coastcross-1' than in 'Coastal' bermudagrass (Cynodon dactylon, L. Pers.). After 72 hours of digestion, inner bundle sheaths, bands between xylem and phloem, metaxylem vessels, sclerenchyma, and

cuticle remained undigested in the two bermudagrasses. Hanna, Monson, and Burton (1973) studied the patterns of digestion in leaves of bermudagrass. The leaf mesophyll was digested first because it had thinner cell walls and less lignification. Phloem, epidermal cells, and then bundle sheath cells were digested in descending order. Coastcross-1 appeared more digestible; that is, more bundle sheath cells were broken down; however, cuticle, trichomes, xylem, and fibers were not digested. Hanna, Monson, and Burton (1976) found no histological differences, in either the stems or leaves, between high- and low-quality bermudagrass. One-week-old stems were more digestible than 4-week-old stems. The band of thick-walled cells which formed in the 4-week-old stems was resistant to digestion. The higher digestibility of high-quality grass appears due to a larger soluble fraction and more digestible cell wall.

Using SEM, Brazle and Harbers (1977) studied the digestion of alfalfa hay. In the leaf, mesophyll was degraded and vascular tissue was partially degraded after 24 hr digestion. In the stem, 48-hr digestion hydrolyzes the dense matrix exposing fiber cells. Fiber cells are the outer limits of vascular tissue. Most of the vascular tissue which was characterized by vessel cells remained after 72 hr of digestion. Stem material extracted from feces showed the same undegraded vascular tissue as material examined after 72 hr digestion. Vascular tissue in alfalfa stems contains most of the lignin found in the plant. Lignin percentage appears to determine the amount of undigested residue in the reticulorumen.

Selection of Digestibility and Chemical Components which Estimate Digestibility

Burton, Hart, and Lowrey (1967) and Burton and Monson (1972) selected highly digestible plants from a cross between 'Kenya' and 'Coastal' bermudagrasses. Selection of the most digestible F_1 hybrid increased digestibility of 'Coastal' bermudagrass by 12 percent. The vegetatively propagated hybrid fed to dairy heifers gave an average daily gain 30% greater than forage from 'Coastal' (Burton, 1972). This hybrid was called 'Coastcross-1'. Increasing digestibility of bermudagrass by a small, but significant, amount increased average daily gains of animals a substantial amount (30%).

Chapman et al. (1971) grazed steers on 'Coastal' and 'Coastcross-1' bermudagrass and found that average daily gain was greater for 'Coastcross-1' (29%). Steers fed pelleted hay of 'Coastcross-1' in a study by Utley et al. (1971) gained 42% faster than steers fed pellets of 'Coastal' bermudagrass. Average daily gain paralleled voluntary intake. More forage was consumed by steers fed 'Coastcross-1' than 'Coastal' bermudagrass. Utley et al. (1978) fed 'Tifton 44', a highly digestible bermudagrass hybrid. Steers grazing 'Tifton 44' gained .80 kg/day compared with .67 kg/day for steers grazing 'Coastal'. When the varieties were pelleted, animals fed 'Tifton 44' gained 19% faster than animals fed 'Coastal' and required 15.9% less feed per unit of gain.

Thomas et al. (1968) selected for both high and low IVDMD of alfalfa (Medicago sativa L.) and compared chemical constituents and animal performance of the selected strains. Digestible DM intake by sheep was 13% higher for the strain high in IVDMD. High strains fed to

sheep had significantly greater dry matter digestibility, protein content, nitrogen free extract, and lower content of crude fiber and lignocellulose. Similar to bermudagrass, increasing IVDMD of alfalfa by a small amount should increase average daily gain substantially.

Mowat, Fulkerson, and Gamble (1967) reported that stem diameter had little effect on IVDMD or ADF or lignin in both 'Vernal' alfalfa (Medicago sativa L.) and several bromegrass (Bromus inermis, Leyss.) entries. Some plant breeders favor fine stems in their selection programs because of the belief that finer stems have less lignin and are more digestible. Mowat et al. (1967) found that fine stems were not necessarily more digestible. Heinrichs and Troelsen (1965) and Heinrichs, Troelsen, and Warder (1969) found that there was considerable variability in chemical constituents among individual alfalfa plants. They also found that the phenotypic characteristic of coarseness or fineness was not related to crude fiber content or digestibility of stems. There was also no association between leaf and stem crude fiber; therefore, crude fiber appears to be inherited independently in the leaves and stems.

Progeny of two cycles of selection for digestibility of alfalfa were studied by Shenk and Elliott (1970, 1971) and Shenk (1969). General and specific combining abilities were significant for yield, leaf content, and NDF content. Progeny high in 6-hr in vitro dry matter disappearance had low NDF, ADF, and ADL, while NDF, ADF, and ADL were high in progeny which have low 6-hr IVDMD.

Allinson, Elliott, and Tesar (1969) found considerable variation from 45.0% to 27.1% in percent dry matter disappearance using the 6-hr IVDMD technique within populations of alfalfa. Clones with high DMD were lower in CWC, ADF, and ADL. This study also found that fineness and coarseness of stems were not related to digestibility.

Gil, Davis, and Barnes (1967) found variances for crosses and for general combining ability were significantly different for IVDMD, CWC, and ADF, but were correlated negatively with yield.

Leaf and Stem Digestibility, Voluntary Intake and Saliva Secretion

Mowat, Kwain, and Winch (1969) found that lignin and fiber increased as grasses and legumes mature, whereas cell wall digestibility decreased. Leaves were lower than stems in lignin and fiber content and higher in in vitro digestibility. In vitro cell wall digestibility was negatively correlated with cell wall content, ADF, and lignin. Legumes were relatively low in cell wall content but the cell walls were more lignified than were the cell walls of grasses. Grasses appear to have different lignin than legumes, but low lignin can still be used as an estimate of digestibility.

An earlier study of Mowat et al. (1965) found that IVDMD of immature stems of timothy (Phleum pratensis L.), orchardgrass (Dactylis glomerata L.), and bromegrass (Bromus inermis Leyss.) was higher than that of their leaves. There was a great decline in digestibility of grass stems with advancing maturity. The digestibility of alfalfa leaves decreased only slightly as the season progressed. The IVDMD of alfalfa stems declined rapidly early in the season.

Tilley and Terry (1964) compared the digestibility of the leaf and stem portion of alfalfa (Medicago sativa L.) and sainfoin (Onobrychis viciaefolia Scop.). The apparent digestibility of sainfoin leaflets (70%) and petioles (73%) was less than that of alfalfa leaves (81%) when compared with alfalfa of similar digestibility. Sainfoin stems were more digestible (75%) than alfalfa stems (59%). Koch, Dotzenko, and Hinge (1972) reported that sainfoin stems were high in digestibility even in late stages of maturity.

The study of Osburn, Thomson, and Terry (1966) discovered that the voluntary intake of sainfoin was greater than that of red clover (Trifolium pratense L.) or alfalfa. The voluntary intake of sainfoin was 87.3 g/kg $W^{0.78}$/24 hr as compared to 74.0 and 63.5 for red clover and alfalfa, respectively. Sainfoin and red clover had similar digestibilities, 62.6 and 64.6, respectively, while alfalfa was 58.3%. Osburn said that intrinsic differences in physical characteristics of forages could affect voluntary intake. Since the digestibility of sainfoin stems was higher than that of red clover and alfalfa, this characteristic could influence voluntary intake.

Heady (1964) said that there was a negative correlation between the amount of crude fiber, and palatability or preference in forage crops. It is known that leaves and the upper portion of plants are preferred over stems by sheep and cattle. Toenjes (1979a) commented that hard stems sliver and make the stem less preferred. Toenjes (1979b) commented that there are three zones in the stem: (1) soft upper portion (2) portion that breaks into strings, and (3) the hard

brittle lower portion. The lower portion is generally refused by cattle. The hardness of the stem is related to the amount of lignification. Voigt et al. (1970) found that lignin influenced palatability of weeping lovegrass (Eragrostis curvula (Schrad.) Nees.). If the stem hardness or lignification was reduced, voluntary intake of stems increased.

Weiss (1953) concluded that stemmy alfalfa stimulated salivary secretion in ruminant animals. The absence of sufficient coarse material in the rumen caused frothy, viscid, ruminal ingesta thus resulting in bloat. When fed chopped alfalfa hay, Mendel and Boda (1961) found that nonbloating animals secreted 5% more saliva while resting and 28% more during feeding than bloating animals. Bloat occurred more frequently during morning hours. Diurnal changes in salivary flow could explain the high frequency of bloat in the morning. Salivary flow was lowest between 6:00-7:00 A.M. (Yarns, Putnam, and Leffel, 1965).

Clark, Jones, and Reid (1974) and Reid et al. (1974) found protein differences in saliva between bloat susceptible and nonsusceptible cattle. Protein in saliva was studied by polyacrylamide gel electrophoresis. Protein bands 4, 6, and 10 differed in intensity when saliva from susceptible and nonsusceptible cattle was tested. Lyttleton (1960) found similar protein differences. McIntosh and Cockrem (1977) and Phillipson and Mangan (1959) reported changes within protein composition in saliva with chemical stimulation. McIntosh reported changes in the amount of protein in bands with saliva stimulation with acetic acid by using gel electrophoresis.

With increasing voluntary intake of coarse-stem tissue, salivary glands may be more stimulated and this may change saliva composition. An increase in individual saliva protein components appears to be more important in reducing the incidence of bloat in ruminants than just increase in total saliva flow.

Chemical Components as Estimators of Nutritive Value

Van Soest (1967) has used chemical assays for determining nutritive value of forage crops for many years. Using a neutral detergent solution, a forage sample can be separated into plant cell content and cell wall content. Plant cell content consists of sugars, starch, fructosans, pectin, protein, nonprotein nitrogen, lipids, water-soluble minerals and vitamins. The cell walls consist of cellulose, hemicellulose, lignin, silica, keratin, waxes, cutin, insoluble minerals, and lignified nitrogen. Using an acid detergent solution, all were removed except lignocellulose, lignified nitrogen, heat damaged proteins, keratin, and silica. Van Soest (1963a) said NDF was essentially hemicellulose, cellulose, and lignin while ADF was cellulose and lignin. Van Soest (1964), Rohweder, Jorgensen, and Barnes (1976), and Morrison (1976) stated that there was a negative correlation between ADL and ADF and digestibility in both grasses and legumes. Van Soest (1964) reported a high negative correlation between voluntary intake and NDF in grasses but not in legumes. Van Soest (1963b) also reported ADF was negatively correlated (-0.79) with digestibility in 18 different forage crops. Lignin was negatively correlated (-0.90) with digestibility

within grasses and legumes. In Van Soest's (1964) study, the correlation between lignin and digestibility for grasses was -0.82 and for legumes it was -0.74. Van Soest (1965) found that the amount of lignin, ADF, and NDF could be used to predict the digestibility and voluntary intake of grasses but only digestibility of legumes. Van Soest and Moore (1965) suggested that the digestibility of NDF was controlled by the concentration of lignin in lignocellulose. Van Soest and Wine (1967) reported that cell-wall content of roughages was significantly correlated with digestibility and voluntary intake in ruminants. Van Soest and Marcus (1964) examined 96 forages, of which nine were legumes, and found a correlation of -0.65 between voluntary intake and CWC. Cell wall constituents were much more closely related to voluntary intake than to digestible DM. Kamstra, Moxon, and Bentley (1958) and Hi Kon Oh, Baumgardt, and Scholl (1966) reported similar correlations between lignin, ADF, and IVDMD.

Forbes and Garrigus (1950) found that small variations in chemical composition of forages may be nutritionally significant. A small increase in lignin decreased organic matter digestibility greatly. Data from steer-feeding trials reveal that for each percentage unit increase in lignin there was a decrease of 5.8% of maximum intake of total organic matter and a decrease of 8.2% in digestible organic-matter intake. Voigt et al. (1970) also stated that small differences in lignin influenced the palatability or intake of weeping lovegrass.

Prediction of Chemical Composition by Near-Infrared Reflectance

Estimation of chemical composition of plant tissue by near-infrared (NIR) reflectance can be an alternative to standard laboratory analysis which are comparatively expensive and time consuming. When a large number of samples is required for genetic studies of chemical constituents, NIR reflectance is advantageous because it is capable of analyzing up to 20 individual samples per hour (Shenk, 1979; Shenk et al., 1977; Fales and Cummins, 1982). Near-infrared reflectance has been used to predict protein, NDF, ADF, lignin, and digestibility of plant products (Shenk, 1979; Williams, 1979; Rotolo, 1979). Shenk, Westerhaus and Hoover (1979) found that filters with wavelengths of 1.672, 1.700, 1.940, 2.100, 2.180, and 2.336 μm could be used for forage analyses for protein, NDF, ADF, and in vitro dry matter disappearance. The wavelengths between 1.60 to 1.70 μm appear important for predicting ADF or IVDMD, whereas the region above 2.00 μm was required for prediction of NDF. Protein was predicted by wavelengths of 2.10 μm and 2.18 μm. Norris et al. (1976) found that when the filters with wavelengths 1.70, 1.94, 2.10, 2.18, and 2.336 μm were used, correlation coefficients and standard errors of estimate were as follows: (1) crude protein -- 0.98 and 1.07, (2) NDF -- 0.90 and 5.3 (3) ADF -- 0.90 and 2.5 and (4) lignin -- 0.72 and 2.1. When the number of filters was increased, the correlation coefficients were higher and standard errors of estimate were lower. By using 8 filters, crude protein had an r value equaling 0.99 and a standard error of estimate of 0.74. The ADF required nine wavelengths to get a correlation coefficient of 0.96 and a standard error

of estimate of 1.56. Lignin had a correlation coefficient of 0.96 and standard error of estimate of 0.80 when seven wavelengths were used. Shenk et al. (1981) found that standard errors of estimate for the calibration of samples were smaller than standard errors of estimate for NIR prediction values when NIR data were compared with laboratory data.

Fales and Cummins (1982) found that the amount of moisture in the sample could affect the NIR reflectance prediction values. As the moisture increased from an oven-dried sample to a sample stored at 100% relative humidity, there was greater variation from the regression line. The correlation coefficient also decreased. The regression line does not intercept near zero in the high-moisture sample. Their results show that the intercept point should also be considered in comparing NIR reflectance values with chemical values.

MATERIALS AND METHODS

A field study was conducted at the University of Arizona Campbell Avenue Farm at Tucson, Arizona in 1979. Field data were collected on April 4, April 30, August 17, and October 26, 1979. Data were collected on specific stem weight of spaced-planted propagules of alfalfa clones selected initially from Mesa-Sirsa alfalfa for vigorous lateral roots and tolerance to leafhoppers, These plants were used to determine the relationship between specific stem weight and other desired agronomic traits, such as dry matter production and fiber content of the forage. Such information would be useful in an alfalfa improvement program.

Ninety-eight clones were planted in a randomized complete block design with three replications (Little and Hills, 1978). Plants were spaced two feet apart in rows 40 in apart. Two propagules of each clone were planted per plot. Plants were harvested June 19, Aug. 20, and Oct. 18, 1980.

The inheritance study experiment was placed in a randomized complete block with three replications. Five progeny were planted per replication for the selfs and ten progeny were planted per replication for crosses and their reciprocals. Each progeny was spaced one foot apart in 40 inch rows in a field plot.

Collection and Preparation of Field Samples

Harvest dates were determined by first-flower appearance. The three tallest stems were collected from each clone for determining

specific stem weight in April, August, October 1979 and August and October 1980. On June 19, 1980 one stem was taken of each propagule. On August and October 1980 only one replication was harvested. In the June harvest of 1980 data on plant height was collected in centimeters. The forage from one plant was harvested per replication and placed in a cloth bag to estimate forage yield, leaf-stem ratio, stem ADF, leaf ADF, stem ADL, and leaf ADL.

Specific stem weight was determined by cutting a 5-cm segment from the base, center, and top of the tallest selected stems. The basal segment was cut from the area immediately above the first node of the cut stem. The top segment was cut from the area immediately below the terminal bud. The mean specific stem weight per plant was determined by averaging the weight of all three stem segments. The apparatus used to cut stems consisted of two sharp microtome blades spaced 5 cm apart on a hard flexiglass block which was hinged and a spring was mounted at the base in such a way that the blades would spring up to make the apparatus ready for the next stem (Figure 1). Stem segments were collected and dried at 80 C and then weighed to the nearest milligram.

Samples for determining forage yield and leaf-stem ratio were dried in a hot-air blower drier. Leaves were separated from stems by the method of Heinrichs et al. (1969). Samples were dried and gently rubbed to remove leaves from stems. This method does add some stem parts to the leaf fraction, but the method was more convenient and considerably faster than the removal of each leaflet one by one from green material to estimate leaf-stem ratio. Total forage yield, and weights

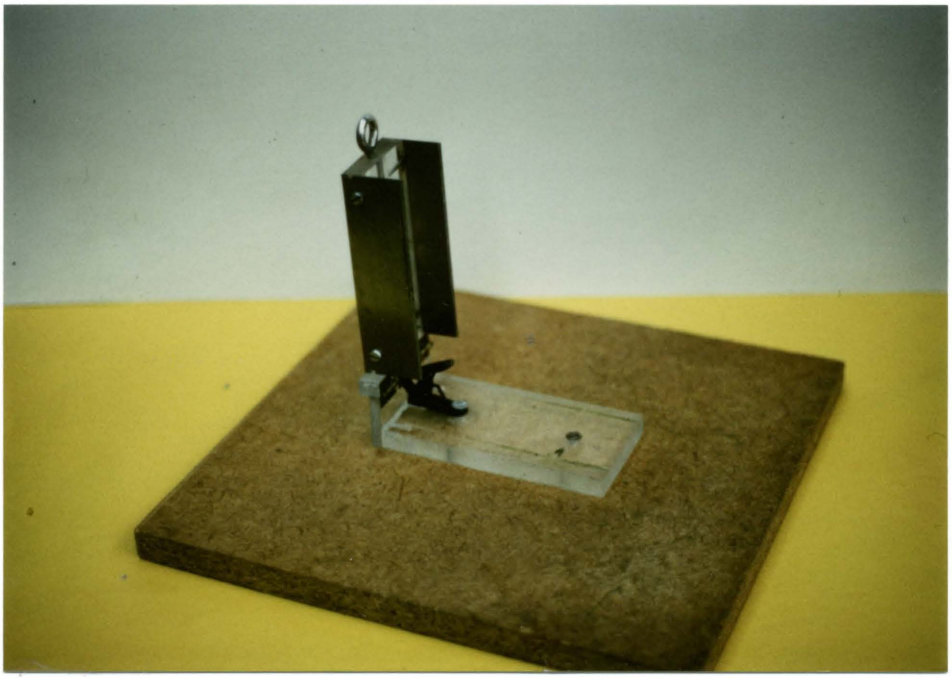

Figure 1. Apparatus for cutting stem segments.

of leaf and stem fractions were recorded in grams. The leaf and stem fractions were passed through a Wiley mill with a 1-mm screen. Ground samples were stored for subsequent laboratory analyses.

Laboratory Analysis

The micro method of fiber analysis described by Waldern (1971) was used to determine ADF and ADL. All reagents used were similar to those outlined by Goering and Van Soest (1970). Digestion units were 50-ml test tubes, 25 X 200 mm. These tubes were placed in holes in an aluminum block and placed on a heavy-duty heater which was connected to a rheostat to regulate the temperature. A large marble served as a condenser. Samples were funnelled through 50-ml, sintered-glass crucibles with coarse porosity.

Reagents were an acid detergent solution (20 g cetyl trimethyl ammonium bromide to liter of 1N sulfuric acid), decalin (reagent grade decahydronapthalene), acetone, and 72% H_2SO_4.

A 0.35 g plant sample, which was passed through a 1-mm sieve, was placed in a test tube, followed by 35 ml of acid detergent solution. Decalin (1 ml) was then added to each tube. A large marble was placed on top of each tube.

Tubes were placed in an aluminum block which was heated to 130 C. This temperature was reached by setting the rheostat at 80 for 1 hour. The rheostat was lowered to 75 to maintain 110 C when gentle boil was reached. The contents were brought to a boil slowly to avoid foaming and to avoid pushing plant particles up the sides of the tube. These particles can be returned by washing with a small amount of the ADF

solution. The solution was boiled for 1 hour. The solution and sample were filtered through a previously-weighed, sintered-glass crucible using a light suction. The crucible and tube were rinsed and washed with hot water. The crucible was then washed with acetone. Samples were dried at 100 C overnight. Crucibles were cooled in a desiccator before weighing. The formula used for calculating acid-detergent fiber was: (Wo-Wt) (100)/S = ADF, where: Wo = weight of oven-dry crucible including fiber; Wt = tared weight of oven-dry crucible; S = oven-dry sample weight. To determine ADL, crucibles were placed in a pyrex tray which contained a water level 1 cm deep in the tray. The crucible was filled half full with 72% H_2SO_4 which was prepared by the technique described by Goering and Van Soest (1970). The contents were then stirred with a glass rod to break up all lumps. The crucibles were refilled with 72% H_2SO_4 as needed and stirred at hourly intervals. After 3 hours, the acid was filtered off with vacuum and the contents were washed with water until free of acid. The stirring rod was rinsed and removed. The crucible was dried at 100 C and weighed. Crucibles were ignited in a muffle furnace at 450 C overnight and then cooled to 100 C. Crucibles were then cooled in a desiccator and weighed. Acid detergent lignin was calculated as follows: (L X 100)/S = ADL, where: L = loss upon ignition after 72% H_2SO_4 treatment; S = oven-dry sample weight.

The Technicon InfraAlyzer with a Hewlett-Packard 9815A microprocessor was the near infrared reflectance instrumentation system used to obtain predicted NIR reflectance values for stem ADF and ADL. Ninety-eight laboratory samples were used to determine a straight line. The stepwise multiple regression was used to determine the best of ten

filters to use for determining the amount of ADF and ADL in alfalfa stems. Filter numbers 4, 6, 10, 12, 14 and 18 were used to predict the NIR reflectance value. These filters have wavelengths of 1.722, 2.100, 2.139, 2.181, 2.330 μm, respectively.

Means, standard deviations, variances, standard errors, and Pearson correlation coefficients were analyzed for all harvests in 1979. An analyses of variance, Pearson correlations, and scattergram regression were conducted on specific stem weight, plant height, leaf-stem ratio, yield, stem ADF, stem ADL, leaf ADF and leaf ADL of plant samples collected from the June 1980 harvest. Pearson correlations and scattergram regression were run on mean specific stem weight and stem weights of the segments. On the August and October harvest 1980 means, standard deviations, standard errors, variances, Pearson correlation, and scattergram regression were run.

Laboratory samples (294) of stem ADF and ADL were then compared with NIR reflectance values by using scattergram regression. The infraAlyzer was then used to determine stem ADF and stem ADL for the August and October harvests in 1980.

RESULTS AND DISCUSSION

The major thrust of this study was to focus attention on a readily observable trait such as SSW in alfalfa and to determine the amount of variation present within a selected group of alfalfa genotypes. Another focus of the study was to determine the inter-relationship between SSW and other agronomic traits and feeding quality of the forage. The relationship between SSW and yield was studied to determine whether or not SSW could be used as a tool by forage crop breeders in their efforts to increase total dry matter production without sacrificing forage quality. The variations in the agronomic and quality traits were studied to see if they could also be used in a selection program.

Specific Stem Weight Experiment in 1979

The variety Mesa-Sirsa was used to study SSW because it is a variety well adapted to the low desert valley areas of southern Arizona. It is resistant to many insects and diseases and is a high forage producer. It has the ability to produce more forage in early spring and late fall (Schonhorst, et al., 1968).

An experiment was conducted in 1979 to determine the optimum time to evaluate a group of alfalfa clones for specific stem weight. Specific stem weight as discussed here is the average of the stem weights of top, center, and basal segments (5 cm each) of three alfalfa stems per clone. Samples were taken on four harvest dates. These harvests were April 4, April 28, August 17, and October 26. The April 4

harvest had the highest mean SSW for the alfalfa population. The August 17 harvest had the lowest specific stem weight (Table 1). Specific stem weight declined as the summer temperature increased. As night temperatures decreased in October, the SSW increased slightly. The April 4 harvest had the highest variance for SSW while the August 17 harvest had the lowest variance. The April 4 harvest had the highest coefficient of variation while the April 28 harvest had the lowest coefficient of variation and a relatively high variance. The August harvest had a lower variance and a coefficient of variation higher than the April 28 harvest. Spring and early summer appears to be the best time to select for SSW in the cultivar Mesa-Sirsa. Mesa-Sirsa produced more forage in early summer and late fall, than in the summer months. This reduction in SSW corresponds with the reduction in forage yield frequently observed in the summer months due to high temperatures. This problem is referred to as "summer slump" (Stanberry et al., 1955).

The experiment was conducted also to identify clones high and low in SSW in this population. One standard deviation or more from the mean was used as the criterion for identifying individuals in the population with high and low SSW (Table 1). The frequency distribution of individuals about the central mean and above plus and minus one standard deviation in all four harvests is shown in Figure 2. The April and October harvests contained the most individuals in the high and low range of the population for SSW. The SSW data for each harvest were also put into frequency histograms (Figures 3, 4, 5, and 6).

Table 1. Means, variances, standard deviation, standard errors, and coefficients of variation for specific stem weight of four alfalfa harvests.[1]

	Harvest Date of 1979			
	Apr 4	Apr 28	Aug 17	Oct 26
	SSW (Mg/5 cm Segment)			
Mean[2]	99.32 [a]	72.06 [b]	46.33 [c]	49.50 [c]
Variance	736.39	124.40	110.9	120.29
Std. dev.	27.14	11.15	10.51	10.97
Std. err.	2.49	1.02	0.98	1.10
C.V.	27.32	15.48	22.69	22.15
n	119	119	115	99

[1] Specific stem weight is the average of the stem weights of top, center, and basal segments of three alfalfa stems per clone.

[2] Means were separated by t-test for unpaired observations. R. G. D. Steel and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc.

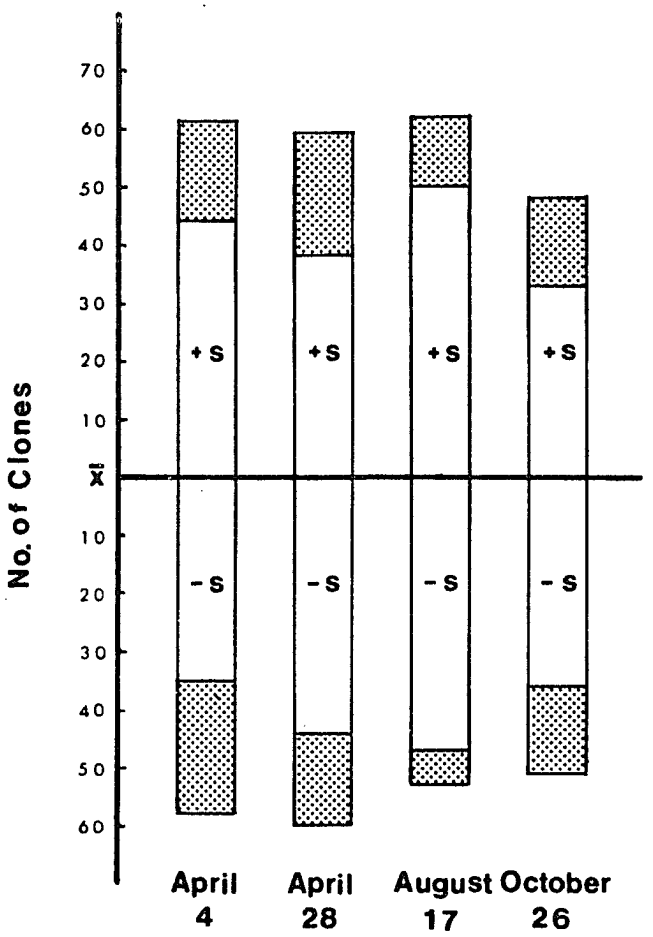

Figure 2. Frequency distribution about the central mean and above plus one standard deviation or below minus one standard deviation for specific stem weight of alfalfa harvests on April 4, April 28, August 17, and October 26, 1979.

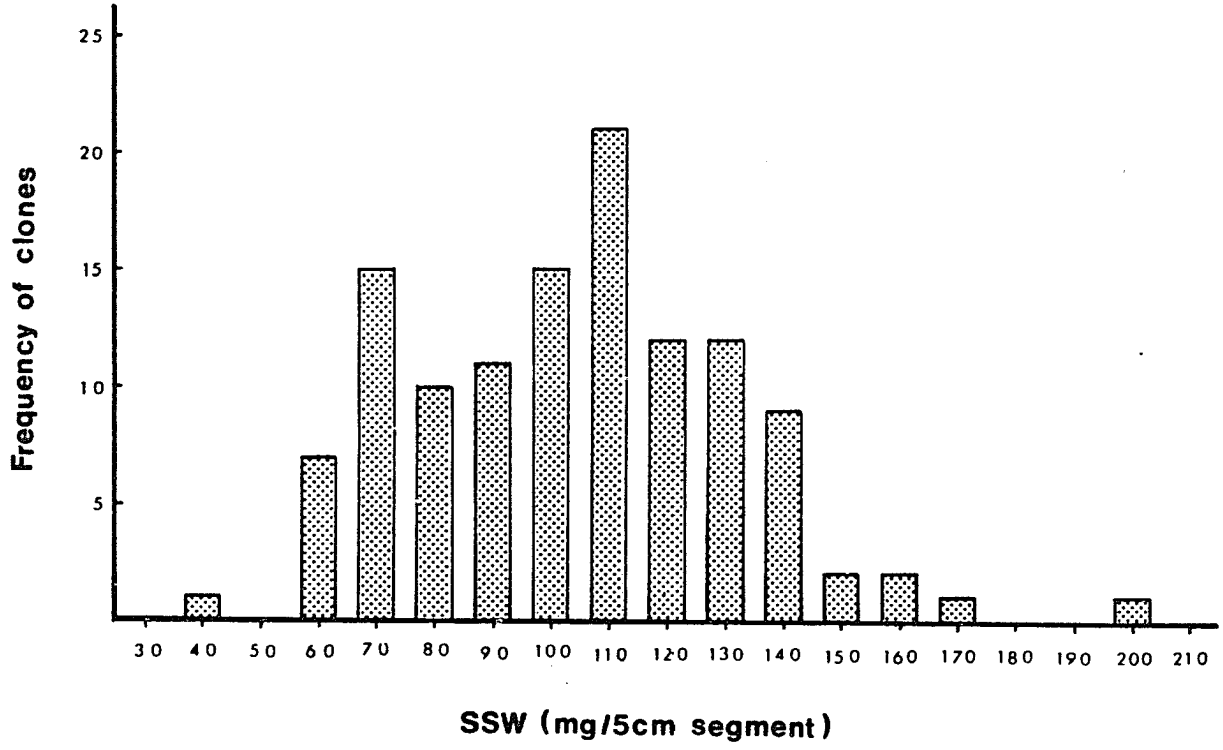

Figure 3. Frequency histogram for specific stem weight of the harvest on April 4, 1979.

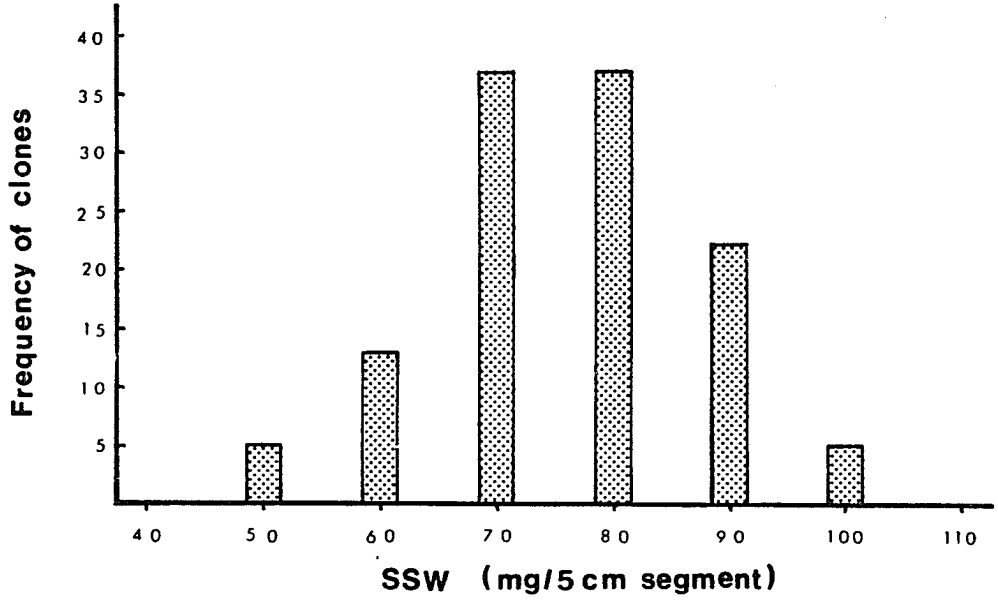

Figure 4. Frequency histogram for specific stem weight of the harvest on April 28, 1979.

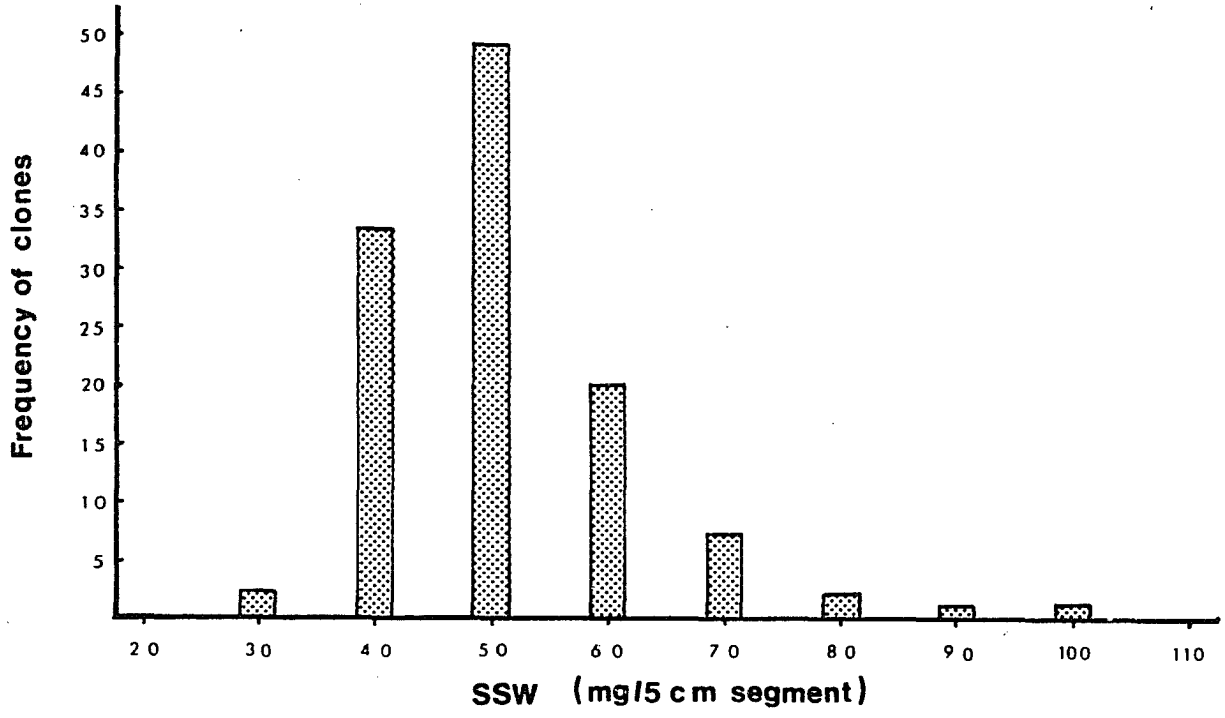

Figure 5. Frequency histogram for specific stem weight of the harvest on August 17, 1979.

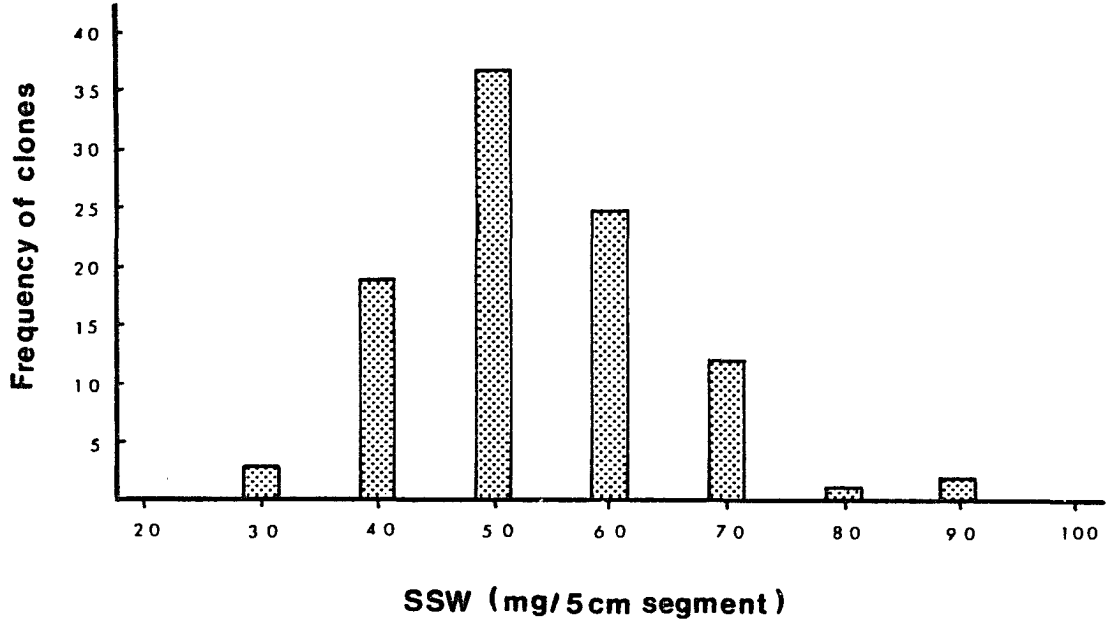

Figure 6. Frequency histogram for specific stem weight of the harvest on October 26, 1979.

Over the four harvest dates there were certain individuals consistently high or low in SSW. These individuals could be used in a breeding program to breed for a high or low SSW alfalfa variety.

Only 21.69% of the variation in average specific stem weight was attributed to stem weight of the top segment, while 88.36% was attributed to the center segment, and 88.36% to the bottom segment (Table 2).

The weights of the stem segments diminish from bottom to top of the stem. The stem weight of the center segment was near to the value of mean SSW which is the average of three stem segments of three alfalfa stems (Table 3). This reduction in SSW is possibly a result of denser chemical components of the stem being laid down from bottom of the stem to the top as it grows. Similar results were obtained by Smith (1970) and Bula (1972).

The average SSW and stem weight of center segment were compared by using a regression line (Table 4). For the April 4, April 28, and October 26 harvests the standard errors of estimate were high and the intercept points were not near zero, but the slope was near one. In the August 17 harvest, the standard error of estimate was 3.29 and the intercept point was 2.76, while the slope was also near one (Table 4).

Table 2. Pearson correlation coefficients between stem weights of segments and mean SSW.[1]

	Harvest Date 1979				Transformation Mean[2]
	Apr 4	Apr 28	Aug 17	Oct 26	
	Mean specific stem weight				
Stem weight of top segment	.41**	.57**	.35**	.53**	.47
Stem weight of center segment	.95**	.92**	.95**	.94**	.94
Stem weight of bottom segment	.98**	.89**	.96**	.86**	.94
n	119	119	115	99	

**Statistically significant at .01 level.

[1]Specific stem weight is the average of the stem weights of top, center, and basal segments of three alfalfa stems per clone.

[2]Transformation for averaging correlation values. Field plot technique. 1962. LeClerg, E. L., W. H. Leonard, and A. G. Clark.

Table 3. Comparison between mean specific stem weight and the weight of segments obtained from the top, center and basal region of the stem.

	Harvest Date 1979			
	Apr 4	Apr 28	Aug 17	Oct 26
	Mean SSW (mg/5 cm segment)*			
Mean**	99.32[a]	72.06[a]	46.33[a]	49.50[a]
Stem weight of top segment	33.49	39.89	17.88	23.63
Stem weight of center segment	96.01[a]	69.59[a]	47.25[a]	49.29[a]
Stem weight of basal segment	167.18	107.40	73.42	76.25
n	119	119	115	99

*Obtained by averaging weight of segments from top, center, and bottom of three stems from individual alfalfa plants.

**Means within same columns were separated by t-test for unpaired observations. R.G.D. Steel and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc.

Table 4. The standard errors of estimate and regression lines between mean specific stem weight and stem weight of center segment for four harvests in 1979.

Harvest Date	Std. Error of Estimate	$\hat{Y} = a + bX$
April 4	8.29	$\hat{Y} = 11.25 + .92X$
April 28	4.46	$\hat{Y} = 8.75 + .91X$
August 17	3.29	$\hat{Y} = 2.76 + .92X$
October 26	3.76	$\hat{Y} = 7.28 + .85X$

Specific Stem Weight Experiment in 1980

Effect of Harvest Date on SSW, Other Agronomic and Quality Traits

One desirable feature of the cultivar Mesa Sirsa is its ability to produce high dry matter yields in early spring and late fall in the low desert valley environment of southern Arizona (Schonhorst et al., 1968). Agronomic traits and quality traits were appreciably affected by harvest date. The variation among harvest dates was mainly due to high temperatures during the summer months.

The specific stem weight declined as the temperature increased in the hot summer months. With cooler night temperatures of late May and early June, the June harvest was higher in SSW than the August harvest. As the night temperature decreased in October the SSW increased (Table 5). The variation in SSW among alfalfa genotypes also decreases with higher temperatures. Plant height declined slightly between June and October harvest but declined more in the August harvest (Table 6). Yield declined sharply between the June and August harvest (Table 7). Between the August and October harvests there was an increase in yield. Variability declined greatly from June to the August harvest but then increased in the October harvest. Again high temperatures appeared to affect stem height and forage yield.

The leaf-to-stem ratio increased in the August harvest (Table 8). This increase was probably due to a reduction in stem production rather than an increase in leaf area. Stem production in June was greater than that of August. The leaf-to-stem ratio declined between the August and October harvest as a result of increased stem production.

Table 5. Means, variances, standard deviations, standard errors, and coefficients of variation for specific stem weight of three alfalfa harvests in 1980.[1]

	Harvest Date 1980		
	June	Aug.	Oct.
	SSW (mg/5 cm segment)		
Mean[2]	69.83[a]	47.33 [b]	70.61[a]
Variance	151.10	70.47	112.03
Std. dev.	12.29	8.39	10.58
Std. err.	.72	.85	1.08
C.V. (%)	17.60	17.74	14.99
n	294	97	96

[1]Specific stem weight is the average of the stem weights of top, center, and basal segments of three alfalfa stems per clone.

[2]Means were separated by t-test for unpaired observations. R. G. D. Steel and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc.

Table 6. Means, variances, standard deviations, standard errors, and coefficients of variation for plant height of three alfalfa harvests in 1980.

	Harvest Date 1980		
	June	Aug.	Oct.
	Plant Height (cm)		
Mean [1]	58.38 [a]	48.27 [c]	53.69 [b]
Variance	52.52	47.38	52.67
Std. dev.	7.25	6.88	7.26
Std. err.	.42	.700	.74
C.V. (%)	12.41	14.26	13.52
n	294	97	96

[1] Means were separated by t-test for unpaired observations. R. G. D. Steel and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc.

Table 7. Means, variances, standard deviations, standard errors, and coefficients of variation for forage yield of three alfalfa harvests in 1980.

	Harvest Date 1980		
	June	Aug.	Oct.
	Yield (g)		
Mean[1]	51.68[a]	22.67[c]	30.98[b]
Variance	263.08	68.74	102.59
Std. dev.	16.22	8.29	10.13
Std. err.	.95	.84	1.03
C.V. (%)	31.38	36.57	32.69
n.	294	97	96

[1]Means were separated by t-test for unpaired observations. R. G. D. Steel and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc.

Table 8. Means, variances, standard deviations, standard errors, and coefficients of variation for leaf-stem ratio of three alfalfa harvests in 1980.

	Harvest Date 1980		
	June	Aug.	Oct.
	Leaf/Stem Ratio		
Mean [1]	1.00 [c]	2.10 [a]	1.55 [b]
Variance	.06	1.12	.19
Std. dev.	.24	1.06	.44
Std. err.	.01	.11	.04
C.V. (%)	24.26	50.40	28.40
n	294	97	96

[1] Means were separated by t-test for unpaired observations. R. G. D. Steel and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc.

The greatest variation in leaf-to-stem ratio occurred in August. The coefficient of variation was near 50% in August; thus, most of variation in leaf-to-stem ratio was due to the environment, mainly high temperature effects. Delaney, Dobrenz, and Poole (1974) also reported an increase leaf-stem ratio from a response to high temperatures in August. Dobrenz et al. (1969) reported a decrease in leaf-stem ratio in June and July but an increase in August.

The ADF and ADL of stems were highest when stem production was the highest in the June harvest. When stem production declined in August, due to high temperature, stem ADF and ADL also declined (Tables 9 and 10). The decline in forage quality generally observed in the summer months could be from a greater loss of leaves during harvest because of drier, hotter harvesting conditions.

Analyses of Variance of June 1980 Harvest

The Partitioning of Variances. The experiment was replicated to remove environmental effects of location and differences in propagule root development. Replication can be used to have more efficiency in selection of genetic factors. In the 1979 experiment, there was one plant per entry and it was noticed that there would be great differences from harvest to harvest due to reaction to environment. There were disease, insect, and temperature effects. There was a comparison of coefficients of variation with and without partitioning the error variance of the different traits for the June harvest (Table 11). The coefficients of variation can be lowered by replicating the experiment.

Table 9. Means, variances, standard deviations, standard errors, and coefficients of variation for stem ADF for three alfalfa harvests in 1980.

	Harvest Date 1980		
	June	Aug.	Oct.
	Stem ADF (%)		
Mean[1]	44.90 [a]	42.35[c]	43.53[b]
Variance	11.87	11.39	5.60
Std. dev.	3.45	3.37	2.36
Std. err.	.20	.34	.24
C.V. (%)	7.67	7.97	5.43
n	294	97	96

[1]Means were separated by t-test for unpaired observations. R. G. D. Steel and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc.

Table 10. Means, variances, standard deviations, standard errors, and coefficients of variation for stem ADL of three alfalfa harvests in 1980.

	Harvest Date 1980		
	June	Aug.	Oct.
	Stem ADL (%)		
Mean [1]	10.10 [a]	9.13[c]	9.49[b]
Variance	.96	.86	.46
Std. dev.	.98	.93	.68
Std. err.	.06	.09	.07
C.V. (%)	9.68	10.15	7.19
n	294	97	96

[1]Means were separated by t-test for unpaired observations. R. G. D. Steel and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc.

Table 11. Coefficients of variation with and without partitioning or variances for June 1980 harvest.

	Without %	With %
SSW	17.60	13.81
Plant ht.	12.41	8.71
Yield	31.38	26.30
Leaf-stem ratio	24.26	21.21
Stem ADF	7.68	6.08
Stem ADL	9.68	7.54
Leaf ADF	9.53	7.55
Leaf ADL	17.98	15.13

This difference shows the importance of removing some of the effects of environment.

Analyses of Variance for Agronomic and Quality Traits. Analyses of variance were conducted on specific stem weight, plant height, forage yield, leaf-to-stem ratio, stem ADF, stem ADL, leaf ADF and leaf ADL for the June harvest (Tables 12 to 19). There was a significant difference among clone means for all traits. There was high variation in SSW, plant height, yield, leaf-to-stem ratio, stem ADF, stem ADL, leaf ADF, and leaf ADL. This variation can be utilized in a selection program. Plants can be selected from either the upper or lower extremes in the population by selecting individuals greater than two standard deviations from the means.

SSW could probably be used as a tool for selection for total dry matter production. Stem ADF or stem ADL, which predict digestibility of the stem, could be used as a tool to develop a more nutritious alfalfa plant. It might be advantageous to select a group of plants with high SSW and low stem ADF. The alfalfa variety with high dry matter production has greater stem production; thus an alfalfa variety with high SSW and low stem ADF might have higher dry matter production without sacrificing digestibility and nutritional quality.

It is common knowledge that the leaf of the alfalfa plant has very high nutritional value. If the amount of leaves could be increased without decreasing yield, nutritive value could be increased. Selection of an alfalfa plant with high SSW and high leaf-to-stem ratio might be a way to increase both quality and dry matter production.

Table 12. Analysis of variance for specific stem weight for June harvest in 1980.

Source of Variation	Degrees of freedom	Mean square	F
Total	293	151.10	
Clones	97	241.57	2.60**
Block	2	1396.59	15.01**
Error	194	93.03	

**Significantly different at the 1% level.

Table 13. Analysis of variance for plant height for the June 1980 harvest.

Source of variation	Degrees of freedom	Mean square	F
Total	293	52.77	
Clones	97	100.58	3.89**
Block	2	347.41	13.45**
Error	194	25.82	

**Significantly different at 1% level.

Table 14. Analysis of variance for forage yield for the June 1980 harvest.

Source of variation	Degrees of freedom	Mean squares	F
Total	293	263.08	
Clones	97	374.10	2.02**
Block	2	2480.50	13.43**
Error	194	184.72	

**Significantly different at 1% level.

Table 15. Analysis of variance for leaf-to-stem ratio for the June 1980 harvest.

Source of variation	Degrees of freedom	Mean square	F
Total	293	.06	
Clones	97	.08	1.81**
Block	2	.39	8.81**
Error	194	.04	

**Significantly different at 1% level.

Table 16. Analysis of variance for stem ADF for the June 1980 harvest.

Source of variation	Degrees of freedom	Mean squares	F
Total	293	11.87	
Clones	97	19.83	2.66**
Block	2	55.29	7.43**
Error	194	7.44	

**Significantly different at 1% level.

Table 17. Analysis of variance for stem ADL for the June 1980 harvest.

Source of variation	Degrees of freedom	Mean square	F
Total	293	.96	
Clones	97	1.71	2.95**
Block	2	.66	1.13
Error	194	.58	

**Significantly different at the 1% level.

Table 18. Abalysis of variance for leaf ADF for the June 1980 harvest.

Source of variation	Degrees of freedom	Mean square	F
Total	293	2.05	
Clones	97	3.29	2.56**
Block	2	15.78	12.28**
Error	194	1.28	

**Significantly different at the 1% level.

Table 19. Analysis of variance for leaf ADL for the June 1980 harvest.

Source of variation	Degrees of freedom	Mean square	F
Total	293	.51	
Clones	97	.81	2.26**
Block	2	.15	.41
Error	194	.36	

**Significantly different at the 1% level.

The high leaf ADF or leaf ADL could be used to develop an alfalfa variety with low bloat incidence. Non-bloating legumes have leaf cells which rupture more slowly than bloating legumes (Howarth et al., 1978). Bloat-prone legumes, such as alfalfa, could possibly be changed into non-bloating types by selecting individuals which have leaf cells more resistant to rupture. A slower rupture of cells releases cell constituents in the rumen more gradually.

Two standard deviations are used to separate the two tails of the population of the June 1980 harvest (Figures 7 and 8). The frequency distribution about the central mean and above or below two standard deviations are shown for SSW, plant height, yield, and leaf-stem ratio in Figure 7. The frequency distributions for stem ADF, stem ADL, leaf ADF, and leaf ADL are shown in Figure 8. The frequency histograms are also shown for the above traits (Figures 9-16). For the June harvest, SSW, leaf ADF and ADL did not have a normal distribution. The frequency distribution of plant height, yield, leaf-to-stem ratio, stem ADF, and stem ADL were near normal.

In the August and October 1980 harvests, one standard deviation from the mean was used to find the high and low values in the population for all traits included in this test. Because replications were not used, means could not be separated by two standard deviations. The frequency distributions for SSW, plant height, yield, leaf-to-stem ratio, stem ADF, and stem ADL of August and October are shown in Figures 17 and 18. The frequency histograms show that distributions for SSW,

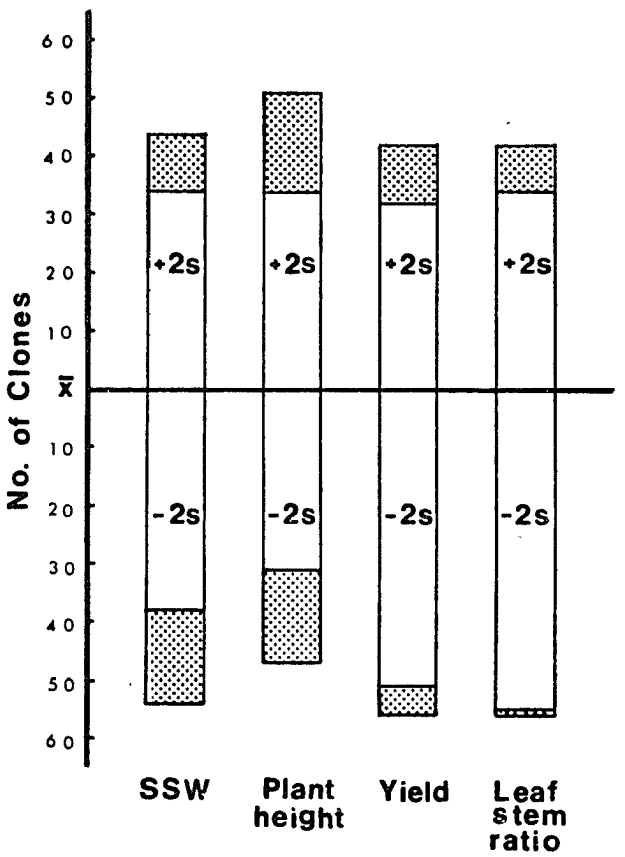

Figure 7. Frequency distribution about the central mean and above plus two standard deviations or below minus two standard deviations for agronomic traits of the June 1980 harvest.

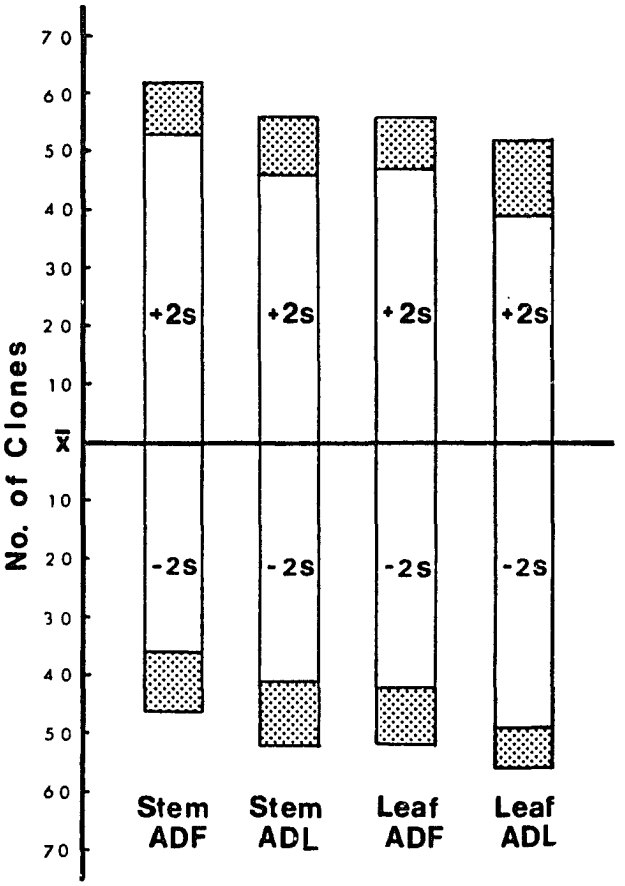

Figure 8. Frequency distribution about the central mean and above plus two standard deviations or below minus two standard deviations for stem and leaf quality traits of the June 1980 harvest.

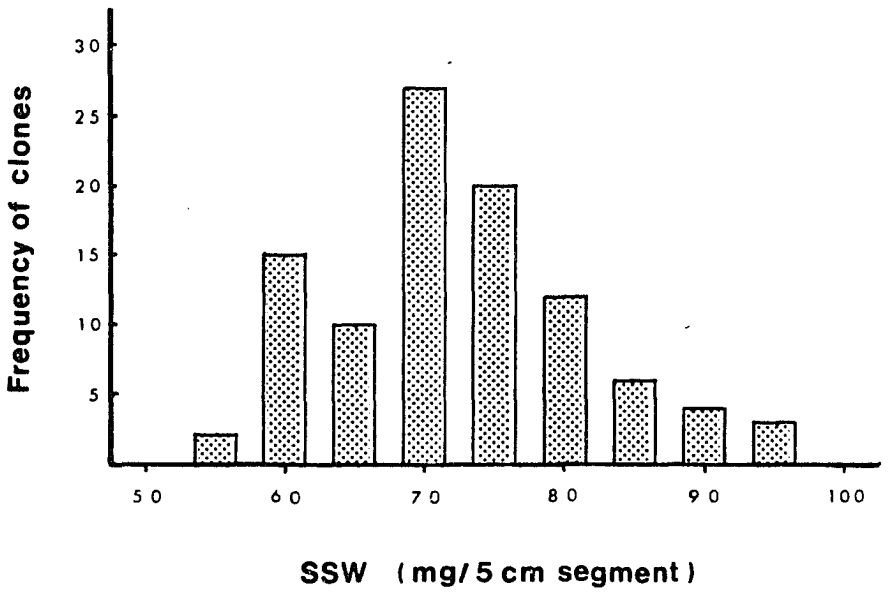

Figure 9. Frequency histogram for specific stem weight of the harvest in June 18, 1980.

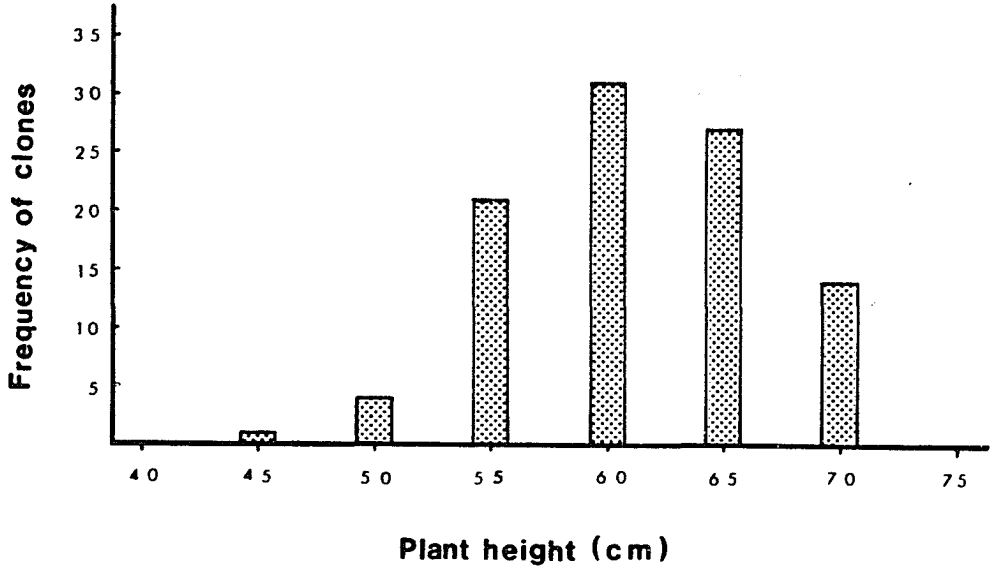

Figure 10. Frequency histogram for plant height of the harvest in June 1980.

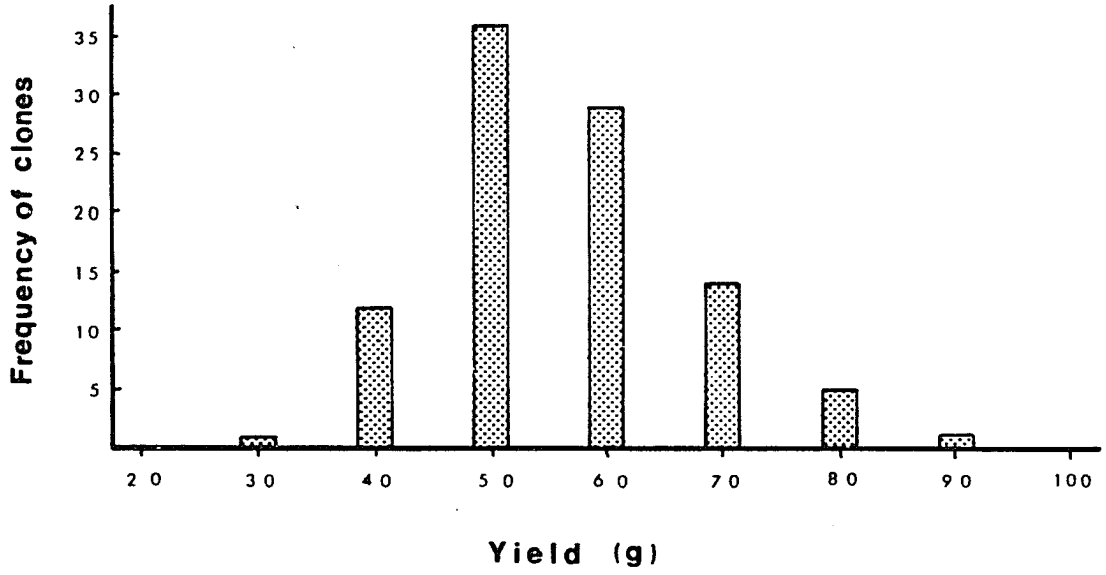

Figure 11. Frequency histogram for forage yield of the harvest in June 1980.

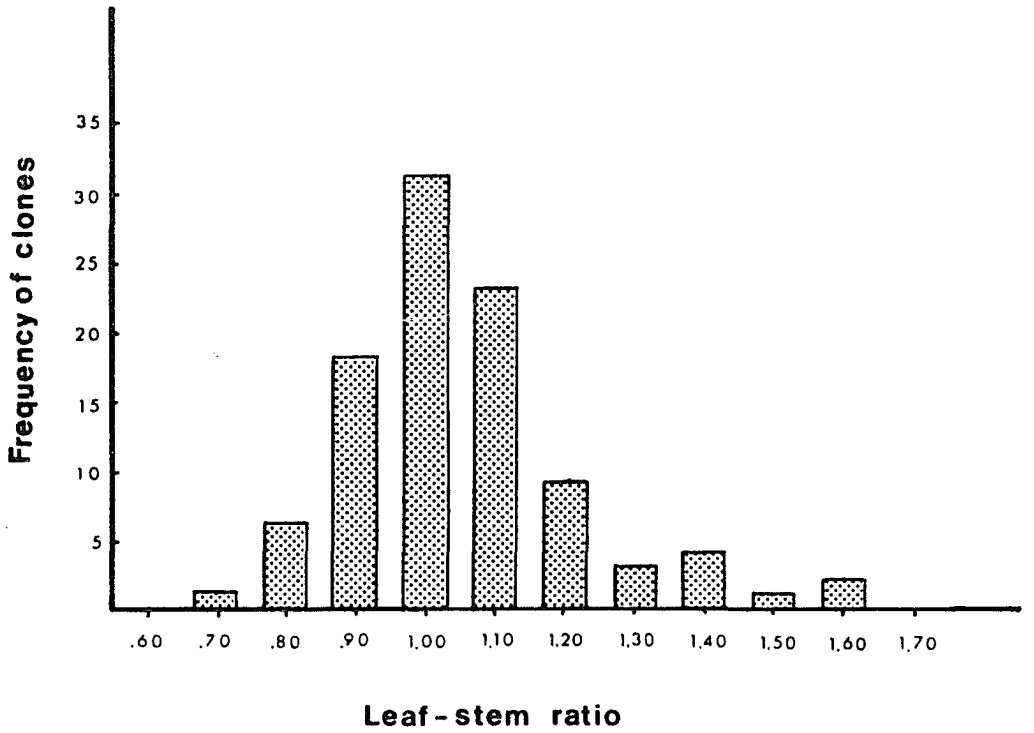

Figure 12. Frequency histogram for leaf-stem ratio of the harvest in June 1980.

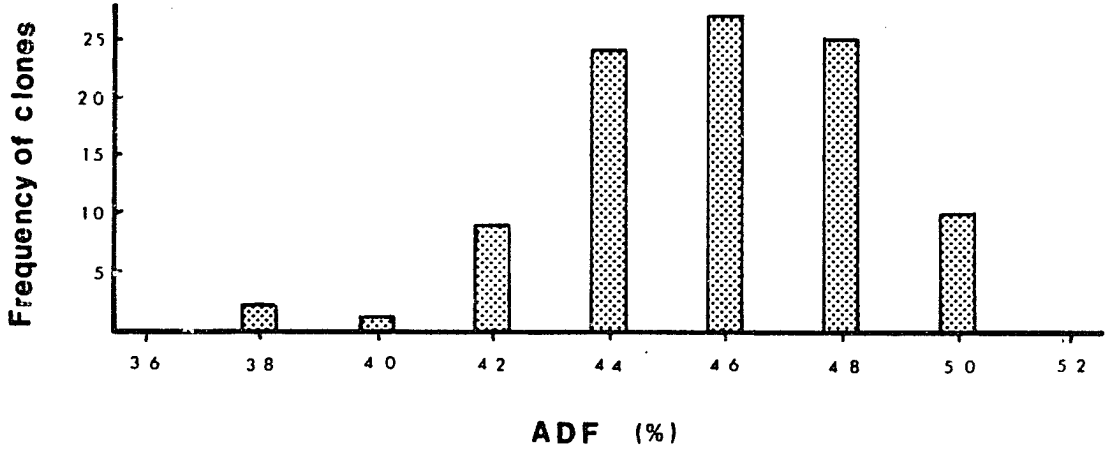

Figure 13. Frequency histogram for stem acid detergent fiber of the harvest in June 1980.

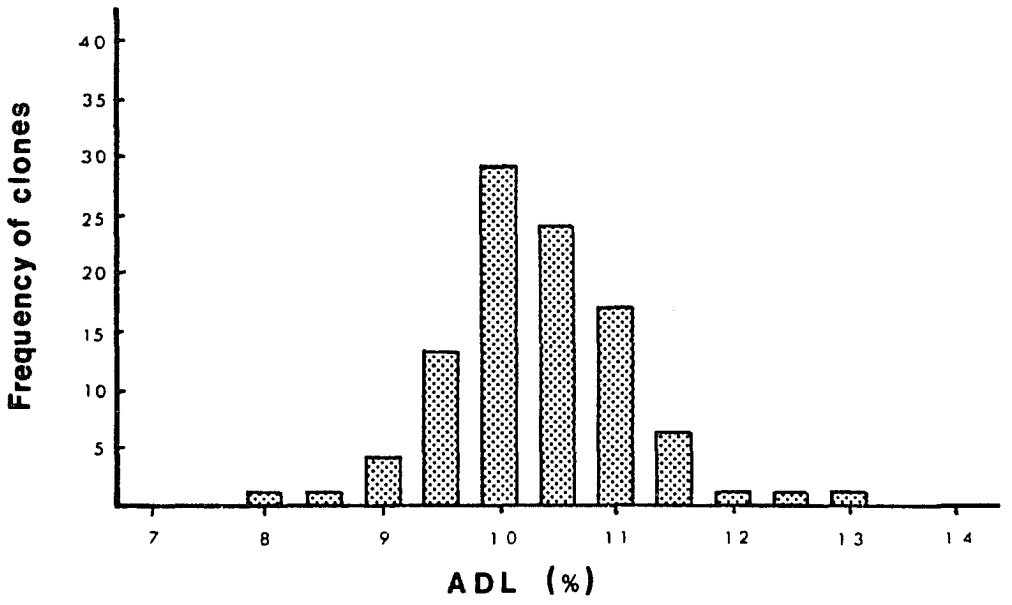

Figure 14. Frequency histogram for stem acid detergent lignin of the harvest in June 1980.

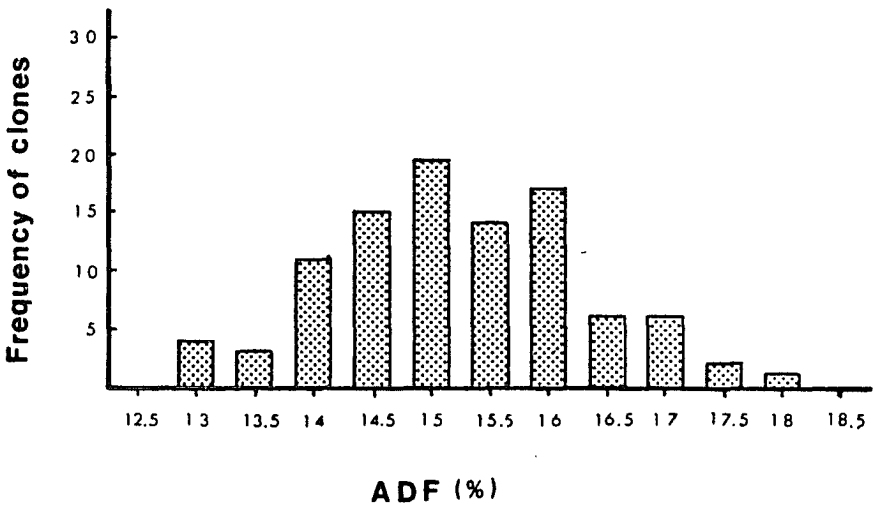

Figure 15. Frequency histogram for leaf acid detergent fiber of the harvest in June 1980.

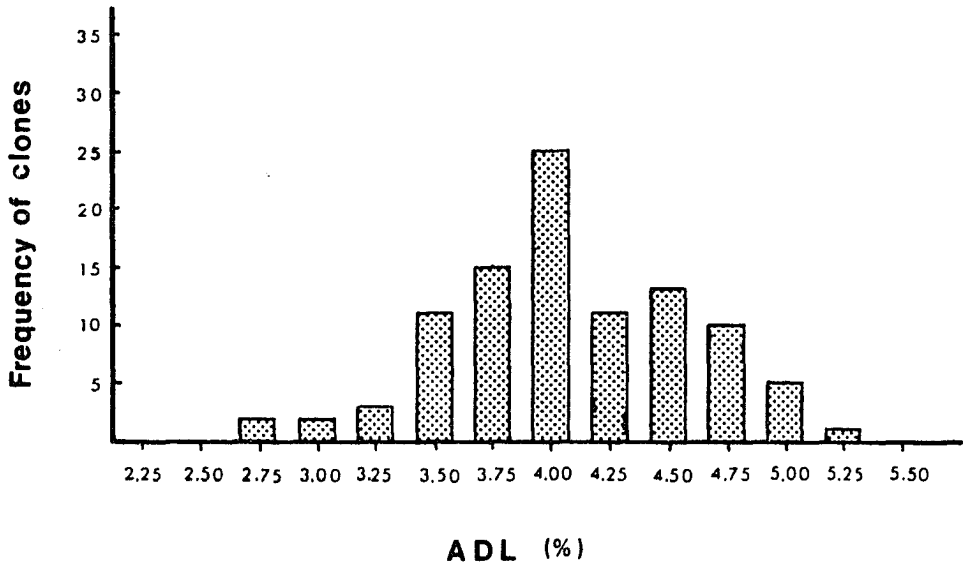

Figure 16. Frequency histogram for leaf acid detergent lignin of the harvest in June 1980.

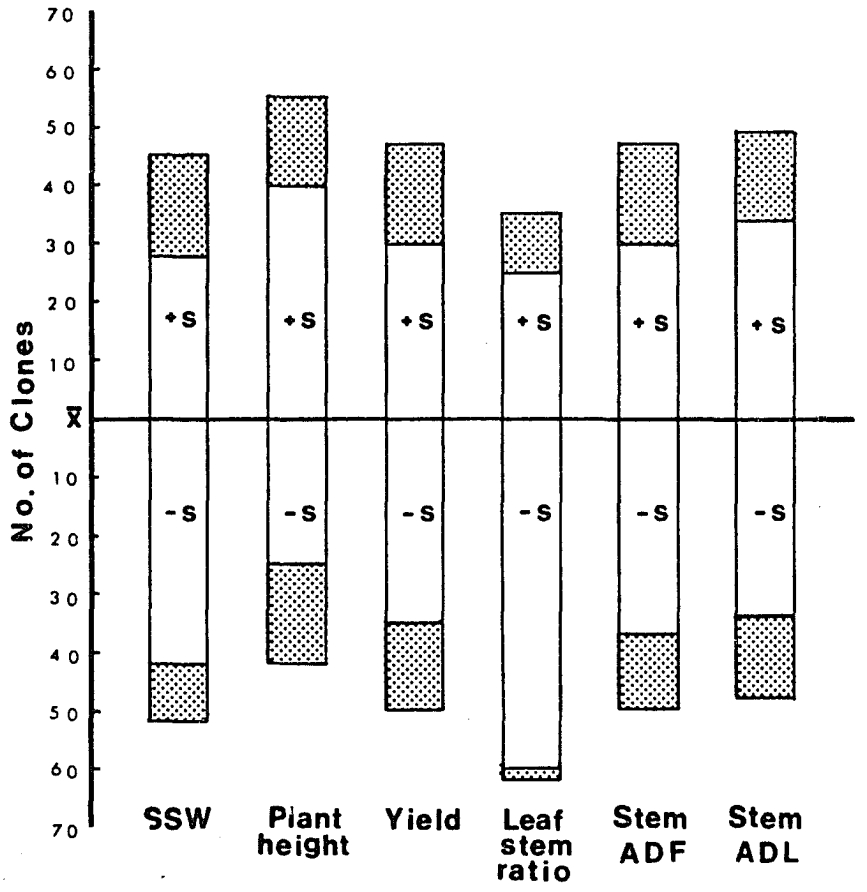

Figure 17. Frequency distribution about the central mean and above plus one standard deviation or below minus one standard deviation for specific stem weight, plant height, yield, leaf-stem ratio, stem acid detergent fiber, and stem acid detergent lignin of the August 1980 harvest.

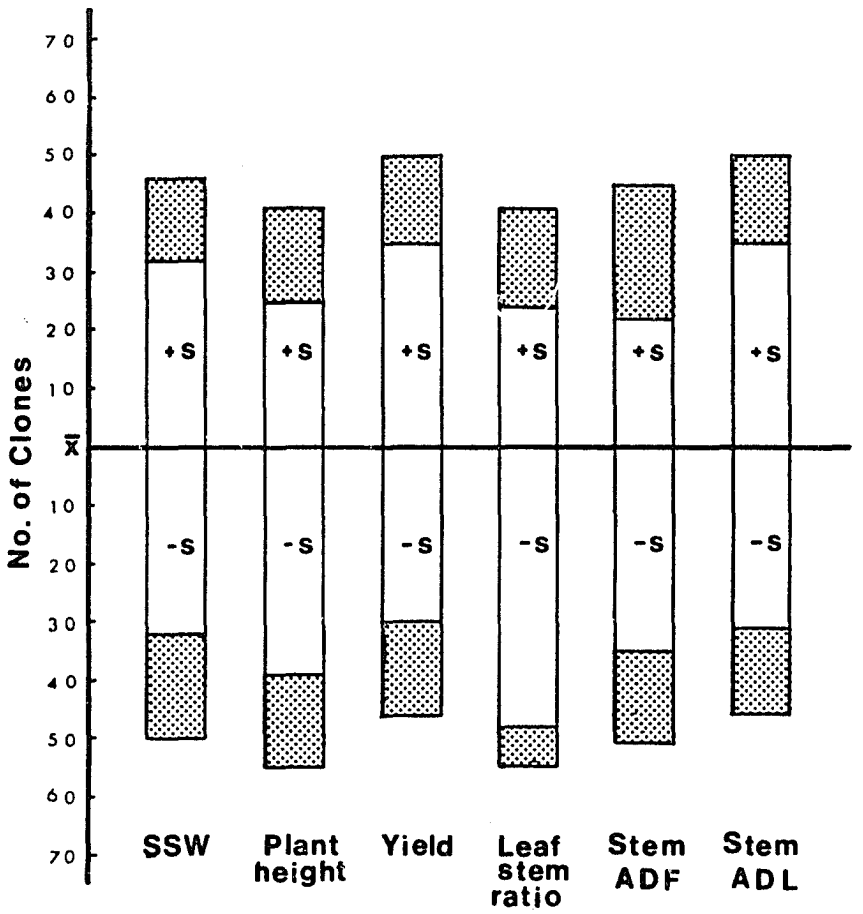

Figure 18. Frequency distribution about the central mean and above plus one standard deviation or below minus one standard deviation for specific stem weight, plant height, yield, leaf-stem ratio, stem acid detergent fiber, and stem acid detergent lignin of the October 1980 harvest.

plant height, and yield of the August harvest are near normal (Figures 19 to 21). The other frequency histograms are somewhat skewed (Figures 22 to 24). The frequency distributions for SSW, plant height, leaf-stem ratio, stem ADF, and stem ADL for the October harvest are also nearly normal (Figures 25 to 30). The yield frequency histogram shows a shewed distribution (Figure 27).

Pearson Correlations between Agronomic and Quality Traits

The Pearson correlation coefficient between the mean SSW and stem weight of the top segment revealed that 27.04% of the variation of the SSW can be attributed to stem weight of the top segment. The amount of 82.81% of the variation can be attributed to the center segment, while 90.25% can be attributed to the bottom segment (Table 20). These relationships mean that the center and bottom segments accounted for most of the specific stem weight. This association can be accounted for because chemical components of cell walls are laid down from the bottom to top of the stem.

Pearson correlation coefficients between variables and their r^2 values in the June 1980 harvest are shown in Tables 21 and 22. It was found that 18.49% of the variation in SSW was attributed to an increase in plant height while only 7.29% variation was attributed to a decrease in leaf-to-stem ratio. Only 11.56% of the variation in stem ADF was attributed to an increase in SSW. An increase in SSW attributes only 6.25% of the variation of yield while an increase in plant height accounted for 20.25% of the variation. A 39.69% variation in stem ADF

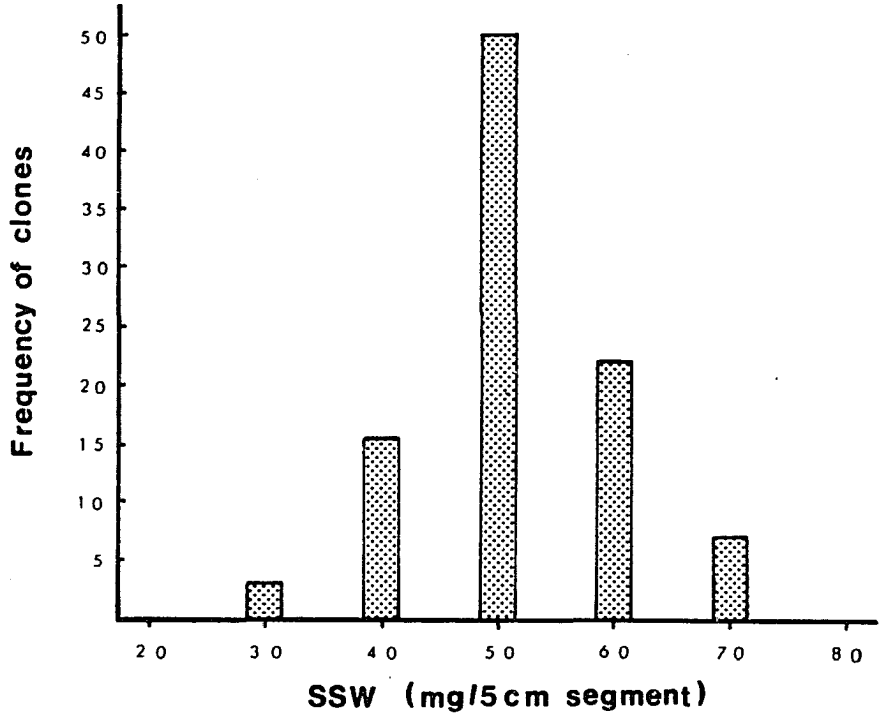

Figure 19. Frequency histogram for specific stem weight of the harvest in August 1980.

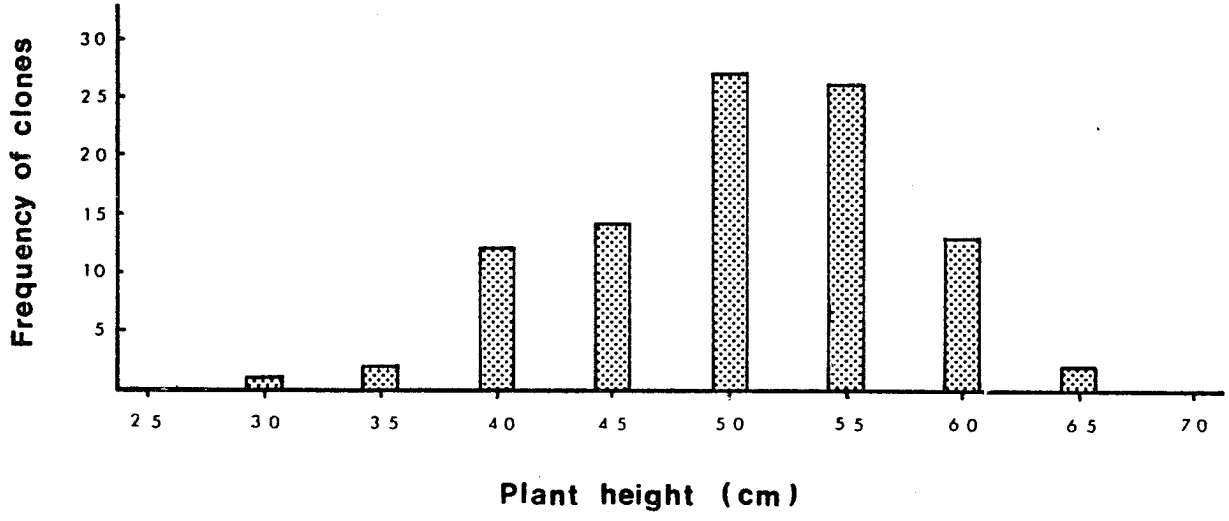

Figure 20. Frequency histogram for plant height in the August 1980 harvest.

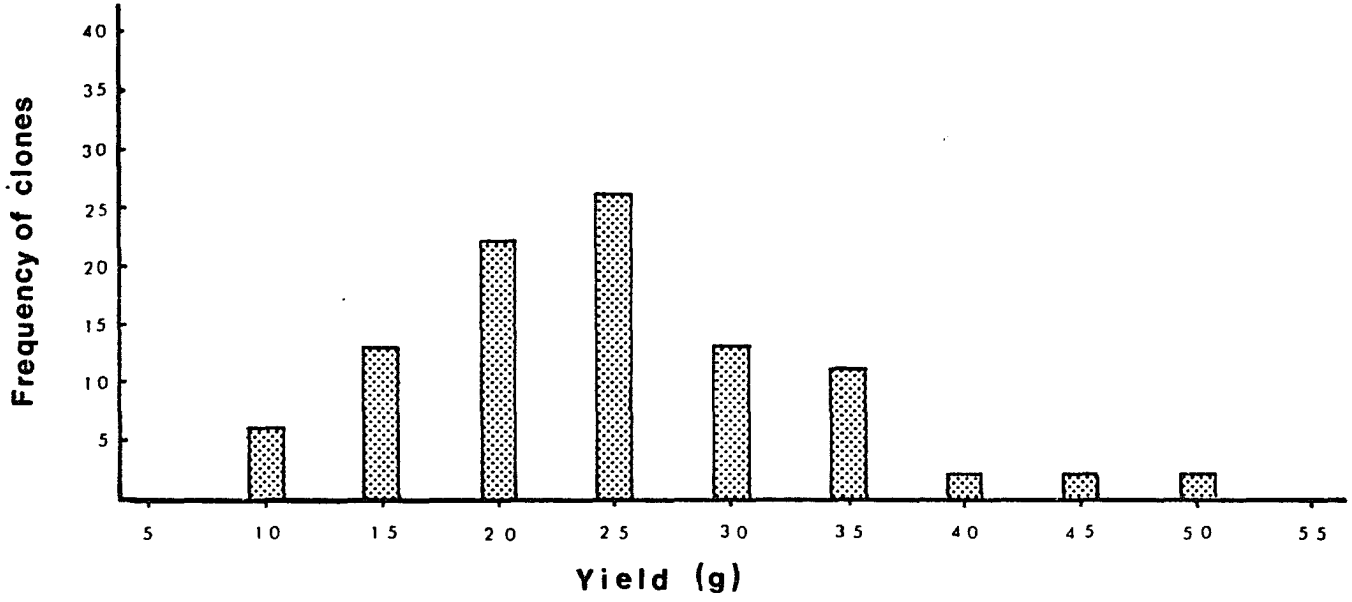

Figure 21. Frequency histogram for forage yield in the August 1980 harvest.

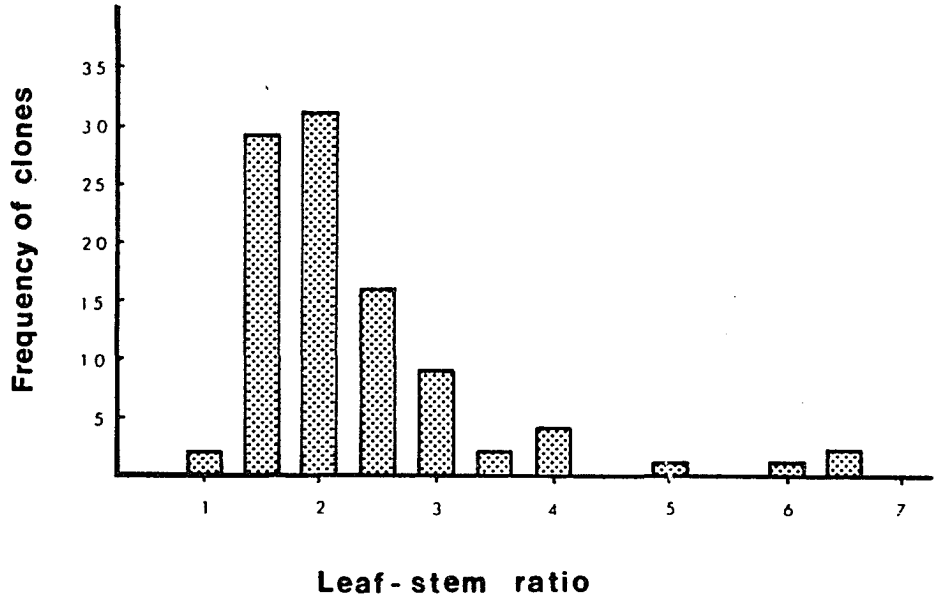

Figure 22. Frequency histogram for leaf-stem ratio in the August 1980 harvest.

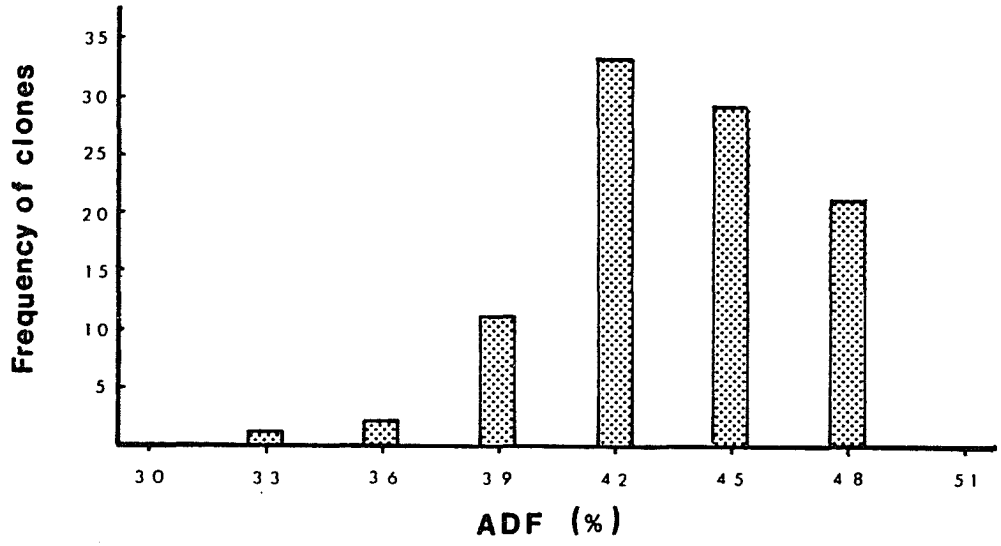

Figure 23. Frequency histogram for stem acid detergent fiber in the August 1980 harvest.

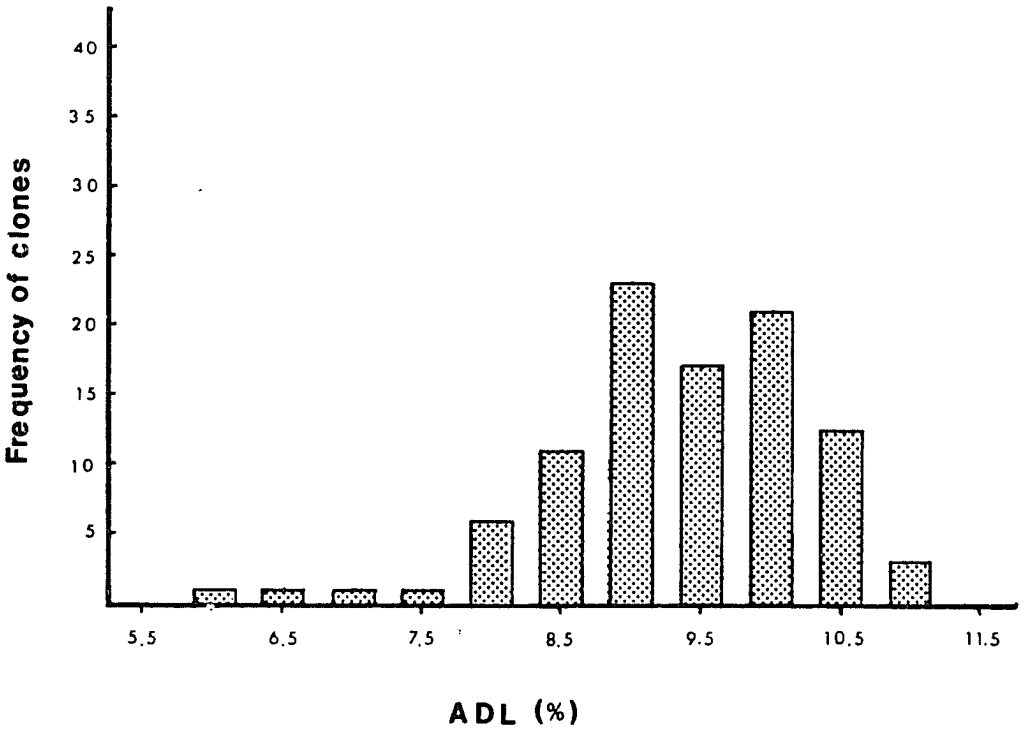

Figure 24. Frequency histogram for stem acid detergent lignin in the August 1980 harvest.

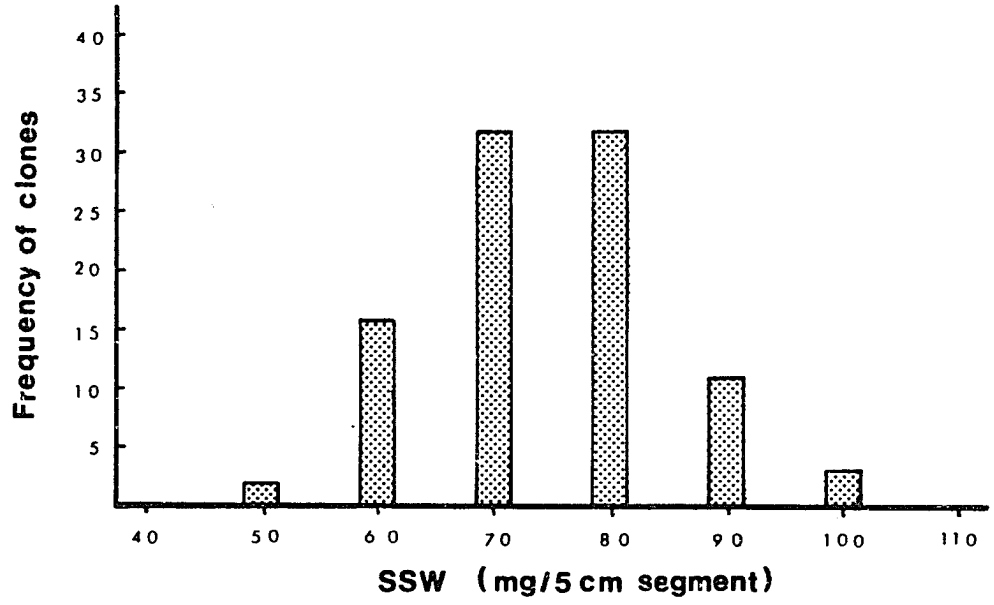

Figure 25. Frequency histogram for specific stem weight in the October 1980 harvest.

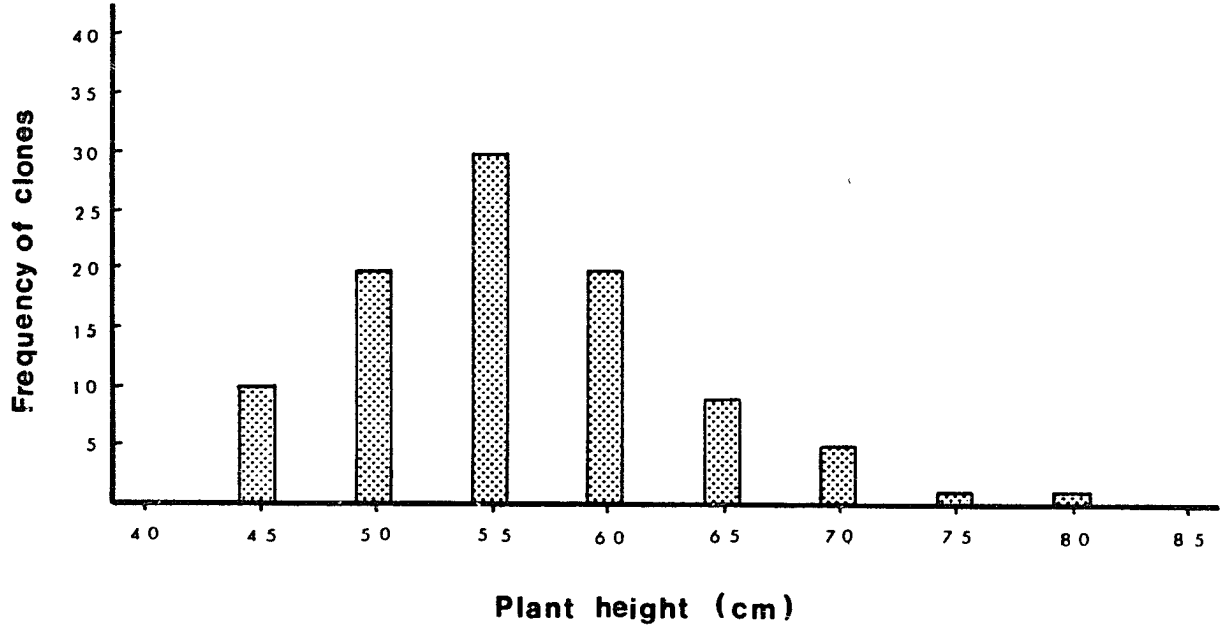

Figure 26. Frequency histogram for plant height in the October 1980 harvest.

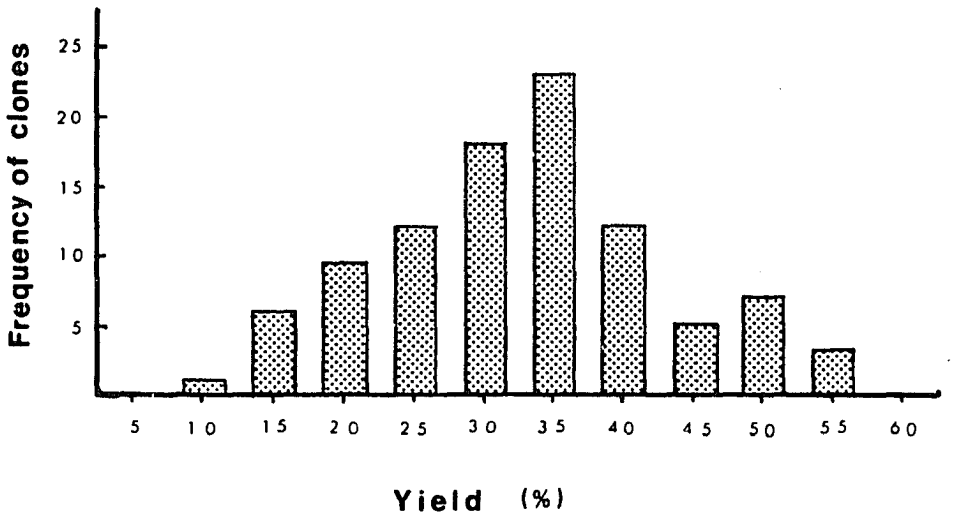

Figure 27. Frequency histogram for forage yield in the October 1980 harvest.

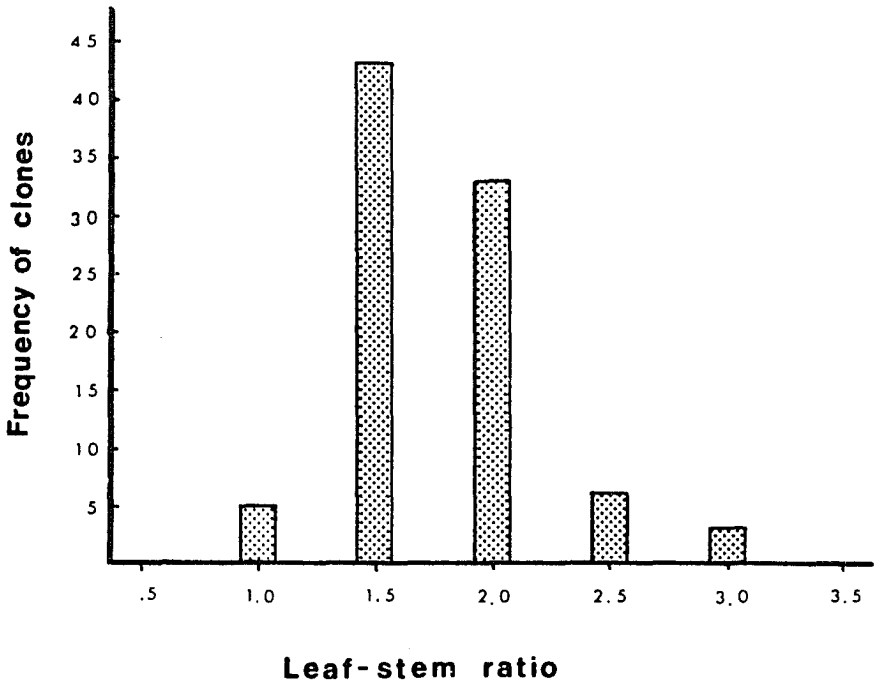

Figure 28. Frequency histogram for leaf-stem ratio in the October 1980 harvest.

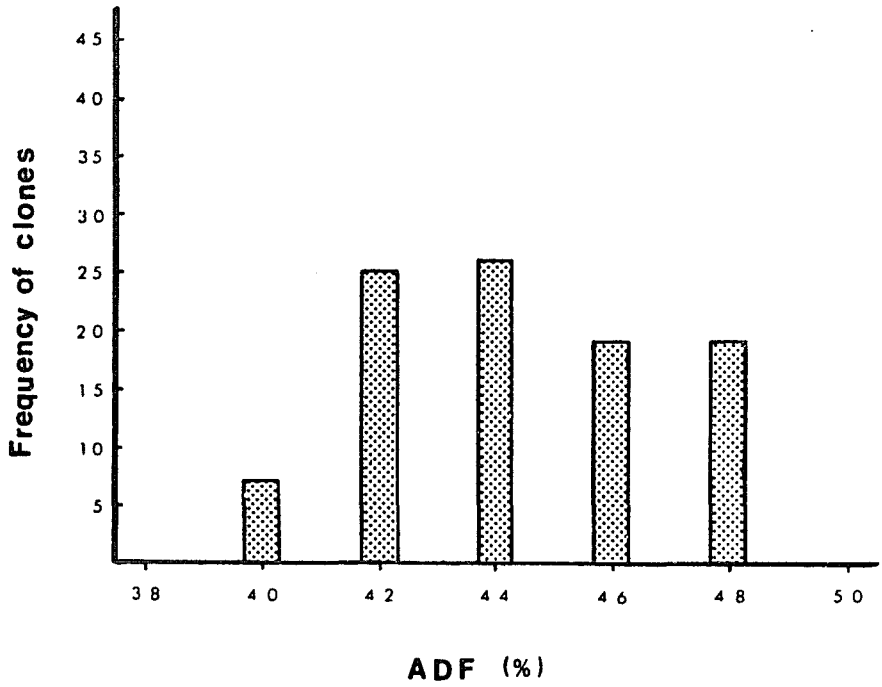

Figure 29. Frequency histogram for stem acid detergent fiber in the October 1980 harvest.

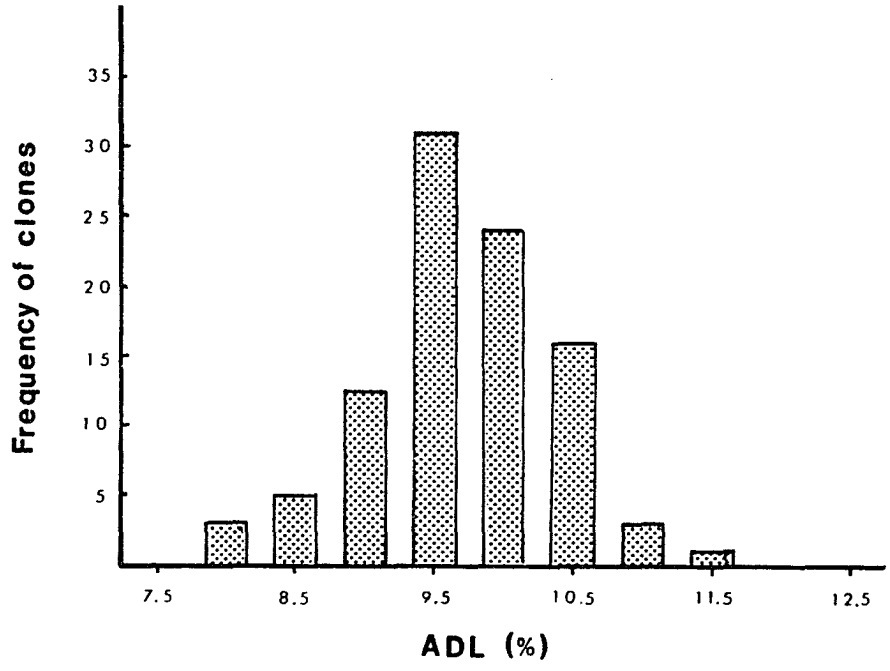

Figure 30. Frequency histogram for stem acid detergent lignin in the October 1980 harvest.

Toble 20. Pearson correlation coefficients between the stem weights of segments and mean SSW of three harvests in 1980.

	Harvest Date 1980			
	June	Aug	Oct	Transformation of the mean[b]
	Mean specific stem weight[a]			
Stem weight of top segment	.47**	.65**	.55**	.52
Stem weight of center segment	.90**	.93**	.92**	.91
Stem weight of bottom segment	.96**	.89**	.94**	.95
n	294	97	96	

**Statistically significant at .01 level.

[a]Mean specific stem weight is the average of the stem weights of top, center, and basal segments.

[b]Transformation for averaging correlation values. Field plot technique. 1962. LeClerg, E. L., W. H. Leonard, and A. G. Clark.

Table 21. Pearson correlation coefficients between variables on Mesa-Sirsa alfalfa harvested at first-bloom in June 1980.

	SSW	Plant height	Yield	Leaf-stem ratio	Stem ADF	Stem ADL	Leaf ADF	Leaf ADL
SSW	1.00	.43**	.25**	-.27**	.34**	.04	.25**	.05
Plant height		1.00	.45**	-.53**	.63**	.37**	.30**	.08
Yield			1.00	-.41**	.31**	.18**	.09	.00
Leaf-stem ratio				1.00	-.31**	-.19**	-.23**	-.12*
Stem ADF					1.00	.60**	.46**	.04
Stem ADL						1.00	.21**	.14**
Leaf ADF							1.00	.40**
Leaf ADL								1.00

**Statistically significant at .01 level.
*Statistically significant at .05 level.
n=294.

Table 22. The r^2 values between variables on Mesa-Sirsa alfalfa harvested at first-bloom in June 1980.

	SSW	Plant height	Yield	Leaf-stem ratio	Stem ADF	Stem ADL	Leaf ADF	Leaf ADL
SSW	100.00	18.49	6.25	-7.29	11.56	0.16	6.25	0.25
Plant height		100.00	20.25	-28.09	39.69	13.69	9.00	0.64
Yield			100.00	-16.81	9.61	3.24	0.81	0.00
Leaf-stem ratio				100.00	-9.61	-3.61	-5.29	-1.44
Stem ADF					100.00	36.00	21.16	0.16
Stem ADL						100.00	4.41	1.96
Leaf ADF							100.00	16.00
Leaf ADL								100.00

was attributed to an increase in plant height. Leaf-to-stem ratio was negatively correlated with plant height, yield, stem ADF, stem ADL, leaf ADF, and leaf ADL, but these relationships were small. The percentage values were as follows: 28.09%, 16.81%, 9.61%, 3.61%, 5.29% and 1.44%, respectively. A 9.61% variation in stem ADF was attributed to an increase in yield. A 21.16% variation in leaf ADF can be ascribed to an increase in stem ADF.

Pearson correlation coefficients between characteristics and their r^2 values of the harvests in August and October were similar to the results obtained from the June harvest (Table 23-26).

Mean SSW had little effect on the total yield of individual clones of spaced plants. Although number of stems per crown was not recorded, there appeared to be a positive relationship between number of stems and forage yield. Plant height also had some effect on total forage yield. Thus, yield appears to be influenced by a combination of different characteristics. Since SSW did not seem to affect total yield for spaced plants, it was decided to check if SSW affected dry matter production of a single stem. Three stems were selected from twenty clones to test for correlation of SSW with the average dry matter production of the three stems. The correlation coefficient value was 0.82. Since the experiment was conducted on spaced plants, the plants spread out under the spaced condition; thus, more stems may be occurring on spaced plants. The SSW may have more of an effect on total dry matter production in dense stand conditions.

There was a low correlation between SSW and stem ADF; thus, stem ADF does not appear to increase greatly with an increase in SSW.

Table 23. Pearson correlation coefficients between variables on Mesa-Sirsa alfalfa harvested at first-bloom in August 1980.

	SSW	Plant height	Forage yield	Leaf-stem ratio	Stem ADF	Stem ADL
SSW	1.00	.34**	.05	-.04	.20*	.08
Plant height		1.00	.45**	-.40**	.42**	.25**
Yield			1.00	-.48**	.38**	.34**
Leaf-stem ratio				1.00	.04	.15
Stem ADF					1.00	.89**

**Statistically significant at .01 level.
*Statistically significant at .05 level.
n=97.

Table 24. The r^2 values between variables of Mesa-Sirsa alfalfa harvested at first-bloom in August 1980.

	SSW	Plant height	Forage yield	Leaf-stem ratio	Stem ADF	Stem ADL
SSW	100.00	11.56	0.25	-0.16	4.00	0.64
Plant height		100.00	20.25	-16.00	17.64	6.25
Yield			100.00	-23.04	14.44	11.56
Leaf-stem ratio				100.00	0.16	2.25
Stem ADF					100.00	79.21
Stem ADL						100.00

Table 25. Pearson correlation coefficients between variables on Mesa-Sirsa alfalfa harvested at first-bloom in October 1980.

	SSW	Plant height	Forage yield	Leaf-stem ratio	Stem ADF	Stem ADL
SSW	1.00	.13	-.10	-.01	.16	.24
Plant height		1.00	.34**	-.32**	.16	.26**
Yield			1.00	-.43**	.12	.17*
Leaf-stem ratio				1.00	-.08	-.06
Stem ADF					1.00	.84**

**Statistically significant at .01 level.
*Statistically significant at .05 level.
n=96.

Table 26. The r^2 values between variables of Mesa-Sirsa alfalfa harvested at first-bloom in October 1980.

	SSW	Plant height	Forage yield	Leaf-stem ratio	Stem ADF	Stem ADL
SSW	100.00	1.69	-1.00	-0.01	2.56	5.76
Plant height		100.00	11.56	-10.24	2.56	6.76
Yield			100.00	-18.49	1.44	2.89
Leaf-stem ratio				100.00	-0.64	-0.36
Stem ADF					100.00	70.56
Stem ADL						100.00

Thus, SSW could be used as a selection tool without expecting an increase in stem ADF. Clones could be selected which were high in SSW but low in stem ADF. Some breeders felt that fineness of the stem was associated with high digestibility or low fiber (Mowat et al., 1967). From these results, coarseness of the stem was not related to high fiber; thus, a coarse stem could be selected with low ADF.

There was a low correlation between stem ADF and leaf ADF; thus, an increase in stem ADF was not necessarily associated with an increase in leaf ADF. These two fibers appear to be inherited separately. Howarth et al. (1978) and Lees et al. (1981) found that the amount of leaf cell rupture was related to legume pasture bloat. Non-bloating legumes have leaves with cells which rupture more slowly. Leaves which contain higher leaf ADF should rupture more slowly. These observations suggest that low-bloating alfalfa plants could be found by selecting for high leaf ADF and low stem ADF. A low stem ADF would make the stem more digestible. With a more digestible stem, the voluntary intake of the stem may be greater. With more coarse material in the rumen, salivary secretion would be stimulated (Wiess, 1953). Such an alfalfa would be higher in nutritional quality and produce faster livestock weight gain.

Regression Lines for Several Selected Variables

Variables that have the highest correlations and that might be of some importance were selected to calculate regression lines (Table 27). The regression coefficient for mean SSW and stem weight of the center segment was 0.95 while the regression coefficient for mean SSW and stem weight of basal segment was 0.48.

Table 27. Regression coefficients between some variables of interest of the three harvests in 1980.

Variables	Regression coefficients		
	June	August	October
SSW - Stem weight of center segment	0.95	0.98	1.00
SSW - Stem weight of basal segment	0.48	0.48	0.48
SSW - Plant height	0.73	0.41	**
Plant height - Leaf-stem ratio	-15.87	-2.60	-5.19
Plant height - Stem ADF	1.34	**	**
Plant height - Forage yield	.20	.37	.24
Stem ADL - Stem ADF	2.11	**	**
Stem ADF - Leaf ADF	1.10	**	**
Yield - Leaf-stem ratio	-27.87	-3.75	-9.92
SSW - Stem ADF	1.22	**	**

**Variables were concerned of no interest or had other methods of analysis.

Plant height decreased 15.87 units per unit change of leaf-stem ratio. The regression coefficient for plant height and stem ADF was -1.34. The relationship between stem ADL and stem ADF was a regression coefficient of 2.11. The slope value of 1.10 units was obtained between stem ADF and leaf ADF.

In the August harvest, the slope value of mean SSW to stem weight of the center segment was 0.98 and the slope value of mean SSW to stem weight of the bottom segment was 0.48. The other regression lines have low r values and high standard errors of estimate.

In the October harvest, the mean SSW and stem weight of the center segment relationship had a slope value of 1.00. The mean SSW and stem weight of the bottom segment relationship had a slope value of 0.48. The other regression lines have low r values and high standard errors of estimate.

Near Infrared Reflectance Prediction of Stem ADF and Stem ADL

The stepwise multiple regression technique was used to determine the best filters to use for determining the relative amount of fiber and lignin in alfalfa stems. The multiple correlation coefficients of stem ADF and stem ADL are shown in Tables 28 and 29. For the calibration regression line, the multiple correlation coefficients of stem ADF and stem ADL were greater than the predicted. The standard errors of estimate were slightly greater for the predicted. This difference means that there was slightly more deviation from the regression line.

The regression was used to compare wet chemical stem ADF with NIR reflectance prediction of ADF. The predicted stem ADF values follow the

Table 28. Correlation values and standard errors of estimate for near-infrared-reflectance prediction of stem ADF in alfalfa.

Parameter	Calibration values	Prediction values
r	.88	.84
r^2	.77	.70
Std. error of estimate	1.36	1.87

Table 29. Correlation values and standard errors of estimate for near-infrared-reflectance prediction of stem ADL in alfalfa.

Parameter	Calibration values	Prediction values
r	.70	.62
r^2	.49	.39
Std. error of estimate	.64	.76

regression line of wet stem ADF values and appear to be accurate predictors of ADF values. For NIR reflectance to accurately predict chemical values, the regression line should fall near a zero intercept and have a slope value near one unit change. The correlation coefficient should also be high. The standard error of estimate should be low. The regression line for wet ADF and NIR reflectance values in this study was $\hat{Y} = -3.45 + 1.08X$. The correlation coefficient was 0.84 and standard error of estimate was 1.87 (Figure 31). The regression line for wet ADL and NIR reflectance values is $\hat{Y} = 0.024 + 1.01X$ (Figure 32). The correlation coefficient was 0.62 and standard error of estimate was 0.76. The correlation coefficient was low but the intercept was near zero and the slope value was near one. The standard error of estimate was also low. NIR reflectance did not predict stem ADL as accurately as stem ADF.

Prediction values obtained in this test were not as accurate as reported by other researchers because the InfraAlyzer used only six filters which were fixed for its prediction. Other researchers found a correlation coefficient of over 0.90. Norris et al. (1976) used 9 filters for predicting ADF and 7 filters for predicting lignin. Norris used an NIR reflectance instrument which can scan for all wavelengths.

An advantage of NIR reflectance is its rapid prediction of chemical components. A disadvantage is that NIR reflectance values are not chemical values but they are predictions of chemical values. But when speed is essential in screening a large genetic population, NIR reflectance might be advantageous as long as the prediction is fairly accurate.

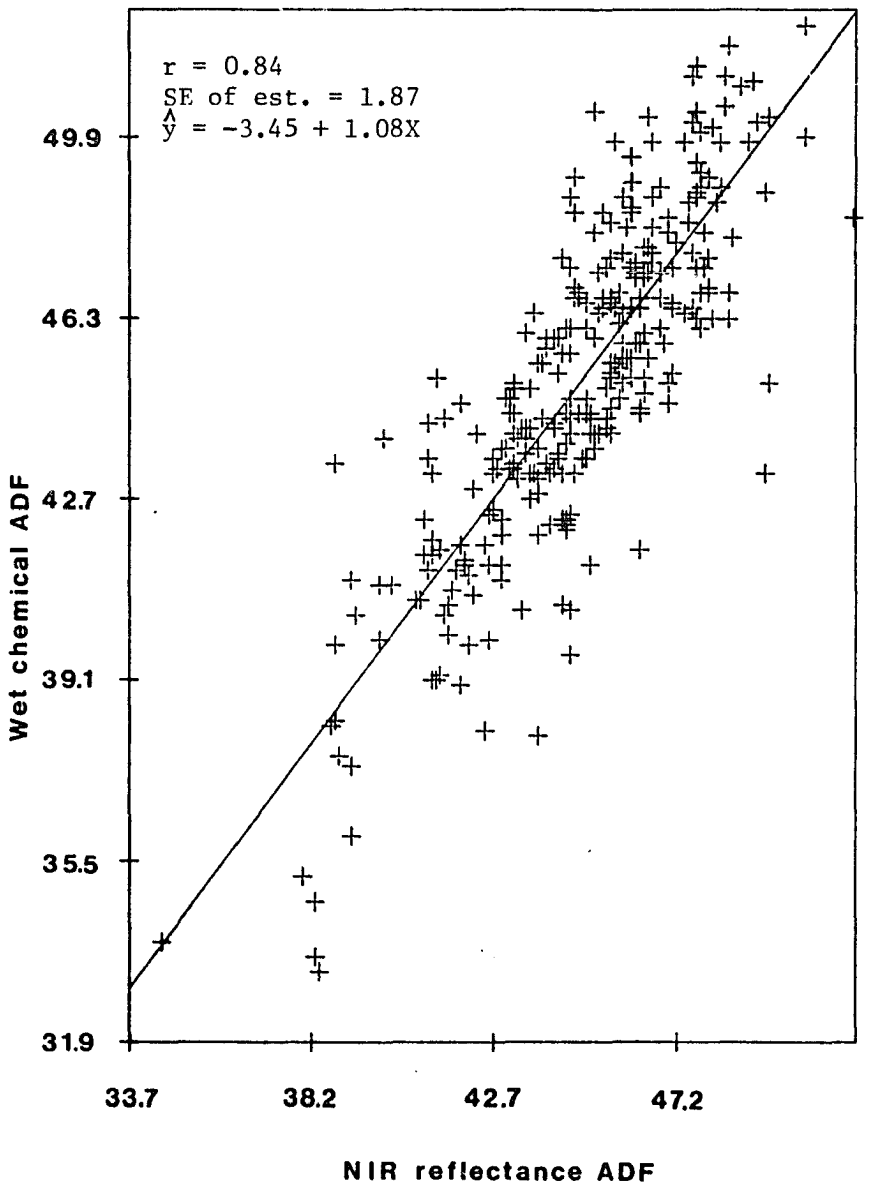

Figure 31. Regression line between laboratory and predicted stem ADF in % of 294 independent samples of alfalfa.

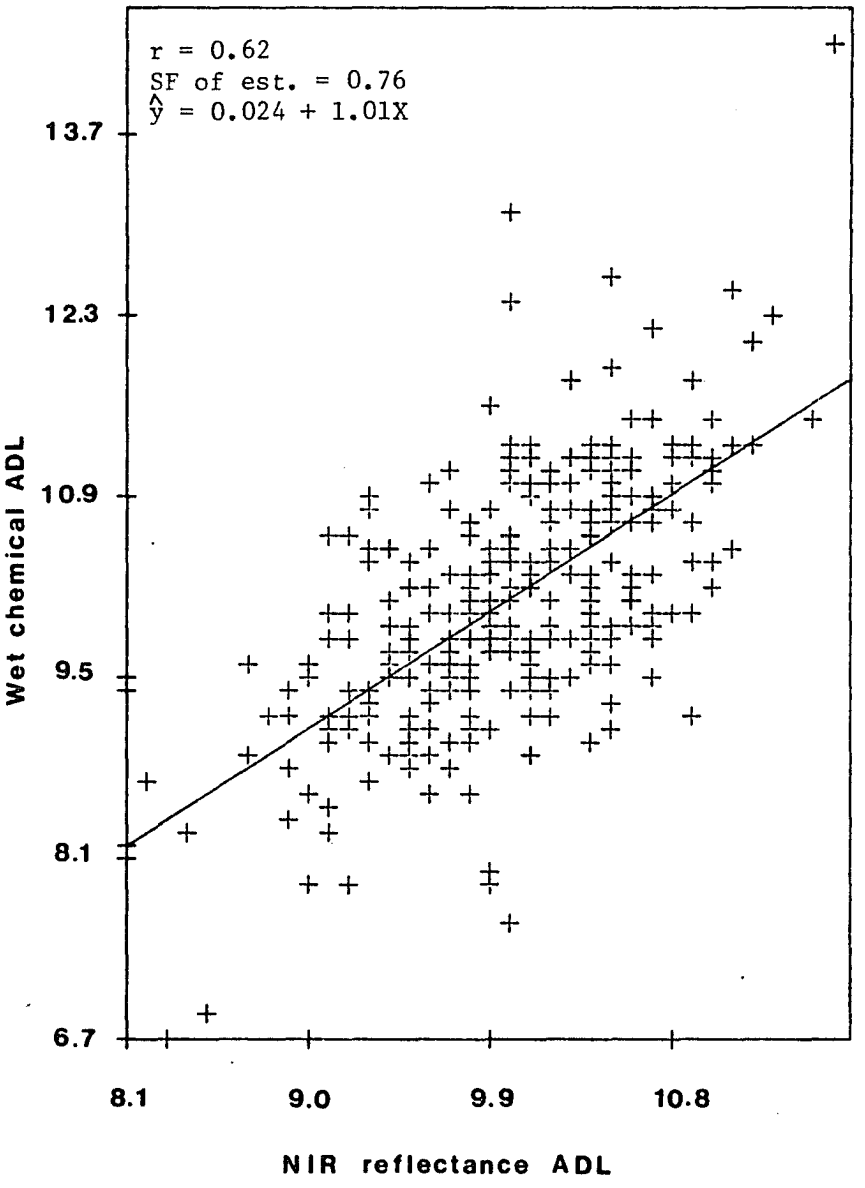

Figure 32. Regression line between laboratory and predicted stem ADL in % of 294 independent samples of alfalfa.

Inheritance of Specific Stem Weight

When an alfalfa plant high in SSW was self-pollinated, its progeny remained high (Table 30). When an individual plant was low in SSW, its progeny had a tendency to remain low in SSW. When a plant high in SSW was crossed with another individual which was also high in SSW, their progeny were also high. When a high SSW genotype was crossed with a low SSW genotype, the progeny were high in SSW. Progeny from the reciprocal cross were also high in SSW. When low by low SSW crosses were made, progeny had a tendency to remain low in SSW. Thus, high SSW appears to be a dominant trait. Since reciprocal crosses gave similar results, it was concluded there was no maternal influence on this characteristic.

Table 30. Means of first filial hybrids and first generation selfs for specific stem weight.

Crosses	Mean SSW mg/5 cm	Combined Crosses	Mean SSW mg/5 cm
$H_1 \times H_1$	120.0		
$H_2 \times H_2$	97.7	H X H	110.9
$H_1 \times H_2$	114.0		
$H_2 \times H_1$	112.0		
$H_1 \times L_1$	111.7		
$H_1 \times L_2$	109.7	H X L	109.4
$H_2 \times L_1$	109.0		
$H_2 \times L_2$	107.3		
$L_1 \times H_1$	116.3		
$L_2 \times H_1$	107.3	L X H	109.3
$L_1 \times H_2$	110.7		
$L_2 \times H_2$	103.0		
$L_1 \times L_2$	99.0		
$L_2 \times L_1$	93.0	L X L	87.7
$L_1 \times L_1$	76.7		
$L_2 \times L_2$	81.7		

H = High SSW

L = Low SSW

CONCLUSION

The mean and variance of SSW (specific stem weight) of alfalfa varies from one harvest to another depending primarily on temperature. Specific stem weights were highest during spring and early summer and lowest in August. Plant height and forage yield also declined with high summer temperatures. The leaf-to-stem ratio increased in August because there is a reduction of stem growth. Stem ADF (acid detergent fiber) and stem ADL (acid detergent lignin) decreased as stem growth decreased.

The analyses of variance revealed that significant differences occurred among 98 alfalfa clones for SSW, plant height, yield, leaf-to-stem ratio stem ADF, stem ADL, leaf ADF, and leaf ADL. Differences were sufficiently great to permit selection in both the high and low ranges for each of these traits when standard deviations were used.

SSW could be used as a tool for selection for total dry matter production since there was considerable variation. SSW had little effect on total dry matter production of spaced plants but SSW increased the total forage yield of single stems. SSW may have more of an effect on total dry matter production in dense stand conditions since there would be less spreading out from the crown.

There was significant variation of stem ADF and stem ADL; both of these traits are useful predictors of digestibility. Selection for low stem ADF would increase digestibility of the forage and might

increase weight gain of ruminants. With more intake and more highly digestible fiber, more energy would be available for animal performance.

The SSW was not correlated with stem ADF; that is, an increase in SSW does not necessarily mean an increase in stem ADF. The coarseness of the stem was not related to high fiber; thus, a coarse stem could be selected with high digestibility and low ADF. Selection of a plant with high SSW and with low stem ADF might be advantageous. Such alfalfa plants would be expected to be more nutritious and would be expected to produce faster animal weight gain. There would be more intake and more digestible nutrients available with lower ADF.

There was significant variation in leaf ADF and leaf ADL and also a positive, but low, correlation between stem ADF and leaf ADF; thus, an increase in stem ADF need not necessarily be associated with an increase in leaf ADF. Leaf ADF could be useful for selection of alfalfa plants with leaf cells that rupture more slowly and thus release soluble protein in the rumen gradually. It has been suggested that such an alfalfa would cause less bloat. The low stem ADF would make the stem more digestible; therefore voluntary intake of stems may be greater. With more coarse material in the rumen, salivary secretion would be stimulated and less bloat would occur.

Since a rapid method for analysis of nutritional characteristics is needed in an alfalfa improvement program. NIR reflectance should be useful as a predictor of chemical values. Since NIR reflectance is a rapid and fairly accurate predictor, large populations of alfalfa could be examined for genetic studies. This would greatly facilitate finding

alfalfa plants low in fiber content and high in digestibility and forage yielding ability.

From a limited inheritance study it appears that high SSW in this population is controlled by one or more dominant genes. Thus, dominance could be taken advantage of in a breeding program because the trait could be selected easily.

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