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The Effect of Maturity on Yield, Quality, and Protein Degradability for Three Temperate Forage Legumes (*Medicago sativa* L., *Trifolium pratense* L., and *Lotus corniculatus*, L.)

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By

Jeremias Rodriguez

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ABSTRACT

THE EFFECT OF MATURITY ON FORAGE YIELD, QUALITY, AND PROTEIN DEGRADABILITY FOR THREE TEMPERATE FORAGE LEGUMES (*Medicago sativa* L., *Trifolium pratense* L., and *Lotus corniculatus* L.)

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Traditionally, forage legumes are harvested according to a calendar date rather than at a specific morphological stage of development. A randomized complete block split-plot experiment was used to evaluate the effect of plant maturity on dry matter yield, forage quality, and *in situ* protein degradability of alfalfa, red clover, and birdsfoot trefoil at three cutting dates over five harvest cycles in a two-year study. Legume maturity was quantified using mean stage by weight (MSW). Dry matter yield, neutral detergent fiber, acid detergent fiber, and escape protein (EP) concentrations increased with advancing maturity as evaluated using MSW, while no regular pattern was observed for crude protein. All three legumes had equal EP concentrations in three harvest cycles. In one harvest cycle, alfalfa and trefoil had higher EP than did red clover. These legumes are potential sources of EP for ruminants, and MSW is a useful tool for quantifying yield and quality of red clover and trefoil.

To my parents, Simeona and Paulino,
I am endlessly grateful to both of you for
everything you have done for me through
my whole life. I love you, señores.

To my wife, Rebeca,
With you the life is much easier. Thanks
for being with me during these last 3 years.

To my brothers and sisters,
Because you are the inspiration for continuing
working for a better tomorrow.

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PREFACE

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INTRODUCTION

Forage legumes are an important source of nutrients for feeding ruminant livestock because animals can efficiently convert these high-quality fibrous materials into milk and meat. In many countries in which cattle production is important, forage legumes are valuable because they produce high dry matter yields.

More than 50 percent of the world's cattle population resides in the Tropics and Subtropics; however, these areas only produce 20 to 30 percent of the world supply of meat and milk (Rotar and Krestchmer, 1985). This is partly because forages are not efficiently managed in tropical areas. In contrast, in the temperate zones of North America and Europe, where the dairy enterprise is important, forages are intensively managed and animal rations are often based upon the use of legumes.

Alfalfa (Medicago sativa L.) and red clover (Trifolium pratense L.) are the primary forage legumes used for animal feeds in the northern and northeastern parts of the USA (Smith, 1962; Barnes and Sheaffer, 1985; Taylor, 1985; MacGraw and Marten, 1986). Birdsfoot trefoil (Lotus corniculatus L.) has become a very important forage crop in the last several years in the northwestern and northcentral parts of the USA (Grant and Marten, 1985).

In recent years in the USA, considerable emphasis has been given to harvesting forage legumes at proper stages of maturity. Traditionally, legumes were harvested according to a calendar date instead of a specific plant morphological stage of development. This kind of legume management produced high herbage yield, but a lower protein concentration than considered optimum (Smith, 1962; Van Soest et al., 1978).

Forage quality and yield are influenced both by harvest schedule and by the plant's morphological stages of development at harvest. Differences in dry matter (DM) yield and crude protein (CP) concentration exist among different plant species harvested on differing schedules (Van Soest et al., 1978). Additionally, stages of plant maturity can influence protein degradability (Brink and Marten, 1970 and 1983; Smith, 1972; Kalu and Fick, 1981 and 1983; Fick and Janson, 1990; Olhson and Wedin, 1989; Sanderson et al., 1989).

At more advanced stages of maturity, forage legumes decrease in CP concentration and increase in neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin concentrations. Herbage dry matter yield also increases as cell-wall components (cellulose, hemicellulose, and lignin) increase with maturity (Van Soest, 1967). Rumen escape protein concentration in legumes also may increase with the increase in herbage cell-wall components as plants mature (Griffin et al., 1991).

To obtain maximum nutritive value from alfalfa, red clover, and birdsfoot trefoil, a sound management approach must be used to relate plant maturity stage at harvest to herbage yield, quality, and protein degradability (Marten, 1970; Van Soest, 1978; Sanderson and Wedin, 1989). The experiment described in this thesis provides

information critical to developing a more efficient approach to harvest management of three temperate forage legume species.

Impact of stages of maturity and harvest schedule on forage yield of alfalfa, red clover, and birdsfoot trefoil

In the northern of the USA, researchers have recommended a three-cut system for harvesting legumes (Smith, 1962 and 1965; Marten et al. 1988) because three cuttings before 1 September gave higher seasonal herbage yields than cutting at more or less frequent intervals. In contrast to the three cut-system recommended by Smith, Tesar (1970) reported that a system with four harvests, the first by late May to early June (bud stage), the second by 5 to 10 July (first flower), the third by 20 to 25 August (first flower), and the fourth by 15 to 30 October yielded 10 percent more herbage than the standard three-cut system (1 June, 15 July, and 15 August). Brink and Marten (1983) showed results similar to those of Tesar when using a four-cut system with the last cut by 1 October. Other researchers have also related morphological stages of legume plant development to total herbage yield and protein content and digestibility (Brink and Marten, 1983; Smith, 1972; Kalu and Fick, 1982 and 1983; Fick and Jason, 1989; Olhsson and Wedin, 1989; Sanderson and Wedin, 1989). Management systems for legumes based on harvest schedule and plant morphological stages of development may result in both high dry matter yield and high nutrient content (protein, minerals, and nonstructural carbohydrates) with plant maturity (Sheaffer et al., 1988).

Legume dry matter yields increase from vegetative to reproductive stages of development of the plant (Kilcher and Heinrichs, 1974). Dry matter yield of stems increases at a constant rate throughout plant development from vegetative to reproductive stages. However, the dry matter yield of leaves increases at a constant rate only during vegetative stages; as plants mature, leaf dry matter increases at a decreasing rate (Kalu and Fick, 1983). Kalu and Fick (1983) found that alfalfa had a higher yield when harvested at later stages of development compared to earlier stages of development. They used a 10-stage numerical system (3 vegetative, 2 bud, 2 flower, and 3 seed-pod stages) to quantify phenological development based on a mean stage by weight system. Similar results were reported by Latheef et al. (1988).

On many soils, at later stages of plant maturity, birdsfoot trefoil produces less total DM yield than is produced either by alfalfa or red clover (Marten and Jordan, 1979). Nevertheless, dry matter yield of birdsfoot trefoil increases with advancing maturity similarly to the other two legume species (MacGraw and Marten, 1986).

Impact of stage of maturity and harvest schedule on forage quality of alfalfa, red clover, and birdsfoot trefoil

Forage quality refers to the nutritive value of the plant material and is commonly determined by the concentration of acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude protein (CP) in the herbage. Typically, forage quality declines as plants progress from vegetative to reproductive stages of development (Smith, 1972). As plants mature, major changes occur in the quality of plant stems (Albrecht et al., 1987), while leaves change little in quality with maturity

(Buxton et al., 1985). Total herbage quality declines with maturity due to the decrease in leaf:stem ratio (Buxton and Horstein, 1986). Highest concentrations of herbage nutrients (crude protein and soluble carbohydrates) are usually obtained with forage harvested at immature stages of development (Brink and Marten, 1983).

Components of forage quality

Neutral detergent fiber

The term NDF is synonymous with plant cell-wall components, which are cellulose, hemicellulose, and lignin (Van Soest and Wine, 1967). Neutral detergent fiber is measured by an extraction procedure with a neutral-pH detergent solution (Goering and Van Soest, 1970) and is the measure of plant structural components most related to forage intake in animals. As NDF concentration of a forage increases, intake potential decreases (Van Soest, 1982).

Sanderson and Wedin (1989) reported a quadratic increase in alfalfa stem and total plant herbage NDF concentration with advanced plant maturity. Sanderson and Wedin stated that the increase in NDF concentration was due to a decrease in the leaf:stem ratio. Thus, it appears that stems contain higher NDF concentration than leaves. According to Sanderson and Wedin (1989), 97 percent of the variation in stem NDF concentration was accounted for by the variation in phenological stages of development as measured by MSW. They also reported that 98 percent of the variation in herbage NDF concentration was accounted for by the variation in phenological stages of development as measured by MSW.

Sanderson and Wedin (1989) also reported a linear increase in NDF concentration of red clover stems and herbage with advanced phenological maturity. They stated that phenological stage accounted for 85 and 94 percent of the variation in red clover stem and herbage NDF concentrations, respectively. Birdsfoot trefoil herbage also increases in NDF concentration as plants mature (Buxton and Brasche, 1991). At later stages of maturity, NDF concentration in birdsfoot trefoil has been reported to be lower than at similar maturity stages in alfalfa (Buxton and Brasche, 1991).

Acid detergent fiber

Acid detergent fiber is the plant cell-wall component related to herbage digestibility, and is composed of cellulose and lignin (Van Soest, 1965). The cellulose component of ADF is a potential source of carbohydrate for rumen microbes. Lignin is not degraded by rumen microbes and provides no nutrients, while also decreasing the degradability of cellulose. Lower ADF concentration in herbage indicates higher herbage digestibility (Van Soest, 1982). An extraction procedure with an acid-pH detergent solution is used to determine ADF concentration (Goering and Van Soest, 1970).

As legume herbage matures, the ADF fraction of plant cell-walls increases (Sanderson and Wedin, 1989). The proportion of ADF composed of lignin also increases with advancing maturity, reducing the proportion of cellulose, and therefore, decreasing forage digestibility (Akin, 1989). Lignification of plant cell-walls is a

major factor limiting legume digestibility. As plants mature, core lignin increases, reducing forage digestibility (Akin, 1989; Jung, 1989).

Kalu and Fick (1983) found that ADF concentration of alfalfa leaves remained unchanged (range of 13 to 22 percent) as plants matured. However, they found that alfalfa stem ADF concentration increased from 34 percent at an early vegetative stage to 55 percent at the late seed pod stage. Aman (1985) reported that red clover herbage digestibility decreased with maturity and that the component responsible for this decrease was an increase in ADF concentration of stems. Birdsfoot trefoil ADF concentration also increases as plants mature. Despite the increase in lignin, birdsfoot trefoil has been reported to contain similar or increased digestibility compared to alfalfa and red clover as plants mature (Marten and Jordan, 1979).

Crude protein

Crude protein is composed of both rumen degraded and rumen escape protein, and contains N from both cell-soluble contents and cell-walls (Van Soest, 1967). Crude protein is determined by measuring the amount of total nitrogen in a legume sample using a Kjeldahl modified nitrogen technique (AOAC, 1975), and then multiplying total nitrogen by a factor of 6.25. The factor of 6.25 is based upon the fact that proteins contain 16 percent nitrogen (AOAC, 1975).

Crude protein concentration in forage legumes is, like the fiber fractions, correlated to plant morphological stages of development (Onstad and Fick, 1988; Sanderson et al., 1989; Ohlsson and Wedin, 1989). Crude protein concentration of legumes decreases as plants mature (Moline and Wedin, 1969; Latheef et al., 1988).

Along with other researchers, Smith (1981) confirmed that alfalfa has a higher CP concentration at earlier than at later stages of development. Other researchers have quantified the decline in alfalfa CP concentration with maturity. Kalu and Fick (1983) quantified the decline in alfalfa CP concentration using an index based on the mean stage by weight (MSW) of the plant. Plant MSW is an average of the individual stages present in the herbage sample weighted for the dry weight of the herbage in each stage number (Kalu and Fick, 1983). Kalu and Fick (1983) reported that CP concentration of alfalfa leaves decreased 3 to 9 percentage units between stage 0 (early vegetative) and stage 2 (late vegetative) and 5 percentage units between stage 3 (early bud) and stage 9 (ripe seed pod). Furthermore, Kalu and Fick found that CP concentration in alfalfa stems declined from 2 to 3 percentage units between stage 0 and stage 2, and alfalfa stems decreased less than 2 percentage units in CP concentration beyond stage 2.

The decline in crude protein concentration with alfalfa maturity is, as with fiber, due in large measure to a decrease in the leaf:stem ratio of the plant (Marten et al., 1988). These researchers documented that although alfalfa leaves retained a high CP concentration during the growing season, at later morphological stages of growth the plant stems became the predominant part of the plant, causing a decline in CP concentration of the total herbage. Buxton et al. (1985) found that CP concentration of alfalfa leaves declined continuously from vegetative to early seed stages.

Faberger (1988) reported a linear decline in CP concentration of red clover herbage as plant phenological stages advanced. Faberger found that at early maturity, red clover had 27% CP, but at late maturity, CP concentration was 16%. He also

found that DM yield of red clover increased with maturity, concluding that the increase in DM yield was associated with the decline in CP concentration of the plants. At advanced stages of maturity, birdsfoot trefoil may have a similar protein concentration to alfalfa (Marten and Jordan, 1979). MacGraw and Marten (1986) compared protein concentration of leaves and herbage in alfalfa and birdsfoot trefoil and found that, at early stages of maturity, alfalfa and birdsfoot trefoil leaves had 38 and 31 percent CP, respectively. As plants advanced in maturity, however, both alfalfa and birdsfoot trefoil herbage contained 29 percent CP.

Escape protein

Part of the value of forage protein fed to ruminants is dependent on the amount that escapes ruminal fermentation because this escape protein is directly available for intestinal digestion and absorption (Broderick, 1978; Mullahey et al., 1992). After meeting the requirement of microbes for rumen degraded protein, legumes ideally would provide undegraded or escape protein to supply any protein deficit of the animal (Erasmus et al, 1988). Escape protein is believed to be mostly associated with nitrogen located in the plant cell-walls (Griffin et al., 1991), which represents 5% of the total herbage N (Howarth, 1988).

One way to measure escape protein is to quantify the amount of protein remaining in a sample using an in situ digestion technique. The in situ technique consists of suspending forage material in the rumen, thus creating contact with the rumen environment. This technique allows determination of forage EP by measuring nitrogen disappearance over time (Sauer et al., 1983; Stern and Calsamiglia, 1991).

Undegraded forage N is then determined by subtraction from the original herbage sample (Anderson et al., 1988). The in situ technique is widely used for forage digestibility estimates because it is inexpensive, simple, rapid, and reproducible (Mehnrez and Orskov, 1977). Due to its increased popularity, this technique has been put under continuous evaluation and criticism. Factors such as bag pore size, sample size, and sample particle size have been evaluated using the in situ digestibility technique (Nocek, 1988).

Legume escape protein concentration has been reported to increase at advanced stages of plant maturity (Griffin et al., 1991). Griffin et al. (1991) reported that alfalfa EP concentration was a linear function of MSW, increasing from 5 to 15% of the CP as plants matured. Stern and Calsamiglia (1991) agreed with those results, suggesting that legumes had higher rumen degradable protein at earlier stages of plant maturity than at later stages. Other researchers also have suggested that escape protein increases with plant maturity because of the decrease in the leaf:stem ratio (Marten, 1970; Kuhbauch and Pletl, 1981).

Although red clover and birdsfoot trefoil are probably the most important forage legumes in the USA after alfalfa (Tesar, 1978; Barnes and Sheaffer, 1985; Grant and Marten, 1985; Van Keuren and Hoveland, 1985), few reports are available about their escape protein values. There is also little data on the effect of maturity on EP concentrations of legumes. However, Griffin et al. (1991) found that alfalfa EP concentration increased with advancing maturity and suggested that the increase in EP may be because of an increase in maturing stems of alfalfa. Alfalfa has also been suggested to have lower escape protein than red clover at similar stages of maturity

(Albrecht et al. 1991). There is no published information about EP concentration of birdsfoot trefoil. However, as in alfalfa and red clover, EP concentration of birdsfoot trefoil is likely to increase with advancing maturity.

Due to the lack of data on EP concentration for these legumes with advancing maturity, we undertook an experiment to evaluate EP concentration of three temperate forage legumes. The specific objectives of this experiment were:

1. to evaluate the effect of harvest schedule and plant maturity stage at harvest on yield and forage quality of alfalfa, red clover, and birdsfoot trefoil; and 2. to compare protein degradability of the three forage legumes at different stages of maturity by using an in situ digestibility technique.

MATERIALS AND METHODS

This study was conducted in 1991 and 1992 at the Michigan State University Research Farm at East Lansing, Michigan. The soil was a well-drained Conover Loam (Ochraquafls, fine loamy, mixed, mesic) with pH 7.4. Soil tests indicated 134 kg of phosphorus, 403 kg of potassium, 3961 kg calcium, and 484 kg of magnesium per hectare.

Three forage legumes, 'Big Ten' alfalfa, 'Arlington' red clover, and 'Norcen' birdsfoot trefoil, were established on 15 May 1991. Alfalfa and red clover were seeded at 18 kg ha^{-1} and birdsfoot trefoil was seeded at 11 kg ha^{-1} in plots measuring 0.9 by 7.6 m. Alfalfa and red clover seeds were inoculated with Rhizobium meliloti (Nitragin Co., Milwaukee, WI). Birdsfoot trefoil was inoculated with Rhizobium trifolii (Nitragin Co., Milwaukee, WI).

The experiment was designed as a randomized complete block design (RCBD) with four replications and two factors in a split-plot arrangement. Factor A (main plots) was legume species (alfalfa, red clover, and birdsfoot trefoil) and factor B (subplots) was cutting date (early, medium, and late).

On 16 April 1991, prior to plowing, the field was treated with glyphosate (N-phosphonomethyl glycine) at 2.2 kg a.i. ha^{-1} to control existing legumes and perennial grasses in the field. The field was then moldboard plowed on 2 May. On 13 May, the field was treated with EPTC (S-ethyl dipropylthiocarbamate), preplant incorporated at 3.3 kg a.i. ha^{-1}. To control broadleaf weeds, butyrac 200 (4-(2,4-dichlorophenoxy) butyric acid) was applied at 1.1 kg a.i. ha^{-1} on 14 June. Irrigation

(25.4 mm) was applied to the field on 20 June due to lack of normal precipitation. Permethrin ((3-phenoxyphenyl)methyl(+)-cis,trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate)) and carbaryl (1-naphthyl-N-methylcarbamate) were applied at 0.1 and 1.1 kg a.i. ha^{-1} on 27 June and 13 August, respectively, to control leafhopper (Empoasca fabae Harris). On 19 August 1992, the field was again sprayed, this time with permethrin at 0.1 a.i. kg^{-1} to control leafhopper. Weather data (Table 1) and accumulation of growing degree days (GDD) (Appendix Table A1) were obtained from the Regional Weather Service Office at Michigan State University at East Lansing, MI.

Over the two years (1991 and 1992) all forage legumes were subjected to five harvest cycles. Within each harvest cycle, species were cut at 3 cutting dates (early, medium, and late). Cutting dates for the seeding year (1991) were 10, 19, and 31 July 1991 for harvest cycle 1; 13, 22, and 29 August 1991 for harvest cycle 2. Cutting dates for the following year (1992) were 15 and 26 May and 6 June 1992 for harvest cycle 3; 1, 9, and 18 July 1992 for harvest cycle 4; and 10, 19, and 28 August 1992 for harvest cycle 5.

Legume plots were harvested using a Jari sickle-bar mower (Jari Division of Year-A-Round Cab Corp., Mankato, MN). At the last cutting date of each harvest cycle, all plots were uniformly clipped and those plots were not used for data collection again that year. Yields were determined on a dry matter basis. Approximately 700 g of herbage was subsampled from each harvested plot from each replication for determination of dry matter and forage quality. Forage subsamples

were dried at 65°C for 72 hr. Forage subsamples were then ground (2 mm) in a Wiley mill and used for forage quality determination.

Mean stage by weight (MSW), as defined by Kalu and Fick (1983), is the average of the individual stages present in the herbage sample, weighted for the dry weight of the herbage at each stage number. Alfalfa, red clover, and birdsfoot trefoil MSW values for this experiment were obtained by classifying 40 stems randomly selected from a subsample composited across replications for each harvest cycle, species, and cutting treatment combination (Appendix Table A2). Alfalfa MSW was quantified according to the system of Kalu and Fick (1983), which classifies stages of development as: vegetative (0=early vegetative, 1=mid vegetative, and 2=late vegetative), bud (3=early bud and 4=late bud), flowering (5=early flower and 6=late flower), and reproductive (7=early seed pod, 8=late seed pod, and 9=ripe seed pod). An eight-number staging system similar to Kalu and Fick's system was developed for the staging of red clover and birdsfoot trefoil. Red clover stages of maturity were: no flowers (vegetative=1), flowers (flower stage=5), and seed pods (reproductive stage=8). Birdsfoot trefoil stages of maturity were: no flowers (vegetative=1), 1 to 2 flowers (early flower=5), 2^+ flowers (late flower=6), 1 to 2 immature seed pods (early seed pod=7), and 2^+ seed pods (late seed pod=8).

Acid detergent (ADF) and neutral detergent fiber (NDF) for the three legumes were determined according to Goering and Van Soest technique (1970). Ground forage samples (0.50 g) were boiled in acid detergent or neutral detergent solution for 1 hr and then rinsed with hot water (90°C) and acetone. Samples were dried (100°C) for dry residue determination and then ashed at 500°C for 12 hr. Acid

detergent and neutral detergent fibers were expressed on a dry matter basis, and they were corrected for the ash component.

Crude protein concentrations for the three legume species were obtained by a modified Kjeldahl procedure using hydrogen peroxide (Watkins et al., 1987). Total crude protein was then calculated as N x 6.25 in the herbage sample on a dry matter basis.

Ruminal EP concentrations of the three legume species at each cutting date were determined by the in situ bag technique described by Blasi et al. (1990). However, in contrast to their technique, a different bag pore size and preincubation time was used in this experiment. In 1991 and 1992, two ruminally cannulated Holstein steers, averaging 550 and 480 kg in the two years, respectively, were used during the in situ experiment. They were fed an ad libitum mixed alfalfa/brome (Bromus inermis L.) hay ration (58% NDF, 46% ADF, and 23% CP) twice a day at 0900 and 1700 and 0.5 kg animal^{-1} soybean meal [Glycine max L. (Merr.)] once a day at 1700. Steers consumed an average of approximately 14 kg DM day^{-1} in 1991 and 11 kg DM day^{-1} in 1992. During both years, the steers were housed indoors. Water was available at all times and the basal diet was given to steers for two weeks prior to the in situ bag incubation.

Quadruplicate sets of nylon bags (pore size 53 μm) containing the forage samples (5.0 g) were put into the steers' rumens at 1700 before feeding. Forage treatment combinations were randomly assigned to steers at 36 bags steer^{-1} run^{-1}. The bags were placed into the steers for two consecutive days followed by three days off

between incubations. Before ruminal incubation, the bags were soaked in 39°C tap water for 15 min. Bags were removed from the rumen 16 hr after insertion.

In an associated experiment using the same Holstein steers, blank bags were placed into the steers to assess ruminal microbial attachment to the sample bag during in situ incubation. However, the percent of microbial CP found in the blank bags was less than 0.5 percent of the total percent of EP calculated from the herbage samples (1.5 %CP) (K. Cassida, 1993, personal communication); therefore, no correction was made for microbial contamination on this in situ experiment.

After removal from the rumen, bags were immediately rinsed with tap water (approximately 1.5 hr) until the rinse water remained clear. Bags were dried in a forced-air oven at 55°C for 24 hr. Percent residual dry matter was calculated from the dry forage residue samples. Escape protein concentration on the residual herbage sample was calculated as N x 6.25. Total EP concentration of the original forage sample measured as a percentage of the original crude protein of the herbage was calculated using the following formula:

$$EP_{cp} = EP_{dm}/CP_{dm} \quad [1]$$

where EP_{dm} = escape protein concentration of the original sample as a percent of dry matter, CP_{dm} = crude protein concentration of the original sample as a percent of dry matter.

$$EP_{dm} = (\%EP \text{ of } in\ situ \text{ residue} \times (1\text{-ISDMD})) \times 100 \quad [2]$$

where %EP of in situ residue = escape protein concentration of in situ residue, ISDMD = in situ dry matter digestibility or degradable dry matter of the herbage sample, calculated using:

ISDMD = ((original sample weight-in situ dry residue weight)/original sample weight)) x 100 [3].

Analysis of variance (ANOVA) for DM yields, NDF, ADF, CP, and EP concentrations, both by harvest cycle and by legume species within cycles, was conducted using SAS (SAS, 1986). Means for DM yield, CP, ADF, NDF, and EP concentrations were separated by Fisher's Protected Least Significance Difference (LSD) test at the 5% level of significance. Simple linear regression analysis (SAS, 1986), using means pooled across harvest cycles within species, was conducted to describe the relationship of DM yield, NDF, ADF, CP, and EP concentrations with maturity stages as quantified by MSW and GDD. The significance of linear regressions ($P \leq 0.05$) were determined using Pearson's correlation coefficient (SAS, 1986). Predicted mean values for DM yield, NDF, ADF, CP, and EP concentrations were calculated from the regression equations.

RESULTS AND DISCUSSION

Mean stage by weight and growing degree days

In this study, MSW was calculated to quantify maturity of the legumes at each cutting date (early-, medium-, and late-cutting date) within harvest cycle. Three MSW systems were used for the legumes (Appendix Table A2) and there was no replication for MSW within cutting date for each specie. However, there was a numerical increase in MSW with advancing cutting date. For alfalfa, the highest MSW value (5.0) was obtained at late-cutting date in the fourth harvest cycle, while a low MSW value (1.5) was obtained at early-cutting date in the third harvest cycle. For red clover, the highest MSW value (2.8) was obtained at late-cutting date in the fourth harvest, and a low MSW value (1.0) was obtained at early-cutting date in the first, third, fourth, and fifth harvest cycles. For birdsfoot trefoil, the highest MSW value (4.1) was obtained at late-cutting date in the fourth harvest cycle, while a low MSW value (1.0) was obtained at early-cutting date in the third and fifth harvest cycles.

Dry matter yields, NDF, ADF, CP, and EP concentrations were regressed on GDD across harvest cycles within species to determine the relationship between GDD and the mentioned legume parameters (Appendix Table A3). However, results from regression analyses indicated a poor relationship between GDD and DM yields, NDF, ADF, and EP concentrations for all three legumes. Crude protein concentration was found to be related to GDD, but the regressions had very low r^2 values for all the

legumes. Therefore, GDD will not be discussed in the results and discussion section of this study.

Dry matter yield

This study was carried out with soil conditions that were favorable for growing alfalfa, red clover, and birdsfoot trefoil. Dry matter yields of the legumes for cutting dates, averaged over legumes species, are reported in Table 2. Results for which there was no significant species x cutting date interaction will be discussed first. Interactions will be discussed subsequently. There was a significant effect of cutting date on DM yield in the second and third harvest cycles. In these two harvest cycles, DM yield increased with advancing cutting date. A significant effect of cutting date on dry matter yield also was found in the fourth harvest cycle; however, in contrast to the second and third harvest cycles, DM yield was higher at late- than at early- and medium-cutting date. Moline and Wedin (1969) also reported DM yield of alfalfa to increase 1.5 Mg ha^{-1} from pre-bud to first flower and 1.1 Mg ha^{-1} from one half bloom to early seed pod stage of development. Latheef et al. (1988) reported DM yield of alfalfa to increase 0.9 Mg ha^{-1} from early to late bloom stage of development. The results for alfalfa do not agree with those reported by Fulkerson et al. (1967), who found DM yield of alfalfa to decrease 0.4 Mg ha^{-1} as herbage passed from bud to early seed pod stage of development. Our results for red clover agree with those reported by Colville and Torrie (1962), who found that DM yield of red clover increased 0.34 Mg ha^{-1} as red clover passed from mid-vegetative to late bud stage of development. Other researchers have also reported increases in DM yield with

advancing maturity of alfalfa (Nelson and Smith, 1968; Weir et al., 1960; Winch et al., 1970), red clover (Van Keuren and Hoveland, 1985) and birdsfoot trefoil (Gasser and Lachance, 1969; MacGraw and Marten, 1986).

There was no significant effect of species on DM yield during the second, third, and fourth harvest cycles (Table 3). Other researchers have reported differences in DM yield when comparing alfalfa and red clover (Smith, 1962) or alfalfa and birdsfoot trefoil (Smith and Nelson, 1967; MacGraw and Marten, 1986), with alfalfa producing higher DM yield than either red clover or birdsfoot trefoil. Dry matter yields of alfalfa and red clover in this experiment are lower than DM yields expected under similar growing conditions. However, DM yields of birdsfoot trefoil are in the normal range reported by other researchers. Tesar (MSU, unpublished data) reported DM yield for alfalfa of 13.3 Mg ha^{-1} in a two-year study. Similar DM yield for alfalfa was reported by Hesterman et al. (1989) in a three-year study. Sheaffer (1986) reported DM yield for red clover between 9.5 and 10.3 Mg ha^{-1}, with a 2 to 4 cut system. Birdsfoot trefoil has been reported to yield 8.6 Mg ha^{-1} by Tesar (1978) in a four-year study, while Leep and Lempke (1986) reported DM yields of 7.6 Mg ha^{-1} for birdsfoot trefoil in two years of a three-year study. All these studies, except the experiment for red clover by Sheaffer, were conducted at East Lansing, MI.

In the first and fifth harvest cycles there was an interaction between species and cutting date ($P \leq 0.05$) for DM yield (Table 4). In the first harvest cycle, DM yield of red clover and birdsfoot trefoil increased significantly with advancing cutting date. However, DM yield of alfalfa was not significantly affected by cutting date. In

the fifth harvest cycle, DM yield of red clover and birdsfoot trefoil increased significantly at each cutting date level, while DM yield of alfalfa plateaued at medium-cutting date. MacGraw and Marten (1986) also reported an interaction between cutting date and legume species for DM yield. However, they found that DM yield increased with advancing maturity of alfalfa, while it plateaued at late flower stage for birdsfoot trefoil.

Regression analysis of DM yield data pooled across harvest cycles within species revealed that MSW was an inadequate predictor for DM yield of alfalfa (Appendix Table A4). However, MSW was a significant linear predictor for DM yield of red clover and birdsfoot trefoil (Figure 1).

Neutral detergent fiber concentration.

There was a significant effect of cutting date on NDF concentrations in the first, second, and fourth harvest cycles (Table 2). In the first cycle, NDF concentration was greater at medium- and late- than at early-cutting date. In the second harvest cycle, NDF concentration was greater at late- than at early-cutting date. In the fourth harvest cycle, NDF concentration was higher at late- than at either early- or medium-cutting date. These results agree with those reported for alfalfa and birdsfoot trefoil by Buxton and Brasche (1991), who reported NDF concentration to increase 7.4 and 6.2 percentage units from early- to late-maturity, respectively, for alfalfa and birdsfoot trefoil during the first year of a two-year study. Sanderson and Wedin (1988 and 1989) also reported increases in NDF concentration with maturity for alfalfa and red clover, as did Griffin et al. (1991) for alfalfa.

A significant effect of species on NDF concentration was found in the first and fourth harvest cycles (Table 3). In the first harvest cycle, alfalfa had higher NDF concentration than either red clover or birdsfoot trefoil. In the fourth harvest cycle, NDF concentration of alfalfa was greater than red clover, while no differences in NDF concentration were found between alfalfa and birdsfoot trefoil and between red clover and birdsfoot trefoil. These results do not agree with those reported by Buxton and Brasche (1991), who found no significant difference in NDF concentration between alfalfa and birdsfoot trefoil when using an equal MSW system for both species.

There was an interaction between species and cutting date ($P \leq 0.05$) for NDF concentration in the third and fifth harvest cycles (Table 4). In the third harvest cycle, NDF concentration significantly increased with cutting date for all three legumes. However, the extent of increase in NDF concentration of alfalfa and birdsfoot trefoil was higher (13 percentage units for both legumes) than for red clover (7.5 percentage units). In the fifth harvest cycle, NDF concentration of alfalfa increased at each succeeding cutting date, while NDF concentrations for red clover and birdsfoot trefoil were significantly higher at late- than at early- and medium-cutting date. Other researchers have also reported interactions between legume species and cutting date for NDF concentration (Buxton and Horstein, 1986; Buxton and Brasche, 1991). Buxton and Brasche (1991) reported an interaction for NDF concentration between sampling dates for alfalfa and birdsfoot trefoil in a two year study. They found that during both years, NDF concentration for alfalfa had a larger rate of increase than for birdsfoot trefoil.

Results from regression analysis indicated that MSW was a significant indicator of NDF concentration for alfalfa, red clover, and birdsfoot trefoil when data across all harvest cycles within species were pooled (Figure 2). These results clearly agree with the results in our ANOVA table, and further show that the increase in NDF concentration with cutting date was linear. These results agree with those reported by Olhsson and Wedin (1989) and Sanderson and Wedin (1989), who found a linear increase in NDF concentration for red clover with MSW. Buxton et al. (1985) suggested that an increase in NDF concentration with increasing MSW for red clover may be because red clover leaves do not readily abscise after they mature, resulting in a greater proportion of old leaves with advancing maturity. Griffin et al. (1991) reported a linear increase in NDF concentration for alfalfa with MSW. However, our results do not agree with results reported by Kalu and Fick (1983) and Sanderson and Wedin (1989), who found a quadratic increase in NDF concentration for alfalfa with increasing MSW. They looked at NDF concentration for alfalfa over a larger range of MSW values (0 to 9) than we did (1.5 to 5) (Appendix Table 2). Therefore, with the narrow MSW range found in this study, legumes did not mature enough to show as great changes in NDF concentration as those found by Kalu and Fick.

Acid detergent fiber concentrations

A significant effect of cutting date on ADF concentration was obtained in the first, second, and third harvest cycles for the three species as reported in Table 2. In the first and second harvest cycles, ADF concentration was higher at medium- and

late- than at early-cutting date. In the third harvest cycle, there was a significant increase in ADF concentration at each cutting date. These results agree with those reported by Kalu and Fick (1983), who found that ADF concentration in alfalfa leaves increased 9 percentage units from early vegetative to early bud stage, while concentration in alfalfa stems increased 17 percentage units from early vegetative to early bud stage and 1 to 2 percentage units from early bud stage to seed pod stage. Horstein et al. (1989) supported Kalu and Fick's results, finding that an increase in ADF concentration of legumes was due to a decrease in the leaf:stem ratio with advancing maturity.

There was a significant effect of species in the third harvest cycle on ADF concentration (Table 3). Alfalfa had a higher ADF concentration than either red clover or birdsfoot trefoil, while these later two legumes had equal concentrations of ADF. Despite the increase in fiber, NDF and ADF concentrations of the legumes are lower than those reported by other researchers at comparable stages of maturity of the legumes. Neither NDF nor ADF concentrations increased as much as expected with advancing cutting date of the legumes, perhaps because of the short cutting interval between cutting date. Paling (1992) reported NDF and ADF concentrations for alfalfa of 41.7 and 32.3%, respectively, in a two-year study near the site where this experiment was established. Hesterman et al. (1989) reported NDF and ADF concentrations for alfalfa of 43.3 and 35.9%, respectively, during a three-year study at East Lansing, MI. In both preceding experiments, alfalfa was harvested at 10% bloom stage of maturity.

During the fourth and fifth harvest cycles, there was an interaction ($P \leq 0.05$) between species and cutting date for ADF concentration; therefore, significant cutting date differences within species for these harvest cycles are reported in Table 4. In the fourth harvest cycle, ADF concentration of alfalfa and birdsfoot trefoil increased significantly from early- to medium-cutting date, where it plateaued. For red clover, ADF concentration did not increase from early- to medium-cutting date, but it increased sharply from medium- to late-cutting date. In the fifth harvest cycle, ADF concentration for alfalfa increased significantly from early- to medium-cutting date and it plateaued at medium-cutting date. For red clover and birdsfoot trefoil, ADF concentrations were significantly lower at early and medium- than at late-cutting date.

When pooled data were regressed across harvest cycles for each of the three legume species, results indicated that MSW was not a significant predictor of ADF concentration for alfalfa (Appendix Table 4). However, MSW was an adequate predictor of ADF concentration for red clover and birdsfoot trefoil (Figure 2). These results for alfalfa do not agree with those reported by Kalu and Fick (1983) and Griffin et al. (1991), who found a linear relationship between MSW and ADF concentration for alfalfa. However, results for red clover agree with those reported by Sanderson and Wedin (1989), who found a linear relationship between ADF concentration and MSW for red clover.

Crude protein concentration

There was a significant effect of cutting date on CP concentration in the third, fourth, and fifth harvest cycles (Table 2). In the third harvest cycle, CP

concentration was greater at early- and medium- than at late-cutting date. These results agree with those reported by Silkerson et al. (1967), who found CP concentration for alfalfa to decrease 4.5 percentage units from bud to first flower stage and 0.6 percentage units from full bloom to early seed pod stage. Similarly, Moline and Wedin (1969) found CP concentration for alfalfa to decrease 2.9 percentage units from pre-bud to first flower stage, 1.4 percentage units from one-half bloom to three-fourth bloom stage, and 0.8 percentage units from full bloom to early seed pod stage. Sanderson and Wedin (1989) also reported CP concentration for alfalfa to decrease with advancing maturity. However, in the fourth and fifth harvest cycles, CP concentration was significantly higher at medium- and late-cutting date than at early-cutting date. From a practical point of view, these small increases in CP concentration (0.4 and 0.6 percentage units) with increasing maturity are probably unimportant.

Possible reasons for the increase in CP concentration were cool temperature during the fourth and fifth harvest cycles. Air temperature in both harvest cycles was 3°C below normal (Table 1), and this relatively low temperature could delay maturation of the legumes and reduce the decline in quality (Walgenback, 1983). Other authors have also reported alfalfa CP concentration to increase under warm temperatures compared to cool temperatures (Smith, 1969; Marten, 1970). However, during the fourth and fifth harvest cycles, MSW values indicated that the legumes matured faster during these harvest cycles than during the first, second, and third harvest cycles (Appendix Table A2).

No significant effect of species was obtained for CP concentration during any of the five harvest cycles (Table 3). Also, there was no species by maturity interaction for CP concentration for the legumes during any of the harvest cycles. The response of CP concentration of the legumes was not consistent during the experiment. However, for both years (1991 and 1992), CP concentrations were equal for the three legumes (23%), and these CP concentrations are higher than CP percentages reported for alfalfa by Hesterman et al. (1989) (21.7%) and Paling (1992) (19.1%). Carlson et al. (1983) reported CP concentrations for birdsfoot trefoil between 16.2 and 20% when harvested three times in a two year study.

When regression analysis was performed across harvest cycles within species (Appendix Table A4), mean stage by weight was not a good predictor of CP concentration for alfalfa, red clover, or birdsfoot trefoil. These results do not agree with results reported by Kalu and Fick (1983) and Faberger (1988), who reported a quadratic relationship between MSW and CP concentration for alfalfa and red clover, respectively. Griffin et al. (1991) also found a quadratic increase in CP concentration with increasing MSW for alfalfa, but they found a wider range for MSW (from 0 to 8) than the MSW range in this study (from 0 to 5).

Escape protein concentration

In the first, second, third, and fourth harvest cycles, there was a significant effect of cutting date on EP concentration (Table 2). In the first and second harvest cycles, EP concentrations were greater at late- than at early-cutting date, and no differences in EP were found between early- and medium- or between medium- and

late-cutting date. In the third harvest cycle, EP concentration increased with advancing cutting date. In the fourth harvest cycle, EP concentration was greater at late- than at early- and medium-cutting date. These results agree with those reported by Griffin et al. (1991), who found that EP concentration of alfalfa, as a percent of total CP in the original sample, increased as plants passed from early flower (5%) to late seed pod (15%), while there was no increase from early to late vegetative stages of development (1.5%). There are little data on EP concentrations in legumes, but other authors have reported EP concentrations in grass to increase with advancing maturity. Blasi et al. (1991) reported that EP concentrations of bromegrass (Bromus inermis Leyss) and big bluestem (Andropogon gerardii Vitman) increased as they advanced in maturity. Mullahey et al. (1992) reported EP concentration of switchgrass (Panicum virgatum L.) to increase with advancing maturity, while EP concentration of bromegrass did not change with plant maturity.

A significant effect of species on EP concentration was found in the third harvest cycle (Table 3). In the third harvest cycle, alfalfa and birdsfoot trefoil had greater EP concentration than did red clover. There was no significant effect of species on EP concentration for the other four harvest cycles. Results for red clover do not agree with results reported by Albrecht et al. (1991), who found EP concentration of red clover to be higher than EP concentration of alfalfa.

There was an interaction between species and cutting date for EP concentration in the fifth harvest cycle (Table 4). In this harvest cycle, EP concentration of alfalfa was not affected by cutting date. Escape protein concentration of red clover

decreased from early- to medium-cutting date, while EP of birdsfoot trefoil increased from early- to medium-cutting date.

When EP concentration was regressed across all harvest cycles within legume species, results indicated that MSW was an adequate predictor of EP concentration of red clover (Figure 3); however, MSW was not a good predictor of EP concentrations of alfalfa and birdsfoot trefoil (Appendix Table A4). These results for alfalfa do not agree with results reported by Griffin et al. (1991), who reported a linear increase in EP concentration of alfalfa with MSW. There is a lack of information relating concentration of EP to maturity of legumes. However, in general, with advancing maturity the proportion of leaf to stem decreases in herbage (Buxton et al, 1985), increasing the amount of plant cell-wall and, therefore, increasing the concentration of fibers and lignin in herbage. Escape protein concentration is also expected to increase as cell-wall mass increases with plant maturity (Griffin et al., 1991) because most of the N that escapes rumen fermentation is bound to the cell-wall.

CONCLUSIONS AND RECOMMENDATIONS

This experiment indicated that MSW was not a useful tool for estimating DM yield and quality of the legumes with a few exceptions. These exceptions are DM yield, NDF and ADF for red clover and birdsfoot trefoil, NDF for alfalfa, and EP concentration for red clover. However, for all previous mentioned parameters, the r^2 values were pretty low.

During the two-year study, legume quality declined with advancing cutting date, the decline being more accentuated in the second than in the seeding year. This study supports previous research reporting increases in DM yields and declines in herbage quality of alfalfa, red clover, and birdsfoot trefoil with advancing maturity. However, DM yields were not compromised with the quality of the legumes at advancing maturity because maximum MSW values were 4 (late bud) for alfalfa, 2 (late vegetative) for red clover, and 3 (early bud) for birdsfoot trefoil. At these maturity levels, the highest NDF concentration was 41% for alfalfa and 37% for both red clover and birdsfoot trefoil, while the highest ADF was 31% for alfalfa, 29% for red clover, and 28% for birdsfoot trefoil. Crude protein concentrations of the legumes were not affected consistently neither by species nor by maturity, but all three legumes had 23% CP at the late-cutting date.

Van Soest (1982) and Hesterman et al. (1991) have suggested that high quality legumes contain more than 19% CP, and less than 40 and 31% NDF and ADF, respectively. Using this criteria, I conclude that the late-cutting date was the best harvest time for alfalfa, red clover, and birdsfoot trefoil. This maturity stage corresponded to late bud for alfalfa, late vegetative for red clover, and early bud stage for birdsfoot trefoil. The legumes in this experiment were harvested at an earlier maturity level than current harvest recommendations which are: 10% bloom for alfalfa, prebloom to early-bloom for red clover, and full flower for birdsfoot trefoil (Smith, 1972; Leep and Tesar, 1981; Taylor, 1985).

In this experiment, the EP concentrations found in alfalfa, red clover, and birdsfoot trefoil are not high enough to contribute a great deal to the EP recommended for supplementing ruminant diets. Conrad and Klopfenstein (1988) have suggested that cattle rations should contain 50% of the digestible crude protein as EP. However, during this study, there was a continuous increase in EP concentrations for the legumes from early- to late-cutting date. This means that EP concentrations could continue increasing with advancing cutting date, especially for alfalfa and birdsfoot trefoil, which tended to have higher EP concentrations than did red clover. Therefore, I conclude that all three legumes can be of particular interest to animal nutritionists looking for legume EP sources for feeding animals; however, when feeding rations relying solely on legumes, an EP supplement may be required.

I recommend that the MSW system used in this experiment be tested further in order to establish its consistency in predicting DM yield and forage quality of the legumes. Also, for achieving greater precision in quantifying the relationship of yield

and quality parameters to MSW, I recommend that every single plot of legumes be harvested and quantified for maturity instead of a composited sample of the legume. Furthermore, I recommend increasing the cutting interval between maturity stages within harvest cycles in order to better assess the maturity stages of legumes using MSW. Finally, I recommend further studies relating MSW and EP concentrations of the legumes because this is a relatively new field.

Table 1. Air temperature and precipitation† in spring and summer of 1991 and 1992 at the Agronomy Farm at Michigan State University, East Lansing, MI.

	Temperature (°C)				Rainfall (mm)	
	Max	Min	Avg.	30 year mean‡	Total	30 year mean‡
1991						
April	16	5	10	8	104	71
May	24	12	18	14	42	69
June	28	14	21	19	74	90
July	28	16	22	22	91	77
August	27	14	21	21	74	79
1992						
April	11	2	7	8	97	71
May	21	8	14	14	41	69
June	24	10	17	19	64	90
July	24	14	19	22	182	77
August	24	12	18	21	37	79

†Weather data collected from the Horticulture Experiment Station at Michigan State University at East Lansing, MI.

‡Long term (1951-1980) mean from the Regional Weather Office at Michigan State University at East Lansing, MI.

Table 2. Effect of cutting date on dry matter (DM) yield, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and escape protein (EP) concentrations of alfalfa, red clover, and birdsfoot trefoil for five harvest cycles in 1991 and 1992.

	Cutting date			
	Early	Medium	Late	C.V. cutting date
1st harvest cycle†				
DM yield (Mg/ha)	--‡	--	--	--
NDF (% of DM)	33.0[a]	35.6[b]	36.6[b]	6.6
ADF (% of DM)	23.3[a]	25.2[b]	26.2[b]	8.6
CP (% of DM)	23.5	23.6	23.5	0.7
EP (% of CP)	6.4[a]	7.2[ab]	8.4[b]	19.0
2nd harvest cycle				
DM yield (Mg/ha)	1.7[a]	2.7[b]	3.2[c]	5.2
NDF (% of DM)	33.6[a]	35.6[ab]	37.6[b]	8.7
ADF (% of DM)	25.0[a]	29.1[b]	28.7[b]	10.8
CP (% of DM)	23.4	23.5	23.6	0.9
EP (% of DM)	6.2[a]	6.9[ab]	7.7[b]	21.0
3rd harvest cycle				
DM yield (Mg/ha)	2.1[a]	3.9[b]	5.1[c]	9.6
NDF (% of DM)	--‡	--	--	--
ADF (% of DM)	21.8[a]	27.7[b]	32.4[c]	8.6
CP (% of DM)	23.2[a]	23.2[a]	22.1[b]	1.0
EP (% of DM)	3.7[a]	4.7[b]	6.0[c]	17.9

†1st Harvest cycle: 10, 19, and 31 July 1991; 2nd Harvest cycle: 13, 22, and 29 August 1991; 3rd Harvest cycle: 15 and 26 May and 6 June 1991.

‡Significant interaction effect between species and cutting date. See Table 4 for interaction effects.

[abc]Numbers within rows followed by the same letter are not significantly different as determined by Fisher's protected LSD ($P \leq 0.05$).

[Table 2 (con't)].

	Cutting date			C.V. cutting date
	Early	Medium	Late	
4th harvest cycle†				
DM yield (Mg/ha)	2.5[a]	2.8[a]	3.4[b]	17.7
NDF (% of DM)	32.9[a]	37.7[b]	40.7[c]	5.9
ADF (% of DM)	--‡	--	--	--
CP (% of DM)	22.0[a]	22.4[b]	22.4[b]	0.9
EP (% of CP)	4.9[a]	5.9[a]	7.72[b]	24.6
5th harvest cycle				
DM yield (Mg/ha)	--‡	--	--	--
NDF (% of DM)	--‡	--	--	--
ADF (% of DM)	--‡	--	--	--
CP (% of DM)	23.0[a]	23.6[b]	23.6[b]	1.0
EP (% of CP)	--‡	--	--	--

†1st Harvest cycle: 10, 19, and 31 July 1991; 2nd Harvest cycle: 13, 22, and 29 August 1991; 3rd Harvest cycle: 15 and 26 May and 6 June 1991; 4th Harvest cycle: 1, 9, and 18 July 1992; 5th Harvest cycle: 10, 19, and 28 August 1992.

‡Significant interaction effect between species and cutting date. See Table 4 for interaction effects.

[abc]Numbers within rows followed by the same letter are not significantly different as determined by Fisher's protected LSD ($P \leq 0.05$).

Table 3. Effect of forage species on dry matter (DM) yield, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and escape protein (EP) concentrations of alfalfa, red clover, and birdsfoot trefoil for five harvest cycles in 1991 and 1992.

	Species			
	Alfalfa	Red clover	Trefoil	$C.V._{species}$
1st harvest cycle†				
DM yield (Mg/ha)	--‡	--	--	--
NDF (% of DM)	38.3[a]	34.0[b]	32.9[b]	3.6
ADF (% of DM)	26.0	24.7	24.0	5.1
CP (% of DM)	23.5	23.5	23.5	0.2
EP (% of CP)	7.6	6.4	8.0	6.7
2nd harvest cycle				
DM yield (Mg/ha)	2.5	2.7	2.4	5.3
NDF (% of DM)	37.4	34.1	35.2	3.4
ADF (% of DM)	28.5	27.8	26.7	4.5
CP (% of DM)	23.6	23.5	23.6	0.2
EP (% of CP)	7.3	6.7	6.9	3.8
3rd harvest cycle				
DM yield (Mg/ha)	3.8	3.7	3.5	5.6
NDF (%)	--‡	--	--	--
ADF (%)	30.2[a]	25.0[b]	26.6[b]	3.4
CP (%)	22.9	22.8	22.8	0.4
EP (%)	5.8[a]	3.7[b]	5.1[a]	8.3

†1st Harvest cycle: 10, 19, and 31 July 1991; 2nd Harvest cycle: 13, 22, and 29 August 1991; 3rd Harvest cycle: 15 and 26 May and 6 June 1992.

‡Significant interaction effect between species and maturity. See Table 4 for interaction effects.

[abc]Numbers within rows followed by the same letter are not significantly different as determined by Fisher's protected LSD ($P \leq 0.05$).

[Table 3 (con't)].

	Species			
	Alfalfa	Red clover	Trefoil	$C.V._{species}$
4th harvest cycle†				
DM yield (Mg/ha)	2.9	2.7	3.1	5.0
NDF (% of DM)	38.5[a]	35.7[b]	37.1[ab]	1.4
ADF (% of DM)	--‡	--	--	--
CP (% of DM)	22.3	22.3	22.3	0.3
EP (% of CP)	6.8	5.0	6.7	10.1
5th harvest cycle				
DM yield	--‡	--	--	--
NDF (% of DM)	--‡	--	--	--
ADF (% of DM)	--‡	--	--	--
CP (% of DM)	23.4	23.4	23.4	0.5
EP (% of CP)	--	--	--	--

†1st Harvest cycle: 10, 19, and 31 July 1991; 2nd Harvest cycle: 13, 22, and 29 August 1991; 3rd Harvest cycle: 15 and 26 May and 6 June 1992; 4th Harvest cycle: 1, 9, and 18 July 1992 and 5th Harvest cycle: 10, 19, and 28 August 1992.

‡Significant interaction effect between species and maturity. See Table 4 for interaction effects.

[abc]Numbers within row followed by the same letter are not significantly different as determined by Fisher's protected LSD ($P \leq 0.05$).

Table 4. Interaction effect between species and cutting date on dry matter (DM) yield, neutral detergent fiber (NDF), and acid detergent fiber (ADF) concentrations of alfalfa, red clover, and birdsfoot trefoil for five harvest cycles in 1991 and 1992.

Cutting date	Species		
	Alfalfa	Red clover	Trefoil
1st Harvest cycle			
DM yield (Mg/ha)			
Early	2.2[a]	1.8[a]	1.3[a]
Medium	2.9[a]	2.5[b]	2.8[b]
Late	2.9[a]	3.4[c]	3.6[c]
$C.V._{\cdot cutting\ date}$	31.0	15.0	11.8
3th Harvest cycle			
NDF (% of DM)			
Early	31.5[a]	30.3[a]	27.3[a]
Medium	38.9[b]	33.8[b]	36.3[b]
Late	44.6[c]	37.8[c]	40.7[c]
$C.V._{\cdot cutting\ date}$	2.9	4.6	5.6
4th Harvest cycle			
ADF (% of DM)			
Early	27.1[a]	22.5[a]	25.2[a]
Medium	31.7[b]	24.2[a]	31.4[b]
Late	31.9[b]	29.9[b]	29.9[b]
$C.V._{\cdot cutting\ date}$	3.6	6.1	8.5

[abc]Numbers within columns followed by the same letter are not significantly different as determined by Fisher's protected LSD ($P \leq 0.05$).

‡1st Harvest cycle: 10, 19, and 31 July; 2nd Harvest cycle: 13, 22, and 29 August 1991; 3rd Harvest cycle: 15 and 26 May and 6 June 1992; 4th Harvest cycle: 1, 9, and 18 July 1992.

[Table 4 (cont'n)]

Cutting date	Species		
	Alfalfa	Red clover	Trefoil
5th Harvest cycle			
DM yield (Mg/ha)			
Early	1.5[a]	1.2[a]	0.7[a]
Medium	3.5[b]	2.1[b]	1.9[b]
Late	3.5[b]	3.1[c]	2.5[c]
$C.V._{\cdot cutting\ date}$	8.1	11.8	6.2
NDF (% of DM)			
Early	32.4[a]	31.5[a]	31.4[a]
Medium	38.3[b]	30.8[a]	30.6[a]
Late	41.0[c]	36.1[b]	35.0[b]
$C.V._{\cdot cutting\ date}$	3.1	3.6	5.1
ADF (% of DM)			
Early	26.0[a]	20.8[a]	24.5[a]
Medium	31.0[b]	22.4[a]	23.2[a]
Late	33.2[b]	26.0[b]	28.0[b]
$C.V._{\cdot cutting\ date}$	5.4	4.6	6.7
EP (% of CP)			
Early	7.2[a]	8.1[a]	2.4[a]
Medium	5.9[a]	4.6[b]	3.9[b]
Late	5.8[a]	6.2[ab]	2.9[ab]
$C.V._{\cdot cutting\ date}$	19.8	29.0	18.4

[abc]Numbers within columns followed by the same letter are not significantly different as determined by Fisher's protected LSD (P≤0^S' 1

‡1st Harvest cycle: 10, 19, and 31 July; 2nd Harvest cycle: 13, 22, and 29 August 1991; 3rd Harvest cycle: 15 and 26 May and 6 June 1992; 4th Harvest cycle: 1, 9, and 18 July 1992; 5th Harvest cycle: 10, 19, and 28 August 1992.

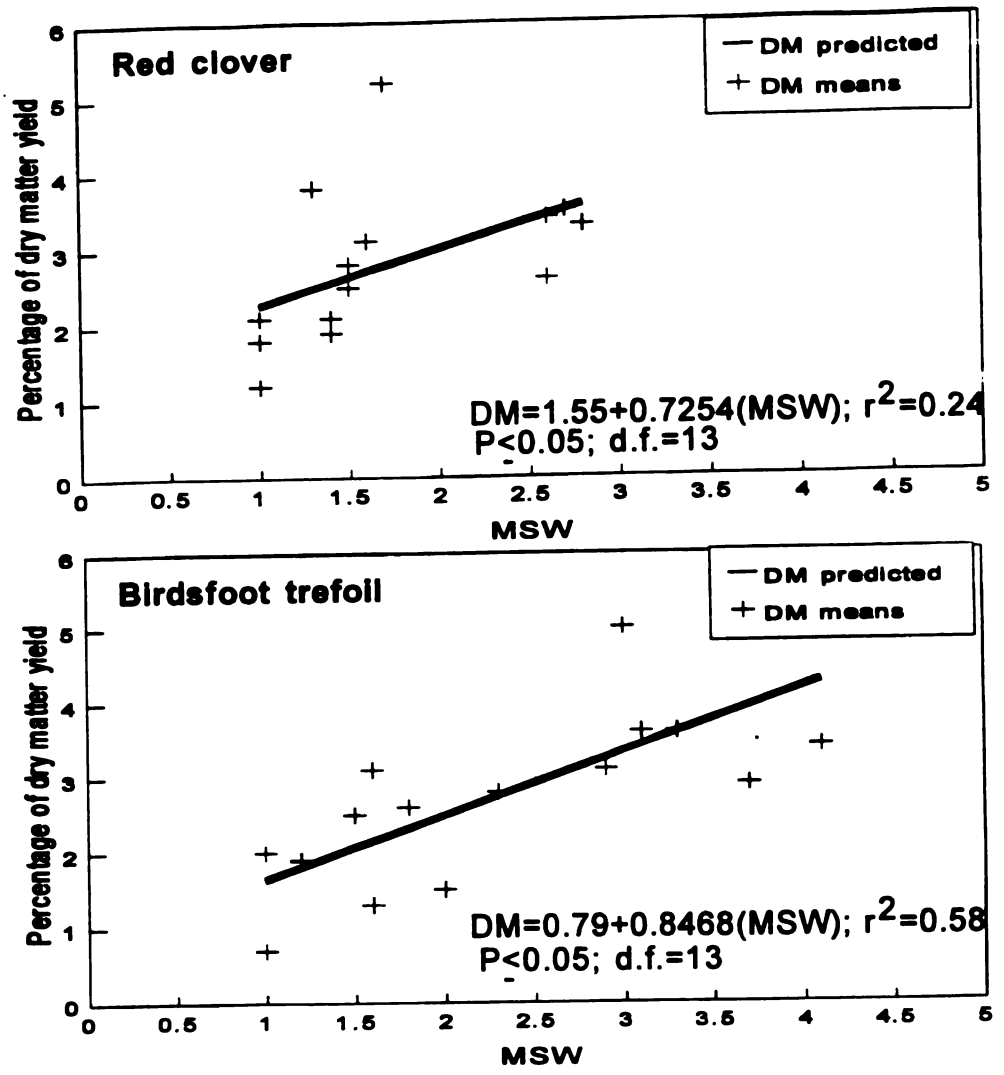

Figure 1. Dry matter (DM) yields of red clover and birdsfoot trefoil regressed on mean stage by weight (MSW).

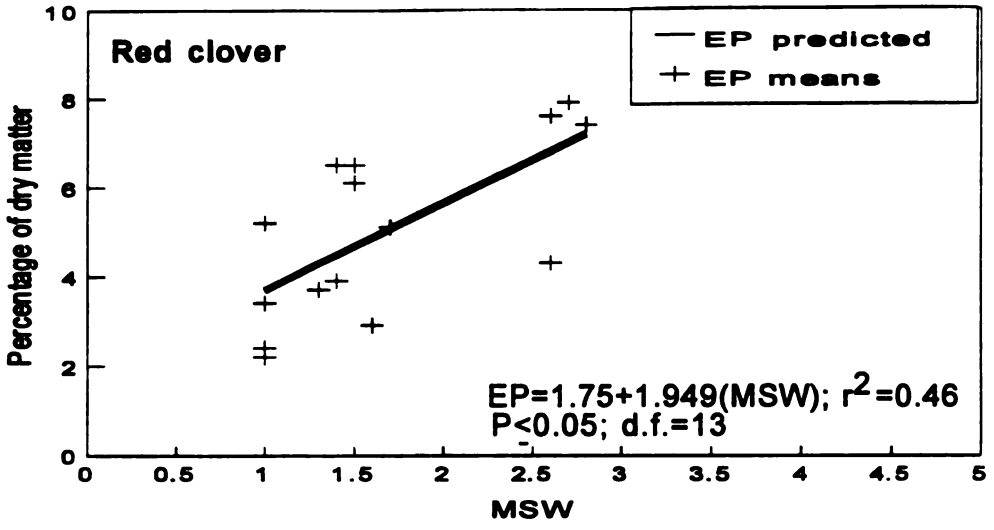

Figure 3. Escape protein (EP) concentration of red clover regressed on mean stage by weight (MSW).

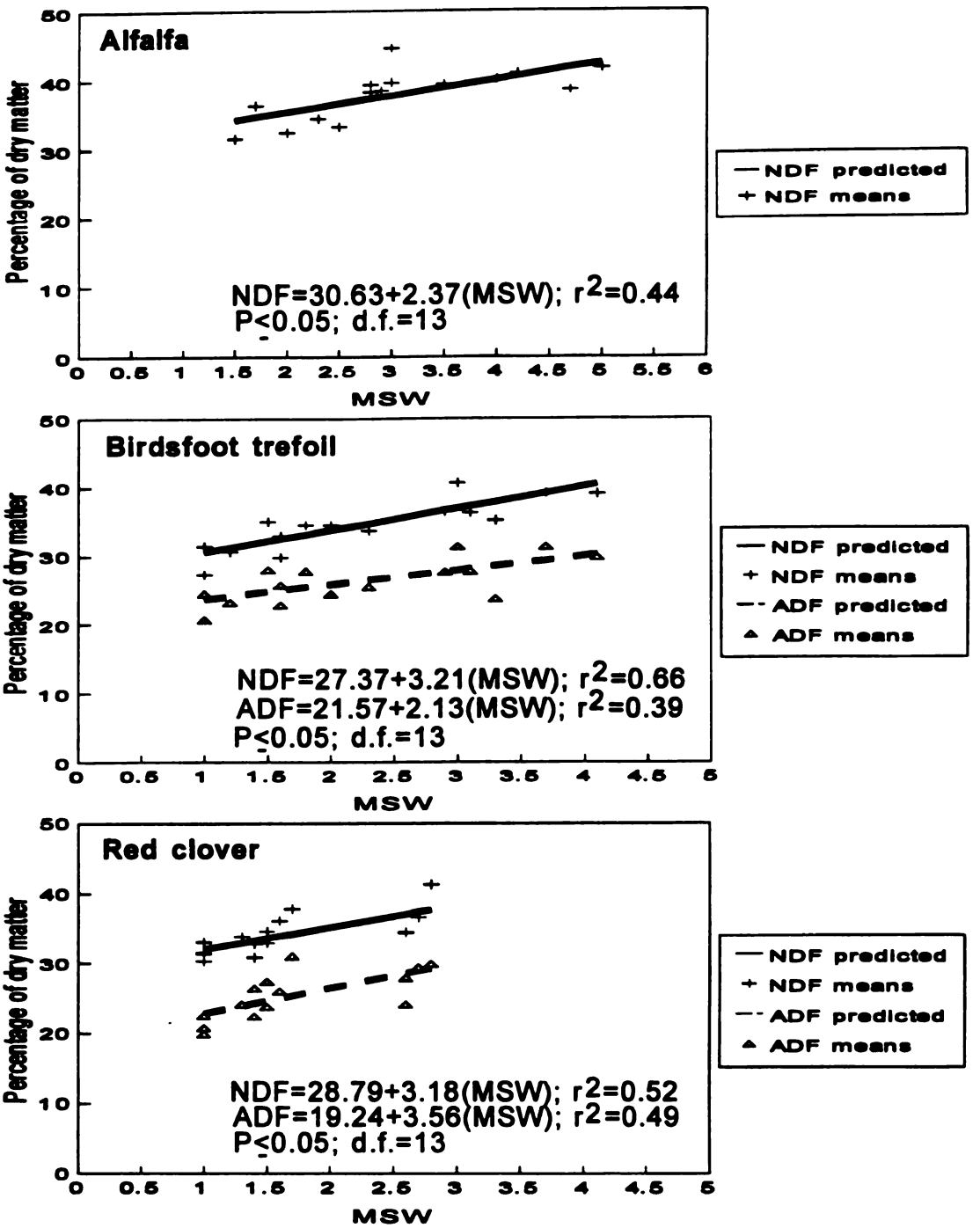

Figure 2. Neutral detergent fiber (NDF) of alfalfa, red clover, and birdsfoot trefoil and acid detergent fiber (ADF) concentrations of red clover and birdsfoot trefoil regressed on mean stage by weight (MSW).

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APPENDIX

Appendix Table A1. Growing degree days (GDD)† for alfalfa, red clover, and birdsfoot trefoil at early-, medium-, and late-cutting date during five harvest cycles in 1991 and 1992.

	Cutting date	Date	GDD(°C)
1st Harvest cycle	Early	July 10	758
	Medium	July 19	768
	Late	July 31	870
2nd Harvest cycle	Early	August 13	1004
	Medium	August 22	1138
	Late	August 29	1303
3rd Harvest cycle	Early	May 15	84
	Medium	May 26	203
	Late	June 6	309
4th Harvest cycle	Early	July 1	412
	Medium	July 9	514
	Late	July 18	635
5th Harvest cycle	Early	August 10	870
	Medium	August 19	938
	Late	August 28	967

†GDD=((maximum daily temperature$_{86}$ °F+minimum daily temperature$_{50}$ °F)/2)-50 °F. Growing degree days in °F was converted to °C using the formula: °C=5/9(°F-32). Growing degree days is cumulative from 1 April 1991 and from 1 April 1992.

Appendix Table A2. Mean stage by weight (MSW)† for alfalfa, red clover, and birdsfoot trefoil at early-, medium-, and late-cutting date for five harvest cycles in 1991 and 1992.

Cutting date	Species		
	Alfalfa	Red clover	Trefoil
	----------# ----------		
1st Harvest cycle			
Early	1.7	1.0	1.6
Medium	4.7	1.5	2.3
Late	4.0	1.6	3.3
2nd Harvest cycle			
Early	2.5	1.4	2.0
Medium	3.8	1.5	1.8
Late	3.0	2.7	2.9
3th Harvest cycle			
Early	1.5	1.0	1.0
Medium	2.8	1.3	3.1
Late	3.0	1.7	3.0
4th Harvest cycle			
Early	2.3	1.0	1.6
Medium	3.5	2.6	3.7
Late	5.0	2.8	4.1
5th Harvest cycle			
Early	2.3	1.0	1.0
Medium	2.9	1.4	1.2
Late	4.2	1.6	1.5

†For alfalfa, the Kalu and Fick's mean stage by weight system was used. For red clover and birdsfoot trefoil, the MSW system described earlier in the Material and Method section of this thesis was used.

Appendix Table A3. Linear and quadratic regression equations and r^2 values of dry matter (DM) yield, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and escape protein (EP) concentrations of alfalfa, red clover, and birdsfoot trefoil regressed on growing degree days (GDD) for five harvest cycles in 1991 and 1992.

Species	Variables	Equations†	r^2
Alfalfa			
Linear	DM	DM=22.58+0.0008(GDD)‡	0.29
	CP	CP=28.21+0.0015(GDD)	0.02
	ADF	ADF=36.65+0.0021(GDD)	0.57
	NDF	NDF=6.76+0.0002(GDD)	0.57
	EP	EP=5.68+0.0015(GDD)	0.28
Quadratic	DM	DM=23.12-0.0019+(GDD)+0.00001(GDD^2)	0.48
	CP	CP=28.50+0.0001(GDD)-0.00001(GDD^2)	0.02
	ADF	ADF=34.29+0.0138(GDD)-0.01379(GDD^2)	0.08
	NDF	NDF=8.60-0.0001(GDD)-0.55666(GDD^2)	0.57
	EP	EP=5.03+0.0041(GDD)-0.00001(GDD^2)	0.33
Red clover			
Linear	DM	DM=22.41+0.0010(GDD)	0.35
	CP	CP=23.50+0.0021(GDD)	0.04
	ADF	ADF=33.54+0.0004(GDD)	0.003
	NDF	NDF=7.19+0.0007(GDD)	0.06
	EP	EP=3.08+0.0027(GDD)	0.24
Quadratic	DM	DM=22.87-0.0012(GDD)+0.000002(GDD^2)	0.45
	CP	CP=23.02+0.0045(GDD)-0.000009(GDD^2)	0.04
	ADF	ADF=31.37+0.0112(GDD)-0.000009(GDD^2)	0.16
	NDF	NDF=8.50+0.0004(GDD)-0.040216(GDD^2)	0.29
	EP	EP=2.95+0.0032(GDD)-0.0000004(GDD^2)	0.24

†All equations were not significant at $P \leq 0.05$ level. All linear regression equation calculations were based on 13 d.f. All quadratic equation calculations were based on 12 d.f.

‡GDD=((maximum daily temperature$_{86}$ °F+minimum daily temperature$_{50}$ °F)/2)-50 °F. Growing degree days on °F was converted to °C using the formula: °C=5/9(°F-32).

[Appendix Table A3 (con't)].

Species	Variables	Equations†	r^2
Birdsfoot trefoil			
Linear	DM	DM=22.30+0.0010(GDD)‡	0.34
	CP	CP=27.41-0.0015(GDD)	0.06
	ADF	ADF=35.52-0.0010(GDD)	0.02
	NDF	NDF=6.31+0.0010(GDD)	0.21
	EP	EP=5.26+0.0018(GDD)	0.19
Quadratic	DM	DM=22.84-0.0008(GDD)+0.001013(GDD^2)	0.40
	CP	CP=29.72-0.0095(GDD)+0.000005(GDD^2)	0.15
	ADF	ADF=37.89-0.0091(GDD)+0.000006(GDD^2)	0.08
	NDF	NDF=8.99+0.0001(GDD)-0.733747(GDD^2)	0.57
	EP	EP=3.56+0.0087(GDD)-0.000005(GDD^2)	0.38

†All equations were not significant at $P \leq 0.05$ level. All linear regression equation calculations were based on 13 d.f. All quadratic equation calculations were based on 12 d.f.

‡GDD=((maximum daily $temperature_{86}$ °F+minimum daily $temperature_{50}$ °F)/2)-50 °F. Growing degree days on °F was converted to °C using the formula: °C=5/9(°F-32).

Appendix Table A4. Linear and quadratic regression equations and r^2 values of dry matter (DM) yield, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and escape protein (EP) concentrations of alfalfa, red clover, and birdsfoot trefoil regressed on mean stage by weight (MSW) for five harvest cycles in 1991 and 1992.

Species	Variables	Equations†	r^2‡
Alfalfa			
Linear‡	DM	DM=1.74+0.3916(MSW)	0.18
	CP	CP=23.11+0.0039(MSW)	0.001
	ADF	ADF=24.23+1.5434(MSW)	0.21
	NDF	NDF=30.63+2.3768(MSW)	0.44
	EP	EP=5.45+0.4288(MSW)	0.19
Quadratic	DM	DM=-0.83+2.1230(MSW)+2.12300(MSW^2)	0.27
	CP	CP=22.97+0.1014(MSW)-0.01487(MSW^2)	0.005
	ADF	ADF=10.02+11.1264(MSW)-1.46200(MSW^2)	0.08
	NDF	NDF=8.60-0.0001(MSW)-0.55665(GDD^2)	0.42
	EP	EP=5.03+0.7117(MSW)-0.04315(MSW^2)	0.19
Red clover			
Linear	CP	CP=23.25-0.0792(MSW)	0.008
Quadratic	DM	DM=-3.14-3.142(MSW)-1.49550(MSW^2)	0.43
	CP	CP=21.80+1.682(MSW)-0.46417(MSW^2)	0.06
	ADF	ADF=5.99+19.60901(MSW)-4.22773(MSW^2)	0.63
	NDF	NDF=24.82+7.98382(MSW)-1.26664(MSW^2)	0.29
	EP	EP=-0.51+4.67955(MSW)-0.71965(MSW^2)	0.47
Birdsfoot trefoil			
Linear	CP	CP=23.49-0.18587(MSW)	0.09
	EP	EP=4.99+0.70959(MSW)	0.21
Quadratic	DM	DM=-0.47+2.09390(MSW)-0.26164(MSW^2)	0.58
	CP	CP=22.79+0.50702(MSW)-0.14536(MSW^2)	0.13
	ADF	ADF=17.95+5.70068(MSW)-0.74951(MSW^2)	0.43
	NDF	NDF=22.66+7.85723(MSW)-0.97591(MSW^2)	0.71
	EP	EP=5.21+0.049237(MSW)+0.04557(MSW^2)	0.21

†All linear regression equation calculations were based on 13 d.f. All quadratic regression equation calculations were based on 12 d.f.
‡All equations are not significant, $P \leq 0.05$.

Appendix Table A5. Table of means of dry matter (DM) yield, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and escape protein (EP) concentrations of alfalfa, red clover, and birdsfoot trefoil at early-, medium-,and late-cutting date for five harvest cycles† in 1991 and 1992.

	Species														
	Alfalfa					Red clover					Birdsfoot trefoil				
Cutting date	DM	CP	ADF	NDF	EP	DM	CP	ADF	NDF	EP	DM	CP	ADF	NDF	EP
1st Harvest	Mg/ha	-----	% of DM	-----	% of CP	Mg/ha	-----	% of DM	-----	% of CP	Mg/ha	-----	% of DM	-----	% of CP
Early	2.2	23.4	24.5	36.3	7.2	1.8	23.6	22.5	33.0	5.2	1.3	23.5	22.8	29.8	6.9
Medium	2.9	23.6	26.4	38.5	7.5	2.5	23.6	23.8	34.6	6.5	2.8	23.5	25.5	33.7	7.8
Late	2.9	23.5	27.0	40.1	8.2	3.4	23.5	27.9	34.4	7.6	3.6	23.5	23.7	35.2	9.5
2nd Harvest															
Early	1.7	23.5	24.3	33.2	6.0	2.8	23.5	26.4	32.8	6.5	1.5	23.3	24.4	34.5	6.1
Medium	2.6	23.5	32.1	39.3	8.2	2.6	23.5	27.4	32.9	6.1	2.6	23.5	27.8	34.6	6.5
Late	3.1	23.6	29.0	39.6	7.7	3.1	23.5	29.4	36.6	7.9	3.1	23.6	27.7	36.5	7.3
3th Harvest															
Early	2.1	23.3	24.6	31.5	4.9	2.1	23.2	20.0	30.3	2.2	2.0	23.0	20.7	27.3	4.2
Medium	4.3	23.1	31.2	38.9	5.7	3.8	23.2	24.1	33.8	3.7	3.6	23.2	27.8	36.3	4.9
Late	5.1	22.3	34.8	44.6	6.8	5.2	22.0	31.0	37.8	5.1	5.0	22.0	31.4	40.7	6.2

†Harvest cycle 1: Early=10 Jul., Medium=19 Jul., Late=31 Jul.; Harvest cycle 2: early=13 Aug., Medium=22 Aug., Late=29 Aug.; Harvest cycle 3: Early=15 May, Medium=26 May, Late=6 June; Harvest Cycle 4: Early=1 Jul., Medium=1 Jul., Late=18 Jul.; Harvest cycle 5: Early=10 Aug., Medium=19 Aug., Late=28 Aug.

[Appendix Table A5 (cont'd)].

Cutting date	Species														
	Alfalfa					Red clover					Birdsfoot trefoil				
	DM	CP	ADF	NDF	EP	DM	CP	ADF	NDF	EP	DM	CP	ADF	NDF	EP
4th Harvest	Mg/ha	----	% of DM	-----	% of CP	Mg/ha	----	% of DM	----	% of CP	Mg/ha	----	% of DM	----	% of CP
Early	2.4	22.0	27.1	34.4	6.2	2.1	21.9	22.5	31.3	3.4	3.1	22.0	25.2	32.9	5.2
Medium	2.8	22.3	31.7	39.4	6.4	2.6	22.4	24.2	34.5	4.3	2.9	22.5	31.4	39.2	6.9
Late	3.6	22.4	31.9	41.6	7.9	3.3	22.6	29.9	41.3	7.4	3.4	22.3	29.9	39.2	7.9
5th Harvest															
Early	1.5	22.9	26.0	32.4	7.2	1.2	23.0	20.8	31.5	2.4	0.7	23.0	24.5	31.4	8.1
Medium	3.5	23.6	31.0	38.3	5.9	2.1	23.7	22.4	30.8	3.9	1.9	23.6	23.2	30.6	4.6
Late	3.5	23.7	33.2	41.0	5.8	3.1	23.6	26.0	36.1	2.9	2.5	23.6	28.0	35.0	6.2

†Harvest cycle 1: Early=10 Jul., Medium=19 Jul., Late=31 Jul.; Harvest cycle 2: early=13 Aug., Medium=22 Aug., Late=29 Aug.; Harvest cycle 3: Early=15 May, Medium=26 May, Late=6 June; Harvest Cycle 4: Early=1 Jul., Medium=1 Jul., Late=18 Jul.; Harvest cycle 5: Early=10 Aug., Medium=19 Aug., Late=28 Aug.

Appendix Table A6. Table of means of In situ dry matter digestibility (ISDMD)† of alfalfa, red clover, and birdsfoot trefoil at early-, medium-, and late-cutting date for five harvest cycles in 1991 and 1992.

	Legumes/cutting date								
	Alfalfa			Red clover			Birdsfoot trefoil		
	Early	Medium	Late	Early	Medium	Late	Early	Medium	Late
	%								
1st Harvest cycle	79	78	76	86	84	80	82	77	76
2nd Harvest cycle	82	75	74	85	83	78	82	79	77
3rd Harvest cycle	84	77	74	90	85	83	87	80	77
4th Harvest cycle	78	74	73	85	84	77	81	75	75
5th Harvest cycle	81	78	74	89	86	85	79	85	78
Grand mean	81	76	74	87	84	81	82	79	77

†ISDMD calculated as percent of DM of the herbage sample.
‡Harvest cycle 1: Early=10 Jul., Medium=19 Jul., Late=31 Jul.; Harvest cycle 2: early=13 Aug., Medium=22 Aug., Late=29 Aug.; Harvest cycle 3: Early=15 May, Medium=26 May, Late=6 June; Harvest Cycle 4: Early=1 Jul., Medium=1 Jul., Late=18 Jul.; Harvest cycle 5: Early=10 Aug., Medium=19 Aug., Late=28 Aug.

Appendix Table A7. Correlation coefficients of dry matter (DM) yield, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and escape protein (EP) concentrations of alfalfa, red clover, and birdsfoot trefoil at five harvest cycles in 1991 and 1992.

	Parameters			
Species/parameters	NDF	ADF	CP	EP
Alfalfa				
DM yield (Mg/ha)	0.85*	0.81*	-0.19	-0.01
NDF (%DM)		0.82*	-0.10	0.40
ADF (%DM)			-0.25	0.07
CP (%DM)				0.03
Red clover				
DM yield (Mg/ha)	0.68*	0.77*	-0.29	0.35
NDF (%DM)		0.81*	-0.39	0.56*
ADF (%DM)			-0.15	0.73*
CP (%DM)				0.20
Trefoil				
DM yield (Mg/ha)	0.74*	0.64*	-0.46	0.07
NDF (%DM)		0.93*	-0.45	0.34
ADF (%DM)			-0.48	0.18
CP (%DM)				0.10

*Significant correlation, $P \leq 0.05$.

Appendix Table A8. Dry matter (DM) yield and crude protein (CP) yield total over a two-year study for three legume species (alfalfa, red clover, and birdsfoot trefoil) at three cutting dates† (early-, medium-, and late-).

	Species		
	Alfalfa	Red clover	Trefoil
	---------- Mg/ha ----------		
DM yield			
Early	10.0[a]	8.5[a]	9.1[a]
Medium	15.8[b]	13.6[b]	13.9[b]
Late	18.2[c]	17.7[c]	18.1[c]
C.V.	8.0	8.7	5.2
CPyield			
Early	2.3[a]	1.9[a]	2.0[a]
Medium	3.4[b]	3.4[b]	3.2[b]
Late	4.2[c]	4.0[c]	4.2[c]
C.V.	13.3	10.4	5.8

†Early cutting date: 10 July and 13 August 1991 and 15 May, 1 July and 10 August 1992; medium cutting date: 19 July and 22 August 1991 and 26 May, 9 July, and 19 August 1992; late cutting date: 31 July and 29 August 1991 and 6 June, 18 July, and 28 August 1992.

[abc]Numbers within column followed by the same letter are not significantly different ($P \leq 0.05$).