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ALFALFA

QUALITY, MATURITY, AND MEAN STAGE OF DEVELOPMENT

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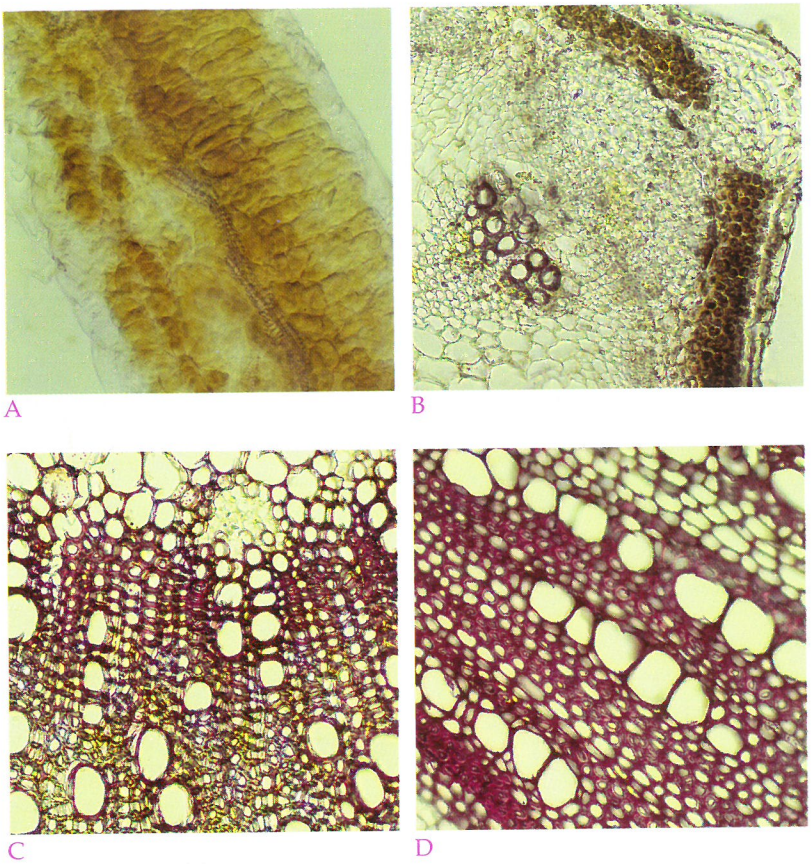


Figure 1. Photomicrographs of alfalfa tissue stained red with phloroglucinol reveal the extent of lignification in the cell walls. Leaf tissue (A, microscopic magnification of 440X) shows only slight lignification (red) in the vascular cells. The dense appearance indicates a high level of cell contents. Young stem tissue from the third visible internode below the growing tip (B, 220X) has thickened cell walls in the vascular bundle and only slight lignification. Older stem tissue from the ninth internode below the tip (C, 220X) shows fibrous secondary vascular tissue and the beginning of general lignification. The oldest tissue from the fifteenth internode below the tip (D, 220X) is almost completely lignified. Sections were taken from a stage 3 stem of 'Oneida' alfalfa in the first growth of the year.

Mary Basl and Dr. J. M. DiTomaso prepared the slide sections for Figure 1. Drawings in Figure 3 were prepared by Lisa Richter and Terry Jancek.

ACKNOWLEDGMENTS

The concept of using stage numbers to calculate the mean stage of development in alfalfa was published in 1972 by Gengenbach and Miller. While working as a Ph.D. student with Dr. Fick at Cornell University, Beverly W. Y. Liu modified that system to allow more detailed study of the vegetative stages. Following Liu's work, Bernard A. Kalu refined the definitions of stages and developed the first equations to predict alfalfa quality from mean stage. As a part of her Ph.D. research with Dr. Fick, coauthor Sharon C. Mueller tested the mean stage concept on alfalfa growing in forage mixtures. Numerous colleagues from across the United States have helped in the continued testing of these methods. Dr. Mueller is now a farm adviser with the University of California Cooperative Extension in Fresno County, California.

INTRODUCTION

Alfalfa is America's most important perennial forage crop. It is usually harvested several times a year and fed as hay, silage, greenchop, pellets, or cubes. Alfalfa may also be used for pasture or grown for seed production. It is high yielding and a good source of available protein for livestock. It can be grown alone or in combination with grasses and other legumes. In crop rotations, alfalfa improves soil structure, builds soil fertility, and reduces pest problems for other crops.

Proper management throughout the life of an alfalfa stand is essential because conditions in one season affect production in following years. Proper management involves several steps:

- Selecting the best variety with pest resistance appropriate for the local environment.
- Proper liming and fertilization (and, in some states, irrigation).
- Timing of cultural practices (such as pest control) for maximum benefit.
- Scheduling harvests to maintain the health and vigor of the stand.

The result of good management is a balance between productivity, quality, and stand persistence.

Although a great deal is known about alfalfa management, production practices must be refined continually to maintain the competitiveness and profitability of enterprises that use alfalfa. All aspects of management are built around the growth and development of the crop. A better understanding of growth and development is the key to better management.

Today, the major limitation of alfalfa management in the humid eastern regions of North America is inadequate control of the nutritional quality of the feed. We have been studying ways to predict the quality of growing alfalfa as a step for improving management. It has long been known that the quality of alfalfa decreases as the crop matures, but the prediction of quality from stage of maturity has awaited precise definition of the mean stage of development. The concept of mean stage should be useful to everyone who works with alfalfa.

This publication briefly reviews the concepts of forage quality and presents detailed descriptions of the stages of alfalfa development. Two methods for calculating mean stage of development are described and related to management and forage quality. The information is intended for use in research, teaching, and extension. Photographs and quality parameters were taken from a two-year-old stand of 'Honeoye' alfalfa grown near Ithaca, New York. Commercial stands from throughout the United States have also been studied.

FORAGE QUALITY

Forage quality determines the contribution of a forage to animal production. It has at least four components:

- Intake—how much is voluntarily consumed.
- Digestibility—how much is absorbed into the animal body through the digestive system.
- Efficiency—the ratio of animal production to nutrient supply.
- Anti-quality factors—components of the feed that inhibit intake, digestibility, or efficiency.

Forage quality is a complex concept that involves both the feed and the animal being fed. It is best determined by testing the feed in an animal feeding trial. Because that is often impractical, researchers have developed methods to estimate forage quality from chemical and physical properties of the feed associated with animal performance.

Forages are particularly difficult to manage in rations because their nutritive value is highly variable. Much of the variation arises from changes that occur between the time the forage is cut and the time it is delivered to the animal. Rain damage after mowing, or molding and heating in storage, can have great negative effects on forage quality.

The nutritional value of growing forage also decreases each day as the crop matures. Hence there is a negative association between quality and maturity. In young pre-bud alfalfa, the concentration of nutrients in the herbage is high but the yield is low. The yield of useful nutrients is maximized in most cases by waiting to harvest until flower buds can be seen at the stem tips. Until that time, yield increases faster than quality falls. By the time blooms are abundant, yield increases no longer keep up with quality declines.

There is another reason for harvesting about the time of first flowering. Ruminants, especially animals used for reproduction and lactation, have a requirement for nutritional fiber. Fiber is usually regarded as a negative factor in forage quality, but harvesting alfalfa too early in the cool, humid springs that sometimes occur in the Northeast can result in inadequate fiber levels. Less nutritious forage with more fiber must then be added to the diet.

Using a microscope, it is possible to see the structural and chemical constituents of plant cells that are associated with forage quality. The most prominent feature is the relative amount of cytoplasm and cell wall (Figure 1, inside front cover). Cytoplasm is usually called "cell contents" in forage quality work, and it contains most of the crude protein, soluble carbohydrates, and lipids of the cell. Cell contents are highly digestible and contribute to high intake and efficient forage utilization. Leaves and stem tips are always high in quality and cell contents.

The cell wall encloses the cytoplasm and is composed of mostly cellulose, hemicellulose, and lignin. These are the chemicals of cellular fiber and they provide structural strength for the plant. Thick secondary walls form in vascular strands and stems. As cell walls mature, especially in older stems and toward the base of the plant, they are impregnated with lignin, which adds more strength to the fiber. Cellulose and hemicellulose are only partially digestible in the ruminant digestive system. The presence of lignin further reduces digestibility. That is why stemminess and maturity are usually associated with lower alfalfa quality.

FORAGE QUALITY ANALYSIS

Modern forage quality analysis reflects the distinction of cell wall and cell contents. Boiling a forage sample in neutral detergent gives a fibrous residue (the neutral-detergent fiber or NDF) that is nearly identical to the cell wall. The remainder (1 – NDF) approximates the cell contents (Figure 2). The NDF fraction contains mostly cellulose, hemicellulose, and lignin. The original cell wall also has a small amount of pectin, which is soluble in neutral detergent, and protein.

Boiling in acid detergent gives a residue called acid-detergent fiber (ADF), which is mostly

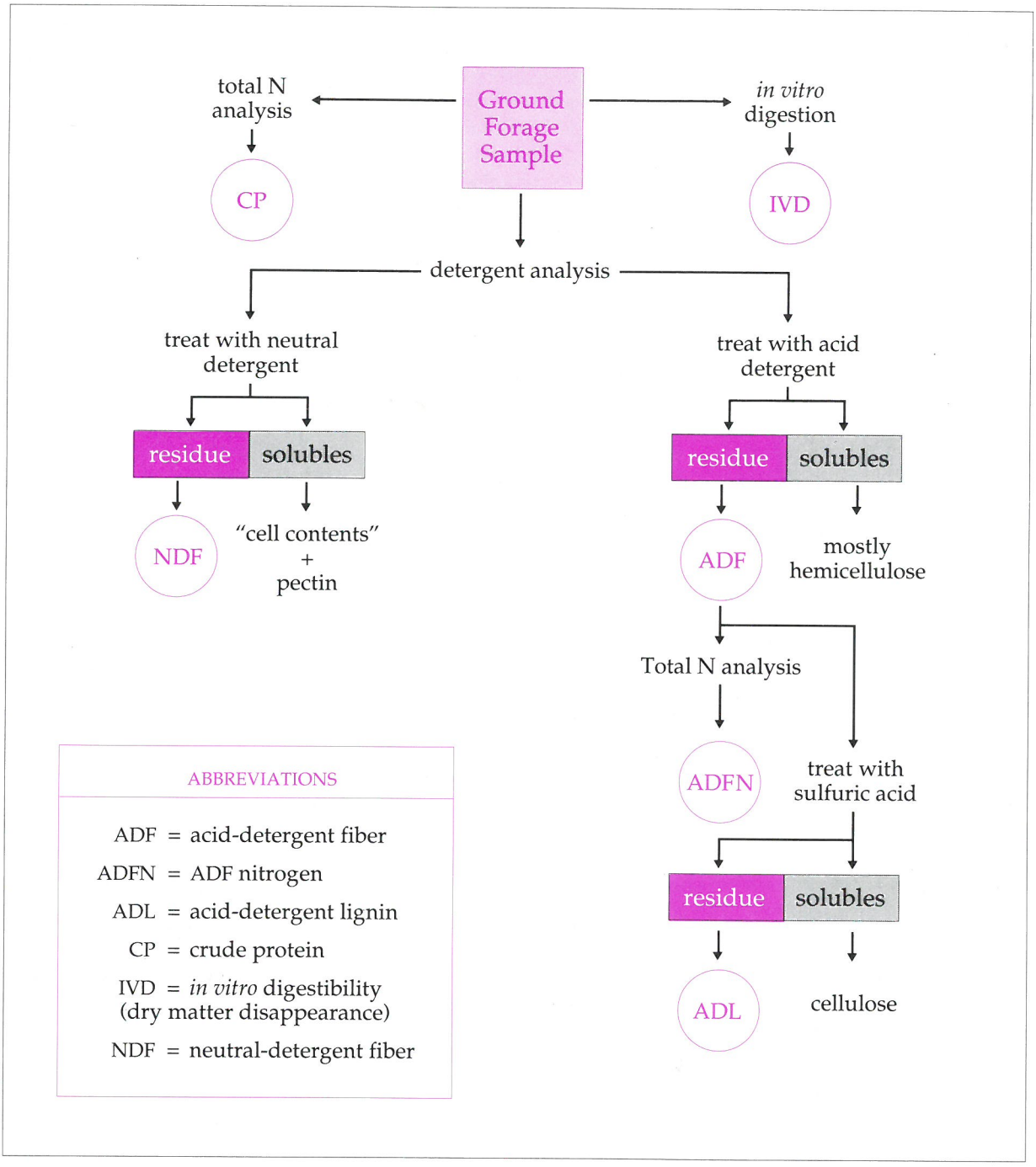


Figure 2. The laboratory procedures to determine forage chemical composition and quality are related to one another as shown here. The diagram is based on the methods of Goering and Van Soest (1970).

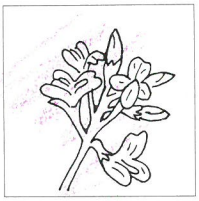
cellulose and lignin. The lignin fraction of the cell wall can be assayed with either permanganate, which dissolves the lignin, or with sulfuric acid, which leaves the lignin as residue. The lignin residue is called acid-detergent lignin (ADL).

The cell contents contain most of the crude protein (CP) of the herbage. The CP content is measured by determining the amount of elemental nitrogen (N) in a herbage sample and multiplying by 6.25. Crude protein includes true protein, amino acids, and other nitrogen-containing compounds such as chlorophyll and nucleic acids. The CP fraction may be subdivided by measuring the nitrogen content in the ADF fraction.

Acid-detergent-fiber nitrogen (ADFN) represents nitrogen that is unavailable to digestion through binding in the cell wall or through chemical alterations in heat damage to forage. Sometimes soluble CP is also determined. Soluble CP provides rapidly available ammonia, which can be used by rumen microorganisms to synthesize microbial protein. Excessive levels of rumen ammonia transfer to the blood and are converted to urea and excreted via the urine. Extremely high levels of soluble CP can result in excess ammonia and reduced animal production.

The feed fiber fractions (NDF, ADF, and ADL) are negatively associated with forage quality. The best predictor of intake and efficiency seems to be NDF. Digestibility can be estimated more directly by *in vitro* fermentation of a feed sample with microorganisms collected from the rumen (Figure 2). ADF and *in vitro* digestibility (IVD) have been used to estimate the total digestible nutrients (TDN) or net energy of the feed. Crude protein (CP) is routinely estimated in quality analysis because it represents one of the primary nutrients to be balanced in most livestock rations.

Taken together, these five parameters of quality (ADF, ADL, CP, IVD, and NDF) provide a good basis for estimating forage nutritive value in the laboratory. Other laboratory procedures, such as near infrared spectroscopy (NIRS), must be correlated with these basic forage properties to be useful in predicting quality. The mean stage of development, as defined in the following sections, quantifies alfalfa maturity so the relationship between maturity and quality can be used to make predictions as well.



THE STAGES OF ALFALFA DEVELOPMENT

As the crop develops, changes in the plant can be seen on individual stems. As alfalfa matures, the stems progressively pass through vegetative, flower bud, flower, and seed pod stages. Several stems at various stages of development can frequently be found on one plant. If the crop is uncut, as in seed production, later flushes of young growth intermingle with older stems.

The stages of alfalfa development are determined by examining the stems. Characteristics used to evaluate each stage are shown in Figure 3. Photographs in the center spread show stems at identifiable stages of morphological development. They are classified according to the staging system defined by Kalu and Fick.

For many years, the stage of alfalfa development has been estimated using the reproductive status of the most mature stems in the canopy. Thus, alfalfa is said to be at "late bud" or "early bloom." Such information can be helpful, but the new method using numbers allows a mean stage to be calculated. The mean stage more precisely relates maturity to forage quality.

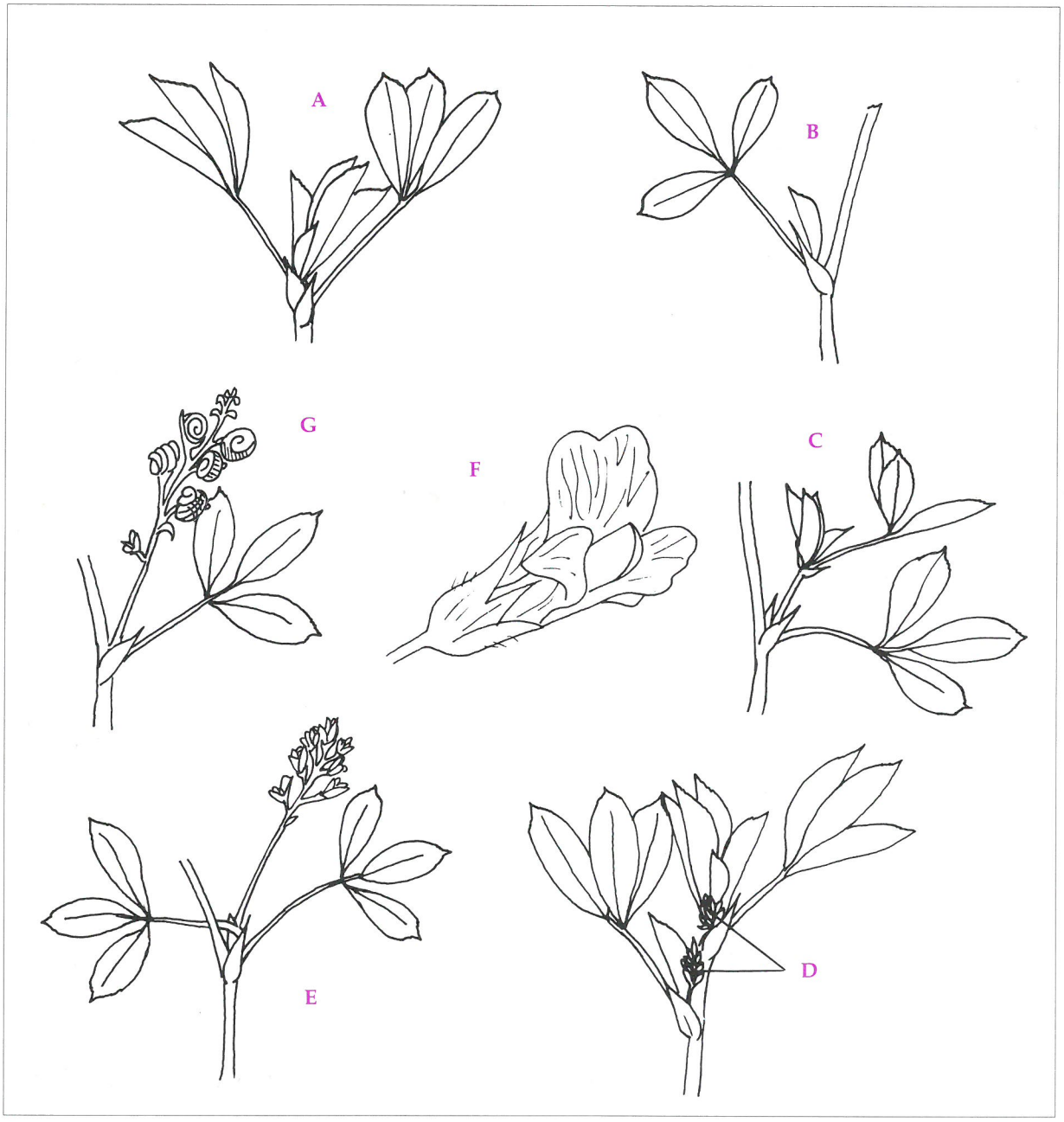


Figure 3. The growing tip of a vegetative shoot (A) is a cluster of folded and unfolding leaves. Vegetative stems usually develop branches as they mature. The first sign of a branch is a folded leaf in the axil where a more mature leaf is attached to the stem (B). The branch develops with a typical clump of folded and unfolding leaves (C), and can also form flowers. Flower buds develop near the shoot tip, hidden in the cluster of unfolding leaves. They become visible (D) as their basal stalk elongates. At bloom, alfalfa flowers are clustered in a loose raceme at the end of a branch (E). The individual flower (F) has five petals, of which the standard is the largest and first to unfold. Seed pods develop after pollination in a characteristic coil of up to four spirals (G). With maturity, seed color changes from green to brown. Because the shoot continues to grow and form new flowers after the first pollination, the first seed pods are found near the middle of the stem.

Vegetative Stages

At early stages of development, reproductive structures are not visible on alfalfa stems. Leaf and stem formation characterize vegetative growth.

Stage 0: Early Vegetative

Stem length ≤15 cm (6 inches)

No visible buds, flowers, or seed pods

The junction between the main stem and a leaf or branch is called the axil. An axillary bud is present in each leaf axil; however, they are so small at this stage that they are not easily seen.

Stage 1: Mid-Vegetative

Stem length 16–30 cm (6–12 inches)

No visible buds, flowers, or seed pods

As the stems continue to develop, axillary branch formation begins with the appearance of one or two leaves in the axil. Development of axillary leaves is more pronounced in the mid-portion of the stem than at the base or apex.

Stage 2: Late Vegetative

Stem length ≥31 cm (12 inches)

No visible buds, flowers, or seed pods

Elongating branches are often found in the axils of the leaves at this stage. It may be possible to feel buds at the growing apex but they are not visible without peeling back the enclosing leaves. Stage 2 stems are often rare in midsummer because of the rapid appearance of buds on shorter stems. This is a result of environmental conditions that hasten maturation.

Flower Bud Development

Flower buds first appear near the growing apex of a stem or an axillary branch. At the transition from the vegetative stages to the bud stages, flower buds can be difficult to identify. At first, buds are small, distinctly round, and appear hairy or fuzzy. In contrast, new leaves are flattened and oblong.

Stage 3: Early Bud

1–2 nodes with visible buds

No flowers or seed pods

Flower buds appear clustered at the stem tip because of the closely spaced nodes in that part of the shoot. As the nodes elongate during development into the next stage, it becomes easier to distinguish individual nodes for the purpose of counting.

Stage 4: Late Bud

≥3 nodes with visible buds

No flowers or seed pods

This stage differs from the previous one only in the number of nodes with flower buds. The structure of the developing inflorescence becomes visible with elongation and clearer separation of individual flower buds in the raceme.

Flowering

When environmental conditions meet specific requirements for temperature and photoperiod, flower buds develop into flowers. Flowering normally occurs in the field, but in the autumn when there are fewer than 12 hours of daylight, buds may abort without forming flowers. Flowers may be purple, blue, cream, yellow, white, or variegated combinations of those colors.

Stage 5: Early Flower

One node with one open flower

No seed pods

To be counted as an "open" flower, the standard petal of the flower must be unfolded. One or more flowers within the raceme may be open; however, the definition of stage 5 describes open flowers at only one node. Because one raceme arises from each node, the number of racemes with open flowers is actually what is counted. Flowering usually begins near the apex of the stem while buds are still developing rapidly above and below the point of initial flower opening.

Stage 6: Late Flower

≥2 nodes with open flowers

No seed pods

This stage differs from stage 5 in that stage 6 has more racemes with open flowers. Nodes with flowers are spread throughout the mid-portion of the stem.

Seed Production

If flowers are pollinated, they will ordinarily develop seed pods. In some environments, pollination is poor and only a few flowers form seed. Typically, alfalfa is harvested for feed before the seed-bearing stages, when quality is lowest.

Stage 7: Early Seed Pod

1–3 nodes with green seed pods

The spiral-shaped green seed pods are not always easy to see. Typically, seed pods first appear from the mid-portion to the base of the main stem while the upper nodes are still flowering. Again, one or more seed pods may be found on each raceme, but only 1–3 racemes with seed pods are present on stems in stage 7.

Stage 8: Late Seed Pod

≥4 nodes with green seed pods

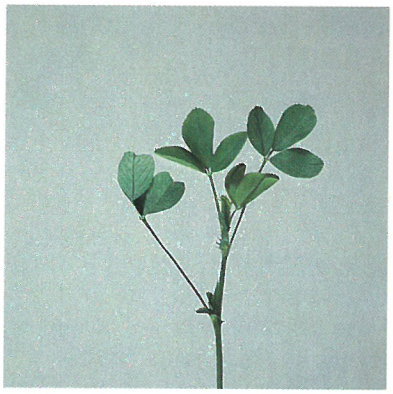
At this stage the older stems are highly branched and they may be lodged. Many leaves have fallen off the plant and many of those that remain are yellow.

Stage 9: Ripe Seed Pod

Nodes with mostly brown mature seed pods

As the seed pods ripen, they turn brown and dry. At this stage, most of the leaves on the lower portion of the stem have been lost and the stem is quite thick and fibrous. Alfalfa that is grown for seed production is harvested at this stage, when most of the seed is mature.

STAGES OF ALFALFA DEVELOPMENT


Stage 0: Early Vegetative


Stage 1: Mid-Vegetative


Stage 2: Late Vegetative


Stage 3: Early Bud


Stage 4: Late Bud

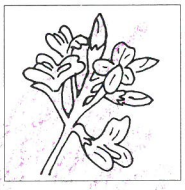

Stage 5: Early Flower


Stage 6: Late Flower


Stage 7: Early Seed Pod


Stage 8: Late Seed Pod


Stage 9: Ripe Seed Pod



CALCULATING THE MEAN STAGE OF DEVELOPMENT

Collecting a Sample

Two methods have been used to calculate the mean stage of development for alfalfa herbage. Mean Stage by Weight (MSW) is based on the dry weight of herbage in each stage. Mean Stage by Count (MSC) uses the number of stems in each stage to quantify maturity. Both procedures require a random sample of at least 40 alfalfa stems.

A representative sample of alfalfa can be collected by any of several different methods. Stems can be taken from either a randomly selected square area (0.1 m^2 or 1 ft^2) in the field, or a specified distance along an obvious drill row. Cut stems, leaving a 3 cm (1 1/4 inch) stubble. It is important to collect all the harvestable alfalfa stems in the sample area. Remove weeds and dead material from the alfalfa sample.

Samples can be stored temporarily in plastic bags in a cooler, but longer storage requires a freezer. If frozen, the sample should be allowed to thaw before the stems are examined and sorted. Otherwise, frozen tips will break off and cannot be associated with the original stem.

"Staging" the Stems

Separate individual stems into the ten stages of development. Our experience using the system indicates that the most difficult aspects are the relatively subjective decisions involved in classifying individual stems. The most important concern is consistency. We have not counted buds until they are visible. An elongated flowering branch is counted as a flower even if the flowers have fallen. Later in development, however, an elongated flowering branch without flowers is counted as a seedpod if seedpods are present on other stems. In New York State, the most mature stems of an alfalfa stand usually pass through one stage per week during the first growth of the season.

If the shoot apex is removed or damaged for any reason, axillary buds below the point of injury form branches and development continues. The most mature characteristic of the shoot, whether on the main stem or a branch, is used for classification. In the case of deer grazing, the damaged stems are discarded if there are only a few in the entire herbage sample. If there are many, we have classified the damaged stems according to the most developed characteristic found on the axillary buds or lateral branches.

The stem classification system developed by Kalu and Fick is not entirely morphological because the three vegetative stages are distinguished by length. As a consequence, stage 2 is sometimes skipped in the developmental sequences of summer, when stems are usually shorter than in the spring.

Counting the nodes for the vegetative stages would be an entirely morphological system, but it would also require a great deal more care and effort. Studies in Indiana by J. J. Volenec and his colleagues showed that the number of nodes at a common stage depends on plant population, but stem length does not. Thus counting nodes probably will

not improve the precision of this system.

The time it takes to classify a herbage sample varies depending on the number and range of stages present. Most young samples have stems in only one or two categories. Older samples can have stems in each stage from vegetative through seed pod, therefore taking longer to classify (Figure 4). On the average, 10 to 20 minutes per sample is required once the criteria for each stage are learned.

It is important to remember that all but the youngest herbage samples contain several stages. To avoid confusion, one should carefully distinguish developmental stage 3 (those stems in a sample at the early bud stage) from mean stage 3.0 (the weighted average stage for all stems in a sample). We recommend that a decimal point be included with mean stage values to avoid ambiguity.

Determining the Mean Stage

Stems from each stage should be counted to determine the mean stage by the MSC procedure. For MSW, stems from each stage should be dried in individual bags in a forced-draft oven until they reach a constant weight. We have used a temperature of 65°C so quality could be analyzed on the same samples.

Mean Stage by Count (MSC) is calculated as the average of the individual stage categories present in the herbage sample, weighted for the number of stems at each stage:

$$MSC = \sum_{S=0}^{9} (S \cdot N)/C$$

Mean Stage by Weight (MSW) is calculated similar to the way MSC is, except the average of the individual stages is weighted for the dry weight of stems in each stage:

$$MSW = \sum_{S=0}^{9} (S \cdot D)/W$$

where

S = Stage number (0–9)

N = Number of stems in stage S

C = Total number of stems in the herbage sample

D = Dry weight of stems in stage S

W = Total dry weight of stems in the herbage sample.

For example, if a sample of alfalfa had 10 stems in stage 3, 25 stems in stage 4, and 6 stems in stage 5, MSC would be calculated as follows:

$$MSC = \frac{(10 \cdot 3) + (25 \cdot 4) + (6 \cdot 5)}{(10 + 25 + 6)}$$

$$= 160/41$$

$$= 3.90$$

Our studies indicate that mean stage increases about 0.05 to 0.15 units per day while the crop is growing rapidly, with

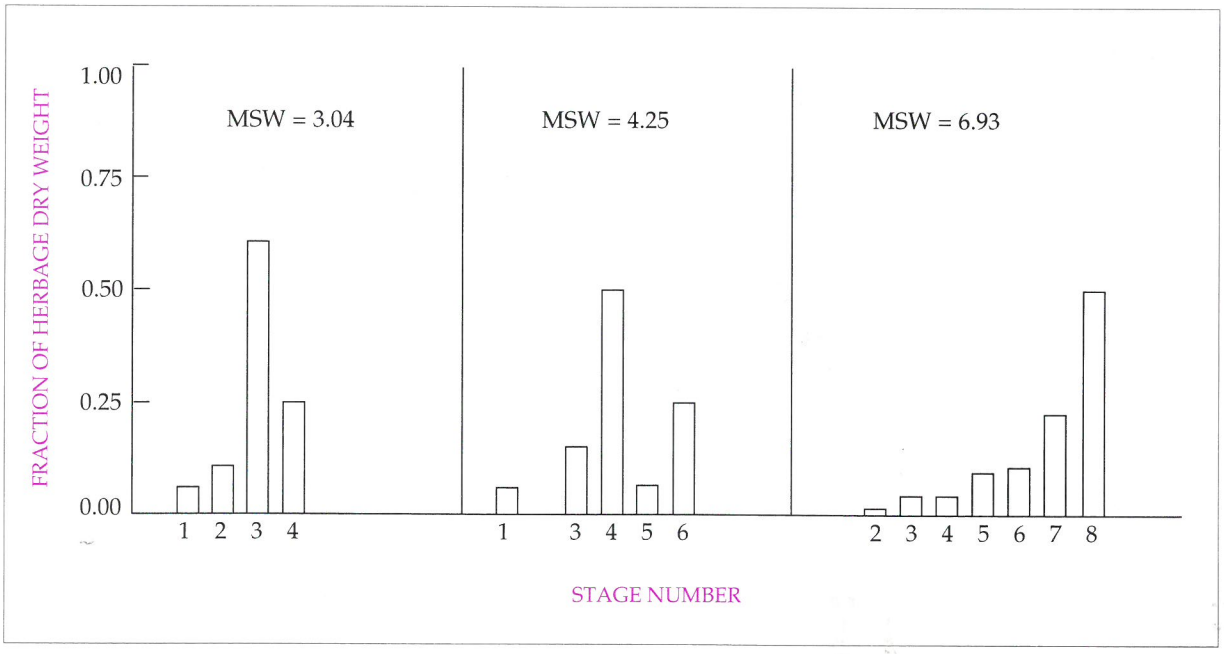


Figure 4. Each random forage sample has stems distributed through several stage classes, and MSW is the mean stage weighted for the fraction of herbage dry matter in each stage. The samples described here were collected by Mueller in Central New York State.

MSW increasing slightly faster than MSC. Four samples from a uniform area of alfalfa had MSC or MSW values with standard deviations of 0.1 to 0.4 units until the time flowers appeared. With more mature samples, the standard deviation tended to be higher, up to 0.6 units. When regrowth began before the herbage was harvested, MSC values declined and standard deviations for MSC became very large (up to 1.5 units). Standard deviations for MSW remained less than 0.8 units.

Both MSC and MSW quantify morphological development of alfalfa. Most users will prefer MSC because it is less tedious. However, only MSW is closely related to forage quality once crown buds start to elongate into an older canopy. In New York, crown bud elongation usually begins after about seven to eight weeks of development. Therefore, older canopies contain both young and old stems. Because MSC is weighted for stem numbers, the increase in the number of younger stems leads to a leveling off and then a decline of MSC (Figure 5). The younger stems also enter into the calculation of MSW, but their small mass limits the impact. Therefore, MSW applies to alfalfa of all ages, and is the preferred method for alfalfa grown for seed production. For practical hay crop management, MSC should be adequate. If it is necessary to convert MSC values to MSW, use this equation, which has worked in New York State:

$$MSW = 0.456 + 1.153 \cdot MSC;$$
$$n = 569;\ r^2 = 0.98;\ RMSE = 0.311$$

This equation applies to samples up to eight weeks of age.

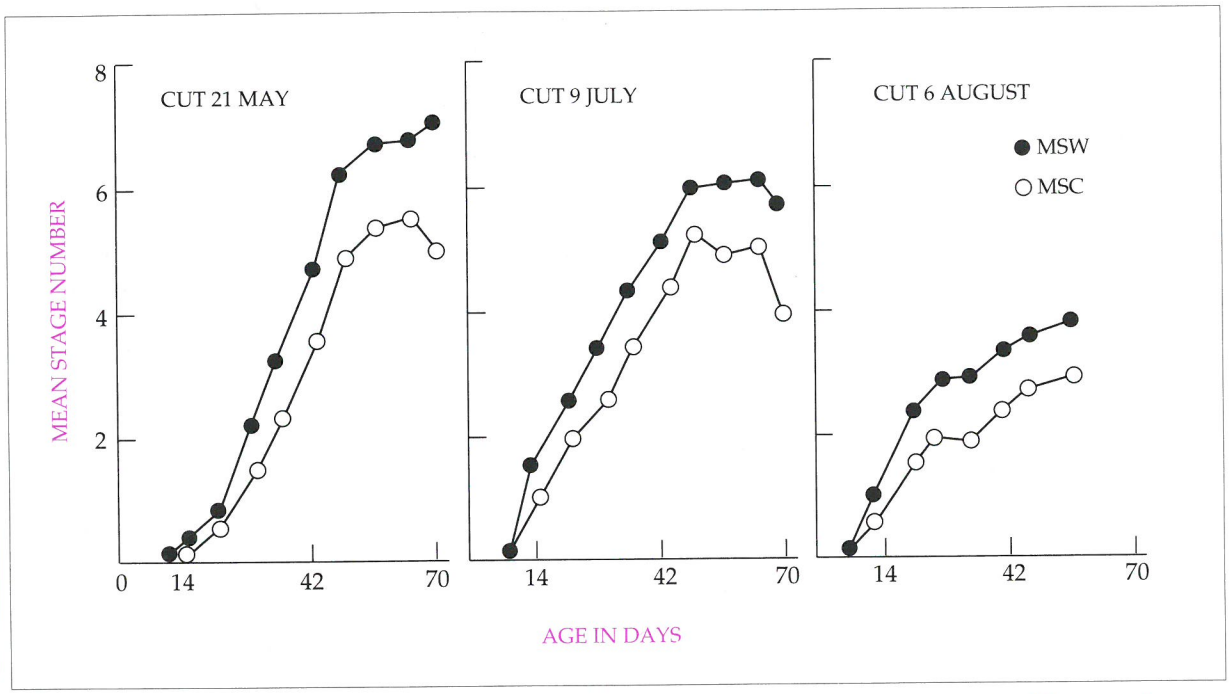


Figure 5. The rate of change in MSW is faster than in MSC, and MSW is less likely to decline in older canopies. These data come from Kalu and Fick (1981) and show regrowth following harvest on the indicated dates.

THE RELATIONSHIP BETWEEN MEAN STAGE AND FORAGE QUALITY

Because mean stage and quality are closely associated in alfalfa (Figure 6), we can predict the quality of the standing crop by measuring mean stage. The following MSW prediction equations have been tested nationally:

CP = 36.15 – 6.09 · MSW + 0.48 · MSW^2; n = 43; RMSE = 2.8

IVD (true) = 93.67 – 4.29 · MSW; n = 42; RMSE = 3.3

NDF = 20.62 + 8.03 · MSW – 0.59 · MSW^2; n = 43; RMSE = 3.6

ADF = 17.05 + 3.85 · MSW; n = 44; RMSE = 3.0

ADL = 2.77 + 1.01 · MSW; n = 44; RMSE = 0.9

Each equation predicts the percentage of a quality component in alfalfa dry matter. If needed, MSC can be converted to MSW so the equations can be used.

The prediction error is estimated by RMSE, which is also indicated by the scattering of points around the lines in Figure 6. Although the predictions are not perfect, mean stage appears to have a very important correlation to the quality of alfalfa. It is also an improvement over the old system. For example, in one study, samples at "first flower" at different

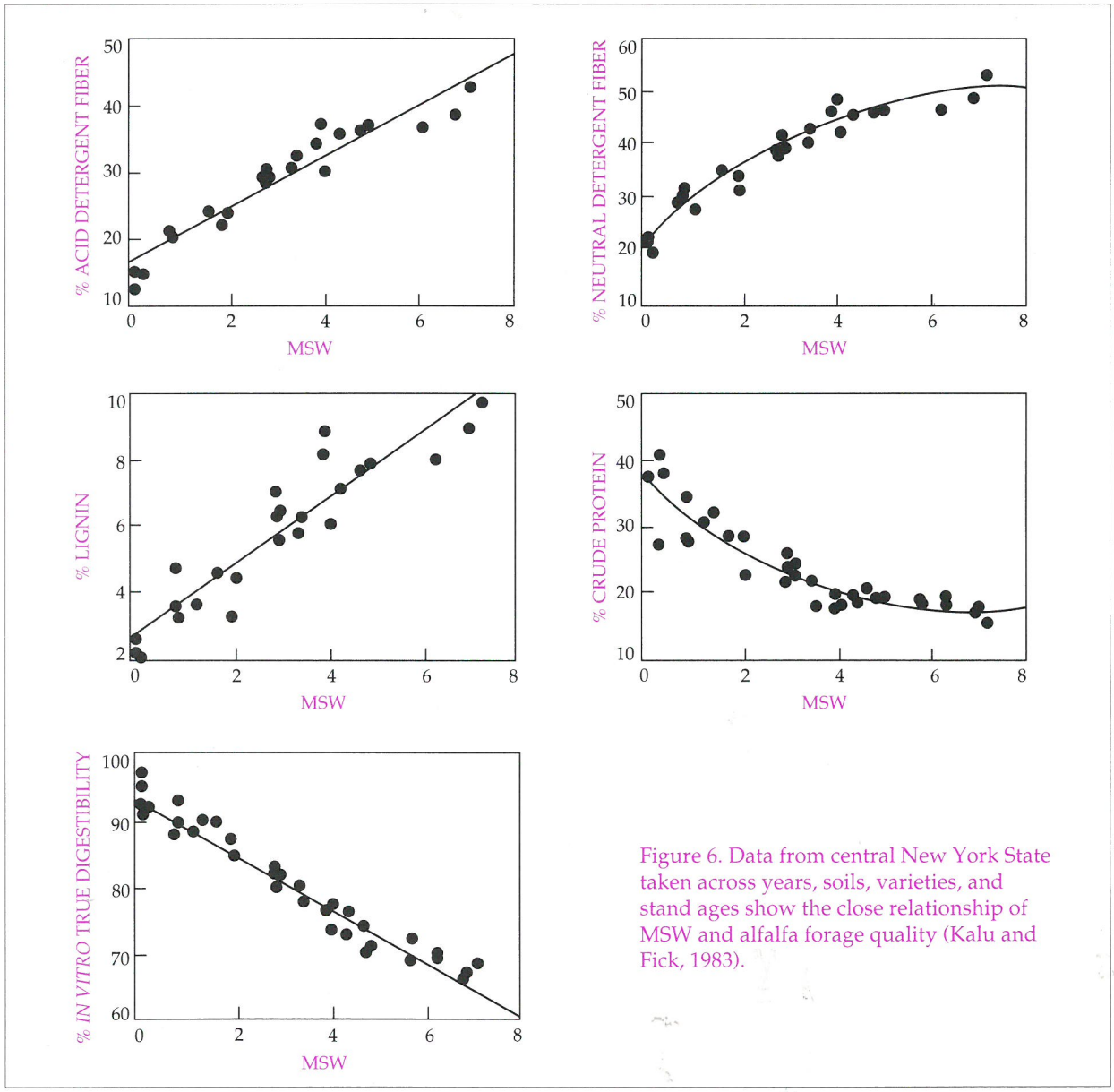
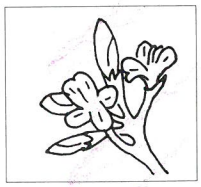


Figure 6. Data from central New York State taken across years, soils, varieties, and stand ages show the close relationship of MSW and alfalfa forage quality (Kalu and Fick, 1983).

times of year had MSW values ranging from 1.7 to 3.4. Thus, "first flower" in the old system corresponds to a range in estimated CP levels of 27.2 to 21.0 percent, respectively.

Mean stage (MSC or MSW) can be used to predict the quality of the standing herbage in the field. Many of the major changes in alfalfa quality occur after the herbage is cut. Thus, mean stage is not a good predictor of hay or silage quality. Instead, it provides a starting-point prediction indicating what the quality might have been under ideal management without post-cutting losses. Predicting quality of the standing crop can help one decide when to harvest and illustrate how much is lost through post-cutting management.

We believe the system can be improved by developing quality prediction equations based on MSC. If equations are developed for regional or local use, prediction error can probably be reduced. States, counties, or even individual farms might have their own MSC equations for alfalfa quality. This manual will help local workers collect the stage data needed for these improvements.

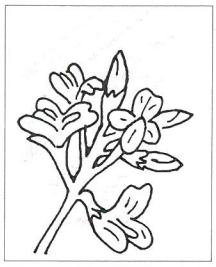


CONCLUSIONS

Planning will become an increasingly important aspect of farm management, but planning requires prediction. The utilization of forages in livestock production is limited by uncertainty about the quantity and quality of forage supplies. To take full advantage of many desirable attributes of alfalfa, producers will need to manage the crop so the yield and quality of the herbage are more predictable.

Great progress has been made in understanding forage quality and the nutritional requirements of livestock that utilize alfalfa as feed. Similar progress needs to made in understanding alfalfa development and the chemical changes that control alfalfa quality.

The determination of mean stage of development is a starting point in refining our understanding of alfalfa and in relating our management programs to crop maturity and quality change. We recommend the mean stage concept to researchers as a description of the state of the crop and to managers as a reference point for planning.



RELATED STUDIES

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