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CELL-WALL CONSTITUENTS AND DIGESTIBILITY OF FOUR ALFALFA (MEDICAGO SATIVA L.) CULTIVARS

by

Terrance Jerome Vorachek

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GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Terrance Jerome Vorachek entitled CELL-WALL CONSTITUENTS AND DIGESTIBILITY OF FOUR ALFALFA (MEDICAGO SATIVA L.) CULTIVARS be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

Albert K. Dobrenz   3/29/73
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After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

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SIGNED: Terrance Jerome Vrachek

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES—Continued

LIST OF ILLUSTRATIONS

Figure Page

ABSTRACT

Several yield and nutritive quality related factors were determined on four alfalfa (*Medicago sativa* L.) cultivars under field conditions at the Soil Conservation Service Plant Materials Center, Tucson, Arizona. The cultivars were 'El Unico', 'Sonora', 'Moapa', and 'Mesa-Sirsa'. The cultivars were harvested at 1/10 and full-bloom stages of maturity. The following parameters were evaluated for each cultivar grown under the two harvest regimes. Percent *in vitro* digestible dry matter, apparent digestible (*in vitro*) dry matter production, consumptive water-use efficiency, dry forage yield, percent neutral-detergent fiber (initial and residual), and leaflet to stem-petiole ratios were determined and histological comparisons were made.

Within-harvest cultivar parameters were not generally different. When compared over the entire collection period, cultivars varied significantly for dry forage yield, apparent digestible dry matter production and leaflet to stem-petiole ratios for both stages of maturity. Mesa-Sirsa produced the most dry forage and apparent digestible dry matter per unit area for both harvest regimes.

Water-use efficiency differences among cultivars were also apparent. Mesa-Sirsa was also the most efficient cultivar with regard to water-use efficiency, while Sonora and Moapa were least efficient for the 1/10 and full-bloom stages, respectively.

INTRODUCTION

Alfalfa (Medicago sativa L.) is the most important forage crop in Arizona. In 1972 the Arizona Crop and Livestock Reporting Service reported there were approximately 80,000 hectares of alfalfa grown in the state. The alfalfa producer today is primarily concerned with yield, with little emphasis on nutritive quality, due to the reluctance of the consumer to pay a premium for quality alfalfa. This may be due in part to the belief among certain alfalfa producers and consumers (livestockmen) that alfalfa is a highly desirable forage from the standpoint of quality and needs little improvement. However, when dealing with large tonnages such as those represented by alfalfa in Arizona, small increases of 1 to 2% in digestibility may be very important.

This lack of interest in alfalfa quality on the part of the industry also results in problems for the plant breeder and physiologist in their forage quality programs. Without the support of the growers and feeders, the researcher has difficulty in justifying and funding forage quality studies.

New innovations in the determination of nutritive quality of forages have made the evaluation of this factor relatively easy and inexpensive. One of the newest and most promising methods for estimating the digestibility and nutritive values of forages is the cell-wall constituents, cell-content technique. This separates the plant material into the readily soluble cell contents and the insoluble fiber and lignin portions. These fractions may be correlated to such factors as leaf to stem-petiole ratio, cultivar differences and stage of maturity at harvest.

This study was initiated to provide some basic information on the *in vitro* digestibility of forage produced by four alfalfa cultivars grown in Arizona. The objectives of this study were: (a) to determine the variation in dry matter yield and *in vitro* digestibility of four alfalfa cultivars harvested at two stages of maturity and (b) to correlate dry matter yield, *in vitro* digestibility, leaf to stem ratios, neutral detergent fiber, and histological features of the four cultivars, when harvested at two stages of maturity throughout the growing season.

LITERATURE REVIEW

Stage of Maturity, Yield and Quality Interrelationships

Crop yields have generally been reported as kilograms or metric tons of plant material per hectare. Such yields were measured solely on the basis of total dry matter production of the economically important portion of the crop. Total yield, long used as virtually the sole factor in the evaluation of a species or cultivar, was frequently unrelated to nutritive value (Hazell, 1965; Hughes, Heath and Metcalfe, 1962; Leach, 1968; Smith, 1965). Recently, more emphasis has been placed on the relationship of dry matter yield and nutritive quality of a forage, as influenced by cultivar differences, management practices and seasonal variation. These studies have included comparisons of various nutritive aspects indicative of quality, e.g., protein, fiber, and soluble carbohydrate content and digestibility estimates (Blaser, 1964; Carlton et al., 1968; Cole et al., 1970; Winch, Sheard and Mowat, 1970; Wright and Dobrenz, 1970).

Changes in the nutritive value and ultimate worth of numerous forages in ruminant diets have also been illustrated by numerous studies in the past (Balk et al., 1972; Blaser, 1964; Carlton et al., 1968; Meyer et al., 1960; Winch et al., 1970). Higher dry matter yields, along with much lower nutritive quality, were observed with advances in the stage of maturity. Dry matter yields had the most significant positive correlations when compared to the fiber and lignin contents of the forages under study.

Forage digestibility has been directly correlated to the intake and palatability of a given species. These factors have been largely evaluated in animal performance studies, generally based on weight gain (Colburn, Evans and Ramage, 1968). Dry matter yields and in vitro and in vivo digestibility tended to show strong negative correlations with the fiber and lignin content (Blaser, 1964; Baumgardt and Smith, 1960; Colovos et al., 1969; Harkness and Alexander, 1969; Van Dyne and Weir, 1964). Bland and Dent (1964) found that in early spring, early maturing cultivars with succulent leaves were much preferred by grazing animals and reported that preference appeared to be significantly related to digestibility and the soluble carbohydrate content of the forage. Heady (1964) defined palatability as plant characteristics or conditions which stimulate a selective response by animals. Campling (1964), however, questioned the term palatability and stated that palatability has become equated with voluntary intake or the quantity of forage consumed. He suggested that forage intake was closely related to the digestibility and nutritive value of the plants.

Many of the variations in chemical composition and digestibility as affected by stage of maturity, are directly related to changes in the leaf to stem ratio. This relationship has been illustrated by numerous palatability and digestibility studies carried out through the years. Researchers working with grazing animals often used palatability as a criteria in the selection of high quality, highly digestible forages. Grazing animals select specific plant parts, primarily the leaves and floral parts, when allowed to graze freely on a range in good condition. This selection of specific plant parts was reportedly based on the chemical variation of the plant parts, namely the higher concentration of the soluble nutrients and lower fiber and lignin content of the leaves versus the stems (Cook, Harris and Stoddart, 1948; Garner, 1963; Hardison et al., 1954; Heady and Torell, 1959; Van Dyne, 1963). Galt et al. (1969) reported similar results based on a preference study on Arizona rangeland. They found the crude protein of rumen samples to be much higher than that of hand clipped samples and concluded that the difference was due to the selective grazing of plant parts by the animal.

Several studies have shown a progressive decline in *in vivo* and *in vitro* digestibility as forages matured. This change in digestibility was associated with a significant decrease in soluble cell-contents and an increase in cell-wall constituents. These variations were closely associated with decreased leaf to stem ratios (Farhoomand and Wedin, 1968; Sosulski, Patterson and Law, 1960; Terry and Tilley, 1964; Zaleski and Dent, 1960). Dobrenz, Schonhorst and Thompson (1969) also found a direct correlation between leaf to stem ratio and the protein content of several alfalfa cultivars adapted to the desert southwest. When the leaf to stem ratios were highest during the growing season, the protein content of the forage was also highest. Luckett and Klopfenstein (1970) and Koch, Dotzenko and Hinze (1972) reported that changes in the *in vitro* dry matter disappearance (DMD) of alfalfa and sainfoin (*Onobrychis* spp.) were closely related to changes in the fibrous components of stems and to changes in the leaf to stem ratio. They found a decreased protein content, with advanced plant maturity, along with high negative correlations between DMD and fiber components of the stems and whole plants. Alfalfa harvested at the bud stage of maturity was consistently higher in percent leaves, protein content, and DMD than alfalfa harvested at either 1/10-bloom or full-bloom.

Meyer et al. (1960) published data obtained from a feeding trial which supported the findings of the previous researchers. Weight gains of lambs fed hay harvested at different stages of maturity were significantly correlated with lignin content. The critical point with respect to nutritive value occurred at approximately 1/10-bloom. However, upon reaching 1/10-bloom, the nutritive value changed at a much slower rate than at the earlier stages of maturity. Correlations of lignin and protein contents with daily weight gains were -.94 and 0.85, respectively. Blaser (1964) also reported a progressive decline in digestibility as plants changed from leafy vegetative to stemmy growth as they matured. This change in leaf to stem ratio was accompanied by a rapid growth of structural materials (cellulose and lignin) and a moderate to slight increase of the soluble materials (protein, lipids, and carbohydrates). Blaser's work also showed variations in animal performance because of forage digestibility differences, which were directly correlated to the chemical constituents of the plants. Similar changes were noted by Sherrod (1971) who evaluated the effects of the stage of maturity on the chemical composition of *Kochia scoparia* (L.) Schrad.

Management and environmental factors which effect plant growth and morphology have also been shown to significantly influence nutritive quality and digestibility. Vough and Martin (1971) found that forages grown under high soil moisture stress had a higher percentage of leaves, a higher *in vitro* digestibility, and lower acid-detergent fiber and lignin content than the forages grown under low moisture stress. However, significant differences in crude protein between the two moisture regimes were not noted.

The effects of temperature on nutrient content of alfalfa has also been studied. Jensen, Massengale and Chilcote (1967), Smith (1969) and Marten (1970) reported that alfalfa grown under high temperatures contained significantly more crude protein. Smith (1969) also found a decrease in the non-structural carbohydrates and *in vitro* digestibility with high temperatures. Marten (1970) reported similar results for the non-structural carbohydrates, but noted that *in vitro* digestibilities were not significantly influenced by temperature.

Seasonal Variation in Plant Structural Material

Van Soest (1967) stated that forage dry matter could be broken into two fractions on the basis of nutritional availability. The first fraction, the cell-wall contents, consisted primarily of cellulose, hemicellulose and lignin, which were directly related to the chemical digestibility of a forage. The second fraction, cell-contents, was composed of the soluble compounds, i.e., lipids, soluble carbohydrates, proteins and other water soluble compounds. These compounds were reported to be 98% digested by the animal. As a result, Van Soest suggested that in forage digestibility estimation the major emphasis should be placed on cell-wall constituents with little emphasis on this readily available fraction. The soluble portion may account for 60% or more of the forage dry matter and up to 90% of the dry matter in concentrates. These data agreed with investigations conducted by Stanley, Beaty and Palmer (1968) using clover (Trifolium vesiculosum (Savi)) in which they found the cell-walls of young forage comprised less than 40% of the total yield. During flowering, however, the cell-wall constituents were 40 to 47% and reached a high of 50+% at maturity. A subsequent decrease in the cell contents (soluble fraction) was also reported during this period. This relationship was also noted in a study conducted by Deinum and Van Soest (1969) in which they found that the percent of apparently digested cellular contents, as determined by an in vitro technique, was closely related to the percent cellular contents.

Fiber contents in most forages were negatively correlated with leaf to stem ratio and positively correlated to stage of maturity. Increased fiber resulted in a decreased in vitro and in vivo digestibility of the forage (Johnson, de Faria and McClure, 1971; Keys, Van Soest and Young, 1970; Sullivan, 1966; Tomlin, Johnson and Dehority, 1965). Generally, advances in the stage of maturity of a forage resulted in significant increases in the fiber fraction and subsequent decreases in the soluble fraction of plant material (Colovos et al., 1969; Luckett and Klopfenstein, 1970; Van Soest, 1965; Wilkinson, Adams and Jackson, 1970). Numerous researchers have reported the effects of specific plant constituents on digestibility (Hogan and Weston, 1969; Hogan, Weston and Lindsey, 1969; Sosulski and Patterson, 1961; Terry and Tilley, 1964; Winch et al., 1970). These researchers found that the fiber fraction, primarily cellulose and lignin, was negatively correlated to forage digestibility while the soluble fraction, namely protein in this instance, showed a positive correlation.

Animal intake and preference studies have also shown the interrelationship between the two fractions. Keys et al. (1970) found a decreased digestibility of both hemicellulose and crude protein as a result of an increased fiber intake. Balch (1960) found that the digestion rate of fiber was much slower than that of the non-fibrous fractions, which resulted in much slower rates of passage for high fibrous feeds and thus much lower rates of consumption. The point at which the fiber fraction became a limiting factor was thought to be when the cell-wall content was 50 to 60% of the forage dry matter content (Van Soest, 1965). Collaborative data were obtained in a study conducted by Welch and Smith (1969), in which they reported that poor quality roughage with high fiber and low cell contents resulted in a greater rumination time and lower digestibility. Bland and Dent (1964) found significant positive and negative correlations between animal preference and the soluble carbohydrate content and fiber content, respectively. In early spring, early maturing varieties with succulent leaves were much preferred, which suggested digestibility and soluble carbohydrates significantly influenced animal preference.

Lignin has been described as an amorphous material which in close association with the fibrous carbohydrates (cellulose and hemicellulose) makes up the cell-wall fraction. The effects of lignin upon forage digestibility are not clear, although various theories have been proposed. Kamstra, Moxon and Bentley (1958) proposed a 'physical barrier' theory, which indicated that lignin encrustation limited the exposure of the various carbohydrate fractions to the rumen microorganisms and their hydrolytic enzymes. This theory gained support as a result of studies conducted by Dehority and Johnson (1961) and Dehority, Johnson and Conrad (1962) in which improved cellulose digestibility (in vitro) was brought about by wet-ball milling. Van Soest (1967) reported that the availability of holocellulose (cellulose and hemicellulose) was a function of lignification and structural features of the cell-wall. He discounted the effects of lignification on the digestibility of the soluble cell-contents. Although the physical inhibition theory has gained the most support, some type of physicochemical inhibition should not be ruled out. This theory suggested some type of effect on enzymatic reactions which may be due to either a chemical inhibitor or the encrustation of other cell-wall constituents by lignin.

Van Soest (1969) suggested that the actual mechanism responsible for decreased cell-wall constituent digestibility may be the formation of lignin-polysaccharide complexes. This concept appears to be of greater importance in legumes than grasses since they are generally much higher in lignin content when compared at the same stage of maturity and dry matter content. Therefore, he suggested that based on *in vitro* evaluation the proportion of fiber digested in legumes may be less than in grasses. Tomlin, Dehority and Johnson (1961) reported similar differences in the relationship of lignin to *in vitro* digestibility in grasses and legumes. Their study showed a definite linear trend between the cellulose digestibility of orchardgrass (*Dactylis glomerata* L.), smooth bromegrass (*Bromis inermis* Leyss.) and reed canarygrass (*Phalaris arundenacea* L.) hay as the grasses matured. However, the same relationship was not observed for alfalfa, red clover (*Trifolium pratense* L.) and birdsfoot trefoil (*Lotus corniculatus* L.). Based on *in vitro* studies, Van Soest (1971), as cited by Johnson (1972) concluded that the differences in the digestibility of grasses and legumes were due to the influence of the fiber fraction. He indicated that the lignin in grasses influenced the digestibility of the available cellulose fraction, whereas the lignin in legumes influenced the amount of the available cellulose fraction.

Many researchers have indicated that the quantitative differences in lignification are not simple and may require the development of new techniques to detect these differences. These complex factors were illustrated by work carried out by Ghose and King (1963) who reported that differences in *in vitro* cellulose digestion occurred in forages with approximately the same lignin content. Quicke et al. (1959) also reported that differences in the lignin content of mature forages were too small to account for observed differences in cellulose digestibility. They concluded that differences in digestibility of equally lignified cell-wall fractions may have been due to a selective availability of the various fractions to the ruminant animal. This difference in availability was suggested to be the major reason for the lack of consistency in lignin and forage quality relationships. Deinum and Van Soest (1969) agreed with the previous statements as a result of their 'summative equation' study which was based on a chemical procedure. This study produced very inconsistent results when attempts were made to correlate the lignin content and chemical degradation of the cell-wall constituents. The lack of uniformity was theorized to be due to the low lignin content of the forages studied, which resulted in an increased influence of plant silica and soil contaminants. However, when the chemical procedure was replaced with an *in vitro* technique, fairly accurate correlations between forage digestibility and lignin content were observed.

Van Soest (1969) pointed out that silica may have profound effects on the nutritive value of plants. Although it is widely important in monocotyledonous plants, silica is not generally thought to be taken up by legumes and is of little importance when evaluating these species.

Influence of Species and Cultivar on Digestibility

Few data are available on the nutritive variations among plants below the species level. Considerable research has shown significant differences in digestibility among forage species. Riewe and Lippke (1970) reported that legumes had significantly less cell-wall material than grasses over a wide range of maturities. Although the total cell-wall constituents were lower in the legumes, the lignin content was much higher in the grasses. Differences between the chemical constituents of warm and cool-season grasses were also noted. When compared at similar stages of maturity, warm-season annual grasses were found to have higher cellular contents than cool-season grasses. When grasses with similar cellulose and hemicellulose contents were compared, the warm-season grasses also had significantly higher lignin contents. Supporting data were reported by Sullivan (1966) which indicated that the hemicellulose content of cell-walls in many warm-season perennials exceeded the cellulose content, while the reverse was true for cool-season perennial grasses. Duble, Lancaster and Holt (1971) also found significant differences in the silica and lignin contents among species in their study of warm-season grasses. Krueger et al., (1969) found significant differences in the dry matter digestibility, crude protein, and acid-detergent fiber among orchardgrass, smooth bromegrass and timothy (Phleum pratense L.). They attributed most of the differences to variations in the leaf to stem ratio and subsequent variations in chemical composition. Species variations were also reported by Terry and Tilley (1964) using perennial ryegrass (Loluim perenne L.), cocksfoot (Dactylis glomerata L.), timothy, lucerne (Medicago sativa L.), tall fescue (Festuca elatior var Arundinacea (Schreb.) Wimm.) and sainfoin. In vitro digestibilities of these forages were directly associated with a reduction in the water-soluble and protein constituents. They also noted a reduction in fiber digestibility, resulting from a decreased leaf to stem ratio. Other researchers have reported species differences in in vitro and in vivo digestibility and chemical composition as influenced by leaf to stem ratios (Farhoomand and Wedin, 1968; Gangstad, 1966; Rabas, Schmid and Marten, 1970; Walters et al., 1967).

Significant differences have been reported in the digestibility of forage cultivars due to variations in chemical composition. The chemical components suggested most often to be responsible for these differences were crude protein, simple sugars, fiber and lignin. Digestibility (in vivo and in vitro) has been found to be positively correlated to crude protein and simple sugars and negatively correlated to the fiber and lignin contents (Bland and Dent, 1962; Buckner et al., 1967; Dent, 1963; Sosulski et al., 1960; Wedin, 1970). Differences in the in vitro digestibility of several cultivars of cocksfoot have been reported (Cooper et al., 1962; Dent and Aldrich, 1968). Supporting data illustrating differences in chemical composition of alfalfa cultivars suited to growth in the desert southwest were reported by Dobrenz et al. (1969). They found significant differences in the crude protein content among cultivars on the whole plant basis. The average percentage protein based on the whole plant analyses ranged from 19.8% in Hairy Peruvian to 17.4% for M-56-11-T.C., an experimental line. The protein values tended to follow the same seasonal pattern as the leaf to stem ratios, which indicated a direct relationship between the two factors.

Studies have also been conducted using individual genotypes and clones. Researchers have reported positive correlations between the various chemical constituents and digestibility and have concluded that forages can be bred for quality factors. Clonal and genotypic variations have been observed in relationship to dry matter digestibility and the fiber and crude protein contents. The heritability of these factors have been high enough to allow selection for these characteristics in order to produce a higher quality forage (Burton, Hart and Lowrey, 1967; Burton, Knox and Beardsley, 1964; Carlson et al., 1969; Cooper et al., 1962; Griffith and Cooper, 1959; Hernan Chaverra, Davis and Barnes, 1967; Monson, Powell and Burton, 1972; Rieck, Croy and Davies, 1972; Wurster, Kamstra and Ross, 1971). However, Burton and Monson (1972) in a study of dry matter disappearance (DMD) of a world collection of 500 bermudagrasses (Cynodon dactylon L.) concluded selection at the clonal level was not feasible. Although genotypic differences were noted, DMD appeared to be conditioned by a number of genes that exhibited very little dominance. This lack of dominance made selection for this characteristic difficult.

MATERIALS AND METHODS

This field study was conducted at the Soil Conservation Service Plant Materials Center at Tucson, Arizona. The field data were collected between April and October 1971, on two borders of alfalfa (*Medicago sativa* L.) established in 1967. Cultivars of 'Mesa-Sirsa', 'El-Unico', 'Sonora' and 'Moapa' were planted in a randomized complete block design with four replications. Each plot consisted of an area 3.4 m by 8.5 m (Figure 1).

The soil at the experimental site consisted of Comoro fine sandy loam. This is a deep, well-drained soil with a fine sandy loam to sandy loam surface and subsoil to approximately 76 cm (Joy, 1970). The borders were irrigated when 50% of the available soil moisture was utilized. An Oakfield probe was used to check the borders for moisture to 91.4 cm to determine when irrigation was required.

Collection and Preparation of Field Samples

Forage yields were determined by harvesting an area 91.4 cm by 7.6 m within each replicated plot. Harvests were made when the alfalfa reached the 1/10 or full-bloom stage on the respective borders. The 1/10-bloom stage was defined as the point when 1/10 of the stems had one or more blossoms. A Milbradt mower was used to cut the alfalfa approximately 6 cm above ground level. A 500 g sample was taken from

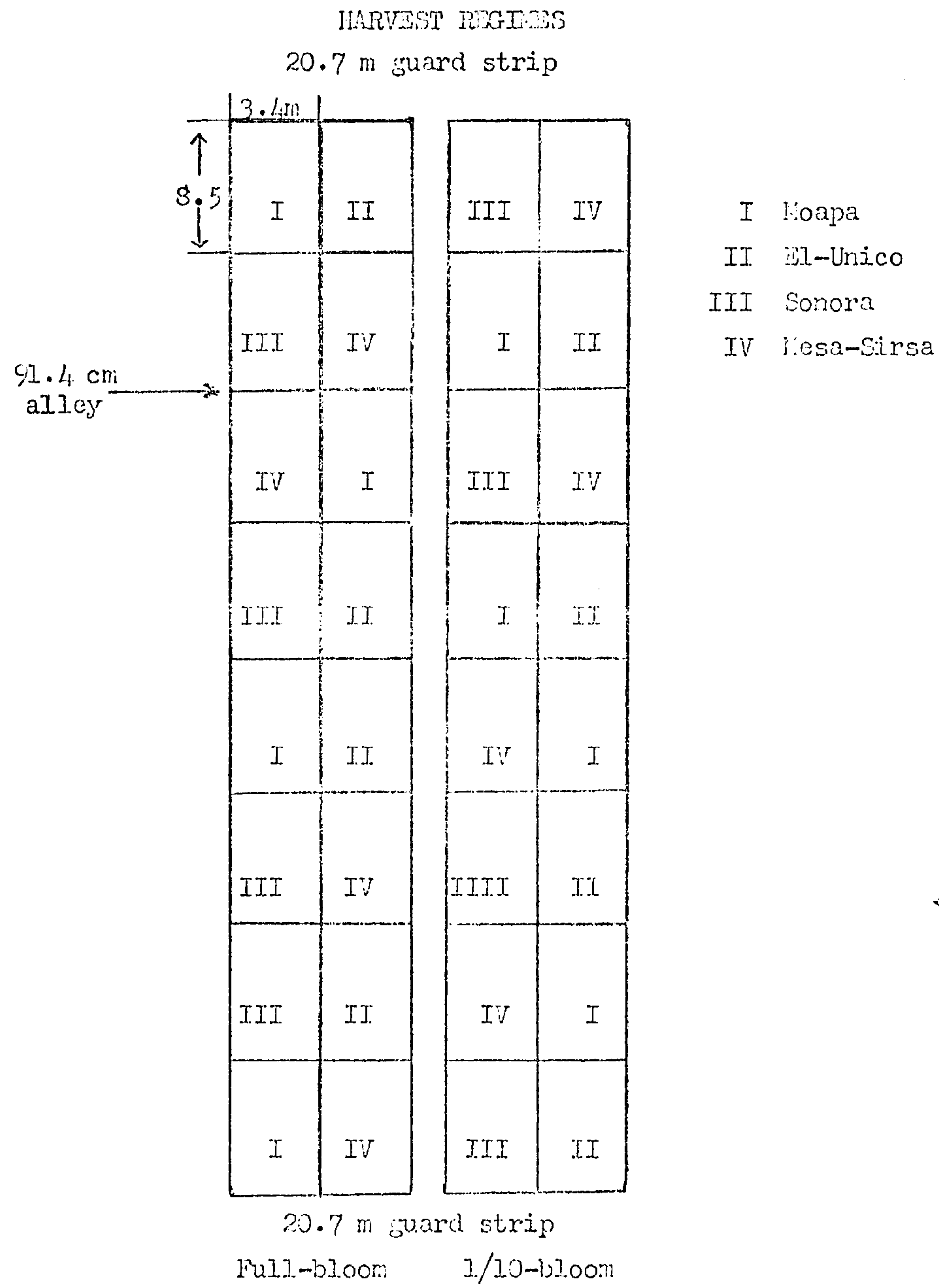

Figure 1. Field design used to study several characteristics of four alfalfa cultivars harvested at two stages of maturity in 1971.

each plot for moisture determination at each harvest. Yields were calculated on an oven dry (80 C for 12 hr) basis. The remainder of the forage from the entire clipped area was weighed and placed in large burlap bags. The material was dried at 45 C for 96 hr and ground through a hammer mill. The forage was then passed through a Wiley mill with a 2 mm screen. Subsamples (approx 100 g) were obtained and stored in square glass jars for laboratory analysis.

Samples were also taken from each replication immediately after cutting for leaf to stem-petiole ratio determinations. The leaflets were separated from the stem and petioles and dried at 80 C for 24 hr. The leaflet weight was divided by the stem-petiole weight to obtain the leaf to stem-petiole ratio.

Histological samples were randomly collected from each of the cultivars prior to each harvest. These stem cross-sections were taken at the sixth node of each stem. The sections were dehydrated, embedded, mounted and stained according to standard histological techniques (Johansen, 1940).

Laboratory Analysis

The forage samples (100 g) previously stored were mixed once again, subsampled and ground through a Wiley mill with a 40 mesh screen in final preparation for laboratory analysis.

A modified Van Soest, Wine and Moore (1966) technique was utilized to determine the cell-wall constituents (CWC) and cell-contents (CC). Alfalfa digestibility values were determined by (1) initial neutral-detergent fiber, (2) *in vitro* digestion, (3) residual neutral-detergent fiber, and (4) calculation of the apparent digestible dry matter (ADDM) values. The *in vitro* digestion procedure utilized was a modified Tilley and Terry (1963) technique in which the pepsin digestion was omitted.

Initially, 0.5 g of the ground plant material was added to a 250 ml digestion flask, followed by the addition of 100 ml neutral-detergent solution, 2 ml decahydronapthalene, and 0.5 g sodium sulfite in that order. The contents of the flasks were refluxed for 30 min at a gentle boil. The digested plant material was then poured into tared Gooch crucibles and filtered via suction. The residue was thoroughly washed with hot water and acetone during filtration, and dried at 100 C for 12 hr. Calculation of the initial neutral-detergent fiber content (cell-wall constituents) was determined by subtracting the tared crucible weight from the crucible plus residue weight.

The artificial rumen (*in vitro*) technique required the collection of rumen innoculum. Rumen fluid collections were made at approximately noon for each incubation trial. This allowed the 4 to 6 hr post-prandium necessary for adequate concentrations of rumen microorganisms. The rumen ingesta were strained through four layers of cheese cloth into a preheated thermos bottle for transport back to the laboratory. Immediately upon reaching the laboratory, 15 ml of the strained rumen fluid and 15 ml of the pH 7.0 buffer solution were added to the 100 ml Nalgene test tubes which contained 0.5 g plant material (Figure 2). Care was taken

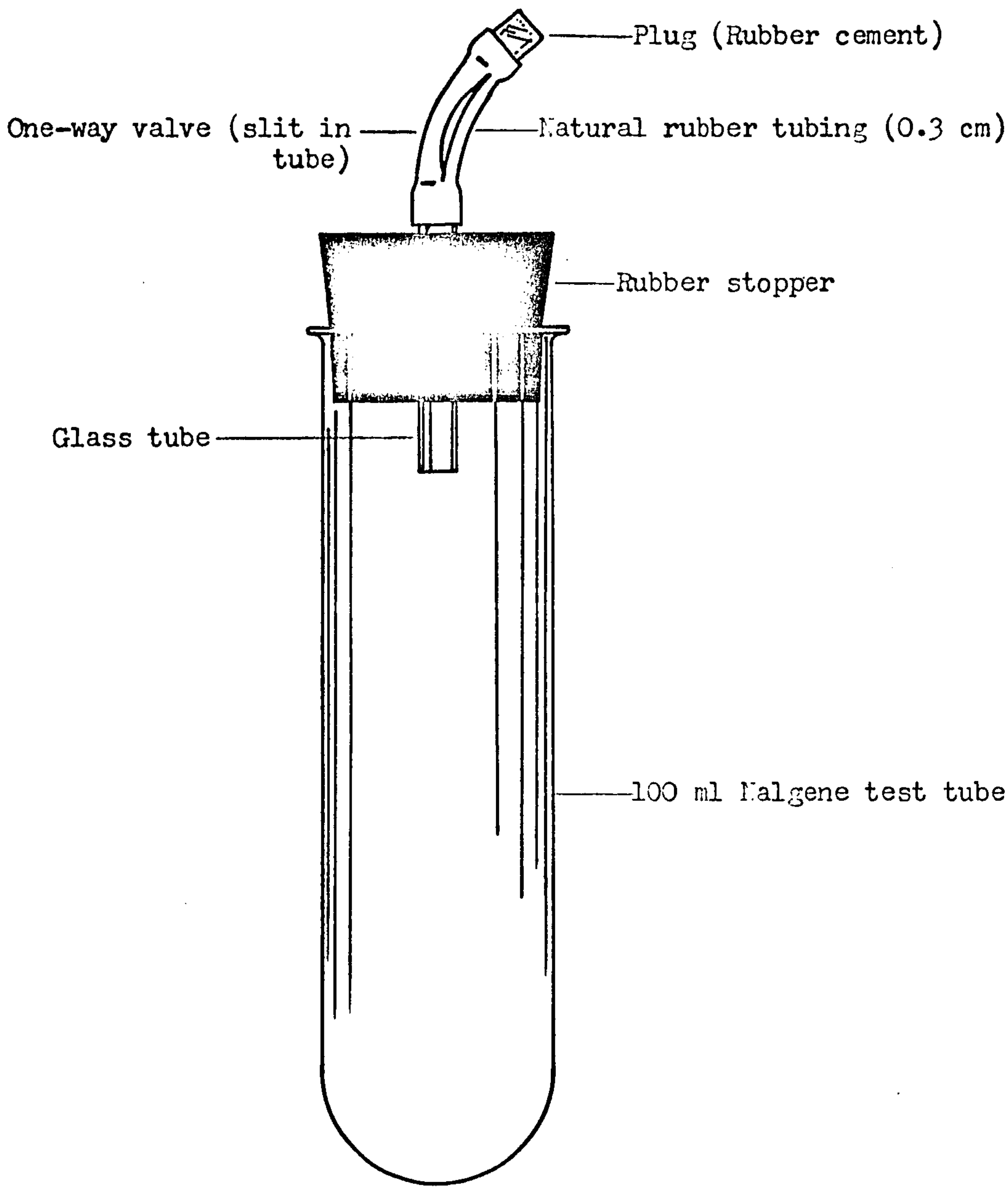

Figure 2. Schematic diagram of the artificial rumen (in vitro) digestion apparatus.

throughout the entire procedure to maintain the thermal environment necessary for maximum microbial survival. The tubes were then flushed with CO_2 gas, capped with a one-way valve and stoppered. This allowed gases to escape, while maintaining an anaerobic environment. The tubes were then incubated in a 39 C water bath for 24 hr, during which each tube was hand agitated at approx 4 hr intervals. Upon completion of the incubation period, the tubes were removed and 1 ml of a 1% mercuric chloride solution was added to the digested sample to immediately stop all further microbial action. These samples were then frozen in the tubes until analysis for residual neutral-detergent fiber was conducted.

The final portion of this study involved the determination of undigested cell-wall constituents (residual neutral-detergent fibers). The frozen samples (including rumen innoculum and buffer) were allowed to thaw and were emptied directly into the reflux flasks. These samples were then analyzed by the same procedure outlined for the determination of initial cell-wall constituents. Plant material added via the rumen innoculum was corrected for each sample through use of a blank in the *in vitro* digestion procedure.

The ADDM values were calculated according to Van Soest's (1967) summative equation: ADDM (%) = [% total CWC x % digestible CWC + (% CC x .98)] - 12.9%. The 98% correction factor for cell-content digestibility was discussed earlier. The 12.9% value was determined by Van Soest (1967) as a correction for endogenous and bacterial matter.

The numerical data in this study were analyzed according to a split-plot in time, with main plots in a randomized block design (Steel and Torrie, 1960). Analyses of variance were determined for all variables studied both on an individual and combined harvest basis. Mean separations were made according to the Student-Newman-Keul's Test. Correlation coefficients between all variables were also calculated.

RESULTS

Dry Forage Yield

Average total dry forage yield (kg per plot) of the four cultivars ranged from 17.2 (Mesa-Sirsa) to 14.9 (Sonora) for the 1/10-bloom stage of development and from 18.1 (Mesa-Sirsa) to 16.5 (Moapa) for the full-bloom stage (Figure 3). Forage production generally followed the same trends over the collection period for both stages of maturity. Approximately two-thirds of the total forage production was harvested within the first half of the growing season, April through July (Table 1). Average dry forage production of the four cultivars was not significantly different within-harvests at either the 1/10 or full-bloom stages of maturity (Table 2).

Average forage yield at the 1/10-bloom stage ranged from a mid-season high (July 7) of 3.6 to a low of 1.6 kg per plot for the final harvest (October 7). This peak was somewhat later than the mid-June peak observed by Joy, Poole and Dobrenz (1972). The fact that seasons were different and stands were older could account for the differences. Average production per harvest for the individual cultivars, cut at 1/10-bloom ranked in order from highest to lowest, were Mesa-Sirsa, El-Unico, Moapa and Sonora, with values of 2.9, 2.7, 2.7, and 2.5 kg per plot, respectively (Table 3). These cultivar differences agreed with previous data (Poole, 1971). Dry forage production per harvest was significantly higher for Mesa-Sirsa than Sonora.

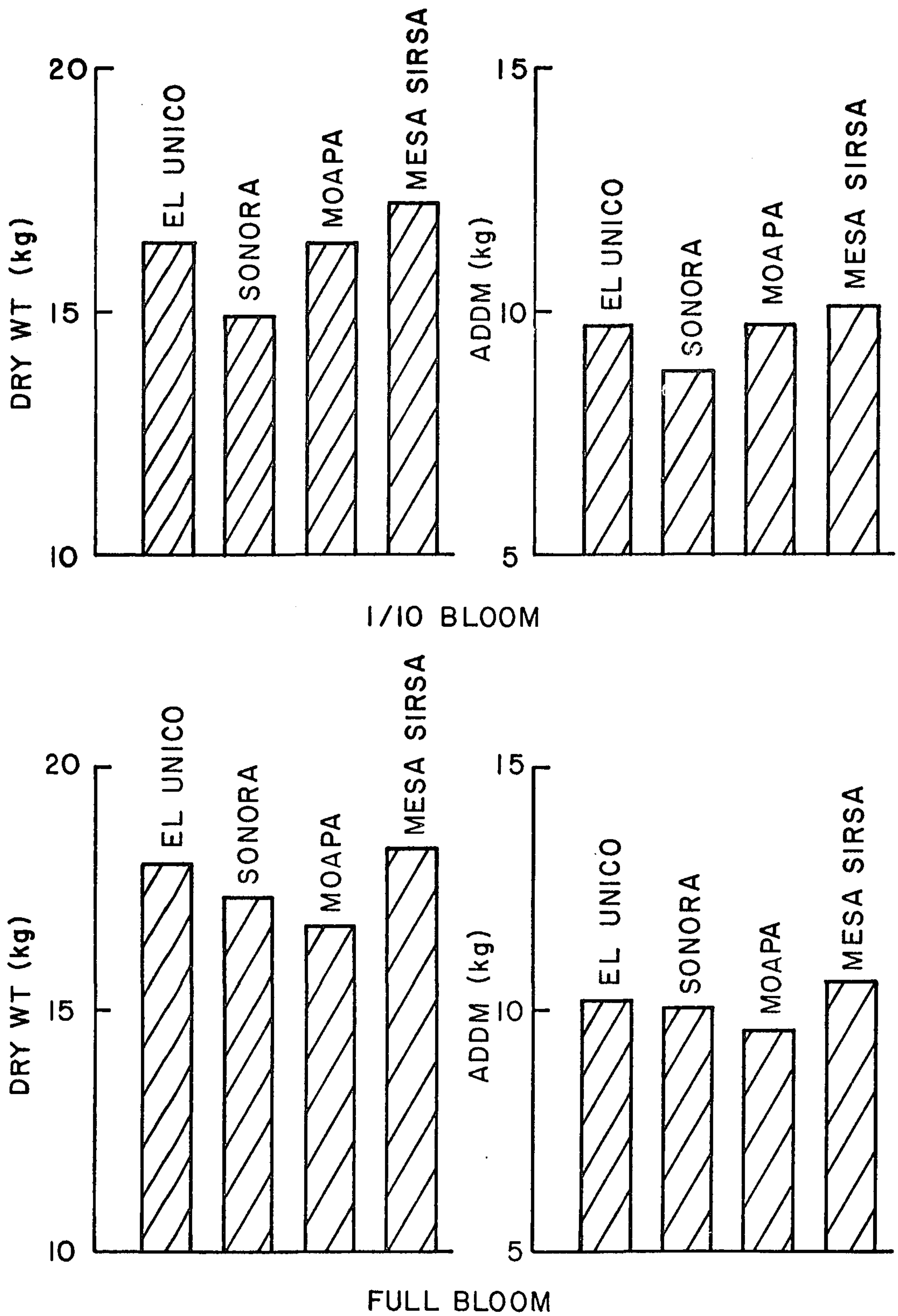

Figure 3. Total dry forage yield and apparent digestible dry matter production per plot of four alfalfa cultivars harvested at two stages of maturity in 1971.

Table 1. Mean values for several factors measured on alfalfa at two stages of maturity in 1971.

Variables	Harvest dates					
1/10-bloom	May 5	June 5	July 7	Aug. 9	Sept. 9	Oct. 7
Dry weight (kg/plot)	3.2 b*	3.4 ab	3.6 a	2.6 c	1.8 d	1.6 d
Leaflet to stem-petiole ratio	.62 b	.51 d	.36 f	.58 c	.46 c	.79 a
Initial neutral-detergent fiber (%)	39.8 b	36.3 c	39.9 b	40.5 b	41.8 a	37.1 c
Residual neutral-detergent fiber (%)	26.4 c	24.3 d	28.5 ab	29.4 a	28.3 b	24.6 d
Apparent digestible dry matter (%)	59.5 b	61.5 a	57.4 c	56.5 c	57.7 c	61.2 a
Apparent digestible dry matter (kg/plot)	1.9 b	2.1 a	2.1 a	1.5 c	1.1 d	1.0 d
Full-bloom	May 13	June 21	July 27	Sept. 1	Oct. 14	
Dry weight (kg/plot)	3.9 b	4.4 a	3.2 c	3.2 c	2.7 d	
Leaflet to stem-petiole ratio	.54 b	.40 c	.44 c	.44 c	.70 a	
Initial neutral-detergent fiber (%)	39.8 d	41.6 c	43.0 b	44.8 a	39.1 c	
Residual neutral-detergent fiber (%)	26.7 c	29.0 b	30.1 ab	30.8 a	27.3 c	
Apparent digestible dry matter (%)	59.3 a	57.0 b	55.9 c	55.2 c	58.6 a	
Apparent digestible dry matter (kg/plot)	2.3 b	2.5 a	1.8 c	1.8 c	1.6 c	

* Within stage of maturity, means for each variable followed by the same letter are not significantly different at 5% level.

Table 2. Mean forage production of four alfalfa cultivars harvested at two stages of maturity in 1971.

Cultivars	Dry forage production (kg/plot) Harvest dates					
1/10-bloom	May 5	June 5	July 7	Aug. 9	Sept. 9	Oct. 7
El-Unico	3.2 a*	3.4 a	3.8 a	2.6 a	1.8 a	1.7 a
Sonora	2.8 a	3.1 a	3.3 a	2.6 a	1.7 a	1.5 a
Moapa	2.9 a	3.6 a	3.8 a	2.5 a	1.9 a	1.7 a
Mesa-Sirsa	3.7 a	3.6 a	3.5 a	2.8 a	2.1 a	1.6 a
Full-bloom	May 13	June 21	July 27	Sept. 1	Oct. 14	
El-Unico	4.7 a	4.4 a	3.1 a	3.2 a	2.5 a	
Sonora	3.3 a	4.5 a	3.1 a	3.2 a	3.1 a	
Moapa	3.4 a	4.3 a	3.3 a	3.2 a	2.4 a	
Mesa-Sirsa	4.3 a	4.6 a	3.2 a	3.2 a	2.8 a	

* Within harvest and stage of maturity, means followed by the same letter are not significantly different at 5% level.

Table 3. Mean values for several factors measured on four alfalfa cultivars harvested at two stages of maturity in 1971.

Variables	Cultivars				
1/10-bloom	El-Unico	Sonora	Moapa	Mesa-Sirsa	Mean
Dry weight (kg/plot)	2.7 ab*	2.5 b	2.7 ab	2.9 a	2.7
Leaflet to stem-petiole ratio	.55 b	.57 a	.57 a	.52 b	.55
Initial neutral-detergent fiber (%)	39.3 ab	38.5 b	39.0 ab	40.1 a	39.2
Residual neutral-detergent fiber (%)	27.1 a	27.0 a	26.9 a	26.8 a	26.9
Apparent digestible dry matter (%)	58.8 a	58.9 a	59.0 a	59.1 a	59.0
Apparent digestible dry matter (kg/plot)	1.6 ab	1.5 b	1.6 ab	1.7 a	1.6
Full-bloom					
Dry weight (kg/plot)	3.6 a	3.4 a	3.3 a	3.6 a	3.5
Leaflet to stem-petiole ratio	.49 b	.52 a	.54 a	.47 b	.51
Initial neutral-detergent fiber (%)	41.8 a	41.2 a	41.5 a	42.1 a	41.7
Residual neutral-detergent fiber (%)	29.2 a	28.4 a	28.6 a	28.8 a	28.8
Apparent digestible dry matter (%)	56.7 a	57.5 a	57.3 a	57.2 a	57.2
Apparent digestible dry matter (kg/plot)	2.0 a	2.0 a	1.9 a	2.1 a	2.0

* Within stage of maturity, means for each variable followed by the same letter are not significantly different at 5% level.

The average dry forage yield for the full-bloom stage of maturity peaked slightly earlier (approx 2 wks) and was 20 to 30% higher when compared to forage harvested at the 1/10-bloom stage (Table 1). The average yield of the full-bloom harvests ranged from 4.4 (June 21) to 2.7 kg per plot (October 14). The cultivar means for dry forage production per harvest were 3.6 (Mesa-Sirsa), 3.6 (El-Unico), 3.4 (Sonora) and 3.3 (Moapa) kg per plot (Table 3).

Leaflet to stem-petiole ratios when averaged over all harvests for individual cultivars were significantly different (Table 3). Sonora and Moapa had significantly higher leaflet to stem-petiole ratios than did El-Unico or Mesa-Sirsa at both stages of maturity. The leaflet to stem-petiole ratios of the 1/10-bloom harvests averaged over the collection period ranged from 0.52 (Mesa-Sirsa) to 0.57 (Sonora and Moapa), while the full-bloom harvest values ranged from 0.47 (Mesa-Sirsa) to 0.54 (Moapa). However, these ratios generally did not differ significantly among cultivars within harvests for either the 1/10 or full-bloom stages (Table 4).

Apparent Digestible Dry Matter

Significant cultivar variation within harvests was not detected for either initial neutral-detergent fiber (INDF) or residual neutral-detergent fiber (RNDF) (Tables 5 and 6, respectively). Seasonal trends of the fiber values were evident, however, for both the 1/10 and full-bloom stages of development (Table 1). Initial neutral-detergent fiber values when averaged over all cultivars for the 1/10-bloom stage ranged

Table 4. Average leaflet to stem-petiole ratio of four alfalfa cultivars harvested at two stages of maturity in 1971.

Cultivars	Leaflet to stem-petiole ratio					
	Harvest dates					
1/10 bloom	May 5	June 5	July 7	Aug. 9	Sept. 9	Oct. 7
El-Unico	.57 a*	.51 a	.38 a	.56 a	.47 a	.81 a
Sonora	.68 a	.58 a	.38 a	.55 a	.50 a	.75 a
Moapa	.66 a	.50 ab	.35 a	.64 a	.46 a	.81 a
Mesa-Sirsa	.55 a	.45 b	.35 a	.58 a	.41 a	.77 a
Full-bloom	May 13	June 21	July 27	Sept. 1	Oct. 14	
El-Unico	.49 b	.38 a	.43 a	.45 a	.71 a	
Sonora	.59 ab	.44 a	.46 a	.42 a	.71 a	
Moapa	.63 a	.42 a	.46 a	.48 a	.74 a	
Mesa-Sirsa	.46 b	.38 a	.43 a	.42 a	.66 b	

* Within harvest and stage of maturity, means followed by the same letter are not significantly different at 5% level.

Table 5. Initial neutral-detergent fiber of four alfalfa cultivars harvested at two stages of maturity in 1971.

Cultivars	Initial neutral-detergent fiber (%) Harvest dates					
1/10-bloom	May 5	June 5	July 7	Aug. 9	Sept. 9	Oct. 7
El-Unico	40.8 a*	36.6 a	39.7 a	40.2 a	41.5 a	37.0 a
Sonora	38.4 a	35.2 a	39.6 a	40.3 a	42.0 a	35.7 a
Moapa	39.0 a	36.0 a	39.7 a	39.5 a	42.2 a	37.6 a
Mesa-Sirsa	40.8 a	37.5 a	40.6 a	40.1 a	41.6 a	38.2 a
Full-bloom	May 13	June 21	July 27	Sept. 1	Oct. 14	
El-Unico	40.6 a	41.7 a	43.9 a	44.0 a	38.7 a	
Sonora	39.5 a	41.2 a	43.0 a	43.1 a	39.3 a	
Moapa	38.7 a	42.4 a	41.5 a	46.8 a	38.4 a	
Mesa-Sirsa	40.4 a	41.0 a	43.6 a	45.5 a	40.0 a	

* Within harvest and stage of maturity, means followed by the same letter are not significantly different at 5% level.

Table 6. Residual neutral-detergent fiber of four alfalfa cultivars harvested at two stages of maturity in 1971.

Cultivars	Residual neutral-detergent fiber (%)					
	Harvest dates					
1/10-bloom	May 5	June 5	July 7	Aug. 9	Sept. 9	Oct. 7
El-Unico	27.0 a*	23.9 a	28.7 a	29.5 a	28.5 a	25.5 a
Sonora	25.9 a	25.3 a	28.5 a	30.4 a	28.1 a	23.8 a
Moapa	26.3 a	23.9 a	27.9 a	28.5 a	28.5 a	26.1 a
Mesa-Sirsa	26.8 a	24.3 a	28.9 a	29.3 a	28.1 a	23.2 a
Full-bloom	May 13	June 21	July 27	Sept. 1	Oct. 14	
El-Unico	27.4 a	28.7 a	30.6 a	31.7 a	27.7 a	
Sonora	26.6 a	29.4 a	29.5 a	28.9 a	27.7 a	
Moapa	25.9 a	29.7 a	29.6 a	31.5 a	26.1 a	
Mesa-Sirsa	26.8 a	27.7 a	30.7 a	31.2 a	27.6 a	

* Within harvest and stage of maturity, means followed by the same letter are not significantly different at 5% level.

from 36.3 to 41.8%, while those for the full-bloom stage were approximately 3% higher and ranged from 39.1 to 44.8%. The RNDF fractions generally followed the same pattern, with a range of 24.3 to 29.4% for the 1/10-bloom harvests and 26.7 to 30.8% for the full-bloom harvests.

Variation in the percent apparent digestible dry matter (ADDM) closely followed the seasonal trends of the fiber parameters (Table 7). This relationship resulted from the use of the neutral-detergent fiber values in the calculation of the digestibility estimates. No significant differences were found among the within-harvest cultivar means for either stage of maturity. These values ranged from approximately 55 to 63% for the 1/10-bloom stage and from 54 to 60% for the full-bloom stage. Cultivar ADDM values averaged over the entire collection period also failed to show significant differences for either stage of maturity (Table 3). This was indicated by the narrow ranges of 58.8 to 59.1% and 56.7 to 57.5% for the 1/10 and full-bloom harvests, respectively.

Differences in the production of ADDM reflected the patterns observed in dry forage yield and percent ADDM, since these values were mathematical products of the latter two variables (Tables 2, 7, and 8). Average total ADDM production (kg per plot) of the four cultivars ranged from 10.1 (Mesa-Sirsa) to 8.8 (Sonora) for the 1/10-bloom stage of development and from 10.4 (Mesa-Sirsa) to 9.4 (Moapa) for the full-bloom stage (Figure 3).

Table 7. Percent apparent digestible dry matter of four alfalfa cultivars harvested at two stages of maturity in 1971.

Cultivars	Apparent digestible dry matter (%) Harvest dates					
1/10-bloom	May 5	June 5	July 7	Aug. 9	Sept. 9	Oct. 7
El-Unico	59.5 a*	62.0 a	57.2 a	56.4 a	57.4 a	60.3 a
Sonora	60.0 a	60.5 a	57.4 a	55.5 a	57.9 a	62.0 a
Moapa	59.6 a	61.9 a	58.0 a	57.4 a	57.5 a	59.8 a
Mesa-Sirsa	59.1 a	61.5 a	57.0 a	56.6 a	57.8 a	62.7 a
Full-bloom	May 13	June 21	July 27	Sept. 1	Oct. 14	
El-Unico	58.5 a	57.2 a	55.3 a	54.3 a	58.1 a	
Sonora	59.3 a	56.5 a	56.5 a	57.1 a	58.2 a	
Moapa	59.9 a	56.2 a	56.3 a	54.5 a	59.7 a	
Mesa-Sirsa	59.2 a	58.2 a	55.3 a	54.8 a	58.3 a	

* Within harvest and stage of maturity, means followed by the same letter are not significantly different at 5% level.

Table 8. Apparent digestible dry matter of four alfalfa cultivars harvested at two stages of maturity in 1971.

Cultivars	Apparent digestible dry matter (kg/plot) Harvest dates					
1/10-bloom	May 5	June 5	July 7	Aug. 9	Sept. 9	Oct. 9
El-Unico	1.9 a*	2.1 ab	2.2 a	1.5 a	1.0 a	1.0 a
Sonora	1.7 a	1.8 b	1.9 a	1.4 a	1.0 a	1.0 a
Moapa	1.7 a	2.2 a	2.2 a	1.5 a	1.1 a	1.0 a
Mesa-Sirsa	2.2 a	2.2 a	2.0 a	1.6 a	1.2 a	1.0 a
Full-bloom	May 13	June 21	July 27	Sept. 9	Oct. 14	
El-Unico	2.7 a	2.5 a	1.7 a	1.8 a	1.4 a	
Sonora	2.0 a	2.6 a	1.7 a	1.8 a	1.8 a	
Moapa	2.0 a	2.4 a	1.9 a	1.7 a	1.5 a	
Mesa-Sirsa	2.6 a	2.7 a	1.8 a	1.7 a	1.6 a	

* Within harvest and stage of maturity, means followed by the same letter are not significantly different at 5% level.

Generally, significant differences in ADDM production were not detected among cultivars within each harvest. However, overall average ADDM production per harvest for Mesa-Sirsa was significantly greater than for Sonora for the 1/10-bloom plots (Table 3). These cultivar means were 1.7, 1.6, 1.6 and 1.5 kg per plot for Mesa-Sirsa, El-Unico, Moapa and Sonora, respectively. Significant differences in the overall average ADDM production per harvest of the cultivars were not apparent for the full-bloom stage of maturity. These cultivar means were 1.9, 2.0, 2.0 and 2.1 kg per plot for Moapa, Sonora, El-Unico and Mesa-Sirsa, respectively.

Significant differences were noted in ADDM production among harvest dates when averaged over cultivars (Table 1). The harvest means for the 1/10-bloom stage ranged from 1.0 to 2.1 kg per plot. This same trend was evident for the full-bloom harvests in which the values ranged from 1.6 to 2.5 kg per plot. Values for ADDM production at the full-bloom stage of growth were considerably higher than those for the 1/10-bloom harvests even though the percent ADDM was lower for the full-bloom stage of development. This relationship reflected the influence of total dry forage yield on ADDM production.

The two digestibility variables discussed were correlated in all possible combinations with the other variables measured. Comparisons were made for each stage of maturity over all harvests both on the individual cultivars and on all cultivars combined. Definite trends and significant correlations were observed in the individual cultivar comparisons (Tables 9 through 16). Significant negative correlations ($r = -.45^*$ to $r = -.58^*$) were found between leaflet to stem-petiole ratios and dry forage production at both stages of maturity in all cultivars. Other factors closely aligned to the above relationship were the negative correlations between leaflet to stem-petiole ratios and INDF ($r = -.25$ to $r = -.65^*$), RNDF ($r = -.14$ to $r = -.70^*$) and ADDM production (kg) ($r = -.32$ to $r = -.54^*$). These correlations indicate that when leaflet to stem-petiole ratios decreased, the dry forage yield increased with a corresponding increase in INDF. This increased fiber content resulted in lower fiber digestibility (RNDF) and ADDM production.

Similar results were noted in the combined cultivar-harvest date comparisons (Tables 17 and 18). Correlations between leaflet to stem-petiole ratios and the same variables were $r = -.52^*$ to $r = -.53$ (dry forage yield), $r = -.37$ to $r = -.58$ (INDF), $r = -.31$ to $r = -.37$ (RNDF), and $r = -.44^*$ to $r = -.49^*$ (ADDM production, kg), for 1/10 and full-bloom, respectively.

Anatomical Evaluations

Comparisons of stem cross-sections failed to show any obvious differences among cultivars within the same stage of maturity (Figure 4). Anatomical differences at the two stages of maturity appeared to be present when the stem cross-sections were compared on the basis of degree of lignification and concentration of other fibrous materials.

Table 9. Correlation coefficients between variables measured on El-Unico alfalfa harvested at 1/10-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.56*	0.00	0.00	0.00	0.99*
Leaf to stem-petiole ratios		1.00	-.35	-.35	0.35	-.53*
Initial neutral-detergent fiber			1.00	0.52*	-.51*	0.06
Residual neutral-detergent fiber				1.00	-1.00*	-.13
Apparent digestible dry matter (%)					1.00	0.13
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

Table 10. Correlation coefficients between variables measured on Sonora alfalfa harvested at 1/10-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.45*	-.11	0.10	-.11	0.99*
Leaf to stem-petiole ratios		1.00	-.49*	-.41*	0.41*	-.39
Initial neutral-detergent fiber			1.00	0.72*	-.71*	-.22
Residual neutral-detergent fiber				1.00	-1.00*	-.05
Apparent digestible dry matter (%)					1.00	-.04
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

Table 11. Correlation coefficients between variables measured on Moapa alfalfa harvested at 1/10-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.57*	-.28	-.14	0.14	0.99*
Leaf to stem-petiole ratios		1.00	-.29	-.14	0.14	-.54*
Initial neutral-detergent fiber			1.00	0.62*	-.61*	-.35
Residual neutral-detergent fiber				1.00	-1.00*	-.26
Apparent digestible dry matter (%)					1.00	0.26
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

Table 12. Correlation coefficients between variables measured on Mesa-Sirsa alfalfa harvested at 1/10-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.49*	-.03	0.22	-.22	0.99*
Leaf to stem-petiole ratios		1.00	-.25	-.33	0.33	-.46*
Initial neutral-detergent fiber			1.00	0.33	-.32	-.09
Residual neutral-detergent fiber				1.00	-1.00*	0.04
Apparent digestible dry matter (%)					1.00	-.04
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

Table 13. Correlation coefficients between variables measured on El-Unico alfalfa harvested at full-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.58*	-.02	-.25	0.26	0.98*
Leaf to stem-petiole ratios		1.00	-.65	-.22	0.21	-.52
Initial neutral-detergent fiber			1.00	0.44*	-.43*	-.08
Residual neutral-detergent fiber				1.00	-1.00*	-.38
Apparent digestible dry matter (%)					1.00	0.38
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

Table 14. Correlation coefficients between variables measured on Sonora alfalfa harvested at full-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.38	-.08	0.11	-.11	0.97*
Leaf to stem-petiole ratios		1.00	-.61*	-.28	0.27	-.32
Initial neutral-detergent fiber			1.00	0.63*	-.62*	-.23
Residual neutral-detergent fiber				1.00	-1.00*	-.14
Apparent digestible dry matter (%)					1.00	0.14
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

Table 15. Correlation coefficients between variables measured on Moapa alfalfa harvested at full-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.57*	0.23	0.28	-.28	0.97*
Leaf to stem-petiole ratios		1.00	-.58*	-.70*	0.69*	-.42*
Initial neutral-detergent fiber			1.00	0.59*	-.57*	-.10
Residual neutral-detergent fiber				1.00	-1.00*	0.04
Apparent digestible dry matter (%)					1.00	-.04
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

Table 16. Correlation coefficients between variables measured on Mesa-Sirsa alfalfa harvested at full-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.51*	-.28	-.44*	0.44*	0.99*
Leaf to stem-petiole ratios		1.00	-.52*	-.18	-.17	-.44*
Initial neutral-detergent fiber			1.00	0.58*	-.56*	0.35
Residual neutral-detergent fiber				1.00	-1.00*	0.57*
Apparent digestible dry matter (%)					1.00	0.56*
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

Table 17. Correlation coefficients between several variables measured on four alfalfa cultivars harvested at 1/10-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.53*	-.06	-.06	-.06	0.99*
Leaf to stem-petiole ratios		1.00	-.37	-.31	0.30	-.49*
Initial neutral-detergent fiber			1.00	0.52*	-.50*	0.13
Residual neutral-detergent fiber				1.00	-1.00*	-.09
Apparent digestible dry matter (%)					1.00	0.08
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

Table 18. Correlation coefficients between several variables measured on four alfalfa cultivars harvested at full-bloom in 1971.

	Dry weight	Leaf to stem-petiole ratios	Initial neutral-detergent fiber	Residual neutral-detergent fiber	Apparent digestible dry matter (%)	Apparent digestible dry matter (kg)
Dry weight	1.00	-.52*	0.00	-.09	-.10	0.98*
Leaf to stem-petiole ratios		1.00	-.58*	-.37	0.36	-.44*
Initial neutral-detergent fiber			1.00	0.55*	-.53*	-.10
Residual neutral-detergent fiber				1.00	-1.00*	-.27
Apparent digestible dry matter (%)					1.00	0.27
Apparent digestible dry matter (kg)						1.00

* Significant at 5% level.

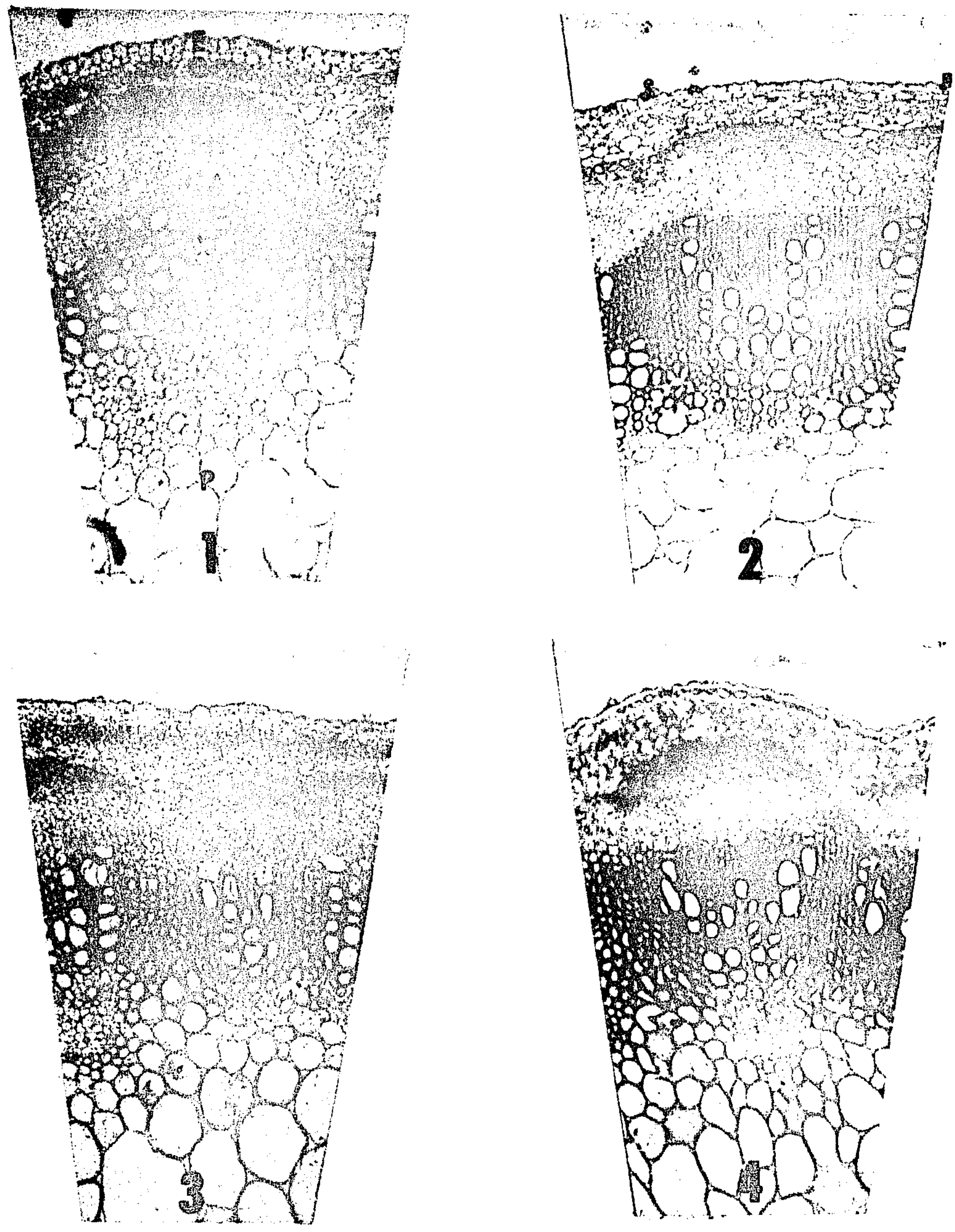

Figure 4. Stem cross-sections of two alfalfa cultivars taken at the 6th node, at two stages of maturity in 1971: (1) El-Unico, 1/10-bloom, (2) El-Unico, full-bloom, (3) Moapa, 1/10-bloom, and (4) Moapa, full-bloom. (E = Epidermis, C = Chlorenchyma, PF = Phloem fibers, PH = Phloem, CA = Cambium, X = Xylem, P = Pith.)

Alfalfa stems sampled at the full-bloom stage of development had more phloem fibers, xylem tissue and a higher degree of lignification than did the stems collected at 1/10-bloom.

Consumptive Water-Use Efficiency

Differences in the consumptive water-use efficiency were found among cultivars and stages of maturity. The borders received 1765 and 2476 kg of irrigation water per m^2 for the 1/10 and full-bloom borders, respectively. Rainfall accounted for an additional 280 kg of water per m^2 for each border during the collection period (Table 19).

The water requirement values (kg water per kg dry forage) for individual cultivars harvested at the 1/10-bloom stage ranged from 825 (Mesa-Sirsa) to 953 (Sonora)(Figure 5). These values were slightly higher than data reported by Poole (1971) on the same plots and cultivars. This additional water requirement probably resulted from an increased evaporation rate (decreased stand density) and a loss in plant vigor because of increased age. Forage harvested at the full-bloom stage of growth required 20 to 30% more water per unit of dry forage than alfalfa harvested at the 1/10-bloom. These values ranged from 1059 (Mesa-Sirsa) to 1161 (Moapa) kg water per kg dry forage. Mesa-Sirsa was the most efficient alfalfa cultivar in terms of water-use efficiency when harvested both at 1/10-bloom and full-bloom.

The water requirements nearly doubled when placed on an ADDM production (kg) basis, since the *in vitro* digestibility of the forage was only 50 to 60%. These values ranged from 1420 (Mesa-Sirsa and

Table 19. Average monthly air temperatures (maximum and minimum) and precipitation recorded at the Plant Materials Center Tucson, Arizona, in 1971.

Months	Temperature (C)		Precipitation (cm)
	max	min	
January	19	0	0.56
February	20	2	1.65
March	26	5	0.00
April	27	7	0.91
May	31	11	0.00
June	37	17	0.00
July	39	23	4.85
August	35	21	13.79
September	35	18	5.49
October	26	11	3.33
November	22	5	1.85
December	15	2	4.23
Means	28	10	Total 36.73

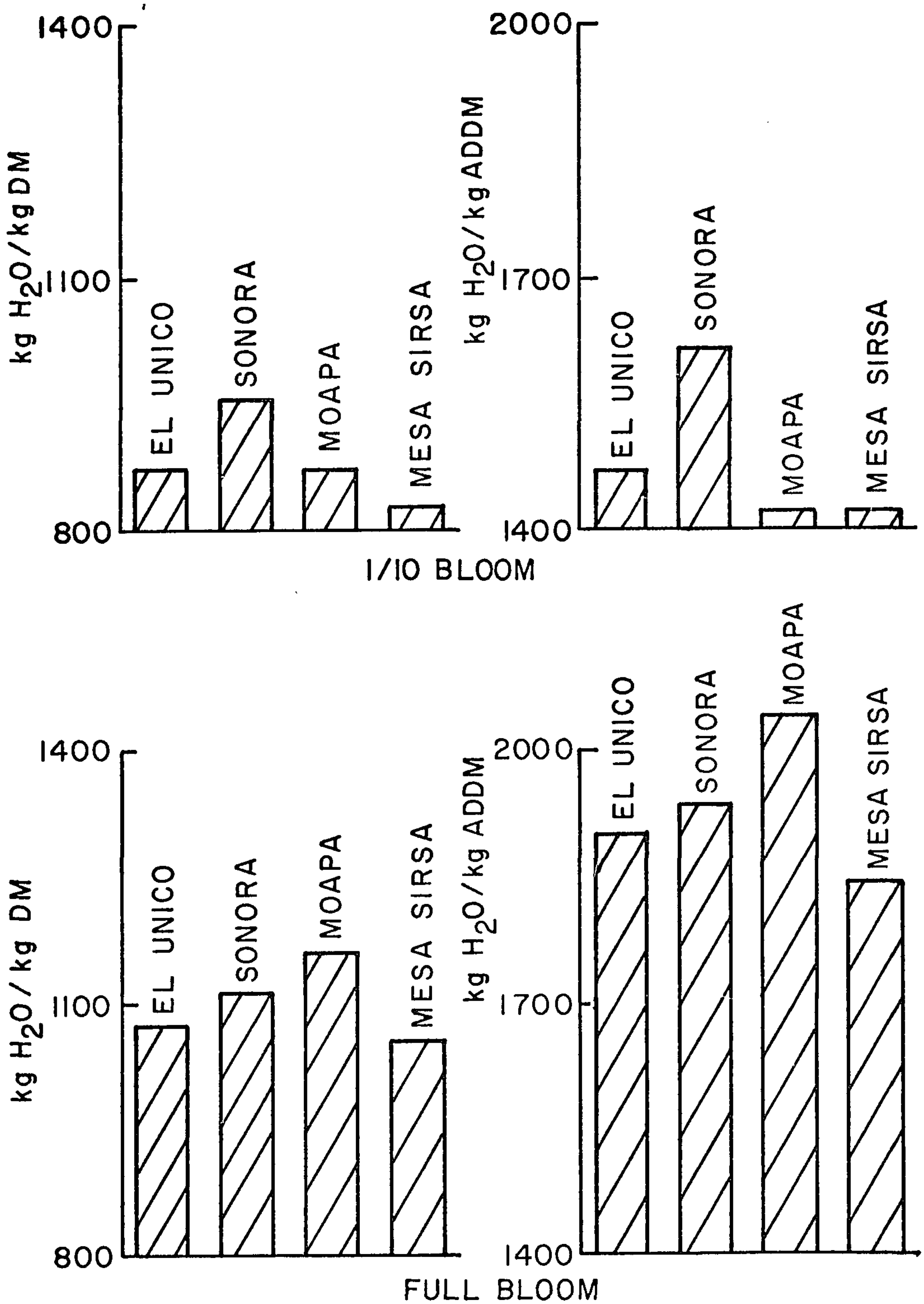

Figure 5. Kilograms of water required per unit of dry forage and apparent digestible dry matter produced from four alfalfa cultivars harvested at two stages of maturity in 1971.

Moapa) to 1613 (Sonora) for the 1/10-bloom forage. The water requirements of the full-bloom plots ranged from 1842 (Mesa-Sirsa) to 2038 (Moapa).

DISCUSSION

The dry forage yield in this study followed the same seasonal production trends observed by other researchers who used identical or closely related cultivars adapted to the arid southwest (Dobrenz et al., 1969; Joy, 1970; Poole, 1971; Thompson and Schonhorst, 1971). These reports showed similar peaks in dry forage harvested per cutting, with approximately 50 to 65% of the total seasonal forage yield harvested between April and the July peak.

Variation in dry forage yield per harvest was evident among cultivars in the current study at the 1/10-bloom stage of maturity (Table 3). Significant differences were not apparent at the full-bloom stage, which may have been due to differences among cultivars in rate of vegetative growth during flowering. Mesa-Sirsa produced the greatest quantity of dry forage per harvest at 1/10-bloom, while Mesa-Sirsa and El-Unico produced the greatest amount of dry forage harvested at the full-bloom stage. Other researchers have also reported cultivar and genotypic differences in dry forage yield of alfalfa (Dobrenz et al., 1969; Joy, 1970; Poole, 1971; Zaleski and Dent, 1960). Mesa-Sirsa has often been reported as one of the highest producing alfalfa cultivars in Arizona. This high production may be due to the intensive selection for disease and insect resistance which are the desirable characteristics of this cultivar.

The alfalfa cultivars harvested at the full-bloom stage of maturity consistently produced approximately 20% more dry forage per plot than those harvested at 1/10-bloom (Table 3). These data are supported by Leach (1968) who noted increased dry matter yields following an increase in harvest intervals. Thompson and Schonhorst (1971) reported that alfalfa harvested at 1/10-bloom produced the largest amount of 'total digestible nutrients', but that the greatest total yield of dry forage was obtained from alfalfa cut later in maturity. Carlton et al. (1968), however, reported that maximum dry matter accumulation occurred between 2 to 45%-bloom for alfalfa.

The leaflet to stem-petiole ratio was the only variable in the present study that showed significant seasonal and among-cultivar differences for both stages of maturity (Tables 1 and 3). Leaf to stem ratios have been suggested by numerous researchers as a measurement of forage quality, palatability and digestibility. Animal preference, as influenced by palatability and digestibility, has been directly related to the leafiness of the plant materials studied (Burton et al., 1964; Gangstad, 1966). Luckett and Klopfenstein (1970) reported that the changes in the cell-wall constituents of the stems and changes in the leaf to stem ratios were responsible for variations observed in the *in vitro* DMD of a forage. Significant negative correlations were observed in the current study between leaflet to stem-petiole ratios and both dry forage and ADDM production for both stages of maturity (Tables 17 and 18). Seasonal changes in leaflet to stem-petiole ratio accounted for much of the variation in INDF (approx 15 to 30%), dry forage yield (approx 25%) and ADDM production (approx 20 to 25%). Walters et al. (1967), however, stated that species and varietal variations in forage *in vitro* digestibility were not closely associated with leafiness.

Data in this study indicated that the *in vitro* digestibility of alfalfa decreased as the fiber content increased (Table 1). Other forage digestibility studies have shown significant positive and negative coefficients for stage of maturity when correlated with fiber content and *in vitro* digestibility, respectively (Baumgardt and Smith, 1960; Duble et al., 1971; Harkness and Alexander, 1969; Lloyd et al., 1961). Generally, significant differences were not present in the among-cultivar comparisons for either the INDF or percent ADDM at either stage of maturity (Table 3). Significant negative correlations between these factors were generally present for all cultivars and stages of maturity when averaged over all harvests (Tables 9 through 16). Percent INDF accounted for approximately 25% of the variation in percent ADDM (Tables 17 and 18). Several researchers have shown significant differences in *in vitro* digestibility when forages were compared on the species and cultivar basis (Dent, 1963; Wedin, 1970; Wurster et al., 1971). These digestibility differences have generally been closely correlated with the fiber fraction (CWC) (Duble et al., 1971; Gaillard, 1966; Ingalls et al., 1965; Keys et al., 1970). However, Carlson et al. (1969) and Tilley et al. (1969) found that the *in vitro* digestibility of forages was not affected by species or clonal selection.

Anatomical evaluations show some promise in the prediction of forage quality, especially in species comparisons and when significant variations among cultivars are present. In this study, anatomical differences were noted only among stages of maturity (Figure 4). A reliable field sampling technique is essential for the collection of a representative sample of plant material for histological evaluation. Extensive morphological differences existed in this study among plants within individual plots. Therefore, the influence of this variation on the anatomical characteristics must be considered. Additional investigation is also needed to develop a quantitative laboratory technique for evaluation of anatomical factors which may effect forage nutritive quality. This may involve the measurement of vascular bundle density and microscopic measurement of fibrous plant tissue.

Cultivar differences in water-use efficiency were noted in the current study. Mesa-Sirsa was the most efficient cultivar in the amount of water required per unit of both dry forage and ADDM produced. Sonora and Moapa were the least efficient at the 1/10-bloom and full-bloom stages of maturity, respectively. Significant cultivar and clonal differences in water requirements of forages have been previously reported, in addition to a direct relationship between water-use efficiency and dry forage yield (Cole et al., 1970; Dobrenz, Cole and Massengale, 1971; Dobrenz et al., 1969; Joy, 1970; Poole, 1971; Wright and Dobrenz, 1970).

The production of digestible forage per unit area in this study was generally not significantly different among cultivars at either stage of maturity (Table 3). However, ADDM production was approximately 20% greater when the alfalfa was harvested at full-bloom vs. 1/10-bloom.

Based upon this study, the alfalfa breeder should select for maximum forage yield and water use efficiency, but should also give consideration to those factors which may effect nutritive quality.

SUMMARY

A study was conducted at Tucson, Arizona to determine the apparent digestible (in vitro) dry matter (ADDM) production, dry forage yield, neutral detergent fiber (NDF) content, leaflet to stem-petiole ratio, anatomical variations and water-use efficiency of four alfalfa cultivars harvested at the 1/10 and full-bloom stages of maturity in 1971. The cultivars studied were El Unico, Sonora, Moapa and Mesa-Sirsa.

Generally, significant differences were not found among cultivars within harvest dates for any of the variables measured in this study. Seasonal trends for NDF content, dry forage yields, ADDM production and leaflet to stem-petiole ratios were evident for both cultivars and harvest dates. Percent ADDM and leaflet to stem-petiole ratios were the highest in the initial and final harvests. Dry forage yield and ADDM production, which were negatively correlated with leaflet to stem-petiole ratios, tended to be higher early in the growing season, peaked in late June or July and then declined steadily.

Individual cultivars compared on a seasonal basis exhibited significant differences for several parameters at the 1/10-bloom stage. Many of these differences were not apparent among cultivars within harvests. The total amounts of forage and ADDM produced for the entire growing season were significantly different among cultivars. Mesa-Sirsa

was the most productive cultivar and Sonora was the least productive, when both of the above factors were considered.

Leaflet to stem-petiole ratios of Sonora and Moapa were significantly higher than those of Mesa-Sirsa or El-Unico. This was the only factor evaluated on an overall harvest basis that maintained the same relationship for both stages of maturity.

No differences in the anatomical features of stems, such as additional phloem and xylem tissue, could be detected among the four cultivars when comparisons were made within harvests. However, differences in specific anatomical features were evident between stages of maturity. Additional phloem fibers, xylem vessels and degree of lignification were evident at the full-bloom stage, when compared to the 1/10-bloom stage of development. Use of this technique in other studies may detect meaningful relationships between anatomical features and digestibility.

Variation in water-use efficiency among individual cultivars was found. The most efficient cultivar with regard to dry forage yield at both stages of maturity was Mesa-Sirsa, while Sonora and Moapa were the least efficient at the 1/10 and full-bloom stages, respectively. Mesa-Sirsa and Moapa were the most efficient cultivars in production of ADDM per unit of water applied for the 1/10-bloom stage of maturity, while Sonora was the least efficient. However, at the full-bloom stage, Mesa-Sirsa and Moapa were the most and least efficient cultivars in ADDM production, respectively. When stages of maturity were compared, the full-bloom stage was considerably less efficient in dry forage yield and ADDM production per unit of water applied. The water requirement of forage crops in the arid southwest is of major importance, both with regard to economics and, more important, availability.

This study should be of interest to the forage producer and researcher whose goal is the maximum production of digestible forage per unit area at a minimum cost. Although seasonal trends were noted for most of the variables studied, dry forage yield was the dominant factor influencing total ADDM production. This was illustrated by the fact that although Mesa-Sirsa generally had a much lower leaflet to stem-petiole ratio than Sonora, it was also generally found to be the superior cultivar in ADDM production. Thus, based upon the four cultivars included in this study, the plant breeder should consistently be aware of those factors that contribute to forage quality. However, selection of genotypes used in new alfalfa cultivars should be based primarily on dry forage yield and water-use efficiency.

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