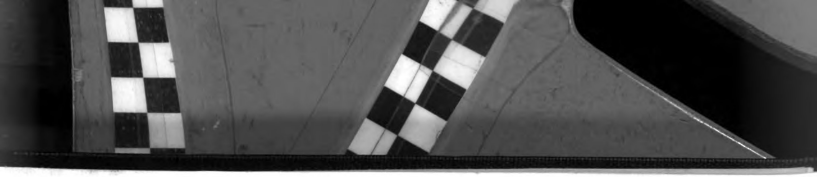
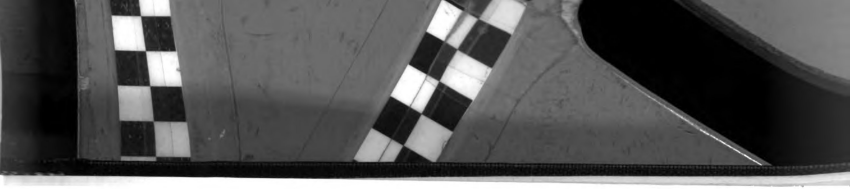




FACTORS AFFECTING THE NUTRITIVE VALUE OF FORAGES

By

Derek W. Allinson

AN ABSTRACT OF A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop Science

1966

ABSTRACT

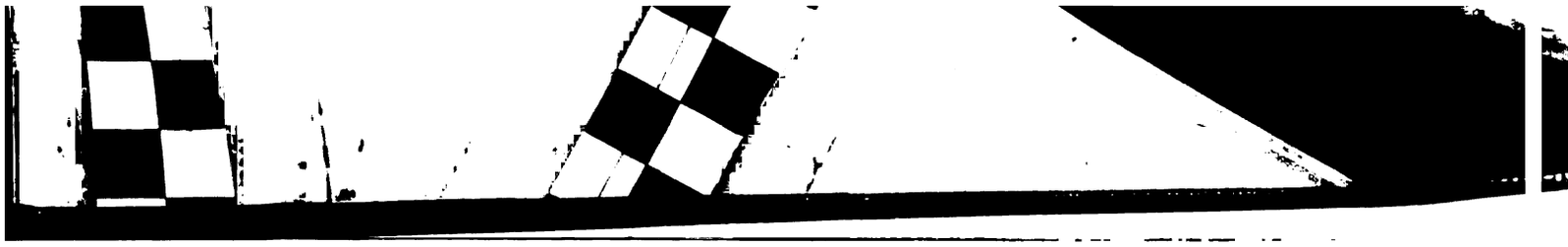
FACTORS AFFECTING THE NUTRITIVE VALUE OF FORAGES

by Derek W. Allinson

The influence of cutting frequency, nitrogen fertilization and moisture level upon the nutritive value of specified forages was investigated. Forages, sampled frequently throughout the growing season in 1964 and subjected to either a four-, three-, two- or one-cutting system, ranked in the following order: alfalfa, birdsfoot trefoil, reed canarygrass, bromegrass and orchardgrass. There was no significant difference between four, three or two cuttings in their effect on nutritive value; all were superior to the one-cutting system. Nitrogen fertilization in the period 1965-1966 showed no consistent effects on nutritive value. In 1965, however, two types of reed canarygrass had superior nutritive value when fertilized at 150 lb N per acre compared to 0 and 450 lb N per acre. Common reed canarygrass was superior to Siberian reed canarygrass in nutritive value. Soil moisture level did not affect nutritive value although significant changes in chemical composition occurred.

Morphological condition and moisture content of forages were adequate indicators of nutritive value only when the forages were not cut until the end of the growing season.

A Medicago population containing varieties of varying genetic



Derek W. Allinson

background was screened for nutritive value on a single clone basis. Variation was considerable; species and variety differences were obtained. Simple correlation coefficients indicated physical fractions of forages were associated with in vitro data at 36 hours rather than at 6 hours.

Compared to clones of low nutritive value, those of high nutritive value were consistently low in lignin, fiber and cell wall constituents, and stimulated increased production of acetic, propionic and butyric acids in in vitro fermentations.

Clones of high or low nutritive value had differing levels of soluble carbohydrates and exhibited variable levels of antibiosis. However, antibiosis and soluble carbohydrates were not apparently related to nutritive value as measured in these experiments. Clones of high nutritive value were characterized by lignin ultraviolet difference spectra such that peak absorptivity values were of the order $250 > 300 > 350$ mμ. Clones of low nutritive value had peak absorptivity values in the order $250 > 350 \geqq 300$ mμ. Lignins were extracted in significantly greater quantities from clones of high nutritive value, even though such clones contained less total lignin. The hypothesis is presented that differences in nutritive value may be ascribed to, at least in part, the maturity of the fiber/lignin complex.

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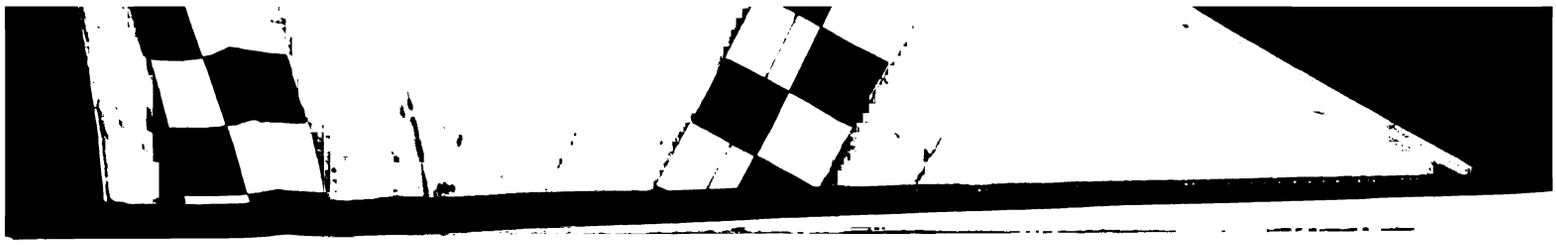

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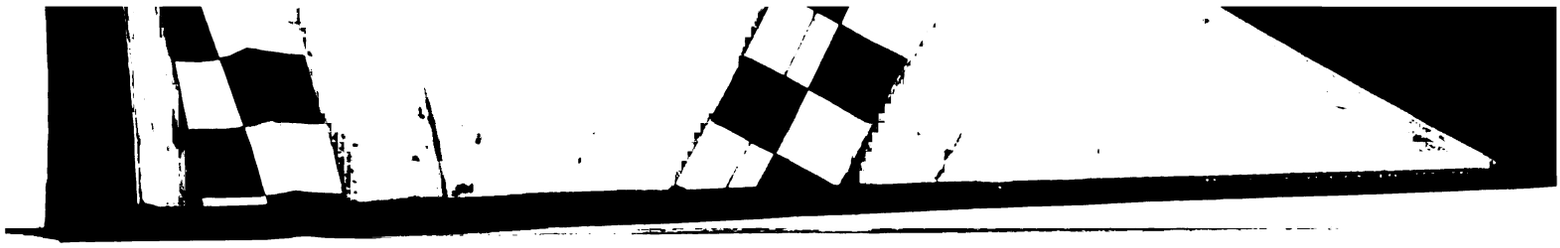

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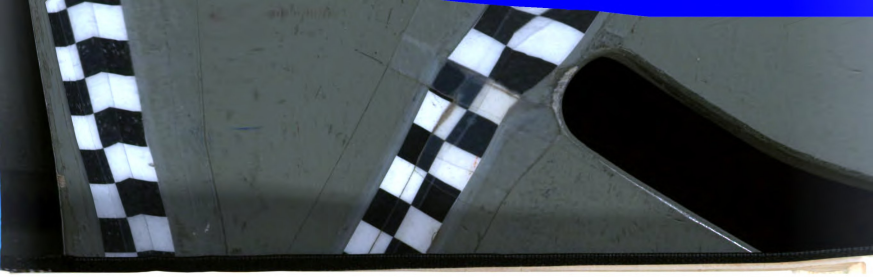

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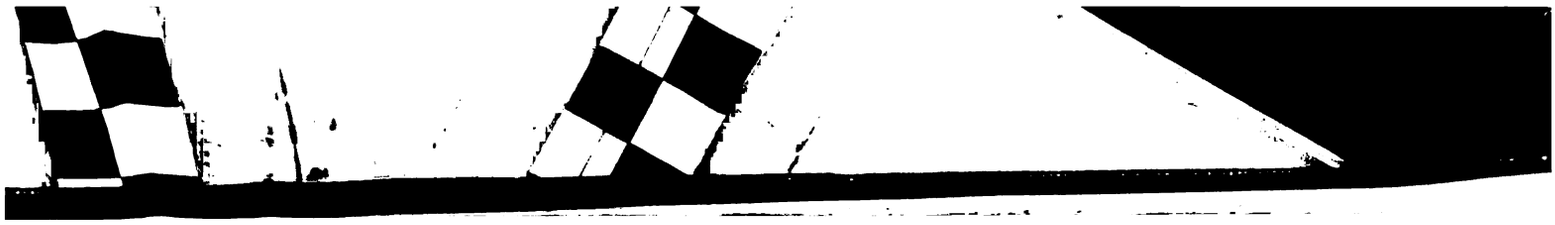


INTRODUCTION

Agronomists are aware of the need for improvements in the techniques of manipulating forage swards, both culturally and genetically. Parameters previously considered of primary importance must be in harmony with the parameter of nutritional usefulness. Improvements in the quantitative aspect are discernible, easily evaluated and readily made. This is not the case with the qualitative aspect.

Qualitative improvements instigated through cultural techniques involve, basically, ecological manipulations. The choice of species and varieties, the time and number of harvests, the rates of fertilizer and the level of available moisture all have influences upon the ecological complex. The forage sward reacts in a positive way, and this is measurable. Such measurement may be in terms of animal output. Alternatively, and more purposefully, such changes may be traced by one of the many in vitro systems shown to have a significant relationship to animal output. Consequently, once the manipulation has been made and the reaction measured, rational predictions of the dynamics of qualitative changes occurring in the forage crop are possible. Such predictions could be applied on a broad basis.

However, these improvements are necessarily restricted in scope, since the genetic background of the crop ultimately sets a limit. The ease with which the nutritional qualities of a genotypic spectrum can be improved has yet to be ascertained.

This study has been aimed at assessing the changes occurring in various forage crops under the influence of standard management practices. A number of evaluatory techniques has been used although the basic tool throughout has been a 6-hour in vitro fermentation. While recognizing cultural techniques to be invaluable, the breeder must ultimately create the population in which the culturalist must work. Therefore, the variation existing within and between genera, species and varieties has been examined. An attempt has been made to explain this variation in terms of chemistry, in terms applicable to the plant physiologist and in terms usable to the agronomist.

LITERATURE REVIEW

Measurement of forage quality

A forage crop is not utilized directly but is first processed by the animal. As such, forage crop usefulness is measured in terms of animal output. This measurement is laborious, expensive, time consuming and requires considerable quantities of forage. Consequently, such measurements are useless for the assessment of breeders' lines, introduced plant materials and forage obtained from small plot cultural studies. Considerable research has indicated a number of techniques as being adequate preliminary substitutes for the animal trial.

The routine chemical analysis used as an indicator of forage quality has been repeatedly repudiated as unsound (Fraser, 1960; Van Soest, 1963a, 1964). Yet as Fraser points out, this routine analysis is the basis upon which feed analysis and feeding tables are constructed. Simple animal digestion trials from which the nutritive usefulness of a forage crop is predicted are also based upon this proximate or Weende system of analysis. Somewhat paradoxically, all new attempts at forage evaluation are ultimately compared to the animal digestion trial, even though the basis of such trials is known

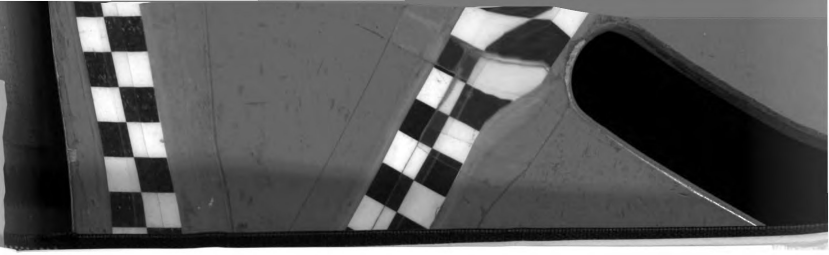
to be unsound.

More recently, several in vitro systems, basically artificial rumens, have been developed. These have been used not only for the evaluation of forages but also, and perhaps more specifically, for the elucidation of rumen reactions. Excellent reviews of these techniques are given by Barnett and Reid (1961), Annison and Lewis (1962), El-Shazly et al. (1960), Barnes et al. (1964), Johnson (1963), and Barnes (1965). In addition to the in vitro techniques, a whole series of associations has been derived between forage nutritive value and specific chemical components of the forage. The work of Van Soest is outstanding in this respect. Solubility in various solvents has also been suggested as a technique for forage evaluation (Dehority and Johnson, 1964; Donefer et al., 1963).

The in vitro systems, offering a rapid, inexpensive evaluation while requiring but minimum quantities of forage, are superficially ideal. They have not yet gained widespread acceptance. Criteria of the fermentations include dry matter disappearance, cellulose disappearance, protein synthesis, production of volatile fatty acids, gas production, starch digestion, vitamin synthesis and amino-acid synthesis (Johnson, 1963). Correlations with in vivo digestibility, feed intake or other animal response may be derived from any of these parameters. Usually only one parameter is used, possibly leading to biased estimates of nutritive usefulness. Barnes (1965) has suggested the incorporation of several measures to give an effective evaluation tool.

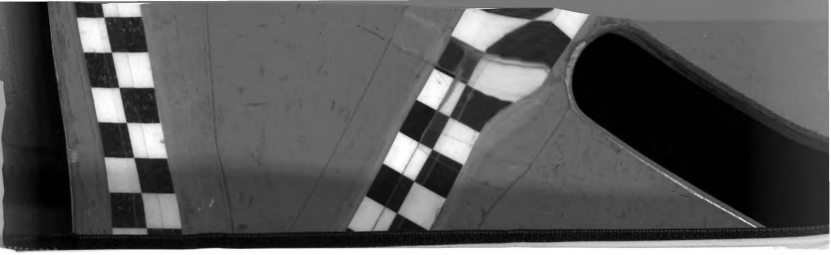
Such derived correlations have generally been significant (Asplund et al., 1958; Bowden and Church, 1962a, 1962b; Baumgardt et al., 1962a; Baumgardt and Hi Kon Ho, 1964; Hershberger et al., 1959; LeFevre and Kamstra, 1960). Such results do suggest that in vitro techniques could be used for a laboratory evaluation of forages. Unfortunately, day-to-day variation may be considerable, and large discrepancies exist between laboratories. The techniques themselves are variable, ranging from all-glass systems to complex continuous-flow dialysis systems. However, the complexity of the in vitro system is no criterion of its usefulness (Bowden and Church, 1962a). El-Shazly et al. (1960) compared three distinct systems, found no major differences, and concluded that the simplest was preferable. Barnes (1965) also compared several systems and decided that substrates were eventually arranged in essentially the same order regardless of the method or the fermentation period. Barnes further indicated the system must be able to measure the effect of the early lag phase in fermentation.

Hershberger et al. (1959), indicated a variable lag period followed by a rapid rate of cellulose degradation, such degradation taking place 6 to 18 hours after the start of fermentation. These workers indicated that, following 18 hours, the rate of cellulose degradation slowed. After 24 hours little change occurred. Rice et al. (1962), also noted a lag phase. Cellulose from oat straw was initially more resistant to bacterial attack than cellulose from alfalfa-bromegrass. However, once digestion of the oat cellulose began



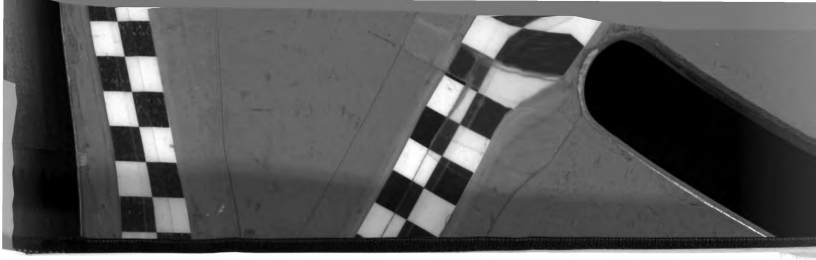
degradation proceeded at such a rate that after 24 hours no significant difference in cellulose digestion was apparent. Perhaps the non-cellulosic constituents of a forage have a definitive effect on ultimate cellulose utilization. High levels of available carbohydrates had a depressing effect on cellulose digestibility; low levels stimulated such degradation (Barnett and Reid, 1961). As Barnes (1965) indicated, strict estimation on cellulose digestion alone may be misleading since a high cellulose loss may be associated with low levels of available carbohydrates. Schillinger (1965) indicated that certain water-soluble antimetabolic factors in alfalfa depressed fermentation through direct inhibition of the cellulose-fermenting bacteria. These facts, then, would indicate the early lag phase in fermentation as being indicative of eventual cellulose degradation.

Baumgardt and Hi Kon Ho (1964), using fermentation periods of 18, 24, 30 and 48 hours, determined in vitro cellulose digestibility to be significantly correlated to in vivo digestible dry matter. The coefficients obtained from these fermentation periods did not differ significantly; however, day-to-day variation decreased with increasing fermentation time. Bowden and Church (1962a) also indicated within-trial variability for dry matter digestibility at 48 hours to be small. Cellulose digestibility was found to be more variable than simple dry matter digestibility. Johnson et al. (1962b), found that a 12-hour fermentation period, measuring in vitro cellulose digestibility, compared closely with the nutritive value index of Crampton et al. (1960a, 1960b). A 6-hour fermentation was discarded



as being too variable. Ingalls (1964) found a 6-hour fermentation measuring dry matter disappearance was significantly correlated with NVI; a 36-hour fermentation period was correlated to animal digestibility. Regarding short fermentation periods, the work of Yadava and Bartley (1964) is informative. Using identical twin cows fed alfalfa hay, nutrients having the greatest digestibility had the fastest removal rates from the rumen. The nitrogen-free extract and crude protein fractions had faster removal rates than the crude fiber fraction. Six hours after feeding, digestion of the crude fiber was under way, suggesting the lag phase of fermentation to be almost complete. In view of the work of Rice et al. (1962), perhaps the shorter fermentations are preferable as indicators of forage value.

Crampton et al. (1960a), indicated digestion data alone have a limited application without the inclusion of intake data. They maintained that the effective nutritive value of a forage was governed by both voluntary intake and the ultimate yield of digestible energy, and that both factors were interrelated. Consequently, Crampton et al. (1960a, 1960b), proposed that the product of relative intake, (expressed per unit of metabolic size per animal and relative to a standard forage), and percent digestibility of its energy be used as an index of nutritive value (NVI). They indicated that NVI was predictable from in vitro data; correlations of 0.91 and 0.89 were obtained between NVI and a 12- and 24-hour in vitro fermentation, respectively. Blaxter (1960), too, believes the value of a feed to be proportional to "its 'effective' energy content" in maintaining energy



equilibrium in ruminants, voluntary feed intake being a factor in determining the energy retention of an animal. The relationship between intake and digestibility, reviewed by both Blaxter (1960) and Crampton et al. (1960a) showed that rumen ingesta loads were reduced at varying rates depending upon their digestibilities. A specific reduction load stimulated hunger with consequent intake. The speed at which hunger occurs may be a forage characteristic. Blaxter indicated that the higher the intake, measured as the ratio of metabolizable energy intake/basal metabolism, the greater its gross efficiency. However, reductions in digestibility were associated with changes in the passage of feed through the gut. Van Soest (1964) on the other hand, suggested that voluntary intake and digestibility are not necessarily related, and may be independent. Consequently, measures of digestibility are not necessarily adequate predictors of intake.

The prediction of forage nutritive value has long relied upon estimations of indigestible fractions. The crude fiber fraction from proximate analyses has been most commonly used. Both Van Soest (1963a, 1964) and Kivimae (1960) have indicated this complex as being of an indefinable nature. Van Soest pointed out that the biological distinction between the nitrogen free extract and the crude fiber fractions was unrealistic, especially since the crude fiber residue, supposedly indigestible, may be more digestible than the supposedly highly digestible NFE fraction. Using either crude fiber, lignin, protein or methoxyl as indicators, Kivimae indicated that the accuracy of the estimation of nutritive value varied with species, stage of

growth and time of cutting. Ideally, then, a residue of indigestible compounds would be a good indicator of nutritive value. Fiber, containing cellulose and lignin only, with no nitrogenous compounds, is such a residue (Van Soest, 1963a, 1963b). Van Soest (1963a, 1963b, 1964) then, has proposed the use of acid and neutral detergents for the analysis of fibrous feeds. The acid detergent fiber (ADF) represents a residue consisting chiefly of lignin and polysaccharides. The ADF residue is used for an estimation of forage lignins i.e., acid detergent lignin (ADL). Significant correlations have been obtained between both ADF and ADL with in vivo dry matter digestibility. The fiber prepared using neutral detergent represents essentially the cell wall constituents (CWC) of forages. The CWC residue differs from the ADF residue chiefly in that CWC retains the hemicellulose fraction. CWC is also significantly correlated to in vivo dry matter digestibility.

The water soluble fraction of a forage represents the portion most readily available for fermentation (Marquardt and Asplund, 1964b). The ability of this fraction to support cellulose digestion would have a strong influence on the nutritive value of the crop. Consequently the solubility of dry matter in various solutions has been indicated as useful in predicting nutritive values (Dehority and Johnson, 1964; Donefer et al. 1963). The rapid availability of fermentative sources is not the only limiting factor, but as suggested by Donefer et al. rapid solubilization of forage constituents may produce many erosion sites facilitating the attachment of cellulolytic organisms to the fiber complex.

Finally, mention should be made of the dacron or nylon bag technique used by Archibald et al. (1961), Van Keuren and Heinemann (1962) and others for forage evaluation. The technique has been significantly correlated to in vivo evaluations and does have good repeatability. However, it offers no advantage over the laboratory in vitro techniques.

The use of short term in vitro fermentation techniques offers a valuable method for laboratory evaluation of forages. The lag phase of fermentation seems to be of importance. It may be that optimum levels of rapidly available carbohydrates which stimulate cellulose fermentation are present in forages of high nutritive value.

Seasonal Fluctuations in the Composition of Forages

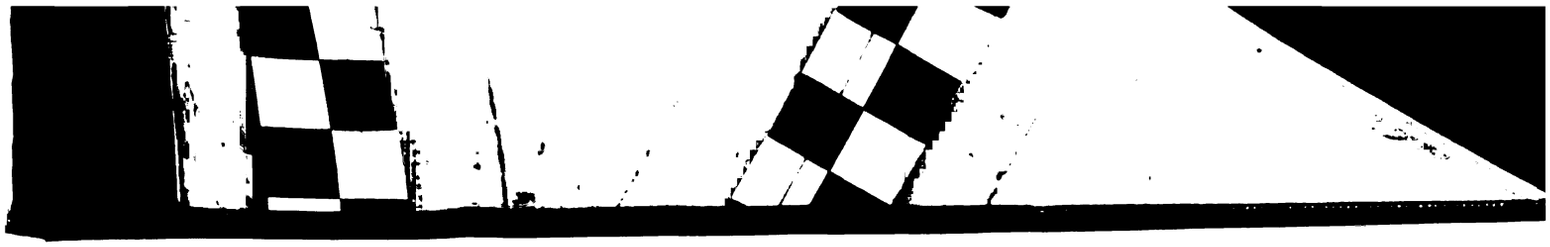
The dynamics of constitutive changes in forages have been well documented. Such changes are associated both positively and negatively with changes in forage nutritive value.

The main purpose of forages is to supply energy to the ruminant animal (Reid et al., 1959). Further, energy supply is the first limiting factor in ruminant nutrition (Crampton et al., 1960a). Generally, 50% of available energy from forages is in the form of complex plant constituents (Rice et al., 1962). These constituents are essentially cellulose and hemicellulose. Factors which affect these energy yields, and in particular the availability of such yields, must necessarily affect the nutritive value of the forage.

Brown (1943) implicated both environment and cultural practices as influential factors affecting kentucky bluegrass herbage. Considerable variations in crude fiber, protein, lignin and ash levels occurred. Variation occurred even in herbage cut semi-monthly at a 1-inch level. Brown indicated measured crude fiber levels were associated with rising summer temperatures, increased lignin levels with a decline in growth activity. These two factors caused a progressive drop in nutritive value.

Nutritive value declined with maturity (Baumgardt and Smith, 1962c). Nutritive value of regrowth following cutting was generally neither as high at early stages nor as low at later stages compared to the first growth. This was especially true for bromegrass, which remained in a vegetative condition.

Maturation of alfalfa is accompanied by increased lignin and holocellulose levels and decreased protein levels (Meyer et al., 1960), the critical point being 1/10th bloom. Following this stage, changes in feeding value were not as marked as preceding stages. Live weight gain of lambs, adjusted to a mean feed consumption, showed alfalfa to be superior at pre-bud compared to flowering stages. Dehydrated hay was superior to sun-cured hay. Curiously enough, the quality of the alfalfa was not greatly influenced by season, the overall comparison of alfalfa harvested at the same maturity stage in May and August having but small differences. Meyer et al. (1957) obtained similar results with oat hay. The milk stage seemed to be



the critical point since after this stage nutritive value decreased sharply.

With advancing maturity, structural constituents increase in quantity. Qualitative changes in the cell wall complex also occur. Phillips et al. (1954) indicated that fiber did not increase continuously up to the seed stages although lignin did. Consequently, with aging of tissues lignification increases and digestibility of the fiber decreases. Norman (1939) found the cellulose/lignin ratio decreased with seasonal development in ryegrass. Bennett (1940) showed pectins remained at a constant low level while hemicellulose and lignin increased in quantity throughout the season. Reder (1954) thought structural constituents in plants were affected more by maturity than by any other factor. Growth was characterized by increasing rates of lignin, cellulose and hemicellulose; senescence was characterized by a decreasing rate of lignification. These changes are readily measured using the parameters suggested by Van Soest (1965) i.e., ADF, ADL and CWC.

Such rather gross quantitative changes seem common over a wide taxonomic spectrum. The influence of environment is considerable, and year-to-year variation may be highly significant (Sullivan et al., 1956; Phillips et al., 1954; Smith, 1964). Recently, in a somewhat more sophisticated way, Elliott (1963) has investigated certain antimetabolic factors affecting nutritional responses.

Cultural Factors Affecting Forage Composition

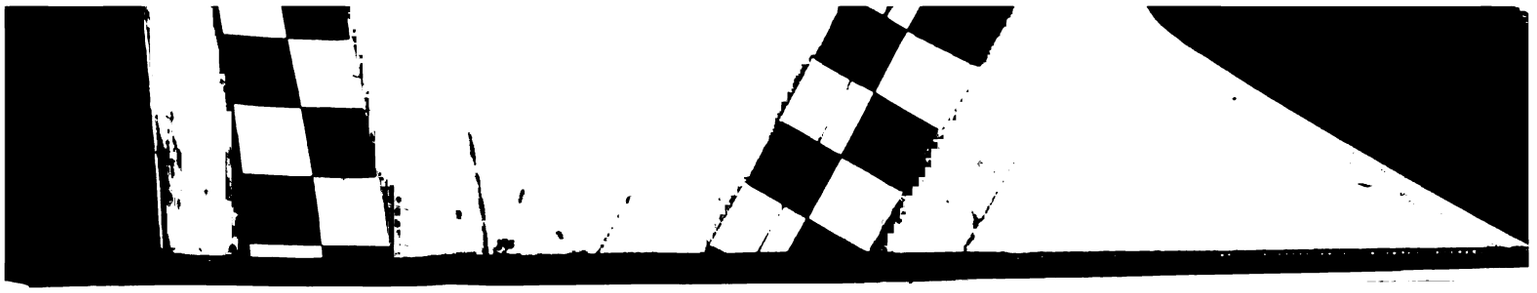
Baumgardt and Smith (1962) showed species differed in their production of estimated total digestible nutrients per acre (ETDN). Most ETDN were produced in the spring growth period of the first crop. This corresponded to the 1/10th bloom stage in alfalfa. The ETDN of the second crop, grown in the summer period, increased up to full bloom. Vernal alfalfa in Wisconsin, cut three times at the 1/10th bloom stage produced the greatest quantities of dry matter, crude protein and ETDN on an acre basis, when compared to a three- or two-cutting schedule involving harvesting more mature herbage. Delaying harvests decreased digestibility much less with second and subsequent crops than with the first crop. The number of days between the first and second cutting was the determining factor.

The stage of maturity influences forage palatability. Granstad (1964) indicated significant positive correlations between animal preference and percentage leaf tissue in sudangrass varieties. Negative correlations existed between animal preference and plant weight, plant height, forage yield per acre, percentage carbohydrates and crude fiber. Animals preferred tender, succulent, turgid plant tissues high in potassium. Similarly, Baker et al. (1960) indicated intake of green sudangrass forage to be negatively correlated with percentage dry matter in the forage. These data are consistent with those of Van Soest (1965) indicating fiber mass inhibited intake in those forages with a high cell wall content.

Norman (1939) and Sullivan et al. (1956) agree that structural constituents are highest in second cuttings. Such growth usually occurs in a period typified by long days. Cellulose and fiber decrease thereafter, while lignin decreases only in the fall. Second-cut materials contain a lower percentage of fructosans than first cuttings when the two are compared on a basis of equal protein content. Reder (1954) indicated crude fiber levels, measured in samples morphologically similar, were higher in samples obtained late compared to early in the season.

These observations agree with those of Reid et al. (1959) who showed that animals consumed more early first-cut than late-cut forage. The time of cutting may be the determining factor deciding the rate of intake of a first-cut herbage. Leaf content of first-cut forage is an excellent indicator of forage energy value. Regrowth leaf content, conversely, is a poor indicator.

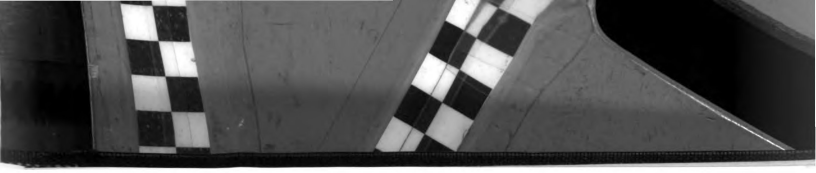
The work of Meyer et al. (1960) cited previously, is somewhat contrary to that of Reid et al. (1959). According to the latter workers, aftermath forage under humid conditions, regardless of stage of growth, has a lower digestible dry matter value than first growth harvested prior to June 1. Meyer et al. did not find this to be the case with alfalfa grown under less humid conditions. Ingalls et al. (1965) observed little difference in dry matter intake of first- and second-cut forages. Over a two-year period, the means of all cutting treatments indicated little difference in the digestion coefficients of alfalfa, birdsfoot trefoil, reed canarygrass and bromegrass.



However, both digestible dry matter intake and NVI, adjusted to body weight, followed a trend exemplified by dry matter intake, i.e., the legumes were superior to the grasses, bromegrass superior to reed canarygrass. First-cut forage had a higher dry matter digestibility than second-cut forage.

Fertilization has been shown to both increase and decrease structural constituents. According to Norman (1939) nitrogen effects on orchardgrass included a reduction in fructosan content and an increase in lignin. Consequently a narrowing of the cellulose/lignin ratio occurred. Washko and Marriott (1960) showed that as fertilizer rates increased from 50 to 400 lb N per acre, forage prcduction and protein content of the herbage increased; digestibility was unaffected up to the 300 lb rate. Hart and Burton (1965) using Gahi-1 pearl millet found that forage yield and crude protein increased with increased rates of nitrogenous fertilizer. However, neither nitrogen rate nor row spacing affected dry matter digestibility as measured by the nylon bag technique. Webster et al. (1965) showed that high rates of nitrogen fertilizer reduced holocellulose and hemicellulose contents in bermudagrass forage. In vitro digestibility estimations indicated nitrogen fertilizer, up to rates of 1,400 lb per acre, to have essentially no effect. Seasonal changes were quite marked, an inverse relationship existing between lignin content and in vitro digestibility.

Reid et al. (1959) indicated that neither high rates of nitrogen fertilizer nor irrigation affected the digestible dry matter value of

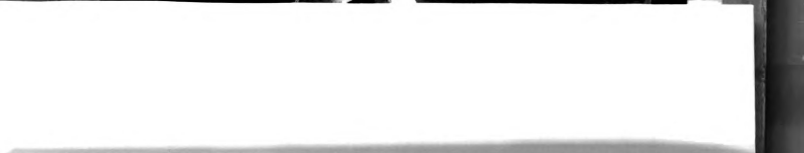


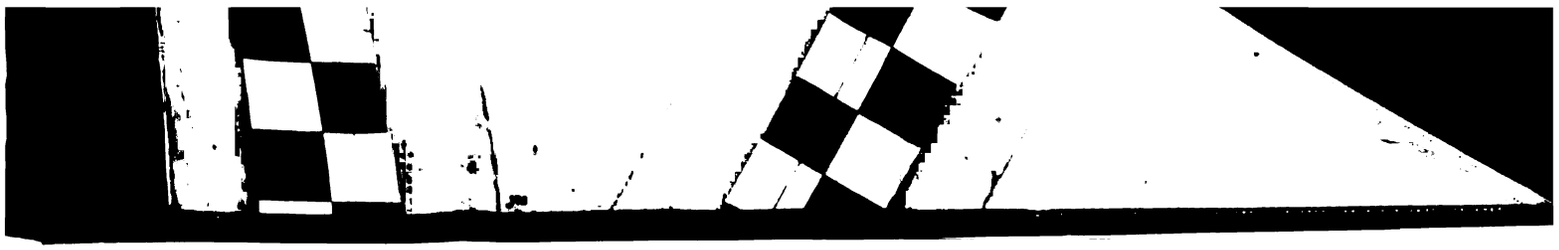
aftermath forages. The work of Chalupa et al. (1961) showed that increased applications of nitrogenous fertilizer to reed canarygrass resulted in increased crude protein, ether extract and gross energy (cal/g), while crude fiber and NFE fractions decreased. However, a reduction in the digestion coefficient of the crude fiber also occurred. The TDN and digestible energy (DE) values were increased. Values of TDN and DE for reed canarygrass fertilized with 200 lb N per acre were significantly higher than those of alfalfa. Ramage et al. (1958) however, estimated lower TDN values for reed canarygrass and orchardgrass fertilized with high levels of nitrogenous fertilizer.

Most of the research on fluctuations of nutritive values in forages has been concerned with estimates, essentially of proximate analysis fractions. These have already been indicated as unreliable. Such research must be regarded as indicative and not as absolute. The fibrous fraction has, however, constantly been sorted out as the most likely determinative factor of forage quality.

The Lignin Complex

The ecological importance of the lignin complex is underestimated. Supplying strength and rigidity to the plant cell, lignin has played a vital role in the evolution of the vascular plants. As a secondary growth substance lignin may also have considerable survival significance, contributing disease and insect resistance through its resistance to decay and microbial enzymatic action (Neish, 1960;





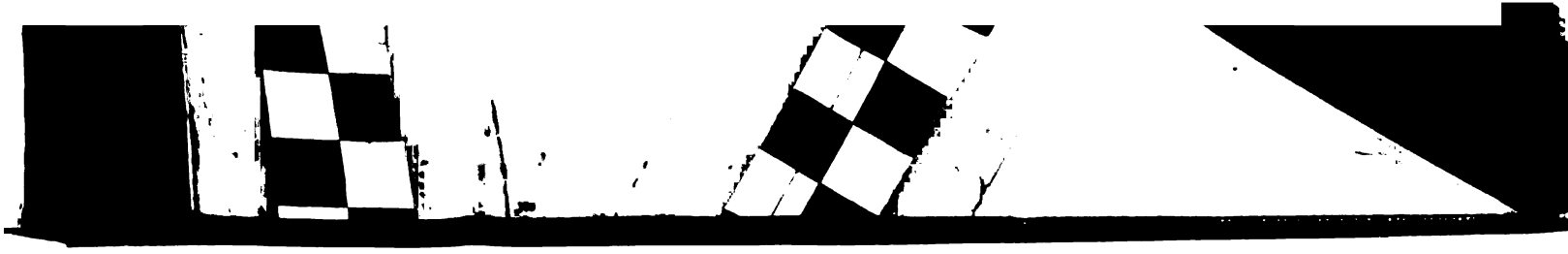
Gortner and Gortner, 1953).

Lignin is a tri-dimensional polymer whose basic structural unit is a phenyl-propane (C_6 - C_3). These monomeric units are joined together by ethereal linkages as well as carbon-to-carbon bonds. It is likely that covalent bonds exist between lignin and cellulose, although x-ray examinations are somewhat contradictory (Bolkner, 1963; Astbury et al., 1935; Davies et al., 1964; Thomas and Hewitt, 1935).

The differing phenylpropane nuclei comprising the lignin complex are apparently characteristic of both the phylogenetic history as well as the morphological development of vascular plants (Higuchi, 1957a, 1957b; Towers and Gibbs, 1953). As tissues mature, the syringyl/guaicyl ratio increases (Alston and Turner, 1953; Higuchi, 1957b). The methoxy content, related to this ratio, similarly increases. Phylogenetically, guaicyl lignins were the first to appear to be followed by the more complex angiosperm lignins containing both guaicyl and syringyl nuclei (Neish, 1960). Consequently, gymnosperms, monocotyledons and dicotyledons have characteristic lignin complexes (Schubert, 1953).

Further, even within a given taxon, considerable variation occurs, notwithstanding variation which results from morphological development (Alston, 1963; Wardrop, 1958; Stafford, 1962). Harney and Grant (1965) have shown that phenolic constituents in the Lotus genus can be used to elucidate the phylogenetic relationships between taxa.

Cellular differentiation, beginning in the meristem and resultant of a controlled production of unique enzyme systems, involves changes



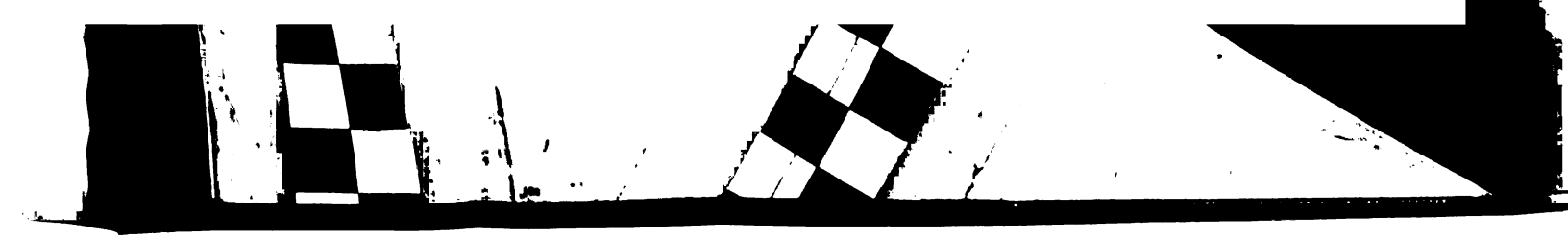
of size, shape, structure and physiology (Street, 1963). The ontogenic sequence in the development of a wood fiber involves cell divisions, cell enlargement, cell wall thickening and lignification (Kremers, 1958; Wardrop, 1958). The cell wall consists of an interwoven matrix of cellulose microfibrils set in an amorphous matrix of pectins, lignins and hemicelluloses. The relative proportions of such components vary with the type of cell and stage of differentiation. Binding may be due to physical forces such as hydrogen bonding and Van der Waals forces, or to chemical bonds (Davies et al., 1964). Stafford (1962) believes lignification to be the final phase in differentiation in any one area of the cell wall. Lignification follows elongation in the metaxylem, although lignification may occur in the secondary walls of the protoxylem even in elongating tissues. In Phleum, the lignin of the metaxylem is similar throughout the plant, but that of the sclerenchyma may vary. Stafford considers these differences may be due to differential biogenesis of mature lignin.

Wardrop (1957) comprehensively discusses the lignification process of both primary and secondary woods. Wardrop observed the complementary distribution of lignin with peroxidase.

A whole spectrum of papers is available regarding the biosynthesis of lignin. The papers of Freudenberg (1950), Siegel (1953, 1954, 1955, 1960), Higuchi (1957a, 1957b), Neish (1960), Pearl (1964), Stafford (1960a, 1960b, 1962, 1964) and Schubert (1965) deal with the subject extensively. Schubert divides the biochemical pathway of lignification into two phases. The first involves the

synthesis of primary building units such as p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. Coniferyl alcohol, rarely found in detectable quantities, is stored as its glucoside coniferin, and is the proposed key intermediate in lignification. Syringin, the glucoside of sinapyl alcohol, is regarded as a precursor of lignin in hardwoods. These intermediates arise from CO_2 via the shikimic acid pathway. The second phase involves the conversion of these intermediates to lignin-like polymers via a laccase or a peroxidase. This polymerization requires the presence of cellulose, or some other cell wall component, to act as an orientation center.

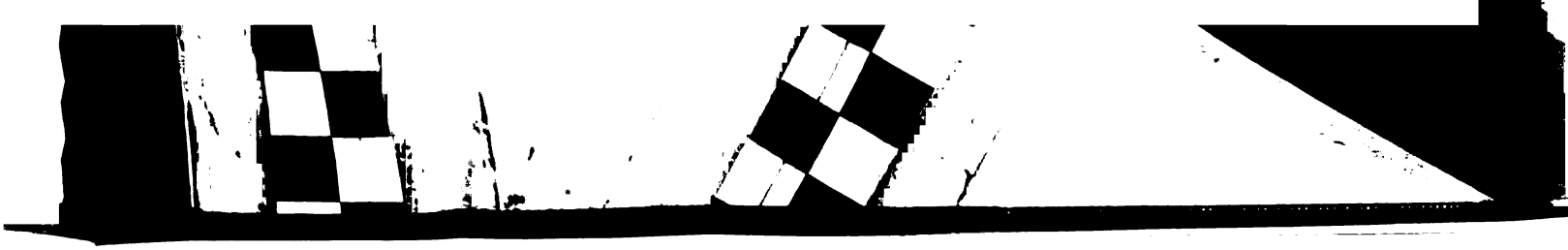
The role of IAA in peroxidase formation has been reviewed extensively by Jensen (1955), Siegel et al. (1960), Leopold (1955), Ray (1958), Galston (1955) and Morgan (1963, 1964) among others. Treatment with IAA induced the enzyme IAA oxidase of which peroxidase is a component (Andrea and Andrea, 1953). A concept of gradients of auxin, IAA oxidase and IAA oxidase inhibitor led Galston to suggest that at the point where auxin concentration is so low, growth ceases and cell maturation occurs. Jensen indicated only the vascular tissues were able to induce the formation of peroxidase. It may be that the formation of balanced auxin gradient, involving IAA, IAA oxidase and IAA oxidase inhibitors, with an inverse peroxidase gradient, is a prerequisite to lignification.



Lignin, Cellulose and Digestibility

Lignification is not a sudden process. The large decrease in digestibility following seed formation may be associated with but a small increase in lignin content. This change, however, must vitally affect energy availability. Stafford (1965) indicated that with approaching maturity, proteins were degraded. This released amino acids of which tyrosine and phenylalanine were sources of lignin precursors. This may account for the heavy lignification occurring in grasses just prior to flowering. Norman (1939) showed that flower formation was also accompanied by a lowered fructosan content and a cellulose increase.

Fine grinding of forages was shown by a number of investigators to increase in vitro cellulose digestibility (Baumgardt and Hi Kon Ho, 1964; Dehority, 1961) and pectin and hemicellulose fermentation (Dehority et al., 1962), by reducing the lag phase. Dehority suggested the available sites for the attachment of cellulolytic bacteria to the substrate was increased. Delignification increases the digestibility of cellulose both in vitro and in vivo. The importance of the type of cellulo-lignin complex is indicated by Halliwell and Bryant (1963) who maintain that the capacity of an organism to attack degraded cellulose is no criterion of its ability to attack undegraded types of cellulose. These observations have led to the commonly held theory that lignin forms a barrier surrounding the cellulose, thus preventing microbial enzymatic action. Physical



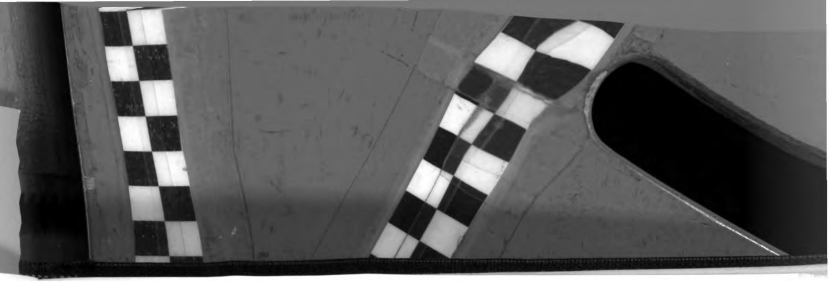
and chemical degradation serve to remove encrusted lignin. It must be remembered that the breakdown, and eventual benefit to the ruminant animal, of complex fibrous materials, is facilitated by the microbial complex contained in the rumen.

The quantity of lignin may be of relatively small importance. Alfalfa has a higher lignin content than grasses of a similar digestibility. Using anaerobic bacteria, Ghose and King (1963) showed alfalfa stems to be five times as digestible as jute when both species had the same lignin content. Acid treatment enhanced and alkali treatment decreased the resistance to degradation of both jute and cotton fiber. Ghose and King suggested that since lignin and hemicellulose are known to have ester linkages, protection of the fiber by stereochemical inhibition of enzymatic action may also occur.

Selection for Nutritional Characters

Almost no work has been done on variation between genotypes within a single variety. Digestibility estimates and intake studies have generally been carried out at the species level. Miles et al. (1963) reported on a wide varietal and seasonal variation occurring in factors closely associated with quality in herbage. They suggested that varieties, since they have not been screened for qualitative characters, may well be carrying a considerable range of genetic variability.

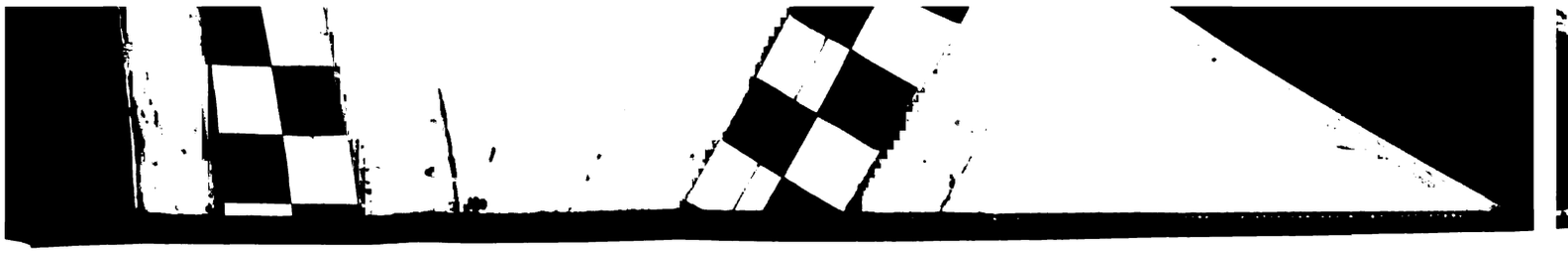
Elliott (1963) has indicated the need for exploration of genetic



controls for factors having both growth prompting and inhibitional properties. At the clonal level, this could lead to varieties of increased nutritional usefulness.

Beevers (cited by Cooper, 1960) revealed considerable differences between genotypes of ryegrass in regard to carbohydrate and nitrogen fractions and also in the content of individual amino acids and organic acids. Cooper indicated this affords a suitable basis for selection for increased nutritive value.

Finally, Leng (1962) indicated that long-term selection for high and low levels of protein and oil in corn provided high levels of response to occur without great decreases in genetic variability. Consequently, while desired responses were obtained, undesirable genetic fixation did not occur.



MATERIALS AND METHODS

The evaluation of changes in the nutritive value of specified species, induced through cultural manipulation, was made using a number of laboratory techniques. Substrate sources were obtained from one greenhouse and two field experiments.

A. Environmental Variation Induced Through Cultural Techniques

1. The Influence of Cutting Frequency on the Nutritive Value of Specified Forages

Stands of Vernal alfalfa (*Medicago sativa*), Viking birdsfoot trefoil (*Lotus corniculatus*), Lincoln bromegrass (*Bromus inermis*), Pennlate orchardgrass (*Dactylis glomerata*), and Common reed canarygrass (*Phalaris arundinaceae*), were established on a sandy-loam soil on the Michigan State University experimental farm, East Lansing, in the fall of 1962. In the fall of 1963, the legumes were liberally supplied with potassium and phosphate.

In the spring of 1964, the grasses were given the equivalent of 100 lb N per acre in the form of ammonium nitrate. Plot sizes were 24 x 14 feet. Each plot was subdivided into ten subplots.

Each species was cut on a one-, two-, three- or four-cutting system between May 1 and September 1 of 1964. Consequently, each species was represented by four plots in the experiment. The

one-cutting and two-cutting plots for all species were within one block; the three-cutting and four-cutting plots for all species were in a second block. The dates for the harvests were as follows:

4 cuttings: May 19, June 23, July 29, September 1

3 cuttings: June 2, July 15, September 1

2 cuttings: June 23, September 1

1 cutting: September 1

Samples representing one cutting treatment and one species were taken at two-week intervals beginning May 5. These samples were obtained from a subplot within a main plot. Consequently, no area within a main plot was sampled more than once. These samples were dried, ground and evaluated for nutritive value. Second samples obtained simultaneously were used for moisture determination. Where possible, cutting and sampling dates were coincidental.

2. The Effect of Varying Rates of Nitrogen Fertilizer upon the Nutritive Value of Two Types of Reed Canarygrass (Phalaris arundinacea)

Established stands of Common reed canarygrass and Siberian reed canarygrass were used. The experiment was conducted over two years, 1965 and 1966, on the Michigan State University experimental farm, East Lansing.

The first cutting was windrowed and baled on May 29, barn dried, and subsequently used for a sheep feeding trial.

Samples of both types and all nitrogen levels were taken at regular intervals between May 16 and November 1. These samples were obtained from assigned subplot areas. The samples were dried, ground and evaluated for nutritive value and chlorophyll content.

In 1966, each of the nitrogen plots from the previous year was subdivided into two equal halves. One half received two-thirds of the nitrogen rate of the previous year, except that the treatment having no nitrogen in 1965 received 300 lb N per acre in 1966. This enabled a carry-over effect to be evaluated, both for continued high and low fertility. The ammonium nitrate was applied in one application on May 6. Actual acre rates of nitrogen application were 0, 100, and 300 lb to the respective plots.

One harvest was taken in the growing season on July 5. Four samples were taken for nutritive evaluation. These were obtained on June 2, June 25, August 18 and October 16.

3. The Effect of Moisture Levels upon Nutritive Value

Sudangrass clones, genotypically identical, were grown in 10-inch pots in the greenhouse. The soil was a mixture of three parts sand to one part peat. Two moisture levels, applied to three clones per level, were subjectively maintained. A high moisture level was established to supply all the water the plants required. The low moisture plants were supplied water only when severe wilting was evident. The clones were harvested when all were in a morphologically

identical stage, i.e., late flowering.

Evaluatory techniques

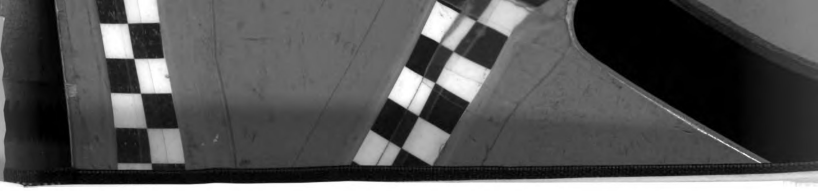
Nutritive evaluation of samples obtained from the sources indicated included the following techniques:

(a) In vitro fermentations

Samples, harvested by hand, were dried in a forced-draft oven at approximately 60C for 48 hours. Ground through a Wiley mill (40 mesh), the samples were stored in sealed plastic bags in a cold chamber until required. The actual fermentation technique, measuring dry matter disappearance (DMD), expressed as a percentage, was based upon that of Bowden and Church (1962a) and Baumgardt et al. (1962a). Modifications incorporated by Ingalls (1963) were used.

Fermentation periods of 6 and 36 hours, shown to be significantly correlated to in vivo NVI and digestibility respectively, were used. All samples were evaluated at least in duplicate. Day-to-day variation was assessed by incorporating a standard forage in each fermentation. Adjustments for this variation were made using the technique of Spragg (1920).

The inoculum, strained through three layers of cheesecloth, was obtained from a fistulated Holstein cow maintained on an alfalfa hay diet. Collection of the inoculum was made two hours after the morning feeding. One hour previous to collection, feed and water were removed from the cow. Ingesta were removed from the ventral part of the rumen.



The inoculum, placed in one-liter flasks, was immersed in a water bath at 39C for 30 minutes. The fluid separated into two distinct fractions; the lower layer, siphoned off and bubbled with carbon dioxide for two minutes, constituted the active inoculum.

A 1 g sample was weighed into a 125 ml erlenmeyer flask. Approximately one hour before the rumen inoculum was to be added, the flask was charged with 20 ml of a phosphate-urea-carbonate buffer. The flasks, capped with a stopper and bunsen valve, were placed in a water bath at 39C. The buffer was prepared by dissolving in 2,000 ml water, 8.2 g monobasic potassium phosphate, 17.4 g dibasic sodium phosphate, 4.0 g urea and 7.4 g monohydrate sodium carbonate.

A 24 ml inoculum sample was added to the flask to initiate fermentation. Each flask was immediately flushed with carbon dioxide and the flask resealed. At the end of the fermentation period, a few drops of a 20% thymol solution were added to stop microbial activity. The flasks were stored in the refrigerator until required.

To determine the dry matter disappearance of each sample, the fermentation mixture was filtered through a preweighed filtering crucible. High form 50 ml crucibles fitted with a frittered glass disc of coarse porosity were found to be most suitable. Filtration was further facilitated by covering the disc with a quarter-inch layer of Solka floc. Sample residues were washed with distilled water. The crucibles were dried in a forced draft oven at 80C for 36 hours. At the end of this period, the crucibles were reweighed, and the loss of dry matter obtained by difference. Duplicate samples

of inoculum were similarly filtered and endogenous dry matter determined. Adjustments for endogenous material were made in the calculation of dry matter disappearance.

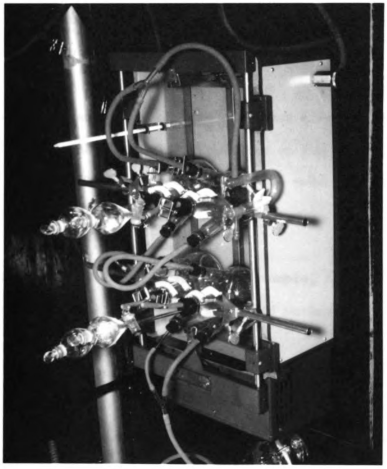
The dry matter content of respective samples was determined separately. A 1 g sample, placed in an open metal boat, was dried in an electric oven at 100C for five hours. Following cooling in a dessicator, the samples were reweighed and the dry matter content determined. All DMD values were corrected and expressed as the percentage of dry matter lost in the fermentation period.

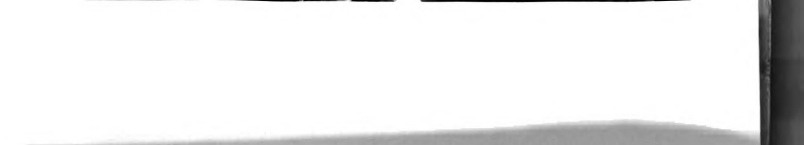
(b) Volatile Fatty Acid (VFA) Analysis

The production of acetic, propionic and butyric acids in the in vitro fermentation was assessed using gas chromatography. Samples for VFA analysis were obtained from the fermentation mixtures either at the end of or at intervals throughout the fermentation period. In the former case, the regular in vitro technique was used; in the latter case, the system illustrated in Figure 1 was used. Four double-armed 500 ml fermentation flasks were mounted in series in a water bath on the arms of a shaker attachment. Carbon dioxide was blown through continuously. Ports for the admittance of buffer solution and for obtaining samples of the fermentation mixture were incorporated. Measurement of the pH changes could be made by inserting pH electrodes through the side arms of the flasks.

Samples for VFA analysis were diluted to a known volume and acidified to a pH of approminately 3.0. A 2.0 μl sample was injected into an aerograph model A 600-D, "Hi Fi" gas chromatograph

Figure 1. Artificial rumen apparatus used in studying the production of volatile fatty acids from the fermentation of alfalfa clones.





with a hydrogen flame ionization detector coupled to a Sargent SRL recorder. The injection port temperature was 135C. Nitrogen was used as the carrier gas. VFA peaks could be compared to those produced by a standard solution of barium acetate, sodium propionate and sodium butyrate. Replicates were run until uniform peak combinations were obtained. VFA concentrations were expressed in micromoles per ml of fermentation mixture.

(c) Analysis of Fibrous Fractions

Samples were analyzed for acid detergent fiber (ADF) and acid detergent lignin (ADL) using the technique of Van Soest (1963). Analysis of cell wall constituents (CWC) was made using Van Soest's neutral detergent method (1964).

(d) Chlorophyll determinations

Using a 0.25 - 0.50 g dried, ground sample, total chlorophyll was determined by the A.O.A.C. (1955) specified method.

B. Variability Within Genotypes

An estimate of environmental variability and environmental phenotypic interaction was made using sudangrass (Sorghum sudanense, Stapf). Three clones were developed from seed in the greenhouse in June 1964. The clones were arbitrarily named genotypes 6,5 and 3. Using tiller initials, six propagules of each were established. Approximately six months' were allowed following vegetative propagation to allow the propagules to become fully established. The first harvest

for evaluation was taken May 8, 1965. The propagules were in the full-head stage; anthesis had begun. Each propagule was evaluated for DMD, yield and stem/leaf ratio. Following harvest, the propagules were given the equivalent of 200 lb N per acre, in the form of ammonium nitrate, in split applications. A second harvest, evaluated as before, was obtained on June 28. In this instance, the propagules were morphologically immature, being in the boot or early-head stage.

The regrowth of genotype 6 was allowed to continue to the flowering condition in isolation. Pollination, effectively self-pollination, took place. The seed was collected, thus constituting an inbred S_1 generation.

The S_1 seed had uniform black seed coats and 99% germination. The S_1 seed was planted on May 12, 1965; twenty plants were allowed to flower in isolation and to set seed. This constituted the S_2 generation. The remaining S_1 plants were allowed to establish themselves over a 6-month period through periodic cutting and nitrogen fertilization. A harvest for evaluation was obtained April 18, 1966.

The S_2 seed, which showed considerable segregation for seed color and which had poor germination, was planted and established in December 1965. Following establishment these clones were harvested for evaluation in October 1966.

A small population of Piper sudangrass was established. This served both as a check for the S_1 and S_2 generations and also for the genotype 6 population.

C. The Nutritional Usefulness of Species and Varieties Within the Medicago genus

Nutritional evaluation was made upon a number of clones growing in a spaced-plant alfalfa nursery. Initially, seeds were sown in the greenhouse in March, 1964. Seedlings were transplanted into the field (the experimental farm, East Lansing) on May 19-20, 1964. The plants surviving the severe winter of 1964-1965 constituted the population from which samples were obtained in 1965. Species included M. sativa and M. falcata, representing genetic material incorporated into several commercial varieties. Two small populations of M. glutinosa and M. coerulea were also present.

The individual plants were harvested by hand between June 14 and 18, then bagged, dried, ground through a Wiley mill and stored. Each individual population was harvested within a matter of hours and immediately placed in the drier. Morphologically, the plants were in early bloom. Yield per plant was determined. Plants showing extreme disease symptoms and stunted growth were discarded.

A primary screening involved an estimation of each plant's nutritive value using the 6-hour in vitro fermentation technique. On the basis of these results, approximately 30 clones from each of the Vernal, DuPuits and Wisconsin 460 "varieties" were selected. These clones were analyzed for in vitro DMD using a 36-hour fermentation period. Analyses were also made for ADF, ADL and CWC(CSC). Visual estimation of these results led to the selection of six clones for

further study. A pair of clones was selected from each of the three varieties, each clone representing an extreme of apparent nutritive usefulness.

The ability of these clones to stimulate VFA production was examined next. Time production estimates and estimates after 36 hours of fermentation were made. The same six clones were also evaluated using the Tilley-Terry (1963) technique -- the in vitro procedure having wide acceptance in Europe and in the United States.

Attempts to elucidate mechanisms causing differences on a plant-to-plant basis were made. Three possible causes have been suggested, viz., (a) the availability of carbohydrates, (b) the presence of antimetabolic factors affecting cellulolytic micro-organisms, and (c) masking of cellulose by the lignin complex, thus preventing the microbial degradation.

(a) Estimation of carbohydrates

Preliminary water extractions of individual clones were made. Ten g of ground forage were packed into a glass column. Approximately 1,000 ml of cold water at 34F were dripped through the forage over a 48-hour period. At the end of this period, the residue was filtered, the filtrate combined with the extract, and the residue dried in an electric oven at 100C overnight. The effect of the water extraction on DMD evaluation was made. Reconstitution of extract and residue and its effect upon evaluation was also attempted. Glass wool placed over the fritted glass disc at the base of the column prevented blocking during the extraction process. Filtration through four

layers of cheesecloth facilitated separation at the end.

Water extractions of individual clones were made in an attempt to estimate quantitative and qualitative carbohydrates present. Extracts were made in two ways. The first technique involved extracting 2 g of forage in 25 ml water in the refrigerator for 16 hours. The mixture was filtered, first through cheesecloth, then through Whatman No. 1 filter paper. The filtrate was evaporated over a steam bath when a gummy mass precipitated out. The filtrate was refiltered through Whatman No. 1 filter paper and the filtrate made up to 10 ml with distilled water. This filtrate was used for paper chromatographic and total anthrone carbohydrate estimation. In the second technique, 2 g of forage were extracted in 20 ml water in a water bath at 39C for six hours. The mixture was separated as before. The filtrate was made up to 100 ml with distilled water. To facilitate better separation of the sugars, this filtrate was passed through weakly basic, polyamine anion exchange resin in a 1 cm x 10 cm column. Amberlite, 1A-4B 20-50 mesh with a total exchange capacity of 2.5 m eq/ml/minute by volume was used. The filtrate was then evaporated in the flash evaporator to a volume of 10 ml. This extract was also used for paper chromatographic and anthrone carbohydrate analysis.

Descending paper chromatography using Whatman No. 1 chromatographic paper and a solvent system composed of n-butanol, acetic acid and water in a 4:1:5 v/v ratio was used. Large quantities of extract were spotted, i.e., 100-200 λ. The chromatograms were

allowed to develop in the chromatocabs for periods of 12 to 64 hours. The latter period gave the better separation but no Rf values could be obtained. An ammoniacal silver nitrate detectant was used, and the paper was developed in the oven at 105C for 5 to 10 minutes.

Anthrone estimations were made according to the method outlined by Yemm and Willis (1954). A reaction period of 10 minutes at 100C was adopted. Optical density readings were taken at 620 mμ and 540 mμ using a Bausch and Lomb Spectronic 20.

(b) Estimation of forage antibiosis

The work of Elliott (1963a) and Schillinger (1965) would indicate that certain water soluble antimetabolic factors present in alfalfa effect a wide spectrum of organismic reactions. This might indicate these factors to react upon basic reaction pathways.

Water extracts of individual clones were made. 2 g of the forage were placed in an ice bath with 40 ml distilled water for 16 hours. The mixture was filtered, the filtrate made up to 100 ml with water. The residue was re-extracted with 20 ml distilled water at 39C for 24 hours. The mixture was filtered and the filtrate made up to 100 ml. Both extracts were used for DMD determinations. The cold water extract was designated extract 1, the hot water extract designated extract 2.

The effects of these extracts were tested on the germination of corn and bean seeds. The corn seeds were soaked for one hour at 39C in the extracts, placed upon blotting paper in petri dishes, soaked in the appropriate extract, and germinated for three days in an incubator. The bean seeds were treated similarly, except they were not presoaked, and

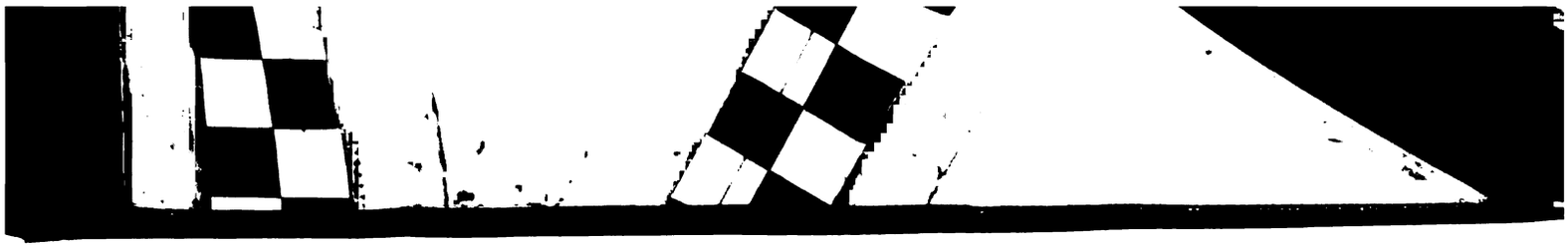
incubated for four days, rather than three days.

Following germination, the vegetative parts produced were cut from the seeds, dried in an electric oven at 100C for 24 hours and weighed. Seeds soaked and grown in water were used as a control. Twelve seeds were germinated per petri dish.

(c) Lignin analysis

A lignin analysis was made on a limited number of alfalfa clones. The lignin source was the residue remaining from the ADF determination. Extraction of the lignin was made in a manner similar to that of Stafford (1960a). A 0.0500 g sample was initially extracted for 16 hours in 4 ml of 0.5 NaOH, at 70C. The supernatant was pipetted off. The residue was washed twice with 0.5N NaOH, each time the solution being centrifuged down for five minutes at 5,000 x G to precipitate the residual fiber. The wash in each case was added to the original supernatant. The residue remaining from this initial 16-hour extraction was re-extracted with 5.0 ml of 0.5N NaOH for another 50 hours. At the end of this period, the supernatant was removed as before, the residue washed as above, and the supernatant and washes combined. A third extraction of 22 hours, using the residue from the second extraction, using 5.0 ml of 0.5N NaOH was also made. Again, the supernatant was removed, the residue washed and the supernatant and washes combined.

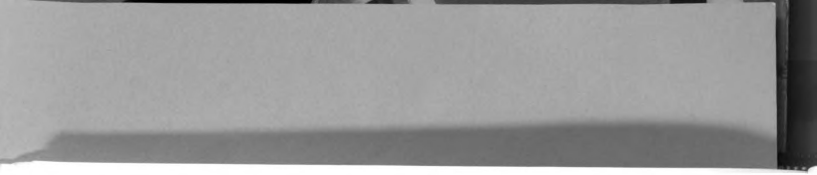
The treatment of supernatant and washes was similar following each extraction. The first extract was diluted with distilled water up to 50 ml; the second and third extracts diluted to 15 ml. A 10 ml

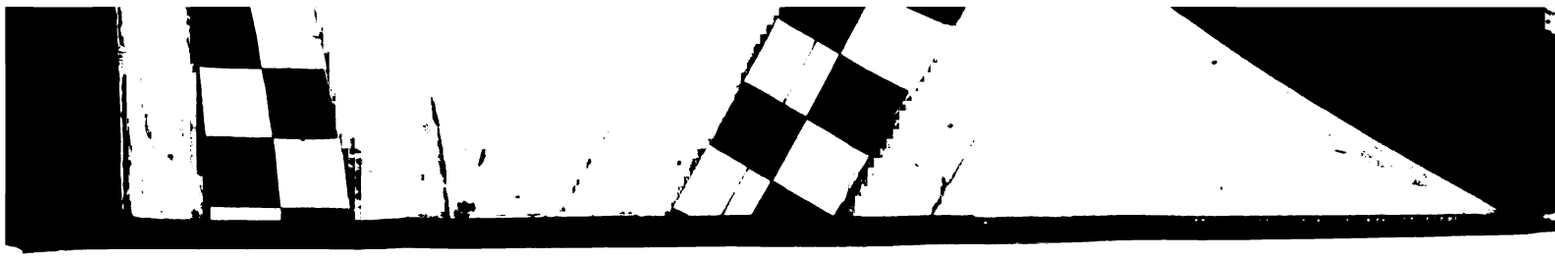


aliquot of the first extract and a 5 ml aliquot of the second and third extracts were then taken. Each aliquot was adjusted to a pH of 8.5 - 9.0. Each aliquot from each extract was halved, one half neutralized to a pH 7.1 using 6.0 ml of a pH 7.1 phosphate buffer, the other half diluted to pH 12.0 using 6.0 ml .05 NaOH. For each extraction, dilutions were identical, the dilution of the second and third extracts also being identical.

Ultraviolet absorption spectra were determined on both neutral and base aliquots. The phosphate buffer was used in the standard cell. A Beckman DU single-beam spectrophotometer with a DU power supply and a Dual Source Lamphouse was employed. Matched 1-cm silica cells were used for all measurements. Optical density readings were taken at intervals of 10 mμ from 230 to 440 mμ. By subtracting the neutral values from the alkaline values, thereby eliminating the nonionizable chromophores, the difference spectra were obtained. The ultraviolet spectra were obtained within one hour following extraction.

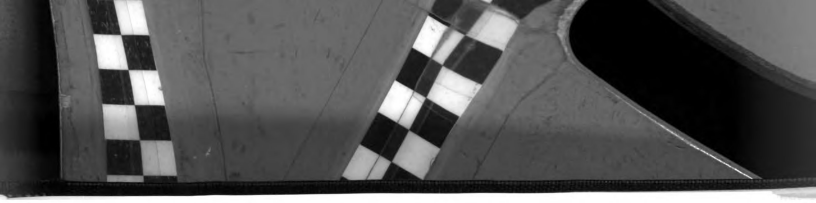
Estimation of the weight loss of lignin, i.e., its extractability, from the fiber lignin complex was also made. A sample of 0.0500 g of ADF residue was incubated in 4.0 ml of 0.5 NaOH for a period of 16 hours at 70C as before. At the end of this period, the mixture was filtered through frittered glass crucibles, dried at 100C overnight and weighed. The weight of lignin lost via the extraction was obtained by difference. Using the method outlined for the qualitative estimation of the lignins, lignin extractability was also





estimated for 66 and 88 hours. Estimations for each extraction period and each clone were made in triplicate.

A further attempt at qualitative analysis of the lignins was made using descending paper chromatography. The lignins, from first, second and third extractions, were spotted in quantities of 20-60 λ on Whatman No. 1 chromatography paper. The chromatograms were developed in one of three solvents: (a) a n-butanol:acetic acid: water in the proportions 4:1:5 v/v; (b) a n-butanol:acetic acid: water in the proportions 8:2:2 v/v; or (c) ethyl methyl ketone:2N ammonium hydroxide in the proportions 2:1 v/v (Block, Durran and Zweig, 1958). The chromatograms were developed in the chromatocabs for periods of 12 to 48 hours. The shorter time periods were used for obtaining Rf values. Two detectants were used. The most useful was ammoniacal silver nitrate, made up of equal volumes of 0.1N $AgNO_3$ and 5N NH_4OH. A second detectant was made up of two grams of sucrose mixed with 10 ml concentrated HCL and 90 ml of absolute alcohol. The sucrose detectant was developed by heating for one minute at 85-95C. The ammoniacal silver nitrate was developed by heating in the oven for 5-10 minutes at 105C.



RESULTS

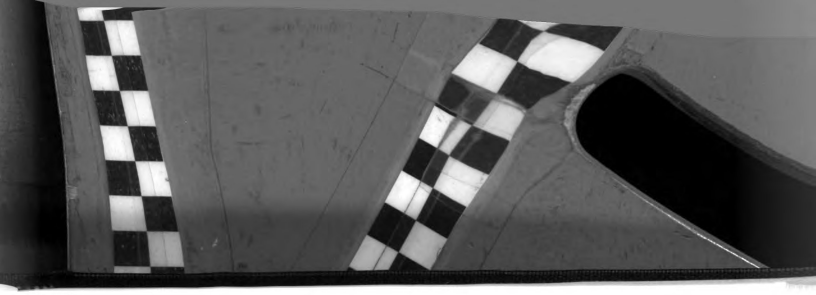
All results are expressed in terms of DMD. Highly significant correlations have been derived for NVI and in vivo digestibility with DMD at 6 and 36 hours, respectively. DMD values reported are those obtained from a 6-hour fermentation period.

The Influence of Cutting Frequency on the Nutritive Value of Specified Forages

The changes in DMD and moisture content of alfalfa, birdsfoot trefoil, bromegrass, orchardgrass and reed canarygrass occurring throughout the season, when subjected to either a four-, three-, two- or one-cutting system, are illustrated in Figures 2-6, respectively.

Any cutting treatment usually caused an increase in DMD to occur. The extent of such increases depended upon the particular species and the frequency of any previous cuttings in that season. Analysis of variance indicated that main effect differences, i.e., species, cuttings and season and all two-way interactions between these were significant at the 1% level. Consequently, individual species must be considered independently.

Figure 2. Changes in DMD and moisture content of alfalfa occurring throughout the growing season as influenced by cutting frequency. The dates for the harvests were as follows:
4 cuttings: May 19, June 23, July 29, September 1
3 cuttings: June 2, July 15, September 1
2 cuttings: June 23, September 1
1 cutting: September 1.



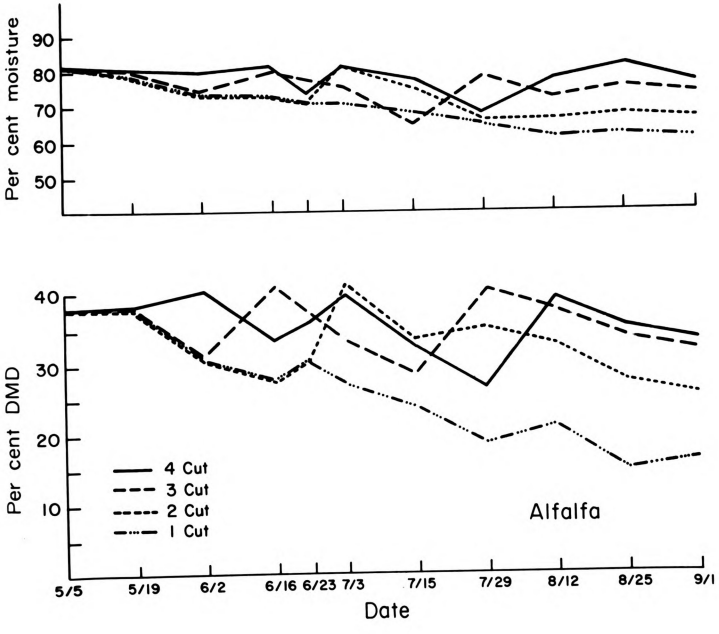
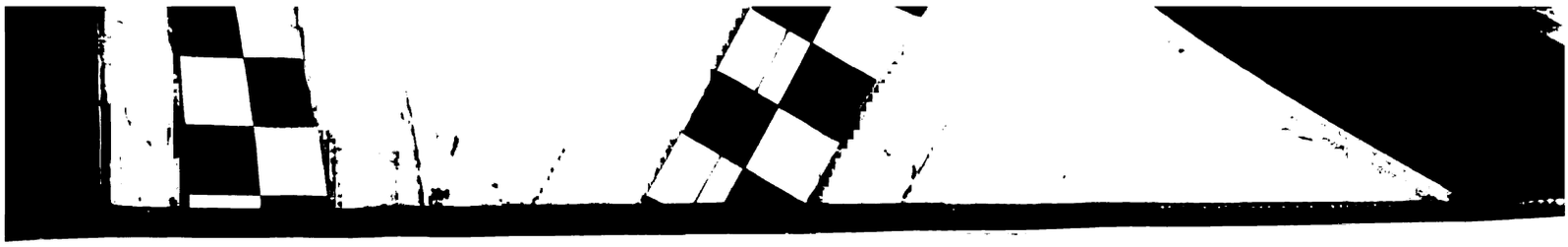
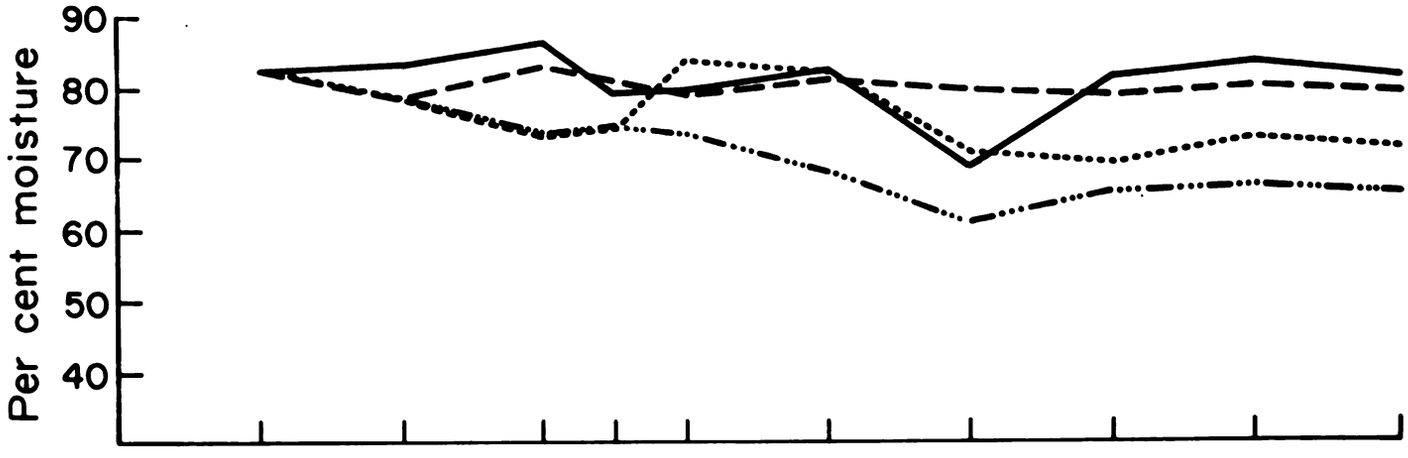

Per cent moisture
90
80
70
60
50
Per cent DMD
40
30
20
10
4 Cut
3 Cut
2 Cut
1 Cut
Alfalfa
5/5
5/19
6/2
6/16
6/23
7/3
7/15
7/29
8/12
8/25
9/1
Date

Figure 3. Changes in DMD and moisture content of birdsfoot trefoil occurring throughout the growing season as influenced by cutting frequency. The dates for the harvests were as follows:
4 cuttings: May 19, June 23, July 29, September 1
3 cuttings: June 2, July 15, September 1
2 cuttings: June 23, September 1
1 cutting: September 1.




90
80
70
60
50
40
Per cent moisture

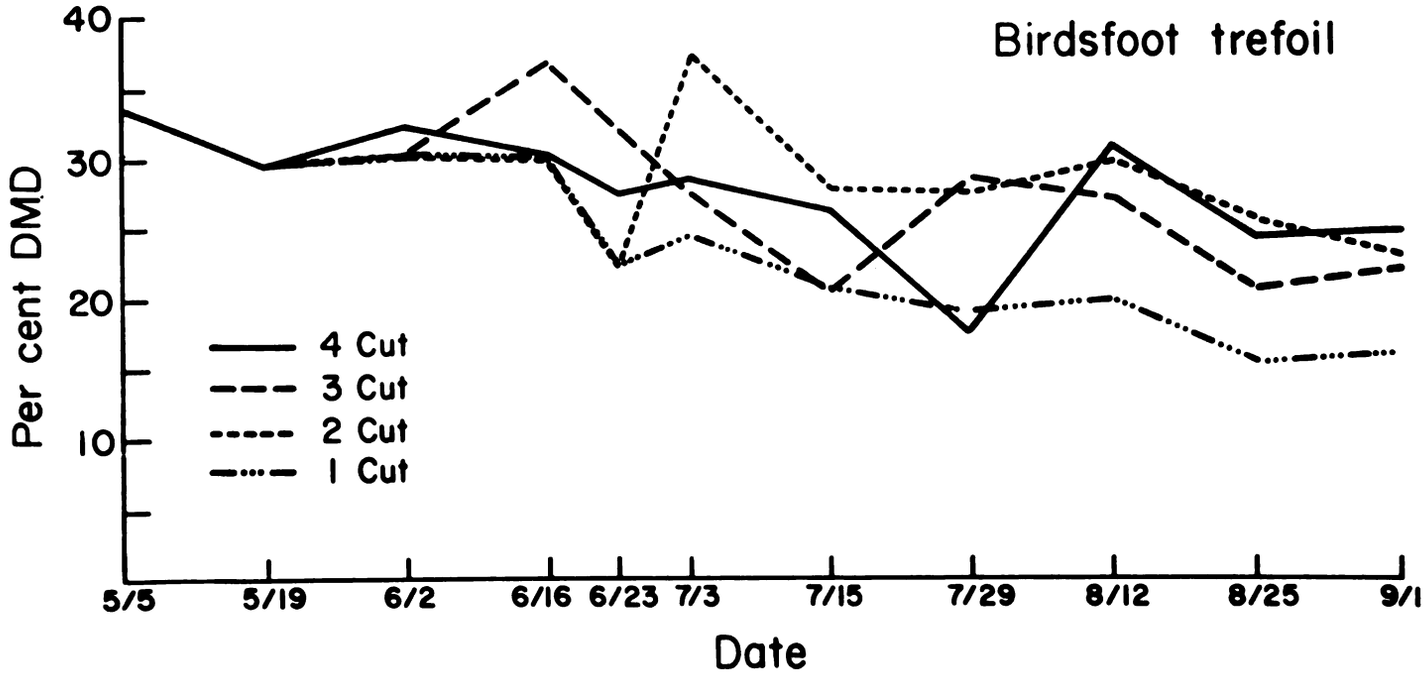
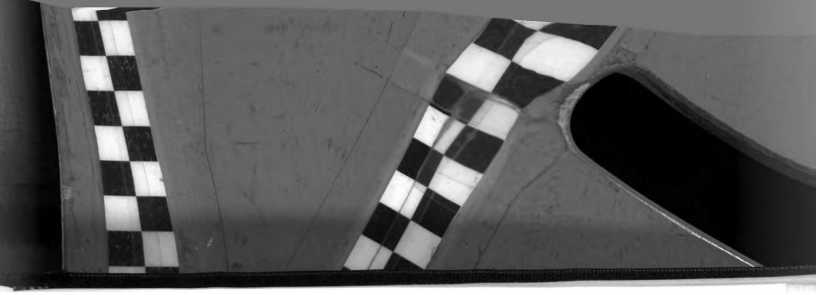

Birdsfoot trefoil
40
30
20
10
Per cent DMD
4 Cut
3 Cut
2 Cut
1 Cut
5/5
5/19
6/2
6/16
6/23
7/3
7/15
7/29
8/12
8/25
9/1
Date

Figure 4. Changes in DMD and moisture content of bromegrass occurring throughout the growing season as influenced by cutting frequency. The dates for the harvests were as follows:

4 cuttings: May 19, June 23, July 29, September 1
3 cuttings: June 2, July 15, September 1
2 cuttings: June 23, September 1
1 cutting: September 1.



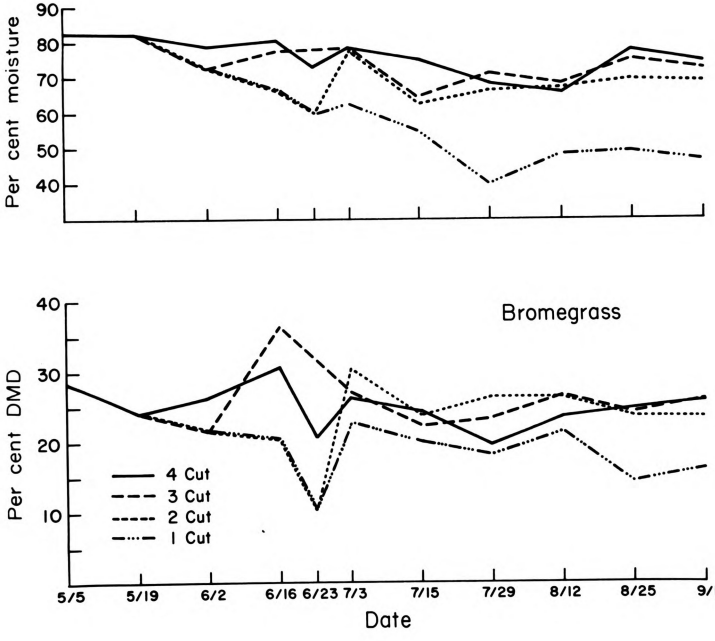
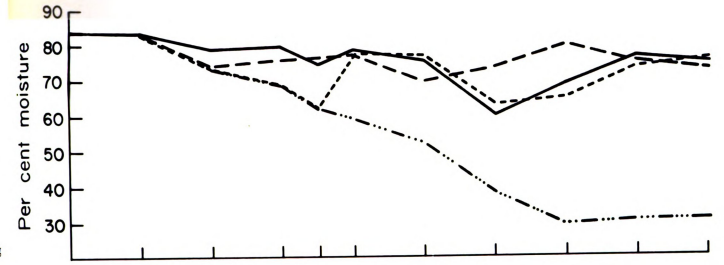

Per cent moisture
90
80
70
60
50
40
Per cent DMD
40
30
20
10
Bromegrass
4 Cut
3 Cut
2 Cut
1 Cut
5/5
5/19
6/2
6/16
6/23
7/3
7/15
7/29
8/12
8/25
9/1
Date

Figure 5. Changes in DMD and moisture content of orchardgrass occurring throughout the growing season as influenced by cutting frequency. The dates for the harvests were as follows:

4 cuttings: May 19, June 23, July 29, September 1
3 cuttings: June 2, July 15, September 1
2 cuttings: June 23, September 1
1 cutting: September 1.


Per cent moisture
90
80
70
60
50
40
30

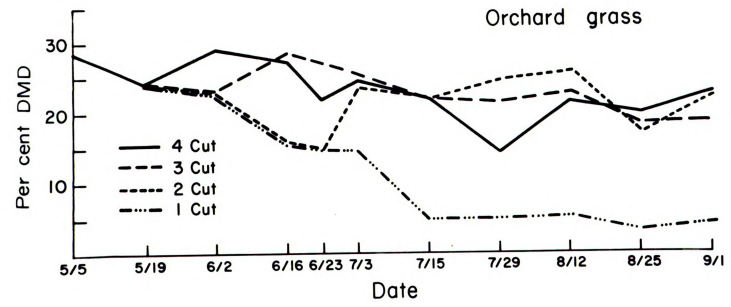
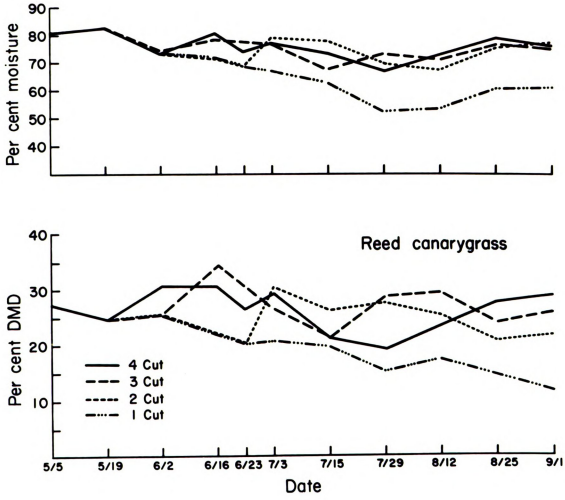

Orchard grass
Per cent DMD
30
20
10
4 Cut
3 Cut
2 Cut
1 Cut
5/5
5/19
6/2
6/16
6/23
7/3
7/15
7/29
8/12
8/25
9/1
Date

Figure 6. Changes in DMD and moisture content of reed canarygrass occurring throughout the growing season as influenced by cutting frequency. The dates for the harvests were as follows:
4 cuttings: May 19, June 23, July 29, September 1
3 cuttings: June 2, July 15, September 1
2 cuttings: June 23, September 1
1 cutting: September 1.


Per cent moisture
90
80
70
60
50
40
Reed canarygrass
Per cent DMD
40
30
20
10
4 Cut
3 Cut
2 Cut
1 Cut
5/5
5/19
6/2
6/16
6/23
7/3
7/15
7/29
8/12
8/25
9/1
Date

Species cut two or more times in the growing season had DMD values on September 1 approximately equivalent to those obtained at the beginning of the season. Increases in DMD following a cutting attained or exceeded those values obtained from initial spring growth. Such increments occurred any time in the growing season. However, the extent of these increases in DMD, and also the period of time for which they were maintained, were dependent upon the species.

Forage which remained uncut until September 1 lost nutritive value gradually but progressively. The rate of this decline appeared to be a characteristic of the species. Orchardgrass, especially, but also birdsfoot trefoil and reed canarygrass suffered drastic declines in nutritive value. Bromegrass appeared quite resistant to drastic changes. Alfalfa had the greatest decline in nutritive value but it still had the highest DMD value on September 1 because it had the highest nutritive value of all species in the spring. Species reacted to cuttings in a similar way, although the quantitative response differed. Species reacted consistently, however, in terms of increased DMD values immediately following the first cutting of the two-cutting system. Despite a limited decline in DMD which occurred in most species, the nutritive value changed but slightly during the remainder of the growing period. Mean DMD values for all species except alfalfa, following this cutting, were equal to or superior to mean DMD values for the forages subjected to the four cutting system.

The DMD values for each harvest, the dates of which were representative of those in practical use, and the respective averages per forage, are given in Table 1. The indicated means for all cutting systems may be used to compare species. Alfalfa, with a mean DMD value of 27.3% was superior, orchardgrass with a value of 16.6% inferior, while the remaining forages were intermediate. Similarly, the indicated means for all species may be used to compare cutting treatments. The four- and three-cutting systems were similar (25.4 and 24.9%), the two-cutting system intermediate (21.6%) and the one-cutting system inferior (13.0%).

Reed canarygrass maintained surprisingly high values. It was equal to bromegrass overall, and equal to birdsfoot trefoil under the three- and four-cutting systems. The data indicated considerable species/cutting interaction.

The use of such isolated values may, however, be misleading. The average DMD values obtained throughout the growing season from samples taken at regular intervals, regardless of cutting frequency, may be more informative. These average DMD values, i.e., seasonal means are given in Table 2. As in Table 1, alfalfa is clearly superior. These seasonal means, however, showed that birdsfoot trefoil with an overall mean DMD value of 27.0% was better than either reed canarygrass or bromegrass. The two-cutting system for birdsfoot trefoil and the three-cutting system for bromegrass, orchardgrass and reed canarygrass gave the highest DMD values. Mean values for either four-, three- or two-cutting systems were not

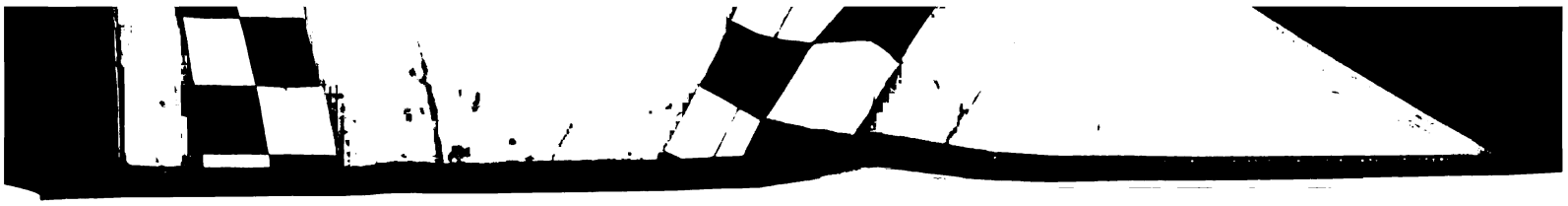


Table 1. Percent DMD values obtained for five forages cut one, two, three or four times per season.

Number of Cuttings	Alfalfa	Birdsfoot trefoil	Brome-grass	Orchard-grass	Reed Canary-grass	Average
			4-cutting system			
1	38.5	29.5	24.0	24.3	24.7	
2	36.3	27.2	20.3	22.4	26.4	
3	26.7	17.5	19.5	14.8	19.0	
4	33.6	24.6	25.8	23.3	28.9	
Average	33.8	24.7	22.4	21.2	24.8	25.4
			3-cutting system			
1	31.4	30.4	21.7	23.7	25.6	
2	28.6	20.1	22.2	22.6	21.3	
3	32.2	21.9	26.6	19.2	25.8	
Average	30.7	24.1	23.5	21.8	24.2	24.9
			2-cutting system			
1	30.9	22.4	10.9	15.1	20.4	
2	25.7	23.0	23.5	22.6	21.7	
Average	28.3	22.7	17.2	18.9	21.1	21.6
			1-cutting system			
1	16.5	16.0	16.1	4.5	11.8	13.0
			Average for all cutting treatments			
Average	27.3	21.9	19.8	16.6	20.5	

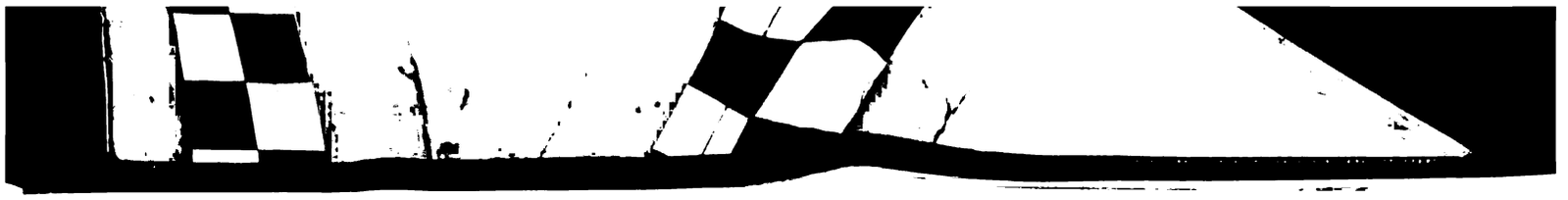


Table 2. Seasonal mean or average % DMD values obtained throughout the growing season from samples taken at regular intervals from five forages cut one, two, three or four times per season.

Species	Cuttings per season				Average t value	
	4	3	2	1		
Alfalfa	35.9	35.6	33.1	26.4	32.8	8.29**
Birdsfoot trefoil	27.7	27.6	28.8	23.7	27.0	3.92**
Reed canarygrass	26.6	26.8	24.7	19.9	24.5	5.47**
Bromegrass	24.9	26.1	23.6	19.8	23.6	2.66*
Orchardgrass	23.5	23.7	22.4	13.4	20.8	
Average	27.7	28.0	26.5	20.6		

2-cut vs. 1-cut
"t" value - 3.42**

** Significant at the 1% level
* Significant at the 5% level

significantly different.

The relationship between stage of growth and nutritive value was evident when species remained uncut until September 1. Such a relationship was not apparent among forages that were harvested frequently. The data in Table 3 show that as the forages matured, their nutritive value decreased. In alfalfa under the one-cutting system, the change from bud to early bloom brought about the greatest change in nutritive value -- from 38.5 to 31.4%. Orchardgrass following heading similarly declined in nutritive value. With increasing maturity, the morphological stage of growth was indicative of lowered nutritive value. Frequently harvested forage did not behave this way. In the four-cutting system, decreases in DMD

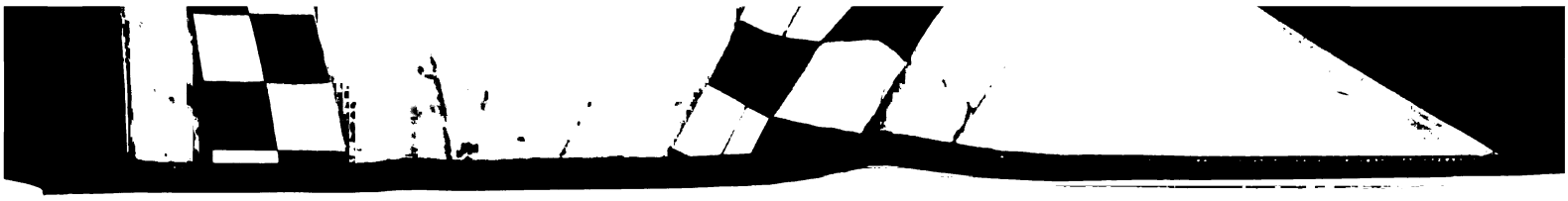


Table 3. The relationship between stage of growth and nutritive value in alfalfa and orchardgrass.

Date	Cutting	Orchardgrass		Alfalfa	
		Stage of growth	% DMD	Stage of growth	% DMD
		4-cutting system			
5/5		Vegetative	28.0	Vegetative	38.1
5/19	First	Boot	24.3	Bud	38.5
6/2		Regrowth	28.6	Regrowth	40.7
6/16		Vegetative	27.8	Vegetative	33.8
6/23	Second	Vegetative	22.4	Bud	36.3
7/3		Regrowth	24.9	Vegetative	39.9
7/15		Vegetative	22.2	Vegetative	32.5
7/29	Third	Vegetative	14.8	Vegetative	26.7
8/12		Regrowth	21.9	Vegetative	39.7
8/25		Vegetative	20.1	Vegetative	35.2
9/1	Fourth	Vegetative	23.3	Vegetative	33.6
		1-cutting system			
5/5		Vegetative	28.0	Vegetative	38.1
5/19		Boot	24.3	Bud	38.5
6/2		Head	23.7	Early bloom	31.4
6/16		Milk	16.6	50% bloom	27.9
6/23		Dough	15.1	100% bloom	30.9
7/3		Seed	15.2	50% pod	27.1
7/15		Seed set	5.7	75% pod	24.1
7/29		Straw	5.1	100% pod	18.7
8/12		Straw	5.5	Straw	21.7
8/25		Straw	3.7	Straw	15.3
9/1	First	Straw	4.5	Straw	16.5

occurred following a cutting even though the forage was vegetative; for orchardgrass, such declines were frequently rapid. Consequently, forage in a vegetative state often had a lower nutritive value than forage in a more advanced stage of growth harvested earlier in the growing season.

The effect of cutting upon forage moisture levels is shown in Figures 2-5. Simple correlations, derived between DMD and the corresponding moisture levels, are given in Table 4. With the exception of alfalfa, highly significant correlations were obtained only when extremes of both DMD and moisture content were compared. On this basis, the use of moisture content as a predictor of nutritive usefulness may be limited.

Table 4. Correlations between forage % DMD and moisture content at the time of harvest.

Species	Cuttings per season			
	1	2	3	4
Alfalfa	0.68*	0.80**	0.74**	0.96**
Bromegrass	0.64*	0.38	0.62*	0.64*
Orchardgrass	0.79**	0.49	0.41	0.95**
Reed canarygrass	0.56	0.22	0.29	0.85**
Birdsfoot trefoil	0.77**	0.32	0.56	0.86**

** Significant at the 1% level
* Significant at the 5% level

The Effect of Varying Rates of Nitrogen Fertilizer upon the Nutritive Value of Two Types of reed canarygrass

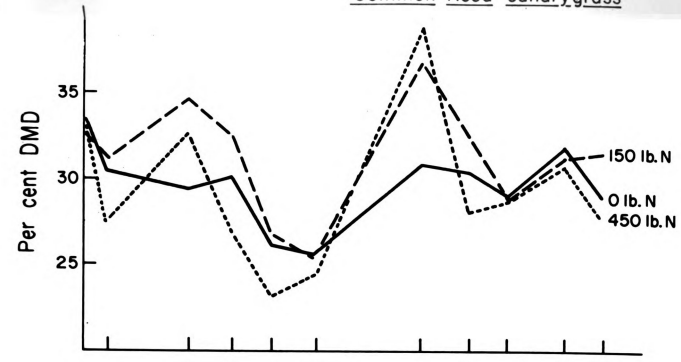
The changes occurring in the nutritive usefulness of common reed canarygrass and Siberian reed canarygrass, fertilized at three levels of ammonium nitrate, from May to November 1965, are presented in Figure 7.

General seasonal trends, regardless of fertilizer level, were identical for both forages. Cutting was apparently of greater significance in its effect on DMD than was fertilizer. Nitrogen application delayed the development of the spring growth of common reed canarygrass; paradoxically, at this stage both unfertilized grasses, especially those of the Siberian type, had superior DMD values. The first field cutting on May 27, early in the growing period, initiated a rapid increase and subsequent decrease in DMD. Regrowth was slow because precipitation was light. Following the second cutting, nitrogen increased DMD, especially on common reed canarygrass. As in the previous experiment, DMD values in the fall equalled those in the spring.

Common reed canarygrass maintained its nutritive usefulness in the fall. Both types "burned" although the common type was less susceptible and "burn" was most severe in nonfertilized plots. The onset of "burn" and declining DMD values occurred simultaneously.

Following the first field cutting all forages remained in a vegetative condition. Table 5 indicates mean values for DMD and

Figure 7. Changes in DMD of two reed canarygrass types occurring throughout the growing season as influenced by nitrogen application.



pes occurring
ogen application.

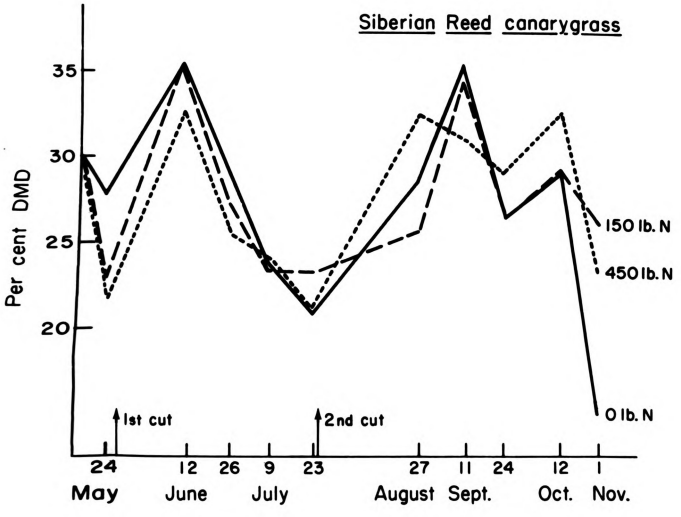


Table 5. Mean seasonal DMD and chlorophyll values for two canarygrass types at three levels of nitrogen.

Nitrogen level lb per acre	Common reed canarygrass		Siberian reed canarygrass	
	DMD %	Chlorophyll mg/g DM	DMD %	Chlorophyll mg/g DM
0	29.6	3.24	27.4	2.31
150	31.3	3.96	27.5	3.68
450	29.3	4.66	27.6	4.21
Average	30.1	3.95	27.5	3.40

forage chlorophyll content throughout the entire growing period. Chlorophyll content increased linearly and significantly with increments of nitrogen fertilizer. Only the treatment of 150 lb N per acre applied to reed canarygrass appeared to have any effect in terms of DMD. Analysis of variance for the 1965 data is presented in Table 6.

Varieties were significantly different; common reed canarygrass in the fall maintained its nutritive value longer than did Siberian, and had improved DMD values when fertilized with 150 lb N per acre. The effects of nitrogen fertilization appeared to reduce nutritive usefulness in the spring, but not following the second cutting.

Estimates of the nutritional qualities of barn-dried, first-cut forage which had responded quantitatively in terms of yield to the applied fertilizer were obtained from an animal feeding trial using

year-old sheep.[1] The results are shown in Table 7.

Table 6. Analysis of variance for 1965 DMD data obtained from two canarygrass types subjected to three levels of nitrogen fertilization.

Sources of Variance	df	MS
Types	1	108.2**
Nitrogen levels	2	6.1*
Seasonal effects	10	70.2**
Types x Nitrogen	2	6.4*
Types x Season	10	18.0**
Nitrogen x Season	20	9.8**
Types x Season x Nitrogen	20	1.4

** Significant at 1%
* Significant at 5%

Common reed canarygrass was superior to Siberian reed canarygrass on both an intake and weight gain basis. The treatment of 150 lb N per acre was superior for both varieties on an intake and weight gain basis.

Nitrogen fertilization did not affect dry matter digestibility. Simple correlations derived between DMD values and total chlorophyll content are presented in Table 8. Generally low, these values show

[1] Conducted by Dr. J.W. Thomas, Department of Dairy, Michigan State University.

Table 7. The effect of nitrogen fertilization on the nutritive value of common and Siberian reed canarygrass fed to year-old sheep.

Type	lb N per acre	Intake g/Kg$^{.75}$	Gain lb/day	DM Digestibility
Siberian reed canarygrass	0	45.21[1]	-.048	63.38
	150	53.32	0.152	63.11
	450	50.95	-.015	63.72
Common reed canarygrass	0	79.98[2]	0.279	--
	150	86.86	0.347	--
	450	75.90	0.324	--

1 30-day trial
2 14-day trial

Table 8. Simple correlations derived between % DMD and total chlorophyll, expressed as mg chlorophyll per g dry matter.

Type	Nitrogen treatment, lb per acre		
	0	150	450
Common reed canarygrass	0.31	0.35	0.25
Siberian reed canarygrass	0.72*	0.28	0.56

* Significant at 5% level.

that chlorophyll content, as a predictor of forage quality, is valueless.

The analysis of variance for the 1966 data is given in Table 9. As in 1965, common reed canarygrass was superior to Siberian reed

anarygrass. The types/season interaction was marked. Mean values
f all nitrogen levels used to illustrate this interaction are shown
n Table 10. Both varieties showed lowered nutritive value with
ncreasing maturity. Siberian reed canarygrass had lower DMD values
n the fall. This was, as in 1965, associated with winter "burn."
y October 16, all the Siberian reed canarygrass, but no common reed
anarygrass, had "burned."

Table 9. Analysis of variance for 1966 DMD data obtained from two reed canarygrass types subjected to three levels of nitrogen fertilization.

Source of Variance	df	MS
Types	1	365.2**
Nitrogen treatment	5	4.8
Seasonal effects	3	231.1**
Types x Nitrogen	5	6.1
Types x Season	3	175.9**
Nitrogen x Season	15	5.4
Types x Season x Nitrogen	15	9.4

* Significant at the 1% level

The nutritive value of reed canarygrass grown with and without nitrogen fertilizer is summarized in Table 11. Nitrogen did not consistently affect the nutritive value of these forages. However, the nitrogen fertilizer did influence the agronomic characters of yield and seed production. No lodging occurred in grass on plots receiving high

rates of nitrogen fertilizer in 1965, but no nitrogen in 1966. This was especially noticeable on plots with heavy seed yields. Siberian reed canarygrass produced a rather open sward, susceptible to ingression of competitive species. The Siberian type was apparently better adapted to low nitrogen fertilizer rates from the standpoint of yield and seed production than was common reed canarygrass.

Table 10. Combined nitrogen treatment means (% DMD) for two reed canarygrass types in 1966.

Date	Common reed canarygrass	Siberian reed canarygrass	Morphological condition
June 2	26.8	24.1	Vegetative
June 25	20.0	16.2	Seed
August 18	25.6	26.6	Regrowth vegetation
October 16	26.6	10.1	Vegetative
Average	24.8	19.3	

Table 11. The effects of varying rates of nitrogen fertilizer upon the nutritive value (% DMD) of two reed canarygrass types over a two-year period.

	lb N per acre					
1965	0	0	150	150	450	450
1966	0	300	0	100	0	300
Common reed canarygrass	25.0	24.1	25.1	25.6	24.6	24.1
Siberian reed canarygrass	19.7	20.0	17.2	20.3	17.5	20.6

The Effects of Moisture Level upon Nutritive Value

Table 12 shows the changes that occurred in the nutritive value of sudangrass clones grown under two moisture levels under greenhouse conditions. High moisture levels promoted significantly higher crude fiber levels and lower protein levels than did low moisture levels. Statistically, changes which occurred in the DMD values were not significant. Since crude fiber levels were increased under the high moisture, it is of interest that DMD changes were not significant. Under these conditions as measured by digestibility, crude fiber was apparently not related to DMD.

Table 12. The effects of two moisture levels upon the nutritive value of sudangrass.

Source	% DMD 36 hours	% Crude Fiber	% Crude Protein	% Ether Extract
		High Moisture Level		
Clone 1	64.5	28.94	11.46	0.56
Clone 2	60.7	29.56	10.02	0.47
Clone 3	59.5	29.41	10.67	0.48
Average	61.1	29.30	10.72	0.50
		Low Moisture Level		
Clone 4	58.4	27.27	13.55	0.50
Clone 5	58.1	26.78	15.15	0.50
Clone 6	58.5	26.93	16.91	0.52
Average	58.3	27.00	15.20	0.51
t value	2.135	10.39**	12.69**	0.134

** Significant at 1% level

Variability Within Genotypes

Propagules of three sudangrass clones were harvested at two stages of maturity, boot and anthesis. DMD, stem/leaf ratio and yield showed considerable within-genotype variability (estimated environmental variability). Variability, both within and between genotypes, was expressed not only for DMD, but also for stem/leaf ratio and yield. The extent of such variability changed between harvests. Since nitrogen fertilizer was applied following the first harvest, increased mean yields were obtained. Because the second harvest was taken at a morphologically earlier stage, means for DMD increased and stem/leaf ratio decreased. Variation within genotypes was greater in the second harvest than in the first. The results are shown in Table 13.

Figures 8 and 9 show the morphological variation within and between genotypes.

Figure 8. Genotype 6 left, genotype 3 right.

Figure 9. Two propagules of genotype 3.

Table 13. Yields, stem/leaf ratio and DMD from two successive harvests of sudangrass propagules.

Stage of Harvest	% DMD		Stem/leaf ratio		Yield g	
	SD	Mean	SD	Mean	SD	Mean
			Genotype 6			
Boot	1.53	30.82	0.200	1.83	7.02	43.1
Anthesis	1.17	27.65	0.584	2.67	2.69	16.8
			Genotype 5			
Boot	1.54	29.38	0.200	1.55	6.27	38.3
Anthesis	0.80	27.82	0.584	2.75	2.93	19.8
			Genotype 3			
Boot	2.24	28.30	0.142	1.63	7.53	35.8
Anthesis	1.36	24.62	0.680	2.17	5.65	13.1

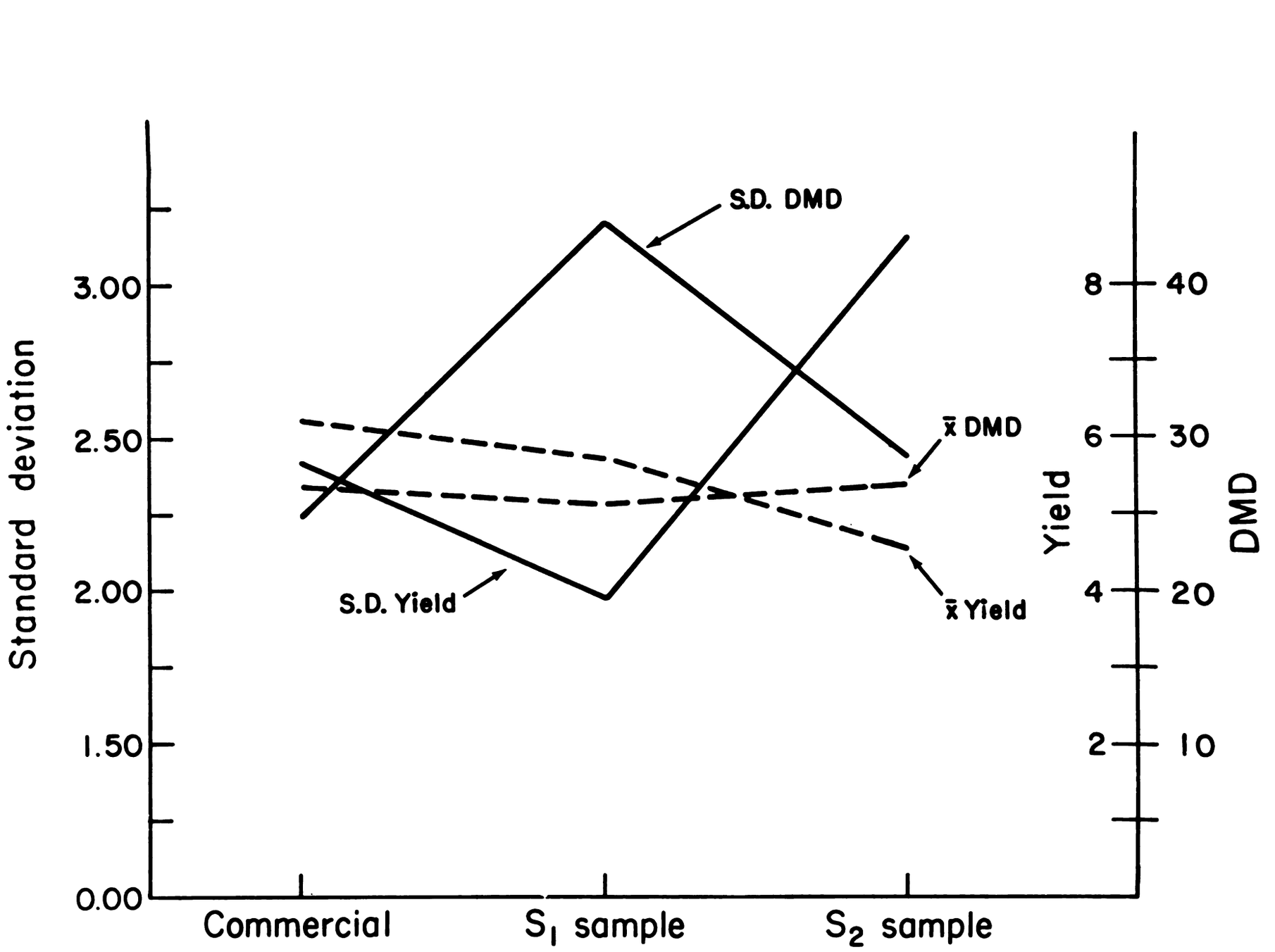
SD - Standard Deviation

The relationship of such environmental genotypic interaction is of significance when compared to population variability. Figure 10 illustrates variability, expressed as standard deviations and means of DMD and yield, obtained from three small populations of sudangrass. The S_2 population (43 clones), obtained from random pollination within the S_1 population, should have shown a return to the parental type. DMD values behaved this way. Yield, however, continued to decrease, indicative of some inbreeding, even though a release of variability did occur. These data indicate that the genetic systems concerned with yield characters and nutritive characters are dissimilar. Low negative correlations between DMD and yield for both the commercial Piper sudangrass and the S_1 populations were obtained. These were -.05 and -.23, respectively. A positive correlation of 0.30 was obtained between these parameters for the S_2 population.

The Nutritive Usefulness of Species and Varieties Within the Medicago Genus

The results of a preliminary screening of various alfalfa populations are illustrated in Table 14. The range of DMD values obtained in any given population was large. Population means were significantly different. A low but consistently negative correlation was obtained within populations between % DMD and yield in grams per plant. This correlation could be raised to a value of -.87, significant at 1%, by using as pairs, mean % DMD and yield values per

Figure 10. Changes in yield and DMD parameters of sudangrass clones as influenced by inbreeding.


S.D. DMD
x̄ DMD
S.D. Yield
x̄ Yield
Standard deviation
3.00
2.50
2.00
1.50
0.00
Yield
8
6
4
2
DMD
40
30
20
10
Commercial
S1 sample
S2 sample

Tab

Pop

DP

M.

Dul

Tut

Ver

Hai

Wie

E J

Cul

1
2
3
**

Table 14. Analysis of Alfalfa Populations

Population	Parameter	No[1]	Mean	SD	% CV	Range	Correlation DMD vs. Yield
DPI[2]	% DMD	41	32.8	3.87	11.8	41.9- 25.5	0.0865
	Yield	41	126.6	43.88	34.7	285.6- 48.2	
M. glutinosa	% DMD	12	30.1	1.98	6.6	33.3- 27.7	-.5126
	Yield	12	212.8	61.76	29.0	294.9-106.4	
DuPuits	% DMD	171	35.4	3.74	10.6	45.0- 27.1	-.0223
	Yield	171	122.1	42.71	35.0	254.7- 49.8	
Tuna	% DMD	110	31.9	3.28	10.3	40.2- 22.6	0.0211
	Yield	110	173.3	49.80	28.7	390.4- 90.1	
Vernal	% DMD	219	32.6	3.32	10.2	41.0- 25.4	-.2503**
	Yield	219	179.8	57.10	31.8	337.6- 45.2	
Hardigan	% DMD	39	32.6	2.57	7.9	38.3- 25.3	-.2095
	Yield	39	161.6	61.96	38.3	325.8- 50.5	
Wisconsin 460	% DMD	29	26.3	2.09	7.9	30.5- 22.2	-.0810
	Yield	29	295.5	83.04	28.1	482.4-152.9	
E 21569[3]	% DMD	17	33.1	2.42	7.3	36.7- 28.6	-.2513
	Yield	17	184.7	61.23	33.1	345.8-121.4	
Culver	% DMD	268	33.4	3.31	9.9	42.2- 25.0	-.1172
	Yield	268	201.9	66.48	32.9	417.9- 54.1	

1 Number of clones
2 DPI - DuPuits, National Institute, Versailles, France
3 E 21569 - M. coerulea
** Significant at 1% level.

population. The distribution of DMD values was normal. Variation for the parameter of yield was equally considerable. Selected clones from the DuPuits and Vernal varieties and the entire Wisconsin 460 population were selected and evaluated for physical characteristics. DuPuits, a M. sativa variety, and Wisconsin 460, a M. falcata selection represented apparent high and low extremes of nutritive usefulness, respectively, in the alfalfa population. Vernal, a synthetic variety containing both M. sativa and M. falcata germplasm, was intermediate. Table 15 indicates the characters assessed and the mean values obtained. DuPuits, with the highest DMD values at both 6- and 36-hour fermentation periods, had the lowest percentage of CWC, ADF and ADL and yield. Wisconsin 460 had the lowest DMD values at the 6-, but not at hhe 36-hour fermentation period, and the greatest yields and percentages of CWC, ADF and ADL.

The same trends were present both within and between varieties. Table 16 shows these same character means grouped on the basis of nutritive value. Those clones consistently high in nutritive value were constantly low in CWC, ADF and ADL. Consequently, forages with high cell soluble contents, as opposed to high fibrous contents, were of a superior nutritive value.

Table 15. Physical and nutritive parameters measured in clones of Vernal, DuPuits and Wisconsin 460 alfalfa.

Parameter	DuPuits(28)[1]		Vernal(22)		Wisconsin 460(29)	
	Mean	SD	Mean	SD	Mean	SD
% DMD	36.9	6.6	33.2	6.7	26.3	2.1
Yield	118.4	50.4	203.8	51.8	295.5	83.0
% CWC	42.7	3.5	47.9	4.2	51.2	3.2
% ADF	32.6	4.2	35.6	3.8	38.8	2.4
% ADL	7.6	1.1	7.7	0.9	8.9	1.0
% ADL/% ADF	0.24	0.03	0.22	0.02	0.23	0.02
% DMD(36 hr)	52.8	3.4	47.7	3.3	49.5	2.5

[1] Values in parentheses indicate the number of clones analyzed

Table 16. Physical and nutritive parameters measured in clones of DuPuits and Vernal alfalfa differentially grouped according to high or low nutritive value.

Parameter	DuPuits		Vernal	
	High	Low	High	Low
% DMD	42.0	29.1	39.1	26.1
% CWC	41.2	45.1	45.7	50.6
% ADF	30.9	35.3	34.1	37.4
% ADL	7.4	8.1	7.4	8.0
% ADL/% ADF	0.24	0.23	0.22	0.21
% DMD(36 hr)	54.4	50.2	49.5	45.5

Simple correlations were derived between all variables for these selected clones. These are illustrated in Table 17. The correlation between DMD and physical forage fractions increased with increased fermentation time. This would indicate fibrous fractions to be determinative of digestibility rather than NVI. Fibrous fractions such as CWC, and ADF, ADF and ADL were significantly correlated. The lignin fiber ratio was not correlated to either DMD at the 6- or 36-hour fermentation periods. Consequently, increased lignin levels did not have any effect per se even though data in Table 16 indicates clones of low nutritive value from both DuPuits and Vernal alfalfa had higher values of lignin than clones of high nutritive value. Correlations were not constant, either in value or sign, on a variety or species basis.

On the basis of these results, two clones were selected from each of the three populations. One clone was high in nutritive value, the other low. The clones and their indicated nutritive value are shown in Table 18. Regardless of the evaluatory technique, the clones rated highest in DMD, DuPuits 20 (DP 20), Vernal 72 (V 72), and Wisconsin 460-24 (W 24), also appeared superior for other factors contributing to nutritive value as compared to the lower-rated clones DuPuits 69 (DP 69), Vernal 133 (V 133), and Wisconsin 460-8 (W 8), respectively. These data validate the use of the 6-hour in vitro fermentation period.

Table 17. Simple correlations derived between percentage of DMD (6- and 36-hours), ADF, ADL and CWC and yield of three selected alfalfas.

	Yield	CWC	ADF	ADL	ADL/ADF	DMD(36 hr)
			DuPuits[1]			
DMD(6 hr)	-.07	-.52**	-.53**	-.33	0.19	0.64**
Yield		0.46*	0.20	0.08	-.11	-.11
CWC			0.79**	0.39*	-.40*	-.69**
ADF				0.63**	-.34	-.86**
ADL					0.51**	-.54**
ADL/ADF						0.31
			Vernal[2]			
DMD(6 hr)	-.58**	-.62**	-.45*	-.34	0.12	0.60**
Yield		0.65**	0.57**	0.39	-.20	-.37
CWC			0.86**	0.57**	-.35	-.66**
ADF				0.78**	-.21	-.68**
ADL					0.44*	-.73**
ADL/ADF						-.16
			Wisconsin 460[3]			
DMD(6 hr)	-.08	-.32	-.29	-.23	-.08	0.13
Yield		0.15	0.10	-.06	-.16	-.27
CWC			0.86**	0.40*	-.10	-.30
ADF				0.61**	0.08	-.42*
ADL					0.84**	-.40*
ADL/ADF						-.19

1 df - 26
2 df - 20
3 df - 27
** Significant at 1% level
* Significant at 5% level

Table 18. The nutritive value of six alfalfa clones selected on the basis of three in vitro fermentation techniques and three chemical analyses.

Clone	In vitro fermentation			Chemical analysis		
	% DMD		Terry-Tilley[1]	% ADF	% ADL	% CWC
	6-hr	36-hr	% DMD			
DuPuits 20	45.0	52.4	66.5	33.1	7.5	43.1
DuPuits 69	27.1	43.4	62.6	45.8	10.9	51.6
Vernal 72	39.1	53.6	66.4	30.7	5.8	44.8
Vernal 133	26.4	41.9	53.8	41.0	9.4	53.2
Wisc. 460-24	29.8	54.8	63.5	34.2	8.0	45.2
Wisc. 460-8	22.2	46.5	55.2	41.6	11.3	54.9

[1] Terry, R.A., and K.M.A. Tilley. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassl. Soc. 18:104-111.

The nutrititive value of these six clones and two M. glutinosa clones, as assessed by the production of volatile fatty acids (VFA) during a 36-hour in vitro fermentation, is indicated in Table 19. VFA production was consistently higher from those clones previously indicated of superior nutritive value. There were only slight differences, however, in the proportions of acids produced. Acetic, propionic and butyric acids were produced in the proportions of 8.24 to 2.24 to 1.00 with but slight deviations. Butyric acid was

Table 19. Volatile fatty acid production in a 36-hour in vitro fermentation from selected alfalfa clones.

Clone	VFA Production, μM/ml			Total
	Acetic	Propionic	Butyric	
DP 20	Aa 106.5	A 32.6	Aa 15.5	A 154.2
DP 69	Bc 83.4	21.9	bc 10.2	Bc 115.6
V 72	ABbc 90.0	b 23.7	bc 11.0	Bbc 124.8
V 133	76.1	21.4	c 8.3	c 105.7
W 24	c 81.7	20.8	Ab 11.7	Bc 114.2
W 8	79.6	21.7	bc 9.0	Bc 110.3
G 24	AaBb 100.2	Ab 28.4	bc 10.6	ABb 139.1
G 36	72.6	19.4	7.4	99.4

Capitals - Highest value is significantly different at the 1% level from any other value without similar capital.
Small - Highest value is significantly different at the 5% level from any other value without similar small letter.

consistently produced in slightly greater proportions in those clones of superior nutritive value.

The production of VFA from these clones was also studied at time intervals ranging from 6 to 48 hours with the apparatus shown in Figure 1. The Vernal and Wisconsin 460 clones were evaluated together and the DuPuits clones later. Consequently, absolute comparisons cannot be made concerning the quantities of VFA produced between runs, since the inoculum source was different.

The data in Table 20 show that only the major volatile fatty acids, acetic, propionic and butyric, were produced in quantity. Only traces of iso-butyric, valeric and iso-valeric acids were obtained. Previously indicated superior clones stimulated VFA production. The DuPuits clones continued to stimulate actively acetic, propionic and butyric acid production for the full 48 hours. The Vernal and Wisconsin 460 clones, however, definitely showed a cessation of acid production following the 36-hour fermentation period. The fermentation of DP 69 (low DMD) showed a rather spectacular burst of activity following 36 hours of fermentation, resulting in the final 48-hour values of acetic and propionic being higher and butyric acid being equal to that of DP 20 (high DMD). There were no consistent differences in the proportions of acids produced. Acetic, propionic and butyric acids were produced in the average ratio of 7.66 to 2.34 to 1.00, respectively.

consistently produced in slightly greater [illegible] times
of superior nutritive value.

The production of VFA from [illegible]
intervals ranging from 0 to 72 hours at [illegible]
Figure 1. The Vernal and [illegible]
and the DuPuits clones [illegible]
cannot be made concerning the [illegible]
since the inoculum source [illegible]

The data in Table 20 show that [illegible]
acetic, propionic and butyric [illegible]
of iso-butyric, valeric and iso-[illegible]
Previously inoculated substrate clones stimulated VFA production. The
DuPuits clones continued to stimulate acetic, propionic and
butyric acid production for the full 72 hours. The Vernal and
Wisconsin 660 clones, however, definitely showed a cessation of acid
production following the 36-hour fermentation period. The
fermentation of DM to (low BMD) showed a rather spectacular burst of
activity following 36 hours of fermentation, reaching in the final
48-hour values of acetic and propionic being higher and butyric acid
being equal to that of DM 30 (high BMD). There were no consistent
differences in the proportions of acids produced. Acetic, propionic
and butyric acids were produced in the average ratio of 3.60 to 1.30
to 1.00, respectively.

Table 20. The production, in μM/ml, of acetic, propionic, butyric acids, during the in vitro fermentation of selected alfalfa clones, at time periods of 6 to 48 hours.

Clone	Hours				Accumulative total[1]
	6	26	36	48	
			Acetic acid		
DP 20	53.4	60.1	63.5	71.8	248.8
DP 69	46.8	61.8	55.1	80.2	243.9
V 72	25.6	35.6	50.4	41.8	153.4
V 133	28.8	34.9	44.3	32.4	140.0
W 24	33.7	45.8	52.6	39.5	171.6
W 8	33.2	47.4	43.9	38.0	162.5
			Propionic acid		
DP 20	18.4	21.7	21.7	21.7	83.5
DP 69	16.7	18.4	18.4	23.4	76.9
V 72	8.0	10.9	14.7	13.0	46.6
V 133	9.2	10.4	11.4	11.9	42.9
W 24	10.7	12.8	14.2	11.5	49.5
W 8	11.0	11.8	12.1	11.1	46.0
			Butyric acid		
DP 20	6.5	6.8	6.8	7.7	27.8
DP 69	5.2	6.0	5.5	7.7	24.4
V 72	3.4	6.2	6.6	8.8	25.0
V 133	4.8	4.3	5.5	3.7	17.3
W 24	4.8	5.7	6.4	4.4	21.3
W 8	4.1	5.7	9.4	5.7	24.9

[1] The sum of values at 6, 26, 36 and 48 hours

(a) Estimation of carbohydrates

Preliminary water extractions were made on sudangrass clones. Evaluations were then made on both extracted and reconstituted forage. Reconstituted forage consisted of a sample of extracted forage plus an aliquot of water extract equivalent to that previously removed. The effects of water extraction on the nutritive value of these clones are shown in Table 21.

Table 21. Evaluation of sudangrass clones subjected to water extraction and subsequent reconstitution.

Source	% DMD(6 hr)	Source	% DMD(24 hr)
Clone 1	22.0	Clone 4	50.1
Extracted clone 1	2.7	Extracted clone 4	32.6
Reconstituted clone 1	0.0	Reconstituted clone 4	36.8
Clone 3	16.5	Extracted clone 4 plus 0.32 g glucose	21.0
Extracted clone 3	1.6	Reconstituted clone 4 plus 0.32 g glucose	20.9
Reconstituted clone 3	0.0	Clone 4 plus 0.32 g glucose	48.8
		Clone 4 plus water extract	48.2

Water extractions effectively reduced DMD. Six-hour fermentations showed zero values for DMD using reconstituted forage. Analysis of clone 3 showed the percentage of crude fiber increased from 29.4 to 41.8%, presumably at the expense of the soluble constituents of the forage.

Extracted forage was, however, "digested" to the extent of two-thirds that of unextracted material in a 24-hour fermentation. Glucose reduced this "digestion" by one third -- presumably a glucose fermentation took place rather than cellulose degradation. The addition of glucose or water extract to unextracted material had only a slight inhibitory effect. It would appear that water extraction removed most of the soluble components of the forage, effectively lengthening the lag phase. Reconstituted clone 4 did not behave in a manner analogous to extracted material plus glucose. A stimulated, rather than an inhibited, digestion took place.

An estimation of the carbohydrates in water extracts of the six alfalfa clones previously selected, was made, utilizing the anthrone reaction. Hot and cold water extracts were evaluated. The results are shown in Table 22.

Regardless of whether hot or cold water extracts were used, the relationships within and between variety pairs remained identical. The values for the hot water extract were superior to those for the cold water extracts at the 5% level ("t" value - 3.57*). The relationship between varieties was of the order DuPuits> Wisconsin 460> Vernal. Carbohydrate values did not align themselves with other values indicative of nutritional usefulness on the individual clone basis.

Descending paper chromatography was used to attempt a tentative identification and separation of the individual sugars in the water extract. Separations using a 16-hour irrigation period were poor and

Extracted forage was, however, [illegible] two-thirds that of unextracted [illegible] Glucose reduced this [illegible] glucose fermentation [illegible] The addition of glucose [illegible] only a slight inhibitor [illegible] removed most of the [illegible] lengthening the lag phase [illegible] manner analogous to [illegible] rather than an inhibitor [illegible]

An estimation of the [illegible] alfalfa clones previously selected, was made, utilizing the anthrone reaction. Hot and cold water extracts were evaluated. The results are shown in Table 22.

Regardless of whether hot or cold water extracts were used, the relationships within and between variety pairs remained identical. The values for the hot water extract were superior to those for the cold water extracts at the 5% level ("t" value - 3.574). The relationship between varieties was of the order DuPuits > Wisconsin 460 > Vernal. Carbohydrate values did not align themselves with other values indicative of nutritional usefulness on the individual clone basis.

Descending paper chromatography was used to attempt a tentative identification and separation of the individual sugars in the water extract. Separations using a 16-hour irrigation period were poor and

Table 22. Soluble carbohydrate levels as mg per g forage in the water extracts of six alfalfa clones as measured by the anthrone reaction.

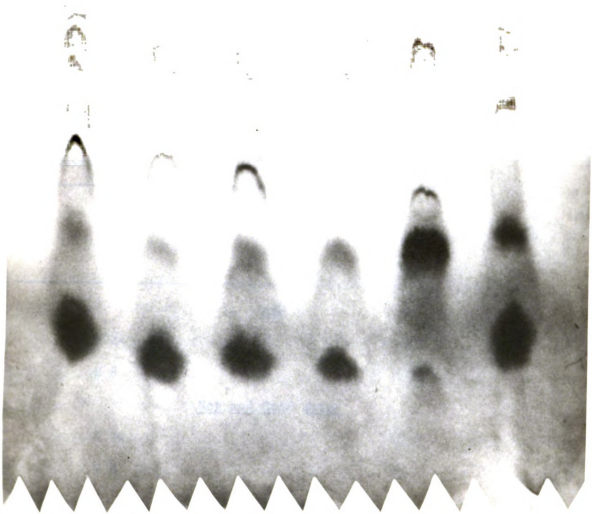
Clone	Water Extracts	
	Cold	Hot
DP 20	49.1	79.5
DP 69	57.0	104.3
V 72	42.6	57.5
V 133	34.5	35.0
W 24	45.0	71.3
W 8	35.0	53.7

inconclusive. Standard sugars indicated Rf values were higher than published values. Galactose and glucose, for example, gave Rf values of 0.20 and 0.22, respectively. The published values for these sugars are 0.16 and 0.18, respectively(Block, Durram and Zweig, 1959).

Figure 11 illustrates a chromatogram of water extracts of the six alfalfa clones. A 64-hour irrigation period was used. Considerable separation of the sugars occurred. Tentative identification of the final two spots in each column based upon standards chromatographed simultaneously were galactose and fructose, respectively. The circular spots, evident in DP 20, V 72 and W 24, and, to a lesser extent, in DP 69 and V 133, appeared to be a glucosamine -- glucosamine hydrochloride was used as the standard. No identification was made on the remaining spots.

Figure 11. A chromatograph of six water extracts, containing soluble carbohydrates, obtained from alfalfa clones of differing nutritive value.

DP20 DP69 V72 V133 W24 W8



The most obvious difference was between the two M. falcata clones. The W 24 extract, based on the concentration of the individual spots, was almost devoid of fructose with considerable quantities of galactose. The W 8 extract apparently had greater quantities of fructose than galactose. The remaining clones were quite similar with fructose being the predominant sugar present.

Both W 24 and W 8 residues remaining after hot and cold water extraction as well as acid detergent fiber residues were evaluated by the in vitro fermentation technique.

Table 23. DMD of residues of W 24 and W 8 following water and acid extraction.

Clone	Treatment	% DMD	
		36 hr	24 hr
	ADF		
W 24		6.0	1.9
W 8		2.8	0.0
	Hot and Cold Water		
W 24		22.7	21.5
W 8		17.7	13.9
	Cold Water		
W 24		29.4	
W 8		22.3	

Differences in nutritive value between these two clones, previously indicated in Table 18, were still evident in the acid detergent fiber residue. The fiber residues were of differing susceptibility to microbial fermentation. Similarly, forage extracted with consecutive washings of cold and hot water still maintained characteristic differences even after 36 hours of fermentation.

(b) Estimation of Forage Antibiosis

Preliminary assessments of the phytotoxic substances present in water extracts of individual alfalfa clones were made using W 24 (high DMD) and W 8 (low DMD). Seeds of corn (Zea mays) were germinated after soaking in hot and cold water extracts of these two clones. Results are presented in Table 24.

Inhibition was greatest for the W 8 extracts compared to the corresponding W 24 extracts. Cold water extracts inhibited development more than did hot water extracts. This difference in phytotoxicity between hot and cold water extracts was not necessarily due to thermal inactivation of the phytotoxic principle since all seeds were soaked in the extracts for one hour at 39C (the hot water extraction temperature) previous to incubation.

Actual germination was not inhibited by any of the extracts. However, dry matter production was differentially affected. The cold water extract of W 8 stimulated radicle development, but completely inhibited the development of adventitious roots.

Table 24. Growth determinations resulting from the germination of corn seeds soaked in hot and cold water extracts of clones W 24 and W 8.

Water Extract	Mean radicle length mm	Coleoptiles		Adventitious roots		Total dry weight mg	% Inhibition
		Mean length mm	No	Mean Length mm	No		
			Clone W 24 - High DMD				
Cold water	33.3	5.5	6	12.6	16	107.1	34.0
Cold and hot water	29.7	12.9	8	19.0	29	145.6	10.3
			Clone W 8 - Low DMD				
Cold water	42.6	8.5	2	0.0	0	77.2	52.4
Cold and hot water	24.6	11.3	8	13.4	21	110.1	32.2
			Water - Check				
Water	55.2	13.7	9	22.6	18	162.3	

The experiment was amplified using bean seeds (Phaseolus vulgaris var. Sanilac) and a different line of corn seeds, incorporating extracts from previously selected alfalfa clones, i.e., DP 20, DP 69, V 72, V 133, W 24 and W 8. Table 25 indicates the results. A factorial design replicated three times with randomized blocks was used.

The analysis of variance for both beans and corn indicated the inhibitory properties of the two extracts were different at the 1% level of significance. In the case of the beans, the clonal differences were significant at the 5% level. The clone/extract interactions were not significant. Cold water extracts compared to hot water extracts exhibited greater inhibitory properties regardless of the seed source. Little relationship existed between clones of indicated superior nutritive quality and those exhibiting the greatest inhibitory properties. Water extracts obtained from clones W 24 and W 8 reversed their effects depending upon the line of corn seed used (Tables 24 and 25). While the extracts showed considerable phytotoxicity regarding germination, this phytotoxicity seemed related to the seed source as much as to the source of extract.

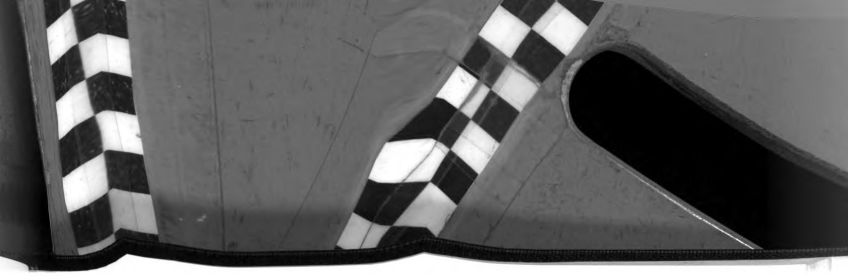


Table 25. Milligrams of dry matter in new tissues produced by the germination of corn and bean seeds soaked in hot and cold water extracts of six alfalfa clones.

Clone	Corn			Beans		
	Cold Water Extract	Cold and Hot Water Extract	Total	Cold Water Extract	Cold and Hot Water Extract	Total
DP 20	266.7	579.0	845.7	252.0	490.8	742.8
DP 69	306.4	324.3	630.7	190.8	286.7	477.5
V 72	274.6	547.9	822.5	304.1	206.7	510.8
V 133	300.7	386.9	687.6	373.7	437.1	810.8
W 24	290.4	440.1	730.5	245.8	448.1	693.9
W 8	339.5	472.1	811.6	315.3	750.8	1066.1
Total	1778.3	2750.3	--	1681.7	2620.2	--

LSD (5%) for extract totals - corn 467.8
beans 612.7
LSD (5%) for clone totals - beans 354.0

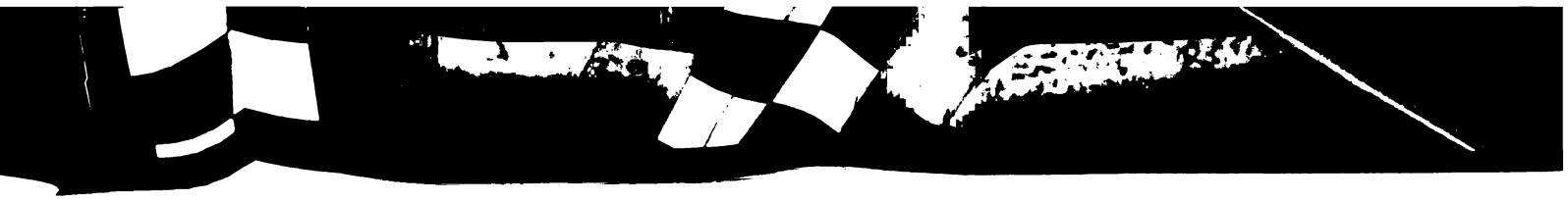
(c) Lignin analysis

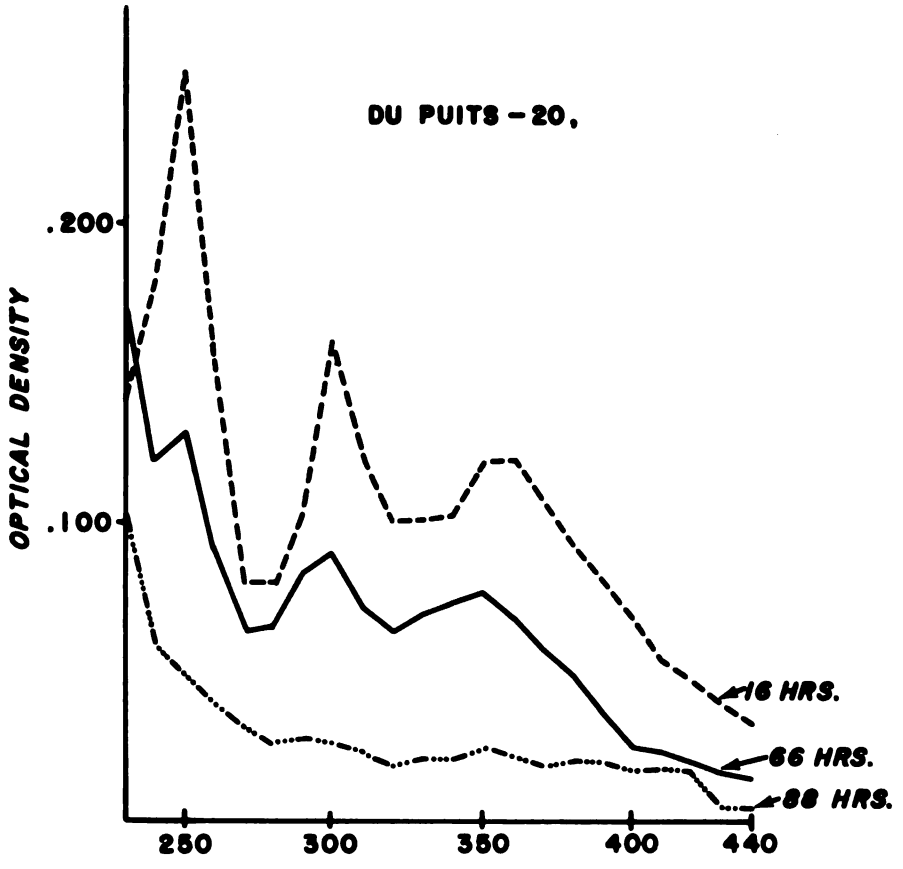
The difference ultraviolet absorption spectra of the lignin extracted from clones DP 20, DP 69, V 72, V 133, W 24 and W 8 are shown in Figures 12-14. These difference spectra in which nonionizing chromophores are eliminated were characteristic of lignin spectra as described by Stafford (1960, 1964) and Wexler (1964). Optical density (OD) maxima peaks occurred at 250, 300 and 350 mμ. Strong peak minima generally were obtained in the region between 270 to 285 mμ. The peaks at 250 and 300 mμ correspond to unconjugated phenol groups; the peak at 350 mμ corresponds to conjugated phenols or, as Stafford has suggested, to a composite series of peaks due to different phenols.

Since dilutions for all clones were identical, clone spectra are comparable. The 16-hour extract was diluted 1.67 times that of the 66- and 88-hour extracts whose dilutions were identical.

Observed spectra were different not only in spectral conformation, but also quantitatively. These differences were present not only between clones, but especially between clones differentially grouped on the basis of nutritive value. The OD values for the 16-hour extraction periods were characteristically higher in those clones of high nutritive value, i.e., DP 20, V 72 and W 24. These same clones had, however, a lower lignin content compared to those clones of a low nutritive value. With longer extraction periods, the spectra changed both with regard to the absorption peaks and the OD values obtained.

Figure 12. Difference absorption spectra of lignin preparations, obtained from two DuPuits clones, involving extraction periods of 16, 66 and 88 hours.




DU PUITS - 20,
.200
.100
OPTICAL DENSITY
16 HRS.
66 HRS.
88 HRS.
250
300
350
400
440

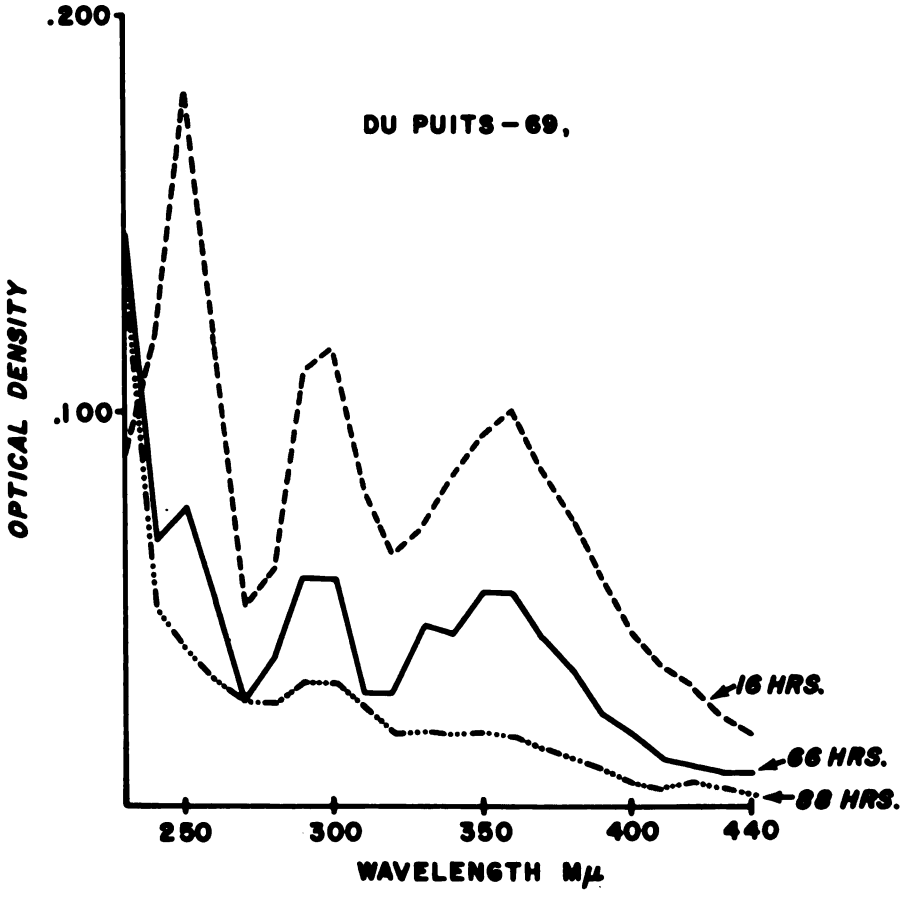
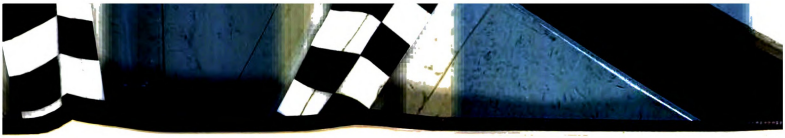
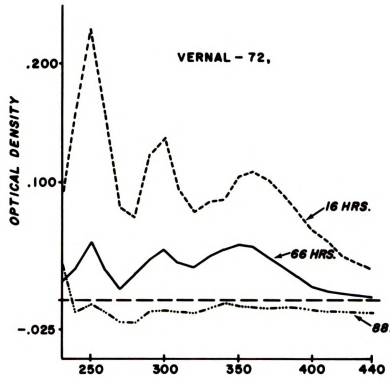

DU PUITS - 69,
.200
.100
OPTICAL DENSITY
16 HRS.
66 HRS.
88 HRS.
250
300
350
400
440
WAVELENGTH Mμ

Figure 13. Difference absorption spectra of lignin preparations, obtained from Vernal clones, involving extraction periods of 16, 66 and 88 hours.




VERNAL – 72,
.200
.100
-.025
OPTICAL DENSITY
16 HRS.
66 HRS.
88 HRS.
250
300
350
400
440

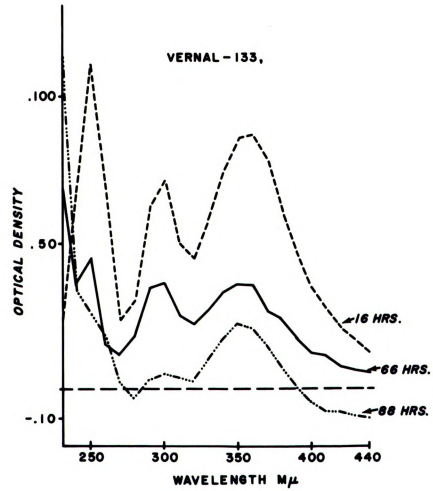
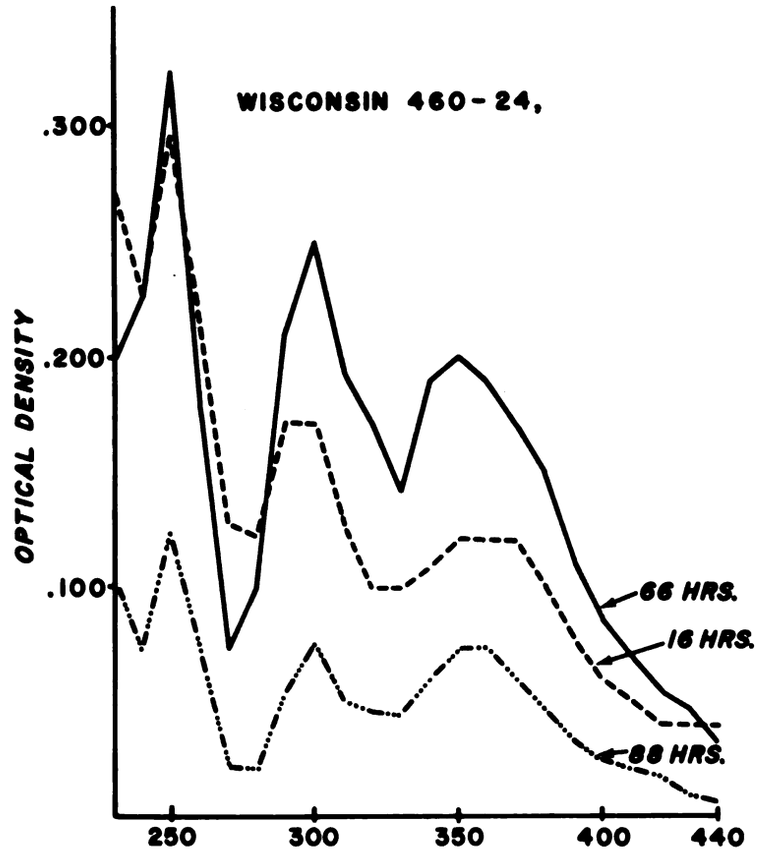
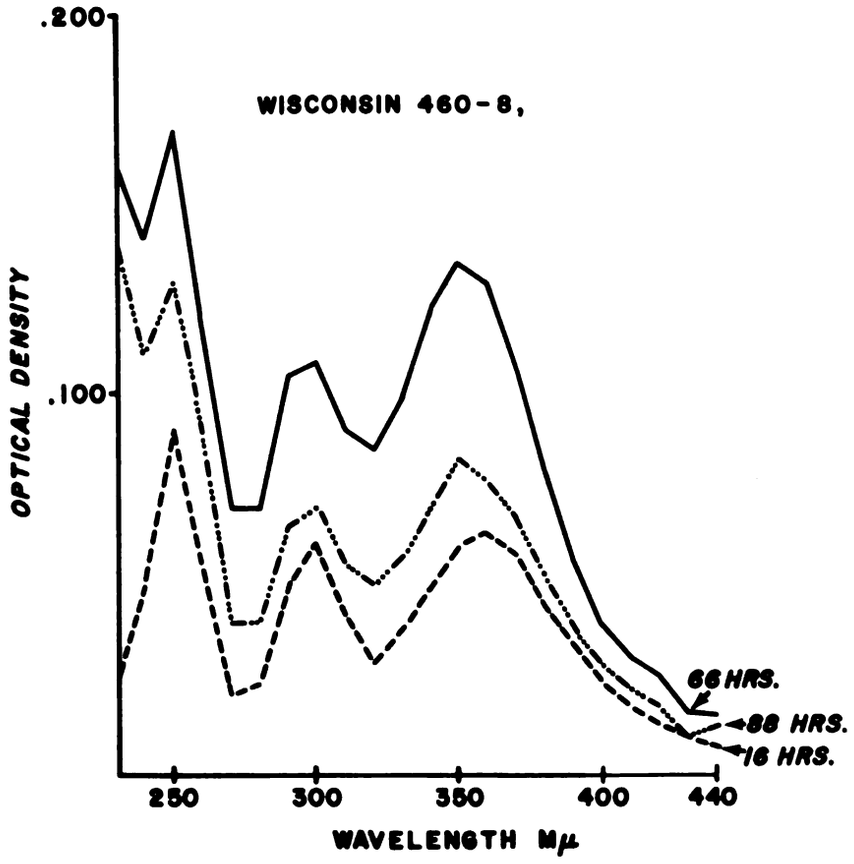

VERNAL – 133,
.100
.50
-.10
OPTICAL DENSITY
16 HRS.
66 HRS.
88 HRS.
250
300
350
400
440
WAVELENGTH Mμ

Figure 14. Difference absorption spectra of lignin preparations, obtained from two Wisconsin 460 clones, involving extraction periods of 16, 66 and 88 hours.


WISCONSIN 460-24,
.300
.200
.100
OPTICAL DENSITY
66 HRS.
16 HRS.
88 HRS.
250
300
350
400
440


.200
WISCONSIN 460-8,
.100
OPTICAL DENSITY
66 HRS.
88 HRS.
16 HRS.
250
300
350
400
440
WAVELENGTH Mμ

The initial lignin extracts, obtained from clones of superior nutritive value, gave difference absorption peaks at 250, 300 and 350 mμ, the OD values of which maintained a relationship of 250 mμ values > 300 mμ values > 350 mμ values. The OD values for the 300 mμ wavelength were approximately 60% those for the 250 mμ wavelength; the 350 mμ wavelength values were approximately 75% those for the 300 mμ wavelength. Continued extraction of these clones resulted either in (a) a complete loss of absorbance, as exemplified by V 72, (b) a gradual loss of peak values at the 250, 300 and 350 mμ wavelengths, or (c) a continued absorption and peak production such that the 350 mμ peaks increased greatly in proportion compared to initial extraction.

The clones of poor nutritive value, i.e., DP 69, V 133 and W 8, did not, in the initial extraction, produce the absorption peaks in the descending order characterized by the clones of high nutritive value. The absorption peaks at 350 mμ were characteristically of greater magnitude, equal or exceeding the absorption OD values at the 300 mμ wavelength. Continued extraction resulted in extracts which continued to give absorptivity, particularly at the 350 mμ wavelength.

The changes in absorptivity within paired clones, i.e., DP 20 and DP 69, V 72 and V 133, and W 24 and W 8, are indicated in Table 26, using OD ratios for values obtained at 250, 300 and 350 mμ.

Table 26. The ratio of optical density values obtained from ultraviolet difference spectra, at three wavelengths, of lignins extracted from six alfalfa clones of differing nutritive value.

Clone	Extraction Time-Hours	Ratio		
		300/250 mμ	350/250 mμ	350/300 mμ
DP 20	16	0.64	0.48	0.75
	66	0.69	0.58	0.83
	88	0.52	0.48	0.92
DP 69	16	0.64	0.51	0.81
	66	0.76	0.71	0.93
	88	0.75	0.45	0.60
V 72	16	0.59	0.47	0.80
	66	0.84	0.88	1.05
	88	----[1]	----	----
V 133	16	0.64	0.77	1.20
	66	0.82	0.80	0.97
	88	0.19	0.85	4.40
W 24	16	0.59	0.41	0.70
	66	0.78	0.63	0.70
	88	0.63	0.60	0.96
W 8	16	0.67	0.66	1.05
	66	0.65	0.79	1.23
	88	0.54	0.64	1.19

[1] No absorption

Within each pair for the initial extraction, all the ratios increased when the clones of high nutritive value were compared to the clones of poor nutritive value. Second and third extractions resulted in proportionally increased levels of lignins absorbing in the 300 mμ and 350 mμ wavelengths. These increases were especially noticeable in those clones of low nutritive value.

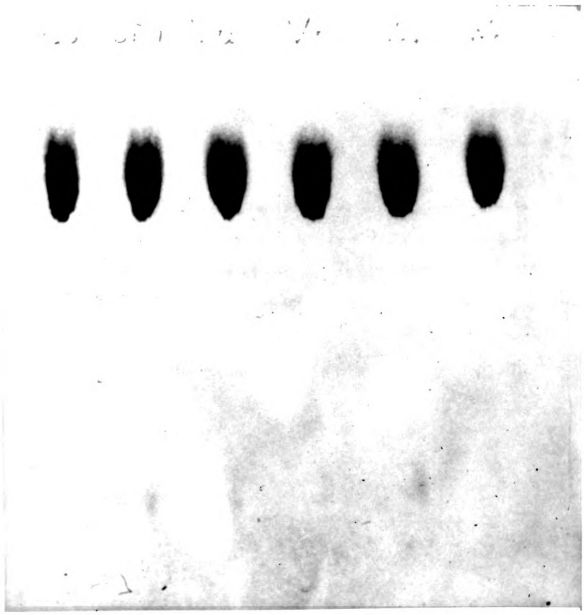
The weight of lignin lost in these extractions verified the results indicated by the optical density values (Table 27). Analysis of variance indicated both clone and extraction period differences were significant at the 1% level. DP 20, V 72 and W 24 had lignin/fiber complexes from which the lignin was significantly more extractable than the corresponding clones of low nutritive value. The lignin from these latter clones was not completely removed even after an extraction period of 88 hours. The clones of low nutritive value retained their lignin, and despite containing greater quantities of lignin, the actual weight lost was less than that lost from clones of superior nutritive value.

Separation and tentative identification of the components of lignin contained in these extracts were made using descending chromatography. Figure 15 illustrates a chromatogram of a 16-hour extract of each clone stained with ammoniacal silver nitrate after a 14-hour irrigation with n-butanol, acetic acid and water in a 4:1:5 v/v ratio. The upper row of spots had Rf values of 0.32 and the lower row of spots, barely visible, had Rf values of 0.85. A butanol, acetic acid and water solvent in the proportions 8:2:2 v/v,

Table 27. The ADL and weight of lignin removed in mg and as a percentage of total lignin present with three successive extraction periods.

Clone	Extraction period, Hours						Total % ADL
	16		66		88		
	%	mg	%	mg	%	mg	
DP 20	76.0	8.65	81.4	9.30	112.7	12.70 A	7.5
DP 69	58.7	7.15	65.6	7.85	93.1	11.40 B	10.9
V 72	99.2	8.85	118.1	10.45	155.2	13.70 A	5.8
V 133	61.7	6.80	71.8	8.10	97.3	10.90 B	9.4
W 24	70.6	8.60	90.0	10.40	116.9	14.10 A	8.0
W 8	48.7	6.55	58.4	7.85	82.3	11.15 B	11.3

Figure 15. A chromatograph of six lignin extracts obtained from alfalfa clones of differing nutritive value.



ts obtained from

and a sucrose hydrochloride acid detectant, similarly revealed the upper row of spots but not the lower row. The use of an ethylmethyl-ketone:2N ammonium hydroxide solvent again revealed only the upper row of spots with Rf values of 0.10. These two Rf values gave a tentative identification of the upper spots as being amino-phenyl-sulfuric acid. The spots with the Rf value of 0.85 were tentatively identified as protocatechuic acid.

Chromatography of lignin extracts obtained from these clones using extraction periods ranging from ½ hour to 88 hours resulted in chromatograms generally with one row of spots. These spots had consistent Rf values of 0.31 to 0.35.

DISCUSSION

Forages subjected to various cultural practices, have been extensively analyzed for many chemical and agronomical characters. The nutritional usefulness of such crops has been frequently disregarded, and broad assumptions made concerning sporadic nutritional analyses and the more readily measured fractions of the proximate analysis. Similarly, relationships between forage crop maturity and nutritional usefulness have been generalized, nutritive value consequently being often expressed as a function of forage maturity.

Nutritional usefulness until of late has been regarded as a character, not of independent expression, but as a function of other agronomic parameters, i.e., stem/leaf ratio, earliness of flowering, and consequently, as indicated above, of maturity since these agronomic characters are linked to seasonal growth patterns. Because of this, all nutritional investigations of forages have been made on a "field" basis -- bulked samples obtained from field or plot studies. The inheritance of characters favoring high nutritive value has been barely investigated. Alternatively, the inheritance and incorporation of characters such as insect and disease resistance, which may be diametrically opposed to nutritive value, have been considerable.

Elliott (1963) has indicated that the individual clone in heterozygous species and the relatively homozygous line in self-fertilizing species "represents the level at which direct manipulation of heredity may be applied in the upgrading of nutritional quality or efficiency."

There is no reason to suppose that the nutritional quality of a forage crop cannot be improved. The screening of large populations from which clones of superior nutritive value can be selected is complicated by the lack of available techniques. Similarly, the estimation of forage value and the changes forages undergo in the field are hampered by the lack of such techniques. In vitro fermentations, allied with other more definitive assays are useful tools for such preliminary estimations.

The 6-hour fermentation is a tool capable of predicting the nutritional character of a forage. Two samples of a standard forage, evaluated in a number of fermentations over a two-year period, were used to estimate day-to-day variations. The overall mean of the standard in all fermentations was 31.0% and the standard deviation of the mean was 3.86. The standard error of the mean for an individual determination was 2.73; the standard error of the mean for the overall mean was 0.57. The standard deviation for the difference values between standard samples within a fermentation was 1.17. Standard errors of the mean for samples run in quadruplicates within a single fermentation were between 0.49 and 0.56. These values are comparable with those given in the literature (Tilley and Terry, 1963; Barnes, 1965; Hershberger et al., 1950; Bowden and Church, 1962).

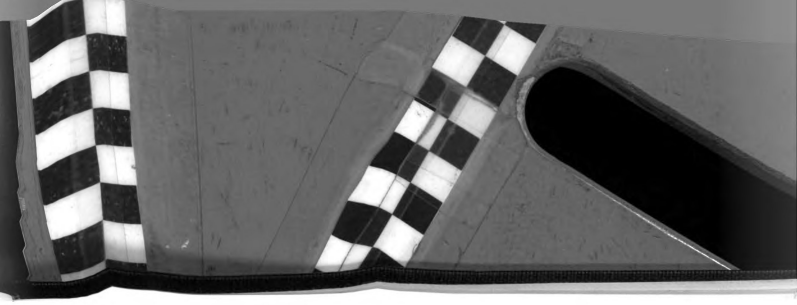
Analysis of variance for standards indicated most variance was between fermentations as opposed to within fermentations. This would indicate that samples should be evaluated in as many fermentations as possible at the expense of duplication within fermentations. Consequently, single estimates of bulked forage from the field plot must be looked at askance unless they are backed up with other more accurate estimates of nutritional usefulness.

A. The Environment -- Variation Induced Through Cultural Techniques

The culturalist at the basic level has the choice of species and/or variety, and the number and time of harvests. That species have differing utilizable or available nutritional qualities is clear.

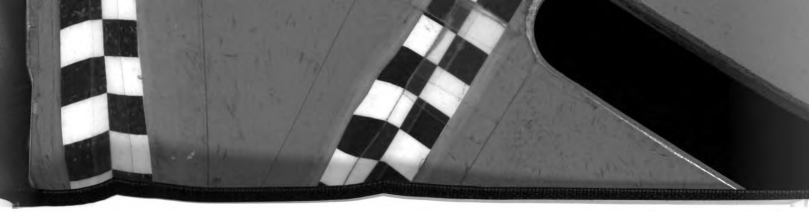
In the 1964 experiment, alfalfa had the highest nutritive value of all the species evaluated, regardless of the cutting frequency. However, as the number of cuttings decreased on a seasonal basis, this nutritional superiority decreased. There was, then, a species/cutting interaction.

As Baumgardt and Smith (1962) state, declines in nutritive value were associated with advancing maturity. This statement has, however, but limited application since under practical conditions forage crops are rarely permitted a full cycle of development. In the experiments reported herein, forage which remained uncut throughout the growing season lost nutritive value at a rate characteristic of the individual species. Bromegrass, birdsfoot trefoil, alfalfa, reed canarygrass and



orchardgrass retained 57.0, 47.0, 43.3, 43.0 and 16.0%, respectively, of their nutritive value when they remained uncut from initial spring growth to September 1. Bromegrass retained a high proportion of its initial nutritive value. This retention was not due to a delayed maturity sequence. Bromegrass and orchardgrass headed, flowered and set seed simultaneously; both had similar DMD values during spring growth. The ADL on September 1 was 19.9 for orchardgrass and 10.4% for bromegrass. Yet alfalfa forage sampled at the same time contained 13.8% ADL and its DMD value was higher than that of bromegrass. Similarly, reed canarygrass with a lignin content of 10.6% had a lower DMD value than that of bromegrass. Total lignin content, obviously, was not the sole contributing factor determining nutritive value. Ingalls et al. (1965) indicated legumes contained two to three times as much lignin as grasses harvested simultaneously, and reported those legumes still had a higher dry matter nutritive value index than did the grasses. Consequently, we might infer that a qualitative change takes place in the lignin complex perhaps at a time coincidental to increasing quantitative changes of lignin, and that this has a definitive effect on forage nutritive value. Certainly, only minor quantitative changes occur in lignin content at the time of most rapid change in nutritive value.

Increases in nutritive value immediately following a cutting were such that frequently DMD values were obtained superior to those of initial spring growth. This was in contrast to the findings of Reid et al. (1959) who indicated aftermath forage was inferior to initial



spring growth. The determinative factor must be the time interval between cutting and the sampling of regrowth, since declines in the nutritive value of regrowth, especially for forages cut three or four times per season, was frequently very rapid. These declines in nutritive value were typically minor in the regrowth of forages following the first cutting of the two-cutting system. In this system the nutritive value of the legumes remained high until flowering occurred in early August. The grasses remained vegetative following the first cutting and a substantial loss of nutritive value did not take place until six to seven weeks later. Consequently, this decline was not associated with a change in morphological condition. This change may be associated with increased levels of structural constituents since Norman (1939), Sullivan et al. (1956) and Reder (1954) have indicated such constituents to be higher in second cuttings compared to first cuttings. Again it is tempting to suggest that while quantitative changes undoubtedly occur, these are not the determinative factors, but that associated qualitative changes have the definitive effect.

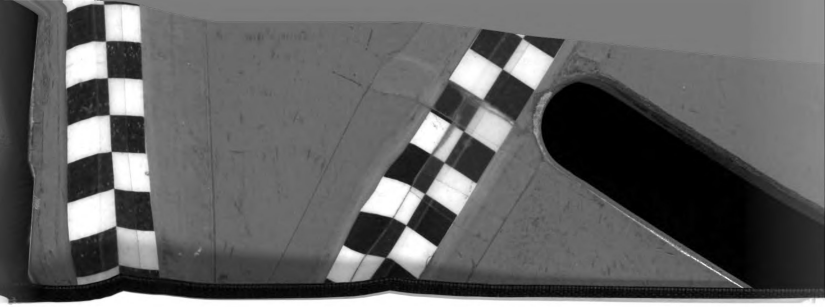
The rapidity with which changes in nutritive value occur in forages cut frequently, compared to those cut less frequently, indicates that the time interval between growth initiation and the first and subsequent cuttings may also have a definitive function. Doubtless, structural changes are associated with this time interval.

Forages cut at least once prior to September 1 had high nutritive value in the fall. The grasses under the four-cutting system were

still increasing in nutritive value at the time of the final sampling. The legumes under the same cutting system lost nutritive value from August onwards. The actual nutritive value in the fall was a characteristic of both species and cutting system. Depending upon these factors, the nutritive value of the forages in September was equivalent to that of the same forage in May. These data indicate that forage of high nutritional usefulness could be prepared and economically utilized through late fall grazing. Rather and Harrison (1938) showed that cutting of alfalfa after October 15 or especially after October 30 had but slight effects on the following year's yield. The pattern set by all the examined forages when the two-cutting system was used indicated that earlier fall cutting would produce a forage of high nutritional usefulness. This effectively lengthens the grazing season in lower Michigan under actual farm practice.

The effects of nitrogen fertilizer application on the nutritive value of two reed canarygrass types were examined over a two-year period -- 1965 to 1966. The common type, thick stemmed and coarse leaved, was consistently superior in nutritive value to the fine leaved, fine stemmed Siberian type. Evaluation by sheep indicated the common type superior to the Siberian type both on the basis of intake and weight gain. Dry matter digestibility of the Siberian type was relatively high, but intake was low whereas the common type was acceptable to sheep. The in vitro data consistently indicated common to be superior to Siberian reed canarygrass.

Nitrogen fertilizer consistently affected agronomic characters



such as yield and chlorophyll content, but its effect of forage nutritive value was inconsistent. For both types, sheep intake and weight gain were greatest on forage fertilized at the 150 lb N per acre level. Alternatively, dry matter digestibility of the Siberian type was unaffected. On this basis nitrogen would appear to exert its influence on intake rather than on digestibility per se of forages. The 1965 fermentation data showed the 150 lb N per acre treatment superior to the others for common reed canarygrass. The combined 1965/1966 fermentation data showed nitrogen fertilizer to have no consistent effect on nutritive value. This is in itself consistent with the variable reports in the literature.

Analysis of variance for both 1965 and 1966 fermentation data showed that forage type, season and the two-way interaction between these effects were significant. The interaction was most marked in 1966 (Table 10). The Siberian type lost nutritive value rapidly at the time of heading and also in the fall while still vegetative. The common type was much less variable. The mean DMD values for all nitrogen levels at heading and in the fall, respectively, were 20.0 and 26.6% for the common type and 16.2 and 10.1% for the Siberian type.

Nitrogen application did not delay "burning" on the Siberian type in 1966, although it did in 1965. Differential losses in nutritive value, associated with winter "burn" did not occur until November 1 in 1965, when the respective mean DMD values for the common and Siberian types was 29.3 and 21.1% respectively. Such year-to-year variation has been observed both with regard to structural constituents of

forages and their nutritive values (Phillips et al.,1954; Sullivan et al., 1956; Smith, 1954).

The nutritive value of the common reed canarygrass was as high on November 1, 1965, as values obtained in late May of the same year. This confirms the suggestion that quality forage can be produced in lower Michigan for late fall grazing. Both the common and Siberian types, fertilized at the 150 lb N per acre level in 1965, had DMD values on November 1 of 31.5 and 25.9% respectively, compared to values of 31.2 and 22.8 for the same forages on May 24.

Neither chlorophyll content nor moisture content was a consistently accurate predictor of forage nutritive value. Simple correlations between chlorophyll content and DMD values were low, the greatest dicsrepancies between the two parameters occurring in the spring and fall. The relationship between forage moisture content and nutritive value was not consistent for either species or cutting system. Derived correlations were consistently significant for only the one-cutting system and for alfalfa. In the former case, the greatest fluctuations in both nutritive value and moisture content occurred. Consequently, as with morphological condition, moisture content as a predictor of nutritive value is of value only if forage remains uncut.

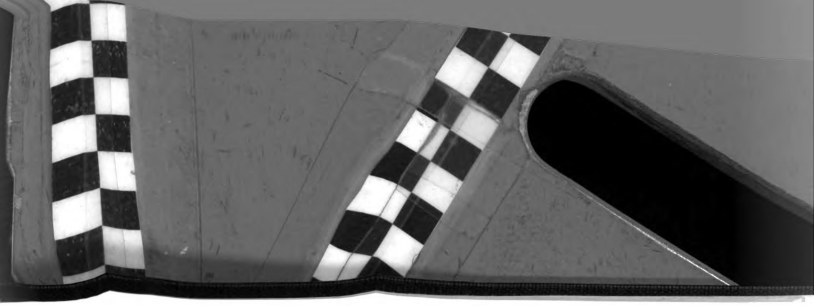
Sudangrass clones grown under a high moisture regime had higher yields (64.1 g per clone) and crude fiber and lower protein levels than clones grown under a low moisture regime (22.3 g per clone). Nutritive value was not significantly affected by moisture level although DMD values were higher by about 3.3% for clones grown under

high moisture. These results are in agreement with those of Reid et al. (1959).

That clones with increased fiber content should also have increased fermentation values is surprising. However, proximate analysis values given by Gaessler and McCandlish (1918) for sudangrass hay at a similar stage of maturity (heading) indicated the values obtained in this experiment to be low for fiber and high for crude protein. This would indicate that fiber levels obtained under the high moisture regime were lower than those normally obtained in forage at a similar stage of maturity. Consequently, fiber may not have influenced the nutritive value of the clones. Alternatively, these clones had grown rapidly and while increased fiber levels were obtained, "fixation" in the form of lignification may not have occurred beyond that occurring in the clones under the low moisture regime.

B. Variability Within Genotypes

Variation between clones of a heterozygous nature is expected. Variation between propagules of a given clone, genetically identical, is surprising. Figure 9 illustrates the morphological variation between two such propagules. While tillers of differing initial dominance were necessarily used to establish propagules, six months were allowed following establishment to permit full development of each propagule. That initial differences would persist over this period is unlikely. However, these propagules were different in such



agronomic characters as vigor, stem/leaf ratio and nutritive value. Since propagules were grown in the greenhouse, environmental influences were expected to be minimized, yet maximum genetical and environmental expression was indicated.

The standard deviations of the DMD values obtained from each set of propagules harvested at anthesis, i.e., 1.17, 0.80 and 1.36, were probably of no greater magnitude than that expected from random error inherent in the fermentation technique. The corresponding standard deviations for the propagules harvested at the boot stage, i.e., 1.53, 1.54 and 2.24, were, however, 24 to 48% higher. This excess variation cannot be attributed to the fermentation technique, nor to genotypic differences since theoretically there were none, but must be attributed to developmental/environmental interaction within, and genotypic/environmental interaction between propagules.

The standard deviation of the DMD values from the Piper sudangrass commercial population was 2.25. Approximately 80% of these clones were at the boot stage and 20% at the flowering stage when they were harvested.

On the basis of the lowest standard deviation obtained from a set of propagules, i.e., genotype 5 at anthesis -- 0.80, and the highest standard deviation obtained, i.e., genotype 6 at the boot stage -- 2.24, environmental/developmental variation may contribute between 36.0 to 99.6% of the total variability encountered in a heterozygous population.

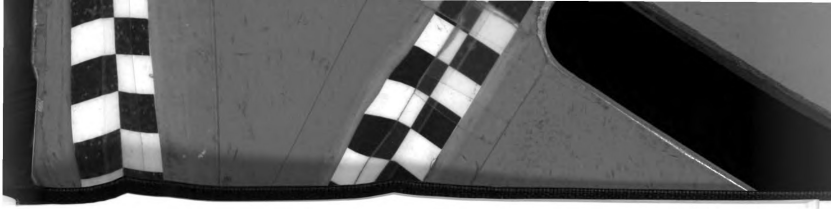
The population mean DMD values in the commercial, S_1 and S_2

populations changed only slightly (Figure 10). In the same population yield decreased steadily. Since these populations were small, conclusions are not warranted. Yet apparently the genetic system controlling nutritional characters is dissimilar to that controlling yield.

C. The Nutritional Usefulness of Species and Varieties Within the Medicago Genus

Nine alfalfa populations, evaluated for nutritive value on an individual clone basis, indicated considerable variation to exist both within and between populations. The range of nutritive value within populations, in terms of DMD, was 17.9% for the M. sativa var. DuPuits at one extreme and 5.6% for the M. glutinosa population at the other extreme. However, estimates of some populations were necessarily restricted in accuracy since the clones available were low in number. The DuPuits population had the highest mean DMD -- 35.4%; the Wisconsin 460 population the lowest mean DMD -- 26.3%.

The considerable consistent variation obtained from the DuPuits, Tuna, Vernal, Hardigan and Culver varieties indicates that upgrading of the nutritional quality of forages may be possible. The environmental/developmental variation has been shown to be large; no estimates of heritability of factors governing nutritional quality have been made. Very probably such factors will be buffered, thus restricting gain. Consequently long-term selection and recombination



of desirable genotypes, hopefully without genetic fixation and loss of other desirable characters, is indicated.

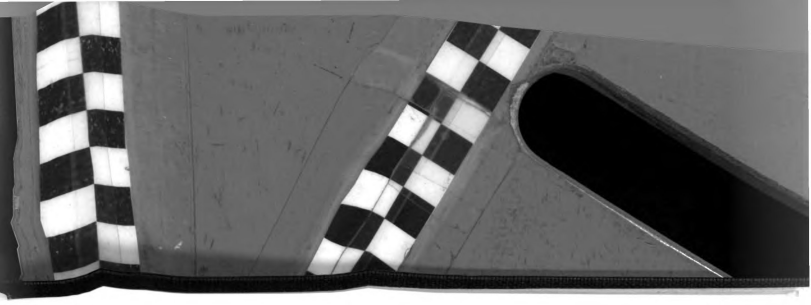
The examination of clones from DuPuits, Vernal and Wisconsin 460 "varieties," differentially grouped on the basis of nutritive value, indicated that while differences in physical characteristics existed within a "variety," extrapolations could not be made quantitatively from one "variety" to another. Those clones high in nutritive value typically were low in CWC, ADL and ADF, and vice versa, regardless of variety. However, clones of Vernal and DuPuits with identical ADF and ADL values did not have identical nutritional qualities. These data confirm the views of Elliott (1962) that the level at which nutritional upgrading of varieties may be initiated is the individual plant. The view of Van Soest (1965), that generalizations between chemical composition and nutritional quality are unfounded, is also confirmed.

Simple correlations derived between DMD values for 6- and 36-hour fermentation periods and characters such as ADF, ADL and CWC, further indicate that alfalfa varieties differ in chemical constitution. The relationships between such values are also dependent upon the genotypic background of the forage concerned (Table 17). DuPuits and Vernal clones exhibited significant correlations between DMD at 6 and 36 hours, while Wisconsin 460 did not. Further, while the ADL, ADF and CWC fractions of DuPuits and Vernal clones were generally significantly correlated to DMD values (especially 36-hour fermentation values), the Wisconsin 460 clones were not so correlated. Van Soest (1965) has stated that interrelationships between intake and digestibility and

chemical composition are species oriented. On the basis of the cited results, taxonomically, species is not specific enough, and narrower taxa must be categorized. Differences are certainly present at the variety level. Physical fractions were more closely related to digestibility than NVI. Correlation values between ADL, ADF and CWC and DMD were higher for the 36-hour fermentation period than those for the 6-hour period. The former period is related to digestibility, the latter period to NVI.

Yield was consistently negatively correlated to nutritive value (Tables 14, 17). The most vigorous, disease-free plants may reach a critical fiber/lignin complex stage at an earlier date than those of less vigor. Such a stage would not necessarily be indicated by either a visual morphological change, or a change in chemical fraction as measured by commonly used analyses.

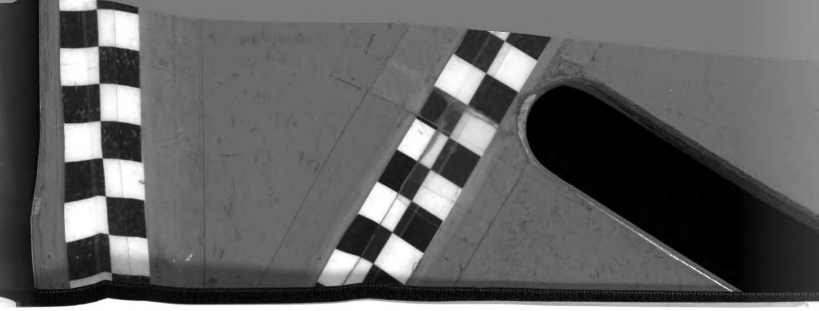
Forage material is fermented in the rumen by a microbial population, the chief products of which are the volatile fatty acids (VFA) acetic, propionic and butyric. Individual alfalfa clones, selected on the basis of their DMD and physical fraction values, differentially stimulated VFA production. Those clones, previously indicated to be of superior nutritive value, were able to stimulate the microbial production of greater quantities of VFA compared to clones of inferior nutritive value. The proportions of VFA produced were not significantly different. Variety differences occurred. DuPuits clones stimulated VFA production for up to 48 hours. Vernal and Wisconsin 460 clones stimulated VFA production for only 36 hours.



Lack of forage substrate was not the cause for the cessation of VFA production. The DMD values for the 48-hour period were greater for the DuPuits clones than the others. The DuPuits clones could apparently sustain a cellulosic fermentation at a rate favorable to microbial activity. This suggests that the fiber complex is not as readily susceptible to microbial degradation in those clones of poor nutritive value.

Differences in the nutritive value of individual clones were still evident following water extraction. Reconstitution of the clones did not re-establish the original clonal nutritive value. Since water extraction essentially removed soluble carbohydrates, the addition of these to the extracted clonal material should logically have re-established the initial nutritive value. Since this did not occur, perhaps the rate at which such carbohydrates became available to the fermentation media had a definitive effect. Rapidly available carbohydrates may have depressed cellulose fermentation, especially when the levels of such carbohydrates, though readily available, were low and the fibrous fraction was considerable. This infers that forages of a high nutritional efficiency have a well-established balance between readily available carbohydrates and cell wall constituents.

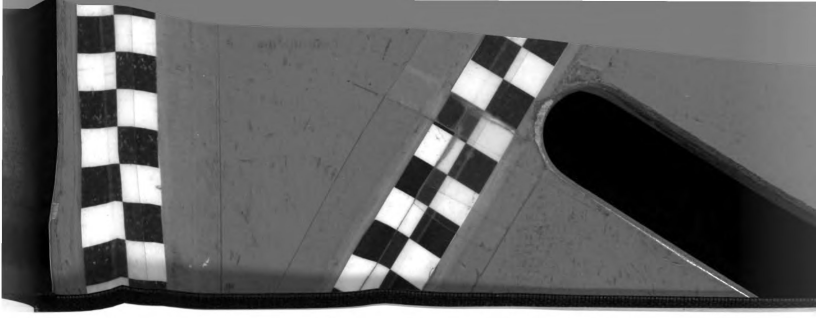
The persistency of differing nutritive values was considerable. The ADF residues of clones W 24 and W 8 still exhibited differing DMD values in a 36-hour fermentation; these values were 6.0 and 2.8%, respectively. The extraction of these clones by progressively more



severe treatments reduced the DMD values obtainable, but the difference between the clones was consistently shown.

The levels of soluble carbohydrates extractable from alfalfa clones were different. These quantitative differences were not, however, indicative of the nutritive value of the clones. Qualitative differences as exemplified by chromatography of the carbohydrate extracts were minimum. The chromatograms of DP 20, DP 69, V 72, V 133 and W 8 were virtually identical. With the exception of W 24, fructose was tentatively identified as the predominant sugar present.

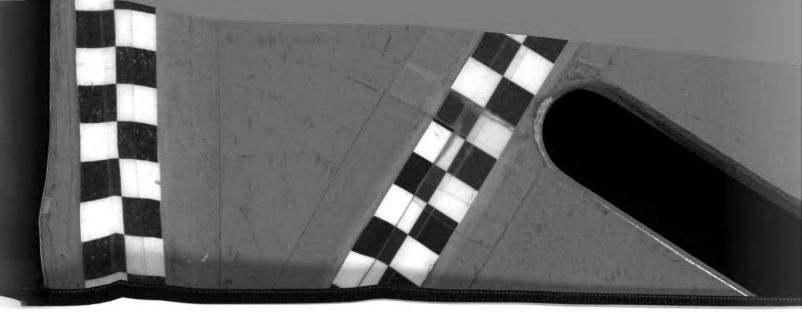
The presence of water soluble phytotoxic principles in alfalfa forage has been indicated previously (Schillinger, 1964; Elliott, 1963a, 1963b; Guenzi et al., 1964). These phytotoxic principles inhibited seed germination of both corn and beans in this study. Water extracts made from clones DP 20, DP 69, V 72, V 133, W 24 and W 8 exhibited antibiosis. However, such levels of antibiosis were not coincidental with the nutritive value of the clones as indicated by DMD and physical fraction values. The phytotoxic principle was readily extracted. A primary cold water extract was significantly greater in its antibiosis properties than was a re-extract of the residual clonal material made with hot water. A seed/extract interaction occurred; seed of various backgrounds did not react similarly to the various extracts. While the levels of antibiosis exhibited by water extracts obtained from the above-mentioned clones were not coincidental with their indicated nutritive value, it must be questionable that any level of antibiosis is permissible if optimum



nutritional efficiency of forages is to be realized.

The quantities of lignin extracted from ADF residues of alfalfa clones were significantly different. Although clones of high nutritive value contained less lignin than the corresponding clones of low nutritive value, they were more susceptible to lignin extraction.

The ultraviolet difference spectra of these extracted lignins were apparently related to clonal nutritive value. Initial extractions of lignins from clones of superior nutritive value had characteristic UV difference spectra such that peak lignin absorption at different wavelengths was of the order $250 > 300 > 350$ mμ. Since these lignins, as indicated by actual weight loss, were readily extracted, these peak values may be taken as indicative of the constitutional make-up of the lignin complex. The UV difference spectra for clones of low nutritive value had peak conformations such that absorption was of the order $250 > 350 \geqq 300$ mμ. Continued lignin extraction of these clones resulted in considerable continued absorption, especially at the 300 and 350 mu wavelengths. These absorptivities would indicate the structural composition of the two lignin types to be different. The greatest difference existed in the lignins having absorptivity in the 350 mu range. These lignins, composed of conjugated phenolic groups, are characteristic of mature lignin (Stafford, 1964). Initial extractions of lignins from clones of low nutritive value not only had increased proportions of these 350 mμ absorbing lignins, but these lignins continued to be extracted for up to 88 hours. This suggests that these lignins are formed in much higher quantities in clones of low



nutritive value than in clones of high nutritive value. The hypothesis may be presented that in fact, nutritional differences between clones, are, at least in part, due to such differences in the lignin complex.

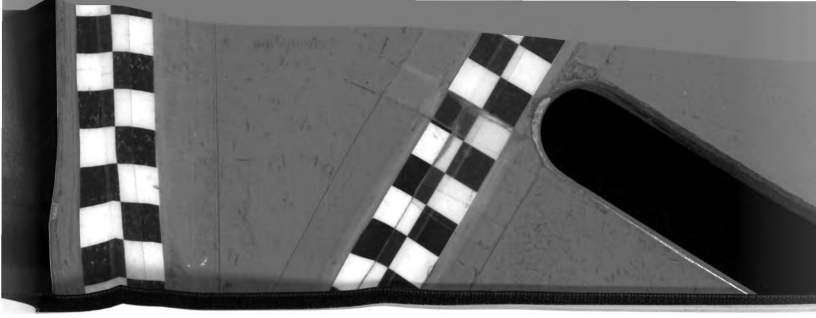
This hypothesis is compatible with the ease of lignin extraction in clones of high nutritive value in which the lignin complex, unstable or immature, is readily degraded. Clones of inferior nutritive value may have a stable lignin/fiber complex resistant to degradation. The degradation of these lignin/fiber complexes by fermentative bacteria may follow similar lines. Immature lignin/fiber complexes are readily fermented, this not being the case with mature complexes. The qualitative difference suggested earlier is satisfied by this hypothesis.

Chromatography of lignin extracts was unsatisfactory since essentially no separation of lignin components occurred. Only two areas could be very tentatively identified, these being protocatechuic acid and the other amino phenylsulfuric acid. The latter compound was obtained consistently regardless of whether a ½-hour or 88-hour extract was chromatographed. Since no separation occurred, apparently the lignin extract was internally bound.

The agronomic relationship of the parameter of nutritional usefulness to the more readily evaluated quantitative characteristic is not known. Certainly the systematic importance or evolutionary significance of factors contributing to or devaluating from the nutritive usefulness of a given taxon has yet to be investigated. A gain in nutritional qualities may be accompanied by a loss of some

other important agronomic characteristic, perhaps even a characteristic which contributes to survival in the field. If this is so, then a reattaining of balance, contributing to overall fitness may have to be induced.

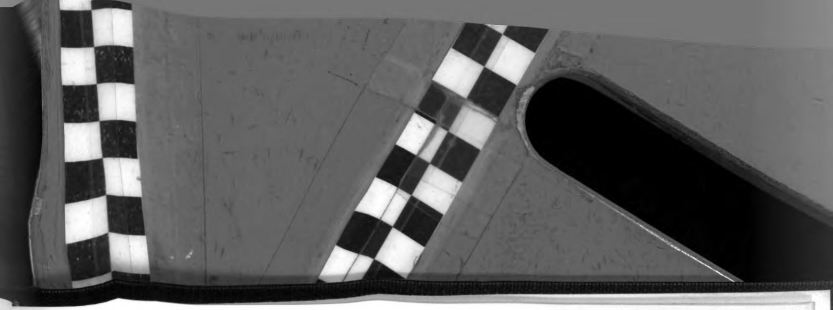
The genetic incorporation and fixation of characters into the genotype of a given taxon may have resulted from some stress during the phylogenetic history of the taxon. The change may have contributed adaptation to the particular environment existing at that time. Under cultural conditions, where a manipulation of the environment is attempted, these characters may have no use and may be detrimental to the eventual usefulness of such organisms. The levels of antibiosis and the differences in lignin structure may be such characters. Lignin has a structural role in plant existence. However, the pliability of this structure may be considerable. The biogenesis of lignins is apparently an orderly process, part of which is enzymatically controlled. Associations between the process of lignification and indoleacetic acid destruction have been made (Jensen, 1955; Siegel et al., 1960). This relationship may offer a mechanism by means of which the lignification process may be controlled. The production of a desired lignin complex which would allow regular plant development while remaining susceptible to ultimate degradation would change the dynamics of grassland utilization.



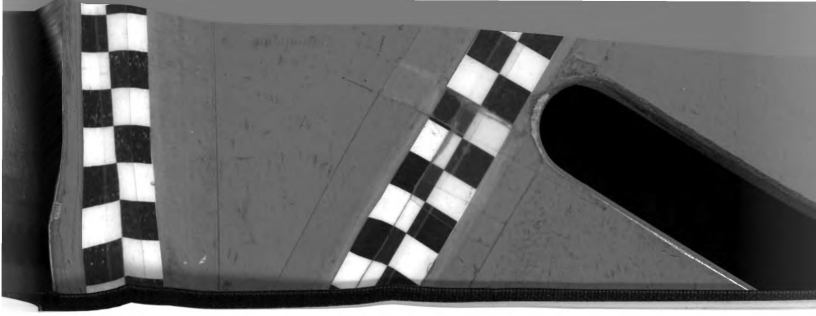
SUMMARY AND CONCLUSIONS

Cultural factors affecting the nutritive value of several forage species were investigated using one greenhouse and two field experiments. The variability of nine populations within the Medicago genus with regard to nutritional usefulness was examined at the individual clone level. Estimates of environmental variability on the individual genotype were made using propagules of genetically identical sudangrass clones. The importance of antibiosis mechanisms, carbohydrate levels and the lignin complex in determining the nutritional usefulness of individual clones were assessed. The study yielded the following conclusions.

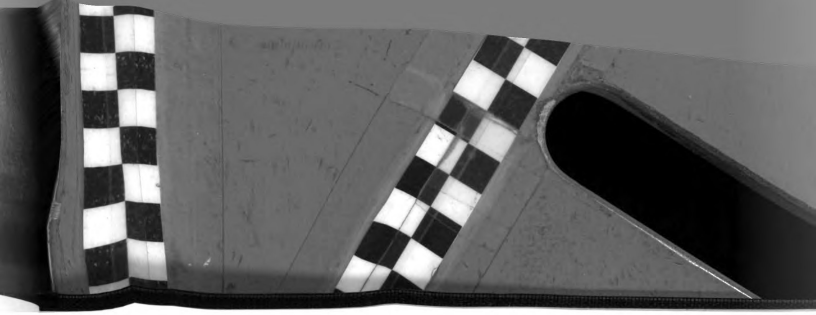
1. Species differed in their nutritive usefulness. Mean values obtained ranked the forages in the order alfalfa, birdsfoot trefoil, reed canarygrass, bromegrass and orchardgrass. Reed canarygrass and bromegrass were not significantly different.
2. Four, three and two cuttings per season were not significantly different in their mean effect upon nutritive value. All were superior to the one-cut system.
3. A species/cutting interaction occurred.



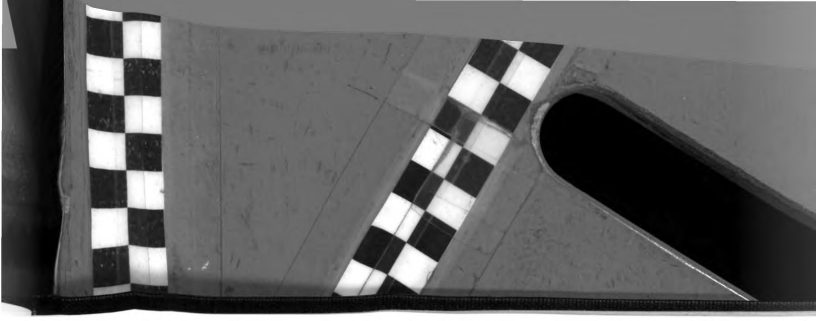
4. Morphological condition and forage moisture content are predictors of nutritive quality only when forage remains uncut throughout the season.
5. Nitrogen effects on forage nutritive value were inconsistent. In 1965 sheep intake and weight gain were higher on two reed canarygrass types fertilized with 150 lb N per acre compared to fertilization rates of 450 and 0 lb N per acre. Grasses fertilized with nitrogen had higher DMD values in 1965 following the second field cutting on July 24, but nitrogen depressed DMD values prior to the cutting. Two-year fermentation data indicated nitrogen had no consistent effect.
6. Common reed canarygrass was consistently superior to Siberian reed canarygrass on the basis of in vivo and DMD results. The type/season interaction was considerable; the Siberian type lost its nutritive value in the fall.
7. The time interval between initial spring growth, the first field cutting and subsequent cuttings was the determining factor associated with cutting effects on nutritive value.
8. The production of high-quality forage for late fall grazing is feasible in lower Michigan.
9. Moisture levels did not significantly affect nutritive value as indicated by DMD data from a 36-hour fermentation.
10. Year-to-year variability in nutritive value was considerable.
11. The genetic systems concerned with yield and nutritional characters are dissimilar in sudangrass.



12. Considerable variation existed between and within alfalfa populations when nutritional value was evaluated on an individual clone basis. DuPuits had the highest population mean DMD value of 35.4%, Wisconsin 460 the lowest -- 26.3%. Within populations, ranges in DMD values of 17% were obtained. The developmental/environmental interaction may be high -- from 36 to 99% of total variability within a sudangrass population was accounted for on the basis of this interaction. This indicated the necessity for repeated estimates of individual clonal value before incorporation of such clones into a breeding program.
13. Fractions such as acid detergent fiber, acid detergent lignin and cell wall constituents were consistently and negatively correlated to DMD values for a 36-hour fermentation period. Simple correlation coefficients were lower when DMD values based on 6-hour fermentations were used. Extrapolations within varieties were valid, but not between varieties within a species.
14. Differences in soluble carbohydrate levels and levels of antibiosis exhibited by individual clones were considerable, but these differences were not necessarily related to nutritive value. Differences in nutritive value between individual clones were still apparent when the clones were reduced to an acid detergent fiber residue.
15. Clones of different nutritive value were differentially susceptible to lignin extraction. Lignin was significantly more extractable from clones of a high nutritive value.



16. Lignin extracts from clones of high nutritive value were characterized by ultraviolet difference spectra. These extracts had absorptivity peaks such that $250 \geq 300 \geq 350$ mμ. Extracts from clones of low nutritive value had absorptivity peaks such that $250 \geq 350 \geqq 300$ mμ. The latter values indicate nutritionally poor clones had a more mature or stable lignin complex than clones of high nutritional value.
17. The suggestion is made that control of lignin development in the forage crop could infinitely improve forage crop utilization.



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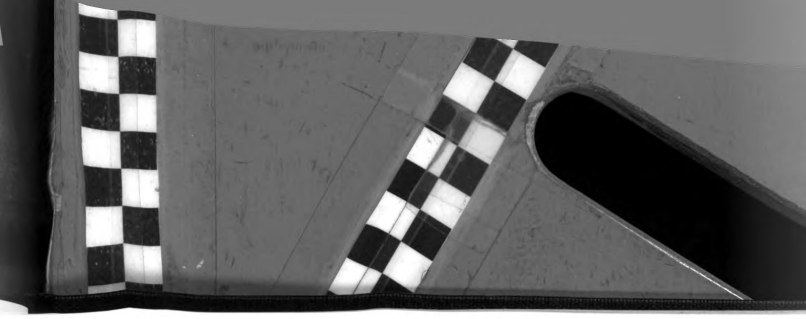

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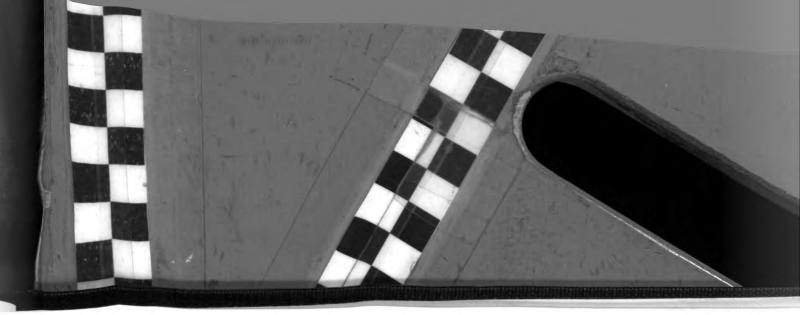

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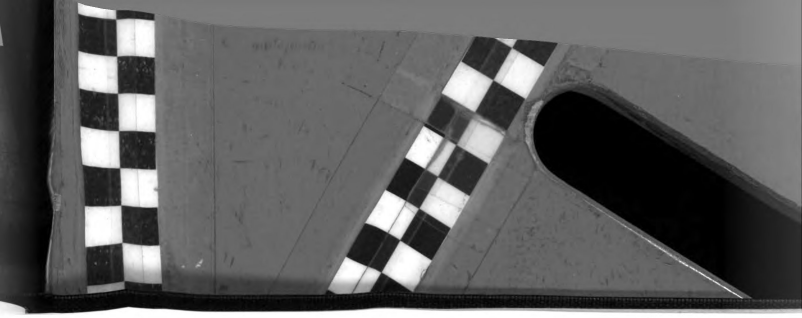

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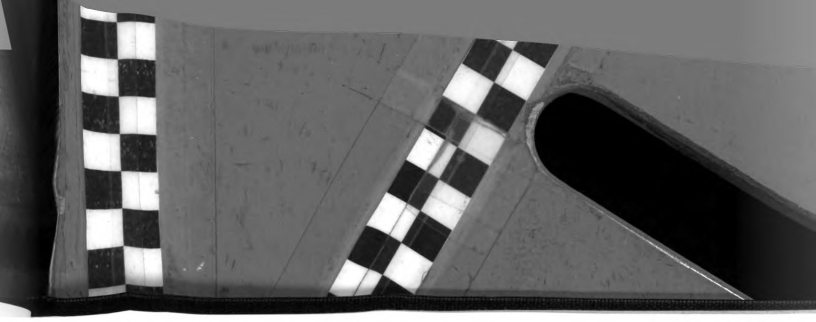

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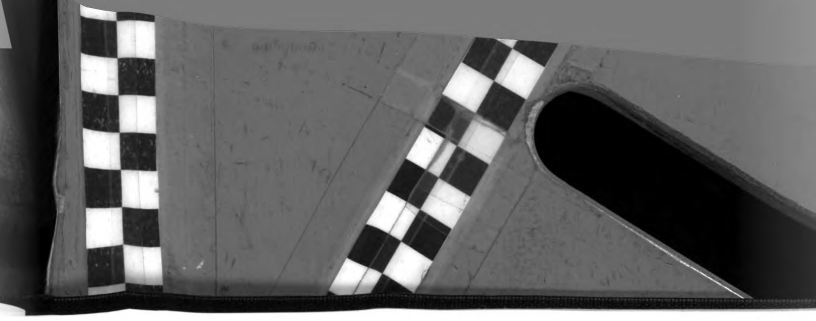

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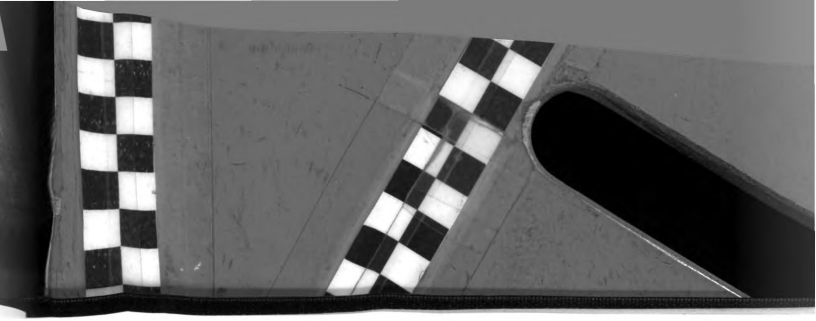

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