SEASONAL EFFECTS ON THE ALCOHOL-SOLUBLE SUGARS AND STARCH CONTENTS AND THE DIGESTIBILITY OF SEVERAL BERMUDAGRASS CLONES

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CHAPTER I

INTRODUCTION

Over one half of the total land area of the United States, about one billion acres, produces forages which, when marketed through livestock, have an annual value of about $8 billion (Hodgson, 1968). Most of these forages are utilized as ruminant feed.

Hodgson (1968) cited U.S.D.A.'s Long Range Study, which predicted that the demand for beef in the United States will increase 45 percent in the next ten years. This is due to increases in population and per capita meat consumption in this country.

By the year 2000 the United States will need some 44 million livestock on feed for slaughter and 128 million other cattle, which is twice the present number (Hodgson, 1968). Obviously, the need for forages will also increase.

The land area producing forages, however, continues to decrease because grassland is being diverted to production of food and fiber crops, construction of new buildings, houses, roads, etc. (Staten, 1952). Consequently, we will need to increase the efficiency of forage production through

both higher productivity and increased quality (higher nutritive value).

One way to achieve this is through forage breeding programs resulting in the development of new varieties having the following characteristics: (a) higher yield and better distribution of yield throughout the growing season, (b) stability of production from year to year, (c) adaptability to various systems of management and use for which the species is desired, (d) ability to grow satisfactorily in association with other grasses and legumes, (e) ease of harvesting and management, and (f) high nutritive value (Wheeler, 1950).

The above characteristics have been shown to be greatly influenced by climatic factors such as light intensity, temperature, rainfall, etc. and by soil conditions. Elder and Murphy (1961) provided evidence that forage quality was influenced by season in that animal gains were excellent in Spring and early Summer but poor in late Summer and early Fall.

The objectives of this experiment were: (a) to ascertain the quality of selected bermudagrass clones across the season, (b) to determine the seasonal effects on quality as evidenced by moisture content, alcohol-soluble sugars, starch content and dry-matter digestibility of the leaves and the stems of the clones, and (c) to determine if a relationship existed between those chemical constituents and the dry-

matter digestibility. These data would indicate that one of the greatest contributions which could be made in a bermuda-grass breeding program would be to identify clones which would contribute to higher quality in the late Summer and the Fall.

CHAPTER II

LITERATURE REVIEW

Forage Improvement through Breeding

Hanson (1950) indicated that forage breeding work did not receive much attention in the United States until the turn of the 19th century. In forage breeding programs, plant introduction is very important. Today's researchers try continually to find new germ plasm to be used to develop new hybrids and varieties superior to existing ones. The initial step in such a program is selection from local material which is already well adapted for survival. If there is a shortage of such material, introduction from other areas of similar climatic conditions will be very useful. Introduction from other areas of different climatic conditions may also give valuable results (Cooper, 1966). Many of the major legumes and forage grasses were introduced into the United States from foreign lands (Hanson, 1959). Bermudagrass is one such grass.

Bermudagrass, Cynodon dactylon (L.) Pers., is found throughout the tropical and subtropical parts of the world

(Burton, 1951). Today it occurs northward as far as Maryland, Kansas and the warmer valleys of Washington and Oregon (Wheeler, 1950), and is one of the most important pasture grasses in the South (Holt et al., 1951; Staten, 1952; Wheeler, 1950).

Breeding programs have produced improved cultivars of bermudagrass. "Coastal" and "Suwannee" cultivars are the results of hybridization of superior clones from South Africa and Georgia (Burton, 1965). "Midland" bermudagrass, a winter hardy strain which is better adapted and higher yielding than Coastal in Oklahoma, was developed by crossing Coastal with a cold hardy Indiana type (Harlan, et al., 1954).

Burton et al. (1967) pointed out that very little has been done to improve bermudagrass quality due to the problems associated with characterizing and measuring this attribute. In a breeding program with improved quality as the objective they developed "Coastcross-1" bermuda which is 11 to 12 percent more digestible than Coastal and theoretically should give up to 30 percent better cattle daily gains (Burton and Southwell). Coastcross-1 is a hybrid between Coastal and an introduced selection from Kenya, Africa.

Experiments toward the improvement of forage crops through breeding have also been undertaken for several other species. The nutritive value of pasture plants can be im-

proved by: (a) improving the palatability, (b) reduction in the amounts of plant constituents harmful to stock, and (c) improving the contents of carbohydrates, proteins and accessory food substances essential to animal health (Corkill, 1965).

Law and Anderson (1940) observed that leafiness is an important selection criteria in big bluestem (Andropogon furcatus Muhl.). Their experiment produced highly significant increases in leaf area after 4 generations of selection in open-pollinated lines.

In orchardgrass (Dactylis glomerata L.) the low palatability and digestibility were caused by: (a) high lignin content, (b) an unfavorably high K/Ca ratio, and (c) the harshness of the leaf due to the presence of silicified dentations (Van Dijk, 1958). It is not easy to select directly for a lower lignin content, but the problem can be approached by breeding for a higher leaf/stem ratio since the lignin content of the leaf is significantly lower than that of the stem. Lower K/Ca ratio may be achieved by carrying out breeding programs on soils poor in lime and selecting for orchardgrass with better capacity to absorb calcium (Van Dijk, 1958).

A large variation in digestibility existed among individual plants (Julen and Lager, 1966). Digestibility appear-

ed to be inherited and a breeding program for producing varieties with higher digestibility appeared feasible.

Low palatability in tall fescue (Festuca arundinacea Schreb.) may be due to leaf inflexibility (Gillet and Jadas-Hecart, 1965). Chemical composition was not related to flexibility. On the other hand, the size of the transverse section of the fibro-vascular bundles seemed to be related to flexibility. Tall fescue cultivars with improved flexibility have now been obtained through selection.

Sullivan and Myers (1939) used colchicine to obtain tetraploid Lolium perenne L. plants which were higher than the diploid plants in reducing sugars, sucrose, total sugars and the proportion of dry-matter which was soluble in 80 percent alcohol.

Interspecific hybrids within Lolium and Festuca and intergeneric hybrids between the genera Lolium and Festuca may be established to combine the desirable features of both genera: persistence, yield, palatability, winter-hardiness, drought-tolerance and disease-resistance (Hertzsch, 1966). Hertzsch (1966), working with Lolium perenne, L. multiflorum, Festuca pratensis and F. arundinacea obtained the best results by crossing the interspecific Lolium hybrids (L. perenne x L. multiflorum) with F. pratensis.

Buckner et al. (1967), produced an amphiploid of annual

ryegrass-tall fescue hybrid which proved to be superior to the backcross progenies of (perennial ryegrass x tall fescue) x tall fescue hybrids and "Kenwell" and "Kentucky 31" tall fescue varieties in sugars, protein, digestibility and palatability.

Burton and his associates (1966, 1968) investigated the improvement potential in pearl-millet (Pennisetum typhoides (Burm.) Stapf and C. E. Hubbs). Photoperiodism was used to develop short-day sensitive, late-maturing varieties which were superior to early ones in forage quality, seasonal distribution and ease of management. Late varieties were also more persistent, leafier, higher in protein content and more digestible.

Estimates of Forage Quality

The usefulness of a grass as an energy source for animal is determined mainly by its digestibility which indicates the utilizable proportion of the feed (Raymond, 1966). Other factors such as chemical composition and the rate of intake are also important, the rate of intake being influenced by forage acceptability, the rate of passage, the quantity of forage available to the animal and the effect of environment upon the grazing animal (Mott, 1962).

Burton et al, (1967), noted that daily gains of steers, dry-matter intakes and in vivo digestibility methods cannot

be used to screen large numbers of genotypes in a forage breeding program directed toward quality improvement because of the large quantities of forage required (14,500 kg., 2,700 kg. and 1,450 kg., respectively). Other methods, the *in vitro* dry-matter digestibility, dry-matter percent, crude protein content, percent leaves and crude fiber content, which use small amounts of forage, are suitable for use by plant breeders in the early screening stages.

In general, there are three main laboratory methods used to estimate the nutritive value of forages: (a) chemical analysis, (b) *in vitro* cellulose, or dry-matter digestibility techniques using rumen microorganisms and (c) solubility techniques (Johnson et al., 1964). The solubility techniques involve the determination of the solubility of forage or its components in several types of solvents such as cupriethylenediamine (CED), 1.0 N H_2SO_4, etc. (Johnson et al., 1964).

Methods for measuring forage quality by chemical analysis (crude protein, lignin, crude fiber, ether extract, ash, nitrogen-free extract, etc.) were evaluated by Sullivan (1962). He considered the crude protein content to be an acceptable criterion of quality. Raymond (1966), however, indicated that relationship between herbage digestibility and its crude protein content has been shown to be associative rather than causal, while Raymond implied that chemical

determinations (protein, fiber, silica, etc.) are influenced by morphologic age of the plant and that age is really the causal factor of quality.

The separation of carbohydrates into crude fiber and nitrogen-free extract may be misleading (Sullivan, 1962). Crude fiber of different composition may be found in different kinds of forage or in different samples of the same species of forage. It consists primarily of cellulose, lignin and some xylans. The cellulose may have a high digestibility if it is not highly lignified. Correlations between the fiber content and its digestibility are high only in samples containing extremes of plant maturity and lignification (Sullivan, 1962).

The lignin content appeared to be a good criterion of forage quality (Buckner et al. 1967; Phillips et al., 1954; Sullivan, 1962; Forbes and Garrigus, 1948; Webster et al., 1965; Raymond, 1966). Lignin has a very low digestibility. Highly significant negative correlations between the lignin percentages and the digestion coefficients of dry-matter, organic matter and energy have been reported (Sullivan, 1962). Van Soest (1964), however, pointed out that lignin determination is in some ways objectionable because it is tedious and time consuming, and may yield residues that contain more artifact than true lignin.

The moisture content of the standing crop before harvest can also be used to measure forage quality. It is inversely associated with silica and crude fiber and is a characteristic of the species of grass, and negatively correlated with the lignin content. Moisture content was generally higher in good quality forages (Buckner et al., 1967; Sullivan, 1962; Sullivan et al., 1956). Similarly, Reid et al. (1959) noted that the dry-matter content at the time of harvesting was superior to the leaf percent as an index of quality, and there was a relationship between the dry-matter content and digestible dry-matter percentage.

Another reference material which can be used to calculate the digestibility of other feed constituents is naturally occurring silica. The percentage change in nutrient/silica ratio of the feed in passing through the digestive tracts gave an indirect measure of the apparent digestibility of the corresponding nutrient (Gallup et al., 1945). Buckner et al. (1967) found that silica was highly related to fiber and inversely related to moisture, sugar, digestibility and protein, indicating that silica was inversely related to the nutritional value of forages.

Buckner et al. (1967) also suggested that sugar content might be a good indicator of the nutritional value of grasses. Ryegrass-tall fescue hybrids, "Kenwell" tall fescue and

"Kentucky 31" tall fescue increased in digestibility as well as in sugar content and palatability during the fall.

Energy and protein make up the major portion of the feed requirements of ruminants. An 1,100 lb. cow producing 45 lbs. of milk required about eight times as much total digestible nutrients or energy as protein. An 800 lb. fattening beef yearling required almost ten times more total digestible nutrients than digestible protein (Blaser, 1964). When ruminants were restricted to forage from well-managed grasses and legumes, animal output was generally limited by a shortage of energy in the ingested herbage rather than by digestible protein (Blaser, 1964). Consequently, there was a need to develop grasses and legumes high in digestible energy which animals would consume in large amounts, and forage breeding programs should be directed toward increasing carbohydrates such as starch or fructosan (Blaser, 1964). Higher sugar content in grasses would be desirable because: (a) the protein content is high enough, (b) this constituent would be advantageous in silage making, and (c) the grass should be more palatable (Alberda, 1966). Buckner, et al. (1967) noted that sugar content determinations would permit the screening of large populations of plants for improved nutritional value in a breeding program.

Several <u>in vitro</u> digestibility techniques have been developed by scientists recently. In 1963 Tilley and Terry

(1963) described a two-stage *in vitro* method. The use of pepsin for protein digestion improved this method over others. It gave a very close agreement with the digestibilities measured *in vivo*. The method not only permitted the use of very small amounts of forage so that a large number of samples could be run, but also the digestibilities of plant parts (leaf, stem, sheath, etc.) could be determined separately.

Digestibility studies of the whole plant plus its respective parts were conducted by Pritchard, et al. (1963) and Terry and Tilley (1964). The latter pointed out that this kind of experiment is relevant to plant breeding studies. Digestible dry-matter content of a forage crop may be raised by: (a) selection for a larger proportion of leafy material in mature growth. Burton et al. (1964) explained that grass leaves, particularly the blades, are generally considered the most nutritious part of the plant. Leaf percentages can be raised several fold by genetically shortening the stem internodes, and such varieties may still be very leafy when in full flower.

(b) selection for a higher digestibility of stem or other parts of the plant. This can be achieved by selection of either (i) plants with a low content of fibrous material and a corres-

> pondingly high content of non-fibrous digestible materials such as soluble carbohydrates, proteins and other nitrogeneous substances, organic acids, etc., or (ii) those in which the content of digestible fiber is unusually high (Terry and Tilley, 1964).

Factors Affecting Forage Quality

The chemical composition and nutritive value of a forage grass depends primarily on the degree of maturity of the grass (Alexander et al., 1961; Blaser, 1964; Fuller, 1964; Holt et al., 1951; Patterson et al., 1963; Phillips et al., 1954; Prine and Burton, 1956). Bermudagrass is highly nutritious and highly palatable at stages of growth up to early maturity, but as it matures the lignin, crude fiber and cellulose contents increase and the grass gets tough and wiry, while the ether extract, acid soluble ash, and protein contents decrease along with its palatability and nutritive value (Fuller, 1964; Holt et al., 1951; Phillips et al., 1954).

In Coastal bermudagrass increasing the clipping interval from one to eight weeks increased hay yield, stem length, leaf length, plant height and seed head frequency. Clipping frequency had little effect on total protein yield and percentage nitrogen recovery, but protein and leaf percentages

decreased (Prine and Burton, 1956). Patterson et al. (1963) reported that compared to six-week harvest intervals, clipping every three weeks resulted in a 26 percent reduction in forage yield but a 30 percent increase in protein content. High quality bermudagrass hay with about 26 percent crude fiber on a dry matter basis can be produced if a cutting interval of between 3 to 4 weeks is used (McCullough and Burton, 1962). Carotene and xanthophyll contents also dropped as cutting interval was increased; forage cut every six weeks contained only about half as much as that cut every three weeks (Burton, Wilkinson and Carter, 1969).

Pritchard et al, (1963) and Terry and Tilley (1964), working with several grasses and legumes, found that all plant parts had high digestibility in early stages of growth, but the digestibilities decreased with increasing maturity, but at different rates. The digestibility of the heads and stems fell much more rapidly than the leaf. Pritchard et al. found that the stem segments close to the base had similar digestibility, but the upper portion of the stem had lower digestibility. Tilley and Terry concluded that the decline in digestibility is associated with a reduction in the content of water-soluble carbohydrates and protein constituents of the plant and a reduction in the digestibility of fiber.

Alexander et al. (1961) reported that the decline in

digestibility caused by maturity can be reduced by nitrogen application. Protein digestibility decreased from 47 to 32 percent with maturity when 50 lbs. N/acre were added but with 100 lbs. N there was only a slight decrease in protein digestibility. These data were from heifers, but similar results were obtained with cows and sheep. The dry-matter and energy digestibility followed a trend similar to the protein digestibility; and if the stage of maturity at the time of harvesting or grazing exceeded eight weeks at least 100 lbs. of nitrogen per acre should be used to maintain high digestibility.

The importance of light intensity and temperature on dry-matter production was discussed by Alberda (1965, 1966). He pointed out that both factors influence the rate of net assimilation. The optimum temperature for dry-matter production was dependent on the light intensity, and the effect of light intensity was different at different temperatures. Light intensity influenced only the rate of photosynthesis, but temperature influenced the rates of both photosynthesis and respiration.

Light energy levels have major effects on photosynthesis and chemical composition. At low light energies the rate of photosynthesis is proportional to the light energy, but as the energy input is increased, photosynthesis does not increase proportionally and eventually reached saturation

(Cooper, 1966).

Burton et al. (1959) reported that heavy shade reduced the efficiency of Coastal bermudagrass in dry-matter production. Under heavy shade the heavily fertilized grass (1600 lbs. N per acre) contained 25.7 percent more crude protein but 29.6 less available carbohydrates compared to 200 lbs. N per acre rate. Shade also increased lignin content significantly, the increase being greatest at low N level.

With perennial ryegrass, Lolium perenne L, the soluble carbohydrate content increased with increasing light intensity, but the nitrate and protein content decreased (Alberda, 1965).

Bathurst and Mitchell (1958), working with several forage species, found that the sugar content was highest with full light and low temperature and lowest with shade and high temperature. Nitrate content was almost always highest in plants grown at lower temperature in full light.

Smith (1968), working with timothy, Phleum pratense, noted that the dry-matter production at early anthesis was considerably greater under cool (18.5 C day, 10 C night) than under the warm (29.5 C day, 21 C night) temperatures, while the percentages of sugar and starch were much alike at both temperatures at all stages of growth. There was a negligible fructosan accumulation in the leaf blade under the

warm temperatures, but a very high content (21.5 percent) accumulated under the cool temperatures.

Temperature optima have been noted for growth, photosynthesis and translocation rates for a number of species. Burton (1951) noted that bermudagrass grew best when the mean daily temperatures were above 75 F. Very little growth occurred at temperatures between 60 and 65 F, and temperatures of 26 to 28 F usually killed the stems and the leaves back to the ground. The rate of apparent photosynthesis of seaside bentgrass, Agrostis palustris, a grass adapted to cooler regions, and bermudagrass, a grass adapted to warmer regions of the United States, increased from 15 to approximately 25 C. As the temperature was raised from 25 to 40 C the rate of apparent photosynthesis of the bentgrass was decreased while that of the bermudagrass continued to increase to about 35 C (Miller, 1960).

The U-3 bermudagrass strain requires over 1232 degree hours F per day (0 F base) for good growth, provided the day temperatures are over 50 F. This grass will still grow at nearly freezing night temperatures if the day temperatures are sufficiently high. Very low temperature concurrent with high light intensity resulted in the discoloration of the grass because the rate of chlorophyll degradation in the blades exceeds the rate of chlorophyll synthesis (Youngner, 1959).

The effect of the air and root temperatures upon translocation of C^{14} photosynthate in sugarcane was investigated by Hartt (1965). She found that the air temperatures directly affected the photosynthate translocation from the blade, the percentage of photosynthate moved up and down the stem, and the velocity of translocation down the stem. In plants grown at a root temperature of 17 C translocation from the leaves was decreased compared to the control temperature of 22.4 C, resulting in the smaller plant size. The decrease in translocation associated with low root temperature was considerably more severe than that associated with low air temperature. At 20, 24 and 33 C the velocities of translocation were 1.40, 1.56 and 2.00 cm per minute, respectively.

Diurnal variation in the content of various carbohydrate fractions have been observed. Water-soluble carbohydrate percentages in alfalfa generally followed a curvilinear diurnal trend from a low at 6:00 a.m. to maximum levels at 12:00 noon and decreased slightly by 6:00 p.m. Non-structural carbohydrate content followed a non-linear daily trend with the most rapid increase occurring in the afternoon. Conversion from a water-soluble, low molecular weight carbohydrate to a water-insoluble, temporary storage polysaccharide was apparently more rapid in the afternoon than in the morning (Holt and Hilst, 1968).

Apart from climatic limitations, in most environments

forage production is limited by soil nutrients, especially nitrogen (Cooper, 1966). Increasing N rates have been reported to increase hay yield, protein content, as well as the length of stem, internode and internode number (Alexander et al., 1961; Prine and Burton, 1956).

Although N is usually the first limiting factor for growth, phosphorus and potash applications may also be needed since satisfactory levels of P_2O_5 and K_2O must be maintained (Fisher and Caldwell, 1959; Holt et al., 1951). Langer (1966), using combinations of 3 levels of N, P and K obtained highly significant N, P and K effects as well as positive NP, NK and NPK interactions. Where there is a shortage of N, increases in P and K levels gave very little effect on leaf area. On the other hand, N had some effect even when P and K were limiting.

Elder and Tucker (1968), working with Midland bermudagrass, also found that phosphorus was a limiting factor. The highest production came from a 100-160-160 lbs./acre application of N, P_2O_5 and K_2O. Splitting applications of K are also advisable to avoid winterkilling bermudagrass in areas where minimum temperatures approach 0 F (Adams et al., 1967c).

Burton (1954) suggested that in the Coastal Plain of Georgia a 4-1-2 ratio of N, P_2O_5 and K_2O with 400 lbs. of N

applied annually would be adequate for Coastal bermudagrass hay production. Gausman and Cowley (1954), on the other hand, found that under their experimental conditions N was the only element which significantly increased yield in Coastal bermudagrass; P did not give any significant response.

With the increasing rate of N soil may become increasingly acidic with a consequent reduction in bermudagrass yield; and lime can be applied to maintain favorable pH of 6.0 to 6.5 (Adams, et al., 1967a; Adams et al., 1967b; Fisher and Caldwell, 1959).

CHAPTER III

MATERIALS AND METHODS

Thirteen hybrid bermudagrass clones were selected on the basis of preliminary dry-matter digestibility data obtained in 1967. Five clones: x317 (H), x326 (H), x820 (H), x896 (H), and x898 (H), were high in dry-matter digestibility in June and July; six clones, x 331 (M), x71 (M), x341 (M), x347-1 (M), x347-2 (M), and x347-3 (M), were high in digestibility in June but low in July; and two clones, x734 (L) and x878 (L), were low in both June and July. The clone identification codes, parentage and origin of the clones are listed on Table I. The clone identification codes, which will be used throughout this report, are combinations between the cross numbers (x217, x326, etc.) and their digestibility data in 1967: (H) for those high in June and July; (M) for those high in June but low in July; and (L) for those low in June and July. The clones had been growing in Stillwater for several years, and samples were taken in 1968. The soil received 60 lbs. of N per acre in early spring and another 60 lbs. in mid-June. Adequate levels of P and K were maintained.

On July 18, 15 days before harvesting the first samples, the grasses were cut to about two inches from the ground. Cuttings from each clone were taken at five harvest dates at 15-day intervals: August 2, August 17, Sept. 1, Sept. 16 and October 1. All cuttings were taken between 2:00 p.m. and 4:00 p.m., placed in plastic bags and stored in ice while in the field. They were brought to the laboratory as soon as possible.

The plants were divided into two morphological constituents: the leaf (blade) and the stem plus sheath (henceforth will be referred to as the leaf and the stem). These were cut to about 1/5 inch long. Three samples weighing two grams each were taken for each plant part from each clone for the analyses. One sample was dried in an oven at 70 C for 24 hours. It was cooled in a dessicator and reweighed to find the dry-matter content and to calculate the results of analyses on a dry-weight basis. The dried samples were later used for *in vitro* digestibility trials. The rest of the samples were put into small jars and 15 ml. of boiling 95 percent ethanol were poured into each jar to stop the enzyme activity in the plants.

Alcohol-Soluble Sugar Extraction

Alcohol soluble sugar extraction was carried out following the method suggested by Hassid and Neufeld (1964) and

Murphy (1958) with some modifications. Each sample was ground with a homogenizer (3 minutes for the leaf, 6 minutes for the stem plus sheath), transferred back into the jar, heated in a boiling water bath for 30 minutes, filtered into a 100 ml. volumetric flask using No. 42 Whatman filter paper. The residue was washed with small portions of 80 percent ethanol and made to volume. Aliquots were taken to determine the alcohol-soluble sugar content, using the anthrone method, which were measured at 620 mμ on a Bausch & Lomb Spectronic 20 spectrophotometer (Ashwell, 1957; Guimberteau, 1960; Hassid and Neufeld, 1964).

Starch Extraction

Starch extraction was done according to the method suggested and discussed by Denny (1934), Sullivan (1935), and Weinmann (1947) as modified. The residue from the alcohol-soluble sugar extraction was transferred into plastic tubes, and 15 ml. of deionized water were added. The tube was heated in a boiling water bath for 60 minutes to gelatinize the starch, and cooled to room temperature. Deionized water was added to bring the volume in the tubes to 19 ml. Nine ml. of acetate buffer (0.40M, pH 4.45) were added plus 2 ml. of amylase enzyme mixture (1000 mg. in 200 ml. of acetate buffer, 0.13 M, pH 4.45). The amylase was obtained

from Sigma Chemical Company, and the enzyme activity was as follows: alpha-amylase; 1 mg. would liberate approximately 4.2 mg. of maltose from starch at pH 6.9 at 20 C; beta-amylase, 1 mg. would liberate approximately 1.1 mg. of maltose from starch at pH 4.8 at 20 C. Potato starch standard was prepared fresh with each run. The tubes were covered with parafilm plastic paper and incubated for 46 hours at 38 C, with periodic shaking. A clear solution was obtained (with centrifugation if necessary), and aliquots were taken to determine glucose content with the anthrone method. The glucose readings were multiplied by starch factor 0.9 to obtain the starch percentage (Lindahl, et al., 1949; Sullivan, 1935; Weinmann, 1947).

In Vitro Dry-Matter Digestibility

The in vitro dry-matter digestibility was determined using the method described by Tilley and Terry (1963). The dried samples were ground with a Wiley mill to pass a 40-mesh screen.

For rumen digestion 0.5 gram of each sample was placed in a 250 ml. centrifuge bottle. Eighty ml. of buffer solution of McDougall's (1948) artificial sheep saliva and 20 ml. strained rumen liquor were added to each bottle. Anaerobic conditions were created inside the bottles using CO_2;

they were sealed with cork gas release valves. A pH of 6.7 to 6.9 was maintained with 1.0 N Na_2CO_3, and incubation in darkness at 38 C took 48 hours. The samples were shaken every four hours.

For pepsin digestion, all bottles were placed under refrigeration to stop bacterial activity, then centrifuged 15 minutes at 1,800 g. The supernatant was discarded; 100 ml. pepsin solution (2 grams 1:10,000 pepsin in 1,000 ml of 0.1 N HCL) was added, and the samples incubated at 38 C for 48 hours, with occasional shaking. Most of the supernatant was discarded; the residue and the remaining super were transferred to a tared weighing container, and dried at 67 C. The weight of the blank was then subtracted from the sample, and the percent digestibility was calculated.

Samples were taken from the same clones and Midland bermudagrass at Chickasha in 1968 with the exception that only one clone of cross x347 was taken. The clones were replicated twice. Samples were harvested five times at monthly intervals from June 15 to October 15. They were oven-dried at 60 C for 24 hours as they reached Stillwater, and ground with a Wiley mill to pass through a 40-mesh screen. One gram was taken from each sample for alcohol-soluble sugar determination and one gram samples were used for dry-matter digestibility trials.

CHAPTER IV

RESULTS AND DISCUSSION

Stillwater Samples

Moisture content as percentage of fresh weight ranged from 41.0 to 65.3 percent in the leaves and from 43.2 to 76.0 percent in the stems (Table II). The average values for the stems were higher than those for the leaves. Moisture content of the clones was rather irregular on August 2, August 17 and September 1, declined somewhat on September 16 and increased on October 1. This pattern was generally true for both the leaves and the stems.

Clone x820 (H) showed the highest moisture content in the leaves and the stems in all cuttings except on September 16, where the values were second highest. Other clones high in moisture content were x347-2 (M), x347-3 (M), x331 (M) and x878 (L).

The alcohol-soluble sugar content (% dry-matter) of the clones ranged from 5.2 to 19.7 percent in the leaves and from 3.6 to 28.6 percent in the stems (Table III, Figures 1 to 5). Except for x317 (H) on August 17 and x896 (H) on October 1,

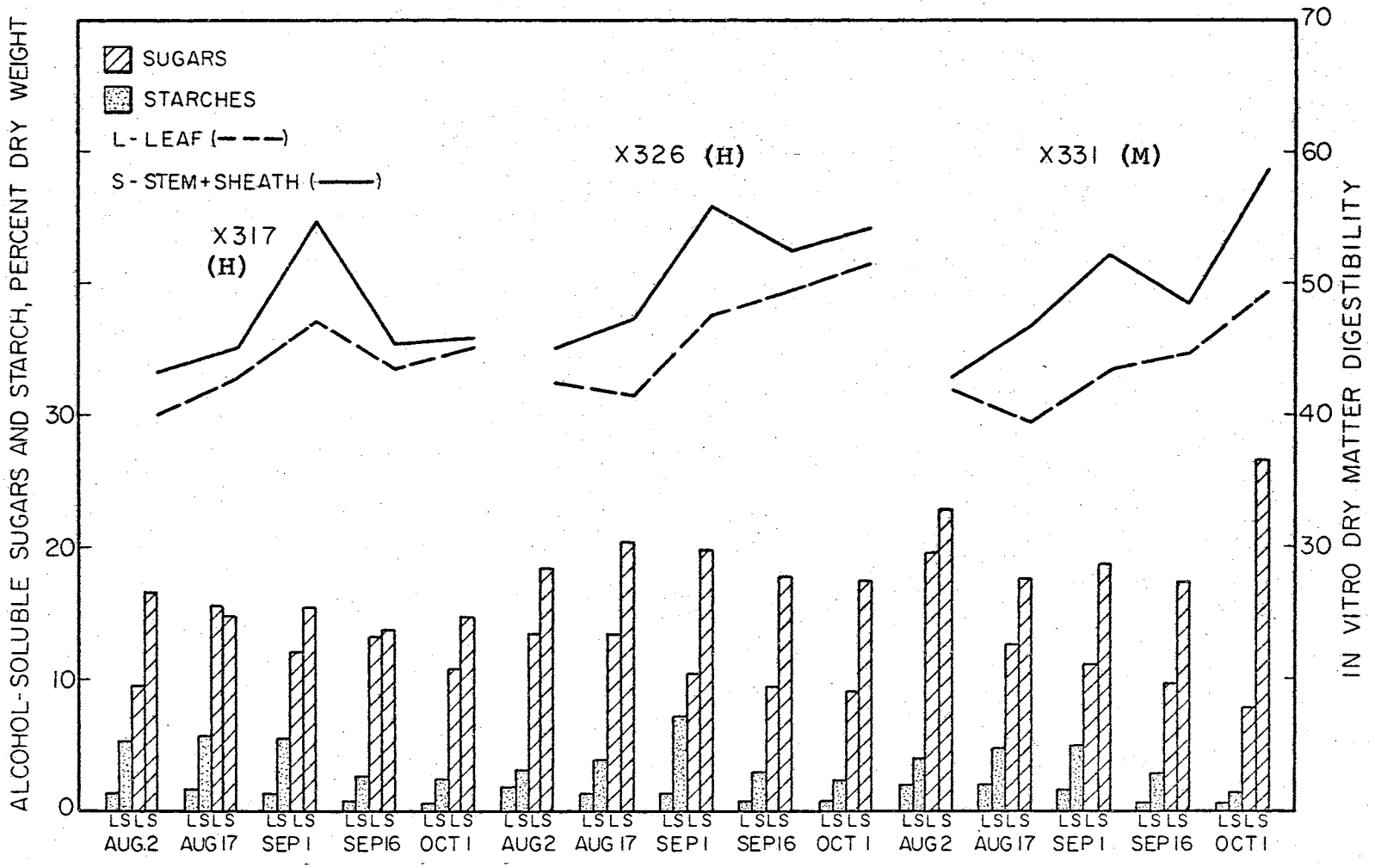

Figure 1. Sugars, Starch and Digestibility for Clones x317 (H), x326 (H), and x331 (M) at Stillwater.

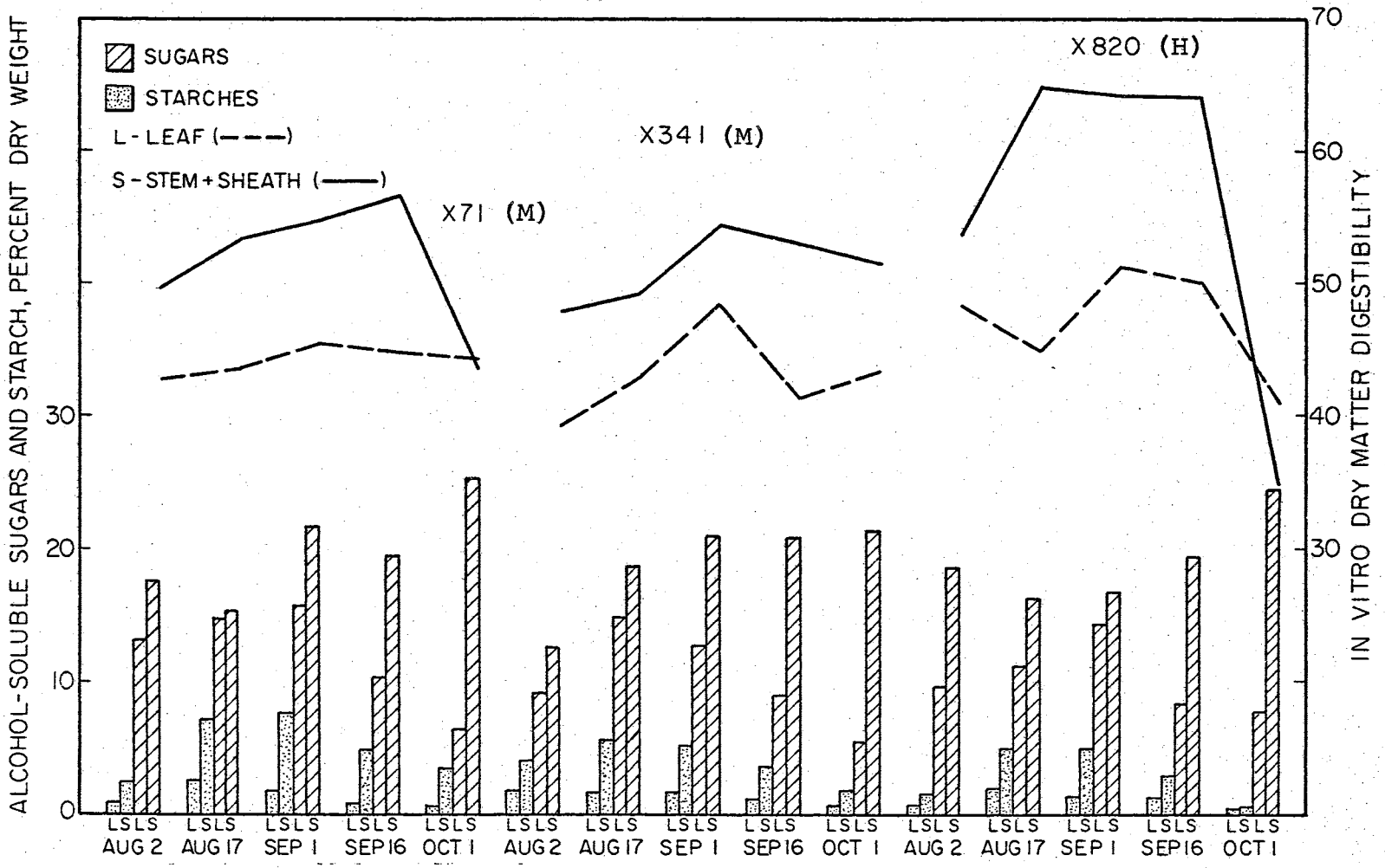

Figure 2. Sugars, Starch and Digestibility for Clones x71 (M), x341 (M), and x820 (H) at Stillwater.

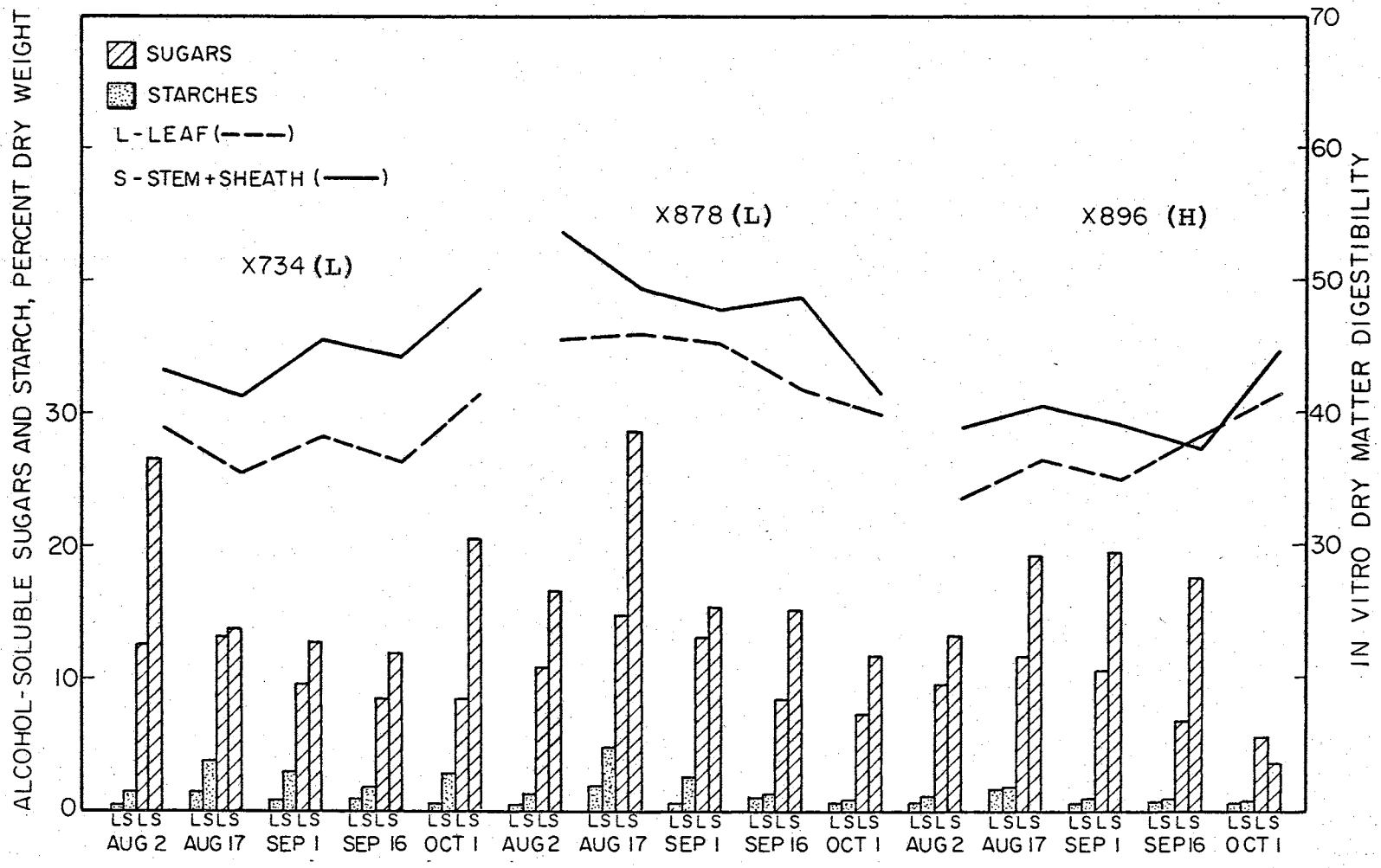

Figure 3. Sugars, Starch and Digestibility for Clones x734 (L), x878 (L), and x896 (H) at Stillwater.

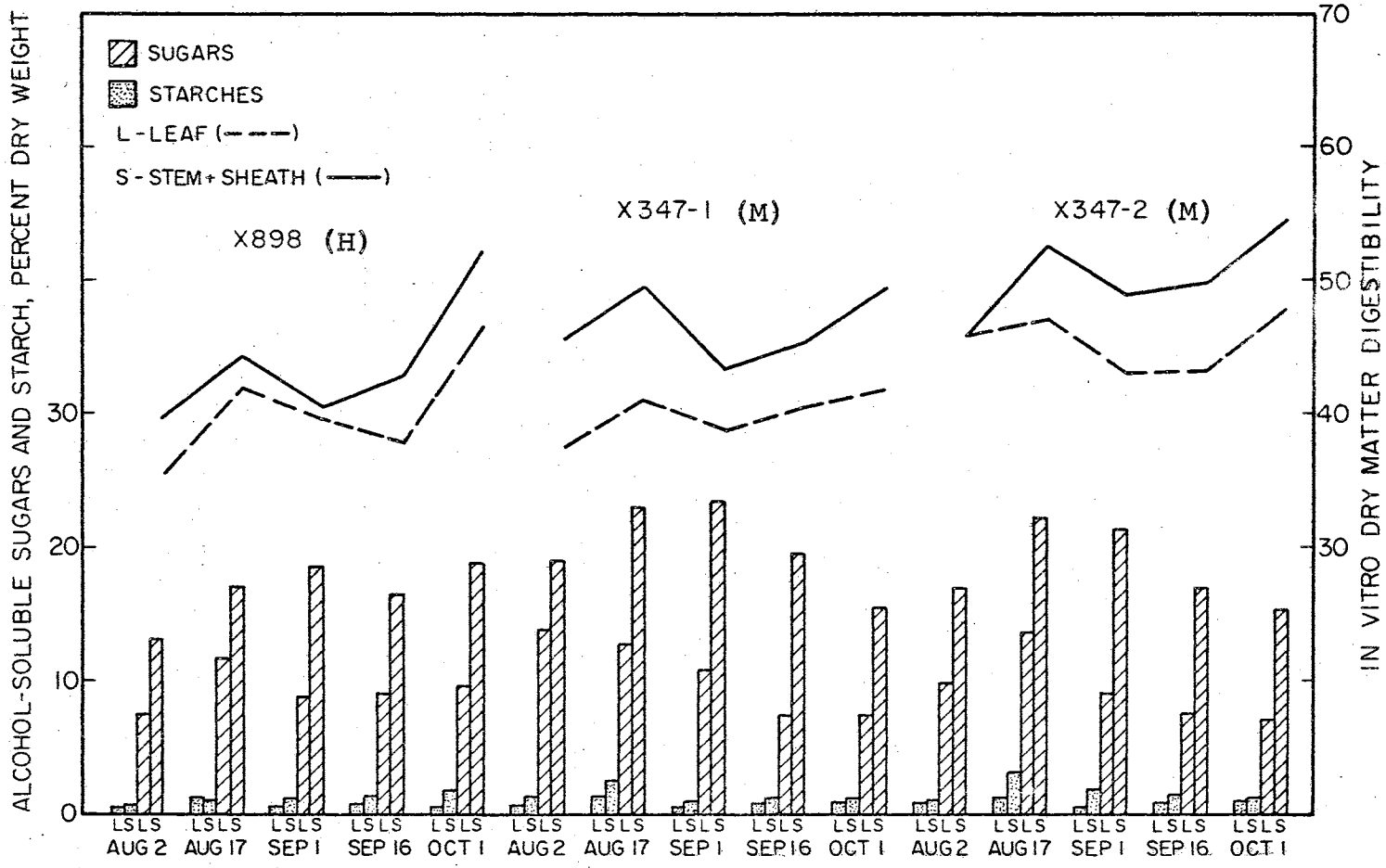

Figure 4. Sugars, Starch and Digestibility for Clones x898 (H), x347-1 (M), and x347-2 (M) at Stillwater.

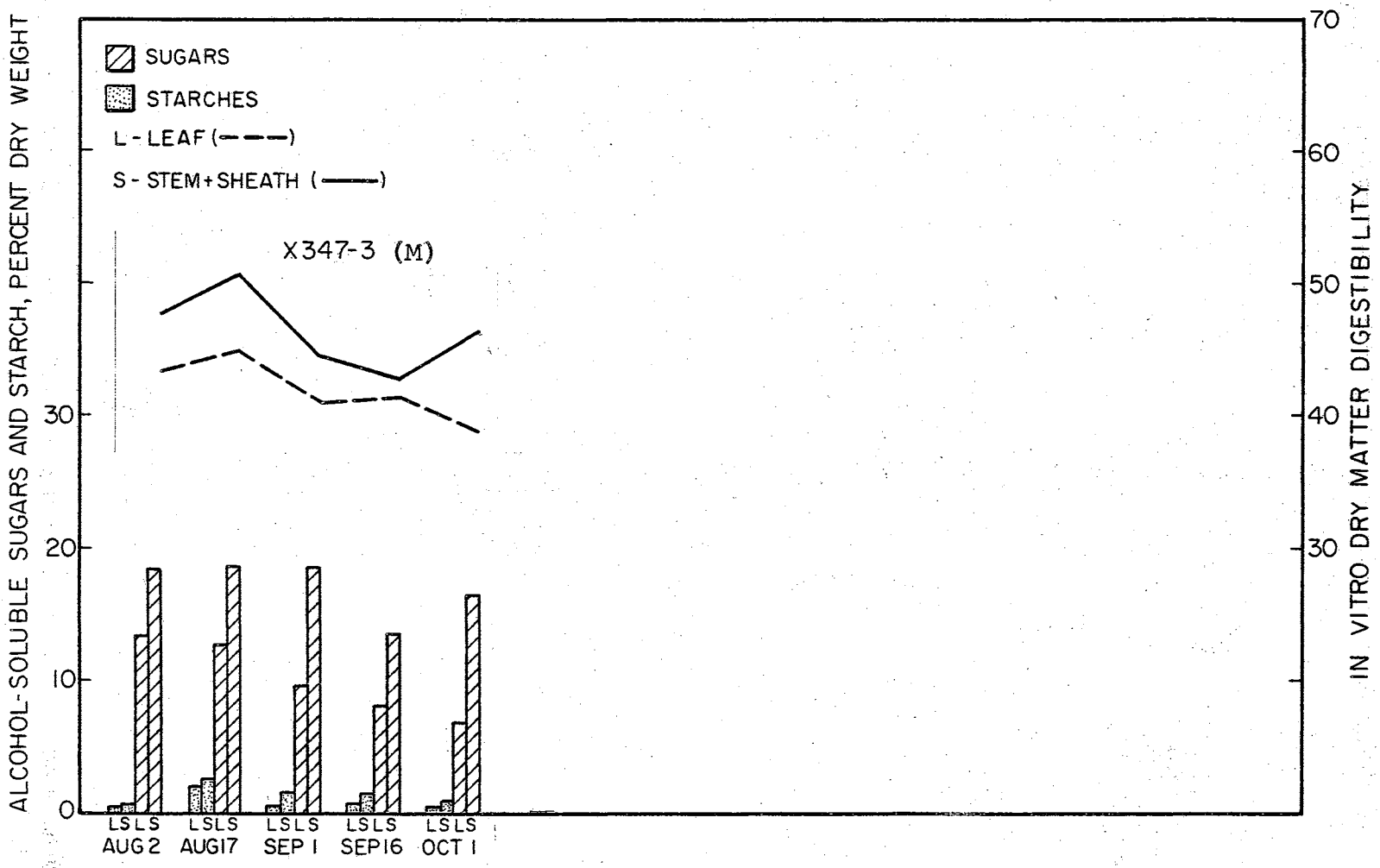

Figure 5. Sugars, Starch and Digestibility for Clone x347-3 (M) at Stillwater.

all cuttings had higher sugar content in the stems than in the leaves.

There was no general clonal pattern in the alcohol-soluble sugar content, especially in the stems, as the season progressed. In most clones the sugar content in the leaves were low in the first cutting, peaked on August 17 or September 1 and decreased again in the final cuttings; in others, the content was high in the first cutting and decreased in the successive ones. The sugar content in the stems was erratic in most clones.

Waite and Boyd (1953) planted a number of forage species and harvested them every time they reached the height of 8 to 10 inches from early May to October 31. They found the sugar content was generally higher in the stems than in the leaves.

Starch content (% dry-matter) ranged from 0.2 to 2.5 percent in the leaves and from 0.3 to 7.5 percent in the stems (Table IV, Figures 1 to 5). At almost all cutting dates the starch percentage of the clones was higher in the stems than in the leaves.

The starch content showed a more uniform pattern than the sugar content. Generally it was low in the August 2 cuttings, peaked on August 17 or September 1, and decreased again on September 16 and October 1. The highest starch

content was obtained in x71 (M) in the leaves on August 17 and in the stems on September 1. Other clones high in leaf starch were x331 (M), x341 (M) and x317 (H), while clones x317 (H), x341 (M) and x326 (H) had a high starch content in the stems.

Since sugar and starch represent the readily digestible carbohydrates, these were combined, and their combined values were related with dry-matter digestibility rather than relating digestibility to either sugar or starch alone. These values are comparable to the "total available carbohydrate" as defined by Weinmann (1947). Combined sugar-starch levels ranged from 5.7 to 21.6 percent in the leaves and from 4.3 to 33.0 percent in the stems (Table V). The stems had higher values than the leaves in all cuttings except for x896 (H) on October 1.

The starch content of the clones constituted a relatively small component of the total available carbohydrate, and as a result, the combined sugar-starch values for the season did not differ greatly from that of the sugar content alone.

Dry-matter digestibility determination for the clones ranged from 33.4 to 47.1 percent in the leaves and from 40.0 to 56.3 percent in the stems (Table VI, Figures 1 to 5). In almost all cuttings higher values were observed in the stems than in the leaves. The higher digestibility percentages in

the stems than in the leaves were probably the result of short growth periods (2 weeks) between harvests.

No general pattern of digestibility across the season was observed to represent all clones. In 6 clones: x331 (M), x734 (L), x896 (H), x898 (H) x347-1 (M) and x347-2 (M), the digestibility values were relatively low on August 2 and peaked on October 1 cuttings for the leaves and the stems, and five clones had peaks on either August 17, September 1 or September 16.

High average digestibility values for the Stillwater samples in 1968 were obtained for x326 (H), x71 (M), x341 (M) and x820 (H); medium values for x317 (H), x331 (M), x878 (L), x898 (H), x347-1 (M), x347-2 (M) and x347-3 (M); and low values for x734 (L) and x896 (H). Some clones were in the same digestibility class as in 1967; other clones shifted up or down one class, while x896 (H) shifted down 2 classes. These class groupings for digestibility were arbitrary, and the lack of replication makes it impossible to obtain an estimate of random variability in this material.

Sullivan (1962) suggested that moisture content of forages could be used as a criterion of quality, and since quality was determined mainly by its digestibility (Raymond, 1966) there was a possible relationship between moisture content and digestibility. The dry-matter digestibility

data (Table VI) supported this relationship in that the digestibility values were generally higher in the stems than in the leaves. The correlation coefficient between moisture content and dry-matter digestibility are presented on Table VII. The highest correlation coefficients (0.82) was obtained for clone x347-2 (M). Correlation coefficients for clones x331 (M), x347-1 (M), x896 (H), x341 (M), x734 (L), x878 (L) and x 347-3 (M) ranged from 0.79 to 0.60, but the rest of the clones had very small r values. However, other chemical constituents could depress digestibility. Buckner et al. (1967) obtained a correlation coefficient on -0.97 between silica and moisture, while silica was inversely related to digestibility.

The correlation coefficients are not high between sugar plus starch content and dry-matter digestibility. The components were higher in the stems than in the leaves in almost all cuttings, however, an increase in sugar plus starch among the clones within either the leaves or the stems was not always followed by an increase in digestibility. The r values ranged from -0.20 to 0.91. High values of 0.91 and 0.86 were obtained for x347-3 (M) and x341 (M), respectively. Clones x347-2 (M), x878 (L), x347-1 (M) and x71 (M) had r values ranging from 0.71 to 0.61. Other clones had r values too small to suggest any relationship between available carbohydrates and dry-matter digestibility. Buckner et al. (1967) found

a positive association between sugars and digestibility in ryegrass-tall fescue hybrids and tall fescue varieties. Terry and Tilley's (1964) study with Dactylis glomerata and Lolium perenne with five cuttings taken between late April and early July (all first cuts) showed that when the grasses were young the stems and the leaf sheaths had higher soluble carbohydrate contents than the leaves. As the plant matured, the soluble carbohydrate content was still higher in the stems and sheaths, but the digestibility dropped sharply such that they had lower digestibility than the leaf.

In general, the data do not support a strong relationship between moisture content and dry-matter digestibility; however, there were fair correlations in some clones. The clones x347-1 (M), x347-2 (M) and x347-3 (M) were crosses from the same parentage and their correlation coefficients for these respective factors were fairly good.

Chickasha Samples

The alcohol-soluble sugar content and the in vitro dry-matter digestibility values of the samples collected from Chickasha are presented on Tables VIII and IX and illustrated in Figures 6, 7 and 8. The highest sugar content of 5.9 percent was obtained by x317 (H) in June cutting, but the lowest value of 2.3 percent was also obtained by x317 (H) in September. The average sugar content across the season ranged from

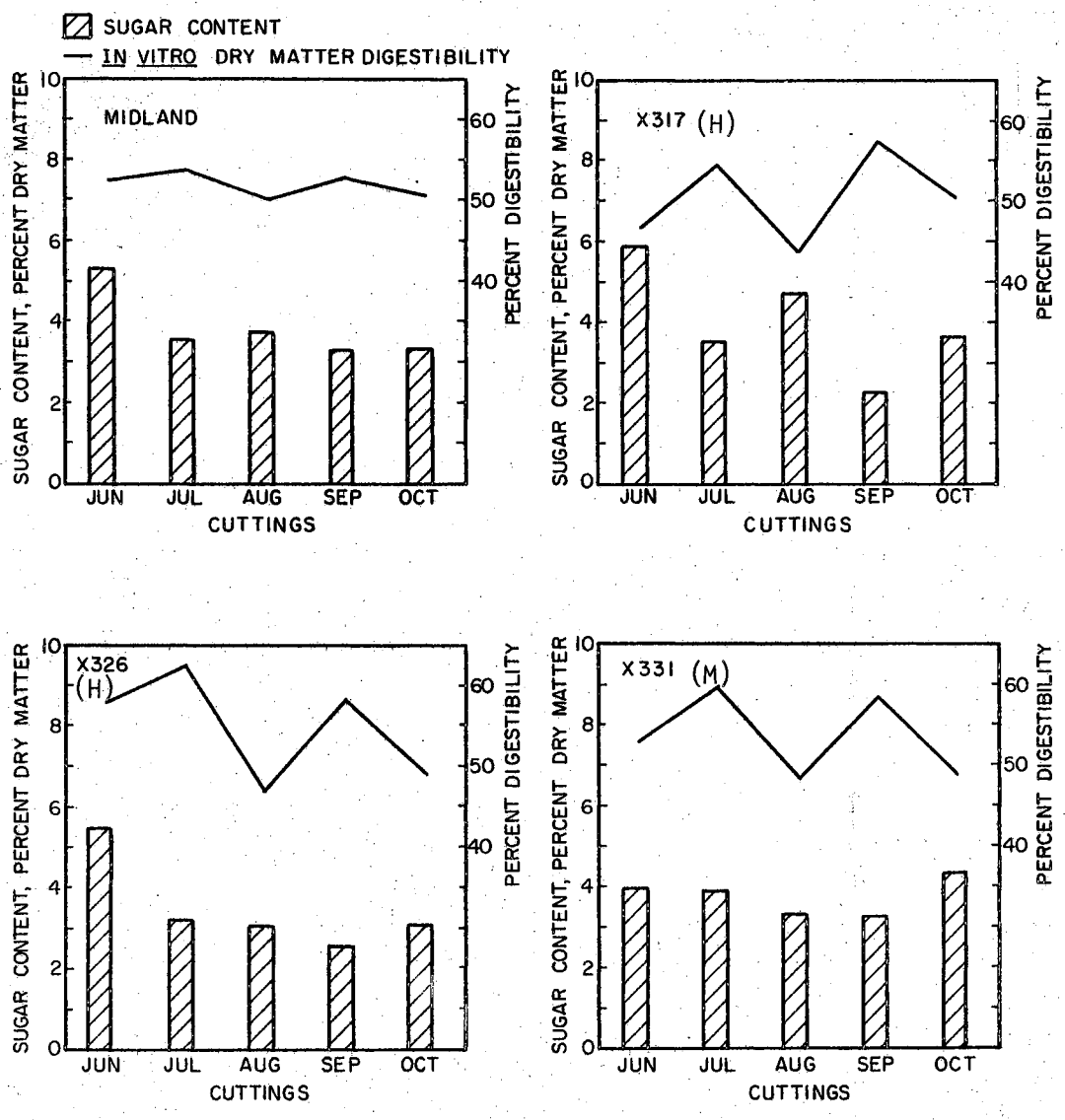

Figure 6. Sugar Content and Digestibility for Clones Midland, x317 (H), x326 (H), x331 (M) at Chickasha.

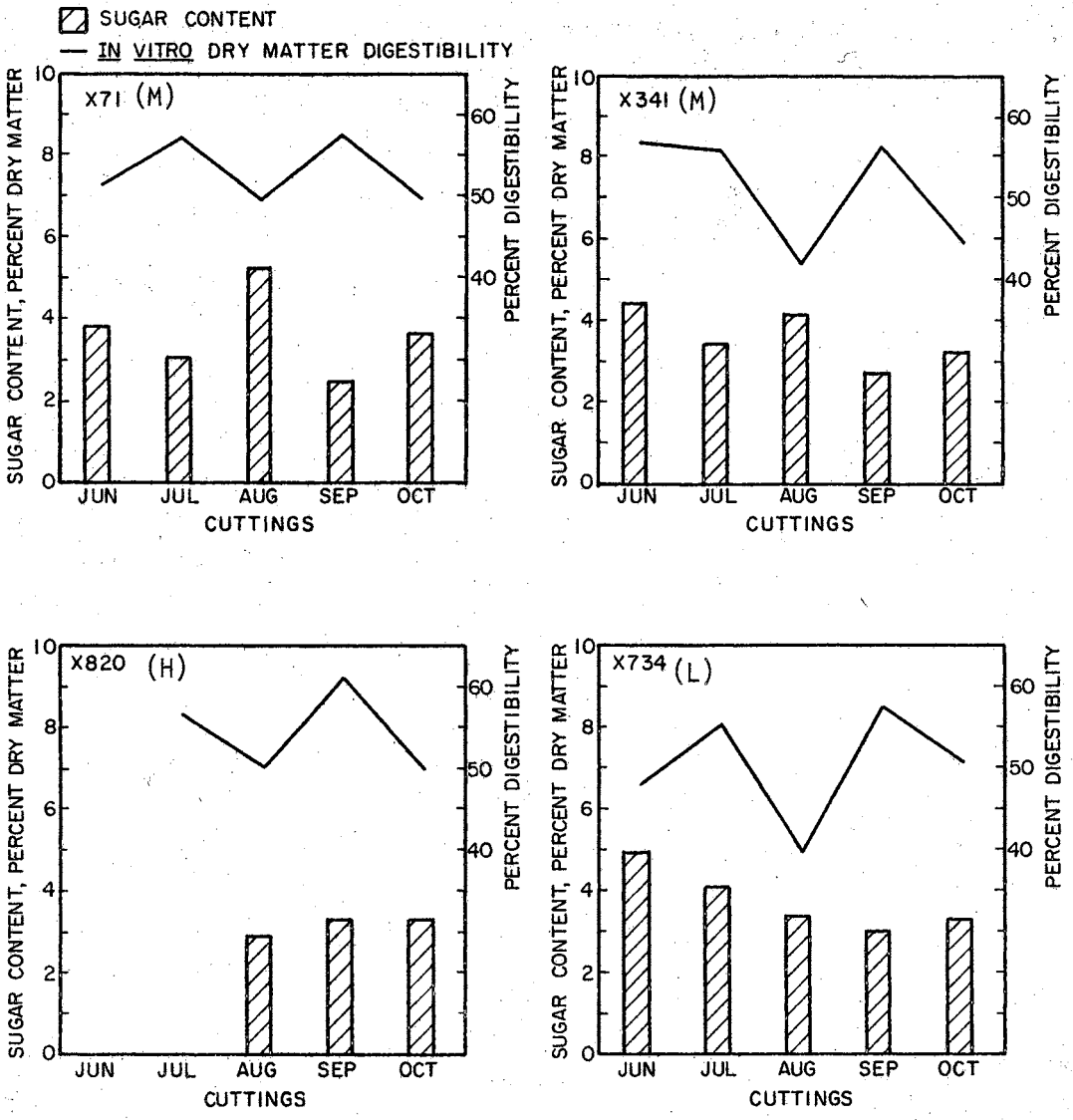

Figure 7. Sugar Content and Digestibility for Clones x71 (M), x341 (M), x820 (H), and x734 (L) at Chickasha.

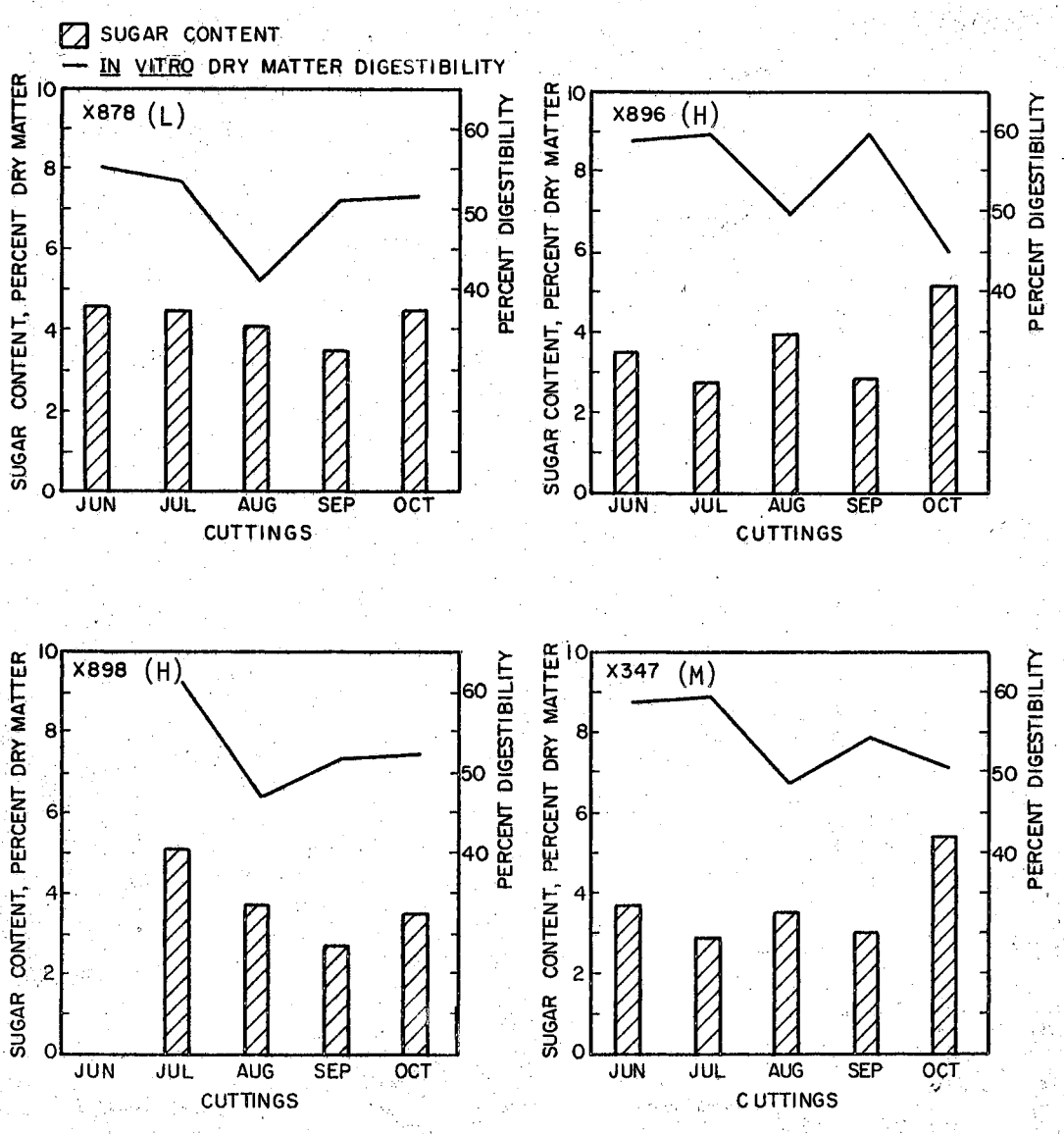

Figure 8. Sugar Content and Digestibility for Clones x878 (L), x896 (H), x898 (H), and x347 (M) at Chickasha.

3.1 percent in x820 (H) to 4.2 percent in x878 (L).

The sugar content of the clones showed a rather uniform pattern, indicating that environmental conditions have significant effects on its occurrence. It was generally high in June, decreased in July, increased in August, decreased again in September and increased again in October. The average sugar content of all clones across the harvests showed a similar pattern.

The Chickasha samples had much lower sugar content than the Stillwater samples. This may be due to several reasons: (a) Chickasha samples were harvested at an advanced stage of maturity (1 month old) compared to those from Stillwater (2 weeks); (b) Stillwater samples were placed in hot alcohol very shortly after harvest while Chickasha samples were dried in the oven, (c) it took about four hours to transfer the samples from Chickasha to drying oven in Stillwater, during which time some sugar in the tissues could have been converted to other chemical constituents, (d) the drying process also could have resulted in the conversion of sugar to other constituents, and (e) there could have been environmental differences (soil conditions, fertilization, precipitation, temperature, light intensity) between the two locations.

No statistically significant difference in sugar content between the clones was found (Table XI). There is, however, a highly significant difference (0.01 level) between cut-

tings, indicating a large seasonal variation in sugar content.

The *in vitro* dry-matter digestibility ranged from 41.4 percent in x878 (L) in August to 62.3 percent in x326 (H) in July. The average digestibility for the clones across the harvests ranged from 50.4 to 54.8 percent. Digestibility of Midland bermudagrass was very uniform across the harvests, while digestibility of all other clones varied greatly from one cut to the other. The clone x820 (H), x326 (H), x896 (H), x71 (M), and x347 (M) showed higher average digestibility than Midland (Figures 6 to 8).

Clones grown at Chickasha which had high average digestibility values were x326 (H) x331 (M), x71 (M), x820 (H), x896 (H) and x347 (M), and those with medium values were x317 (H), x341 (M), x734 (L) and Midland. Almost all clones had either the same or higher classification in 1968 than in 1967 except x317 (H), which was down one class. The data from Chickasha where the clones can be compared with Midland and data from Stillwater indicate that some of these clones have the potential to improve quality of bermuda forage (as evidenced by dry-matter digestion)during the late summer and fall. The seasonal effects will probably continue to be a factor in reduction in quality. For example, digestibility percentages at Chickasha were generally high in July and September and low in June, August and October. There were

significant (0.01 level) differences in digestibility among cuttings indicating seasonal effects on digestion (Table XII).

The correlation coefficients between the sugar content and the digestibility are too small to suggest any relationship between the two factors (Table X). One clone had a positive correlation but many clones had negative values. In general correlation coefficients for Chickasha samples were not comparable to the Stillwater samples.

The limited data lend reasonable encouragement that digestibilities can be used as a selection criteria. It would be quite helpful to have data from other laboratory determinations to assist in quality estimations of breeding material. Moisture, sugar and starch contents are also supporting data; however, variability is quite great among data from these tests. This variability can probably be reduced by closer attention to sampling and by using a larger number of replications.

Other tests (protein, lignin, silica, fiber content and detergent fiber separation) should be evaluated for screening tests for large sample numbers. Protein can be run for large number of samples; however, detergent fiber separations, lignin and silica determinations may be too tedious and time consuming for screening tests.

CHAPTER V

SUMMARY AND CONCLUSIONS

Laboratory analyses were conducted in 1968 to determine the seasonal effects on bermuda forage quality as evidenced by moisture content, sugar and starch content, dry matter digestibility of the whole plant, and leaves and stems of 13 bermudagrass clones grown at Stillwater and Chickasha.

Stillwater samples showed a generally higher moisture content in the stems than in the leaves. The moisture content had a rather irregular pattern across the season. Several clones showed some correlation between moisture content and dry matter digestibility, while others showed very low correlations.

The sugar content of Stillwater samples was also generally higher in the stems than in the leaves. There was no uniform pattern of the sugar content across the season. The Chickasha samples, on the other hand showed a quite uniform pattern: high in June, August and October, and low in July and September. Chickasha samples had lower sugar content when compared to those of Stillwater samples. No consistent relationship between the sugar content and the digestibility

was observed.

The starch content in Stillwater samples had a rather uniform pattern, being highest on August 17, Septmber 1, or September 16. Starch content was higher in the stems than in the leaves.

Some clones maintained good digestibility throughout the studies. Stillwater samples had higher digestion in the stems than leaves. The higher digestion for the stems probably occurred because the forage was only two weeks old. Digestibility was reduced by adverse environmental conditions.

The data indicate that digestibilities can be used as a selection criteria. Other determinations such as protein content should be determined to support the digestibility, moisture, sugar and starch data.

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APPENDIX

TABLE I

THE CROSS NUMBER, ACCESSION NUMBER, SPECIES AND ORIGINS OF 13 BERMUDAGRASS CLONES USED IN THE EXPERIMENT

Cross Number	Female Accession Number	Species	Origin	Male Accession Number	Species	Origin
x317 (H)	10339	C. da	S. Africa	10163	C. da	India
x326 (H)	10339	C. da	S. Africa	10581	C. da	India
x331 (M)	10339	C. da	S. Africa	10982	C. da	Israel
x71 (M)	10020b	C. da	India	8152	C. af	Afghanistan
x341 (M)	10339	C. da	S. Africa	8153	C. af	Afghanistan
x820 (H)	8800	C. af	Afghanistan	10421	C. ro	Rhodesia
x734 (L)	10979	C. da	Hawaii	9961	C. da	Kenya
x878 (L)	9948	C. af	Afghanistan	-	C. da	Oklahoma
x896 (H)	9956	C. da	India	10248	C. da	S. Africa
x898 (H)	9958	C. da	Italy	10416	C. ae	Malaya
x347-1 (M)	9959	C. da	Yugoslavia	9219a	C. da	Ethopia
x347-2 (M)	9959	C. da	Yugoslavia	9219a	C. da	Ethopia
x347-3 (M)	9959	C. da	Yugoslavia	9219a	C. da	Ethopia

Species:

C. ae : Cynodon aethiopicus
C. af : Cynodon afghanicus
C. da : Cynodon dactylon
C. ro : Cynodon robustum

TABLE II

PERCENT MOISTURE CONTENT FOR 13 BERMUDAGRASS CLONES COLLECTED ON 5 SAMPLING DATES FROM STILLWATER

Clones	Cutting Dates: Aug. 2 leaf	Aug. 2 stem	Aug. 17 leaf	Aug. 17 stem	Sept. 1 leaf	Sept. 1 stem	Sept. 16 leaf	Sept. 16 stem	Oct. 1 leaf	Oct. 1 stem	Mean leaf	Mean stem
x317 (H)	45.1	53.3	49.2	57.9	53.6	57.3	41.4	53.9	52.2	64.6	48.2	57.4
x326 (H)	51.8	59.1	49.2	54.3	49.3	58.0	42.6	51.4	55.7	63.1	49.7	57.2
x331 (M)	55.3	61.8	53.5	59.7	56.5	61.7	50.0	59.4	59.9	68.5	55.1	62.2
x71 (M)	49.0	46.2	53.9	54.4	56.3	60.1	48.9	54.0	55.8	63.6	52.8	55.6
x341 (M)	52.5	56.9	51.5	56.5	56.6	63.2	50.0	56.8	60.8	68.1	54.3	60.3
x820 (H)	63.2	69.4	58.9	68.3	59.2	71.6	52.8	63.8	65.3	76.0	59.9	69.8
x734 (L)	48.3	56.4	46.1	50.3	47.5	55.6	53.1	43.2	50.6	64.7	49.1	54.0
x878 (L)	51.6	65.5	55.3	62.5	53.8	62.7	49.8	60.8	54.8	63.3	53.1	63.0
x896 (H)	51.9	61.5	51.4	61.1	55.5	65.3	48.9	58.2	57.6	68.4	53.0	62.9
x898 (H)	51.7	61.7	50.5	58.6	51.8	62.6	45.7	57.5	53.5	64.1	50.6	60.9
x347-1 (M)	54.1	60.0	51.8	58.1	50.3	58.4	46.8	56.2	53.8	64.1	51.4	59.4
x347-2 (M)	57.2	66.7	56.6	65.1	55.8	67.6	50.6	65.3	60.0	72.3	56.0	67.4
x347-3 (M)	62.8	68.9	55.8	64.1	54.2	63.2	44.5	56.2	59.8	67.9	55.4	64.1
Mean	53.4	60.6	52.6	59.3	53.9	62.1	48.1	56.7	56.9	66.8		

Each observation is the mean of duplicate samples.

TABLE III

ALCOHOL-SOLUBLE SUGAR CONTENT (% DRY-MATTER) FOR 13 BERMUDAGRASS CLONES COLLECTED ON 5 SAMPLING DATES FROM STILLWATER

	Cutting Dates											
	Aug. 2		Aug. 17		Sept. 1		Sept. 16		Oct. 1		Mean	
Clones	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem
x317 (H)	9.6	16.6	15.6	14.9	12.0	15.5	13.1	13.7	10.5	14.7	12.1	15.1
x326 (H)	13.2	18.4	13.1	20.2	10.3	19.8	9.3	17.9	9.1	17.6	11.0	18.8
x331 (M)	19.7	22.9	12.4	17.8	11.0	18.5	9.7	17.3	7.9	26.5	12.1	20.6
x71 (M)	12.8	17.8	14.5	15.2	15.6	21.9	10.1	19.5	6.3	25.2	11.8	19.9
x341 (M)	9.1	12.6	14.7	18.5	12.6	20.9	8.8	20.9	5.2	21.3	10.1	18.9
x820 (H)	9.5	18.4	11.0	16.1	14.2	16.5	8.2	19.5	7.8	24.3	10.1	19.0
x734 (L)	12.5	26.4	12.9	13.9	9.5	12.5	8.2	12.0	8.1	20.4	10.2	17.0
x878 (L)	10.7	16.4	14.7	28.6	13.1	15.4	8.1	15.2	7.0	11.7	10.7	17.4
x896 (H)	9.4	13.1	11.6	19.2	10.4	19.4	6.7	17.8	5.6	3.6	8.7	14.6
x898 (H)	7.7	13.0	11.5	17.0	8.4	18.4	8.8	16.4	9.4	18.8	9.1	16.7
x347-1 (M)	13.8	19.1	12.5	23.0	10.7	23.4	7.3	19.5	7.3	15.2	10.3	20.0
x347-2 (M)	9.9	16.8	13.7	22.1	8.9	21.2	7.6	16.9	6.9	17.1	9.4	18.8
x347-3 (M)	13.3	18.2	12.7	18.4	9.6	18.5	8.1	13.6	6.7	16.2	10.1	17.0
Mean	11.6	17.7	13.1	18.8	11.3	18.6	8.8	16.9	7.5	17.9		

Each observation is the mean of duplicate samples.

TABLE IV

STARCH CONTENT (% DRY-MATTER) FOR 13 BERMUDAGRASS CLONES COLLECTED ON 5 SAMPLING DATES FROM STILLWATER

	Cutting Dates											
	Aug. 2		Aug. 17		Sept. 1		Sept. 16		Oct. 1		Mean	
Clones	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem
x317 (H)	1.4	5.2	1.7	5.8	1.4	5.5	0.6	2.5	0.4	2.1	1.1	4.2
x326 (H)	1.8	3.0	1.2	3.8	1.2	7.0	0.4	2.8	0.5	2.1	1.0	3.7
x331 (M)	2.0	3.9	2.0	4.6	1.5	4.7	0.6	2.7	0.5	1.2	1.3	3.4
x71 (M)	0.9	2.3	2.5	7.0	1.6	7.5	0.7	4.7	0.3	3.4	1.2	5.0
x341 (M)	1.8	4.0	1.5	5.6	1.7	5.1	0.9	3.4	0.5	1.6	1.3	3.9
x820 (H)	0.6	1.2	1.9	4.8	1.2	4.8	1.2	2.8	0.2	0.3	1.0	2.8
x734 (L)	0.4	1.4	1.3	3.8	0.6	2.9	0.7	1.9	0.3	2.6	0.7	2.5
x878 (L)	0.3	1.0	1.9	4.5	0.3	2.3	0.7	1.0	0.4	0.7	0.7	1.9
x896 (H)	0.3	0.8	1.6	1.7	0.4	0.8	0.7	0.9	0.4	0.7	0.7	1.0
x898 (H)	0.4	0.5	1.4	1.3	0.3	0.9	0.7	1.1	0.3	1.7	0.6	1.1
x347-1 (M)	0.4	1.0	1.1	2.3	0.3	0.8	0.6	1.0	0.6	1.0	0.6	1.2
x347-2 (M)	0.5	0.9	1.0	2.9	0.2	1.6	0.6	1.2	0.8	0.9	0.6	1.5
x347-3 (M)	0.4	0.5	2.0	2.5	0.3	1.4	0.4	1.2	0.3	0.8	0.7	1.3
Mean	0.9	2.0	1.6	3.9	0.8	3.5	0.7	2.1	0.4	1.5		

Each observation is the mean of duplicate samples.

TABLE V

SUGAR AND STARCH CONTENT (% DRY-MATTER) FOR 13 BERMUDAGRASS CLONES COLLECTED ON 5 SAMPLING DATES FROM STILLWATER

	Cutting Dates											
	Aug. 2		Aug. 17		Sept. 1		Sept. 16		Oct. 1		Mean	
Clones	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem
x317 (H)	11.1	21.8	17.3	26.0	13.4	20.9	13.6	16.1	10.9	16.8	13.3	19.3
x326 (H)	15.1	21.4	14.3	24.1	11.5	26.8	9.7	20.7	9.5	19.7	12.0	22.5
x331 (M)	21.6	26.9	14.4	22.4	12.5	23.3	10.3	20.0	8.4	27.7	13.4	24.0
x71 (M)	13.7	20.1	17.0	22.2	17.2	29.5	10.7	24.2	6.6	28.6	13.0	24.9
x341 (M)	10.9	16.6	16.2	24.1	14.2	26.0	9.8	24.4	5.7	22.9	11.4	22.8
x820 (H)	10.1	19.5	12.9	20.9	15.4	21.4	9.4	22.3	7.9	24.7	11.1	21.7
x734 (L)	12.8	27.8	14.2	17.7	10.2	15.4	9.0	13.9	8.4	23.0	10.9	19.5
x878 (L)	11.0	17.3	16.6	33.0	13.4	17.7	8.8	16.2	7.4	12.4	11.4	19.3
x896 (H)	9.7	13.8	13.1	20.9	10.7	20.2	7.5	18.6	6.0	4.3	9.4	15.6
x898 (H)	8.0	13.5	12.9	18.3	8.7	19.4	9.5	17.5	9.7	20.5	9.8	17.8
x347-1 (M)	14.1	20.0	13.6	25.3	11.1	24.1	7.8	20.5	7.9	16.3	10.9	21.2
x347-2 (M)	10.4	17.7	14.6	25.0	9.1	22.8	8.2	18.0	7.6	18.0	10.0	20.3
x347-3 (M)	13.6	18.7	14.7	20.9	9.9	19.9	8.5	14.9	7.0	17.0	10.7	18.3
Mean	12.5	19.6	14.8	23.1	12.1	22.1	9.4	19.0	7.9	19.4		

Each observation is the mean of duplicate samples.

TABLE VI

PERCENT DRY-MATTER DIGESTIBILITY FOR 13 BERMUDAGRASS CLONES COLLECTED ON 5 SAMPLING DATES FROM STILLWATER

	Cutting Dates											
	Aug. 2		Aug. 17		Sept. 1		Sept. 16		Oct. 1		Mean	
Clones	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem	leaf	stem
x317 (H)	40.0	43.3	42.7	45.0	47.1	54.8	43.3	45.4	45.1	46.0	43.7	46.9
x326 (H)	42.3	45.1	41.4	47.4	47.7	55.9	49.5	52.5	51.5	54.2	46.5	51.0
x331 (M)	42.0	42.8	39.7	46.8	43.4	52.1	44.5	48.3	49.8	58.6	43.9	49.7
x71 (M)	42.6	49.7	43.6	53.2	45.4	54.7	44.8	56.5	44.1	44.0	44.1	51.6
x341 (M)	39.1	47.9	42.9	49.2	48.3	54.2	41.5	53.0	43.4	51.5	43.1	51.1
x820 (H)	48.2	53.6	44.9	64.7	51.2	64.1	50.0	64.0	41.3	34.8	47.1	56.3
x734 (L)	38.9	43.1	35.6	41.2	38.0	45.6	36.3	44.1	41.3	49.3	38.0	44.7
x878 (L)	45.6	53.5	45.9	49.2	45.1	47.9	41.9	48.6	40.0	41.9	43.7	48.2
x896 (H)	33.4	38.8	36.2	40.3	34.9	39.1	38.0	37.2	41.5	44.5	36.8	40.0
x898 (H)	35.4	39.9	42.0	44.2	39.8	40.3	37.9	42.7	46.5	52.3	40.3	43.9
x347-1 (M)	37.3	45.3	40.9	49.4	38.9	43.4	40.2	45.2	41.9	49.6	39.9	46.6
x347-2 (M)	45.9	45.8	47.0	52.4	43.0	48.9	43.1	49.9	47.7	54.3	45.3	50.2
x347-3 (M)	43.4	47.7	44.8	50.6	41.0	44.6	41.4	42.8	38.7	46.2	41.8	46.4
Mean	41.1	45.9	42.1	48.7	43.4	49.7	42.5	48.5	44.1	48.2		

Each observation is the mean of duplicate samples.

TABLE VII

STILLWATER CORRELATION COEFFICIENTS

Moisture Content vs. Digestibility		Sugar plus Starch Content vs. Digestibility	
Clone:	r value:	Clone:	r value:
x317 (H)	0.53	x317 (H)	0.41
x326 (H)	0.39	x326 (H)	0.32
x331 (M)	0.79	x331 (M)	0.36
x71 (M)	0.09	x71 (M)	0.61
x341 (M)	0.68	x341 (M)	0.86
x820 (H)	0.01	x820 (H)	0.37
x734 (L)	0.64	x734 (L)	0.59
x878 (L)	0.62	x878 (L)	0.64
x896 (H)	0.72	x896 (H)	-0.20
x898 (H)	0.53	x898 (H)	0.59
x347-1 (M)	0.79	x347-1 (M)	0.64
x347-2 (M)	0.82	x347-2 (M)	0.71
x347-3 (M)	0.61	x347-3 (M)	0.91

TABLE VIII

ALCOHOL-SOLUBLE SUGAR CONTENT (% DRY-MATTER) FOR 12 BERMUDA-GRASS CLONES COLLECTED ON 5 SAMPLING DATES FROM CHICKASHA

Clones	Cutting Dates June	July	Aug.	Sept.	Oct.	Mean
Midland	5.3	3.6	3.7	3.3	3.4	3.9 a**
x317 (H)	5.9	3.5	4.8	2.3	3.7	4.0 a
x326 (H)	5.5	3.2	3.0	2.6	3.1	3.5 a
x331 (M)	4.0	3.9	3.4	3.3	4.3	3.8 a
x71 (M)	3.8	3.1	5.3	2.5	3.7	3.7 a
x341 (M)	4.4	3.5	4.2	2.7	3.2	3.6 a
x820 (H)	- *	- *	2.9	3.3	3.2	3.1 ***
x734 (L)	5.0	4.0	3.4	3.0	3.3	3.7 a
x878 (L)	4.6	4.5	4.1	3.5	4.4	4.2 a
x896 (H)	3.5	2.8	4.0	2.9	5.1	3.7 a
x898 (H)	- *	5.1	3.7	2.7	3.5	3.8 ***
x347 (M)	3.7	2.9	3.6	3.0	5.4	3.7 a
Mean	4.6 a**	3.6 bc	3.8 ab	2.9 c	3.9 ab	

* No data available

** Means marked by the same letters are not significantly different at .05 level according to Duncan's Multiple Range Test

*** Not included in the Multiple Range Test

Each observation is the mean of duplicate samples from 2 replications.

TABLE IX

PERCENT IN VITRO DRY-MATTER DIGESTIBILITY FOR 12 BERMUDAGRASS CLONES COLLECTED ON 5 SAMPLING DATES FROM CHICKASHA

Clones	Cutting Dates					
	June	July	Aug.	Sept.	Oct.	Mean
Midland	52.4	53.6	50.1	52.7	50.4	51.8 abc**
x317 (H)	46.6	54.4	43.4	57.1	50.3	50.4 c
x326 (H)	57.7	62.3	46.8	58.0	49.1	54.8 a
x331 (M)	52.8	59.6	48.1	58.1	48.7	53.5 abc
x71 (M)	51.3	57.1	49.4	57.4	49.7	53.0 abc
x341 (M)	56.8	55.8	41.8	56.2	44.5	51.0 abc
x820 (H)	- *	56.4	50.0	61.1	49.3	54.2 ***
x734 (L)	48.0	55.4	39.9	57.9	50.9	50.5 bc
x878 (L)	55.2	53.5	41.4	51.1	51.8	50.6 bc
x896 (H)	58.9	59.4	49.9	59.6	45.0	54.6 ab
x898 (H)	- *	61.2	47.0	51.7	52.2	53.0 ***
x347 (M)	58.7	59.3	48.6	54.3	50.8	54.3 abc
Mean	53.8 b**	57.3 a	46.4 d	56.3 ab	49.4 c	

* No date available

** Means marked by the same letters are not significantly different at .05 level according to Duncan's Multiple Range Test

*** Not included in the Multiple Range Test

Each observation is the mean of duplicate samples from 2 replications.

TABLE X

CHICKASHA CORRELATION COEFFICIENTS

Sugar Content vs. Dry-Matter Digestibility

Per Clone		Per Cut	
Clone:	r value:	Cut:	r value:
Midland	0.14	June	-0.54
x317 (H)	-0.85	July	-0.10
x326 (H)	0.23	August	-0.13
x331 (M)	-0.21	September	-0.22
x71 (M)	-0.82	October	-0.07
x341 (M)	-0.16		
x820 (H)	0.56		
x734 (L)	-0.17		
x878 (L)	0.34		
x896 (H)	-0.95		
x898 (H)	0.72		
x347 (M)	-0.50		

TABLE XI

ANALYSIS OF VARIANCE FOR SUGAR CONTENT (% DRY-MATTER) OF SAMPLES COLLECTED FROM CHICKASHA, EXCLUDING x820 (H) AND 898 (H)

Source	d.f.	S.S.	M.S.	F.
Total	99	125.254		
Replications	1	0.355	0.355	0.325
Clones	9	4.434	0.492	0.452
Cuttings	4	30.531	7.632	7.002**
Clone x Cut.	36	36.522	1.014	0.930
Rep. x Clone	9	7.891	0.876	
Rep. x Cut.	4	6.263	1.565	
Rep. x Clone x Cut.	36	39.255	1.090	

** Significant at .01 level

TABLE XII

ANALYSIS OF VARIANCE FOR DIGESTIBILITY (% DRY-MATTER) OF SAMPLES COLLECTED FROM CHICKASHA, EXCLUDING x820 (H) AND 898 (H)

Source	d.f.	S.S.	M.S.	F.
Total	99	3654.71		
Replications	1	84.63	84.63	5.58*
Clones	9	287.39	31.93	2.10*
Cuttings	4	1805.48	451.37	29.79**
Clone x Cut.	36	734.52	20.40	1.34
Rep. x Clone	9	93.55	10.39	
Rep. x Cut.	4	154.23	38.55	
Rep. x Clone x Cut.	36	494.91	13.74	

* Significant at .05 level
** Significant at .01 level

VITA

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